JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Discrimination of Storage Conditions and Freshness in Virgin Olive Oil

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

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

Virgin olive oil samples stored in the light at ambient temperature, in the dark at ambient temperature, and at low temperature in the dark for 12 months both with and without headspace were separated into recognizable patterns with stepwise linear discriminant analysis. The discrimination with variables volatile and phenolic compounds, free fatty acid (FFA), peroxide values, K_{232}, and K_{270} revealed a departure of stored oil from freshness and showed significant ($p < 0.01$) differences between storage conditions. Virgin olive oil stored at low temperature had characteristics closest to fresh oil while oil stored in the light showed the largest departure from freshness. Parameters that exclusively and significantly ($p < 0.01$) discriminated storage conditions were identified as potential markers of the storage condition. In the presence of oxygen, hexanal was a marker of storage in the light, FFA was a marker for dark storage, and markers of low-temperature storage were acetic acid and pentanal. In the absence of oxygen, octane was the marker for storage in the light whereas tyrosol and hexanol were markers of virgin olive oil stored in the dark, with no marker indicative of low-temperature storage. *E*-2-Hexenal, K_{232}, and K_{270} were identified as markers of virgin olive oil freshness.

KEYWORDS: Virgin olive oil; stepwise linear discriminant analysis (SLDA); oxidation markers; freshness markers; storage conditions

INTRODUCTION

As soon as virgin olive oil is extracted from the olive fruit, there is potential for the quality to deteriorate. Commercially, olive oil is stored with minimal oxygen exposure to protect quality; however, during domestic consumption, oxygen ingress is inevitable and may hasten oxidation and loss of freshness. To maintain the quality of virgin olive oil, it is paramount to control the oxidation status so that oil composition is consistent from production to consumption. This can be achieved through an understanding and control of both external factors, i.e., oxygen concentration, temperature, and light (*1*, *2*), and internal factors, i.e., major and minor constituents of virgin olive oil that influence oxidation (*1*−*5*).

Light exposure, temperature, and oxygen concentration influence virgin olive oil quality and freshness during transportation, storage, and consumption (*2*). Commercially, virgin olive oil is usually stored and transported in the dark but often packaged in transparent bottles in response to consumer preferences (*2*, *3*), thereby exposing the oil to light before and after purchase. Temperature variation during virgin olive oil storage and transportation is common and may be attributed to natural climatic changes and in some cases to intentional temperature control. Virgin olive oil is rarely stored at low temperature commercially, although low-temperature storage before laboratory analysis has been widely reported to preserve the freshness of olive oil (*6*−*11*). An understanding of olive oil oxidation at low temperature may find wide industrial applications in numerous areas, including oil-rich frozen food products.

Monitoring of oxidation in virgin olive oil during storage has been based on the change in major and minor constituents of virgin olive oil usually investigated through univariate statistical approaches using a variety of oxidation indicators. Phenolic and volatile compounds are the common minor constituents that are measured and shown to change during virgin olive oil storage (*11*−*15*). Understanding and control over phenolic compounds, which act as antioxidants (*11*, *16*), can prevent oxidative deterioration of virgin olive oil. Antioxidants maintain levels of volatiles that impart positive sensory characteristics (*17*) and deter the generation of C7−C12 volatile compounds responsible for sensory defects (*17*). To date, the focus in determining the extent of oxidation has been on the volatile compounds that are formed, and not necessarily on the compounds that are lost, as virgin olive oil loses its freshness.

Many studies on oxidation of virgin olive oils (*4*, *13*, *16*, *18*−*20*) have reported good correlations between changes in compounds and stability, as measured by accelerated tests, and consequently, such compounds have been identified as markers of oxidation. However, the application of accelerated studies to real-time (nonaccelerated) shelf life studies remains question-

* To whom correspondence should be addressed. Tel: +61-2-6933 2978. Fax: +61-2-6933 2737. E-mail: pprenzler@csu.edu.au.

10.1021/jf061038j CCC: $33.50
Published on Web 08/18/2006

able (*21*). The extreme conditions in accelerated tests—high temperatures and with air bubbled into the oil—do not simulate actual storage conditions and may lead to qualitative and quantitative changes to the oil that are not related to real-time storage. This may lead to difficulties in choosing markers of oxidation that could be used to indicate deterioration of quality under different storage conditions. Here, we define a marker as a parameter (compound or physical measurement) that is uniquely and significantly correlated with a particular treatment of an oil.

Regardless of the drawbacks of accelerated tests in shelf life investigations, studies based on real-time shelf life conditions are rare, and where they exist they are usually extrapolated to apply to storage conditions not used in the original shelf life study. Studies based on single storage conditions such as light (*22*) and dark (*23*) have been reported. Some studies have combined different storage conditions, for instance, dark and low temperatures (*24*) and dark storage, uncontrolled light, and uncontrolled temperature (*12*). Univariate statistical approaches were applied in most of these shelf life studies except for Pagliarini et al. (*12*) where a multivariate statistical approach was used. Univariate statistical analyses limit consideration of the interactions that may occur between several external and internal factors. Multivariate statistical analysis can be applied to simultaneously explore factors influencing oxidation of virgin olive oil when stored under different conditions. The use of exploratory and classification statistical approaches such as stepwise linear discriminant analysis (SLDA) and principal component analysis (PCA) can identify patterns in samples and variables contributing to the clustering of samples (*25*).

The objective of this study was to investigate how different storage conditions affect oil quality relative to that of fresh oil. A multivariate statistical approach with SLDA was applied to simultaneously compare the effect of light, dark, and low-temperature storage, in the presence and absence of oxygen, on virgin olive oil. To the best of our knowledge, this is the first study of its kind that has identified parameters that significantly ($p < 0.01$) discriminate oil storage conditions in a real-time shelf life study lasting 1 year. From this study, parameters that were uniquely associated with storage conditions were identified, and these may be used as markers for particular storage conditions.

MATERIALS AND METHODS

Materials. Standards and reagents from the indicated sources were used without further purification. Phenolic standards were 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). Prior to high-performance liquid chromatography (HPLC) analysis, standards were prepared in methanol + water (50 + 50 v/v) and filtered through 0.45 μm plastic nonsterile filters. Grade 1 water (ISO3696) purified through a Milli-Q water system was used for chromatographic preparations.

Volatile standards were 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 used were chloroform, acetic acid, and potassium iodide (Biolab); sodium thiosulphate (Asia Pacific Speciality 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, methanol (Mallinckrodt Chemicals), acetonitrile (J.T. Baker, Phillipsburg, NJ), and formic acid (Sigma) were used in phenolic compounds analysis.

Olive Oil Samples. Fresh extra virgin olive oil samples (3 × 5 L), commercially extracted from *Paragon* olive fruit during the 2003 harvest season, were supplied by Riverina Olive Grove. Prior to packaging in the 5 L containers, the oil was allowed to settle for at least 1 month, during which time any suspended material deposited, leaving a completely clear oil. The sensory description of the fresh extra virgin olive oil, as provided by the supplier, was pronounced banana fruit, mild pepper, and pungency. Aggregated quantitative data monitored over a 12 month period for common olive quality indices and major volatile and phenolic compounds are provided in **Table 1**.

Methods. *Virgin Olive Oil Storage Conditions.* Virgin olive oil (approximately 100 mL) was transferred into clear pharmaceutical bottles (6 × 100 mL/storage condition) and stored in the light at ambient temperature (24 ± 3 °C), dark at ambient temperature, and at low temperature in the dark. Virgin olive oil bottles for dark and low-temperature storage were wrapped in aluminum foil to exclude light. Virgin olive oil samples for light storage were placed on a laboratory shelf out of exposure to direct sunlight, and low-temperature samples were stored in a refrigerator (1.0 ± 1.0 °C). Virgin olive oil was analyzed at bottling to provide data on fresh oil. Thereafter, one bottle per storage condition was analyzed every 2 months for 12 months. Virgin olive oil was stored both without headspace and with a 50% headspace.

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 HPLC—diode array detection (DAD), respectively, as described earlier (*26*).

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 papers (*26*, *27*).

Determination of Quality Parameters. Determination of FFA, PV, and UV absorbances (*K* values) was performed according to the standard EC and International Olive Oil Council (IOOC) methods (*28*, *29*). These parameters (PV, FFA, K_{232}, K_{270}, and ΔK) are the common quality indices used to assess olive oil (*29*) and are used as variables in the characterization of storage conditions (**Table 2**).

Statistical Data Analysis. Patterns that best separated storage conditions were identified by SLDA using quality indices and concentrations of volatile and phenolic compounds (**Table 2**) as independent variables with SPSS 12.0 (SPSS Inc., Chicago, IL). Linear discriminant analysis is a standard statistical technique for projecting data from a high dimensional space onto a perceivable reduced subspace such that the data can be separated by visual inspection (*30*). For instance, in our case, 31 variables with over 4500 data points were significantly reduced to 15 representative variables depicting only data points that identify trends and patterns in the original 4500 points, which may not be evident from the use of univariate statistics.

Significant ($p < 0.01$) differences for parameters measured under different storage conditions (**Table 1**) were identified by one-way analysis of variance posthoc multiple comparison analysis using Duncan's test.

RESULTS AND DISCUSSION

Virgin olive oil is best when consumed fresh. Storage has the potential to lower the quality of virgin olive oil. In order to more fully understand the impact of different storage conditions, multivariate analysis with SLDA was used to recognize storage patterns with scatter plots (**Figures 1—3**). Discriminating variables (*31*) were identified based on a stringent criterion ($p = 0.01$) to ensure selection of the most likely predictors of freshness and storage conditions: light, dark, and low temper-

Table 1. Quantitative Data for Virgin Olive Oil When Fresh and When Stored Under Different Conditions[a]

		without headspace (absence of oxygen)			with headspace (50% air)		
variables	fresh virgin olive oil	cold-stored oil	dark-stored oil	light-stored oil	cold-stored oil	dark-stored oil	light-stored oil
				volatile compounds			
acetic acid	1.8 ± 0.3 c	1.2 ± 0.3 b	1.1 ± 0.3 b	1.3 ± 0.3 b	0.13 ± 0.02 a	0.12 ± 0.01 a	0.15 ± 0.01 a
1-penten-3-ol	0.49 ± 0.07 c	0.23 ± 0.09 b	0.21 ± 0.09 b	0.22 ± 0.07 b	0.02 ± 0.01 a	0.049 ± 0.008 a	0.052 ± 0.006 a
pentanal	2.1 ± 0.3 d	0.9 ± 0.5 b	1.0 ± 0.4 b	1.5 ± 0.3 c	0.08 ± 0.01 a	0.08 ± 0.01 a	0.05 ± 0.04 a
hexanal	4.9 ± 0.6 c	2.7 ± 0.8 b	3.1 ± 0.9 b	6 ± 1 d	0.08 ± 0.01 a	0.09 ± 0.01 a	0.37 ± 0.05 a
E-2-hexenal	7.2 ± 0.7 c	4 ± 2 b	4 ± 2 b	4 ± 2 b	1.4 ± 0.2 a	2.1 ± 0.3 a	3.5 ± 0.4 b
E-2-hexen-1-ol	9 ± 1 c	5 ± 2 b	5 ± 2 b	5 ± 2 b	1.9 ± 0.2 a	2.2 ± 0.3 a	1.8 ± 0.1 a
hexanol	4.5 ± 0.4 b	2.7 ± 0.9 a	3 ± 1 a	2.6 ± 0.9 a	2.7 ± 0.3 a	2.7 ± 0.4 a	2.7 ± 0.2 a
octane	0.38 ± 0.04 c	0.19 ± 0.08 b	0.19 ± 0.08 b	0.7 ± 0.2 d	0.049 ± 0.009 a	0.04 ± 0.02 a	0.06 ± 0.02 a
octanal	< 0.16	< 0.16	< 0.16	< 0.16	0.31 ± 0.02 a	0.36 ± 0.06 b	0.28 ± 0.09 a
E-2-nonen-1-ol	< 0.07	< 0.07	< 0.07	< 0.07	0.2 ± 0.1 a	0.2 ± 0.1 a	0.13 ± 0.03 a
2-pentyl furan	0.80 ± 0.07 c	0.5 ± 0.2 b	0.5 ± 0.2 b	0.5 ± 0.2 b	0.11 ± 0.01 a	0.14 ± 0.03 a	0.11 ± 0.04 a
				phenolic compounds			
hydroxytyrosol	25 ± 2 d	14 ± 9 a	21.2 ± 0.9 c	21 ± 1 b,c	14.2 ± 0.6 a	17 ± 2 a,b	19 ± 1 b,c
tyrosol	35 ± 3 d	27 ± 1 b	31 ± 2 c	29 ± 2 c	20.7 ± 0.9 a	25 ± 2 b	30 ± 2 c
ligstroside dialdehyde	98 ± 8 c	36 ± 14 a	31 ± 19 a	38 ± 7 a	54 ± 10 b	31 ± 7 a	31 ± 4 a
(+)-acetoxypinoresinol	185 ± 17 c	97 ± 20 b	99 ± 32 b	102 ± 30 b	74 ± 11 a	53 ± 8 a	62 ± 6 a
oleuropein aglycon	82 ± 16 d	43 ± 21 b,c	34 ± 20 a,b,c	52 ± 26 c	22 ± 7 a	22 ± 4 a	32 ± 7 a,b
				quality indices			
FFA[b]	0.302 ± 0.007 a,b	0.37 ± 0.07 c	0.39 ± 0.08 c	0.37 ± 0.09 c	0.30 ± 0.01 a,b	0.35 ± 0.02 b,c	0.27 ± 0.03 a
K_{232}	1.72 ± 0.01 a	1.77 ± 0.05 a,b	1.9 ± 0.1 c	1.85 ± 0.04 b,c	1.78 ± 0.03 a,b	2.4 ± 0.2 e	2.13 ± 0.04 d
K_{270}	0.15 ± 0.01 b	0.142 ± 0.009 b	0.144 ± 0.009 b	0.20 ± 0.01 d	0.13 ± 0.01 a	0.168 ± 0.008 c	0.237 ± 0.005 e
PV[c]	12.0 ± 0.4 a	17 ± 1 b	18 ± 2 b, c	18 ± 1 b, c	20 ± 2 c	26 ± 3 d	36 ± 2 e

[a] Different superscripts in a row indicate significantly ($p < 0.01$) different means ± standard deviation in mg/g of 12 analyses over a year. [b] FFA as % oleic acid. [c] PV expressed as milliequiv oxygen/kg oil.

Table 2. Variables for the Characterization of Freshness and Storage Conditions

volatile compounds	phenolic compounds	other variables
acetic acid	hydroxytyrosol	FFA
1-penten-3-one	tyrosol	PV
1-penten-3-ol	caffeic acid	K_{232}
pentanal	3,4-DHPEA-DEDA[a]	K_{270}
pentan-1-ol	ligstroside dialdehyde	ΔK
Z-2-penten-1-ol	ligstroside acetals	
octane	oleuropein derivatives	
hexanal	(+)-pinoresinol	
E-2-hexenal	(+)-acetoxypinoresinol	
E-2-hexen-1-ol	oleuropein aglycone	
hexanol	hemiacetal of oleuropein	
6-methyl-5-hepten-2-one		
2-pentyl furan		
octanal		
E-2-nonen-1-ol		

[a] 3,4-Dihydroxy phenyl ethyl alcohol–decarboxymethyl elenolic acid dialdehyde.

ature, both in the presence and in the absence of oxygen. This approach gave insights into the chemical changes occurring in the oil during storage.

Discrimination of Storage Conditions Relative to Freshness. Fresh virgin olive oil and olive oil stored in the light, dark, and at low temperatures were significantly ($p < 0.01$) separated (**Figures 1−3**) showing distinct differences in the quality of olive oil under different storage conditions. The separation based on the first two discriminant functions had a significant ($p < 0.05$) Wilks' λ statistic on both functions (**Table 3**) indicating the suitability of SLDA in discriminating the different storage conditions. The separation of different storage conditions in this study differs markedly from an earlier report (*12*) in which storage conditions (uncontrolled light, temperature, and dark storage) for up to 14 months did not show a statistically significant influence.

Table 3. Discrimination of Storage Conditions Showing % Variance Explained and Significance of Discriminant Functions

	% variance explained		
discriminated groups	function 1, V_1	function 2, V_2	cumulative
fresh oil relative to storage condition	46.7[a]	26.2[a]	73.0
storage without headspace	91.8[a]	8.2[a]	100.0
storage with headspace	80.0[a]	20.0[a]	100.0

[a] Wilks λ statistic significantly ($p < 0.05$) different.

The successful separation of the different storage conditions is illustrated in **Table 3** where the cumulative variance explained of 100% was achieved for the first two discriminant functions with storage conditions alone and 73% for fresh oil relative to different storage conditions. The lower cumulative variance explained (73%) for fresh oil relative to different storage conditions as compared to 100% for the storage conditions alone indicate the closeness of some storage conditions to fresh oil. The presence of oxygen causes the most significant departure from fresh oil for all storage conditions (**Figure 1**). Furthermore, the presence of oxygen enhances the separation of the different storage conditions from each other.

The observations above indicate how differences in storage conditions can cause variations in olive oil composition and quality. A wider departure from fresh oil was observed with oil exposed to oxygen (with headspace) showing higher significant ($p < 0.01$) differences than oil stored in the absence of oxygen (without headspace). It was also observed that oil stored at low temperature was comparable to fresh oil (**Figure 1**). In addition, olive oil stored at low temperature and in the dark formed a cluster in the absence of oxygen but the oils stored under the same conditions in the presence of oxygen were separated and significantly ($p < 0.01$) different from each other. The formation of a cluster for virgin olive oil stored in the dark

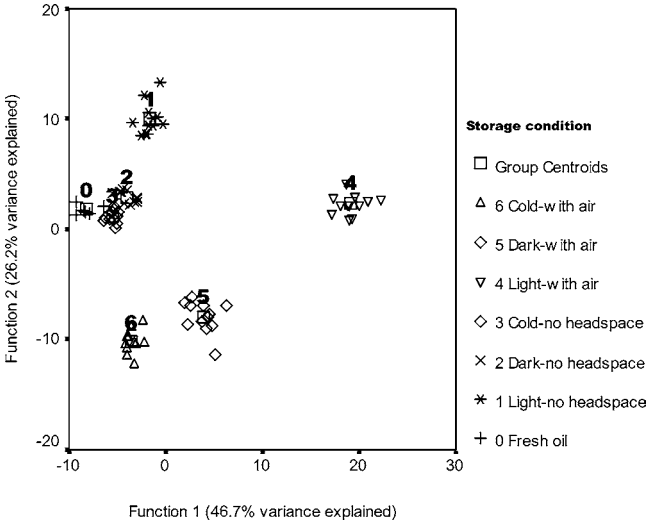

Figure 1. Scatter plot for the first two canonical discriminant functions separating fresh and stored olive oil.

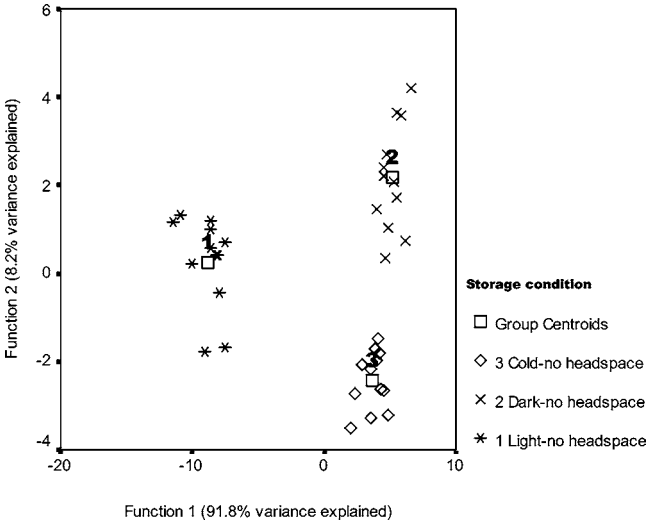

Figure 2. Scatter plot for the first two canonical discriminant functions separating storage conditions in the absence of oxygen.

and at low temperature in the absence of oxygen (**Figure 1**) indicates minimal differences in composition and quality, which later emerge when the bottles are opened and exposed to oxygen.

Discrimination of Storage Conditions in the Absence of Oxygen. Storage of olive oil in the absence of oxygen simulates the conditions during transportation and storage at commercial level, before consumption at the household level. Even though virgin olive oil is not exposed to oxygen, which would promote oxidative rancidity (*2*, *32*), the discrimination in **Figure 1** establishes significant ($p < 0.01$) differences when the oil is exposed to light and when it is subjected to different storage temperatures. The differences are further explored below (**Figure 2**) to identify the discriminating variables that characterize storage conditions of virgin olive oil in the absence of oxygen.

Not all parameters measured in virgin olive oil (**Table 2**) discriminated storage conditions. Only those parameters that had a significant ($p < 0.01$) contribution in separating the storage conditions were entered in the functions of the scatter plot (e.g., **Figure 2**). The discrimination of storage conditions along function 1 of **Figure 2** is given in the linear discriminant equation (V_1, eq 1) below.

$$V_1 = 2.56[\text{hexanol}] + 0.83 \times K_{232} + 0.22[\text{tyrosol}] - 1.76[\text{octane}] - 1.05 \times K_{270} - 0.52[\text{acetoxypinoresinol}] \quad (1)$$

Octane, acetoxypinoresinol, and K_{270} (parameters with negative coefficients) discriminated olive oil stored in the light, which is on the negative side of function 1 in **Figure 2**. Olive oil stored in the dark and at low temperature, which lie on the positive side of function 1 in **Figure 2**, were discriminated with hexanol, tyrosol, and K_{232} (positive coefficients). Olive oil samples stored in the dark and at low temperature, which were not discriminated on function 1, were further separated from each other on function 2 in **Figure 2**. Following the deduction of discriminating variables above but now for function 2, olive oil stored at low temperature was discriminated with acetoxypinoresinol and K_{270} while octane, hexanol, tyrosol, and K_{232} discriminated dark- and light-stored olive oil.

Discrimination of Storage Conditions in Presence of Oxygen. The introduction of headspace during storage simulates the conditions during consumption at household level. As noted above, clusters **4**, **5**, and **6** (absence of oxygen) were significantly ($p < 0.01$) different from **1**, **2**, and **3** (presence of oxygen) in **Figure 1**. This implies that given the same storage conditions, the composition of olive oil differs at commercial distribution and storage level (oxygen exposure minimized) and at household consumption level (oil exposed to oxygen). The higher % variance explained, on the *y*-axis of **Figure 3** (see also **Table 3**), for virgin olive oil stored in the presence of oxygen (**Figure 3**) indicates that the differences in composition and quality with storage conditions are more pronounced at the household consumption level than at the commercial level.

The presence of oxygen had a pronounced effect on the variables that significantly ($p < 0.01$) discriminated storage conditions as revealed by comparing eq 1 with eq 2, the linear discriminant equation for function 1 of **Figure 3**.

$$V_1 = 1.63[\text{hexanal}] + 0.42 \times K_{232} + 0.62 \times K_{270} + 0.54[\text{hydroxytyrosol}] - 0.44[\text{acetic acid}] - 0.66[E\text{-2-hexenal}] - 0.50 \times \text{FFA} \quad (2)$$

Equation 2 indicates that hexanal, hydroxytyrosol, K_{232}, and K_{270} (parameters with positive coefficients) discriminated virgin olive oil stored in the light in presence of oxygen (**Figure 3**). These parameters contrast with those found for light storage in the absence of oxygen (eq 1)—octane, acetoxypinoresinol, and K_{270}. Virgin olive oil stored in the dark and at low temperature (**Figure 3**) was discriminated by acetic acid, *E*-2-hexenal, and FFA (parameters with negative coefficients). There was poor separation of dark and low-temperature storage conditions with respect to function 1 in **Figure 3**, but function 2 clearly discriminated these storage conditions. Virgin olive oil stored in the dark was characterized with *E*-2-hexenal, hydroxytyrosol, K_{232}, K_{270}, and FFA while acetic acid and hexanal discriminated light and low-temperature olive oil storage.

Parameters that significantly ($p < 0.01$) discriminated storage conditions as presented above are compiled in **Table 4**. These parameters are investigated further, below, to determine which are unique to a particular set of storage conditions and hence can be considered to be a marker of those conditions. Furthermore, quantitative changes in these parameters may reveal insights into oil chemistry relative to storage conditions.

Parameters That Characterize Low-Temperature Storage. *Without Headspace.* Low-temperature storage maintains the quality of olive oil close to that of fresh oil as observed by the proximity of the group centroids in **Figure 1**. These conditions

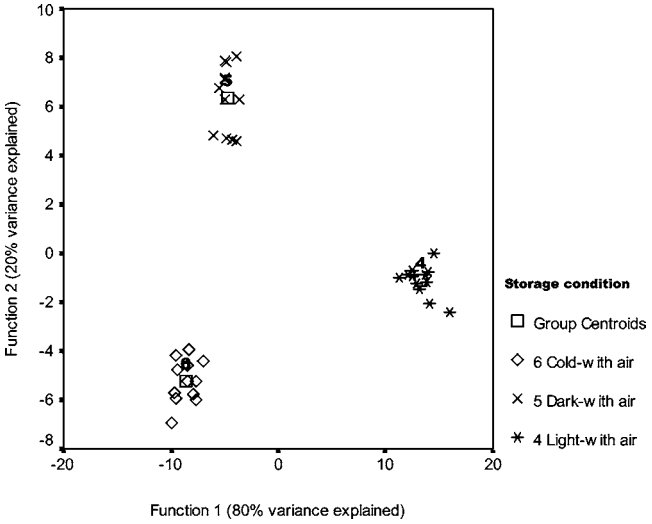

Figure 3. Scatter plot for the first two canonical discriminant functions separating storage conditions in the presence of oxygen.

Table 4. Variables Separating the Different Storage Conditions of Virgin Olive Oil

	discriminating variables		
storage condition	without headspace	with headspace	headspace-independent
low temperature	K_{270}	acetic acid	
	E-2-hexen-1-ol	pentanal	
	ligstroside dialdehyde	PV[a]	
	(+)-acetoxypinoresinol		
dark	*E*-2-hexenal	*E*-2-hexenal	*E*-2-hexenal
	K_{232}	K_{232}	K_{232}
	PV[a]	K_{270}	
	tyrosol	FFA[b]	
	hexanol	hydroxytyrosol	
light	octane	hexanal	K_{270}
	E-2-hexen-1-ol	K_{232}	
	K_{270}	K_{270}	
	ligstroside dialdehyde	PV[a]	
	(+)-acetoxypinoresinol	hydroxytyrosol	

[a] PV expressed as milliequiv oxygen/kg oil. [b] FFA as % oleic acid.

resulted in the least significant ($p < 0.01$) increase in PV and lower values of K_{270} than fresh virgin olive oil (**Table 1**). This suggests that hydroperoxides (as measured by PV) increase slowly over a 12 month storage period (presumably due to oxygen present at bottling or adventitious ingress through incomplete seals) and that their decomposition to secondary oxidation products is inhibited (low K_{270}). The slow oxidation rate of olive oil at low temperature is consistent with the report of Velasco and Dobarganes (*2*), who state that at low or moderate temperatures, hydroperoxides are the major compounds formed.

All phenolic compounds significantly ($p < 0.01$) decreased in concentration during low-temperature storage. While conditions that lead to oxidative damage to oils (light, heat, and O_2) were kept to a minimum, it is apparent that oxidative chemistry was still occurring, leading to a decrease in levels of the antioxidant compounds. SLDA identified ligstroside dialdehyde and acetoxypinoresinol as the phenolic compounds whose change in concentration was most characteristic of low-temperature storage in the absence of oxygen. However, because these compounds also discriminate light storage, without headspace (**Table 4**), they are not unique to one set of storage conditions and, therefore, cannot be classified as markers.

Table 5. Potential Oxidation Markers of Virgin Olive Oil Stored in the Light and Dark and at Low Temperature

	markers		
storage condition	without headspace	with headspace	common
low temperature		acetic acid	
		pentanal	
dark	tyrosol	FFA[a]	*E*-2-hexenal
	hexanol		K_{232}
light	octane	hexanal	K_{270}

[a] FFA as % oleic acid.

E-2-Hexen-1-ol was the only volatile compound whose concentration was found to be discriminating by SLDA (**Table 4**) for low-temperature storage without headspace. The concentration of *E*-2-hexen-1-ol decreased during the storage period. This C6 compound is associated with the lipoxygenase pathway—a series of enzyme-catalyzed transformations leading to volatile compounds with favorable "green" aromas (*33*, *34*). Because this particular compound is reported to have a "green" odor, loss of this compound could lead to a "flattening" of the aroma of olive oil. *E*-2-Hexen-1-ol was not uniquely associated with low-temperature storage (**Table 4**) and therefore cannot be classified as a marker of this storage condition. In fact, low-temperature storage in the absence of oxygen showed no marker (**Table 5**) supporting the observation (**Figure 1**) on the similarity of fresh oil to that stored at low temperature.

With Headspace. Low-temperature storage brought about the least change in the oil (**Figure 1**) as compared to the other storage conditions in the presence of oxygen. Not surprisingly, the presence of headspace O_2 resulted in PV being identified as a discriminating variable by SLDA (**Table 4**). However, it is not uniquely associated with low-temperature storage and is hence not a marker (**Table 5**). As above (without headspace discussion), low temperature appeared to slow the rate of conversion of hydroperoxides to secondary oxidation products as indicated by the low value of K_{270}.

Levels of phenolic compounds decreased during storage, similar to that observed in the absence of headspace (see above). SLDA did not identify any phenolic compounds as discriminating variables for the storage of oil at low temperature and with headspace (**Table 4**).

The volatile compounds acetic acid and pentanal exclusively discriminated low-temperature storage (**Table 4**) and hence can be classified as markers of this storage condition (**Table 5**). As with *E*-2-hexen-1-ol (above), these compounds decrease in concentration during storage. It is not yet known whether they are lost chemically during the storage period or whether they are lost during opening of the containers for sampling. Sensory evaluation would be required to investigate what impact, if any, loss of these compounds would have on stored oil.

Parameters That Characterize Dark Storage. *Without Headspace.* As with low-temperature storage, virgin olive oil kept in the dark in the absence of oxygen showed a significant ($p < 0.01$) increase in PV (**Table 1**) as compared to fresh oil. Under these conditions, a significant ($p < 0.01$) increase in K_{232} was also observed. These results are consistent with an earlier study (*24*), where slight increases in PV and K_{232} were observed for virgin olive oil stored under similar conditions. Storage in the dark leads to maximum values of K_{232} (**Table 1**), independent of the presence or absence of headspace. This suggests that nonphotoassisted, autoxidation reactions, leading to primary oxidation products, are prominent at ambient temperatures. This

is further reflected in the fact that K_{232} was a discriminating variable for oils stored in the dark both in the presence and in the absence of headspace (**Table 4**). As such, it constitutes a marker for dark storage for both conditions (**Table 5**).

Oils stored in the dark showed decreased levels of all phenolic compounds (**Table 1**), indicating ongoing oxidation reactions during the storage period. However, only tyrosol was found by SLDA to discriminate this storage condition (**Table 4**) and, as it was uniquely associated with this storage condition, is classified as a marker compound for dark storage in the absence of oxygen (**Table 5**).

SLDA identified hexanol and *E*-2-hexenal as volatile compounds that discriminated oils stored in the dark. Loss of these C6 compounds (see above) during storage may lead to oil with a less favorable aroma. Hexanol was uniquely associated with this storage condition and is therefore a marker (**Table 5**) compound. *E*-2-Hexenal was also associated with dark storage with headspace (**Table 4**) and is a marker for dark storage regardless of the presence or absence of oxygen (**Table 5**).

Cavalli et al. (*14*) reported a reduction in *E*-2-hexenal content and an increase in C6 alcohols and C5 ketones in olive oil stored in the dark at ambient temperature, and these compounds have been proposed as markers of virgin olive oil freshness. In this study, a decrease in *E*-2-hexenal was observed, but no increase in C6 alcohols nor C5 ketones was detected. In fact, in our study, the C6 alcohol hexanol decreased in concentration during storage.

Another study (*15*) on dark storage of virgin olive oil, but this time under accelerated conditions (60 °C), reported an increase in a number of volatile compounds. Nonanal was proposed as the most sensitive marker to oxidative deterioration. Under the nonaccelerated conditions used in this study, nonanal was not detected during storage. This highlights the need to carefully interpret oxidation markers evaluated under different conditions.

With Headspace. In the current study, K_{232} was a common discriminating variable for virgin olive oils stored both in the presence and in the absence of headspace (**Table 4**). Quantitatively, K_{232} values were significantly ($p < 0.01$) higher in the presence of headspace than in the absence of headspace (**Table 1**) indicating increased oxidation, consistent with this storage condition.

FFA was identified as a discriminating variable that was significantly ($p < 0.01$) greater in virgin olive oil stored in the dark with headspace (**Table 1**) than fresh oil, suggesting possible hydrolytic reactions. Oxidative reactions leading to a rise in FFA have been attributed to the production of volatile acids from the decomposition of hydroperoxides and oxidation of aldehydes (*20*). This was not consistent with our findings (**Table 1**) where no concomitant increase in volatile acids with FFA was observed.

E-2-Hexenal was the only volatile compound to be found to be discriminating for dark storage in the presence of headspace (**Table 4**). As discussed above, it is a general marker for dark storage (**Table 5**) since it was also discriminating for dark storage in the absence of headspace. *E*-2-Hexenal is reported to be one of the most important volatile compounds contributing to the pleasant aroma of extra virgin olive oil (*14*). Loss of this C6 aldehyde during storage will lead to oil that has less desirable sensory properties as compared to the fresh oil.

The phenolic compound hydroxytyrosol was selected by SLDA as a discriminating variable for dark storage in the presence of headspace (**Table 4**). However, it is not unique to this storage condition and is hence not able to act as a marker (**Table 5**). It is interesting that this *ortho*-diphenol is a discriminating variable in the presence of headspace (under both dark and light storage conditions) and may reflect its reactivity as an antioxidant in the presence of oxygen (*35, 36*). Earlier studies (*12, 24*) have reported a significant influence of hydroxytyrosol in maintaining virgin olive oil quality.

Parameters That Characterize Light Storage. *Without Headspace.* Olive oil stored in the light showed the most significant departure from fresh oil (**Figure 1**). Photoassisted oxidation is a well-known cause of defective oil (*2*); yet, colorless, glass containers are common, even though they may be exposed to light 24 h per day on a supermarket shelf. Interestingly, the only quality index that discriminated this storage condition was K_{270}. Its value significantly increased (**Table 1**) during storage in the light in the absence of oxygen (**Table 4**). K_{270} is associated with secondary oxidation (*3, 28*), and the fact that it was the discriminatory quality index would suggest that photoassisted secondary oxidation, rather than primary oxidation (as indicated by K_{232} and PV), is the dominant mechanism for oil deterioration under these storage conditions.

That secondary oxidation is an important process under these storage conditions is reinforced by considering the increase in octane levels (**Table 1**). Octane has been linked to the breakdown of 10-hydroxyperoxide of oleic acid and correlated with sensory defects in olive oil (*37*). Light storage in the absence of oxygen was the only condition that led to the increased concentrations of octane in the oil (**Table 1**). As octane exclusively discriminated this storage condition, it qualifies as a marker compound (**Table 5**).

E-2-Hexenol was another volatile compound that was identified as a discriminating variable by SLDA (**Table 4**). Its concentration decreased during storage in the light in the absence of oxygen (**Table 1**). In general, the C6 compounds all decreased under this storage condition, pointing to a loss of freshness in the oil as discussed above. The exception was hexanal, which increased in concentration (**Table 4**). Hexanal is known to be associated with the oxidation of oil (*38*); however, the increase observed under the storage conditions employed here did not result in it being identified as a discriminating variable.

Levels of all phenolic compounds significantly decreased (**Table 1**) during light storage, as was the case for the other storage conditions. SLDA identified acetoxypinoresinol and ligstroside dialdehyde as discriminating variables for this storage condition in the absence of oxygen (**Table 4**); however, as they were not uniquely discriminating, they are not marker compounds. Loss of both phenolic and volatile compounds suggests that both aroma and taste were affected by storage.

With Headspace. All quality indices associated with oxidation, i.e., PV, K_{232}, and K_{270}, significantly ($p < 0.01$) increased (**Table 1**) when virgin olive oil was stored in the light with a headspace. The presence of oxygen therefore resulted in a rate of formation of hydroperoxides that was faster than the decomposition rate as signified through increased concentrations of primary oxidation products (i.e., PV and K_{232} values). The data in **Table 1** suggest that secondary oxidation products (i.e., K_{270} values) are linked to light exposure regardless of whether oxygen is present or not. Thus, K_{270} is a common marker for light storage (**Table 5**).

All volatile compounds found in fresh oil decreased during storage in the light in the presence of oxygen (**Table 1**). Hexanal was found to be a discriminating variable (**Table 4**), and because it was uniquely linked to this storage condition, it may be classified as a marker (**Table 5**). The low level of hexanal found in oil stored in the light and with a headspace is indicative of

oil that has lost its original freshness. Such oils would rate low on sensory scores where flavor intensity is rated (*17*).

As discussed above (dark storage with headspace), hydroxytyrosol is a discriminating variable (**Table 4**) for oils stored with a headspace. The amount of light exposure did not affect the levels of hydroxytyrosol found during storage (**Table 1**). This suggests that hydroxytyrosol is not directly photodegraded but rather reacts with other species that are generated in the presence of oxygen.

Effect of Oxygen Exposure during Virgin Olive Oil Storage. Oxygen is usually introduced by accelerated methods in an effort to enhance lipid oxidation and, for example, attempt to correlate an oil's resistance to oxidation with levels of endogenous antioxidants (*16*). We are not aware of studies where oxygen is deliberately introduced as a variable in a real-time storage trial. Yet, oxygen exposure is an inevitable consequence of consumer use and storage, and chemical changes occurring during this period are an important consideration in a product's quality and reliability. This aspect of the "supply chain" has received little attention. Here, the inclusion of oxygen coupled with storage at ambient temperatures, i.e., nonaccelerated conditions, allows some insights into this the final stage of the supply chain.

The major difference between oils stored with or without headspace is the appearance of longer chain volatile compounds, vis octanal and *E*-2-nonen-1-ol, which were only detected in oils exposed to oxygen (**Table 1**). The formation of octanal is linked to the breakdown of 13-hydroxyperoxy oleic acid (*15*), and *E*-2-nonen-1-ol is formed from 9-hydroxyperoxy linoleic acid (*39*, *40*). The higher concentration of oxygen is expected to increase the formation of peroxides, and this is generally supported by an increase in PV, but the appearance of octanal and *E*-2-nonen-1-ol suggests that the breakdown of hydroperoxides is also linked to levels of oxygen. Longer chain volatile compounds are typically reported in accelerated studies, e.g., Gutierrez et al. (*20*); however, such studies also lead to high levels of hexanal and acetic acid. Under the conditions employed in this study, concentrations of hexanal and acetic acid decreased with storage in the presence of oxygen. This reinforces our earlier observation that results from accelerated oxidation of oil must be extrapolated with caution to real-time shelf life studies.

As noted earlier, loss of particular compounds may be just as important an indicator of loss of freshness as the generation of new compounds during storage. The presence of oxygen during storage significantly ($p < 0.01$) lowered concentrations of acetic acid, 1-penten-3-ol, *E*-2-hexen-1-ol, and acetoxypinoresinol relative to storage in the absence of oxygen (**Table 1**). In addition to monitoring the generation of new compounds, monitoring the loss of these compounds may be important when investigating the effect of oxygen exposure during real-time virgin olive oil storage.

Potential Oxidation and Freshness Markers of Virgin Olive Oil. The change of oxidation markers with storage conditions (**Table 5**) may explain why diverse oxidation markers have been previously reported for virgin olive oil. Some proposed markers include nonanal (*15*) and the ratio of hexanal/nonanal (*38*, *41*). While most studies have used nonanal as a primary indicator of rancidity, Solinas et al. (*42*) observed that 2-pentenal and 2-heptenal were the main rancidity indicators. Neither nonanal nor 2-pentenal or 2-heptenal were identified as oxidation markers in this study. Although hexanal levels change with olive oil storage and it was identified statistically (**Table 5**) as a marker of storage in the light with headspace, hexanal is not favored as a marker compound. This is because the amount of hexanal does not distinguish oxidized oils from virgin oils, since hexanal originates from both enzymatic and chemical oxidation (*15*, *17*, *38*).

Gutierrez et al. (*20*) proposed the use of phenolic compounds to establish the average life of olive oils subjected to oxidation with the Rancimat method. In our study, all phenolic compounds decreased in concentration regardless of storage conditions. This suggests that oxidative processes are occurring even under mild conditions. It is interesting to note that in an oxygen-limited environment, SLDA identified monohydroxy compounds as discriminating variables (ligstroside dialdehyde and (+)-acetoxypinoresinol, **Table 4**), whereas in the presence of oxygen, the *ortho*-diphenol hydroxytyrosol was a discriminating variable (**Table 4**). Thus, phenolic compounds are not all equally affected by storage conditions and tyrosol was the only phenolic compound attributed as a marker compound (**Table 5**).

Parameters—*E*-2-hexenal, K_{232}, and K_{270}—that significantly ($p < 0.01$) discriminated virgin olive oil stored both with and without headspace (**Table 4**) were identified as common oxidative markers for oils stored at ambient temperature—dark and light (**Table 5**). The absence of any common oxidative markers for low-temperature stored oil (**Table 5**), which was shown earlier (**Figure 1**) to be closest to fresh oil, indicates that departure from freshness may be detected by changes in levels of *E*-2-hexenal, K_{232}, and K_{270}. Among these parameters, K_{232} and K_{270} are included in the classification of virgin olive oil quality (*28*, *29*) and *E*-2-hexenal was previously reported (*14*) as a marker of olive oil quality and freshness. Currently, *E*-2-hexenal is not included in the classification of virgin olive oil quality, with variations in concentrations in fresh oil attributed to cultivar and maturity effects (*26*). However, *E*-2-hexenal may be included as a parameter for classification of virgin olive oil quality and freshness with reference to its odor activity value to set a minimum value for its sensory impact.

This study has shown that real-time storage trials result in different changes to an oil than during accelerated studies. The use of SLDA allows reduction of a large body of data to identify oil parameters that are uniquely associated with different storage conditions. Loss of phenolic and volatile compounds occurs before parameters such as PV, K_{232}, and K_{270} reflect the changes to the oil. This is important for extra virgin olive oil, which is promoted for its sensory properties as well as potential health benefits. Moreover, changes to the oil accelerate when the oil is exposed to oxygen. Further work is required to establish the effective life of a virgin olive oil once it has come into domestic use.

ABBREVIATIONS USED

PV, peroxide value; FFA, free fatty acid; UV, ultraviolet; 3,4-DHPEA-DEDA, 3,4-dihydroxy phenyl ethyl alcohol—decarboxymethyl elenolic acid dialdehyde; IOOC, International Olive Oil Council; SLDA, stepwise linear discriminant analysis; 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.

ACKNOWLEDGMENT

We acknowledge the provision of olive samples by Riverina Olive Grove. We thank Malcolm Allen for helpful comments on an early draft of the manuscript and Daniel Jardine for his assistance in the LC-MS work.

LITERATURE CITED

(1) Rahmani, M.; Csallany, A. S. Role of minor constituents in the photo-oxidation of virgin olive oil. *J. Am. Oil Chem. Soc.* **1998**, *75*, 837−843.

(2) Velasco, J.; Dobarganes, C. Oxidative stability of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 661−676.

(3) 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.

(4) Aparicio, R.; Roda, L.; Albi, M. A.; Gutierrez, F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.

(5) Salvador, M. D.; Aranda, F.; Fregapane, G. Contribution of chemical components of Cornicabra virgin olive oils to oxidative stability. A study of three successive crop seasons. *J. Am. Oil Chem. Soc.* **1999**, *76*, 427−432.

(6) Tovar, M. J.; Motilva, M. J.; Luna, M.; Girona, J.; Romero, M. P. Analytical characteristics of virgin olive oil from young trees (Arbequina cultivar) growing under linear irrigation strategies. *J. Am. Oil Chem. Soc.* **2001**, *78*, 843−849.

(7) Beltran, G.; Aguilera, M. P.; Rio, C. D.; Sanchez, S.; Martinez, L. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chem.* **2005**, *89*, 207−215.

(8) Salvador, M. D.; Aranda, F.; Gomez-Alonso, S.; Fregapane, G. Cornicabra virgin olive oil: A study of five crop seasons. Composition, quality and oxidative stability. *Food Chem.* **2001**, *74*, 267−274.

(9) Mousa, Y. M.; Gerasopoulos, D.; Metzidakis, I.; Kiritsakis, A. Effect of altitude on fruit and oil quality characteristics of 'Mastoides' olives. *J. Sci. Food. Agric.* **1996**, *71*, 345−350.

(10) Ranalli, A.; Modesti, G.; Patumi, M.; Fontanazza, G. The compositional quality and sensory properties of virgin olive oil from a new olive cultivar—I-77. *Food Chem.* **2000**, *69*, 37−46.

(11) Psomiadou, E.; Konstantinos, X.; Blekas, K. G.; Tsimidou, M. Z.; Boskou, D. Proposed parameters for monitoring quality of virgin olive oil (Koroneiki *cv*). *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 403−409.

(12) Pagliarini, E.; Zanoni, B.; Giovanelli, G. Predictive study on Tuscan extra virgin olive oil stability under several commercial conditions. *J. Agric. Food Chem.* **2000**, *48*, 1345−1351.

(13) Tsimidou, M.; Papadopoulos, G.; Boskou, D. Phenolic compounds and stability of virgin olive oil-Part I. *Food Chem.* **1992**, *45*, 141−144.

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

(15) 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.

(16) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(17) Angerosa, F. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 639−660.

(18) Tsimidou, M. Polyphenols and Quality of virgin olive oil in retrospect. *Ital. J. Food Sci.* **1998**, *10*, 99−116.

(19) Litridou, M.; Linssen, J.; Schols, H.; Bergmans, M.; Posthumus, M.; Tsimidou, M.; Boskou, D. Phenolic compounds in virgin olive oils: Fractionation by solid phase extraction and antioxidant activity. *J. Sci. Food. Agric.* **1997**, *74*, 169−174.

(20) Gutierrez, F.; Villafranca, M. J.; Castellano, J. M. Changes in the main components and quality indices of virgin olive oil during oxidation. *J. Am. Oil Chem. Soc.* **2002**, *79*, 669−676.

(21) Okogeri, O.; Tasioula-Margari, M. Changes occurring in phenolic compounds and alpha-tocopherol of virgin olive oil during storage. *J. Agric. Food Chem.* **2002**, *50*, 1077−1080.

(22) Psomiadou, E.; Tsimidou, M. Stability of virgin olive oil. 2. Photo-oxidation studies. *J. Agric. Food Chem.* **2002**, *50*, 722−727.

(23) Psomiadou, E.; Tsimidou, M. Stability of virgin olive oil. 1. Autoxidation studies. *J. Agric. Food Chem.* **2002**, *50*, 716−721.

(24) Cinquanta, L.; Esti, M.; Di Matteo, M. Oxidative stability of virgin olive oils. *J. Am. Oil Chem. Soc.* **2001**, *78*, 1197−1202.

(25) Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed.; Pearson Education Limited: England, 2005.

(26) 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.

(27) 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.

(28) EC 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. *Off. J. L* **1991**, *248*, 0001−0083.

(29) IOOC Trade standard applying to olive oil and olive pomace oil. *COI/T. 15/NC no. 3/Rev 1*; 2003.

(30) Li, Y.; Jiang, J.-H.; Chen, Z.-P.; Xu, C.-J.; Yu, R.-Q. Robust linear discriminant analysis for chemical pattern recognition. *J. Chemom.* **1999**, *13*, 3−13.

(31) Field, A. *Discovering Statistics Using SPSS for Windows*, 1st ed.; SAGE Publications Ltd.: London, 2000.

(32) Hamilton, R. J.; Kalu, C.; Prisk, E.; Padley, F. B.; Pierce, H. Chemistry of free radicals in lipids. *Food Chem.* **1997**, *60*, 193−199.

(33) 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.

(34) Ridolfi, M.; Terenziani, S.; Patumi, M.; Fontanazza, G. Characterization of the lipoxygenases in some olive cultivars and determination of their role in volatile compounds formation. *J. Agric. Food Chem.* **2002**, *50*, 835−839.

(35) Gutierrez, F.; Jimenez, B.; Ruiz, A.; Albi, M. A. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *J. Agric. Food Chem.* **1999**, *47*, 121−127.

(36) Gutfinger, T. Polyphenols in olive oils. *J. Am. Chem. Soc.* **1981**, *58*, 966−968.

(37) Di Giovacchino, L. Technological aspects. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J., Aparicio, R., Eds.; Aspen Publications, Inc.: Gaithersburg, MD, 2000; pp 17−59.

(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) Fisher, C.; Scott, R. S. *Food Flavours: Biology and Chemistry*; The Royal Society of Chemistry: Cambridge, United Kingdom, 1997.

(40) Frankel, E. N. Hydroperoxide decomposition. In *Lipid Oxidation*; Frankel, E. N., Ed.; The Oily Press Limited: Dundee, Scotland, 1998; pp 55−77.

(41) Kiritsakis, A. K. Flavor components of olive oil—A review. *J. Am. Oil Chem. Soc.* **1998**, *75*, 673−681.

(42) Solinas, M.; Marsilio, V.; Angerosa, F. Behaviour of some components of virgin olive oil flavour in relation to maturity. *Riv. Ital. Sost. Grasse* **1987**, *64*, 475.

Received for review April 12, 2006. Revised manuscript received July 15, 2006. Accepted July 20, 2006. Financial support from Rural Industries Research & Development Corp. (RIRDC) and Charles Sturt University is gratefully acknowledged.

JF061038J