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Abscisic Acid Sprayed on Olive Tree (*Olea europaea* L.) Affects the Phenolic Composition of Olive Fruit Cultivars

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Abstract

The aim of this research was to study the effect of abscisic acid pre-harvest treatment on the phenolic composition of olive fruits. To that end we applied abscisic acid (*i.e.*, 50 mg/L and 100 mg/L) on *Arbequina* and *Picual* olive trees. Two different days of harvesting (*i.e.*, day 3 and 6 after treatment) were also included in the study. Although the results obtained depended on the cultivar and on the day of harvesting a general trend was established. The treatment with 50 mg/L of abscisic acid resulted in higher total phenol content but significant decrease in the DPPH activity. In contrast, olives treated with 100 mg/L abscisic acid resulted in higher total phenol content, DPPH activity and contents of oleuropein, hydroxytyrosol and phenolic acids as compared with controls. The best values of total phenol content and IC_{50} were obtained for treated *Picual* olives (727.75 mg gallic kg^{-1} and 889.72 µg/ml, respectively) whereas the highest values of oleuropein and hydroxytyrosol were measured for treated *Arbequina* olives (508.94 and 559.67 mg kg^{-1}, respectively). Phenolic acid content was also higher in *Picual* olives treated with 100 mg/L of abscisic acid. Particulary, values ranged from 7.26 mg kg^{-1} for caffeic acid to 92.38 mg kg^{-1} for chlorogenic acid. Exogenous abscisic acid applied to olive trees is a promising agronomic practice to obtain olives enriched in antioxidants.

Keywords: elicitors, preharvest treatment, olives, phenolics, antioxidant, functional

1. Introduction

Olive (*Olea europaea* L.) fruits and oil are known for their nutritional properties and therapeutic interest (Kiritsakis, 1998). The benefits of olive fruits and oil are attributed to a convenient fatty acid profile and to the presence of some minor compounds including phenolic compounds (Teissedre, Frankel, Waterhouse, Peleg, & German, 1996; Visioli, Poli, & Galli, 2002). Studies in the literature speculate on the responsibility of phenolic compounds for the oxidative stability of olive oil (Visioli & Galli, 1995).

It is widely known that oleuropein and related compounds such as hydroxytyrosol are the main phenolic compounds in *Olea europaea* L. (Arslan & Özcan, 2011). Both oleuropein and hydroxytyrosol are secoiridoid compounds that possess relevant human health effects. In particular, they have been described to exert antioxidative (Benavente-Garcia, Castillo, Lorente, Ortuno, & Del Río, 2000), antimicrobial (Pereira et al., 2007), antiviral (Micol, Caturla, Pérez-Fons, Mas, Pérez, & Estepa, 2005), anti-inflammatory (Visioli, Bellosta, & Galli, 1998), cardioprotective (Andreadou et al., 2006) and neuroprotective (Jemai, Bouaziz, Fki, El Feki, & Sayadi, 2008) properties. Other important phenolics in olives are phenolic acids. Although their level is considerably lower than that of oleuropein and hydroxytyrosol, their therapeutic effect is equally remarkable. Phenolic acids have demonstrated protection against a variety of diseases, including cancer, heart diseases and diabetes (Morton, Caccetta, Puddey, & Croft, 2000; Johnston, Clifford, & Morgan, 2003). The positive effects of most phenolics have been mainly attributed to their excellent antioxidant properties which also contribute to the oxidative stability of olive oil and olives.

On the other hand, the recent demand for healthy foods by consumers has brought about the increasing search for foods capable of preventing aging related diseases. In this sense, it has already been demonstrated that the chemical elicitation of plant foods is an interesting approach to obtained foods enriched in health promoting compounds.

Although there are a number of elicitors to stimulate the accumulation of bioactive compounds in plant foods; abscisic acid (ABA) has been demonstrated to be one of the most effective lately. ABA is a phytohormone that is directly involved in response to stress (Nakashima & Yamaguchi-Shinozaki, 2013). There are reports in the literature that prove that ABA is successful in the production of tanshinones and phenolic acids (Yang et al., 2012), anthocyanins (Cui, Liang, Y. Liu, F. Liu, & Zhu, 2012) and other phenolics (Cantín, Fidelibus, & Crisosto, 2007; Huang et al., 2016) in derived plant foods.

Research has been done in the enrichment of plant foods in phenolics by chemical elicitation during the last past years. In particular, these studies describe the postharvest application of elicitors other than ABA to enhance the biosynthesis of phenolics (de la Peña Moreno, Monagas, Blanch, Bartolomé, & Ruiz del Castillo, 2010; Ruiz del Castillo, Flores, & Blanch, 2010; Flores & Ruiz del Castillo, 2014; Flores, Blanch, & Ruiz del Castillo, 2017). Occasional works have also been performed on the effect of the pre-harvest elicitation (*i.e.*, on the plant instead of on the food) (Flores & Ruiz del Castillo, 2016; Blanch, Flores, Gómez-Jiménez, & Ruiz del Castillo, 2018). In view of the results obtained in these studies, ABA seems a promising approach to obtain olive fruits enriched in certain phenolic compounds and, therefore, improve their oxidative stability.

The objective of this investigation is to evaluate the effect of exogenous pre-harvest ABA on the total phenolic content (TPC), DPPH activity and contents of oleuropein, hydroxytyrosol and phenolic acids in the olive fruits obtained.

2. Materials and Methods

2.1 Samples and Chemicals

HPLC-grade MeOH was supplied by VWR Inc. (Bridgeport, PA, USA). Ultrapure water was collected from a purification system (Millipore Milford, MA, USA). Acetic acid was obtained from Probus (Barcelona, Madrid). For the elicitation, ABA was purchased from Across Organics (New Jersey, USA). Sodium carbonate and Folin-Ciocalteu reagent were supplied by Merck (Darmstadt, Germany). Oleuropein, hyrdroxytyrosol, 1,1-diphenyl-2-picrylhydrazil (DPPH) and phenolic acid standards (*i.e.*, gallic, vanillic, *p*-coumaric, caffeic, chlorogenic, and ferulic acids) were acquired by Sigma-Aldrich (Steinheim, Germany). Olive fruits (*Arbequina* and *Picual* cultivars) were hand-picked from the trees in November and December 2016 in the University of Extremadura (Badajoz, Spain).

2.2. Pre-Harvest ABA treatment

Twenty-year-old olive tree (*Olea europaea* L.) of two cultivars, *Arbequina* and *Picual*, grown under trip irrigation and fertirrigation in the same orchard near Badajoz (Spain) were included in the study. The irrigation was performed by addying suitable fertilizers in the solution and all the study was carried out under the same agronomical and environmental condition. Fruit treatments were performed *in planta* and on olive trees (5 trees per treatment) of *Arbequina* and *Picual* cultivars. Two branches per tree were selected for experiments for uniform size and fruit load. Two different concentrations of ABA were tested and, therefore, two different treatments were applied. For each treatment 5 branches (1 branch per tree) were sprayed. Water was applied to the controls and aqueous solutions of 50 mg/L (*i.e.*, so-called ABA50) and 100 mg/L (*i.e.*, so-called ABA100) were respectively applied to the treated samples at the time of harvest (500 mL per branch). To avoid contamination during spraying, at least one guard tree was used to separate each of the tested trees, and the trees were sprayed with the solutions only when there was a weak or no wind.

Olives (300 fruits) were collected on two different days of harvesting, days 3 and 6 after treatment, from each tree for each cultivar. A total of 1500 fruits were collected for each treatment and time point of each cultivar. Only undamaged fruits without any kind of infection of physical injury were selected for the experiments. From a visual point of view, all fruits exhibited apparently the same maturity stage. After harvesting, untreated and treated olive fruits were immediately kept in cool bags for a couple of days up to the analysis.

2.3 Analysis of Phenolic Compounds

2.3.1 Extraction

Isolation of the phenolic compounds in the untreated as well as ABA50 and ABA100 treated olive fruits was carried out by following the method described by Vinha et al. (2005). Prior to the extraction, all olive fruits were

de-stoned. The stone was discarded and just the pulps were used for the analyses. Subsequently, a 60 mL-volume of 80:20 (v/v) methanol:water was added to a 5 g-weight of sample, the mixture was homogenized by using an Ultraturrax (IKA, Sigma-Aldrich, Madrid, Spain) and centrifuged at 1500 rpm for 10 min at room temperature. Then, the supernatant was filtered through filter paper and an additional 60 mL of methanol:water was added to the extract, which was re-extracted. After that, 30 mL of hexane was added to the resulting extract to eliminate the remaining oil. Once discharged the hexane layer, the combined methanolic extracts were collected, filtered through Whatman No. 1 filter paper and analyzed by HPLC. All extractions including controls and treated with ABA50 and ABA100 were accomplished in duplicate. As explained below, the extracts obtained were used to determine TPC, the free radical scavenging activity and contents of oleuropein, hydroxytyrosol and phenolic acids.

2.3.2 Determination of TPC

TPC measurements were performed by using a Beckman Coulter DU-800 spectrophotometer (Barcelona, Spain) and following the method described by Singleton & Rossi (1965). Basically, the method is based on the oxidation of the hydroxyl groups of phenols in basic media by the Folin-Ciocalteu reagent. A 0.1-mL volume of the extract, 0.5 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate solution (75 g/L) were mixed. The final mixture was made up to 25 mL with distilled water. Then, the absorbance was measured at 750 nm after 1 h against a blank, which was prepared equally but without adding the reagent. A calibration curve was prepared by using gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents per kg of olive fruit. Analyses were performed in triplicate.

2.3.3 1,1-Diphenyl-2-Picrylhydrazyl Free Radical (DPPH*) Scavenging Assay

The ability of the extracts to scavenge DPPH* radicals was performed according to a slight modification of the method developed by Smith, Reeves, Dage, and Schnettler (1987). Each extract was further diluted to final concentrations of 15.6, 62.5, 125, 250 and 500 µg/mL before being transferred to a 96-well microtiter plate. Each extract solution, before adding DPPH, was used as a blank. Each well contained 50 µL aliquot of the sample and 150 µL of DPPH (400 µM). Decrease of absorbance, with respect to DPPH solution measured immediately, was monitored at 517 nm after 30 min of incubation at 37 °C. The percentage inhibition of the DPPH by each dilution of samples was calculated considering the percentage of the steady DPPH in solution after reaction. Results were expressed as IC_{50}, which is defined as the concentration of extracts where the response is reduced by half (50% reduction in the DPPH). To determine IC_{50}, we calculated the concentration of extract corresponding to half DPPH in the linear regression. The experiments were performed in triplicate.

2.3.4 Determination of Oleuropein and Hydroxytyrosol

To analyze oleuropein and hydroxytyrosol, a Konik-Tech model 560 (Barcelona, Spain) liquid chromatograph fitted with a manual injection valve (model 7725i, Konik-Tech, Barcelona, Spain) and having a 20-µl sample loop was used. The separation was accomplished on a ODS reverse phase (C18) column (250 nm × 4.6 mm i.d., 5-µm particle size, ACE, Madrid, Spain). A mixture of water/acetic acid (95/5, v/v) and methanol were used as solvents A and B, respectively and the flow rate was 1 mL/min. A linear gradient was programmed as follows: initial composition 95/5% A/B, 85/15 A/B at 3 min, 80/20 A/B at 13 min, 75/25 A/B at 25 min, 70/30 A/B at 35 min, 65/35 A/B at 40 min, 60/40 A/B at 45 min, 55/45 A/B at 47 min, 53/47 A/B at 50 min, 52/48 A/B at 60 min, 50/50 A/B at 64 min, 50/50 A/B at 70 min, 95/5 A/B at 75 min. Chromatograms were recorded at 280 nm. Blanks between consecutive runs were performed to assure the washing of the equipment. Three HPLC runs were performed for each single extract. Stock solutions of the standard compounds were prepared in 70% (v/v) methanol to final concentration of 1 mg/mL. Each stock solution was further diluted to obtain six concentrations of the standard. Calibration curves of the standards were established on six data points, and each standard dilution was injected in triplicate. Peak areas for the extracts and standards were integrated by use of Konikrom Plus (KNK-725-240). Analyses were performed in triplicate.

2.3.5 Determination of Phenolic Acids

For phenolic acids, we used the same equipment and method as that described above for oleuropein and hydroxytyrosol. Chromatograms were recorded at two different wavelengths. Gallic acid and vanillic acid were detected at 280 nm whereas caffeic, *p*-coumaric, ferulic and chlorogenic acids were measured at 320 nm. To guarantee the washing of the chromatograph, blanks were performed between consecutive runs. Three HPLC runs were performed for each single extract. Stock solutions of the standard compounds were prepared in 70% (v/v) methanol to final concentration of 1 mg/mL. Each stock solution was further diluted to obtain six concentrations of the standard. Calibration curves of the standards were established on six data points, and each

standard dilution was injected in triplicate. Peak areas for the extracts and standards were integrated by use of Konikrom Plus (KNK-725-240). Analyses were performed in triplicate.

2.4 Statistical Analysis

The results are presented as the average of the all values obtained and standard deviation (± SD). The two varieties *Arbequina* and *Picual* are included in the statistical analysis. The data were analyzed using one-way analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$.

3. Results and Discussion

Statistical comparison was made between controls and treated and between *Arbequina* and *Picual*. Days of harvesting 3 and 6 were not statistically compared.

TPC varied in a wide range, from 114.91 to 657.12 mg gallic acid kg^{-1} (Table 1). These values are in the same interval as those previously reported for olives in the literature (Arslan & Özcan, 2011). By comparing controls from the two cultivars, *Picual* exhibited significantly ($p < 0.05$) higher TPC than *Arbequina*. Specifically, 479.66 mg gallic acid kg^{-1} *vs* 114.91 mg gallic acid kg^{-1} on day 3 and 465.81 mg gallic acid kg^{-1} *vs* 194.47 mg gallic acid kg^{-1} on day 6. This reflects that varietal differences in TPC in olive fruits were established, which is in agreement with Vacca, Fenu, Franco, and Sferlazzo (1993). It is also worth mentioning TPC was unaffected by the day of harvesting.

Table 1. Total phenol content (mg gallic acid kg^{-1}) in olive fruits from olive trees (*Olea europaea* L.) untreated-control and treated with ABA50. Olive samples were picked on days 3 and 6 after treatment. Data from two varieties (*Arbequina* and *Picual*) are included

Total Phenols (mg gallic acid kg^{-1})	*Arbequina*	*Picual*
Day 3		
Control	114.91Aa	479.66Ba
ABA50 Treated	149.75Aa	657.12Bb
Day 6		
Control	194.47Aa	465.81Ba
ABA50 Treated	263.82Ab	276.32Ab

Note. Data are presented as means ($n = 3$), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same column between control and ABA50 treated samples within the same cultivar indicate differences at $p < 0.05$.

Concerning the treatment effect, ABA50 tresulted in significantly ($p < 0.05$) higher TPC in *Arbequina* olives on day 6 (from 194.47 to 263.82 mg gallic acid kg^{-1}) and in *Picual* olives on day 3 (from 479.66 to 657.12 mg gallic acid kg^{-1}) than those in the corresponding controls. Surprisingly for *Picual*, the opposite effect was observed when olives were picked on day 6 after treatment. As seen, TPC decreased significantly ($p < 0.05$) from 465.81 in controls to 276.32 mg gallic acid kg^{-1} after ABA50 treatment. TPC has been described to decrease during the ripening process by Ryan and Robards (1998). Therefore, ABA50 treatment might be accelerating the ripening process in such a way that *Arbequina* olives picked on day 6 after treatment were close to the commercial ripening stage whereas *Picual* olives were closer to overripe.

It is important to remember that ABA is a stress plant hormone which is rapidly accumulated in response to stress and that can balance the plant endogenous hormones (Nakashima and Yamaguchi-Shinozaki, 2013). On the other hand, ABA has been demonstrated to be particularly effective in the accumulation of secondary metabolites (Cantín, Fidelibus, & Crisosto, 2007; Cui, Liang, Y. Liu, F. Liu, & Zhu, 2012). Taking this into account, it is believed that ABA might be affecting directly or indirectly the enzymes involved in the biosynthesis of phenolic compounds in olives through the phenylpropanoid pathway. In fact, exogenous ABA50 would be activing the enzymes regulating phenolics and, this way, accelerating the ripening process. The distinct response of *Arbequina* and *Picual* olives to ABA50 agrees with the differences previously observed in both cultivars. *Picual* olives appear to ripen quickly than those of *Arbequina*. In fact, although both cultivars were harvested at the same time, *Picual* olives exhibited a slightly more advanced maturity stage. Therefore, when *Picual* olives are pre-harvest treated with ABA50, they should not be harvested later than 3 days after treatment to guarantee their quality.

IC_{50}, expressed as µg/ml, in control olives were statistically different ($p < 0.05$) between cultivars when measured on day 3 (Table 2). In particular, values of 814.88 and 632.69 µg/mL were obtained for *Arbequina* and *Picual*, respectively. However, no statistical differences between cultivars ($p > 0.05$) was found when the measurements were made on day 6 (845.68 µg/mL *vs* 889.19 µg/mL). Concerning the ABA50 effect, the IC_{50} values were significantly ($p < 0.05$) lower in treated samples than in controls for both cultivars. Actually, in most cases the free radical scavenging activity decreased up to half after treatment.

Table 2. DPPH scavenging activity expressed as IC_{50} (µg/ml) of olive fruits from *Arbequina* and *Picual* olive trees (*Olea europaea* L.) treated with ABA50. Olive samples were picked on days 3 and 6 after treatment. Data from two varieties (*Arbequina* and *Picual*) are included

IC50 (µg/mL)	*Arbequina*	*Picual*
Day 3		
Control	814.88Aa	632.69Ba
ABA50 Treated	451.81Ab	570.57Ba
Day 6		
Control	845.68Aa	889.19Aa
ABA50 Treated	392.21Ab	339.94Ab

Note. Data are presented as means ($n = 3$), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same column between control and ABA50 treated samples within the same cultivar indicate differences at $p < 0.05$.

It is interesting to point out that no correlation between TPC and the free radical scavenging activity was established. In this regard, *Picual* olives exhibited higher TPC than those of *Arbequina* whereas this trend was not observed for DPPH activity. The disagreement between TPC and DPPH activity suggests the exclusive presence of certain phenolics in *Arbequina* whose concentration might be sufficiently low not to contribute to TPC but with potent free radical scavenging activity. It is noteworthy to keep in mind that the biological activity of any chemical is not directly related to its concentration. Actually, some minor constituents are usually described as particularly active compounds (Braicu, Pilecki, Balacescu, Irimie, & Berindan Neagoe, 2011).

TPC values varied from 265.61 mg gallic acid kg^{-1} in *Arbequina* to 324.75 mg gallic acid kg^{-1} in *Picual* on day 3 and from 155.83 mg gallic acid kg^{-1} in *Arbequina* to 338.22 mg gallic acid kg^{-1} in *Picual* on day 6 (Table 3). As also previously observed (see Table 1), differences in TPC with the day of harvesting were not determined either. As far as the ABA100 treatment is concerned, significant ($p < 0.05$) increases in TPC were observed in both *Arbequina* and *Picual* with exception of *Picual* olives picked on day 3, which exerted similar TPC in controls and treated.

Table 3. Total phenol content (mg gallic acid kg^{-1}) in olive fruits from olive trees (*Olea europaea L.*) untreated-control and treated with ABA100. Olive samples were picked on days 3 and 6 after treatment. Data from two varieties (*Arbequina* and *Picual*) are included.

Total Phenols (mg gallic acid kg^{-1})	*Arbequina*	*Picual*
Day 3		
Control	265.61Aa	324.75Ba
ABA100 Treated	430.53Ab	380.12Ba
Day 6		
Control	155.83Aa	338.22Ba
ABA100 Treated	434.62Ab	727.75Bb

Note. Data are presented as means ($n = 3$), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same column between control and ABA100 treated samples within the same cultivar indicate differences at $p < 0.05$.

By comparing the TPC results obtained from the exposition of olive trees to ABA100 (see Table 3) with those provided by the exposition to ABA50 (see Table 1), ABA100 treatment was clearly more effective. On the one hand, the TPC increments in treated samples as compared with controls were considerably higher and, on the other hand, the maximum TPC values were higher. In particular, for *Arbequina*, TPC reached values of 434.62 mg gallic acid kg^{-1} in ABA100 treated samples on day 6 *vs* 263.82 mg gallic acid kg^{-1} measured from olives treated with ABA50. In the same way for *Picual*, TPC reached values of 727.75 mg gallic acid kg^{-1} in ABA100 treated olives picked on day 6 *vs* 657.12 mg gallic acid kg^{-1} in ABA100 treated olives picked on day 3.

IC_{50}, expressed as µg/ml, in controls were statistically different ($p < 0.05$) between cultivars on both days of harvesting, although the difference was more pronounced on day 3 (Table 4). Regarding the ABA100 effect, the treatment appeared to be successful, particularly on day of harvesting 3 for *Arbequina* and on day 6 for *Picual*. Specifically, the IC_{50} values increased from 271.43 µg/ml in controls to 501.16 µg/ml in treated for *Arbequina* on day 3 and from 445.47 µg/ml in controls to 757.72 µg/ml in treated for *Picual* on day 6.

Table 4. DPPH scavenging activity expressed as IC_{50} (µg/ml) of olive fruits from *Arbequina* and *Picual* olive trees (*Olea europaea* L.) treated with ABA100. Olive samples were picked on days 3 and 6 after treatment. Data from two varieties (*Arbequina* and *Picual*) are included

IC50 (µg/mL)	*Arbequina*	*Picual*
Day 3		
Control	271.43Aa	870.50Ba
ABA100 Treated	501.16Ab	889.72Ba
Day 6		
Control	533.80Aa	445.47Ba
ABA100 Treated	508.24Aa	757.72Bb

Note. Data are presented as means ($n = 3$), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same column between control and ABA100 treated samples within the same cultivar indicate differences at $p < 0.05$.

A comparison between ABA50 treatment (Table 2) with ABA100 (Table 4) in terms of the DPPH activity reflected higher effectiveness of ABA100 than ABA50. Whereas olives treated with ABA50 exerted lower free radical scavenging activity after treatment, the exposition to ABA100 resulted in significantly ($p < 0.05$) higher DPPH activity, particularly on day 3 for *Arbequina* and on day 6 for *Picual.* It is also worth highlighting that, in the same way as ABA50 treatment, a direct relation between TPC and the DPPH activity was not established. This supports the theory of the presence of specific phenolics whose concentration is not directly associated with their free radical scavenging activity.

In view of the results obtained from the ABA50 and ABA100 treatments, the study of the effect of preharvest ABA on the contents of oleuropein, hydroxytyrosol and phenolic acids was carried out by using exclusively olive fruits treated con ABA100.

Varietal differences in the oleuropein content were also clear (Table 5). As seen, the comparison between *Arbequina* and *Picual* control samples showed statistical differences ($p < 0.05$) between cultivars on both days of harvesting. It was observed that *Arbequina* exhibited significantly ($p < 0.05$) higher oleuropein content than *Picual* on day 3 (325.87 mg kg^{-1} *vs* 141.50 mg kg^{-1}) whereas the contrary was observed on day 6 (131.58 mg kg^{-1} in *Arbequina vs* 179.94 mg kg^{-1} in *Picual*). Controversial information concerning the evolution of oleuropein during ripening have been decribed in the literature. Some authors have fournd decreases in oleuropein concentrations during fruit maturitry (Ortega-García, Blanco, Peinado, & Peragón, 2008) whereas others have reported complete degradation of oleuropein during ripening of olives and, therefore, its almost undetectable presence when the fruit darkens (Soler-Rivas, Epsin, & Wichers, 2000). The results here found showed decrease of oleuroein in *Arbequina* olives whereas a slight increase in *Picual*. This supports the previously observation on the different ripening behavior of both cultivars.

Table 5. Oleuropein contents (expressed as mg kg^{-1} weight) in olive fruits from olive trees (*Olea europaea* L.) treated with ABA100. Data from olive samples picked on days 3 and 6 after ABA100 application and from two varieties (*Arbequina* and *Picual*) are included

Oleuropein Content (mg kg$^{-1)}$	*Arbequina*		*Picual*	
	Control	ABA100 Treated	Control	ABA100 Treated
Day 3	325.87Aa	232.12Aa	141.50Ba	323.62Bb
Day 6	131.58Aa	508.94Ab	179.94Ba	147.47Ba

Note. Data are presented as means (n = 3), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same row between control and ABA100 treated samples within the same cultivar indicate differences at $p < 0.05$.

The oleuropein content in controls on days 3 and 6 correlated well with the values obtained for TPC (see Table 3). In this regard, both the oleuropein content and TPC in *Arbequina* controls decreased from day 3 to day 6 whereas both parameters ranged within the same interval from day 3 to day 6 for *Picual* controls. This is somehow expected since oleuropein is one of the major phenolics in olives and, therefore, it contributes greatly to TPC.

The exposition of *Arbequina* and *Picual* trees to ABA100 resulted in a significant ($p < 0.05$) increase in the oleuropein content, although this increase was mostly measured on day 6 for *Arbequina* (from 131.58 mg kg^{-1} to 508.94 mg kg^{-1}) and on day 3 for *Picual* (from 141.50 mg kg^{-1} to 323.62 mg kg^{-1}). The effectiveness of the ABA100 treatment is most likely due to the activation of polyphenol oxidase (PPO) which is a specific enzyme in the phenylpropananoid pathway regulating the formation of oleuropein (Ortega-García, Blanco, Peinado, & Peragón, 2008). For *Arbequina*, the activation was more effective when olives were picked on the day 6 after the treatment whereas for *Picual* PPO was more activated in only 3 days after ABA100 treatment.

Similarly to oleuropein, varietal differences in hydroxytyrosol contents in olive controls were also found (Table 6). Specifically, the hydroxytyrosol content of 166.68 mg kg^{-1} was determined for *Arbequina* on day 3 whereas it could not be detected in *Picual* on the same day. Something similar happened on day 6, *Arbequina* control olives exerted hydroxytyrosol content of 125.13 mg kg^{-1} while 556.70 mg kg^{-1} was measured for *Picual*.

Table 6. Hydroxytyrosol contents (expressed as mg kg^{-1} weight) in olive fruits from olive trees (*Olea europaea* L.) treated with ABA100. Data from olive samples picked on days 3 and 6 after ABA100 application and from two varieties (*Arbequina* and *Picual*) are included

Hydroxytyrosol Content (mg kg^{-1})	*Arbequina*		*Picual*	
	Control	ABA100 Treated	Control	ABA100 Treated
Day 3	166.68Aa	163.68Aa	n.d.Ba	179.17Ab
Day 6	125.13Aa	559.67Ab	556.70Ba	126.88Bb

Note. Data are presented as means (n = 3), where n refers to three independent samples. Different upper-case letters in the same row in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same row between control and ABA100 treated samples within the same cultivar indicate differences at $p < 0.05$.

Concerning the ABA100 effect, the treatment resulted in significantly ($p > 0.05$) higher hydroxytyrosol content on day 6 for *Arbequina* (from 125.13 mg kg^{-1} to 559.67 mg kg^{-1}) and on day 3 for *Picual* (from not being detected to 179.17 mg kg^{-1}). It is necessary to bear in mind that hydroxytyrosol is a product derived from hydrolysis of oleuropein (Granados-Principal, Quiles, Ramirez-Tortosa, Sanchez-Rovira, & Ramirez-Tortosa, 2010). For this reason, both oleuropein and hydroxytyrosol exhibit similar evolution during the ripening process and similar response to ABA.

On the basis of our previous experience (data submitted for publication), the metabolism of olive phenolic acids is not affected by the day of harvesting (Table 7). For this reason, only olives picked on day 3 after the treatment were studied. As observed in Table 7 the contents of phenolic acids varied from 0.07 mg kg^{-1} for *p*-coumaric acid to 92.38 mg kg^{-1} for chlorogenic acid. As also seen, *Picual* exhibited significantly higher ($p < 0.05$) contents than *Arbequina*, confirming the knwon varietal differences in phenolics described in plant-derived foods.

Table 7. Phenolic acid contents (expressed as mg kg^{-1} weight) in olive fruits from *Arbequina* and *Picual* olive trees (*Olea europaea* L.) treated ABA100 and picked on day 3 after ABA100 application

Samples	Gallic Acid	Chlorogenic Acid	Vanillic Acid	Caffeic Acid	*p*-Coumaric Acid	Ferulic Acid
Arbequina						
Control	4.53Aa	0.75Aa	1.25Aa	0.15Aa	0.11Aa	0.27Aa
ABA100 Treated	3.57Aa	0.63Aa	0.99Aa	0.18Aa	0.07Aa	0.37Ab
Picual						
Control	15.42Ba	33.25Ba	43.41Ba	2.32Ba	27.43Ba	28.34Ba
ABA100 Treated	43.72Bb	92.38Bb	52.39Ba	7.26Bb	82.15Bb	40.52Bb

Note. Data are presented as means ($n = 3$), where n refers to three independent samples. Different upper-case letters in the same column in control samples between cultivars indicate differences at $p < 0.05$. Different lower-case letters in the same column between control and ABA100 treated samples within the same cultivar indicate differences at $p < 0.05$.

As far as the treatment is concerned, varietal differences in the ABA100 effect were found. As seen, the phenolic acids in *Arbequina* olives were not significantly ($p > 0.05$) affected by the treatment whereas in *Picual* olives a significant ($p < 0.05$) increase of phenolic acid content was mostly measured. Previous results about the effect of preharvest chemicial elicitation by using methyl jasmonate as an elicitor have demonstrated drop in the phenolic acid content in treated *Arbequina* olives and no effect in treated *Picual* olives (data submitted for publication). By comparing both elicitors, ABA and methyl jasmonate, the results here presented indicate that preharvest ABA100 applied to the olive tree was more effective than methyl jasmonate in terms of phenolic acids. This is possibly due to the higher influence of ABA than that of methyl jasmonate on the activity of specific enzymes regulating the biosynthesis of olive phenolic acids in the phenylpropanoid pathway. In particular, some of these enzymes might be those belonging PPOs, such as phenolasa and catechol-O-methyl transferasa, involved in the bioformation of caffeic acid and ferulic acid (Piquemal et al., 2002; Yoruk & Marshall, 2003).

In conclusion, the exogenous ABA application to olive tree can be a powerful strategy to enhance antioxidant properties when used in the adequate conditions. In this sense, ABA50 would not be recommendable since the DPPH activity of olive fruits decreased after the treatment. On the contrary, ABA100 applied to the olive tree resulted to be a succesfull procedure to obtaine enriched olive fruits. In general terms, TPC, the free radical scavenging activity and the contents of oleuropein, hydroxytyrosol and phenolic acids in olive fruits increased after the pre-harvest treatment of the olive trees with ABA100. In particular, the specific effects of the pre-harvest ABA100 treatment on olives depend on the cultivar and the day of harvesting considered. Therefore, a specific study including specific conditions of each case is necessary before ABA100 treatment to be applied to the olive tree.

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References

Andreadou, I., Illiodromitis, E. K., Mikros, E., Constantinou, M., Agalias, A., Magiatis, P., … Kremastinos, D. T. (2006). The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *Journal of Nutrition, 136*, 2213-2219. https://doi.org/10.1093/jn/136.8.2213

Arslan, D., & Özcan, M. M. (2011). Phenolic profile and antioxidant activity of olive fruits of the Turkish variety "Sarrulak" from different locations. *Grasas y Aceites, 62*, 453-461. https://doi.org/10.3989/gya.034311

Benavente-García, O., Castillo, J., Lorente, J., Ortuno, A., & Del Río, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* leaves. *Food Chemistry, 68*, 457-462. https://doi.org/10.1016/S0308-8146(99)00221-6

Blanch, G. P., Flores, G., Gómez-Jiménez, M. L., & Ruiz del Castillo, M. L. (2018). Effect of the treatment of the olive tree (*Olea europaea* L.) on the phenolic content and antioxidant properties in olive fruits. *Journal of Food Nutrition Research, 6*, 49-55. https://doi.org/10.12691/jfnr-6-1-8

Braicu, C., Pilecki, V., Balacescu, O., Irimie, A., & Berindan Neagoe, I. (2011). The relationships between biological activities and structure of flavan-3-ols. *Intermolecular Journal of Molecular Sciences, 12*, 9342-9353. https://doi.org/10.3390/ijms12129342

Cantín, C. M., Fidelibus, M. W., & Crisosto, C. H. (2007). Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of "Crimson Seedless" grapes. *Postharvest Biology & Technology, 46*, 237-241. https://doi.org/10.1016/j.postharvbio.2007.05.017

Cui, B., Liang, Z., Liu, Y., Liu, F., & Zhu, J. (2012). Effects of ABA and its biosynthetic inhibitor fluridone on accumulation of phenolic acids and activity of PAL and TAT in hairy roots of Salvia miltiorrhiza. *ZhongguoZhong Yao ZaZhi, 37*, 754-759.

de la Peña Moreno, F., Monagas, M., Blanch, G. P., Bartolomé, B., & Ruiz del Castillo, M. L. (2010). Enhancement of anthocyanins and selected aroma compounds in strawberry fruits through methyl jasmonate vapor treatment. *European Food Research and Technology, 230*, 989-999. https://doi.org/10.1007/s00217-010-1243-5

Flores, G., & Ruiz del Castillo, M. L. (2014). Influence of pre-harvest and post-harvest methyl jasmonate treatments on flavonoid content and metabolomic enzymes in red raspberry. *Postharvest Biology & Technology, 97*, 77-82. https://doi.org/10.1016/j.postharvbio.2014.06.009

Flores, G., & Ruiz del Castillo, M. L. (2016). Accumulation of anthocyanins and flavonols in black currants (*Ribes nigrum* L.) by pre-harvest methyl jasmonate treatments. *J. Sci. Food Agric., 96*, 4026-4031. https://doi.org/10.1002/jsfa.7595

Flores, G., Blanch, G. P., & Ruiz del Castillo, M. L. (2017). Effect of postharvest methyl jasmonate treatment on fatty acid composition and phenolic acid content in olive fruits during storage. *Journal of the Science of Food and Agriculture, 97*, 2767-2772. https://doi.org/10.1002/jsfa.8104

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C. L., Sanchez-Rovira, P., & Ramirez-Tortosa, M. C. (2010). Hydroxytyrosol: From laboratory investigations to future clinical trials. *Nutrition Reviews, 68*, 191-206. https://doi.org/10.1111/j.1753-4887.2010.00278.x

Huang, Y., Cai, S., Ye, L., Hu, H., Li, C., & Zhang, C. (2016). The effects of GA and ABA treatments on metabolic profile of germinating barley. *Food Chemistry, 192*, 928-933. https://doi.org/10.1016/j.foodchem.2015.07.090

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., & Sayadi, S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from ChemLali olive leaves. *Chemico-Biological Interactions, 176*, 88-98. https://doi.org/10.1016/j.cbi.2008.08.014

Johnston, K. L., Clifford, M. N., & Morgan, L. M. (2003). Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: Glycemic effects of chlorogenic acid and caffeine. *The American Journal of Clinical Nutrition, 78*, 728-733. https://doi.org/10.1093/ajcn/78.4.728

Kiritsakis, A. (1998). *Olive oil: From the tree to the table* (2nd ed.). Food and Nutrition Press, Inc., Trumbull, Connecticut, USA.

Micol, V., Caturla, N., Pérez-Fons, L., Mas, V., Pérez, L., & Estepa, A. (2005). The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). *Antiviral Research, 66*, 129-136. https://doi.org/10.1016/j.antiviral.2005.02.005

Morton, I. W., Caccetta, R. A. A., Puddey, I. B., & Croft, K. D. (2000). Chemistry and biological effects of dietary phenolic compounds: Relevance to disease. *Clinical and Experimental Pharmacology and Physiology, 27*,152-159. https://doi.org/10.1046/j.1440-1681.2000.03214.x

Nakashima, K., & Yamaguchi-Shinozaki, K. (2013). ABA signaling in stress-response and seed development. *Plant Cell Reports, 32*, 959-970. https://doi.org/10.1007/s00299-013-1418-1

Ortega-García, F., Blanco, S., Peinado, M. A., & Peragón, J. (2008). Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea* L.) cv "*Picual*" trees during fruit ripening. *Tree Physiology, 28*, 45-54. https://doi.org/10.1093/treephys/28.1.45

Pereira, A. P., Ferreira, I. C. F. R., Marcelino, F., Valentao, P., Andrade, P. B., Seabra, R., … Pereira, J. A. (2007). Phenolic compound and antimicrobial activity of olive (*Olea europaea* L. *cv Cobrancosa*) leaves. *Molecules, 12*, 1153-1162. https://doi.org/10.3390/12051153

Piquemal, J., Chamayou, S., Nadaud, I., Beckert, M., Barriére, Y., Mila, I., … Pichon, M. (2002). Down-regulation of caffeic acid O-methyltransferase in maize revisited using a transgenic approach. *Plant Physiology, 130*, 1675-1685. https://doi.org/10.1104/pp.012237

Ruiz del Castillo, M. L., Flores, G., & Blanch, G. P. (2010). Exogenous methyl jasmonate diminishes the formation of lipid-derived compounds in boiled potatoes (*Solanum tuberosum* L.). *Journal of the Science of Food and Agriculture, 90*, 2263-2267. https://doi.org/10.1002/jsfa.4080

Ryan, D., & Robards, K. (1998). Phenolic compounds in olives. *Analyst, 123*, 31R-44R. https://doi.org/10.1039/a708920a

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Smith, R. C., Reeves, J. C., Dage, R. C., & Schnettler, R. A. (1987). Antioxidant properties of 2-imidazolones and 2-imidazolthiones. *Biochemical Pharmacology, 36*, 1457-1460. https://doi.org/10.1016/0006-2952(87)90110-9

Soler-Rivas, C., Epsin, J. C., & Wichers, H. J. (2000). Oleuropein and related compounds. *Journal of the Science of Food and Agriculture, 80*, 1013-1023. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7%3C1013::AID-JSFA571%3E3.0.CO;2-C

Teissedre, P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H., & German, J. B. (1996). Inhibition of i*n vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture, 70*, 55-61. https://doi.org/10.1002/(SICI)1097-0010(199601)70:1%3C55::AID-JSFA471%3E3.0.CO;2-X

Vacca, V., Fenu, P., Franco, M. A., & Sferlazzo, G. (1993). Primo approccio alla caratterizzazione dei composti fenolici di oli vergini di oliva della Sardegna: Elaborazioni statistiche multivariate dei risultati in HPLC. *Rivista Italian Dell Sostanze Grasse, 70*, 595-599.

Vinha, A. F., Ferreres, F., Silva, B. M., Valentao, P., Gongalves, A., Pereira, J. A., … Andrade, P. B. (2005). Phenolic profiles of Portuguese olive fruits (*Olea europea* L.): Influence of cultivar and geographical origin. *Food Chemistry, 89*, 561-568. https://doi.org/10.1016/j.foodchem.2004.03.012

Visioli, F., & Galli, C. (1995). Natural antioxidants and prevention of coronary heart disease: The potential role of olive oil and its minor constituents. *Nutrition, Metabolism, Cardiovasccular Disease, 5*, 306-314.

Visioli, F., Bellosta, S., & Galli, C. (1998). Oleuropein, the bitter principles of olives, enhances nitric oxide production by mouse macrophages. *Life Science, 62*, 541-546. https://doi.org/10.1016/S0024-3205(97)01150-8

Visioli, F., Poli, A., & Galli, C. (2002). Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews, 22*, 65-75. https://doi.org/10.1002/med.1028

Yang, D., Ma, P., Liang, X., Wei, Z., Liang, Z, Liu, Y., & Liu, F. (2012). PEG and ABA trigger methyl jasmonate accumulation to induce the MEP pathway and increase tanshinone production in Salvia miltiorrhiza hairy roots. *Physiologia Plantarum, 146*, 173-183. https://doi.org/10.1111/j.1399-3054.2012.01603.x

Yoruk, R., & Marshall, M. R. (2003). Physicochemical properties and function of plant polyphenol oxidase: A review. *Journal of Food Biochemistry, 27*, 361-422. https://doi.org/10.1111/j.1745-4514.2003.tb00289.x

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