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Impact of Cultivar, Harvesting Time, and Seasonal Variation on the Content of Biophenols in Olive Mill Waste

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Olive mill waste (OMW) contains substantial amounts of valuable antioxidant biophenols that can be recovered for possible applications in food, pharmaceutical, and cosmetic industries. However, the impact of cultivar, harvesting time, and seasonal variation on the phenolic composition of OMW has not yet been assessed. Total phenols, antioxidant activity, and phenol profiles of OMW extracts from five different Australian-grown cultivars (Barnea, Correggiola, Manzanillo, Mission, and Paragon) were studied at four different harvesting times in the 2004 season. The impact of seasonal variation was assessed by comparing total phenol content, antioxidant activity, and phenol profile of two cultivars (Correggiola and Mission) harvested in the 2004 and 2005 seasons. The phenol content and antioxidant activity at different harvesting times were mainly a function of the olive cultivar. Harvesting time had a quantitative effect rather than a qualitative effect on the phenol profile. Intercultivar and harvesting time variation accounted for a 2−5-fold change in the total phenol and antioxidant capacity, while levels of individual biophenols experienced up to 50-fold change. The phenol content and antioxidant capacity of OMW significantly changed between seasons with different variation patterns for different cultivars.

KEYWORDS: Biophenol classes; biophenol profiles; antioxidant activity

INTRODUCTION

Prior to the 1970s, farmers were the only group with any interest in olive mill waste (OMW) as they faced a significant disposal problem. The expansion of the industry, with increased production of noxious waste, presented a serious environmental concern (*1, 2*). Sustainable development of the industry meant that waste management protocols required careful consideration. Papers examining OMW began to appear in the 1960s motivated by scientific curiosity and the possible agricultural benefits of the waste. Initial research targeted biophenols as undesirable substances and endeavored to dephenolize the OMW (*3*−*5*). Although various uses have been proposed including a potentially renewable energy source (*6, 7*) or fertilizer (*8*), it is value addition that offers the greatest potential. Thus, OMW has been proposed as a low-cost substrate for the production of various chemicals (e.g., xanthan (*9*) and ethanol (*10*)). The production of biologically active compounds from OMW constitutes a viable alternative for valorizing this problematic waste.

Following recognition of the antioxidant activity of OMW (*11, 12*) and the association of oxidative stress with many diseases, it was logical to consider OMW as a potential source of biophenolic antioxidants. Thus, research on the valorization of OMW increased significantly in the 1990s (*6, 12, 13*). The European Union initiated a project, Natural Antioxidants from Olive Oil Processing Waste Waters (*14*), investigating the extraction of biophenols from OMW (*13*). Poor reproducibility of bioactivity upon recollection from plant extracts is not uncommon (*15*). Factors that enhance this variability if not monitored carefully include cultivar, harvesting time, and seasonal variation.

The impact of these variables on phenolic content and antioxidant activity of OMW are assessed in this article. This work is the first study to combine spectrophotometric quantitative measures, chromatographic profiling, and antioxidant activity to examine the variability and stability of OMW biophenols for adding value to OMW. The effects of analysis and extraction conditions that can complicate the results of such studies have been reported previously (*16, 17*).

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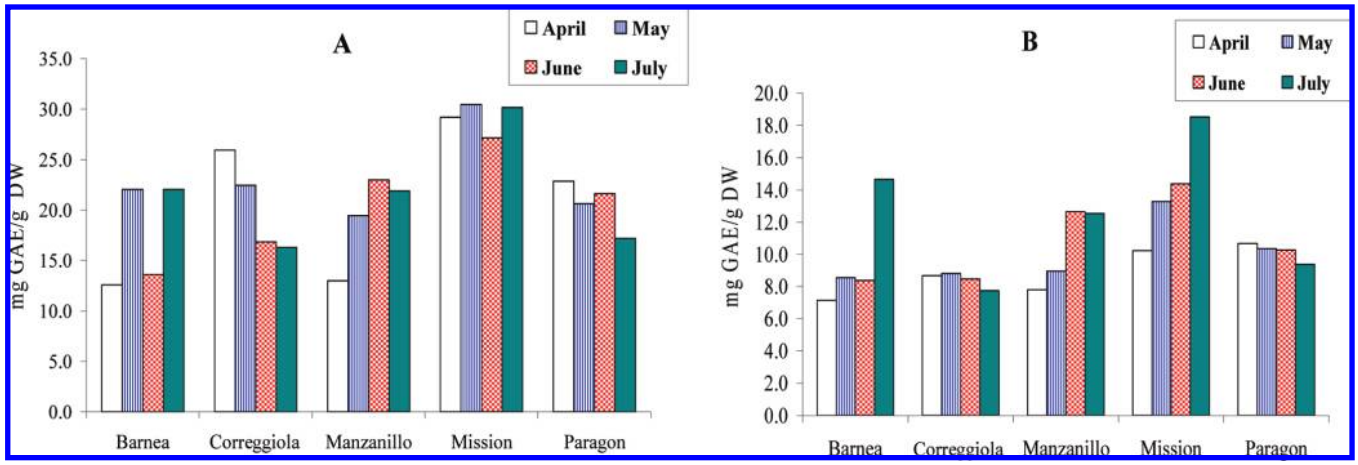

Figure 1. (**A**) Total phenol (Folin−Ciocalteu) content of OMW from different olive cultivars at different harvest points in the 2004 season. (**B**) Total phenol (direct spectrometric measurement at 280 nm) content of OMW from different olive cultivars at different harvest dates in the 2004 season. GAE: gallic acid equivalent. Coefficient of variation was less than 5% in all cases.

MATERIALS AND METHODS

Samples. Olive fruits were harvested from an olive grove at Cookathama near Darlington Point, NSW, Australia and stored at 3 °C until processing. Cultivars Barnea, Correggiola, Manzanillo, Mission, and Paragon were harvested on April 15, May 6, June 1, and July 13 in the 2004 Season. For assessment of seasonal variations, samples from Correggiola and Mission were collected on June 6, 2005.

Generation of Olive Mill Waste. Oil extraction was performed on an Abencor laboratory-scale olive mill comprising a hammer mill, a thermo-malaxer, and a centrifuge that imitated industrial processing. Approximately 1 kg of olive fruit was ground to a paste using the hammer mill, and 700 g of the paste was placed in a mixing jar and malaxed for 20 min at 25 °C in the thermo-malaxer. Boiling water (300 mL) was added, and the paste was remalaxed for 10 min. Centrifugation of the paste resulted in three phases: solid pomace, wastewater, and olive oil. The pomace or OMW was analyzed in the current study.

Biophenol Extraction. OMW (10 g) was extracted with methanol/water/HCl (80/20/1; 15 mL) for 30 min with stirring. After recovery of extract, the process was repeated (15 min) with fresh solvent (10 mL). The combined extracts were filtered through Whatman No. 1 filter paper and defatted by *n*-hexane (30 mL × 2). The defatted extract was filtered through GF/F filter paper, and then refiltered using 0.2 μm Nylon nonsterile syringe filters (Phenomenex, Australia). All extractions were performed at room temperature (20 ± 2 °C). The crude extracts were stored at −18 °C until analyzed.

Dry Weight, Extractable Matter, and pH. These were determined as described earlier (*17*). This involved a gravimetric procedure for dry weight determination while pH was determined on an aqueous suspension (25 mL) of the freeze-dried powder (10 g). Extractable matter (dry matter) was determined using 2 mL of the extracts.

Phenol Content Analysis. *Spectrophotometric Measures.* All analyses were performed in triplicate within 48 h of the extraction. Crude extract (1 mL) was diluted with water to 10 mL, and this diluted extract was used for subsequent spectrophotometric measurements. Quantitative spectrophotometric measures for Folin−Ciocalteu total phenols, phenol classes, and *o*-diphenols were performed as described earlier (*17*) using spectrophotometric measurement and periodically prepared calibration curves of the relevant standards. Total phenols were determined using Folin−Ciocalteu reagent and results expressed as gallic acid equivalents (GAE). Results for *o*-diphenols measured colorimetrically at 370 nm are expressed as caffeic acid equivalents (CAE). Total phenols were also measured directly at 280 nm and results expressed as GAE, while hydroxycinnamic acid derivatives were determined by measuring absorbance at 320 nm and results expressed as CAE; flavonols were estimated as quercetin equivalents QE by measuring the absorbance at 360 nm, and anthocyanins were measured at 520 nm and results expressed as cyanidin chloride equivalents CCE. All results are expressed as mg of the relevant standard per g dry weight of freeze-dried material to avoid differences due to the change of moisture content between different samples.

Table 1. Recovery of Major Antioxidant Biophenols from Australian Olive Cultivars during the 2004 Harvest Season

biophenol	recovery range[a]	highest recovery cultivar[b] (harvest time)	lowest recovery cultivar (harvest time)	cultivar showing (highest average recovery)[a]
hydroxytyrosol glucoside	78–3108[c]	MS (July)	BR (April)	MS (1939)[c]
hydroxytyrosol	108–754	MS (May)	MZ (July)	MS (560)
verbascoside	39–5020	MS (July)	BR (April)	MS (3782)
oleuropein	27–1519	MS (April)	CR (July)	MZ (567)
comselogoside	52–394[d]	MS (April)	MS (July)	MZ (288)[d]
luteolin	14–425	BR (May)	MS (July)	PR (328)

[a] mg/kg DW. [b] BR = Barnea; CR = Correggiola; MZ = Manzanillo; MS = Mission; PR = Paragon. [c] mg hydroxytyrosol equivalent/g. [d] Expressed as *p*-coumaric acid equivalents.

Chromatography and Phenolic Profiles. *HPLC-DAD Analysis of OMW Extracts.* HPLC-DAD was performed with a Varian 9021 solvent delivery system equipped with a Varian 9065 Polychrom UV diode array detector (190−367 nm). Separation was performed (*17*) by gradient elution with 100:1 water/acetic acid (v/v) as solvent A and a mixture of 90:10:1 methanol/acetonitrile/acetic acid (v/v/v) as solvent B on a Luna C-18(2) column, 5 μm particle size (150 mm × 4.6 mm) (Phenomenex, Australia) attached to a SecurityGuard guard cartridge (Phenomenex, Australia).

Antioxidant Capacity. Antioxidant capacity was determined using a DPPH radical scavenging assay involving measurement at 517 nm as described previously (*18*). EC_{50} values were expressed as ppm (μg/mL) of extractable matter, then antioxidant capacity was calculated as $100/(EC_{50})$. The expression of results as antioxidant capacity facilitated graphical presentation and sample comparisons; hence, antioxidant capacity is directly proportional to activity unlike EC_{50}.

Statistical Analysis. Sampling was performed in triplicate, and at least duplicate samples were analyzed. Data are expressed as means ± standard deviations. Data analysis was performed by Microsoft Excel. One-way ANOVA was carried out to test for significant differences using SPSS 11.5 (SPSS Inc., Chicago, IL). Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

OMW was produced from five Australian-grown olive cultivars tentatively identified as Barnea, Correggiola, Manzanillo, Mission, and Paragon. Drupes were collected at four time points (April, May, June, and July) within the olive harvesting

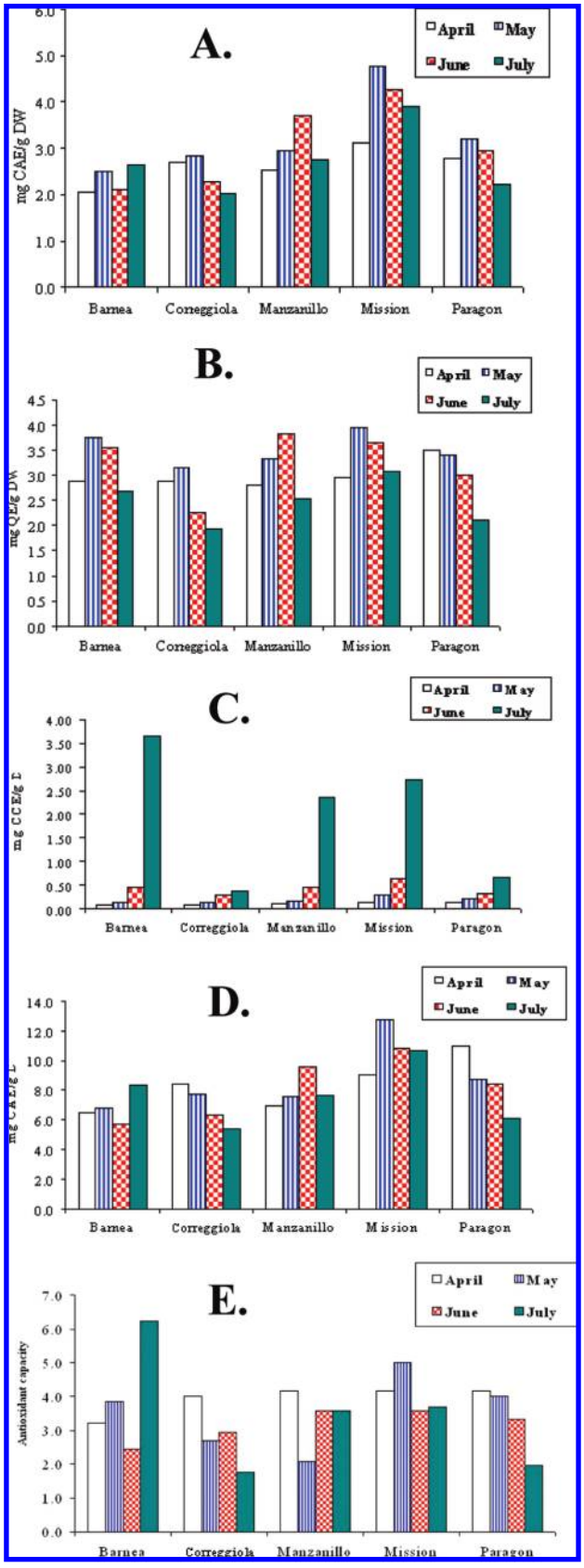

Figure 2. Content of various classes of biophenols plus antioxidant capacity of OMW from different olive cultivars at different harvest times in the 2004 season. (**A**) Hydroxycinnamic acid content. CAE: caffeic acid equivalent. (**B**) Flavonol content. QE: quercetin equivalent. (**C**) Red pigment content. CCE: cyanidin chloride equivalent. (**D**) *o*-Diphenols content. CAE: caffeic acid equivalent. (**E**) Antioxidant capacity. Coefficient of variation was less than 5% in all cases.

season in 2004 in order to assess the influence of cultivar and harvesting time (fruit maturation) on the phenolic content and profile of OMW.

pH, Moisture Content/Dry Weight, and Extractable Matter of OMW. The pH, dry weight, moisture content, and extractable matter data are presented because of their possible correlation with biophenol recovery. The pH of OMW influences the stability and recovery of biophenols and can provide an estimation of the acidic content of the waste. The pH of the aqueous extracts of fresh waste (10 g in 25 mL), for the studied cultivars during different harvest times, was in the acidic range (4.90−5.60), which is similar to the values reported for Spanish OMW (*19*). Generally, the change of pH between different harvest times for the same cultivar was small. For Correggiola and Paragon, the acidity gradually decreased toward the end of the season (July harvest), while for the other cultivars, acidity decreased toward midseason (May/June harvest) and then increased by the end of the season. For all of the studied cultivars apart from Manzanillo, the lowest pH values were reported in April harvest (early season).

Fruit agro-industrial byproducts, in general, are characterized by a high moisture content that constitutes a burden for subsequent value-adding procedures. The moisture (water) content in OMW depends not only on oil extraction technique but also upon the raw material itself (the fruit). The moisture content of the studied samples was between 60−70% w/w. There was little variation in the dry weight of OMW among the five studied cultivars throughout the different harvest times, with minimum dry weight 31.8% for Manzanillo in April and maximum dry weight 41.0% for Barnea in July. Although the overall change in the OMW dry weight was minimal for the same cultivar, the dry weight significantly increased toward the end of the season. Contradictory results were reported by Artajo et al. (*20*).

The variation in the extractable matter per dry weight (DW) between cultivars and in the same cultivar at different harvesting dates was significantly larger than the variation in dry weight. For Paragon, the variation in the extractable matter content was relatively small, 177−197 mg/g DW, while Manzanillo experienced a considerably larger variation, that is, 153−258 mg/g DW. The extractable matter ranged from 12.1% for Barnea in July to 25.8% for Manzanillo in June. Though the pattern of change differed between cultivars, a general trend was to achieve the highest content by midseason with a fall late in the season.

Total Phenols. Folin−Ciocalteu total phenol content of OMW extracts varied widely among different cultivars during the 2004 harvesting season, from 12.6 mg GAE/g DW for Barnea in April up to 30.5 mg GAE/g DW for Mission in May. The average total phenol content was in the following order: Mission > Barnea > Paragon > Manzanillo > Correggiola. The pattern of change in the total phenol content was different among different cultivars, and no obvious general trends were observed (**Figure 1A**). The total phenol content decreased toward the end of the season in the case of Correggiola, Paragon, and Manzanillo. For Barnea and Mission, total phenol content increased at the end of the season after a midseason drop.

The total phenol content showed a higher correlation with cultivar dry weight than with extractable matter, but the correlation was inconsistent. Barnea, Mission, and Manzanillo had a strong positive correlation with dry weight, while Paragon had a strong negative correlation, and Correggiola showed a weak positive correlation.

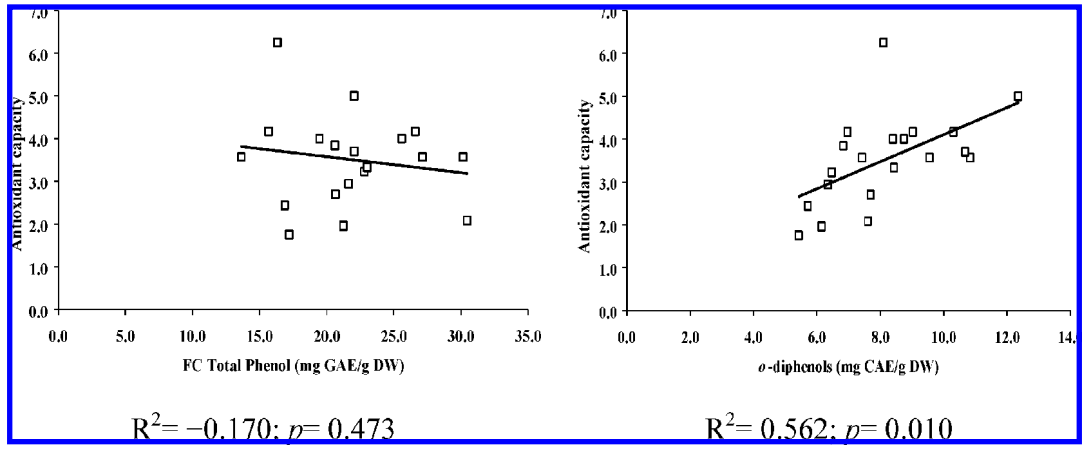

Figure 3. Correlation between antioxidant capacity and Folin−Ciocalteu total phenol and *o*-diphenols contents.

Table 2. Effect of Seasonal Variation on Phenol Content and Antioxidant Activity of Olive Mill Waste Extracts

	COR 2004[h]	COR 2005	MS 2004	MS 2005
dry weight[a]	39.5 ± 2.8 a	33.5 ± 0.9 b	33.6 ± 1.8 b	33.3 ± 2.0 b
extractable matter[b]	236.4 ± 9.5 a	195.5 ± 8.8 b	268.7 ± 16.1 d	231.2 ± 13.0 a
FC total phenols[c]	16.9 ± 1.2 a	22.9 ± 0.5 b	27.2 ± 2.7 d	32.9 ± 2.0 e
antioxidant capacity[d]	2.94 ± 0.09 a	4.73 ± 0.07 b	3.57 ± 0.07 d	5.73 ± 0.02 c
hydroxytyrosol glucoside[e]	0.40 ± 0.02 a	0.62 ± 0.01 b	2.03 ± 0.10 d	2.75 ± 0.24 e
hydroxytyrosol[f]	0.37 ± 0.03 a	0.45 ± 0.06 b	0.47 ± 0.03 b	0.84 ± 0.10 c
verbascoside[f]	1.22 ± 0.01 a	0.92 ± 0.01 b	4.06 ± 0.23 c	1.57 ± 0.09 d
oleuropein[f]	0.11 ± 0.01 a	0.06 ± 0.01 b	0.20 ± 0.02 c	0.11 ± 0.02 a
comselogoside[g]	0.09 ± 0.01 a	0.06 ± 0.01 b	0.15 ± 0.01 c	0.31 ± 0.01 d
luteolin[f]	0.21 ± 0.02 a	0.23 ± 0.01 a	0.07 ± 0.01 b	0.19 ± 0.01 a

[a] % w/w fresh weight. [b] mg/g DW. [c] mg GAE/g DW. [d] Antioxidant capacity = $100 \cdot 1/EC_{50}$ (ppm). [e] mg hydroxytyrosol glucoside/g DW. [f] mg/g DW. [g] mg *p*-coumaric acid equivalent/g DW. [h] COR = Correggiola cultivar; MS = Mission cultivar; 2004 = samples collected in the June 2004 season; 2005 = samples collected in June 2005; different letters in the same row indicate significantly different ($p > 0.05$) mean ± standard deviation of duplicates.

Total phenols were determined also by direct measurement of the absorbance at 280 nm (**Figure 1B**). A strong positive correlation ($R^2 = +0.789$, $p = 0.0001$) was found between Folin−Ciocalteu and direct spectrophotometric measurement of total phenols and, with few exceptions, a change pattern similar to the Folin−Ciocalteu results was found.

Hydroxycinnamic Acid, Flavonol, and Pigment Content. The hydroxycinnamic acid content varied between 2.0 mg CAE/g DW for Correggiola (July harvest) and Barnea (April harvest) and 4.8 mg CAE/g DW for Mission (May harvest). An initial increase in the hydroxycinnamic acid content followed by a gradual decrease toward the end of the season appeared as a general trend, apart from Barnea, which showed a gradual increase toward the end of the season after an abrupt increase in May (**Figure 2A**).

The lowest flavonol content was registered in July for Correggiola (1.9 mg QE/g DW) and the highest content was for Mission in May (4.0 mg QE/g DW). All cultivars studied showed a midseason climax, either in May or June, and a drop in July, except for Paragon, which had a gradual decrease in its flavonol content (**Figure 2B**).

Absorption at 520 nm provides an indication of anthocyanin content. However, other pigments absorb at this wavelength, and we refer to the result of the measurement as red pigment. The lowest (0.08 mg CCE/g DW) and the highest (3.65 mg CCE/g DW) red pigment contents were seen for Barnea in April and July, respectively. With no exceptions, the pigment content increased steadily toward the end of the harvesting season. The accumulation of pigments in olive fruit is a well-known physiological phenomenon characterizing the black maturation stage (*21*). However, the abrupt enormous increase in the pigment content that happened in July was remarkable (**Figure 2C**). While a 4- to 5-fold increase occurred in Correggiola and Paragon, a 20-fold increase was found for Manzanillo and Mission, and a 47-fold increase for Barnea.

***o*-Diphenols Content.** The *o*-diphenols content varied between 5.4−12.8 mg CAE/g DW. The lowest value was reported for Correggiola in July, and the highest was for Mission in May (**Figure 2D**). The pattern of change of *o*-diphenols was fairly comparable to that observed for FC total phenol (**Figure 1**). The highest average *o*-diphenols content was registered for Mission, and the lowest was for Barnea, similar to that for the total phenol content. A strong overall correlation was found between *o*-diphenols content and FC total phenol content ($R^2 = + 0.827$; $p < 0.0001$).

Antioxidant Capacity. All crude OMW extracts exhibited DPPH radical scavenging activity. The antioxidant capacity varied widely between 1.8 ($EC_{50} = 57$ ppm) for Correggiola in July and 6.3 ($EC_{50} = 18$ ppm) for Barnea also in July. Apart from Barnea, the antioxidant capacity showed a decrease toward the end of the season parallel to *o*-diphenols content (**Figure 2E**).

The overall correlation between total phenol (Folin−Ciocalteu) content and antioxidant capacity was poor and insignificant compared with the correlation found between the *o*-diphenols content and antioxidant capacity (**Figure 3**). Even at the level of the same cultivar, poor and insignificant correlation was found between total phenols and antioxidant capacity, with one exception in the case of Correggiola ($R^2 = + 0.999$, $p = 0.01$). Furthermore, the total phenol content had negative correlation with antioxidant capacity in the case of Barnea, Manzanillo, and Paragon. The results from the current study suggest that the good correlation observed between the total phenol content and antioxidant activity for the Tunisian Chemlali (*22*) is

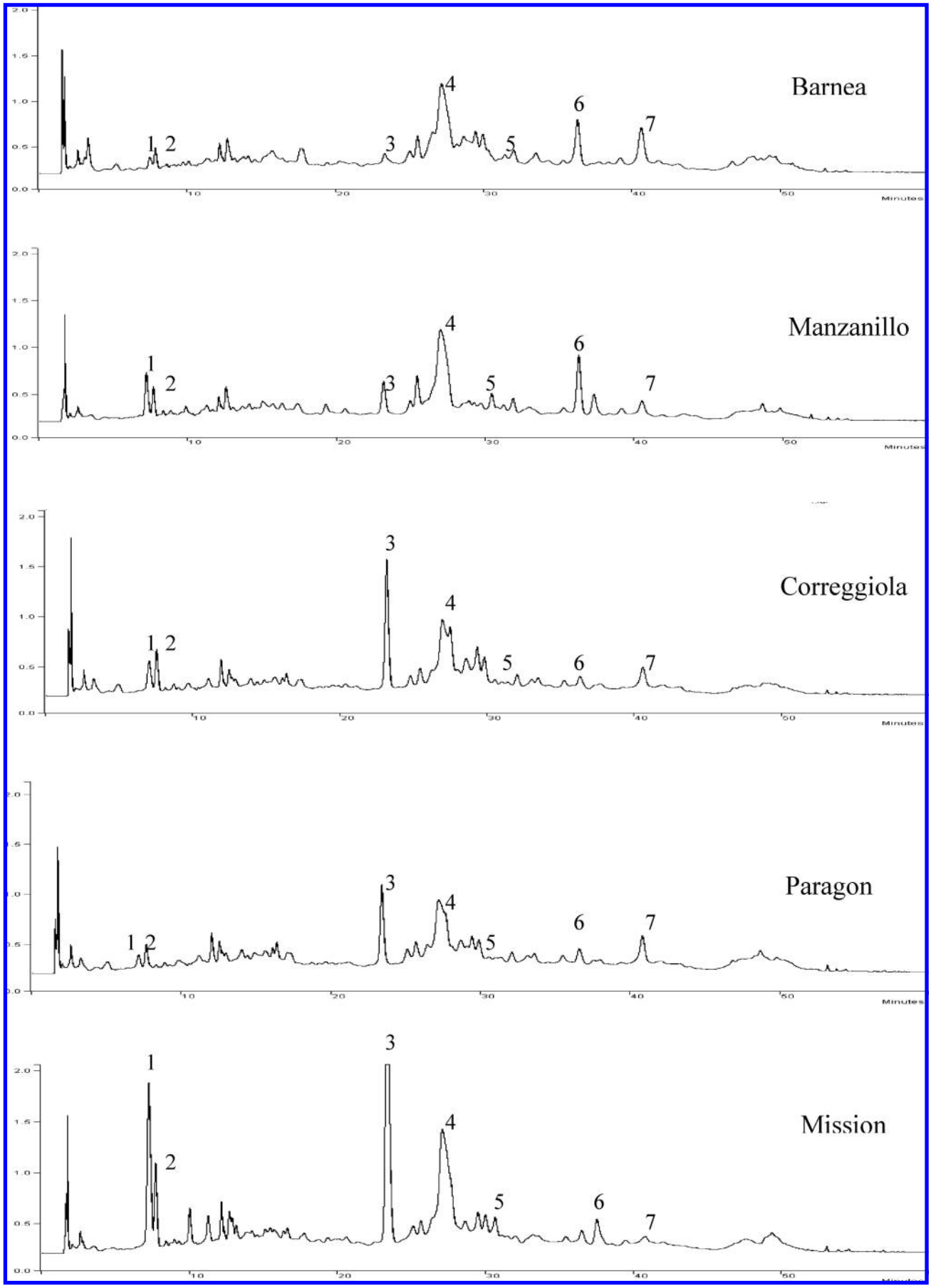

Figure 4. RP-HPLC chromatograms at 278 nm showing the biophenolic profile of OMW from cultivars collected in May 2004 (all normalized to the same scale). (1) Hydroxytyrosol glucoside; (2) hydroxytyrosol; (3) verbascoside; (4) coeluting peaks of rutin, 3,4-DHPEA-EDA, and HT-ACDE; (5) oleuropein; (6) comselogoside; (7) luteolin.

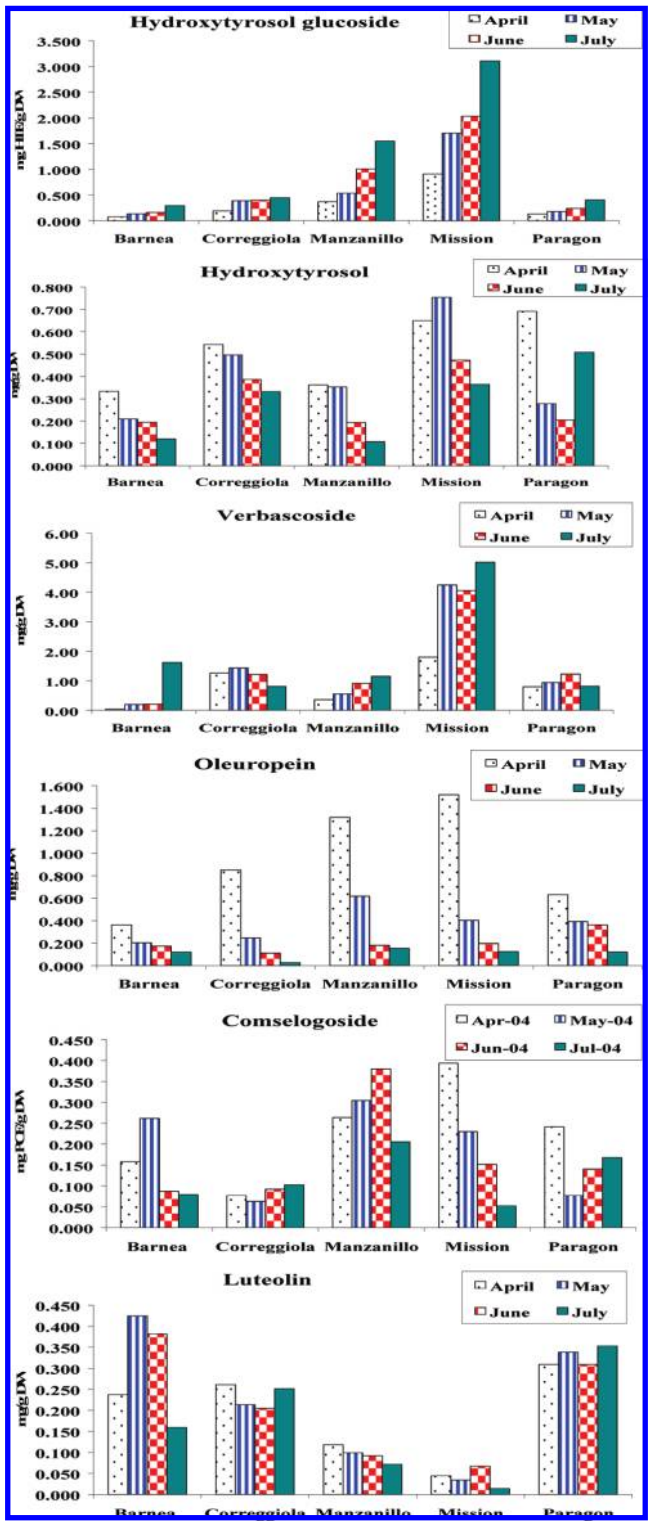

Figure 5. Change of the recovery of major biophenols in different cultivars during the 2004 olive harvesting season: (**A**) hydroxytyrosol glucoside; (**B**) hydroxytyrosol; (**C**) verbascoside; (**D**) oleuropein; (**E**) *p*-coumaroyl secologanoside (comselogoside); (**F**) luteolin. Coefficient of variation was less than 15% in all cases.

cultivar-dependent rather than a general rule. However, strong positive correlations were found between *o*-diphenols content and antioxidant capacity for Barnea, Correggiola, and Paragon. Only Manzanillo had a weak negative correlation. For Mission, *o*-diphenols content had insignificant correlation with antioxidant capacity.

Recovery of Individual Biophenols and Phenolic Profiles. OMW generated from the five cultivars showed very similar phenolic profiles but with differences in the minor constituent biophenols. These OMW samples had a unique phenolic profile compared with that found in the Mediterranean region. A large number of biophenols were identified in Australian OMW (*17, 18, 23, 24*), and six of these were monitored in the present study: hydroxytyrosol glucoside, hydroxytyrosol, verbascoside, oleuropein, comselogoside, and luteolin. Although 3,4-dihydroxyphenylethyl alcohol-deacetoxyelenolic acid dialdehyde (3,4-DHPEA-EDA) and hydroxytyrosol acyclodihydroelenolate (HT-ACDE) were among the major biophenols in all samples, they were not studied quantitatively because of coelution with rutin forming a broad peak (4 in **Figure 4**).

The total concentration of the six studied biophenols for the five cultivars showed poor correlation with the average total phenol content (FC). This could be due to a significant role played by the large number of minor biophenol constituents and/or the low specificity of the Folin−Ciocalteu reagent. Mission had the maximum concentrations of all studied biophenols, apart from luteolin. Mission also had the highest average recovery during the whole season for three biophenols (hydroxytyrosol glucoside, hydroxytyrosol, and verbascoside). At the same time, Mission had the minimum average recovery of comselogoside and luteolin (**Table 1**).

From a harvest date perspective, the concentrations of these compounds showed different variations in different cultivars (**Table 1**) (**Figure 5**) with some exceptions where a certain pattern was observed in all cultivars, for example, hydroxytyrosol glucoside and oleuropein (*vide infra*). From **Table 1**, it can be noted that minimal and maximal recovery of biophenols in different cultivars were often registered either early or late in the season. In other words, midseason harvesting resulted in overall optimum recovery of different biophenols from all cultivars.

Focusing on individual compounds, hydroxytyrosol glucoside content increased gradually toward the end of the season resulting in a maximum recovery in the July harvest; a pattern that was recorded for all cultivars (**Figure 5A**) (*25*). In contrast, the hydroxytyrosol content declined gradually, reaching a minimum by the end of the season (**Figure 5B**). Oleuropein also decreased gradually leaving traces (0.03−0.15 mg/g DW) by the end of the season (**Figure 5D**). The only other study that investigated the variation in biophenol content of OMW during fruit maturation found no clear trend in the variation of oleuropein concentration in olive paste with maturation (*20*). Indeed, most of the oleuropein in the fruit paste was lost during malaxation leaving only traces in the pomace (OMW). Careful sample handling, rapid extraction, and minimal sample preparation may explain the good recovery of oleuropein from OMW in our work. The decrease in oleuropein content with fruit maturation is a widely reported phenomenon in olive fruits (*22, 26, 27*) that was correlated with the activity of hydrolyzing enzymes (*28, 29*). Previous studies recognized an inverse relationship between hydroxytyrosol and oleuropein content during olive harvesting season in olive fruits. Artajo et al. found a gradual increase of hydroxytyrosol in both olive paste and OMW with fruit maturation (*20*). In contrast, the decrease in oleuropein content was accompanied by a parallel decrease in hydroxytyrosol content in the present study. A similar situation has been reported for Arbequina, Farga, and Morrut fruits (*30*), and in different Portuguese olive fruits (*31*).

For the remaining compounds, the effect of harvest date was cultivar-dependent. The verbascoside content in Correggiola and Paragon changed slightly between April and June and dropped

slightly in July (**Figure 5C**). Verbascoside accumulated gradually toward the end of the season in the other three cultivars. Barnea showed a more distinct behavior, in which only traces of verbascoside could be detected in the April harvest, and the concentration increased by a factor of 5 in May, then remained constant in June, and increased dramatically in July, 7−8-fold.

Comselogoside was detected in all cultivars, albeit in relatively small concentrations. For both comselogoside and luteolin (**Figure 5E** and **F**), different cultivars behaved differently, resulting in no general trends. Comselogoside and luteolin because of high molar absorptivities presented as two main peaks in chromatograms at 278 nm, while their concentrations were not that high compared with that of oleuropein (**Figure 4**) (**Table 1**). Luteolin is a universal olive biophenol that was detected in nearly all cultivars from different countries. However, luteolin was the least abundant biophenol among the main biophenols in Australian OMW (**Table 1**).

Though the biophenol profile was qualitatively maintained among cultivars, dramatic quantitative differences existed among cultivars and in the same cultivar at different harvesting times. This may be a cultivar effect or the result of agronomic practices. Nevertheless, for the present samples, intercultivar variation at the same harvesting time resulted in up to a 2-fold difference in total phenol content (Folin−Ciocalteu), 5-fold difference in antioxidant capacity, and 50-fold difference in verbascoside content. Within the same cultivar, the difference due to change in harvesting time can account for 1-fold difference in total phenol content (Folin−Ciocalteu), 4-fold difference in antioxidant capacity, and 40-fold difference in verbascoside content.

Impact of Seasonal Variation. Correggiola and Mission were chosen from the five cultivars studied in 2004 to follow up seasonal variations in the 2005 season. A similar pattern of interseasonal change was observed for both cultivars. Both dry weight and extractable matter content decreased in the 2005 season, though the dry weight decrease for Mission was statistically insignificant. However, total phenol content and antioxidant capacity increased significantly in both cultivars in the 2005 season (**Table 2**). The recovery of hydroxytyrosol and hydroxytyrosol glucoside also increased in the 2005 season. However, variations in total phenol content (FC) did not necessarily reflect a parallel change in the level of individual biophenols as the level of verbascoside and oleuropein decreased in both cultivars for the 2005 season. While the level of comselogoside decreased in Correggiola, it was nearly doubled in Mission. The luteolin content did not significantly change in Correggiola, but it was doubled in the case of Mission. These seasonal variations may result from uncontrollable factors, for example, climatic and pathological conditions or controllable factors such as agricultural practices.

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