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Quantitation of Oleuropein and Related Phenolics in Cured Spanish-Style Green, California-Style Black Ripe, and Greek-Style Natural Fermentation Olives

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Supporting Information

ABSTRACT: Oleuropein, ligstroside, and related hydrolysis products are key contributors to olive bitterness, and several of these phenolics are implicated in the prevention of lifestyle age-related diseases. While table olive processing methods are designed to reduce oleuropein, the impact of processing on ligstroside and related hydrolysis products (e.g., oleacein, oleocanthal, hydroxytyrosol glucoside, ligstroside aglycone, and oleuropein aglycone) is relatively unknown. Herein, levels of these compounds were measured in Spanish-style green (SP), Californian-style black ripe (CA), and Greek-style natural fermentation (GK) olives using rapid ultrahigh-performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS). GK olives had the highest concentration of all compounds measured, with the exception of oleocanthal, which was highest in SP olives (0.081 mg kg^{-1} wet weight (w.wt)). CA olives had the lowest levels of most compounds measured, including ligstroside (0.115 mg kg^{-1} w.wt) and oleuropein (0.974 mg kg^{-1} w.wt). Hydroxytyrosol was the predominate compound in all three styles of commercial olives, with similar concentrations observed for GK and SP olives (134.329 and 133.685 mg kg^{-1} w.wt, respectively) and significantly lower concentrations observed for CA olives (19.981 mg kg^{-1} w.wt).

KEYWORDS: *olives, Olea europaea, phenolics, UHPLC, MS/MS, oleuropein, oleocanthal, ligstroside, oleacein, oleuropein aglycone*

INTRODUCTION

Olives, the edible drupes from the olive tree (*Olea europaea*), are a popular food consumed worldwide. Table olives and olive oil are a major component of the Mediterranean diet: a diet linked to reducing cardiovascular disease,[1] Alzheimer's disease,[2,3] and other age-related conditions.[4] Olives are an important part of this diet not just for their monounsaturated fatty acids, but also a phenolic composition that is unique to *Olea europaea*.[5] Olive oil contains phenolic compounds including oleuropein, hydroxytyrosol, and tyrosol which have demonstrated antioxidant,[6] anti-inflammatory,[7] anticancer,[8] antimicrobial, and antiviral properties.[9] The olive phenolic oleocanthal exhibits ibuprofen-like activity and is active toward inflammatory diseases including neurodegenerative diseases, joint-degenerative diseases, and some specific cancers.[7,10]

Oleuropein (Figure 1) is the most prevalent phenolic present in olives at harvest, and levels in mature olives can reach concentrations as high as 140 mg g^{-1} dry weight (d.wt).[11] Oleuropein is an intensely bitter compound, and levels need to be significantly reduced before olives become edible.[12] Oleuropein and its biosynthetic precursor, ligstroside (Figure 1), are phenolic esters of glycosylated elenolic acid that undergo hydrolysis to form a range of related phenolic compounds. Different styles of olive curing will result in a different complement of hydrolysis products[13] and impact both the flavor and health-promoting properties of various styles of cured table olives.

Oleuropein and ligstroside accumulate during olive maturation.[14] Damage to the fruit during ripening can result in the release of endogenous β-glucosidases and esterases,[13] which can hydrolyze oleuropein and ligstroside into a range of compounds.[15] Hydrolysis can also continue after harvest due to the action of enzymes from lactic acid bacteria that proliferate during storage.[16] Hydrolysis by β-glucosidase results in the formation of oleuropein aglycone and ligstroside aglycone (Figure 1). The aglycones can undergo further ester hydrolysis to produce elenolic acid and hydroxytyrosol or tyrosol. Decarboxylation of the aglycones with no ester hydrolysis results in formation of oleacein and oleocanthal (Figure 1). Direct ester hydrolysis of oleuropein or ligstroside (i.e., loss of hydroxytyrosol or tyrosol) results in formation on oleoside methyl ester (Figure 1).[17]

Although oleuropein is considered the primary bitter compound in olives, ligstroside aglycone, oleuropein aglycone, oleacein, and oleocanthal also correlate with olive oil bitterness.[18] Sensory evaluation of isolated forms of these compounds indicate that they are bitter, astringent, and or pungent with taste thresholds that vary from 0.05 to 1.6 mM, whereas tyrosol is nonbitter.[19] Oleocanthal is characterized as a stinging irritant at the back of the throat and contributes to bitterness perception in olive oil.[20] Isolated ligstroside has not been directly evaluated for sensorial bitterness but the high structural similarity to

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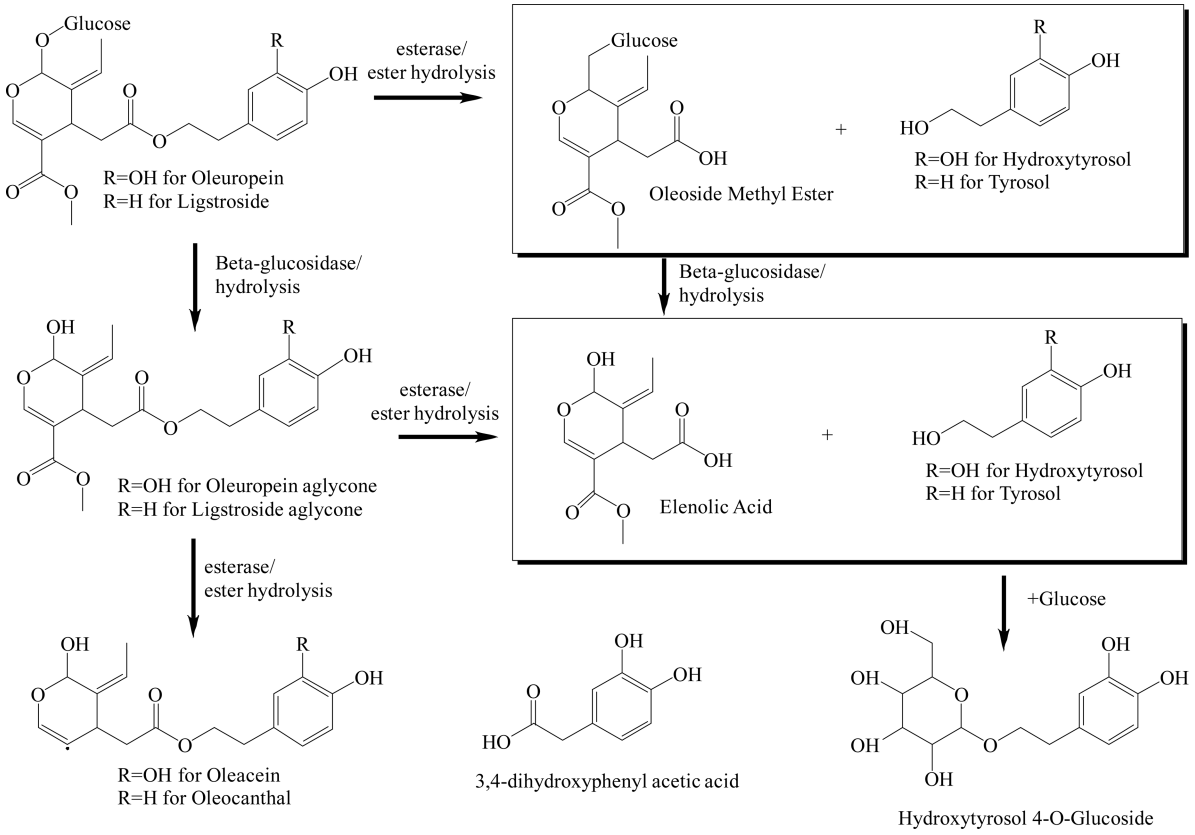

Figure 1. Structures of oleuropein, ligstroside, and related hydrolysis products.

oleuropein and oleocanthal suggest that this compound is also bitter.[21]

Olives are cured (i.e., processed) to reduce bitterness and create edible products. The most common commercial methods used to produce table olives are GK, SP, and CA.[46] GK methods use ripe olives that are either gradually fermented in brine, dry cured with salt, sun cured on the tree, or cured in oil.[22,23] SP methods use unripe green olives that are cured briefly in lye (sodium hydroxide), then fermented, and finally pasteurized.[24,25] CA methods use green unripe olives that are cured over several days in lye, with air oxidation, and then followed by sterilization.[26,27] Lye promotes the base-catalyzed hydrolysis of oleuropein and ligstroside into nonbitter products. Fermentation-based processing relies on the action of endogenous and microbial enzymes in addition to the acid-catalyzed hydrolysis of oleuropein and ligstroside.[28] To date, it is not clear how differing processing methods influence the range of these related phenolic compounds in different olive products.

Phenolic compounds in olives have been quantitated using a variety of instrumentational methods. HPLC methods employing spectrophotometric detection (e.g., ultraviolet/visible (UV/vis) and diode array detection [DAD]) have been used to measure oleuropein, ligstroside, oleuropein aglycone, ligstroside aglycone, oleacein, oleocanthal, hydroxytyrosol, tyrosol, elenolic acid, and hydroxytyrosol glucoside,[29−32] as well as other phenolics present in olives including phenolic acids,[30,33−36] anthocyanins,[29,37] lignans,[30,33,35] and flavonoids.[29,34,35] However, these methods lack the sensitivity needed to measure the full range of hydrolysis products of oleuropein and ligstroside at the concentrations present during table olive processing.[38,39] The limit of detection (LOD) varies greatly between reported analytical methods and is strongly influenced by sample preparation. In general, LODs reported for olive phenolics using mass spectrometric methods are 10−100× more sensitive than spectrophotometric methods,[26,40,42] with the exception of oleuropein which has a LOD of 200−800 μg/L for DAD detection,[35,43] 140 μg/L for fluorescence,[41] and 3 and 10 μg/L for ion trap MS[42] and MS/MS detection, respectively.[26]

Mass spectrometry methods sensitive enough to measure the hydrolysis products of oleuropein and ligstroside at concentrations present in processed table olives have focused primarily on olive oil.[31,32] Although hydroxytyrosol, tyrosol, oleuropein aglycone, oleuropein, hydroxytyrosol glucoside, 3,4-dihydroxyphenyl acetic acid, elenolic acid, and ligstroside aglycone have been measured in processed table olives,[26,44,45] oleacein, oleocanthal, and ligstroside, have not. Oleocanthal and oleacein are bitter compounds, and there is a strong indication ligstroside is as well. Additionally, ligstroside is both a precursor of oleuropein and a source of the bitter compounds ligstroside aglycone and oleocanthal. However, only a direct sensory study can confirm the bitterness of ligstroside.[47−50]

Herein a UHPLC-(ESI) MS/MS method was developed to measure 11 compounds related to olive bitterness at high sensitivity and selectivity. Compounds were quantified in CA, SP, and GK table olives. Characterizing oleuropein and ligstroside as well as the full range of their hydrolysis products in response to curing will provide a greater understanding of how these compounds respond to processing conditions and possibly allow for tighter control of flavor (i.e., bitterness), while developing table olives products with target phenolic profiles that deliver improved health promoting properties.

MATERIALS AND METHODS

Chemicals and Reagents. Oleuropein, tyrosol, and 3,4-dihydroxyphenyl acetic acid were purchased from Sigma-Aldrich (St. Louis, MO,

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U.S.A.). Hydroxytyrosol was purchased from Indofine (Hillsborough, NJ, U.S.A.). HPLC grade acetic acid, acetonitrile, hexane, and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Oleacein (decarboxymethyl oleuropein aglycone), oleocanthal (decarboxymethyl ligstroside aglycone), hydroxytyrosol glucoside, ligstroside aglycone, and oleuropein aglycone were isolated from Thassos olives, separated by preparatory HPLC, identity and stereochemistry confirmed by NMR, and purity isolated to over >98% purity at the University of Athens according to the previously described method.[46]

Olive Samples. A nested sampling design was employed and included three styles of table olives (Manzanillo olives processed in the CA style, Manzanillo olives processed in the SP style, and Kalamata olives processed in the GK style), three producers within each style (CA1, CA2, and CA3; SP1, SP2, and SP3; and GK1, GK2, and GK3), and three lots within each producer (CA1A, CA1B, and CA1C; CA2A, CA2B, and CA2C; and CA3A, CA3B, and CA3C). There were twenty-seven samples in total, all purchased in Northern California Grocery Stores. Each sample of olives was further separated into four composite samples. All composite samples were run in duplicate.

Olive Extraction Method. Olives were blended into a paste using a commercial grinder (Waring WSG30 Commercial Spice Grinder -120 V, China), and ∼20 g of olive pulp was placed in a 50 mL polypropylene conical tube (Fisher Scientific, Fair Lawn, NJ, U.S.A.). Lipids were removed with three successive 10 mL aliquots of hexane. Tubes were vortexed on high for 1 min and centrifuged at 4000 rpm for 5 min. The lipid layer was decanted and the defatted pulp frozen at −80 °C overnight. Samples were freeze-dried for 2 days (Labconco, Kansas City, MO, U.S.A.) to a constant weight, and the resulting powder was sieved through a Tyler standard screen with a 0.0082 in. opening (0.208 mm) and 65 meshes to the inch (2.56 meshes to the mm).

Compounds were extracted with a ratio of 1 g of olive (d.wt) to 40 mL of 60% methanol in deionized water. The sample was vortexed on high for 1 min and centrifuged at 4000 rpm for 5 min. The extract was filtered through a 0.22 μm nylon filter prior to UHPLC-(ESI) MS/MS analysis. Samples were diluted with DI water to be within the linear dynamic range of the methods.

UHPLC-(ESI) MS/MS Analysis. Compound analysis was performed on an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography system (UHPLC) interfaced to a 6460 triple-quadrupole mass spectrometer (MS/MS) with electrospray ionization (ESI) via Jet Stream technology (Agilent Technologies, Santa Clara, CA, U.S.A.). The UHPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A), an autosampler (G4226A) with thermostat (G1330B), and a thermostated column compartment (G1316C). Compounds were separated using a Poroshell 120 C_{18} column (3.0 × 50 mm, 2.7 μm, Agilent Technologies). The mobile phase consisted of a linear gradient of 0.01% acetic acid in DI water (A) and 0.01% acetic acid in acetonitrile (B) as follows: 10% B, 0−2 min; 10−30% B, 2−3 min; 30−65% B, 3−5 min. The column temperature was 20 °C, the flow rate was 0.7 mL/min, and the injection volume was 5 μL. A 2 min post-time flushed the column with 99% acetonitrile and equilibrated back to starting conditions.

Negative ESI mode was used for all compounds. The drying gas temperature and flow rate were 300 °C and 10.0 L/min, respectively. The sheath gas temperature and flow rate were 400 °C and 11.0 L/min, respectively. The nebulizer gas pressure, capillary voltage, and nozzle voltage were 45 psi, 4 kV, and 0 V, respectively. The voltage of the mass filter, precursor ions, collision voltages, product ions, and quantitative transitions were optimized for each compound.

Limit of detection (LOD; defined as 3 × signal-to-noise (S/N)) and limit of quantitation (LOQ; defined as 10 × S/N), were calculated for oleuropein, hydroxytyrosol, oleuropein aglycone, oleacein, oleocanthal, hydroxytyrosol glucoside, ligstroside aglycone, and tyrosol for the dynamic multiple reaction monitoring (DMRM) method. Standards ranging from 0.2 to 20 μg/L were measured in triplicate ($n = 3$), and the S/N ratio was calculated by a peak-to-peak algorithm. As no authentic standards or deuterium labeled isotopes were available for elenolic acid and ligstroside, relative quantitation was performed on these compounds using authentic standards of hydroxytyrosol glucoside and oleuropein, respectively.

To perform a spike and recovery, an "olive blank" was created by washing (removing the aqueous layer and replacing it with DI water daily) blended olives over a period of 5 days to remove endogenous phenolics. The dehydrated "blank olive" pulp was spiked with 100, 500, or 1000 μg/L of oleuropein, oleuropein aglycone, ligstroside aglycone, hydroxytyrosol, hydroxytyrosol glucoside, tyrosol, oleacein, and oleocanthal before extraction. Samples were measured on the UHPLC-(ESI) MS/MS, and the percent recoveries were calculated. Method linear dynamic range (LDR) was obtained by measuring standards at concentrations between 0.5 and 4000 μg/L and determining the linear range where the correlation coefficient >0.997.

RESULTS AND DISCUSSION

This study is the first to quantitate oleuropein, ligstroside, and the full range of related hydrolysis products (i.e., oleuropein aglycone, ligstroside aglycone, oleocanthal, oleacein, hydroxytyrosol, hydroxytyrosol glucoside, tyrosol, elenolic acid, and 3,4-dihydroxyphenyl acetic acid) in cured CA, GK, and SP olives.

UHPLC-(ESI) MS/MS conditions were optimized using multiple reaction monitoring (MRM) for each compound in negative ESI ionization mode (Table 1). Fragmentation patterns and product ions are given in the Supporting Information (see Figures 1S−9S). UHPLC conditions were optimized to achieve resolution of compounds in a short chromatographic run of 5 min using acetonitrile as the organic solvent. Different concentrations of acetic acid or formic acid in the organic and aqueous solvents were evaluated. Optimal ionization was achieved using a mobile phase composition of acetic acid at a concentration of 0.01% in deionized water (A) and in acetonitrile (B). Oleuropein, ligstroside, ligstroside aglycone, oleuropein aglycone, oleacein, and oleocanthal can undergo keto−enol isomerization in aqueous solvents, and peaks corresponding to these compounds consist of these isomers that were not completely resolved. Therefore, quantitation is compound but not isomer specific (Figure 2).

Table 1. Optimized MS/MS Conditions for Determination of Olive Phenolics

compound	RT (min)	fragmentor voltage (V)	precursor ion (m/z)	collision energy (V)	product ions (m/z)
oleuropein	3.7	165	539	20	307
				22	275
ligstroside	3.98	100	523	6	361
				13	112
oleuropein aglycone	4.51	90	377	4	275
				4	307
ligstroside aglycone	4.85	127	361	5	291
				2	259
oleacein	2.11	116	319	0	195
				5	183
hydroxytyrosol glucoside	0.71	80	315	10	153
				31	123
oleocanthal	4.33	62	303	13	137
				0	165
elenolic Acid	1.98	107	241	3	197
3,4-dihydroxyphenyl acetic acid	0.8	90	167	14	123
				14	149
tyrosol	1.21	80	137	12	119
hydroxytyrosol	0.86	106	153	22	95
				13	123

The limits of detection (LODs), limits of quantitation (LOQs), and linear dynamic ranges (LDRs) are given in the

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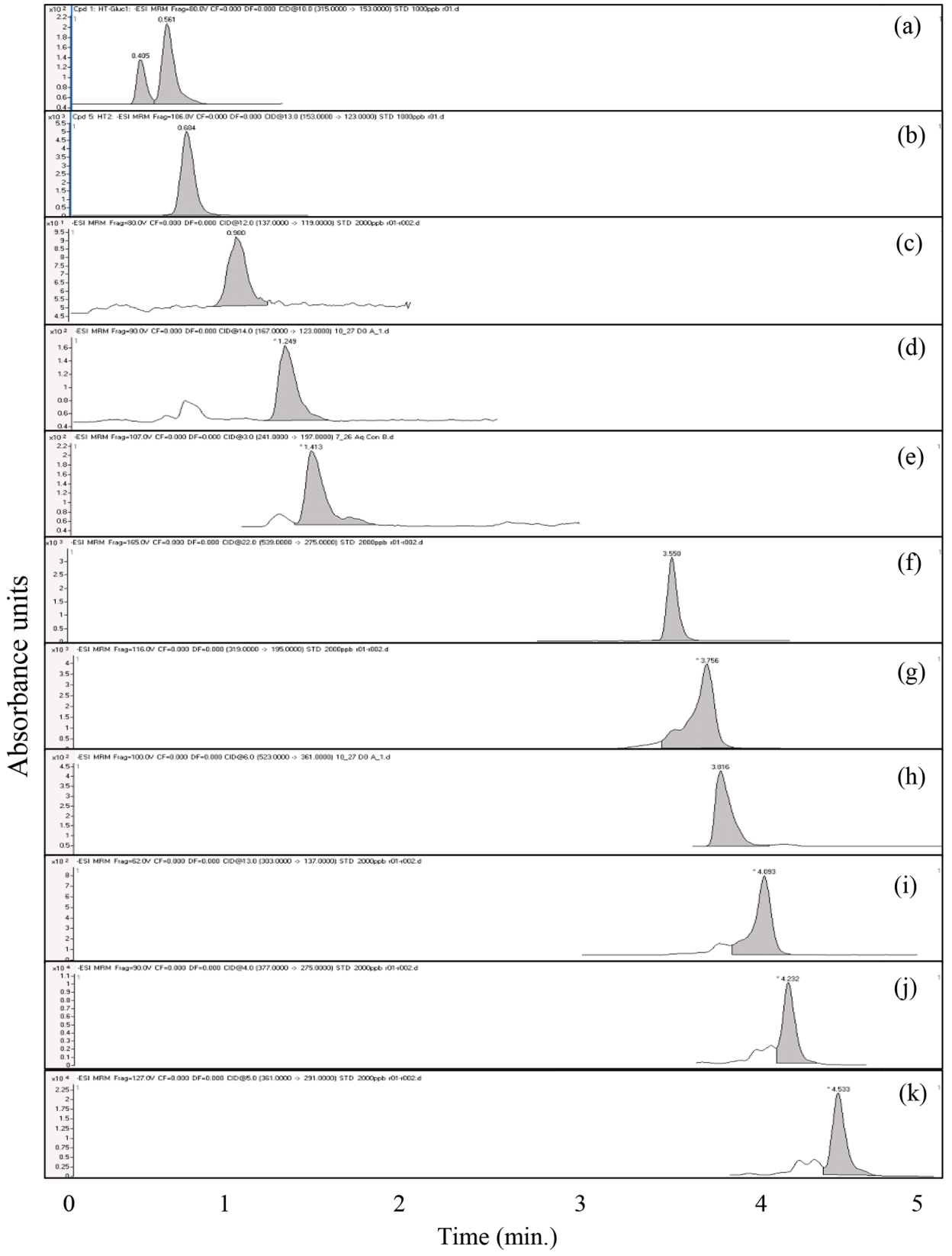

Figure 2. Chromatogram of (a) hydroxytyrosol glucoside, (b) hydroxytyrosol, (c) tyrosol, (d) 3,4-dihydroxyphenyl acetic acid, (e) elenolic acid, (f) oleuropein, (g) oleacein, (h) ligstroside, (i) oleocanthal, (j) oleuropein aglycone, and (k) ligstroside aglycone.

Supporting Information (Tables 1S−3S) and are similar to values reported for MS/MS detection by Melliou et al. Instrumental LOD was determined using pure (>98%) standards in a 60% methanol solution (ng/mL). The method limit of detection was determined in olive extracts (kg d.wt). The instrumental LODs for the eight standard compounds measured ranged from 0.5 to 5 μg/L, and the LOQs ranged between 0.5 and 11 μg/L.

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Table 2. Average mg Compound kg Olive^{-1} (w.wt) for GK, SP, and CA Olives (μ) with Variance due to Producer (αP), Variance due to Lots within a Producer (βP(L)), Variance due to Replication within Lots within Producers (γR(P,L)), and Random Error (ε) (mg kg^{-1} olive w.wt)

compound	CA					GK					SP				
mg kg^{-1}	μ (ave)	αP	βP(L)	γR(P,L)	ε	μ (ave)	αP	βP(L)	γR(P,L)	ε	μ (ave)	αP	βP(L)	γR(P,L)	ε
hydroxytyrosol glucoside	18.603	7.240	19.500	0.000	0.659	25.884	17.605	18.466	1.263	1.294	3.120	0.000	3.412	0.226	0.300
hydroxytyrosol	19.981	0.000	12.970	0.966	0.796	134.329	0.000	21.135	5.740	4.959	133.685	7.731	28.982	7.750	4.553
ligstroside	ND					0.080	0.000	0.043	0.003	0.004	0.026	0.000	0.042	0.005	0.003
ligstroside aglycone	0.115	0.077	0.101	0.000	0.014	1.217	1.037	0.653	0.071	0.096	0.167	0.000	0.297	0.018	0.017
3,4- dihydroxyphenyl acetic acid	0.437	0.420	0.097	0.000	0.021	0.530	0.185	0.110	0.002	0.017	0.328	0.065	0.038	0.018	0.010
oleocanthal	ND					0.038	0.023	0.041	0.004	0.004	0.081	0.000	0.041	0.000	0.016
oleacein	ND					0.080	0.090	0.123	0.018	0.008	ND				
oleuropein aglycone	0.003	0.000	0.009	0.000	0.001	0.449	0.000	0.231	0.017	0.022	0.109	0.019	0.186	0.015	0.006
oleuropein	0.974	0.058	0.672	0.000	0.075	7.303	3.897	2.904	0.283	0.291	3.205	0.000	3.323	0.291	0.210
tyrosol	0.435	0.049	0.216	0.025	0.026	1.315	0.000	0.227	0.000	0.081	0.859	0.071	0.175	0.061	0.035
elenolic acid	ND					ND					ND				

Spike and recovery experiments were concentration and compound dependent. Lower percent recoveries were observed at 100 μg/L (18−75%) as compared to 1000 μg/L (48−94%). These recoveries indicate that matrix effects and ion suppression occur in olive extracts (Table 4S). Data was corrected for recovery when reporting values. Oleuropein, ligstroside, and their related hydrolysis products were measured in CA, GK, and SP commercial table olives (Table 2). A mixed model analysis of variance (ANOVA) was used to establish the average concentration of each compound. The variance associated with producers, lots within a producer, replication (within a lot and producer), and random error was determined. Overall, replication variance and random error were less than 10% of the average.

The results demonstrate that processing methods influence the levels and complement of oleuropein, ligstroside, and related hydrolysis products in table olive fruit (Table 3). Significant differences were observed between CA, GK, and SP olives for hydroxytyrosol glucoside, ligstroside aglycone, 3,4-dihydroxyphenyl acetic acid, oleocanthal, oleuropein, oleuropein aglycone, and tyrosol (Table 3).

Table 3. Tukey HSD Post-hoc Test (One-Way Analysis of Variance ANOVA) Comparing GK, SP, and CA Olives[a]

compound	GK-CA	SP-CA	SP-GK
hydroxytyrosol glucoside	*	***	***
hydroxytyrosol	***	***	ns
ligstroside aglycone	***	***	***
ligstroside	***	ns	***
3,4-dihydroxyphenyl acetic acid	*	*	***
oleocanthal	***	***	***
oleacein	*	ns	***
oleuropein	***	***	***
oleuropein aglycone	***	***	***
tyrosol	***	***	***

[a]Significant set at α < 0.05. ns, not significant and significance level * < 0.05, ** < 0.01, *** < 0.001.

All phenolics measured, with exception of oleocanthal, were significantly higher in GK olives as compared with < SP and < CA olives. Oleocanthal levels were highest in SP olives (0.081 mg kg^{-1} w.wt). CA olives had the lowest concentration of seven of the 11 compounds measured, including ligstroside (0.115 mg kg^{-1} w.wt) and oleuropein (0.974 mg kg^{-1} w.wt). Hydroxytyrosol was the predominate phenolic in all three types of olive, with similar concentrations observed for GK and SP olives (134.329 and 133.685 mg kg^{-1}, respectively), and significantly lower concentrations were observed in CA (19.981 mg kg^{-1}) olives. Elenolic acid was not detected in any of the olives sampled.

Greek style processing methods do not utilize lye; therefore, oleuropein and ligstroside diffuse from the fruit into the brine with limited hydrolysis. In contrast, SP and CA processing methods involve lye treatments. Lye catalyzes the hydrolysis of oleuropein and ligstroside within the olive pulp and in the lye treatment water. Additionally, lye dissolves the epicuticular waxy coating on olives and solubilizes the pectin in the middle lamella causing fruit softening, facilitating greater diffusion of oleuropein, ligstroside, and related hydrolysis products from the olive into the brine. CA processing methods utilize several more lye treatments as compared with SP methods and result in greater hydrolysis and diffusion of olive phenolics. Additionally, no fermentation occurs with the CA olives.

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Oleuropein levels ranged between 7.303 mg kg^{-1} in GK olives, 3.205 mg kg^{-1} in SP olives, and 0.974 mg kg^{-1} in CA olives. The bitterness threshold of pure oleuropein, dissolved on a 1 cm paper square, is 50 μg indicating that, at even low concentrations of oleuropein, bitterness can be detected. Oleuropein is not the only bitter phenolic present in table olives. Oleuropein aglycone, ligstroside aglycone, oleocanthal, and oleacein have all been described as astringent and bitter, with ligstroside aglycone, oleocanthal, and oleacein also described as burning. Based on the bitterness taste threshold reported by Andrewes et al., the expected bitterness detection threshold for oleocanthal and oleacein is 0.12−0.5 mg kg^{-1} whereas for oleuropein aglycone and ligstroside aglycone it is 0.02−0.08 mg kg^{-1}. Examining biter compounds as a group (i.e., oleuropein, oleuropein aglycone, ligstroside aglycone, oleocanthal, and oleocein), the sum level of these compounds is 9.087, 3.562, and 1.092 mg kg^{-1} in GK, SP, and CA olives, respectively. This is well above the established sensory thresholds for these compounds and correlates with the bitterness associated with these different styles of olives.

Hydroxytyrosol was the most prevalent nonbitter phenolic compound measured for all types of olives examined; however, levels were 14.8−14.9% lower in the CA olives as compared with those for the GK and SP olives. Because lye promotes fruit softening, it facilitates greater diffusion of small phenolics from the olive and into the brine. Additionally, oleuropein and ligstroside undergo less hydrolysis during SP and GK style processing. This may influence the overall health promoting attributes of commercial olive products. For example, in vitro studies demonstrate potent anticancer and antioxidative activities for hydroxytyrosol.

In contrast, the hydroxytyrosol glucoside concentration was relatively higher in GK olives and CA olives (25.884 and 18.603 mg kg^{-1}) as compared with the SP olives (3.120 mg kg^{-1}). CA and SP olives both undergo lye treatments. However, unlike CA olives, SP olives undergo an additional fermentation step after lye treatment. The lye treatment facilitates diffusion of phenolic such as hydroxytyrosol glucoside into the brine where the glucoside would act as a substrate for various bacteria and yeast associated with fermentation. Results for oleuropein and hydroxytyrosol concentrations are similar to Marsilio et al., who also observed that GK olives had higher concentrations of oleuropein and hydroxytyrosol (2 and 510 mg kg^{-1} w.wt, respectively) as compared to SP olives (ND and 221 mg kg^{-1} w.wt, respectively), and to Melliou et al., who compared oleuropein and hydroxytyrosol concentrations between GK (1459.5 ± 100.1 and 195.1 ± 7.8 μg g^{-1} w.wt, respectively) and CA olives (36.7 ± 3.1 and 210.0 ± 18.8 μg g^{-1} w.wt, respectively).

Herein oleuropein aglycone and ligstroside are reported for the first time in CA olives in contrast to previous methods in which the levels of these compounds were below the LOD. Measuring oleuropein, ligstroside, and related phenolics compounds during olive curing provides a greater understanding of how these compounds manifest in response to processing conditions, allowing processors to develop commercial products with more health promoting phenolics (e.g., hydroxytyrosol) while controlling bitterness.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b06025.

Table 1S, instrumental LODs and LOQs; Table 2S, method LODs and LOQs, Table 3S, linear dynamic range of phenolics measured; Table 4S, the average percent recoveries of olives spiked at 100, 500, and 1000 ppb; Figure 1S, proposed ESI(−) MS/MS fragmentation of hydroxytyrosol glucoside; Figure 2S, proposed ESI(−) MS/MS fragmentation of tyrosol; Figure 3S, proposed ESI(−)MS/MS fragmentation of hydroxytyrosol; Figure 4S, proposed ESI(−) MS/MS fragmentation of oleocanthal; Figure 5S, proposed ESI(−) MS/MS fragmentation of oleacein; Figure 6S, proposed ESI(−) MS/MS fragmentation of 3,4-dihydroxyphenyl acetic acid; Figure 7S, proposed ESI(−) MS/MS fragmentation of elenolic acid; Figure 8S, proposed ESI(−) MS/MS fragmentation of ligstroside; Figure 9S, proposed ESI(−) MS/MS fragmentation of oleuropein, ligstroside, oleuropein aglycone, ligtroside aglycone oleuropein glucoside and ligstroside glucoside. (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

w.wt, wet weight; d.wt, dry weight; MS, mass spectrometry; UHPLC, ultrahigh performance liquid chromatography; ESI, electron spray ionization; DMRM, dynamic multiple reaction monitoring; MRM, multiple reaction monitoring; LOD, limit of detection; LOQ, limit of quantitation; LDR, linear dynamic range; MS, mass spectrometry; Q-TOF, quadrupole time-of-flight; MS/MS, tandem mass spectrometry; UV/vis, ultraviolet/visible detector; DAD, diode array detector; CA, California-style black ripe Manzanillo olives; SP, Spanish-style green Manzanillo olives; GK, Greek-style natural fermentation Kalamata olives; RT, retention time

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