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ARTICLE

Physical and Chemical Changes during the Maturation of Gordal Sevillana Olives (*Olea europaea* L., cv. Gordal Sevillana)

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A series of physical and chemical changes occur as olives mature on the tree, and these changes are important for the production of oil and table olives. The aim of this study was to increase the understanding of the maturation process of Gordal Sevillana olives, to optimize harvest timing, and to determine the most appropriate harvesting and post-harvesting processing methods. During maturation, the olive size, flesh/pit ratio, and oil content increased, with a maximum oil content of 72 g kg^{-1} (wet weight). Changes in the fatty acid composition are reported. Levels of both total sugars and total phenolic compounds slightly decreased over the maturation period; however, we observed that these compounds were continually being synthesized until full black maturity. The optimal harvest time for the production of Gordal Sevillana as Spanish-style green olives occurred immediately prior to the color change from green to turning color, at which point the sugar levels and flesh/pit ratio were at maximum levels.

KEYWORDS: ***Olea europaea*** **L.; cv. Gordal Sevillana; olive; composition; harvest timing; maturation**

INTRODUCTION

The olive tree (*Olea europaea*) is widely cultivated for the production of both oil and table olives and is of significant economic importance. During olive growth, a number of physical and chemical changes occur, many of which are important for the production of both table olives and olive oil. These changes are influenced by the cultivar, fruit ripeness, irrigation regimes, and environmental factors (geographical area, soil quality, type of cultivation, rainfall, etc.) (*1*−*4*). For example, changes in the fatty acid and phenolic profiles influence the quality, sensorial, and nutritional properties of the oil, and phenolic compounds impart the desired bitterness to table olives. In addition to providing a carbon source for the fermentation of table olives, sugars provide energy for metabolic changes during maturation and contribute to olive fruit texture (*4*, *5*).

This study aims to investigate pertinent physical and chemical (sugars, lipids, total phenolics, and moisture) changes occurring during the maturation of Gordal Sevillana olives (*O. europaea* L., cv. Gordal Sevillana), a variety typically cultivated for the production of table olives. We aim to increase the understanding of the maturation process, which may be useful for optimal harvest timing and the determination of the most appropriate harvesting and post-harvesting processing methods.

MATERIALS AND METHODS

Sampling. Olives were handpicked from all sides of six representative olive trees in a grove at Yandoit Hills, Victoria, Australia. Samples of approximately 1−2 kg were taken periodically from fruit set until full black maturation and stored under vacuum at −18 °C prior to analysis.

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Physical Analysis. The average olive weight was determined by finding the number of olives per 100 g. The olives were dissected to remove the pits, and both fractions were weighed. The flesh/pit ratio was calculated by dividing the flesh weight by the pit weight of these olives.

Moisture. The moisture content was determined on triplicate 100 g samples of homogenized olives with the pits completely removed (except for the first sample, which had no identifiable pit) by vacuum drying (Thermoline Scientific, Smithfield, Australia) until a constant weight (for at least 12 h at 60 °C and approximately −90 kPa). The dried olive mass of the triplicate samples was combined and stored at −18 °C.

Total Lipids and Fatty Acids. Lipids were extracted in triplicate by auto Soxhlet (Buchi 810, Flawil, Switzerland) for at least 2.5 h with 40−60 °C petroleum ether. Extracted oil was stored at −18 °C in amber vials under nitrogen. Fatty acids were quantified by preparing fatty acid methyl esters (FAMEs) (*6*). A total of 1 mL of methyl tridecanoate (Sigma-Aldrich, St. Louis, MO)/hexane internal standard solution (to give 5 mg of methyl tridecanoate) was added to 500 mg of extracted lipids in 2 mL of hexane. The spiked samples were saponified in large test tubes with 1 mL of 2 M NaOH in methanol and were held in a water bath at 75 °C for 20 min. The free fatty acids were methylated by the addition of 1 mL of 3 M H_2SO_4 in methanol, and the tubes were held at 75 °C for 20 min. Upon cooling, 12 mL of Milli-Q water and 1 mL of hexane were added to achieve separation of the layers. The top layer was transferred to amber vials using Pasteur pipettes and was subsequently injected into a Varian 3400 gas chromatograph (GC) fitted with a flame ionization detector (FID) and a 25 m BP1 column (SGE, Ringwood, Australia), with a 0.32 mm internal diameter and a film thickness of 0.5 μm. The column was temperature-programmed as follows: held at 80 °C for 3 min, increased to 185 °C at 15 °C min^{-1}, held for 4 min, increased to 220 °C at 3 °C min^{-1}, heated to 300 °C at 10 °C min^{-1}, and held for 20 min. The carrier gas was nitrogen, and the injector and detector were operated at 230 °C. Individual fatty acids were identified by a comparison of retention times against standard fatty acid mixtures (Supelco, Bellefonte, PA). A 1:100 dilution with hexane was required to resolve the 18 carbon fatty acids.

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Sugars and Phenolic Compounds. Sugars and phenolic compounds were extracted in duplicate with hot ethanol, using a method adapted from Wills et al. (*7*). Each dried, defatted sample (5 g) was weighed into a 250 mL beaker, to which 25 mL of boiling 85% ethanol was added. The solution was frequently stirred and brought to boil for a few minutes on a steam bath, before being filtered (Whatman No. 541) into an Erlenmeyer flask. This extraction procedure was performed a total of 4 times, after which the ethanol was removed by rotary evaporation under vacuum at 45 °C. The remaining solution (approximately 3 mL) was made up to 10 mL with Milli-Q water in a volumetric flask, and the extracts were divided into two 5 mL portions for sugar and phenolic analysis and stored at −18 °C.

The total phenolic content was determined in duplicate on duplicate samples as previously described (*8*), with a further 1:10 dilution to fit the linear range of the assay.

Individual sugars (glucose, fructose, galactose, and sucrose) and the sugar alcohol mannitol were quantitated by gas chromatography (*9*). To remove pigments and other phenolic compounds, the ethanolic extracts were filtered through Sep-Pak C18 cartridges (Waters, Milford, MA). The filter cartridges were activated by flushing with 5 mL of methanol, followed by 10 mL of Milli-Q water, and finally 2−3 volumes of air to remove residual methanol and water. Equal volumes (100 μL) of the filtered extract and a xylose internal standard solution (to give 1 mg of xylose) were allowed to reach mutarotational equilibrium (*10*) and were dried overnight in a fume hood. Although reported in some olive cultivars at low levels, xylose was not detected in our samples and, thus, was a suitable internal standard. The total sugar content of the dried sample was always kept at less than 10 mg. After drying, 1 mL of pyridine was added and the samples were placed in a water bath for 15 min at 60 °C. Trimethylsilyl (TMS) derivatives were prepared by the addition of 200 μL of hexamethyldisilazane (HMDS) (Sigma-Aldrich, St. Louis, MO) and 100 μL of trimethylchlorosilane (TMCS) (Sigma-Aldrich, St. Louis, MO) (*9*). The mixture was shaken vigorously for 30 s and then allowed to stand at room temperature for at least 5 min prior to injection. The derivatives were quantitated using the same GC and column used for fatty acids. Using nitrogen as the carrier gas, the column was held isothermally at 150 °C for 100 min, heated to 300 °C at 10 °C min^{-1}, and then held at this temperature for 20 min to bake off any residues. The injector was operated at 280 °C, and the detector was operated at 300 °C. Prior to injecting samples, the column was conditioned for TMS derivatives by injecting 5 μL of HMDS onto the column 15 times at 2.5 min intervals, while the column was maintained isothermally at 185 °C. For the conditioning, the injector and detector temperatures were 220 and 225 °C, respectively. After the final HMDS injection, the column was held at 300 °C for 10 min and the HMDS maintained the column. TMS derivatives of reducing sugars gave multiple peaks because of the presence of tautomeric and isomeric forms (*11*). Total sugars were calculated by addition.

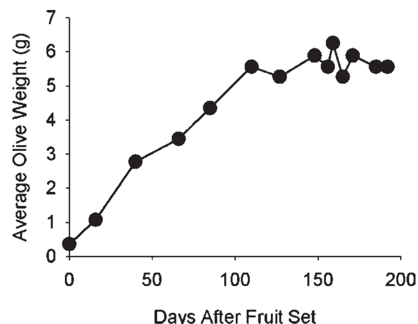

Figure 1. Changes in the mean olive weight during the maturation of Gordal Sevillana olives. Determined as the number of olives per 100 g.

RESULTS AND DISCUSSION

Physical Characteristics. The average olive weight shows the changes in the size of the olives during maturation (**Figure 1**). The weight of the olives increased during the first 110 days, after which there was little change. **Figure 2** is a collection of digital photographs, which shows the changes in color of the olives from green to black. These photographs show easily observable characteristics, which can be related to the physical and chemical composition data. The olives were green for the first 127 days, turning color after 156 days, and black from 171 days. **Figure 3** shows that the flesh/pit ratio increased from 2.3 after 40 days to values of 4.0−4.5 from 159 to 192 days. The slight decrease at the end of the sampling period may be partially attributed to large, mature olives dropping to the ground, leaving only smaller, late maturing fruit on the trees. For the production of naturally black (Greek)-style olives, it is generally advisible to harvest at the point of maximum flesh/pit ratio (*12*), although the Gordal Sevillana cultivar is rarely processed according to the Greek-style method. Furthermore, the Gordal Sevillana cultivar investigated in this study reached its point of maximum flesh/pit ratio at the time that the olives were reaching turning color rather than black (compare **Figures 2** and **3**). This finding suggests that Gordal Sevillana olives are not best suited for the production of naturally black (Greek)-style table olives.

Chemical Composition. During maturation, the moisture content reached a maximum of 803 g kg^{-1} after 40 days (**Figure 4**), followed by a steady decline to a minimum of 681 g kg^{-1}, 185 days after fruit set. The reductions in moisture content during ripening described here are similar to those reported elsewhere (*1*, *13*), with some fluctuations being attributed to seasonal climatic conditions (*1*, *14*, *15*).

Phenolic compounds influence the sensory properties (color, flavor, bitterness, etc.) of olives and their oil, and they protect against oxidative rancidity by acting as antioxidants, which are increasingly being recognized as playing a beneficial role in the diet (*3*, *16*). The phenolic content of olives is typically influenced by a range of factors, such as the maturity level, cultivar, and climate (*2*, *3*). An initial sharp decline in the phenolic content of the olives was observed in this study, followed by a relatively stable total phenolic content. Over the first 40 days, the total phenolic content of the olives decreased rapidly to 1560 ppm gallic acid equivalents (GAE), less than 50% of the original value (3900 ppm GAE) (**Figure 5A**). The total phenolic content was relatively stable from 40 to 171 days, with a slight increase to 2200 ppm GAE. Despite the decrease in percentage terms, plotting the data on a per olive basis shows that phenolic compounds are continually being synthesized during the maturation period (**Figure 5B**). A proportion of the observed initial decrease in total phenolics may be attributed to the pit, which was indistinguishable from the flesh for the first sample, while all subsequent samples had the pit removed from the olive flesh. The olive pit is known to be rich in phenolic compounds (*16*, *17*), whereas the slight increase in total phenolics toward the end of

Figure 2. Digital photographs of representative olives showing the changes in size and color during the maturation of Gordal Sevillana olives. Not to scale.

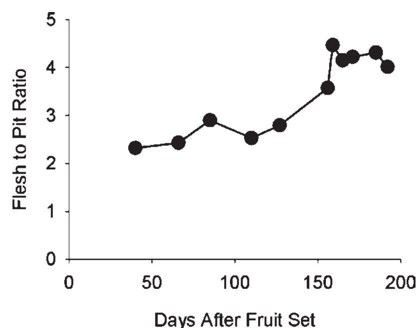

Figure 3. Changes in the flesh/pit ratio during the maturation of Gordal Sevillana olives. Data points are means derived from the masses of flesh and pit in 100 g of olives.

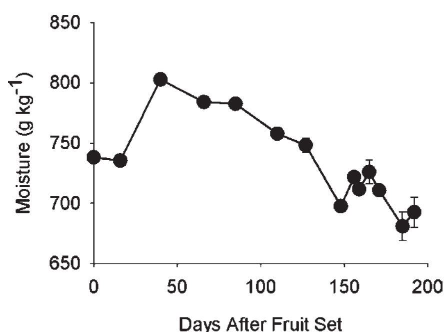

Figure 4. Changes in the moisture content during the maturation of Gordal Sevillana olives (edible portion). Data points are mean values ± standard deviation of triplicate samples.

maturation coincided with the darkening of the fruits (**Figures 3** and **5A**), which is linked to anthocyanin development. Relatively constant total phenolic contents have previously been reported for the Gordal (and Cucco) cultivars (*18*), and the final values reported here were similar to those reported for other cultivars (*16*, *19*).

The concentration of the major sugars (glucose, fructose, galactose, and sucrose) and the primary sugar alcohol (mannitol) were monitored during maturation (panels **A** and **B** of **Figure 6**). The total sugar content showed an initial increase to 53 g kg^{-1} at 16 days, followed by a steady decline as the olives matured, reaching a minimum of 31 g kg^{-1} after 192 days (**Figure 6A**). Glucose was the dominant sugar throughout maturation, decreasing from 32 to 16 g kg^{-1}, and fructose remained relatively stable at just over 10 g kg^{-1}. Mannitol was present at approximately 8 g kg^{-1} over the majority of the maturation period, with a decline to 4 g kg^{-1} over the last 30 days. Galactose was detected at low levels (1 g kg^{-1}), and sucrose was found only in trace amounts (data not shown). Comparable trends in sugar composition during maturation have been reported for other cultivars (*1*, *20*), and the sugar concentration has been proposed as a ripening index for olive oil production, because minimum sugar values have been shown to correspond to maximum oil levels (*21*). While the bulk data (g kg^{-1} sugar; **Figure 6A**) shows a decrease in individual and total sugars during maturation, the per olive data (**Figure 6B**) shows that sugars were continuously synthesized until the olives began darkening (compare **Figures 2** and **6B**). From turning color to full black maturity, the per olive sugar levels decreased by approximately one-quarter. The difference between the two units is most likely due to the fact that, while the individual olives were physically growing in size, they proportionally synthesized less sugar than other fruit constituents,

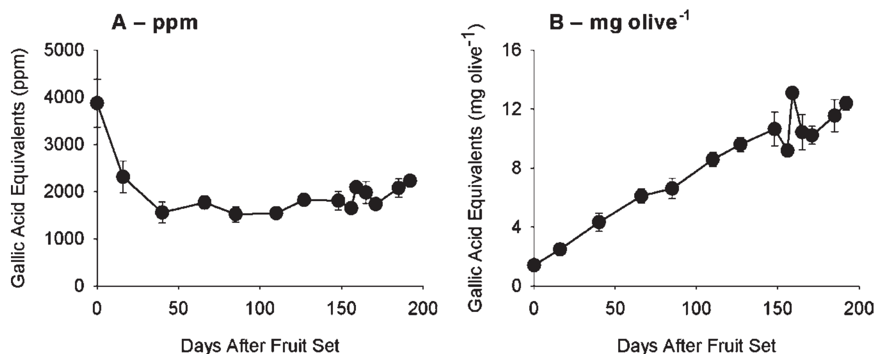

Figure 5. Changes in the total phenolic content during the maturation of Gordal Sevillana olives expressed as both ppm and mg olive^{-1}, for the edible portion, wet weight. Data points are mean values ± standard deviation of duplicate analyses on duplicate samples.

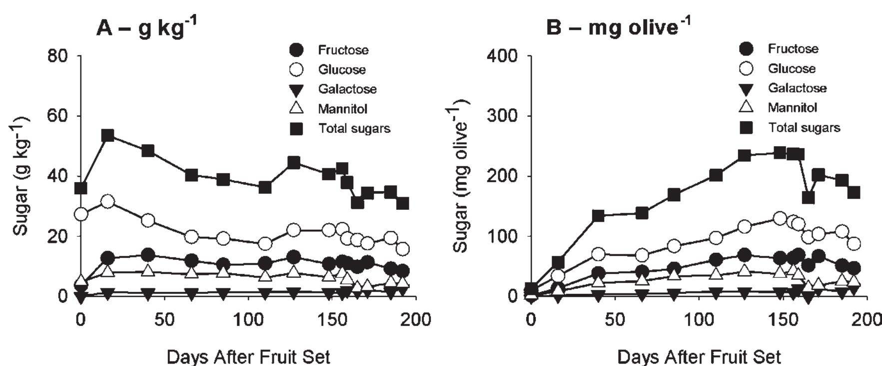

Figure 6. Changes in the total and individual sugars during the maturation of Gordal Sevillana olives expressed as both g kg^{-1} and mg olive^{-1}, for the edible portion, wet weight. Data points are mean values of duplicate samples, which agreed closely.

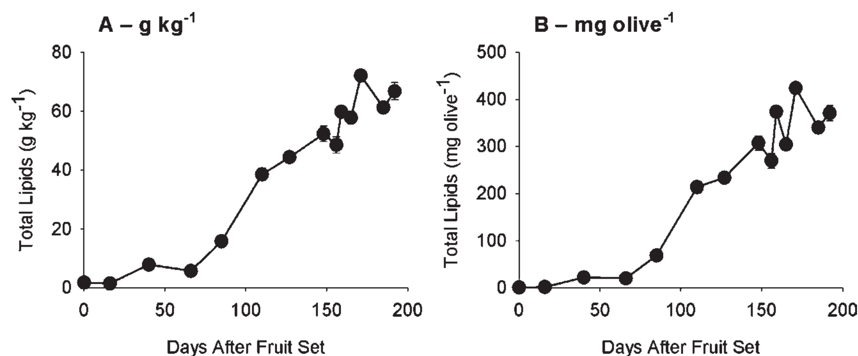

Figure 7. Changes in the total lipids (oil) content during the maturation of Gordal Sevillana olives expressed as both g kg^{-1} and mg olive^{-1}, for the edible portion, wet weight. Data points are mean values ± standard deviation of triplicate samples.

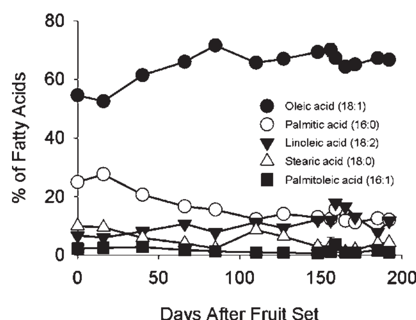

Figure 8. Changes in the major fatty acids during the maturation of Gordal Sevillana olives. Data points are mean values ± standard deviation of triplicate samples.

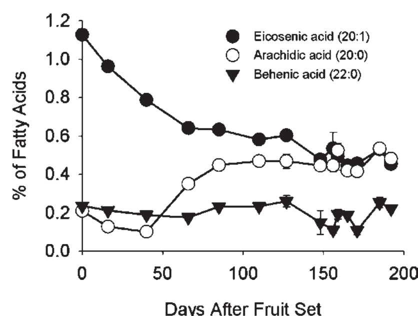

Figure 9. Changes in the minor fatty acids during the maturation of Gordal Sevillana olives. Data points are mean values ± standard deviation of triplicate samples.

resulting in a perceived decrease in sugars per kilogram of olive flesh, while there was still a net increase in sugars in the individual olives.

The lipid content of the olives increased considerably during maturation (panels **A** and **B** of **Figure 7**). At fruit set, the lipid content of the olives was 2 g kg^{-1} (edible portion, wet weight), which increased slowly to 16 g kg^{-1} after 85 days. Over the next 90 days, the lipid content increased rapidly to a maximum of 80 g kg^{-1}. When expressed on a per olive basis, the trend for lipid synthesis mirrored that for total lipid accumulation. That is, there was minimal lipid synthesis up to 85 days, after which point lipids were synthesized at a greater rate. The relatively low oil content ensures that the majority of Gordal olives are processed for table olives and not for oil (*12*). It has been reported that the optimum harvest time for economic oil extraction from olives is at the end of the linear accumulation period and that early harvesting results in a slightly bitter oil with reduced yield, while late harvesting risks damage to fruit for an insignificant gain in oil quantity (*22*). Therefore, the most appropriate harvest time for oil production from the Gordal Sevillana olives would have occurred approximately 171 days after fruit set, with a peak yield of 72 g kg^{-1}. Although different cultivars have varying final oil contents, similar trends in oil accumulation have been reported for other varieties (*1*, *13*, *23*, *24*).

Levels of the major fatty acids (**Figure 8**) and the minor fatty acids (**Figure 9**) were also monitored during ripening. As would be expected, oleic acid was the major fatty acid detected in the olives over the entire maturation period. The oleic acid fraction increased to 71.6% of total fatty acids, 85 days after fruit set, and then decreased gradually to 66.7% at the final sampling point. The linoleic acid content increased during ripening from 6.7% to a maximum of 17.9% after 159 days, before reducing to 11.7%. A small reduction in the palmitoleic acid content was observed, falling from the initial value of 2.2−1.1%, while eicosenic acid decreased steadily from 1.13 to 0.45%. The levels of the major saturated fatty acids decreased during maturation, with palmitic acid decreasing from a maximum of 27.6% after 16 days to 12.1% and stearic acid levels dropping from 10.0 to 4.3%. The proportion of arachidic acid more than doubled during ripening, from 0.21 to 0.48%, while little change was observed in the behenic acid levels, with an initial value of 0.24%, a final value of 0.22%, and a maximum of 0.26%. It is clear that the fatty acid profile of olive oil varies considerably as a result of the harvest time. General decreases in palmitic and oleic acids and the increase in linolenic acid observed in this study are in agreement with results reported by others for different olive cultivars (*1*, *25*).

Here, we have shown the changes in several important compounds during the maturation of Gordal Sevillana olives. During the ripening period, the olive size, flesh/pit ratio, and oil content all increased, while the moisture and total sugar contents decreased. Changes in the fatty acid composition during ripening were reported, and total phenolics initially decreased and then gradually increased during maturation. However, despite the apparent decreases in sugars and phenolics, we have reported that these compounds are continually synthesized during olive maturation. The Gordal Sevillana cultivar is traditionally used for the production of green table olives (*12*). The optimum timing of harvesting would therefore be just prior to the onset of the turning color state. Our observations show that the optimal maturity levels for this cultivar as a green table olive would be when it reaches its maximum pit/flesh ratio (**Figures 2** and **3**). Furthermore, the optimal maturity level coincides with the maximum sugar content achieved during maturation (**Figures 2** and **6B**). Maturing the olives beyond the onset of the turning

color state would result in a decrease in sugars in the fruit, which would minimize the available substrate for the subsequent fermentation of the green table olives.

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