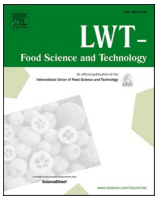
LWT - Food Science and Technology 184 (2023) 115098

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Phenolic and oleosidic compounds in different parts of Spanish olive cultivars: Influence of maturation and processing

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ARTICLE INFO

Keywords:
Olive
Phenolic
Oleoside
Oleuropein
Peel

ABSTRACT

The phenolic and oleosidic compounds in different components (peel, pulp and seed) and zones of olives during maturation were analyzed. These analyses were also carried out on commercial table olives and olive pomace peel. The profile and concentration of the compounds were very different among the components of the raw fruit; flavonoids were only detected in the peel and their content increased with ripening. Oleuropein was concentrated in the peel and the tissue near the peel, whereas verbascoside, secoxyloganin and oleoside 11-methyl ester were mostly detected near the pit. A close relationship between the maturation area within the fruit and the concentration of phenolic compounds was also found. Although the content of phenolic compounds in the peel and seed of commercial table olives was higher than in the pulp, the phenolic profile was similar for all components. Moreover, the concentration of phenolic compounds was low in commercial olive pomace peel.

1. Introduction

According to the International Olive Council figures (IOC, 2023) global production of olive oil and table olives accounted for 3.2 and 2.9 million tons respectively in the year 2022. Spain is one of the main global olive producing countries, with the Picual cultivar being the most employed for olive oil extraction and the Hojiblanca cultivar for table olives. In addition, most of the new olive plantations throughout the world are created with the Arbequina cultivar, due to its suitability for groves farmed at high intensity.

Olive products are obtained from the *Olea europaea* L. fruit and are key components of the valorized Mediterranean diet. The phenolic compounds in olive substances have attracted much attention for years, due to their attributes of beneficial properties for human health and their involvement in the sensory, chemical and physical properties of olive oil and table olives.

The characterization and quantification of these substances of the main Spanish olive cultivars have been studied widely (Gómez-Rico, Fregapane, & Salvador, 2008; Medina, Sanz, León, Pérez, & de la Rosa, 2021; Pérez, León, Sanz, & de la Rosa, 2018; Romero, Medina, Mateo, & Brenes, 2017). However, very few data are available on the phenolic compounds of the peel of these Spanish cultivars (Romero et al., 2017) or even Italian cultivars (Ivancic, Jakopic, Veberic, Vesel, & Hudina, 2022; Servili, Baldioli, Selvaggini, Macchioni, & Montedoro, 1999), even though large differences in the phenolic profile of the peel and pulp of many fruits have been found (Cantos, Espín, & Tomás-Barberán, 2002; Mihailović et al., 2018; Rosalie, Joas, Mertz, Dufossé, & Léchaudel, 2022).

The peel of the olives, particularly the cuticle, functions as a barrier against water loss and serves as protection against pathogenic microorganisms and insect attacks (Malheiro, Casal, Baptista, & Pereira, 2015), and UV radiation (Reynoud et al., 2021). In addition, the osmotic exchanges between olives and brine during the processing of table olives are highly influenced by the characteristics of the peel (Lanza & Di Serio, 2015), and it may also affect the texture and quality of the commercial product (Georget, Smith, Waldron, & Rejano, 2003). Moreover, a paste called Alperujo is obtained during the extraction process of olive oil, which is composed of pulp and pit fragments. Today, the pit fragments are separated from the rest of the Alperujo at olive oil mills for use in combustion. However, pieces of peel and olive pulp adhere to these pit fragments that are then separated to obtain a new product (olive pomace peel), rich in triterpenic acids and in high demand for animal feed (Romero, Medina, Mateo, & Brenes, 2018). The triterpenic composition of this by-product has been characterized, but not its phenolic content that could contribute to a better valorization of this product.

Oleosides do not have a phenolic structure but they can be bound to phenolic compounds including hydroxytyrosol and tyrosol, giving rise to the main secoiridoids found in olive fruit, oleuropein,

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https://doi.org/10.1016/j.lwt.2023.115098
Received 14 April 2023; Received in revised form 28 June 2023; Accepted 17 July 2023
Available online 18 July 2023

demethyloleuropein, and ligustroside (Damtoft, Franzyk, & Jensen, 1993). They are currently extracted as part of the phenolic fraction of oleaceous samples and many of them have been attributed with antimicrobial activity (Heinze, Hale, & Carl., 1975; Medina, Brenes, Romero, García, & de Castro, 2007). Nevertheless, scientists have paid less attention to these substances than to phenolic compounds in olives and olive products. They have been characterized in raw fruit (Klen, Wondra, Vrhovšek, & Vodopivec, 2015; Talhaoui et al., 2015), olive pomace (Cardoso et al., 2005), and table olives (Ambra et al., 2017; Medina et al., 2008), and the evolution of some of them has also been followed during fruit ripening (Ivancic et al., 2022). However, the analysis of these substances in the different parts of the main Spanish olive cultivars during ripening has never been studied.

Therefore, the aim of this work was to quantify, for the first time, the content of non-anthocyanin phenols and oleosidic compounds in different parts of the main Spanish olive cultivars during fruit maturation. Moreover, the analysis of these substances in the pulp and peel of commercial table olives and pomace olive peel was also assessed.

2. Materials and methods

2.1. Raw material

Olives were harvested from olive trees of the Hojiblanca, Picual and Arbequina cultivars, located in the olive tree garden at Instituto de la Grasa (Seville, Spain), in November 2022. Green, purple and black fruits were picked at the same time from three different trees of each cultivar. The trees were 5 years old, grown under standard cultural practices, and irrigated by in-line drip to avoid water stress of plants.

2.2. Analysis of phenolic and oleosidic compounds in pulp, peel and seed of fruits

Three olives of each cultivar and degree of ripening were selected for the duplicated analysis. The peels were separated from the pulp using a surgical scalpel blade nº 23 and small pieces, free of pulp, were cut with scissors. Then, samples of pulp were obtained from the center of longitudinal pieces of mesocarp tissue.

About 0.02–0.09 g of peel, pulp or seed were weighted in a 2 mL centrifuge Eppendorf tube containing 1 mL of dimethyl sulfoxide (DMSO). The mixture was sonicated in an ultrasonic bath for 15 min (Selecta, Barcelona, Spain), vortexed for 2 min and centrifuged at 9000 g for 5 min. Then, 0.75 mL of the supernatant was mixed with 0.25 mL of 0.2 mM syringic acid (internal standard). The mixture was filtered through a 0.22 μm pore size nylon filter and injected (20 μL) into the chromatograph.

2.3. Analysis of phenolic and oleosidic compounds in different areas of the fruit

Three olives of the Picual cultivar with purple color on their surface were peeled and tissue near and far from the pit (0.07–0.09 g) was weighted in a 2 mL centrifuge Eppendorf tube containing 1 mL of dimethyl sulfoxide (DMSO). The rest of the analysis was carried out as described in section 2.2. Analyses were performed in duplicate.

Two olives of the Picual cultivar with green color close to the peduncle area and purple color close to the apex area were analyzed. Longitudinal pieces of mesocarp tissue from the two fruit areas were cut with the scalpel blade. Small pieces of this tissue (0.07–0.09 g) were weighted in a 2 mL centrifuge Eppendorf tube containing 1 mL of dimethyl sulfoxide (DMSO). The rest of the analysis was carried out as described in section 2.2. Analyses were performed in duplicate.

2.4. Analysis of phenolic and oleosidic compounds in different parts of commercial table olives and commercial peel from olive pomace

Two samples of commercial Spanish-style green olives (Jolca and Fragata brands) and another two samples of natural turning color olives (Zambudio and Carrefour brands) were obtained from local markets. The common industrial elaboration of Spanish-style green olives consists in harvesting the fruits with green color, treating with alkali and fermenting in brine for several months, whereas the natural turning color olives are harvested with purple color and put directly in brine where they undergo a fermentation process for months. Finally, all table olives are packed in brine and pasteurized (Sánchez-Gómez, García-García, & Rejano-Navarro, 2006).

The analysis of phenolic and oleosidic compounds in the peel and pulp of the olives was carried out as described in section 2.2.

Commercial peel from olive pomace was obtained from Maslina International Trade S. L. (Santander, Spain) company, and phenolic and oleosidic compounds were analyzed in three independent lots.

About 0.5 g of the commercial peel was weighed in a 5 mL centrifuge Eppendorf tube and 3 mL of DMSO was added. The mixture was sonicated in an ultrasonic bath for 15 min (Selecta, Barcelona, Spain), vortexed for 2 min and centrifuged at 9000 g for 5 min. Then, 0.25 mL of the supernatant was mixed with 0.25 mL of 0.2 mM syringic acid (internal standard) and 0.5 mL of DMSO. The next steps of the analysis were the same as described in section 2.2.

2.5. HPLC analysis

The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump, a Waters column heater module, and a Waters 996 diode array detector (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 μm, 25 × 4.6 i. d., Waters Inc.) column was used. Separation was achieved by gradient elution using water (pH 2.5 adjusted with phosphoric acid) and methanol, with an initial composition of 90% and 10% respectively (Medina et al., 2007). A flow rate of 1 mL/min and a temperature of 35 °C were used in all experiments. The wavelengths selected for phenolic and oleosidic compounds were 280 nm and 240 nm, respectively. Oleuropein, apigenin 7-glucoside, luteolin 7-glucoside, luteolin 4-glucoside, luteolin, apigenin, hydroxytyrosol, verbascoside and rutin were purchased from Extrasynthese S. A. (Lyon Nord, France). Tyrosol, vanillic acid, vanillin, *p*-coumaric acid and caffeic acid were obtained from Sigma-Aldrich. Hydroxytyrosol 4-glucoside and oleoside 11-methyl ester were obtained using a high-performance liquid chromatography preparative system (Romero, Brenes, García, & Garrido, 2002). Secoxyloganin and secologanoside were quantified using the response factor of oleoside 11-methyl ester because of unavailable commercial standards. Besides, all these oleosidic compounds present a similar UV spectrum with maximum absorption at 230–240 nm. Hydroxytyrosol 1-glucoside, caffeoyl ester of secologanoside, comselogoside and demethyl oleuropein were quantified using the response factors of hydroxytyrosol, caffeic acid, *p*-coumaric acid and oleuropein, respectively. Salidroside and nuzhenide were quantified using the response factor of tyrosol because the latter substance is a component of the former compounds.

2.6. Statistical analysis

Statistical analyses were performed using Statistica 8.0 software (Statsoft, Tulsa, OK, USA). One-way analysis of variance, ANOVA (Duncan's test), was employed to compare mean values with a significance level of 95%.

3. Results and discussion

3.1. Phenolic and oleosidic compounds in the peel, pulp and seed

The results reported in Tables 1–3 show that the flavonoids luteolin 7-glucoside, luteolin 4-glucoside, rutin and apigenin 7-glucoside were only found in the peel component of the three Spanish olive cultivars (Picual, Arbequina and Hojiblanca), similar to the findings detected in several Italian cultivars (Moraiolo, Leccino and Coratina) (Servili et al., 1999). However, other researchers have also found these flavonoids in the pulp of raw Italian cultivars (Ivancic et al., 2022). There are also contradictory data about the evolution of these substances during fruit ripening. Servili et al. (1999) did not detect changes in the concentration of these flavonoids with ripening, whereas Ivancic et al. (2022) reported an increase, in particular luteolin 7-glucoside. In our case, the concentration of all flavonoids steadily increased in the peel of the three Spanish olive cultivars with olive maturation (Tables 1–3), and some of them even reached a higher content than 10000 mg/kg, which must be related to the attributed protective action of these substances against UV radiation during maturation (Dias, Pinto, Freitas, Santos, & Silva, 2020; Liakopoulos, Stavrianakou, & Karabourniotis, 2006). It must be noted that the presence of phenolic compounds in the cuticle of several fruits has been identified (Hunt & Baker, 1980; Reynoud et al., 2021), but our peel analyses did not differentiate between those components associated with the cuticle and those originating from the epidermal cells.

The concentration of verbascoside and hydroxytyrosol 4-glucoside also increased in the peel with the ripening of all Spanish cultivars, with the content reached in the Hojiblanca cultivar (>10000 mg/kg) being particularly high. Oleuropein was more concentrated in the peel than in the pulp of the Hojiblanca and Picual cultivars, and it increased with ripening for the former cultivar and an erratic behavior was observed for the latter cultivar, which is in the line with the reported increase of oleuropein in the peel of Italian cultivars with maturation (Ivancic et al., 2022; Servili et al., 1999). As expected, demethyloleuropein was found in the peel of the Arbequina olives instead of oleuropein, as this is the main secoiridoid detected in this cultivar in many previous works (Gómez-Rico et al., 2008; Medina et al., 2021; Romero et al., 2017; Talhaoui et al., 2015). It must be noted that neither hydroxytyrosol nor tyrosol were found in any of the peels analyzed, as opposed to other studies (Ivancic et al., 2022; Servili et al., 1999). With respect to the oleosidic compounds, only secologanoside and oleoside 11-methyl ester were detected in the peel of all cultivars, increasing with maturation in the peel of Hojiblanca and Picual olives.

In the pulp, flavonoids were absent, regardless of the cultivar and degree of ripening. Oleuropein, which was the major secoiridoid in the pulp of Hojiblanca and Picual cultivars, did not show a clear trend with maturation. Several studies have indicated a clear decrease of this compound during olive ripening (Ivancic et al., 2022; Servili et al., 1999), although other researchers did not observe this trend (Fernández-Poyatos, Llorent-Martínez, & Ruiz-Medina, 2021). As expected (Gómez-Rico et al., 2008; Romero et al., 2017), the major secoiridoid in the pulp of Arbequina fruit was demethyloleuropein instead of oleuropein, and it decreased as maturation progressed (Table 3). The oleosidic composition of the pulp was very interesting, as secoxyloganin was detected in all the cultivars analyzed, unlike its absence in the peel, and it clearly decreased with olive ripening (Tables 1–3). Similarly, secologanoside tended to decrease during maturation as opposed to the tendency observed in the peel, whereas the concentration of oleoside 11-methyl was statistically the same for the three ripening stages analyzed. The very little data available on the content of oleoside 11-methyl ester and total oleosidic compounds in olives (Ambra et al., 2017; Ivancic et al., 2022; Talhaoui et al., 2015) indicate a very low concentration of these substances in raw olives (<500 mg/kg fresh fruit) while a range of 4000–8000 mg/kg was found in this work. It is worth mentioning the potential presence of methyl esterases in olives during ripening (Volk et al., 2019) that could transform oleuropein into demethyloleuropein, or alternatively oleoside 11-methyl ester, and secoxyloganin into secologanoside and oleoside that could contribute to the metabolic route of demethyloleuropein (Damtoft et al., 1993).

As previously reported, the main phenolic compound identified in the seed of the fruits was nuzhenide (Romero et al., 2017; Servili et al., 1999), whose concentration did not show a clear trend with olive maturation. By contrast, the content of oleuropein clearly decreased with olive ripening for the Hojiblanca and Picual cultivars, whereas it did not change for Arbequina. Surprisingly, demethyloleuropein was the main secoiridoid detected in the peel and pulp of the latter cultivar, but it was not found in the seed (Table 3).

3.2. Phenolic and oleosidic compounds in different areas of the olives

After peeling three Picual olives, the phenolic and oleosidic compounds were analyzed in two different areas, near and far from the pit. The results reflected in Table 4 indicate that the concentration of oleuropein was much higher in the tissue near the peel than in the pit, which is in accordance with the high content of oleuropein detected in the peel (Table 2). By contrast, verbascoside, secoxyloganin and oleoside

Table 1
Influence of olive maturation on the phenolic and oleosidic compounds content (mg/kg) in the peel, pulp and seed of the Hojiblanca cultivar.

	Peel			Pulp			Seed		
Compound	Green	Purple	Black	Green	Purple	Black	Green	Purple	Black
Hydroxytyrosol 1-glucoside	nd[x]	nd	202	69a[y]	62a	94a	nd	nd	nd
Hydroxytyrosol 4-glucoside	3994b	14747a	11745a	10439a	10326a	9788a	600a	162a	43a
Tyrosol glucoside	nd	nd	nd	85a	74a	104a	nd	nd	nd
Demethyloleuropein	nd	nd	nd	nd	nd	nd	nd	nd	nd
Verbascoside	nd	21b	816a	4109a	3350a	3116a	255a	108a	104a
Nuzhenide	nd	nd	nd	nd	nd	nd	1488a	1782a	1894a
Luteolin 7-glucoside	4991b	9840b	13461a	nd	nd	nd	nd	nd	nd
Oleuropein	6755b	30372a	27207a	5606a	2704a	7444a	915a	345a	158a
Rutin	7291a	8774a	12580a	nd	nd	nd	nd	nd	nd
Caffeic acid ester	nd	nd	nd	11a	8a	4a	nd	nd	nd
Luteolin 4-glucoside	1565b	2189b	2971a	nd	nd	nd	nd	nd	nd
Ligustroside	nd	nd	nd	164a	78b	39b	425a	104a	209a
Apigenin 7-glucoside	350b	1032a	1168a	nd	nd	nd	nd	nd	nd
Comselogoside	nd	nd	nd	20a	13a	13a	nd	nd	nd
Secoxyloganin	nd	nd	nd	7102a	2970b	1499b	nd	nd	nd
Secologanoside	104c	279b	401a	1164a	1101a	653a	nd	nd	nd
Oleoside 11-methyl ester	185b	691a	1061a	4869a	4804a	4975a	nd	nd	nd

[x]Not detected; [y]Row values followed by the same letter for each olive component do not differ at the 5% level of significance according to the Duncan's multiple range test.

Table 2
Influence of olive maturation on the phenolic and oleosidic compounds content (mg/kg) in the peel, pulp and seed of the Picual cultivar.

	Peel			Pulp			Seed		
Compound	Green	Purple	Black	Green	Purple	Black	Green	Purple	Black
Hydroxytyrosol 1-glucoside	nd[x]	51b[y]	113a	13b	16b	23a	nd	nd	nd
Hydroxytyrosol 4-glucoside	531b	1992a	2174a	1234a	1227a	1018b	722a	56b	nd
Tyrosol glucoside	nd	nd	nd	10a	19a	24a	nd	nd	nd
Demethyloleuropein	nd	nd	nd	nd	nd	nd	nd	nd	nd
Verbascoside	nd	99b	598a	168a	242a	380a	167a	85a	88a
Nuzhenide	nd	nd	nd	nd	nd	nd	1947a	1490a	1332a
Luteolin 7-glucoside	629b	1975b	10161a	nd	nd	nd	nd	nd	nd
Oleuropein	4849b	27476a	7780b	5935a	3519b	3315b	1161a	634b	nd
Rutin	3173b	4401b	12231a	nd	nd	nd	nd	nd	nd
Caffeic acid ester	nd	nd	nd	30a	27a	20a	nd	nd	nd
Luteolin 4-glucoside	110b	226b	669a	nd	nd	nd	nd	nd	nd
Ligustroside	nd	nd	nd	176a	106b	113b	93a	141a	184a
Apigenin 7-glucoside	61b	252a	277a	nd	nd	nd	nd	nd	nd
Comselogoside	26b	661a	401a	131a	128a	121a	nd	nd	nd
Secoxyloganin	nd	nd	nd	1448a	341b	412b	nd	nd	nd
Secologanoside	nd	772b	1215a	777a	416a	525a	nd	nd	nd
Oleoside 11-methyl ester	259b	809a	707a	4684a	2538a	4239a	nd	nd	nd

[x] Not detected.
[y] Row values followed by the same letter for each olive component do not differ at the 5% level of significance according to the Duncan's multiple range test.

Table 3
Influence of olive maturation on the phenolic and oleosidic compounds content (mg/kg) in the peel, pulp and seed of the Arbequina cultivar.

	Peel			Pulp			Seed		
Compound	Green	Purple	Black	Green	Purple	Black	Green	Purple	Black
Hydroxytyrosol 1-glucoside	nd[x]	nd	40[y]	6b	12b	29[a]	nd	nd	nd
Hydroxytyrosol 4-glucoside	625c	1503b	1806[a]	1002[a]	1003[a]	1024[a]	357[a]	294[a]	361[a]
Tyrosol glucoside	nd	nd	nd	nd	nd	nd	160[a]	176[a]	170[a]
Demethyloleuropein	1918b	7464[a]	8663[a]	4484[a]	4571[a]	2663b	nd	nd	nd
Verbascoside	119c	281b	647[a]	339[a]	493[a]	369[a]	nd	nd	nd
Nuzhenide	nd	nd	nd	nd	nd	nd	1725c	2975[a]	2075b
Luteolin 7-glucoside	1190b	4377[a]	5772[a]	nd	nd	nd	nd	nd	nd
Oleuropein	nd	nd	nd	461[a]	157b	nd	516[a]	454[a]	549[a]
Rutin	2169c	6288[a]	4120b	nd	nd	nd	nd	nd	nd
Caffeic acid ester	nd	nd	nd	6[a]	7[a]	3[a]	nd	nd	nd
Luteolin 4-glucoside	198b	986[a]	582[a]	nd	nd	nd	nd	nd	nd
Ligustroside	nd	nd	nd	nd	nd	nd	114[a]	63[a]	84[a]
Apigenin 7-glucoside	122b	360[a]	286[a]	nd	nd	nd	nd	nd	nd
Comselogoside	nd	nd	nd	36[a]	53[a]	27[a]	nd	nd	nd
Secoxyloganin	nd	nd	nd	1213[a]	1385[a]	351b	nd	nd	nd
Secologanoside	140b	289[a]	156b	1123[a]	906[a]	674b	nd	nd	nd
Oleoside 11-methyl ester	287b	567[a]	222b	8712[a]	6621b	8233[a]	nd	nd	nd

[x] Not detected.
[y] Row values followed by the same letter for each olive component do not differ at the 5% level of significance according to the Duncan's multiple range test.

Table 4
Influence of the pulp area on the phenolic and oleosidic compounds content (mg/kg) of purple Picual cultivar.

	Olive A		Olive B		Olive C	
Compound	Near peel	Near pit	Near peel	Near pit	Near peel	Near pit
Hydroxytyrosol 1-glucoside	30	24	4	48	12	22
Hydroxytyrosol 4-glucoside	562	540	979	1451	1354	1033
Verbascoside	34	140	7	403	28	113
Oleuropein	2827	1974	10365	4281	12090	3942
Caffeic acid ester	12	11	18	34	16	13
Ligustroside	56	47	114	106	138	102
Comselogoside	62	48	67	54	56	45
Secoxyloganin	288	414	392	594	107	754
Secologanoside	511	329	817	496	1055	579
Oleoside 11-methyl ester	726	3167	861	7518	1147	10058

11-methyl ester were more concentrated in the area near the pit than the peel, which is also in line with the higher content of these substances in the pulp than in the peel (Table 2). The different location of oleuropein and oleoside 11-methyl ester within the fruit is of great interest for the biosynthesis and degradation of the former substance, since it contributes to the bitter taste of table olives and is the precursor of the main secoiridoids detected in olive oil (Damtoft et al., 1993; Volk et al., 2019).

In another experiment, phenolic and oleosidic compounds were analyzed in two Picual olives with green color near the peduncle and

Table 5
Phenolic and oleosidic compounds content (mg/kg) in the fruit areas near the peduncle and apex of two olives of the Picual cultivar. Olives with green color near the peduncle and purple color near the apex.

	Olive A		Olive B	
Compound	Peduncle	Apex	Peduncle	Apex
Hydroxytyrosol 1-glucoside	84b[y]	134a	86b	104a
Hydroxytyrosol 4-glucoside	6028a	5604b	4729a	4197b
Tyrosol glucoside	50a	57a	49a	53a
Verbascoside	717a	611b	850a	487b
Luteolin 7-glucoside	166a	60b	121a	72b
Oleuropein	3615a	2242b	3554a	2339b
Rutin	175a	113b	198a	159b
Caffeic acid ester	4a	1b	5a	3b
Luteolin 4-glucoside	67a	21b	54a	19b
Ligustroside	83a	60b	79a	59b
Apigenin 7-glucoside	30a	10b	39a	17b
Comselogoside	5a	1b	8a	2b
Secoxyloganin	1476a	1221b	2176a	1224b
Secologanoside	539a	389b	352a	276b
Oleoside 11-methyl ester	2130a	2248a	2061a	1918a

[y] Row values followed by the same letter for each olive do not differ at the 5% level of significance according to the Duncan's multiple range test.

purple color near the apex which must be related to the different degree of maturation between these two areas of the fruit (Table 5). Longitudinal pieces of the fruit were cut, and tissue near the peduncle and apex was analyzed. The concentration of oleuropein and most of the phenolic compounds analyzed were higher in the peduncle area than in the apex, which confirms the decrease of these substances with fruit maturation, except for the flavonoids located in the peel of the olives (Table 2). This trend also occurred for the oleosidic compounds secologanoside and secoxyloganin, but not for oleoside 11-methyl ester.

Overall, these results have demonstrated that there is great variability in the phenolic and oleosidic compounds among fruits, but also among the different parts and areas of the olives, which must be taken into account during analysis of these substances.

3.3. Phenolic and oleosidic compounds in commercial olive products

Raw olives cannot be eaten directly after harvesting due to their content of the bitter compound oleuropein, therefore one of the main objectives of the table olive elaboration process is to eliminate this substance. Thus, oleuropein was not detected either in commercial Spanish-style green olives or natural olives (Table 6). The alkali treatment and the acidic conditions contribute to the hydrolysis of this compound during processing (Durante et al., 2018; Medina et al., 2007). Accordingly, hydroxytyrosol was the main phenolic compound detected in the peel and pulp of the commercial products, although in a higher concentration in the peel than in the pulp. Interestingly, hydroxytyrosol was also the main phenolic substance found in the seed of the table olives, which means that there was an exchange of this substance between the seed and the pulp through the pit. In fact, nuzhenide was not detected in the seed of commercial table olives, as opposed to the high concentration found in the seed of raw fruits (Tables 1–3). Thus, hydrolysis of nuzhenide along with ligustroside gave rise to the high content of tyrosol detected in all parts of the processed olives (Table 6). With regards to flavonoids, simple flavonoids including luteolin and apigenin were found in both the pulp and the peel, although in a higher concentration in the latter tissue. Therefore, glucosides hydrolyzed during olive processing and the metabolites diffused from the peel to the pulp. It must be noted that the high concentration of phenolic compounds in the peel of the table olives, in particular *o*-diphenols, has a great influence on the color and quality of the product due to the

Table 6
Phenolic compound content in the different parts of commercial table olives and commercial peel obtained from olive pomace.

	Phenolic compound (mg/kg)								
	Hydroxytyrosol	Tyrosol	Luteolin	Apigenin	Hy 4-Glu[x]	Salidroside	Vanillic acid	*p*-coumaric acid	verbascoside
Spanish-style green olives									
Sample A (peel)	1129a[y]	219a	156a	44a	nd[z]	nd	nd	nd	nd
Sample A (pulp)	656b	77c	4b	1b	nd	nd	nd	nd	nd
Sample A (seed)	1110a	143b	nd	nd					
Sample B (peel)	421a	100a	5	23	nd	nd	nd	nd	nd
Sample B (pulp)	295b	59c	nd	nd	nd	nd	nd	nd	nd
Sample B (seed)	356a	76b	nd	nd	nd	nd	nd	nd	nd
Natural green olives									
Sample A (peel)	1228a	191a	547a	22	nd	nd	nd	30a	399a
Sample A (pulp)	735b	66c	25b	nd	nd	nd	nd	3c	165b
Sample A (seed)	1092a	103b	nd	nd	nd	nd	nd	7b	429a
Sample B (peel)	2653a	426a	119a	23a	nd	nd	nd	92a	408b
Sample B (pulp)	1716b	174b	4b	1b	nd	nd	nd	10c	345b
Sample B (seed)	1984b	225b	nd	nd	nd	nd	nd	31b	729a
Olive pomace peel									
Sample A	nd	nd	94a	36a	51b	35b	35b	10b	16a
Sample B	nd	nd	66b	17b	nd	93a	81a	22a	13a
Sample C	nd	nd	64b	20b	75a	21c	33b	3c	14a

[x] Hydroxytyrosol 4-glucoside.
[y] Column values followed by the same letter for each table olive sample or olive pomace peel samples do not differ at the 5% level of significance according to the Duncan's multiple range test.
[z] Not detected.

oxidation reactions that can occur during elaboration of the commercial product (Ramírez, Gandul-Rojas, Romero, Brenes, & Gallardo-Guerrero, 2015).

With respect to the oleosidic compounds, none were detected in the commercial table olives, which confirmed previous data regarding the continuous hydrolysis of these substances with time during olive fermentation (Ambra et al., 2017; Medina et al., 2008).

With respect to the commercial olive pomace peel, luteolin followed by apigenin, hydroxytyrosol 4-glucoside, salidroside, vanillic acid, vanillin and *p*-coumaric acid were the main phenolic compounds identified in this product (Table 6). However, the concentration of these substances was not so high as to valorize this by-product based on their content. The elaboration of this new by-product from the olive pomace, called Alperujo, comprises several stages, in particular a drying treatment, which could contribute to the oxidation and hydrolysis of the phenolic compounds present initially in the peel of raw olives. Moreover, oleosides were not found in this product as it also occurred in table olives.

4. Conclusions

Despite the high number of studies on olive polyphenols, there are few reports on the characterization of these substances in the different parts and areas of the fruit. In this study, it has been observed that the flavonoids were only present in the peel of the three Spanish olive cultivars analyzed, with their content increasing with olive maturation, which could be related to the protective action of these substances against UV radiation. Oleuropein, the main bitter compound of this fruit, was found in all olive parts (peel, pulp and seed) although it was more concentrated in the peel. Indeed, the concentration of this secoiridoid was also higher in the tissue near the peel than near the pit, opposite to the location of verbascoside, secoxyloganin and oleoside 11-methyl ester. Likewise, with most of the phenolic compounds being found in a lower concentration in the purple area of the fruit than in the green area, it is indicated that the degree of maturation in the fruit zone is crucial for the analysis of these compounds in olives. On the other hand, the composition of the olive by-products analyzed was very different to that found in the raw fruit, due probably to the hydrolysis and oxidation reactions occurring during processing. Hydroxytyrosol was the main phenolic compound in all parts of the commercial table olives, although it was found in higher concentrations in the peel and seed than in the pulp, similar to the data found for tyrosol, luteolin and apigenin. In addition, the low concentration of phenolic compounds in commercial olive peel pomace indicated a very high degradation of these substances during the elaboration of this new by-product, which is rich in triterpenic acids. Overall, these data must be taken into consideration for the analysis of phenolic and oleosidic compounds in olives.

CRediT authorship contribution statement

M. Brenes-Álvarez: Conceptualization, Methodology, Formal analysis, Investigation. **C. Romero:** Investigation, Formal analysis. **E. Medina:** Investigation, Formal analysis. **M. Brenes:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study was financed by the Spanish Government and EU FEDER funds (project PID-2020-1119563RB-I00/AEI/10.13039/501100011033).

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