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Effect of Cultivar and Processing Method on the Contents of Polyphenols in Table Olives

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Polyphenols were determined by HPLC in the juice and oil of packed table olives. The phenolic compositions of the two phases were very different, hydroxytyrosol and tyrosol being the main polyphenols in olive juice and tyrosol acetate, hydroxtyrosol acetate, hydroxytyrosol, tyrosol, and lignans (1-acetoxypinoresinol and pinoresinol) in oil. The type of processing had a marked influence on the concentration of polyphenols in olive juice and little on the content in oil. The analyses carried out on 48 samples showed that turning color olives in brine had the highest concentration in polyphenols (∼1200 mg/kg), whereas oxidized olives had the lowest (∼200 mg/kg). Among olive cultivars, Manzanilla had a higher concentration than Hojiblanca and Gordal. The type of olive presentation also influenced the concentration of polyphenols in olives, decreasing in the order plain > pitted > stuffed. The results obtained in this work indicate that table olives can be considered a good source of phenolic antioxidants, although their concentration depends on olive cultivar and processing method.

KEYWORDS: Packed table olives; polyphenols; cultivar; processing

INTRODUCTION

Table olives are a commodity of great importance in Mediterranean countries and the United States. World production reached 1,456,000 metric tons in the 2001/2002 season, and 39% of this quantity was produced in Spain. There are three main trade preparations of table olives: green or Spanish-style olives (50% of total production), black ripe or Californian-style olives (25% of total production), and naturally black or Greek-style olives (25% of total production). Olives for the Spanish, Californian, and Greek styles are harvested when fruits reach a green-straw, yellow-purple, or black color, respectively (**Figure 1**). Fruits can also be pitted and/or stuffed, and all of these steps give rise to a final product with chemical, organoleptic, and nutritional characteristics different from those of the raw material.

Polyphenols are one of the main secondary metabolites in olives, and they account for ∼1−2% of fresh fruit (*1*). The main polyphenol in green olives is the oleuropein, a glucoside ester of 3,4-dihydroxyphenyl ethanol (hydroxytyrosol) and elenolic acid; its concentration decreases with fruit maturation (*1*) and olive tree irrigation (*2*). In parallel with the oleuropein decrease, there is an increase of hydroxytyrosol glucoside with maturation, and this substance becomes the majority in black mature olives (*3*, *4*). Other natural phenols that have been identified in olive drupes are verbascoside, ligustroside, salidroside, rutin, luteolin 7-glucoside (*5*, *6*), and the anthocyanins cyanidin 3-glucoside and cyanidin 3-rutinoside (*4*). Despite the presence of all these compounds in most olive cultivars, each cultivar has its own polyphenolic profile (*7*). These compounds are also ubiquitous in all parts of olive fruit; the aglycons of oleuropein and ligustroside (*8*, *9*) and the lignans 1-acetoxypinoresinol and pinoresinol (*10*) are the main polyphenols in olive oil, which represents 15−25% of fruits; nüzhenide and salidroside are two phenolic components of the seed (*11*, *12*), and hydroxytyrosol and tyrosol participate in the structure of the stone (*13*).

During olive processing, polyphenols undergo chemical transformations and, in general, diminish their concentration in olives. One of the main steps in the Spanish-style green method is the debittering of fruits under alkaline conditions by which oleuropein is hydrolyzed into hydroxytyrosol (*14*) and elenolic acid glucoside (*15*). The subsequent lactic acid fermentation does not modify the phenolic composition (*16*). Californian-style black olive processing consists of preserving fruits in a brine or an acidified solution and, then, darkening with air under alkaline conditions (**Figure 1**). Polyphenols, mainly oleuropein, diffuse from olive flesh into the surrounding solution during preservation, and their acid hydrolysis occurs (*17*). Subsequently, orthodiphenols are oxidized and polymerized during the darkening step (*18*−*20*). Greek-style black olives are harvested when they are fully ripe and put directly into brine for a yeast fermentation. The main changes in polyphenols are the acid hydrolysis of oleuropein and hydroxytyrosol glucoside and the

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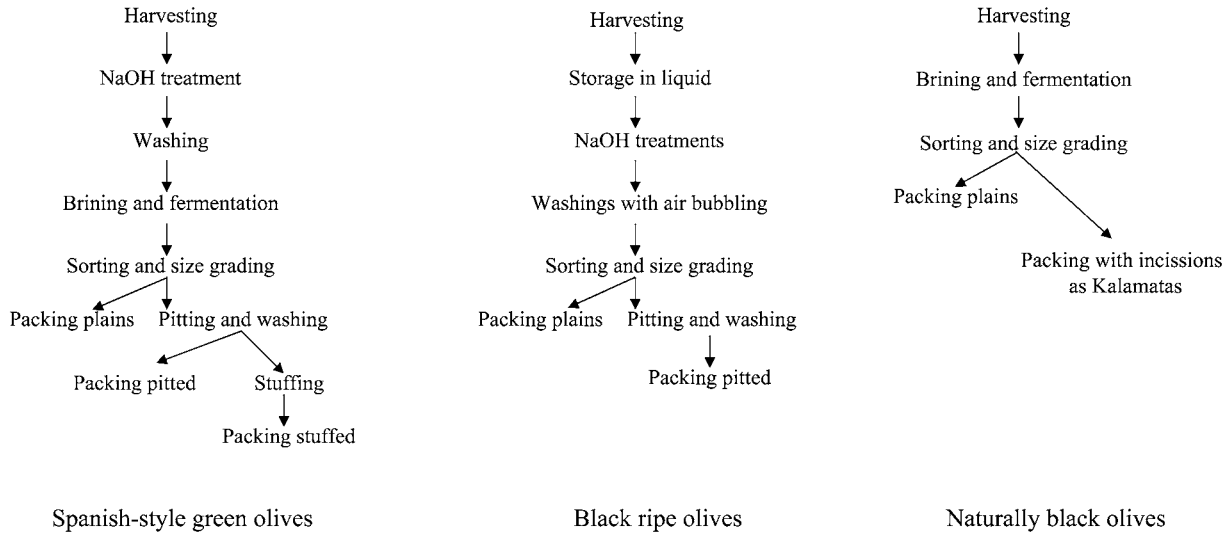

Figure 1. Methods of processing table olives.

polymerization of anthocyanins that contributes to the color stabilization (*21*, *22*).

Therefore, many changes occur during table olive processing, and they affect the chemical and organoleptic properties of the product.

As for olive oil, the main simple phenolic compounds in table olives are hydroxytyrosol and tyrosol (*16*, *18*), and the beneficial properties for human health found in olive oil could also be supposed in table olives. Phenols present in olive oil exert a protective effect against oxidative stress in human cells (*23*) and low-density lipoprotein (LDL) oxidation (*24*). In particular, hydroxytyrosol was reported to inhibit LDL oxidation (*25*) and prevents passive smoking-induced oxidative stress (*26*), among other beneficial actions (*27*). Tyrosol, which is not an ortho-diphenol, can also protect against oxidized-LDL-induced injury in Caco-2 cells (*28*). Other phenolic compounds present in olive oil and table olives such as hydroxytyrosol acetate (*29*) and lignans (*30*) also possess antioxidant properties.

Despite the great importance that phenolic compounds of table olives may have from a nutritional point of view, few data are available (*31*) on the phenolic composition of packed table olives. In addition, the cultivar and processing method must influence to a large extent this content. In this work, we have evaluated for the first time the contribution of both the aqueous and lipid phases of the olive flesh to the total content in phenolic compounds of packed table olives. Therefore, this work will contribute to a wide nutritional database on polyphenols content in the three most important table olive preparations.

MATERIALS AND METHODS

Samples. Forty-six samples of table olives, packed in cans and glass bottles, were purchased in local markets. The olive samples and their characteristic are reflected in **Table 1**.

Moisture content was determined in duplicate in an oven by drying 20 g of crushed olive pulp at 102 ± 2 °C until weight stabilization.

Total fat determination was made by extraction with chloroform/methanol. The procedure was applied according to AOAC official method 983.23 (*32*). Previously, drained olives (80−100 g) were pitted (if appropriate) and triturated with an Ultraturrax T25 (Jante & Kunkel, GmbH) to obtain a homogenized paste.

Extraction of Phenolic Compounds. Phenolic compounds were analyzed in both the aqueous (juice) and lipid phases (oil) of olive pulp. Olives were pitted (if appropriate), and 100 g was blended in a commercial mill. The paste was centrifuged at 6000 rpm for 5 min, and the oil was separated from the juice with a pipet. One milliliter of the juice was centrifuged at 10000 rpm for 8 min, and the supernatant was diluted 1:1 with distilled water. Subsequently, the mixture was centrifuged at 10000 rpm for 8 min and passed through a 0.45 μm nylon filter. Finally, 20 μL was directly injected into the chromatograph.

The phenolic compounds were extracted from the oil phase following the procedure described elsewhere (*33*). Briefly, 0.6 mL of olive oil was extracted by using 3 × 0.6 mL of *N*,*N*-dimethylformamide (DMF), the extract was washed with hexane, and N_2 was bubbled into the DMF extract to eliminate the residual hexane. Finally, the extract was filtered through 0.45 μm pore size and injected into the chromatograph.

Identification and Quantification of Phenolic Compounds. The phenolic compounds were identified in the chromatograms by their retention times, UV spectra, and mass spectrometry responses. Sample extracts were analyzed using a 600E pump (Waters Inc., Milford, MA) and a ZMD4 mass spectrometer (Waters, Inc.) equipped with an electrospray ionization ion source (ESI). The ion spray mass spectra in the negative-ion mode were obtained under the following conditions: capillary voltage, 3 kV; cone voltage, 10 V; extractor voltage, 12 V; desolvation temperature, 120 °C; source temperature, 80 °C. A constant flow of 1 mL/min was used for each analysis, with a split ratio of approximately 5:1 (UV/MS detectors). A Spherisorb-ODS 2 (5 μm, 25 cm, 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved with an elution gradient by using an initial composition of 90% water buffered at pH 4.2 with 0.005 M ammonium acetate and 10% methanol. The gradient has been described elsewhere (*33*).

The detection and quantification of the phenolic compounds were made in olive juice by using a Waters 996 photodiode array detector and those of the phenolic extracts of the oil phase by the same UV detector and a Jasco FP-920 fluorescence detector (Jasco Corp., Tokyo, Japan) connected in series.

Table 1. Samples of Table Olives Analyzed

type of processing	cultivar	style	code
Spanish-style green olives[a]	Gordal	plain	GG
Spanishstyle green olives	Manzanilla	plain	GM
Spanish-style green olives	Hojiblanca	plain	GH
Spanishstyle green olives	Manzanilla	pitted	GMP
Spanish-style green olives	Hojiblanca	pitted	GHP
Spanish-style green olives	Manzanilla	stuffed with anchovy	GMSA
Spanish-style green olives	Manzanilla	stuffed with pimento	GMSP
turning color in brine	Manzanilla	plain	TCM
ripe black olives	Hojiblanca	plain	RH
ripe black olives	Cacereña	plain	RC
naturally black olives	Kalamata	incisioned	BGK
naturally black olives	Thassos	plain	BG

[a] Olives from four different brands were analyzed for each type of commercial table olive except naturally black olives, for which only three brands were sampled.

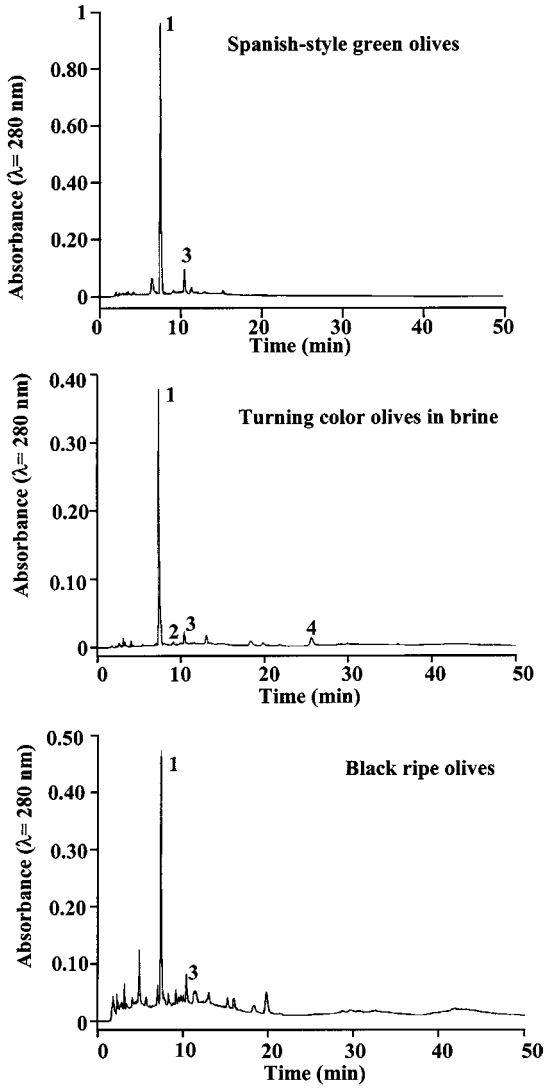

Figure 2. HPLC chromatograms of the phenolic compounds present in the juice of different types of table olives. Peaks: (1) hydroxytyrosol; (2) salidroside; (3) tyrosol; (4) verbascoside.

Quantification of phenolic compounds was made by using the reference compounds obtained from commercial suppliers or preparative HPLC as described elsewhere (*18*), except for salidroside and tyrosol acetate that were quantified as tyrosol.

Total anthocyanins were determined by the bisulfite procedure (*34*) and were expressed in milligrams per liter of cyanidin 3-rutinoside.

RESULTS AND DISCUSSION

All previous analyses of phenolic compounds in olives have been made on the whole olive flesh. Normally, olives are pitted and milled, and phenolic compounds are extracted by liquid/liquid or solid phase methods. However, there are two well-differentiated phases in the olive flesh, juice and oil, and the phenolic contents of each phase are different. This is the first time that the phenolic compositions have been individually characterized and quantified for each phase of olive flesh.

Figure 2 shows the chromatogramas of polyphenols in olive juice of Spanish-style green olives, turning color olives in brine, and black ripe olives. The phenolic chromatogram of naturally black olives was not depicted because it was similar to that of turning color olives in brine. In these two cases were found, as well as the ubiquitous hydroxytyrosol and tyrosol, salidroside (tyrosol glucoside) and verbascoside.

Table 2. Effect of Cultivar and Processing Method on the Polyphenol Content (Micromolar) of Table Olive Juice

type of olive	hydroxytyrosol	tyrosol	salidroside	verbascoside	total
GG[a]	3463 (155)[b]	789 (32)	ND[c]	ND	4252 (140)
GM	7566 (214)	1320 (133)	ND	ND	8886 (339)
GH	4117 (594)	737 (169)	ND	ND	4854 (535)
GMP	4150 (743)	804 (172)	ND	ND	4954 (909)
GHP	2068 (408)	343 (77)	ND	ND	2411 (484)
GMSA	2520 (304)	494 (57)	ND	ND	3014 (343)
GMSP	2776 (288)	470 (64)	ND	ND	3246 (352)
TCM	9306 (1162)	1395 (261)	277 (128)	3 (1)	10981 (1553)
RH	1389 (247)	419 (61)	ND	ND	1808 (295)
RC	1002 (46)	720 (61)	ND	ND	1722 (106)
BGK	5417 (1188)	1716 (226)	510 (115)	4 (1)	7647 (1385)
BG	3475 (482)	815 (338)	500 (216)	2 (1)	4792 (601)

[a] See **Table 1** for identification. [b] Standard error. [c] Not detected.

The Spanish-style green olive processing includes a series of steps during which polyphenols are leached from fruits. Industrial fermenters are filled with 10000 kg of olives and 5000 L of liquid, and if we consider that olive juice is ~65−75% of the olive flesh, the concentration of polyphenols in juice after the alkaline treatment, washing, and brining could be estimated as 20−30% of the initial content. Consequently, the concentration of hydroxytyrosol in olive juice of whole green olives was in the range of 3400−7500 μmol/L, which is much lower than reported in unprocessed olives (*1*, *4*, *17*). Because the concentration of polyphenols in fresh olives depends on cultivar (*4*, *7*), it is reasonable to find these differences in their commercial preparations. Hence, fruits of the Manzanilla cultivar had the highest amount of hydroxytyrosol and tyrosol in olive juice followed by Hojiblanca and Gordal cultivars (**Table 2**).

Two very important steps in the Spanish-style green olive processing are pitting and stuffing. Unfortunately, the success rate of an olive-pitting machine, under normal operational conditions, is ~95%, and nonpitted olives must be separated from pitted olives. This process is made by flotation of olives in a heavy brine and, subsequently, a new washing step is necessary in order to eliminate small fragments of pulp. These operations gave rise to new dilutions of polyphenols in the washing liquids and, therefore, the concentration of hydroxytyrosol in pitted olives was almost half that of the nonpitted olives (**Table 2**). Besides, the concentration of polyphenols in stuffed olives was even lower than that in pitted olives, which may be attributed to a more intense washing.

Turning color olives in brine showed the highest polyphenol concentration in olive juice (**Table 2**), which must be attributed to the type of processing because this implies only two dilution steps (**Figure 1**): brining and packing. However, the level of hydroxytyrosol was 9300 μmol/L, which may seem low, but it must be considered that these olives are harvested at a higher degree of ripeness than Spanish-style green olives and, therefore, with less initial concentration in phenolic compounds (*1*). Along with hydroxytyrosol were also detected tyrosol, salidroside, and a very small amount of verbascoside. Oleuropein was not found because these olives are edible only after a year of fermentation, when the bitter glucoside has almost been hydrolyzed (*17*).

Surprisingly, oxidized olives retained a considerable amount of phenolic compounds, hydroxytyrosol and tyrosol, in juice after the oxidation process (**Table 2**). Both Hojiblanca and Cacereña olives had ~1800 μmol/L, as a sum of hydroxytyrosol and tyrosol, and it represents a considerable quantity despite this level being the lowest found in table olive juices.

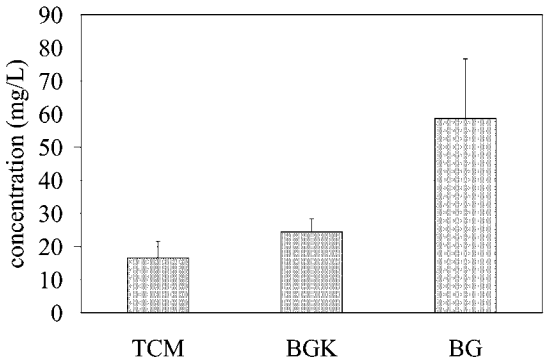

Figure 3. Concentration of total anthocyanin, expressed as cyanidin 3-rutinoside, in turning color olives (TCM), kalamata black olives (BGK), and naturally black olives (BG).

The Greek-style method of processing olives is similar to that of turning color olives in brine, only a fermentation of fruits in brine and packing. However, olives processed as naturally black olives are harvested at a higher degree of ripeness than turning color, and this difference in maturity was also reflected in a different polyphenol concentration in the olive juice. Greek-style olives had half of the concentration of hydroxytyrosol and tyrosol in juice that turning color olives had but were higher in salidroside. The black color of mature olives is due to the phenolic anthocyanins, and it could be supposed that they were present in fermented and packed fruits; as happens in wine and other food commodities, a polymerization process of these compounds occurs during fermentation, and simple anthocyanins were not detected in the final product (*21*, *22*). Thus, the content of total anthocyanins in the olive juice of Greek-style black olives and turning color olives in brine was estimated by a colorimetric method (*34*) (**Figure 3**). A higher concentration in total anthocyanins was observed for naturally black olives than turning color olives, which corresponds with the higher degree of ripeness at which the fruits are harvested when used for the Greek style. Nevertheless, the level of total anthocyanins in olive juice was below 80 mg/L, which can be explained by the relatively low concentration of anthocyanins in black olives (*4*), their dilution during fermentation and packing, and the polymerization reactions.

For the first time the phenolic composition of the oil phase of table olives has been studied. **Figure 4** shows the chromatograms obtained by UV and fluorescence detection of the phenolic compounds present in the oil of Spanish-style green olives. The phenolic profile of table olives oil was very different from that reported for oil obtained from unprocessed fruits (*8*, *9*). In the latter case, the aglycons of oleuropein and ligustroside were the predominant compounds in oil, whereas they were not found in table olive oil, suggesting that aglycons were hydrolyzed during alkaline and fermentation processes. In contrast, some phenolic compounds present in olive oil from fresh olives were also detected in the oil of table olives, such as hydroxytyrosol, tyrosol, vanillic acid, vanillin, hydroxytyrosol acetate, and the lignans 1-acetoxypinoresinol and pinoresinol. In addition, the presence of catechol in table olive oils was confirmed by mass spectrometry; this compound has not been detected in oil from fresh olives (*8*, *9*). Finally, the most representative phenolic compound in the oil of table olives was tyrosol acetate. This compound has a UV spectrum similar to that of tyrosol, and its presence in this oil was confirmed by HPLC-MS. Also, the hydrolysis of isolated peak 7 gave rise to only tyrosol. This compound has previously been identified in virgin olive oil (*35*) but in a very small amount.

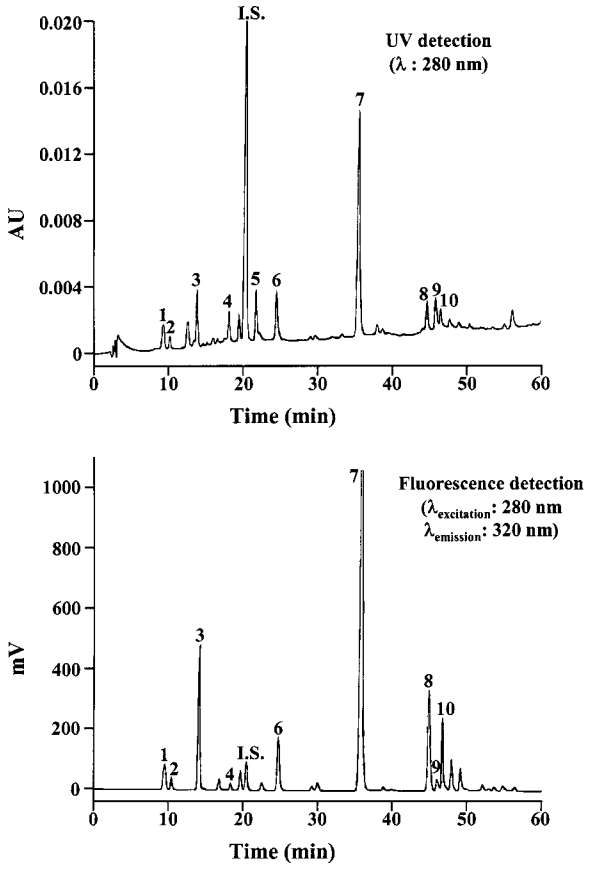

Figure 4. HPLC of the phenolic extract obtained from the oil phase of Spanish-style green olives. Fruits were of the Manzanilla cultivar. Peaks: (1) hydroxytyrosol; (2) catechol; (3) tyrosol; (4) vanillic acid; (5) vanillin; (6) hydroxytyrosol acetate; (7) tyrosol acetate; (8) unknown; (9) 1-acetoxypinoresinol; (10) pinoresinol.

In most table olive samples, tyrosol acetate was the main phenolic compound in the oil (**Table 3**), followed by hydroxytyrosol acetate. The latter compound has been found in most virgin olive oils (*36*) but never in a concentration >100 μM, as it was for the oil of table olives. The rest of the polyphenols analyzed were in a range similar to that found in virgin olive oil.

Differences in total polyphenols among cultivars and types of processing were generally low. The higher concentration in total polyphenols for turning color olives in brines (1382 μM) should be noted, as it also was observed for polyphenols in juice of this olive presentation, and the relatively similar concentrations for the rest of the samples, ranging from 550 to 860 μM. It seems that the lipophilicity of polyphenols present in the oil prevents them from diluting into the aqueous phase during the different steps of olive processing.

Despite the great recent interest in olive polyphenols due to their nutritional properties, there are few data on their contents in packed table olives. In this work, the polyphenols were individually characterized and quantified in the oil and juice of table olives, and the total content in the packed table olives was also estimated, taking into account the percentage of moisture and oil in olive flesh (**Table 4**). Juice of table olives represented ~75% of the flesh except for turning color olives and naturally black olives, for which it was lower because, as is well-known, water content in olives declines with maturity. Also, oil content was higher in these mature olives than in the rest because it increases with maturation.

Table 3. Effect of Cultivar and Processing Method on the Polyphenol Content (Micromolar) in the Oil Phase of Commercial Table Olives

type of olive	hydroxytyrosol	catechol	tyrosol	Hy-AC[a]	Ty-AC[b]	vanillin	vanillic acid	1-acetoxypinoresinol	pinoresinol	total
GG[c]	44 (2)	187 (26)	44 (2)	23 (12)	378 (28)	11 (4)	20 (1)	55 (7)	34 (9)	777 (49)
GM	78 (9)	64 (28)	73 (6)	210 (60)	341 (45)	14 (6)	9 (2)	32 (7)	39 (14)	860 (77)
GH	42 (7)	40 (18)	40 (8)	95 (17)	482 (86)	4 (1)	14 (2)	43 (9)	13 (6)	773 (130)
GMP	26 (12)	59 (36)	42 (9)	273 (150)	385 (29)	2 (1)	10 (2)	31 (5)	7 (2)	835 (228)
GHP	18 (2)	33 (10)	17 (3)	56 (20)	261 (46)	3 (1)	7 (1)	36 (12)	7 (3)	438 (82)
GMSA	21 (2)	19 (7)	24 (2)	203 (40)	314 (41)	2 (1)	4 (1)	26 (5)	7 (2)	620 (62)
GMSP	27 (3)	19 (1)	27 (3)	241 (140)	306 (40)	3 (1)	5 (1)	20 (5)	8 (2)	656 (187)
TCM	177 (15)	83 (42)	117 (16)	654 (160)	232 (28)	6 (2)	13 (1)	56 (8)	44 (20)	1382 (96)
RH	13 (2)	2 (1)	24 (3)	468 (71)	122 (18)	20 (2)	3 (1)	5 (1)	56 (4)	713 (48)
RC	9 (2)	2 (1)	34 (3)	226 (36)	110 (6)	37 (7)	5 (1)	2 (1)	121 (11)	546 (46)
BGK	126 (32)	21 (5)	187 (26)	92 (22)	171 (72)	26 (2)	25 (3)	31 (3)	79 (5)	758 (108)
BG	75 (21)	170 (90)	90 (38)	72 (35)	96 (50)	11 (1)	12 (4)	118 (42)	76 (16)	720 (44)

[a] Hydroxytyrosol acetate. [b] Tyrosol acetate. [c] See **Table 1** for identification of samples.

Table 4. Moisture and Oil Content in the Flesh of Samples Analyzed

type of olive	moisture (%)	oil (%)
GG[a]	76.8 (1.0)[b]	11.9 (0.2)
GM	73.4 (0.9)	15.3 (1.3)
GH	74.1 (1.1)	13.2 (0.8)
GMP	74.9 (1.1)	13.4 (1.4)
GHP	76.8 (1.0)	13.1 (1.4)
GMSA	75.9 (0.8)	12.8 (0.8)
GMSP	75.9 (0.7)	13.3 (0.4)
TCM	68.0 (1.2)	18.6 (0.6)
RH	74.2 (0.6)	15.2 (0.4)
RC	75.7 (0.7)	14.2 (1.2)
BGK	59.9 (1.5)	22.0 (1.1)
BG	58.2 (1.8)	25.9 (1.4)

[a] See **Table 1** for sample identification. [b] Standard error.

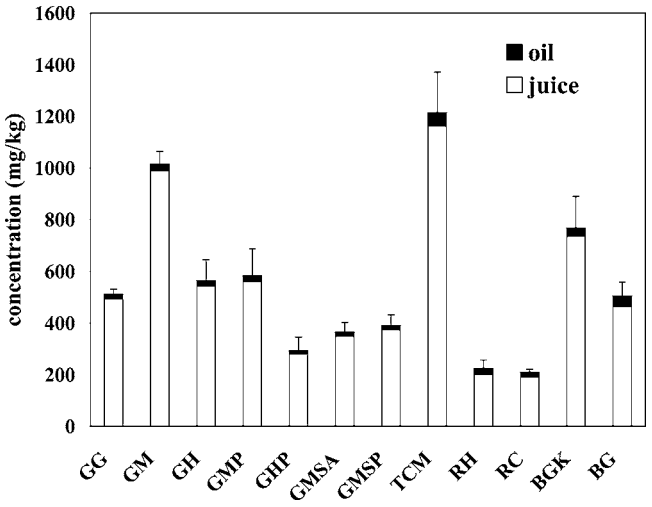

Figure 5. Influence of cultivar and processing method on the total polyphenol content of commercial table olives. Concentration expressed as milligrams per kilogram of olives.

Figure 5 shows the total concentration in phenolic compounds of the different table olive cultivars and processing method samples analyzed and the contribution of both the oil and juice polyphenols to the total amount. Regardless of the relatively high concentration in polyphenols of oil, the low percentage that this phase represents in the whole flesh makes the contribution of oil polyphenols to the total quantity <5%. This also implies that the differences found for juice polyphenols among samples were the same as for total polyphenols. These compounds reached a concentration in turning color olives as high as 1200 mg/kg, and as much as 1000 mg/kg was found in plain Manzanilla olives processed as the Spanish style; both of these levels are much higher than reported before (*31*). However, this amount was much lower for pitted and stuffed green olives and oxidized olives. Overall, it can be said that table olives are a good source of phenolic antioxidants, in some olive preparations being even greater than in virgin olive oil (*36*).

The results found in this work will rekindle interest in table olives from a nutritional point of view and could contribute to a higher consumption of this commodity if their higher polyphenolic antioxidant contents are compared with the low contents of other fermented vegetables such as cucumbers (*37*, *38*) and cabbage (*39*). Likewise, this investigation has also discovered the different compositions in phenolic compounds of the two phases present in the flesh of table olives and their contribution to the total polyphenol content.

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