JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Changes in Virgin Olive Oil Quality during Low-Temperature Fruit Storage

CURTIS M. KALUA,* DANNY R. BEDGOOD, JR., ANDREA G. BISHOP, AND PAUL D. PRENZLER

E. H. Graham Centre for Agricultural Innovation, School of Wine and Food Science, Locked Bag 588, Charles Sturt University, Wagga Wagga 2678, Australia

'Frantoio' olive fruits were stored at low temperature (4 ± 2 °C) for 3 weeks to investigate the effect of postharvest fruit storage on virgin olive oil quality. Volatile compounds and phenolic compounds explained the changes in sensory quality that could not be explained with quality indices (FFA, PV, K_{232}, and K_{270}). Increases in concentrations of (*E*)-2-hexenal and hexanal corresponded to positive sensory quality, whereas increases in (*E*)-2-hexenol and (+)-acetoxypinoresinol were associated with negative sensory quality. Volatile and phenolic compounds were also indicative of the period of low-temperature fruit storage. Oleuropein and ligstroside derivatives in olive oil decreased with respect to storage time, and their significant ($p < 0.05$) change corresponded to changes in bitterness and pungency. (*Z*)-2-Penten-1-ol increased during low-temperature fruit storage, whereas 2-pentylfuran decreased. Changes in volatile compounds, phenolic compounds, quality indices, and sensory notes indicated that virgin olive oil quality was lost within the first week of low-temperature fruit storage and regained at 2 weeks. This research suggests that low-temperature olive fruit storage may be beneficial, with a possibility of increasing oil yield and moderating the sensory quality of virgin olive oils. This study demonstrates that deeper insights into virgin olive oil quality changes during low-temperature fruit storage may be gained by studying volatile and phenolic compounds in addition to quality indices and physical appearance of the fruit.

KEYWORDS: Volatile compounds; phenolic compounds; quality indices; low-temperature fruit storage; virgin olive oil; 'Frantoio' fruit

INTRODUCTION

Most guides to olive processing recommend that oil be extracted as soon as possible after the fruit has been harvested (*1–3*). This is to minimize potential defects in olive oil, such as "mustiness" and "fustiness", which result from microbial damage during fruit storage (*4*). On the other hand, if harvesting capacity exceeds processing capacity, some form of fruit storage is inevitable—be it short-term, days; or medium-term, weeks. Ideally, storage conditions should preserve olive fruit quality and minimize deterioration processes, which might introduce defects in the oil (*3*).

Variables that affect storage of olive fruit include storage temperature, storage time, rate of cooling, relative humidity, maturity, cultivar, storage media (e.g., air, water or brine), and modified atmospheres (e.g., reduced ambient oxygen and/or increased carbon dioxide concentration) (*5–7*). Among these variables, low-temperature fruit storage with temperatures ranging from 0 to 8 °C (*8–12*) and modified atmosphere storage (*5, 7, 13*) have received some attention due to their potential for maintaining olive fruit quality and considerably prolonging the fruit storage period. The effect of storage media on olive oil quality has been reported (*6*) for a study in which olives were kept in seawater (traditional Croatian practice), brine, water, and air at 10, 20, and 30 °C.

Low temperatures (3–5 °C) are usually used to preserve the quality of olive fruit. Low temperatures reduce the rate of chemical reactions and microbial activity that may result in loss of olive fruit quality and subsequent loss of quality of the extracted oil. However, even though low-temperature storage reduces the rate of reactions in the fruit, there is an enhancement of mechanical, physiological, and physicochemical alterations involved during fruit ripening and senescence, such as softening, respiration, ethylene production, and the activity of pectic enzymes (*8, 14*). Whereas low temperatures are associated with mechanical damage of the fruit, temperatures above 5 °C accelerate chemical reactions and promote microbial growth (*5, 7, 9, 12*). This calls for a strict control of fruit storage temperatures to maintain the quality of virgin olive oil.

Low-temperature fruit storage may cause cell structure breakdown resulting from mechanical damage due to frozen extracellular water usually referred to as chilling injury (*14*). The freezing of extracellular water causes cellular dehydration

* Address correspondence to this author at the CSIRO Plant Industry and Food Futures Flagship, P.O. Box 350, Glen Osmond, SA 5064, Australia (telephone 61-8-8303 8605; e-mail curtis.kalua@csiro.au).

10.1021/jf073027b CCC: $40.75
Published on Web 03/06/2008

and physical membrane destruction by ice crystals, resulting in contact between enzymes and their respective substrates (*14*). Softening of fleshy fruit cell wall tissue is characterized by modification and degradation of cell wall components through depolymerization, deesterification, and loss of neutral sugar side chains from the pectic fraction of the cell wall (*15*). Decreases in total phenols and quality indices in olive oil produced from fruit after low-temperature storage have been reported (*5*). Even though there is evidence of degradation of olive fruit cell wall components (*15*) and decrease of total phenols in the oil (*5*), studies on the effect of low temperature on phenolic compounds during fruit storage are rare.

Studies on the quality changes in the fruit during storage have focused on physical parameters such as firmness, decay incidence, fungus development, and visual quality (*5, 7, 9, 12*), which provide information on external quality changes but little information on the changes of specific fruit components that might eventually affect oil quality. The effects of low-temperature fruit storage on olive oil quality have been investigated mainly on the basis of quality indices and sensory quality (*9, 12, 16*) with a few studies looking directly at other components of olive oil such as volatile compounds (*6*), sterol fraction, fatty acid composition, and acidity (*10*). The common quality indices for olive oil (*2, 17–19*) are peroxide values (crude measure of the amount of primary oxidation that has occurred in olive oil), free fatty acids (measurement of hydrolytic breakdown of the fatty acid chains from triglycerides into diglycerides and monoglycerides), K_{232} (crude measure of primary oxidation products that absorb at 232 nm, usually representing peroxides), and K_{270} (crude measure of secondary oxidation products that absorb at 270 nm, usually representing short chain aldehydes and ketones). It is interesting to note that the lack of effect of low-temperature fruit storage on olive oil quality indices compared with the marked changes in phenolic (*14*) and volatile compounds (*6*) has not shifted the focus of researchers toward investigating changes in volatile and phenolic compounds during low-temperature fruit storage.

The limited parameters used as indicators in the reported studies and the differences in the time intervals at which olive oil quality is monitored limit the comprehension of investigations on the effects of low-temperature fruit storage on virgin olive oil quality. For instance, peroxide values (PV) were not significantly ($p < 0.05$) different during low-temperature (5 °C) fruit storage after 30 and 60 days (*5*), whereas monitoring at shorter intervals showed a significant ($p < 0.01$) increase from 0 to 7 days with a decrease in 14 days (*8*). There are also conflicting reports in the literature on the effectiveness of storage of olive fruit prior to oil extraction. For example, García et al. (*16*) found that fruit storage at 5 °C maintained the initial sensorial and chemical qualities of olive oil for 45 days, whereas Pereira et al. (*8*) noted a decline in oil quality (measured by quality indices) after just 7 days of storage at 5 °C. Contrary to the general view that oil quality declines, olive oil extracted after 30 days of air storage at ambient temperature was characterized by better odor properties than oil extracted after 10 and 20 days (*6*). However, these observations did not correspond to qualitative and quantitative changes in volatile compounds. This is an interesting observation that calls for further investigation to find out the cause for changes in odor properties that do not correspond to volatile compounds. With regard to phenolic compounds, which are also related to the sensory quality of olive oil (*20, 21*), their concentrations were reported to decrease in olive oil extracted from frost-damaged fruit, whereas no differences in quality indices of olive oil were observed (*14*). This emphasizes the importance of monitoring phenolic and volatile compounds in both olive oil and fruit (in addition to quality indices) to detect subtle changes that might have an overall effect on the stability and quality of the oil extracted from the stored fruit.

This paper reports on a trial carried out in Australia on the effect of low-temperature fruit storage on virgin olive oil quality extracted from stored fruit based on volatile and phenolic compounds in addition to quality indices. The study was conducted on 'Frantoio' olive cultivar, which is a popular and widely grown olive cultivar in Australia and other parts of the world such as Italy and Spain (*22*). The objective of this study was to investigate the subtle changes in virgin olive oil quality (shown through volatile and phenolic compounds) during low-temperature fruit storage. This study is the first of its kind to investigate phenolic compounds in both the fruit and oil simultaneously with volatile compounds in virgin olive oil during low-temperature fruit storage.

MATERIALS AND METHODS

Materials. Standards and reagents from the indicated sources were used without further purification. Phenolic standards included caffeic acid, *p*-coumaric acid, and gallic acid (Sigma, St. Louis, MO); tyrosol (Aldrich, Milwaukee, WI); hydroxytyrosol (Sapphire Bioscience, Sydney, Australia); and oleuropein (Extrasynthese, Genay, France). Standards were prepared in methanol + water (50 + 50 v/v) and filtered through 0.45 μm plastic nonsterile filters prior to chromatographic analysis. Grade 1 water (ISO3696) purified through a Milli-Q water system was used for chromatographic preparations.

Volatile standards included pentanal, (*E*)-2-hexenal, and nonanol (Merck, Hohenbrunn, Germany); hexanal, heptanal, (*E*)-2-octenal, (*E*)-2-nonenal, 1-penten-3-ol, 2-penten-1-ol, heptanol, octanol, hexyl acetate, methyl isobutyl ketone (MIBK), and 2-nonanone (Aldrich); octanal, octane, nonane, decane, undecane, and dodecane (Sigma); benzaldehyde (Ajax Chemicals, Auburn, Australia); ethanol and acetic acid (Biolab, Sydney, Australia); ethyl acetate (Mallinckrodt Chemicals, Paris, France); and hexanol (Riedel de Haen, Seelze, Germany).

Reagents were as follows: chloroform, acetic acid, and potassium iodide (Biolab), sodium thiosulfate (Asia Pacific Specialty Chemicals Ltd., Seven Hills, Australia), and starch (Scharlau Chemie S. A., Barcelona, Spain) for peroxide values (PV); cyclohexane spectrophotometric grade (Sigma) for UV absorbances (K_{232}, K_{270}, and ΔK); and propan-2-ol (Mallinckrodt Chemicals), sodium hydroxide (Ajax Chemicals), and phenolphthalein indicator (Sigma) for free fatty acid (FFA) determination. Acetic acid (Biolab), hexane and methanol (Mallinckrodt Chemicals), acetonitrile (J. T. Baker, Phillipsburg, NJ), and formic acid (Sigma) were used in phenolic compounds analysis.

Low-Temperature Olive Fruit Storage. 'Frantoio' olive fruit (3 × 100 kg) harvested in the 2005 olive harvest season from Riverina region, New South Wales, Australia, was kept in crates in a cold room (4 ± 2 °C) and industrially extracted with a two-phase decanter every week for 3 weeks. The industrial extraction was done in duplicates (2 × 50 kg) with a homogeneous composite sample from all three crates. Virgin olive oil from the same 'Frantoio' batch (as stored fruit) was used to establish the properties of oil processed from nonstored fruit at 0 weeks. The oil extracted from the olive fruit was stored (<1 week) in the dark at room temperature prior to analysis of quality indices (PV, FFA, K_{232}, and K_{270}), volatile compounds, and phenolic compounds. Virgin olive oil sensory descriptors and oil yield were provided by the processor. Phenolic compounds in the olive fruit were analyzed to monitor changes during low-temperature storage.

Qualitative and Quantitative Analysis of Phenolic Compounds. Qualitative and quantitative analysis of the phenolic compounds in **Table 1** 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 an earlier paper (*23*). Phenolic compounds were extracted with 50 + 50 (v/v) methanol + water solutions (3 × 1 mL)

Table 1. Variables Detected and Measured in Oil and Fruit during Low-Temperature Olive Fruit Storage

fruit phenolic compounds	oil phenolic compounds	volatile compounds	quality and yield
hydroxytyrosol	hydroxytyrosol	acetic acid	free fatty acid (FFA)
tyrosol	tyrosol	1-penten-3-ol	peroxide value (PV)
luteolin rutinoside	vanillic acid	(*Z*)-2-penten-1-ol	K_{232}
caffeic acid	3,4-DHPEA-DEDA[a]	octane	K_{270}
verbascoscide	ligstroside dialdehyde	hexanal	ΔK
luteolin glucoside	ligstroside derivatives	(*E*)-2-hexenal	maturity index (MI)
ligstroside derivatives	oleuropein derivatives	(*E*)-2-hexen-1-ol	oil yield
oleuropein derivatives	(+)-pinoresinol	hexanol	
	(+)-acetoxypinoresinol	6-methyl-5-hepten-2-one	
	oleuropein aglycone	2-pentyl furan	
		(*E*)-2-nonen-1-ol	

[a] 3,4-Dihydroxyphenylethyl alcohol−decarboxymethylelenolic acid dialdehyde.

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. Phenolic compounds in olive fruits were extracted from a sample (1 g) crushed in liquid nitrogen and immediately blended with methanol + water (5 mL, 50 + 50 v/v) and gallic acid (0.5 mL, 100 μg/g) as an internal standard using an Ultra Turax blender. The blended sample was left to stand for 30 min at ambient temperature and filtered (GF/F filter paper) using Büchner filtration apparatus. The solid mass was recovered and re-extracted as above, but now the blended sample was left to stand for 15 min prior to filtering. The filtrates were combined and washed with hexane (3 × 5 mL). The methanolic extracts, from both the fruit and oil, were washed with hexane and filtered through 0.45 μm plastic nonsterile filter prior to qualitative and quantitative analysis. Quantification of oleuropein and ligstroside was combined with those of their respective hemiacetals and dialdehydes and classified as oleuropein and ligstroside derivatives, respectively. Aglycone forms of oleuropein and ligstroside were quantified separately.

Qualitative and Quantitative Analysis of Volatile Compounds. Qualitative and quantitative analysis of the volatile compounds in **Table 1** 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 papers (*23, 24*) with a DVB-CAR-PDMS, 50/30 μm fiber.

Determination of Quality Parameters. Determination of FFA, PV, and UV absorbances (*K* values) was performed according to the EC and IOOC standard methods (*2, 18*). These parameters (PV, FFA K_{232}, K_{270}, and ΔK) are commonly used to assess the quality of olive oil (*2, 17–19*) and were used to investigate the effect of low-temperature fruit storage on the quality of virgin olive oil.

The maturity index (MI) of the olive fruits (**Table 2**) was assessed using the method of the Instituto Nacional de Investigaciones Agronomicas, Estacion de Jaen (Spain), and described by IOOC (*1*). The method assesses the color of the olive skin and pulp with zero representing the lower limit of MI characterized by olive fruits with an intense green or dark green epidermis and a value of seven for the upper limit of maturity characterized by olive fruits with a black epidermis and a totally black pulp (*1*).

Statistical Data Analysis. Parameters that significantly ($p < 0.05$) changed during low-temperature fruit storage (**Table 2**) were determined using one-way ANOVA post hoc multiple-comparison tests using Duncan's test with SPSS 12.0 (SPSS Inc., Chicago, IL). Volatile and phenolic compounds were of different magnitudes (**Table 2**) and to necessitate comparison of trends on a similar reference scale, standardized normal variables (statistical *z* scores over the 3 weeks of storage period) were plotted (**Figures 1−3**) with Sigma Plot 10.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Changes in concentration of volatile compounds in virgin olive oil and in phenolic compounds in both the fruit and oil during low-temperature fruit storage were measured to explore the effect on virgin olive oil quality. Prior to the main study reported here, a preliminary study was conducted for 6 weeks, in the 2003 harvest season (data not shown), to devise an experimental/sampling design and investigate the sampling interval, minimum storage period before changes were registered in quality, and, finally, the parameters that were changing with low-temperature fruit storage.

After the first 2 weeks of the preliminary study, extracted oil showed changes in quality such that a shorter sampling interval of 1 week was used for the subsequent (main) study. The second sampling in the preliminary study, after 4 weeks, showed further quality deterioration for the oil and often resulted in volatile compounds and phenolic compounds decreasing to levels below their limits of detection (LOD). This necessitated the setting of 3 weeks as a study period for the subsequent study in the 2005 harvest season.

The significant changes in the parameters at 0, 2, 4, and 6 weeks of low-temperature fruit storage in the preliminary study were compared to the changes in the main study to identify parameters that effectively described changes during low-temperature olive fruit storage. Parameters that significantly ($p < 0.05$) changed in both the preliminary study and the main study were identified, and their trends were compared and discussed with the application of statistical z-score.

Olive Fruit Trends in Phenolic Compounds during Low-Temperature Storage. Changes in the phenolic compounds of olive fruit during low-temperature storage may have subsequent effects on virgin olive oil composition and quality. Low-temperature fruit storage showed an increase in levels of fruit ligstroside derivatives and tyrosol at weeks 1 and 3 (**Figure 1**), which coincided with oil of poor sensory quality (**Table 2**). These changes in phenolic compounds and other olive fruit components may indicate interactions between intra- and extracellular components culminating in additional oil components, which may assist in understanding virgin olive oil quality. Increase in concentrations of ligstroside derivatives and tyrosol in the fruit at weeks 1 and 3 (**Table 2**) can be associated with their formation before and after cell wall degradation at weeks 1 and 3, respectively. The increasing concentration during the first week of storage might indicate a shift in equilibrium whereby the fruit components try to oppose any change to the initial status. The re-establishment of equilibrium can be observed with fruit hydroxytyrosol (a hydrolytic product of oleuropein) and oleuropein derivatives (**Figure 1**) whereby a change from positive to negative *z* scores from week 0 to 1 for oleuropein derivatives is accompanied by an increase toward more positive *z* scores for hydroxytyrosol (**Figure 1**).

The general trend of increase in *z* scores with duration of low-temperature fruit storage was observed for hydroxytyrosol and luteolin rutinoside in olive fruit (**Figure 1**). Luteolin rutinoside and hydroxytyrosol in the fruit was earlier (*23*) observed to increase with fruit maturity, which corresponds to increase in the porosity of cell wall (*15*).

Table 2. Virgin Olive Oil Quality Indices, Yield, and Volatile and Phenolic Compounds during Fruit Storage[a]

	time				
	0 weeks	1 week	2 weeks	3 weeks	max limit[d]
			Quality Indices and Yield		
FFA[b]	0.12 ± 0.01 a	0.16 ± 0.01 b	0.22 ± 0.01 c	0.14 ± 0.01 ab	0.8
PV[c]	9.50 ± 0.06 a	14.1 ± 0.2 b	13 ± 1 b	12 ± 1 ab	20
K_{232}	1.53 ± 0.01 a	1.84 ± 0.01 c	1.74 ± 0.01 b	1.72 ± 0.01 b	2.50
K_{270}	0.10 ± 0.01 a	0.11 ± 0.01 b	0.10 ± 0.01 a	0.11 ± 0.01 b	0.22
maturity index (MI)	2.65 ± 0.04 b	2.56 ± 0.04 ab	2.46 ± 0.05 a	2.92 ± 0.06 c	NA
yield (% v/w)	21.58	32.84	34.83	17.17	NA
sensory notes	mild fruity, bitterness, pepper, and pungency	flat, bland oil	nice mild fruity, bitterness, pepper, and pungency	fatty, no bitterness, and no fruitiness	NA
			Oil Volatile Compounds[e]		
(*Z*)-2-penten-1-ol	<0.02	<0.02	0.08 ± 0.01 a	0.15 ± 0.04 b	NA
hexanal	2.4 ± 0.1 b	1.8 ± 0.1 ab	3.2 ± 0.3 c	1.3 ± 0.3 a	NA
(*E*)-2-hexenal	7.8 ± 0.6 b	4.0 ± 0.2 a	5.2 ± 0.3 a	4.0 ± 0.9 a	NA
(*E*)-2-hexen-1-ol	<0.03	0.06 ± 0.01 ab	<0.03	0.11 ± 0.02 b	NA
2-pentyl furan	0.22 ± 0.02 a	0.10 ± 0.01 b	0.08 ± 0.01 b	<0.02	NA
total volatiles [f]	10.7 ± 0.1 a	6.7 ± 0.4 b	8.8 ± 0.6 ab	7 ± 1 b	NA
			Oil Phenolic Compounds[e]		
hydroxytyrosol	0.19 ± 0.03 ab	0.36 ± 0.09 c	0.30 ± 0.01 bc	0.13 ± 0.01 a	NA
tyrosol	0.56 ± 0.05 a	1.3 ± 0.1 b	1.3 ± 0.1 b	0.8 ± 0.1 a	NA
vanillic acid	0.18 ± 0.01 a	0.06 ± 0.01 b	<0.05	<0.05	NA
ligstroside derivatives	24.2 ± 0.2 a	23 ± 8 a	18 ± 2 a	3.2 ± 0.5 b	NA
oleuropein derivatives	27 ± 3 a	13.4 ± 0.9 b	12 ± 1 b	4.7 ± 0.1 c	NA
pinoresinol	17 ± 8 a	6 ± 3 ab	3 ± 1 b	2.8 ± 0.3 b	NA
acetoxypinoresinol	82 ± 8 a	97 ± 2 b	77 ± 2 a	94 ± 1 b	NA
oleuropein aglycon	15 ± 3 a	8 ± 1 b	6 ± 3 b	5.1 ± 0.7 b	NA
			Fruit Phenolic Compounds[e]		
hydroxytyrosol	33 ± 2 a	45 ± 15 a	43 ± 1 a	108 ± 3 b	NA
tyrosol	118 ± 11 a	233 ± 42 b	123 ± 47 a	189 ± 4 ab	NA
luteolin rutinoside	146 ± 1 a	276 ± 38 b	299 ± 63 b	638 ± 42 c	NA
luteolin glucoside	138 ± 35 a	269 ± 44 b	114 ± 7 a	530 ± 35 c	NA
ligstroside derivatives	966 ± 81 a	1727 ± 69 b	726 ± 140 a	1530 ± 175 b	NA
oleuropein derivatives	328 ± 54 a	113 ± 6 c	220 ± 34 b	376 ± 27 a	NA

[a] Different letters in a row indicate significantly different ($p < 0.05$) mean ± standard deviation of independent duplicate samples. [b] Free fatty acid as percent oleic acid. [c] Peroxide value expressed as milliequivalents of oxygen per kilogram of oil. [d] Maximum allowable limit as specified by IOOC for extra virgin olive oil. [e] Concentrations of phenolic and volatile compounds are expressed in micrograms per gram. [f] Concentration expressed as micrograms per gram of (*E*)-2-hexenal based on total area counts.

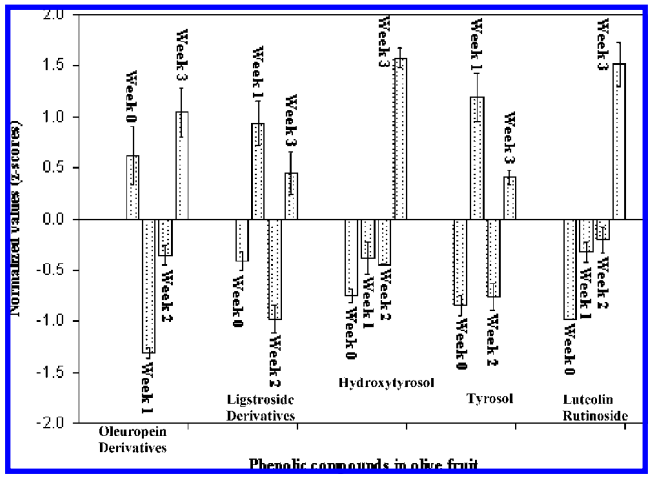

Figure 1. Trends in olive fruit phenolic compounds during low-temperature fruit storage. Error bars represent standard errors for independent duplicate samples.

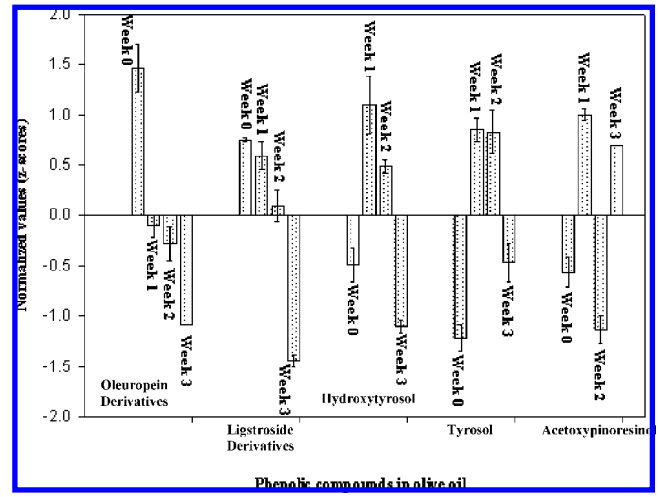

Figure 2. Trends in olive oil phenolic compounds during low-temperature fruit storage. Error bars represent standard errors for independent duplicate samples.

Quantitative data on all fruit phenolic compounds that were significantly ($p < 0.05$) affected during fruit storage (**Table 2**) show that from week 2 to 3, all fruit phenolic compounds increased in their concentrations (**Figure 1**), which provides further evidence of cell structure destruction, whereby bound phenolic compounds are released and interact in the cell sap. The interactions between phenolic compounds showed different effects in olive oil and fruit. Whereas phenolic compounds in the fruit (hydroxytyrosol and luteolin rutinoside) continuously increased (**Figure 1**) with fruit storage, phenolic compounds in the oil (oleuropein and ligstroside derivatives) continuously decreased (**Figure 2**). This main difference of an increase in fruit phenolic compounds and a decrease in oil phenolic compounds might indicate an interaction between reactive phenolic compounds (oleuropein and ligstroside) with other substrates that are released with an increase in the porosity of the cell wall. Accelerated cell wall degradation using enzymes

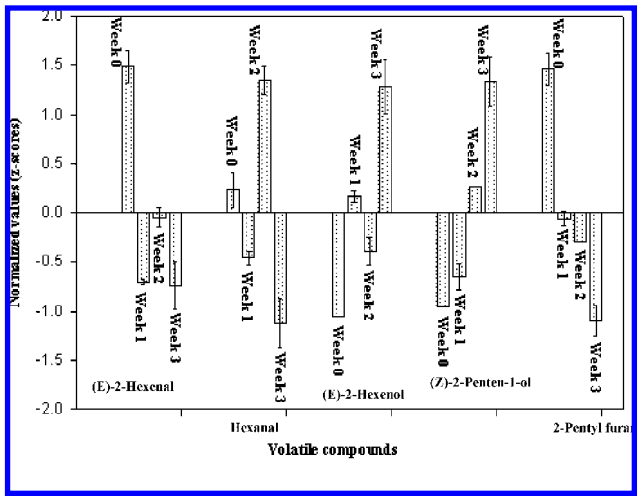

Figure 3. Trends in volatile compounds during low-temperature fruit storage. Error bars represent standard errors for independent duplicate samples.

during mechanical extraction of virgin olive oil has been found to increase the concentration of phenolic compounds in olive paste and oil (*25*). In our case of slow cell wall degradation, the time for interactions between intra- and extracellular components in the fruit is extended and does not always lead to an increase in phenolic compounds (**Figure 1**) in the extracted virgin olive oil.

Two forms of this interaction, antioxidative and hydrolytic emulsifying tendencies, are hypothesized for this lack of proportionality in the increase of phenolic compounds in the fruit and oil. For instance, oleuropein, an *o*-diphenol, could be more active in controlling oxidation (*26*) than ligstroside, a monophenol, explaining the different trends (**Figure 1**) of these two compounds. Additionally, due to the different structures, oleuropein and ligstroside could have different emulsifying properties. Both oxidation and release of emulsifiers through hydrolysis with olive fruit storage are evident from the significant ($p < 0.05$) increase in PV and FFA, respectively, after 1 week of storage (**Table 2**) and could affect the partitioning of phenolic compounds.

Olive Oil Trends in Levels of Phenolic Compounds during Low-Temperature Fruit Storage. It should be noted that phenolic compounds change during the extraction of oil and that not all compounds present in the fruit end up in virgin olive oil. Among the phenolic compounds detected in olive oil, lignans (pinoresinol and acetoxypinoresinol) were not detected in olive fruit (**Table 1**). During fruit storage, an increase in levels of acetoxypinoresinol at weeks 1 and 3 (**Figure 2**) interestingly coincided with poor sensory quality (**Table 2**). This may suggest that the conditions conducive to the formation of acetoxypinoresinol are similar to the conditions for production of poor sensory quality virgin olive oil. Our earlier results (*27*) identified acetoxypinoresinol as a discriminating variable characterizing low-temperature olive oil storage with higher concentrations at low temperature than ambient-temperature storage conditions. This observation might indicate that the formation of lignans, such as acetoxypinoresinol, is favored at low temperatures.

Other phenolic compounds in the oil, such as oleuropein and ligstroside derivatives, progressively decreased at different rates during low-temperature fruit storage (**Figure 2**). The statistical *z* score for oleuropein derivatives was negative after only 1 week of fruit storage compared to 2 weeks for ligstroside derivatives (**Figure 2**), indicating a faster degradation rate of oleuropein derivatives than ligstroside derivatives. The significantly ($p <$ 0.05) higher values for oleuropein and ligstroside derivatives for fresh fruit (week 0) than stored fruit (week 3) relative to sensory quality (**Table 2**) are consistent with earlier reports (*21, 28, 29*) that associated these phenolic compounds with bitterness and pungency. A similar effect of low temperature on the taste of olive oil was reported (*14*) earlier for oils extracted from frost-damaged olives that were less pungent and had no bitterness. The change in sensory properties was attributed to the decrease of oleuropein derivatives and slight rises in concentrations of simple phenolic compounds such as vanillic acid that gave rise to sweeter oils (*14*).

These simple phenolic compounds, for instance, hydroxytyrosol and tyrosol, are formed from the hydrolysis of high molecular weight glycosylated phenolic compounds, such as oleuropein and ligstroside (*30–33*). A shift from high molecular weight compounds to low molecular weight compounds during olive fruit aging was earlier reported (*15*) and attributed to hydrolysis of glycosylated compounds. In our study, an increasing trend for hydroxytyrosol and tyrosol in weeks 1 and 2 (**Figure 2**) suggests a possible increase in the hydrolytic activity in the fruit. This is further supported by an increase in FFA, a hydrolytic product of major lipid component triglycerides, which coincided with a significant ($p < 0.05$) increase in hydroxytyrosol and tyrosol (**Table 2**).

The negative *z* scores for hydroxytyrosol and tyrosol in the oil at 3 weeks of fruit storage (**Figure 2**) indicate the advanced stages of hydrolysis culminating to emulsion formation and consequently the preferential partitioning of the simple phenolic compounds into the hydrophilic waste stream. Further evidence of advanced stages of hydrolysis in the third week can be observed from the low oil yield (**Table 2**), whereby oil is probably lost in the emulsions. In addition to hydrolysis, the negative *z* scores for hydroxytyrosol and tyrosol in the oil at 3 weeks might be due to oxidative losses as the phenolic compounds protect the oil from oxidation, which can be observed from the significantly ($p < 0.05$) higher values of PV in oil extracted from the stored fruit than fresh oil (**Table 2**). The higher decline in hydroxytyrosol than tyrosol from week 2 to 3 (**Figure 2**) is consistent with the higher potential of hydroxytyrosol than of tyrosol in deterring the oxidative formation of volatile compounds (*34*).

Volatile Compounds Trends during Low-Temperature Fruit Storage. Volatile compounds are predominantly generated during virgin olive oil extraction, unlike phenolic compounds, which are components of the fruit (*35, 36*). These compounds are important contributors to olive oil sensory quality (*20, 37, 38*). Changes in olive fruit quality during postharvest handling have been shown to affect the sensory quality of olive oil (*10, 16*). In this study, low-temperature postharvest fruit storage showed a decrease in levels of (*E*)-2-hexenal and hexanal with respect to the mean concentrations (expressed as statistical *z* scores) at weeks 1 and 3 (**Figure 3**), which coincided with oil of poor sensory quality (**Table 2**), and can be associated with a decrease in enzyme activity. Both (*E*)-2-hexenal and hexanal are generated enzymatically (*35, 36*) with the later also formed through chemical oxidation (*38, 39*). A decrease in hydroperoxide lyase (HPL) activity is hypothesized in this situation while chemical oxidation takes precedence. Whereas there was a decrease in levels of (*E*)-2-hexenal and hexanal at weeks 1 and 3, a concurrent increase in (*E*)-2-hexenol (**Figure 3**) was observed, which might indicate a possible enzymatic reduction of (*E*)-2-hexenal to (*E*)-2-hexenol with the aid of alcohol dehydrogenase (*36*). The probable enhancement of alcohol dehydrogenase activity was earlier observed during air storage of olive fruits, during which hexanal was reduced to hexanol (*6*).

The increase in (*E*)-2-hexenal with fruit storage has been rarely reported with the exception of Koprivnjak et al. (*6*), who observed an increase in concentration of (*E*)-2-hexenal with olive fruit storage for 10 days in cool dry air. Our results (**Table 2**) show significantly ($p < 0.05$) higher concentrations for (*E*)-2-hexenal in fresh oil (week 0) than in oil extracted from stored fruit at weeks 1, 2, and 3. However, there was a slight increase for (*E*)-2-hexenal during fruit storage at week 2 (**Table 2**), consistent with the observations of Koprivnjak et al. (*6*).

Hexanal had significantly ($p < 0.05$) higher concentrations at 2 weeks of low-temperature fruit storage than at weeks 0, 1, and 3 (**Table 2**). The increase in concentration for hexanal in this study is not consistent with earlier observations (*6*) based on the 'Bjelica' olive cultivar, which lost 90% of hexanal after storage of olive fruits in the open air for 10 days. However, the high levels of (*E*)-2-hexenal and hexanal, which coincided with positive sensory characteristics (**Table 2**), are consistent with earlier reports (*38, 40*) that associated these volatile compounds with positive sensory characteristics reminiscent of premium virgin olive oil quality.

Apart from (*E*)-2-hexenal, hexanal, and (*E*)-2-hexenol, which changed with the sensory quality of olive oil, (*Z*)-2-penten-1-ol and 2-pentylfuran significantly ($p < 0.05$) changed with the period of fruit storage (**Table 2**). Levels of (*Z*)-2-penten-1-ol increased with weeks of fruit storage, whereas 2-pentylfuran decreased (**Figure 3**). Volatile alcohols with five carbon atoms, such as (*Z*)-2-penten-1-ol, have been reported (*41*) to increase with time during olive oil extraction, whereas an increase in 2-pentylfuran was observed (*39*) with olive oil storage time. The increase in (*Z*)-2-penten-1-ol during fruit storage is similar to its behavior during oil extraction (*41, 42*), whereas a decrease in 2-pentylfuran is the reverse of what happens during olive oil storage (*27, 39*), which illustrates the different effects of time on sensory quality when the oil is within the fruit matrix and after extraction. In the fruit, storage favors interactions between enzymes and substrates because of cell wall degradation, which might promote enzymatic generation of volatile compounds (*6*) associated with positive sensory quality while suppressing the chemical formation of volatile compounds linked to oxidative rancidity, such as 2-pentylfuran (*39*). It can be hypothesized that the trends of (*Z*)-2-penten-1-ol and 2-pentylfuran were from the combination of loss of freshness from chemical oxidation (*27*) and the different enzyme activities from the LOX pathway (*36, 43*). The aldehyde, (*Z*)-2-pentenal, formed from the hydroperoxide lyase (HPL) activity, could have been reduced to alcohol, (*Z*)-2-penten-1-ol, with anaerobic storage conditions. An earlier observation was reported (*27*) for hexanal and hexanol, for which the aldehyde was an oxidation marker with oil storage in the presence of oxygen and the alcohol in absence of oxygen. The reductive nature of the fruit as observed from the reduction of aldehydes to alcohols could explain the decrease of 2-pentylfuran. It can be hypothesized that 2-pentylfuran, usually associated with late oxidation stages (*39*), was quickly consumed under these oxygen-deficient conditions.

Effect of Low-Temperature Fruit Storage on Virgin Olive Oil Quality Indices and Yield. Low-temperature fruit storage changed the quality of virgin olive oil as shown above with volatile compounds and phenolic compounds and further illustrated in **Table 2**, where the common olive oil quality indices, FFA, PV, K_{232}, and K_{270} (*2, 19*), significantly ($p < 0.05$) changed during the 3 week storage period. The quality indices (FFA, PV, K_{232}, and K_{270}) of olive oil in this study had minimum values at time zero, which were below the maximum limits for extra virgin olive oil (**Table 2**). Additionally, this oil extracted at time zero had positive sensory descriptors (**Table 2**).

At 1 week of low-temperature fruit storage, the sensory quality of olive oil deteriorated to flat and bland (**Table 2**), losing all of the aroma and taste of the oil extracted from fresh fruit (week zero). There was a gain in oil yield relative to time zero (**Table 2**) regardless of the loss in quality. A gain in oil yield was earlier reported (*44*) during oil extraction with the aid of enzymes that degraded the cell walls of oil-bearing cells. The gain in oil yield in this study is probably from a similar effect of cell wall degradation due to low-temperature fruit storage.

Virgin olive oil extracted at 1 week had maximum values for oxidation indicators (PV and K_{232}), but all of the quality indices were below the maximum limit for extra virgin olive oil (**Table 2**). These quality indices subsequently decreased at 2 weeks (**Table 2**). An increase in PV within the first 7 days and thereafter a decrease at 14 days of storage, similar to the observation in this study, had been observed earlier (*8*) and was attributed to the probable consumption of minor components, such as phenolic compounds, which would make the formation of peroxides difficult.

Interestingly, good sensory properties were regained at 2 weeks of storage with the re-emergence of the fruity aroma, bitter taste, and pungency (**Table 2**). Most of the quality indices improved with respect to oil extracted from fruit stored for 1 week except for FFA, which reached a maximum (**Table 2**). This maximum FFA value indicated an increased hydrolytic activity, and it coincided with maximum oil yield, which suggests that most of the oil trapped in the cell walls was easily released. Apart from associating cell wall degradation with an increase in oil yield (*44*), the degradation of olive fruit cells during olive oil processing has been reported (*45*) to result in enhanced oil quality with higher hydrolyzable phenolic compounds and sensory scores. Modification and degradation of cell wall components through depolymerization, deesterification, and loss of neutral sugar side chains of the pectic fraction has been reported (*15*) during the aging of olive fruits, resulting in tissue softening. During olive oil extraction with the aid of enzymes, the cell-softening process is accelerated. In contrast, low-temperature storage allows for a slow natural degradation, which can have the negative impact of hydrolyzing triglycerides, leading to high FFA values. In our case, the cell wall porosity probably increased with low-temperature fruit storage and favored interactions between intra- and extracellular components. Hence, the improved sensory quality may be explained by the fact that fatty acid substrates were in contact with enzymes for a longer time. The re-emergence of good sensory attributes for oil extracted from olive fruit stored for 2 weeks can also be evidenced from the higher concentrations of (*E*)-2-hexenal and total volatiles than in oil extracted from 1-week-stored fruit (**Table 2**).

At 3 weeks of fruit storage, sensory quality and oil yield decreased and coincided with a significant ($p < 0.05$) increase in MI (**Table 2**). The low oil yield at 3 weeks indicates advanced stages of hydrolysis in which the hydrolytic products further interacted with triglycerides, forming emulsions. The evidence of advanced stages of hydrolysis was further illustrated with fruit phenolic compounds above; phenolic compounds increased in the fruit but decreased in the oil, suggesting that they might end up in the waste stream.

The sensory notes indicate that the oil extracted from olive fruit at 1 and 3 weeks of storage was of a low quality, whereas the oil extracted from fresh fruits and olive fruits stored for 2 weeks had acceptable, positive sensory properties and quality attributes (**Table 2**). Our observation on the regaining of sensory

quality is consistent with a report (*6*) of olive oil extracted after 30 days of fruit storage in air atmosphere at ambient temperature having better odor properties than samples extracted after 10 and 20 days.

The quality indices (FFA, PV, K_{232}, and K_{270}) did not correspond to the above changes observed for sensory quality. For instance, K_{232}, which is associated with hydroperoxides (*11*), significantly ($p < 0.05$) changed with postharvest fruit storage (**Table 2**), whereas K_{270}, which is associated with volatile compounds from oxidative rancidity (*11, 19*), did not change with oil sensory quality. Studies (*5, 8, 16*) on postharvest fruit storage have reported minimal changes in K_{232} and K_{270} in olive oil extracted from stored fruit, which was consistent with our observation for K_{270} but not for K_{232} (**Table 2**). This study emphasizes the importance of including volatile compounds and phenolic compounds (in addition to quality indices) in understanding the effects of postharvest raw material handling practices in food processing, such as low-temperature storage of fruits. Our results suggest that low-temperature olive fruit storage may be beneficial, with a possibility of increasing oil yield and moderating the sensory quality of virgin olive oils.

ABBREVIATIONS USED

PV, peroxide value; FFA, free fatty acid; MI, maturity index; UV, ultraviolet; 3,4-DHPEA-DEDA, 3,4-dihydroxyphenylethyl alcohol−decarboxymethylelenolic acid dialdehyde; LOX pathway, lipoxygenase pathway; 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; LOD, limit of detection.

ACKNOWLEDGMENT

We gratefully acknowledge Vici Murdoch and Gerard Gaskin of Riverina Olive Grove for coordination and assistance during the study. We also gratefully acknowledge Neville Chaple of Wollundry Grove for supplying the olive fruit and use of the cold room.

LITERATURE CITED

(1) IOOC. *Olive Oil Quality Improvement*; International Olive Oil Council: Madrid, Spain, 1990; p 79.
(2) IOOC. *Trade Standard Applying to Olive Oil and Olive Pomace Oil* IOOC: Madrid, Spain, 2003; Vol. Coi/T.15/NC no. 3/Rev 1, p 9.
(3) Di Giovacchino L. Technological aspects. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J.; Aparicio, R., Ed.; Aspen Publications: Gaithersburg, MD, 2000; pp 17–59.
(4) Morales, M. T.; Luna, G.; Aparicio, R. Comparative study of virgin olive oil sensory defects. *Food Chem.* **2005**, *91*, 293–301.
(5) Kiritsakis, A.; Nanos, G. D.; Polymenopoulos, Z.; Thomai, T.; Sfakiotakis, E. M. Effect of fruit storage conditions on olive oil quality. *J. Am. Oil Chem. Soc.* **1998**, *75*, 721–724.
(6) Koprivnjak, O.; Procida, G.; Zelinotti, T. Changes in the volatile components of virgin olive oil during fruit storage in aqueous media. *Food Chem.* **2000**, *70*, 377–384.
(7) Castellano, J. M.; Garcia, J. M.; Morilla, A.; Perdiguero, S.; Gutierrez, F. Quality of Picual olive fruits stored under controlled atmospheres. *J. Agric. Food Chem.* **1993**, *41*, 537–539.
(8) Pereira, J. A.; Casal, S.; Bento, A.; Oliveira, M. Influence of olive storage period on oil quality of three Portuguese cultivars of *Olea europaea*, Cobrangosa, Madural, and Verdeal Transmontana. *J. Agric. Food Chem.* **2002**, *50*, 6335–6340.
(9) Agar, I. T.; Hess-Pierce, B.; Sourour, M. M.; Kader, A. A. Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. *J. Agric. Food Chem.* **1998**, *46*, 3415–3421.
(10) Gutierrez, F.; Varona, I.; Albi, M. A. Relation of acidity and sensory quality with sterol content of olive oil from stored fruit. *J. Agric. Food Chem.* **2000**, *48*, 1106–1110.
(11) Garcia, J. M.; Seller, S.; PerezCamino, M. C. Influence of fruit ripening on olive oil quality. *J. Agric. Food Chem.* **1996**, *44*, 3516–3520.
(12) Garcia, J. M.; Gutierrez, F.; Barrera, M. J.; Albi, M. A. Storage of mill olives on an industrial scale. *J. Agric. Food Chem.* **1996**, *44*, 590–593.
(13) Dourtoglou, V. G.; Mamalos, A.; Makris, D. P. Storage of olives (Olea europaea) under CO_2 atmosphere: Effect on anthocyanins, phenolics, sensory attributes and in vitro antioxidant properties. *Food Chem.* **2006**, *99*, 342–349.
(14) Morello, J. R.; Motilva, M. J.; Ramo, T.; Romero, M. P. Effect of freeze injuries in olive fruit on virgin olive oil composition. *Food Chem.* **2003**, *81*, 547–553.
(15) Jimenez, A.; Rodriguez, R.; Fernandez-Caro, I.; Guillen, R.; Fernandez-Bolanos, J.; Heredia, A. Olive fruit cell wall: degradation of pectic polysaccharides during ripening. *J. Agric. Food Chem.* **2001**, *49*, 409–415.
(16) Garcia, J. M.; Gutierrez, F.; Castellano, J. M.; Perdiguero, S.; Morilla, A.; Albi, M. A. Influence of storage temperature on fruit ripening and olive oil quality. *J. Agric. Food Chem.* **1996**, *44*, 264–267.
(17) Boskou, D. *Olive Oil Chemistry and Technology*; AOCS Press: Champaign, IL, 2006; p 288.
(18) EEC, Commission Regulation (EEC) no 2568/91 of 11 July 1991 on the Characteristics of Olive oil and Olive-Residue Oil and on the Relevant Methods of Analysis, 1991; Vol. 2568/91, p 83.
(19) Gutierrez, F.; Fernandez, J. L. Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "extra" quality. *J. Agric. Food Chem.* **2002**, *50*, 571–577.
(20) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **2000**, *68*, 283–287.
(21) Andrewes, P.; Busch, J.; de Joode, T.; Groenewegen, A.; Alexandre, H. Sensory properties of virgin olive oil polyphenols: identification of deacetoxy-ligstroside aglycon as a key contributor to pungency. *J. Agric. Food Chem.* **2003**, *51*, 1415–1420.
(22) Aguilera, M. P.; Beltran, G.; Ortega, D.; Fernandez, A.; Jimenez, A.; Uceda, M. Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chem.* **2005**, *89*, 387–391.
(23) Kalua, C. M.; Allen, M. S.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. *J. Agric. Food Chem.* **2005**, *53*, 8054–8062.
(24) Kalua, C. M.; Bedgood, D. R.; Prenzler, P. D. Development of a headspace solid phase microextraction−gas chromatography method for monitoring volatile compounds in extended time-course experiments of olive oil. *Anal. Chim. Acta* **2006**, *556*, 407–414.
(25) Vierhuis, E.; Servili, M.; Baldioli, M.; Schols, H. A.; Voragen, A. G. J.; Montedoro, G. Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. *J. Agric. Food Chem.* **2001**, *49*, 1218–1223.
(26) Cinquanta, L.; Esti, M.; Di Matteo, M. Oxidative stability of virgin olive oils. *J. Am. Oil Chem. Soc.* **2001**, *78*, 1197–1202.
(27) Kalua, C. M.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Discrimination of storage conditions and freshness in virgin olive oil. *J. Agric. Food Chem.* **2006**, *54*, 7144–7151.

(28) Beauchamp, G. K.; Keast, R. S. J.; Morel, D.; Lin, J. M.; Pika, J.; Han, Q.; Lee, C. H.; Smith, A. B.; Breslin, P. A. S. Phytochemistry—Ibuprofen-like activity in extra-virgin olive oil. *Nature* **2005**, *437*, 45–46.

(29) Mateos, R.; Cert, A.; Perez-Camino, M. C.; Garcia, J. M. Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *J. Am. Oil Chem. Soc.* **2004**, *81*, 71–75.

(30) Montedoro, G.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil 3. Spectroscopic characterizations of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228–2234.

(31) Brenes, M.; Garcia, A.; Garcia, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609–5614.

(32) Amiot, M. J.; Fleuriet, A.; Macheix, J. J. Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry* **1989**, *28*, 67–69.

(33) Ryan, D.; Robards, K.; Lavee, S. Changes in phenolic content of olive during maturation. *Int. J. Food Sci. Technol.* **1999**, *34*, 265–274.

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

(35) Salas, J. J.; Sanchez, J.; Ramli, U. S.; Manaf, A. M.; Williams, M.; Harwood, J. L. Biochemistry of lipid metabolism in olive and other oil fruits. *Prog. Lipid Res.* **2000**, *39*, 151–180.

(36) Olias, J. M.; Perez, A. G.; Rios, J. J.; Sanz, L. C. Aroma of virgin olive oil—biogenesis of the green odor notes. *J. Agric. Food Chem.* **1993**, *41*, 2368–2373.

(37) Morales, M. T.; Alonso, M. V.; Rios, J. J.; Aparicio, R. Virgin olive oil aroma—relationship between volatile compounds and sensory attributes by chemometrics. *J. Agric. Food Chem.* **1995**, *43*, 2925–2931.

(38) Morales, M. T.; Rios, J. J.; Aparicio, R. Changes in the volatile composition of virgin olive oil during oxidation: flavors and off-flavors. *J. Agric. Food Chem.* **1997**, *45*, 2666–2673.

(39) Vichi, S.; Pizzale, L.; Conte, L. S.; Buxaderas, S.; Lopez-Tamames, E. Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: modifications induced by oxidation and suitable markers of oxidative status. *J. Agric. Food Chem.* **2003**, *51*, 6564–6571.

(40) Reiners, J.; Grosch, W. Odorants of virgin olive oils with different flavor profiles. *J. Agric. Food Chem.* **1998**, *46*, 2754–2763.

(41) Angerosa, F.; d'Alessandro, N.; Basti, C.; Vito, R. Biogeneration of volatile compounds in virgin olive oil: their evolution in relation to malaxation time. *J. Agric. Food Chem.* **1998**, *46*, 2940–2944.

(42) Kalua, C. M.; Bedgood, D. R.; Bishop, A. G.; Prenzler, P. D. Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *J. Agric. Food Chem.* **2006**, *54*, 7641–7651.

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

(44) Ranalli, A.; Sgaramella, A.; Surricchio, G. The new "Cytolase 0" enzyme processing aid improves quality and yields of virgin olive oil. *Food Chem.* **1999**, *66*, 443–454.

(45) Ranalli, A.; Pollastri, L.; Contento, S.; Iannucci, E. The glyceridic and nonglyceridic constituents of virgin olive oil after use of a novel method of enzyme extraction. *Int. J. Food Sci. Technol.* **2003**, *38*, 17–27.

Received for review October 14, 2007. Revised manuscript received January 24, 2008. Accepted January 24, 2008. Financial support from Rural Industries Research & Development Corporation (RIRDC) and Charles Sturt University is gratefully acknowledged.

JF073027B