Agriculture and Food

AIMS Agriculture and Food, 7(2): 250–264.
DOI: 10.3934/agrfood.2022016
Received: 15 October 2021
Revised: 22 March 2022
Accepted: 26 April 2022
Published: 05 May 2022

http://www.aimspress.com/journal/agriculture

Research article

Effect of cultivar and drying methods on phenolic compounds and antioxidant capacity in olive (*Olea europaea* L.) leaves

Itxaso Filgueira-Garro[1,2,*], Carolina González-Ferrero[3], Diego Mendiola[1] and María R. Marín-Arroyo[2]

[1] Department of Research and Development, URZANTE, 31500 Tudela, Spain
[2] Institute on Innovation and Sustainable Development in Food Chain (IS-FOOD), Public University of Navarre (UPNA), Campus Arrosadía, 31006 Pamplona, Spain
[3] National Centre for Food Technology and Safety, CNTA, NA 134, Km. 53, 31570, San Adrián, Spain

* **Correspondence:** Email: idi2@urzante.com; Tel: +34948850237; Fax: +34948851456.

Abstract: Up to 5% of the total olive weight arriving at the mill is discarded as leaves. Interest in the possible uses of these residues is growing, because they constitute a potential cheap and abundant source of compounds with high total antioxidant capacity (TAC) associated with total phenolic content (TPC) and biophenols such as hydroxytyrosol (HC) and oleuropein (OC), which could be used as nutraceuticals or as natural substitutes for synthetic antioxidants. However, studies that characterize specific cultivars, interannual variability, and different drying methods are lacking. This work investigates the TAC, TPC, HC and OC in olive (*Olea europaea* L.) leaves under four drying methods (vacuum-drying, oven-drying, freeze-drying and air-drying). Leaves were collected from cultivars 'Arbequina' grown under organic methods and from 'Arroniz', 'Empeltre', 'Arbosana', 'Picual' and 'Arbequina' grown under conventional systems. Among fresh samples, 'Arbosana' leaves presented the highest TPC (34.0 ± 1.1 mg gallic acid equivalents/g dry weight (DW)) and TAC (146 ± 20 μmol Trolox equivalents/g DW) and the lowest interannual variability of the TPC (3.2%). The four tested drying methods were also compared as the effect on TPC, TAC, HC and OC. Freeze-drying and air-drying best preserved TPC and TAC in olive leaves. However, air-drying maintained greater OC (14–40 mg/g DW) than freeze-drying (3–20 mg/g DW). Air-dried ecological 'Arbequina' leaves exhibited the highest TPC and TAC. Consequently, this cultivar presented more valorization opportunities as a source of nutraceuticals or natural antioxidants.

Keywords: olive leaves; drying methods; oleuropein; hydroxytyrosol; ‘Arroniz’; ‘Empeltre’

1. Introduction

Annually the olive oil industry produces more than 3 million tons of oil worldwide. The main production area is located in the Mediterranean countries of Spain, Italy, Greece, Tunisia and Turkey [1]. Olive leaves constitute as much as 5% of the total olive (*Olea europaea* L.) weight that enters an olive mill. It is discarded along with another 75% as olive pomace; only the 20% is extracted to produce olive oil [2]. Consequently, the olive oil industry generates large amounts of wastes. Olive leaves, which are separated from the olives before olive oil production, present a high concentration of phenolic compounds among which hydroxytyrosol and oleuropein stand out [3]. Extracts of these compounds could be used in the food industry as preservatives, due to their antioxidant and antimicrobial properties [4]. Consequently, the extraction of phenolic compounds from olive leaves could be a way to add value to this residue; thus, presenting the oil industry with another option for greater sustainability in their processing activities.

Phenol content and antioxidant capacity of olive leaves depend on cultivar type, olive tree age, cultivation area [5] and sampling time [6–8]. Cultivar effect on phenol content and antioxidant capacity has been previously studied [9–11] in ‘Arbequina’ and ‘Picual’ leaves [12–14]. However, there is no scientific data characterizing ‘Arroniz’ nor ‘Empeltre’ leaves and only, scarce information is available about ‘Arbosana’ leaves [5]. Few studies have been published about the interannual variability [15]; none of which contain Spanish cultivars.

Polyphenol oxidases (PPO) are enzymes that cause the degradation of phenol compounds in olive leaves, specifically, oleuropein [16]. To prevent PPO activity, the water content of olive leaves should be decreased. The most studied method for the preservation of phenolic compounds from olive leaves is dehydration. Traditionally, olive leaves have been air-dried, which is the easiest and most economic drying method. However, air-drying is more time-consuming compared to oven and freeze-drying. Even so, several studies [17,18] have suggested air-drying to be effective for the preservation of phenolic compounds in olive leaves.

The effect of oven-drying is unclear; some studies [19,20] have presented polyphenol degradation while other investigations [18,21] concluded that drying olive leaves at high temperatures for a short time does not degrade their polyphenols. Vacuum-drying was studied to prevent the degradation of phenolic compounds; no differences were observed when compared to oven-drying [20,22]. Freeze-drying has proved to be effective in the preservation of olive leaf phenols [18,21]. Despite the quantity of literature that characterizes the phenols in olive leaves dried by the mentioned methods, comparison of them with fresh olive leaf phenol content has not been reported. Therefore, further studies assessing the effect of the drying methods on the phenolic compounds and antioxidant capacity of the olive leaf are required.

To valorize olive leaves by the extraction of their phenolic compounds, all the above-mentioned factors must be kept in mind to produce extracts with homogeneous characteristics. Olive cultivar, interannual variability and drying methods can influence the phenolic content and antioxidant capacity in olive leaves. Thus, the aim of this research was to characterize total antioxidant capacity, total phenol, hydroxytyrosol and oleuropein content in ‘Arroniz’, ‘Empeltre’, ‘Arbosana’, ‘Picual’, and ‘Arbequina’ olive leaves and to assess the effect of four drying methods on these parameters.

2. Materials and methods

2.1. Plant material

Fresh olive leaves were collected from the cleaning process of olives in an oil mill in Tudela (Navarre, Spain). Olive leaves (8 kg of each one) were sampled from five different cultivars ('Arroniz', 'Empeltre', 'Arbosana', 'Picual', conventional 'Arbequina' and ecological 'Arbequina') in two consecutive years during the local harvesting season in November. All the five orchards were cultivated under conventional methods using synthetic pesticides and fertilizers. Leaves collected from ecological 'Arbequina' grove were also used. To prevent influence of agricultural and environmental conditions, the leaves were taken from olive trees of the same age (ten years old) and from the same orchard "La Estanca" (42.015827° N, 1.705438° W, 368 m above sea level) located near Cascante (Navarra). Leaves were detached from branches, vacuum (95%) packaged and stored in refrigeration (4 ℃) and darkness until their analysis, which was carried out within the first five days of storage.

2.2. Reagents and standards

Oleuropein analytical standard (≥98%), Trolox (97%), Folin & Ciocalteu's phenol reagent (2 N) and gallic acid monohydrate (≥98%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Hydroxytyrosol analytical standard (≥98%) was acquired from Extrasynthèse (Genay, France) and 2,2-diphenyl-1-picrylhydrazyl, DPPH (95%), from Alfa Aesar–Thermo Fisher Scientific (Haverhill, Massachusetts, USA). Sodium carbonate anhydrous (100%), acetic acid (100%) and methanol (≥99.9%) were procured from VWR International Eurolab (Barcelona, Spain). Acetonitrile (≥99.9%) was acquired from Merck (Darmstadt, Germany). Type II and type I water were obtained from an Automatic Plus GR (Wasserlab, Barbatain, Spain) purification system.

2.3. Drying methods

The effect of four drying methods (vacuum-drying, oven-drying, freeze-drying and air-drying) on phenolic compounds and antioxidant capacity were compared in six olive leaf samples collected in the second year. Vacuum-drying was carried out in a Memmert VO 400 oven (DD Biolab, Barcelona, Spain) at 40 ℃ and 150 mbar of vacuum for four days. Oven-drying was conducted in the same oven, at 105 ℃, for 4.5 h, without vacuum. Olive leaf samples were freeze-dried in a Lyoalfa-6 laboratory freeze-dryer (Azbil Telstar, Barcelona, Spain) under vacuum (50 mbar) at −72 ℃ for three days. Air-drying was performed by spreading leaves onto a closed surface with natural air at 20–25 ℃ without direct sunlight for fourteen days.

2.4. Water content

Fresh and dried leaves (2 g) were ground with a Grindomix GM 200 (Retsch, Germany) and maintained at 105 ℃ until constant weight using a HR83 Halogen Moisture Analyzer (Mettler Toledo, Barcelona, Spain). The leaf water content was expressed as water loss (%) referred to the initial water content.

2.5. Characterization of olive leaves

2.5.1. Extraction process

Fresh and dried olive leaves were ground in Grindomix GM 200 at 10000 rpm for 2 min and passed through a 1 mm mesh sieve. The ground material (0.50 ± 0.01 g) was mixed with 4 mL of methanol/water 70:30 (v/v) in Genogrinder 2010 (Spex, Spain) at 800 rpm for 15 min, at room temperature (RT). The mixture was centrifuged at 4816 x g for 10 min at 20 ℃ (Sorvall ST40R, Thermo Scientific). The supernatant was removed and saved. The extraction process was repeated from the pellet. Finally, both supernatants were mixed and diluted to a final volume of 10 mL. The olive leaf extract was filtered through a 0.22 μm PVDF Whatman filter and stored for further analysis in refrigeration (4 ℃) and darkness.

2.5.2. Total phenol content

Total phenol content (TPC) of the olive leaf extract was analyzed by the Folin-Ciocalteu method as described by Obied et al. (2005) [23] with some modifications. Briefly, a gallic acid (0–750 mg/L) calibration curve (R2 0.9918–0.9999) was prepared from the standard solution of gallic acid in methanol (1 g/L). Each extract was appropriately diluted with methanol/water 70/30 (v/v). The diluted extract (0.2 mL) was mixed with 5 mL of 10% Folin-Ciocalteu reagent and 2.8 mL of deionized water. This mixture was vortexed, stored 5 min at RT, and added with 2 mL of sodium carbonate solution (20% w/v in deionized water). After an incubation time of 1.5 h, absorbance was measured at 750 nm in a V-530 UV/VIS Spectrophotometer (Jasco, Tokyo, Japan). Deionized water was used as the comparative blank. The results were expressed as mg gallic acid equivalents/g dry weight (mg GAE/g DW) of olive leaf. TPC was determined in fresh olive leaves collected in both years and in dried leaf in the second year. TPC interannual variability was calculated as the relative standard deviation (RSD) of the values of the two years.

2.5.3. Total antioxidant capacity

Total antioxidant capacity (TAC) of the olive leaves extract was assessed using DPPH radical scavenging activity method described by Brand-Williams et al. (1995) [24] with slight modifications. Briefly, to obtain the calibration curve (0–600 μmol/mL), R2 0.9879–0.9990, the standard solution was prepared with 0.03 g of Trolox dissolved in 50 mL of methanol. The DPPH solution was prepared dissolving a DPPH concentrated solution (0.12 g/L) in methanol (approximately 1/5 v/v) until absorbance at 515 nm was adjusted to 0.75 ± 0.05. In a cuvette, 1960 μL of the DPPH adjusted solution was mixed with 40 μL of olive leaf extract, covered with parafilm to prevent solvent evaporation and incubated 1 h at RT. Absorbance was measured at 515 nm against methanol as blank in a V-530 UV/VIS Spectrophotometer. The results were expressed as μmol Trolox equivalents/g leaf DW (μmol TE/g DW). TAC was measured in fresh olive leaves collected in both years and in dried leaves collected in the second year. TAC interannual variability was calculated as the relative standard deviation (RSD) of the values of the two years.

2.5.4. Hydroxytyrosol and oleuropein contents

Determinations of hydroxytyrosol (HC) and oleuropein (OC) contents in olive leaf extracts were performed according to the method described by Suárez et al. (2008) [25] with some modifications. The compounds were separated by HPLC with an e2695 Separation module coupled to a 2998 Photodiode Array Detector (PAD) controlled by Empower software (Waters Alliance, USA) together with an Atlantis dC18 (4.6 x 100 mm, 3 µm) reverse-phased column (Waters Alliance, USA).

Injection volume was 20 µL and the flow rate was 0.8 mL/min. The mobile phase was a gradient of 0.2% acetic acid aqueous solution (A) and 50/50 (v/v) methanol/acetonitrile (B) as follows: 0 min, 96% A, 4% B; 0–40 min, 50% A, 50% B; 40–60 min, 0% A, 100% B; 60–65 min, 0% A, 100% B; 65–67 min, 96% A, 4% B; 67–75 min, 96% A, 4% B. Effluent was monitored under an absorbance detector at A280. Chromatographic peaks were identified by comparing the retention time of samples with those of standard (hydroxytyrosol, 10–1000 µg/mL, R2 0.9967–0.9999; oleuropein, 50–2000 µg/mL, R2 0.9868-0.9997). HC and OC were determined in fresh and dried olive leaf samples collected in the second year.

2.6. Statistical analysis

Experimental data of TPC and TAC from fresh olive leaves collected in both years and water content results were analyzed by one-way ANOVA with a confidence level of 95%. The TPC, TAC, HC and OC data from fresh and dried olive leaf samples collected in the second year were analyzed by two-way ANOVA with interaction and a confidence level of 95%. Tukey test was applied as multiple post-hoc comparison to find means that were significantly different (p-value < 0.05) from each other. The results were expressed as mean values ± standard deviation (SD) of the experimental data obtained in three replications, except for water content data, which was analyzed in duplicate. The statistical analysis was performed using the IBM SPSS Statistics 22 software.

3. Results and discussion

3.1. Water content

Fresh olive leaves contained a water percentage between 41.7 ± 0.4% ('Picual') and 55.8 ± 0.3% ('Arbosana'). Dried olive leaf water content ranged from 1.75 ± 0.03% (freeze-dried 'Arroniz' olive leaves) to 14.95 ± 0.04% (vacuum-dried 'Picual' olive leaves). All the analyzed drying methods were effective since they reached a water loss over 60% (Figure 1), ensuring a water content below 15% in all the dried samples. Oven-drying and freeze-drying were the most effective methods.

3.2. Total phenol content

Fresh olive leaves contained a TPC from 15.0 ± 0.7 to 37.4 ± 2.0 mg GAE/g DW (Figure 2). Other authors reported similar TPC in olive leaves: 17–25 [26], 35 [21] and 10–49 mg GAE/g DW [9]. Among the fresh olive leaf cultivars evaluated, 'Arbosana' leaves had the highest mean TPC value (34.0 ± 1.1 mg GAE/g DW). 'Arroniz' and 'Empeltre' olive leaves had TPCs (25.2 ± 1.8 and 26.2 ± 0.9 mg GAE/g DW, respectively) similar to 'Picual' (24.8 ± 3.0 mg GAE/g DW), conventional

'Arbequina' (26.2 ± 15.9 mg GAE/g DW), and ecological 'Arbequina' (26.6 ± 3.6 mg GAE/g DW) leaves. Significant differences (p-value < 0.05) in TPC were not observed between conventional and ecological 'Arbequina' olive leaves. In ecological cultivation, as synthetic pesticides cannot be used, plants are exposed to greater stress; this normally induces the production of defense substances, such as phenolic compounds [27]. However, this effect was not clearly observed in the present work.

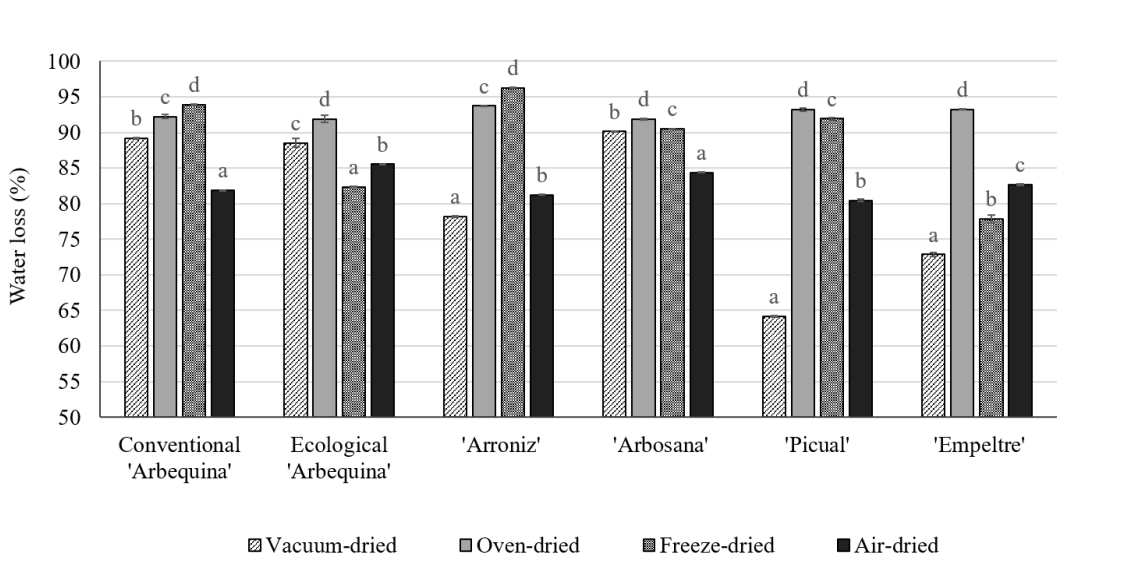

Figure 1. Mean values and standard deviation of olive leaf water loss after drying with four methods (n = 2). Significant differences between drying treatments are expressed with different letters (one-way ANOVA and Tukey test: p-value < 0.05).

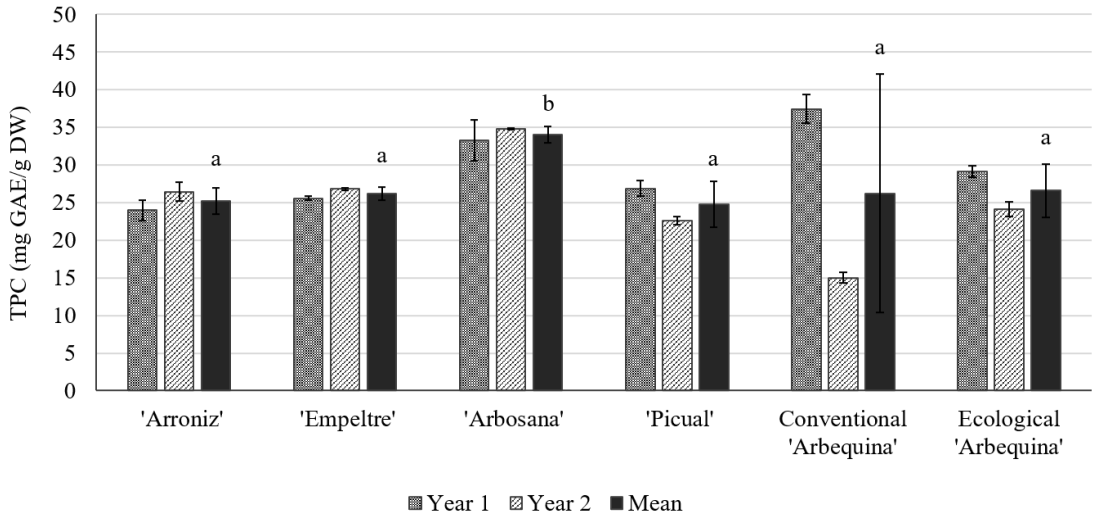

Figure 2. Mean values and standard deviation of total phenol content (TPC) of fresh olive leaves samples (n = 3). Significant differences between cultivars are expressed with different letters (one-way ANOVA and Tukey test: p-value < 0.05). GAE: gallic acid equivalents; DW: dry weight.

'Empeltre' (3.4%) and 'Arbosana' (3.2%) fresh leaves had the lowest TPC interannual variability. Conventional 'Arbequina' fresh leaves presented the highest TPC RSD (60.5%). The only study including TPC interannual variability concluded that it was cultivar dependent and significant (p-value < 0.05) for 'Arbequina' olive leaves [15]. These results could be critical in the selection of the most suitable olive leaves to be valorized, as the supply of phenolic compounds will depend on the interannual variability of TPC. Therefore, among fresh olive leaves 'Arbosana' were the most suitable to valorize because they showed the highest mean TPC and one of the lowest TPC interannual variability.

Vacuum-drying significantly decreased TPC in 'Arroniz' (11.5 ± 0.8 mg GAE/g DW), 'Empeltre' (6.0 ± 1.1 mg GAE/g DW) and 'Picual' (10.0 ± 1.2 mg GAE/g DW) olive leaf samples (Table 1). While in 'Arbosana' (35.4 ± 2.0 mg GAE/g DW) and 'Arbequina' leaves, conventional (28.9 ± 0.9 mg GAE/g DW) and ecological (34.6 ± 3.8 mg GAE/g DW) TPC was maintained or significantly higher after vacuum-drying. The diminution of TPC could be attributed to PPO activity. The vacuum-drying treatment took 4 days throughout which olive leaves were exposed to 40 ℃, conditions that could promote the action of PPO. Browning in 'Arroniz', 'Empeltre' and 'Picual' vacuum-dried olive leaves was visible (Figure 3) indicating that enzymatic reactions may have taken place. Therefore, at these conditions, some phenols could have been degraded by the action of enzymes [18,28].

Oven-drying decreased TPC in all samples except in 'Arbequina' (Table 1). The exposure to high temperatures during oven-drying could have caused browning (Figure 3) and the degradation of phenolic compounds [29]. However, some authors concluded that applying high temperatures for a short time, aiming at dehydrating the olive leaves, does not always decrease their phenol content [18,21]; as shown by the TPCs of oven-dried conventional and ecological 'Arbequina' samples evaluated in the present research.

TPC of the freeze-dried leaves were similar or significantly higher (p-value < 0.05) compared to their fresh leaf counterparts, except for 'Picual' olive leaves, which had significantly lower (p-value < 0.05) TPC (Table 1). Freeze-dried olive leaves did not show any browning (Figure 3), which indicated that PPO was not active as olive leaves were not exposed to temperatures above 10 ℃ [18]. In this research, freeze-drying proved to preserve phenolic compounds confirming the conclusion of other researchers [18,21].

The TPC in the air-dried leaf samples was significantly greater (p-value < 0.05) than that in the fresh leaves (Table 1). The moderate conditions used during this method prevented the degradation of phenolic compounds and maintained the green color of the olive leaves better (Figure 3). Afaneh et al. (2015) [17] and Kamran et al. (2015) [18] also concluded that air-drying was an effective method for the preservation of phenolic compounds. Among air-dried olive leaves (Table 1), ecological 'Arbequina' had the highest TPC (conventional 'Arbequina' ≤ 'Picual' = 'Arroniz' = Empeltre ≤ 'Arbosana = ecological 'Arbequina with 29.2 ± 1.0, 37.8 ± 4.8, 37.9 ± 3.2, 39.8 ± 4.2, 42.6 ± 1.7, and 45.4 ± 6.6 mg GAE/g DW, respectively).

3.3. Total antioxidant capacity

TAC of fresh olive leaves was between 55.3 ± 0.1 and 160 ± 18 μmol TE/g DW (Figure 4). These results agree with the values (60 μmol TE/g DW) observed by Ahmad-Qasem et al. (2016) [21]. 'Arbosana' olive leaves presented one of the highest mean TAC values (146 ± 20 μmol TE/g DW) together with 'Picual' (113 ± 5 μmol TE/g DW) and conventional 'Arbequina' (104 ± 69 μmol TE/g

DW). The TAC of 'Arroniz' (101 ± 18 μmol TE/g DW) and 'Empeltre' (80 ± 8 μmol TE/g DW) olive leaves were not significantly different (p-value > 0.05) from the TAC of 'Picual' (113 ± 5 μmol TE/g DW), conventional 'Arbequina' (104 ± 69 μmol TE/g DW) nor ecological 'Arbequina' (85 ± 4 μmol TE/g DW) olive leaves. Significant differences (p-value < 0.05) in TAC were not reported between conventional and ecological 'Arbequina' olive leaves. Thus, the effect of increasing phenolic compounds production to enhance the natural defense system observed by other authors in ecological cultivars [27] was not observed in the TAC values reported in this research.

'Picual' and ecological 'Arbequina' fresh leaves presented the lowest RSD of TAC (4.4% and 4.7%, respectively). The TAC interannual variability in 'Empeltre' (9.3%) and 'Arbosana' (13.4%) were also low. Conventional 'Arbequina' fresh leaves showed the highest RSD of TAC (66.5%). Therefore, among fresh olive leaves, 'Arbosana' leaves were the most suitable to valorize due to their high TAC and low interannual variability.

Vacuum-drying decreased TAC in olive leaves, except for both conventional and ecological 'Arbequina' samples (Table 1). Differences were only significant (p-value < 0.05) for 'Arroniz' and 'Picual' olive leaves. The reduction of TAC could have been caused by PPO that could be active at vacuum-drying conditions (40 ℃ for 4 days). In Figure 3 the visible browning in 'Arroniz', 'Empeltre' and 'Picual' vacuum-dried olive leaves may indicate that the effect of PPO activity degraded the phenolic compound and their antioxidant capacity [18,28].

Figure 3. Samples of leaves from five olive cultivars fresh collected and after different drying treatments.

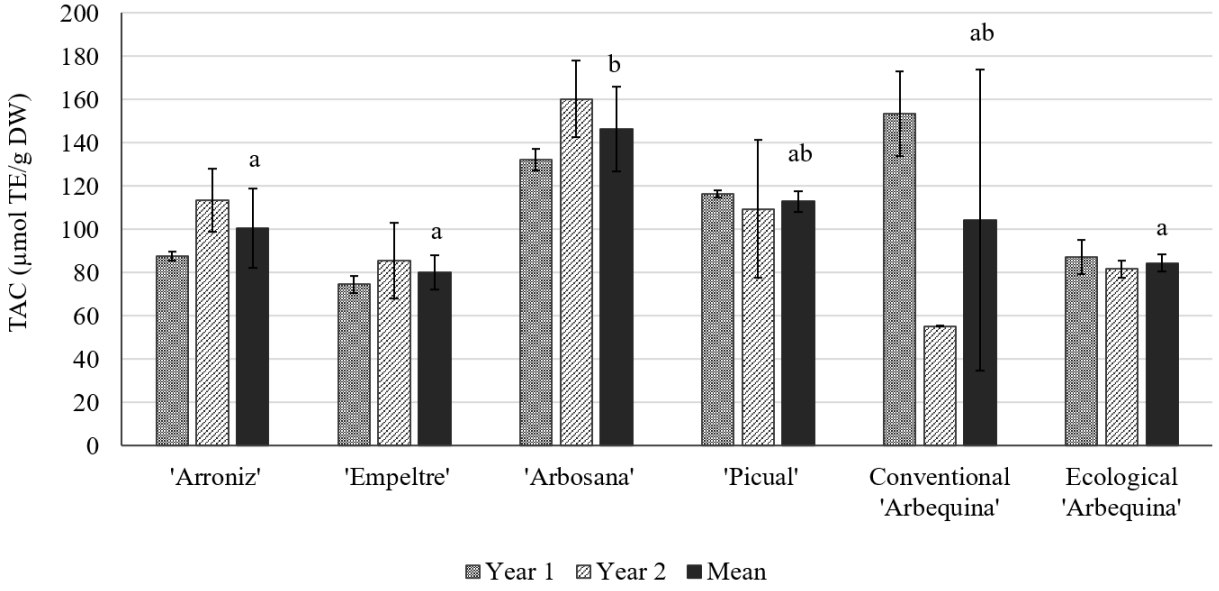

Figure 4. Mean values and standard deviation of total antioxidant capacity (TAC) of olive leaves samples (n = 3). Significant differences between cultivars are expressed with different letters (one-way ANOVA and Tukey test: p-value < 0.05).TE: Trolox equivalents; DW: dry weight.

After oven-drying, TAC did not significantly decrease (p-value > 0.05) in any of the studied olive leaf samples. Other authors also concluded that dehydrating olive leaves by applying high temperatures for a short time did not always decrease their TAC [18,21]. TAC of the freeze-dried leaves analyzed in this work were similar or significantly higher (p-value < 0.05) compared to their fresh leaf sample counterparts, except for 'Picual' olive leaves. In 'Picual' the TAC was significantly lower (p-value < 0.05). In this research, freeze-drying proved to have the ability to preserve TAC, confirming the conclusion of other researchers [18,21].

Air-drying maintained or significantly increased (p-value < 0.05) TAC of all the olive leaf samples. As observed by other authors [17,18], the moderate conditions used during this method prevented the degradation of TAC and maintained the original color of the leaves (Figure 3). Among air-dried leaves the highest TAC ('Picual' = conventional 'Arbequina' ≤ 'Arbosana' = 'Empeltre' = ecological 'Arbequina' ≤ 'Arroniz' with 150 ± 27, 161 ± 10, 198 ± 10, 218 ± 34, 225 ± 53, and 276 ± 42 µmol TE/g DW, respectively) was observed in 'Arroniz' and ecological 'Arbequina' leaves.

Table 1. Total phenol content (TPC), total antioxidant capacity (TAC), hydroxytyrosol content (HC) and oleuropein content (OC) in fresh, vacuum-dried, oven-dried, freeze-dried and air-dried leaves collected from five different olive cultivars.

Cultivar	Fresh	Vacuum-dried	Oven-dried	Freeze-dried	Air-dried	*p*-value
Total phenol content (mg GAE/g DW)						
'Arroniz'	26.5 ± 1.3 Cc	11.5 ± 0.8 Ba	17.4 ± 1.0 Bb	42.2 ± 2.6 CDd	37.9 ± 3.2 ABd	0.000
'Empeltre'	26.8 ± 0.2 Cc	6.0 ± 1.1 Aa	15.3 ± 0.6 ABb	25.6 ± 2.1 Bc	39.8 ± 4.2 ABd	0.000
'Arbosana'	34.8 ± 0.1 Db	35.4 ± 2.0 Db	21.6 ± 0.3 Ca	44.3 ± 1.2 Dc	42.6 ± 1.7 Bc	0.000
'Picual'	22.6 ± 0.6 Bc	10.0 ± 1.2 ABa	13.2 ± 0.3 Aab	16.0 ± 0.7 Ab	37.8 ± 4.8 ABd	0.000
Conventional 'Arbequina'	15.0 ± 0.7 Aa	28.9 ± 0.9 Cb	33.0 ± 0.7 Db	34.5 ± 5.4 Cb	29.2 ± 1.0 Ab	0.000
Ecological 'Arbequina'	24.1 ± 1.0 Ba	34.6 ± 3.8 Db	37.0 ± 2.0 Ebc	46.1 ± 2.8 Dc	45.4 ± 6.6 Bc	0.000
p-value	0.000	0.000	0.000	0.000	0.007	
Total antioxidant capacity (μmol TE/g DW)						
'Arroniz'	113 ± 15 BCb	44 ± 5 Aa	209 ± 10 Bc	204 ± 29 ABCc	276 ± 42 Bd	0.000
'Empeltre'	86 ± 17 ABab	38 ± 5 Aa	114 ± 37 Ab	134 ± 27 ABb	218 ± 34 ABc	0.000
'Arbosana'	160 ± 18 Cab	120 ± 35 Bab	89 ± 27 Aa	201 ± 49 ABCb	198 ± 10 ABb	0.005
'Picual'	109 ± 32 Bbc	31 ± 2 Aa	59 ± 6 Aab	57 ± 6 Aa	150 ± 27 Ac	0.000
Conventional 'Arbequina'	57 ± 1 Aa	137 ± 39 Bab	190 ± 32 Bab	283 ± 112 Cb	161 ± 10 Aab	0.007
Ecological 'Arbequina'	82 ± 4 ABa	147 ± 20 Bab	212 ± 10 Bb	222 ± 31 BCb	225 ± 53 ABb	0.000
p-value	0.000	0.000	0.000	0.004	0.006	
Hydroxytyrosol content (mg /g DW)						
'Arroniz'	0.06 ± 0.01 Aa	0.14 ± 0.00 Aa	0.34 ± 0.01 Bb	0.92 ± 0.06 Cd	0.65 ± 0.09 Cc	0.000
'Empeltre'	1.37 ± 0.11 Dd	0.15 ± 0.00 Aa	0.36 ± 0.05 BCb	0.62 ± 0.02 Bc	0.51 ± 0.05 BCbc	0.000
'Arbosana'	1.53 ± 0.04 Dc	0.80 ± 0.08 Cb	0.44 ± 0.03 Ca	0.82 ± 0.04 Cb	0.41 ± 0.08 Ba	0.000
'Picual'	0.20 ± 0.02 ABab	0.18 ± 0.00 Aa	0.23 ± 0.01 Ab	0.21 ± 0.01 Aab	0.90 ± 0.02 Dc	0.000
Conventional 'Arbequina'	0.96 ± 0.02 Cb	1.01 ± 0.04 Db	1.31 ± 0.02 Dc	0.93 ± 0.06 Cb	0.13 ± 0.02 Aa	0.000
Ecological 'Arbequina'	0.27 ± 0.09 Ba	0.45 ± 0.03 Bb	1.34 ± 0.03 Dd	1.44 ± 0.03 Dd	0.96 ± 0.04 Dc	0.000
p-value	0.000	0.000	0.000	0.000	0.000	

Continued on the next page

Cultivar	Fresh	Vacuum-dried	Oven-dried	Freeze-dried	Air-dried	*p*-value
Oleuropein content (mg/g DW)						
'Arroniz'	0.3 ± 0.1 Ba	1.8 ± 0.1 Aa	0.6 ± 0.1 ABa	20.1 ± 1.8 Cb	36.8 ± 3.1 CDc	0.000
'Empeltre'	<0.05 Aa	1.3 ± 0.0 Ab	0.2 ± 0.0 Aab	4.0 ± 0.1 Ac	32.4 ± 1.0 Cd	0.000
'Arbosana'	<0.05 Aa	30.4 ± 1.7 Cc	1.3 ± 0.1 Ba	20.4 ± 1.0 Cb	23.5 ± 2.0 Bc	0.000
'Picual'	0.8 ± 0.1 Ca	2.5 ± 0.2 Aab	0.5 ± 0.1 ABa	3.4 ± 0.0 Ab	40.1 ± 2.1 Dc	0.000
Conventional 'Arbequina'	<0.05 Aa	20.9 ± 0.6 Bc	23.8 ± 0.5 Dd	14.6 ± 1.8 Bb	14.4 ± 1.2 Ab	0.000
Ecological 'Arbequina'	<0.05 Aa	30.0 ± 2.2 Cd	8.3 ± 0.5 Cb	16.0 ± 0.2 Bc	35.6 ± 1.5 CDe	0.000
p-value	0.000	0.000	0.000	0.000	0.000	

Values are the mean (n = 3) ± standard deviation. Distinct capital letters show significant differences between cultivars and distinct lower-case letters represent significant differences between drying methods (two-way ANOVA; Tukey test: p-value < 0.05). GAE: gallic acid equivalents; TE: Trolox equivalents; DW: dry weigh; SD: standard deviation.

3.4. Hydroxytyrosol and oleuropein content

In 'Empeltre' and 'Arbosana' leaves, the samples with the highest HC in fresh samples (1.37 ± 0.11 and 1.53 ± 0.04 mg hydroxytyrosol/g DW, respectively), HC was significantly lower after vacuum-drying (0.15 ± 0.00 and 0.80 ± 0.08 mg hydroxytyrosol/g DW, respectively), oven-drying (0.36 ± 0.05 and 0.44 ± 0.03 mg hydroxytyrosol/g DW, respectively), freeze-drying (0.62 ± 0.02 and 0.82 ± 0.04 mg hydroxytyrosol/g DW, respectively) and air-drying (0.51 ± 0.05 and 0.41 ± 0.08 mg hydroxytyrosol/g DW, respectively). In 'Arroniz', 'Picual', conventional and ecological 'Arbequina' olive leaves HC was maintained or higher after the different drying methods, except for conventional 'Arbequina' leaves after air-drying (0.13 ± 0.02 mg hydroxytyrosol/g DW). When the investigated drying methods were applied, OC of the olive leaf samples was stable, or significantly higher (Table 1).

Among air-dried leaves, ecological 'Arbequina' presented the highest HC (conventional 'Arbequina' < 'Arbosana' ≤ 'Empeltre' ≤ 'Arroniz' < 'Picual' = ecological 'Arbequina' with 0.13 ± 0.02, 0.41 ± 0.08, 0.51 ± 0.05, 0.65 ± 0.09, 0.90 ± 0.02, and 0.96 ± 0.04 mg hydroxytyrosol/g DW, respectively). Whereas, 'Picual', 'Arroniz' and ecological 'Arbequina' leaf samples contained the highest OC (conventional 'Arbequina' < 'Arbosana' < 'Empeltre' ≤ ecological 'Arbequina' = 'Arroniz' ≤ 'Picual with 14.4 ± 1.2, 23.5 ± 2.0, 32.4 ± 1.0, 35.6 ± 1.5, 36.8 ± 3.1, and 40.1 ± 2.1 mg oleuropein/g DW, respectively).

In general, vacuum-drying and oven-drying could be effective drying methods for some olive leaf cultivars as long as the olive leaves are not exposed to high temperatures for long periods of time [18,21]. Freeze-drying proved to preserve phenolic compounds in leaves, confirming the conclusion of other researchers [18,21]. The moderate conditions used during air-drying prevented the degradation of phenolic compounds, as reported by other authors [17,18].

In the present study, the TPC, TAC, HC and OC of several olive leaf samples were significantly higher (p-value < 0.05) after drying methods. This could have been caused by the damage on plant tissues caused by drying. This damage enhances the transference of matter allowing better penetration of the solvent and therefore a higher concentration of phenolic compounds in the extracts [17,28].

4. Conclusions

This research characterizes 'Arroniz' and 'Empeltre' olive leaves for the first time and compares them with olive leaves from other cultivars like 'Arbosana', 'Picual' and 'Arbequina'. Among fresh olive leaf samples, 'Arbosana' stood out for its high TPC, TAC and HC and low TPC and TAC interannual variabilities. With the data illustrating the effect of drying methods on the TPC, TAC, HC and OC, the most suitable drying method for each olive leaf cultivar can be easily selected. For almost all the studied olive leaves, with exception of conventional 'Arbequina', air-dried olive leaves had higher TPC, TAC and OC than vacuum-dried, oven-dried or freeze-dried leaves. Thus, air-drying was selected as the most suitable drying method. Among air-dried leaves, ecological 'Arbequina' were selected as the most suitable to valorize because they stood out in all the studied parameters (TPC, TAC, HC and OC).

Acknowledgments

This work was supported by the Government of Navarra (grant numbers 0011-1365-2017-000227, 0011-1408-2017-000023, 0011-1411-2018-000032).

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. International Olive Council (2019) International Olive Council Newsletter, The international market, 2019. Available from: https://www.internationaloliveoil.org/wp-content/uploads/2019/12/newsletter_144_english.pdf.
2. Molina-Alcaide E, Yáñez-Ruiz DR (2008) Potential use of olive by-products in ruminant feeding: A review. *Anim Feed Sci Technol* 147: 247–264. https://doi.org/10.1016/j.anifeedsci.2007.09.021
3. Žugčić T, Abdelkebir R, Alcantara C, et al. (2019) From extraction of valuable compounds to health promoting benefits of olive leaves through bioaccessibility, bioavailability and impact on gut microbiota. *Trends Food Sci Technol* 83: 63–77. https://doi.org/10.1016/j.tifs.2018.11.005
4. Nunes MA, Pimentel FB, Costa ASG, et al. (2016) Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. *Innov Food Sci Emerg Technol* 35: 139–148. https://doi.org/10.1016/j.ifset.2016.04.016
5. Talhaoui N, Taamalli A, Gómez-Caravaca AM, et al. (2015) Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. *Food Res Int* 77: 92–108. https://doi.org/10.1016/j.foodres.2015.09.011
6. Talhaoui N, Gómez-Caravaca AM, Roldán C, et al. (2015) Chemometric analysis for the evaluation of phenolic patterns in olive leaves from six cultivars at different growth stages. *J Agric Food Chem* 63: 1722–1729. https://doi.org/10.1021/jf5058205
7. Şahin S, Ahmed Malik NS, Perez JL, et al. (2012) Seasonal changes of individual phenolic compounds in leaves of twenty olive cultivars grown in Texas. *J Agric Sci Technol* 2: 242–247.
8. Özcan MM, Fındık S, AlJuhaimi F, et al. (2019) The effect of harvest time and varieties on total phenolics, antioxidant activity and phenolic compounds of olive fruit and leaves. *J Food Sci Technol* 56: 2373–2385. https://doi.org/10.1007/s13197-019-03650-8
9. Nicolì F, Negro C, Vergine M, et al. (2019) Evaluation of phytochemical and antioxidant properties of 15 Italian *Olea europaea* L. cultivar leaves. *Molecules* 24: 1998. https://doi.org/10.3390/molecules24101998
10. Hülya-Orak H, Karamać M, Amarowicz R, et al. (2019) Genotype-related differences in the phenolic compound profile and antioxidant activity of extracts from olive (*Olea europaea* L.) leaves. *Molecules* 24: 1130. https://doi.org/10.3390/molecules24061130
11. Olmo-García L, Bajoub A, Benlamaalam S, et al. (2018) Establishing the phenolic composition of *Olea europaea* L. leaves from cultivars grown in Morocco as a crucial step towards their subsequent exploitation. *Molecules* 23: 2524. https://doi.org/10.3390/molecules23102524

12. Guinda Á, Castellano JM, Santos-Lozano JM, et al. (2015) Determination of major bioactive compounds from olive leaf. *LWT-Food Sci Technol* 64: 431–438. https://doi.org/10.1016/j.lwt.2015.05.001
13. Romero C, Medina E, Mateo MA, et al. (2017) Quantification of bioactive compounds in Picual and Arbequina olive leaves and fruit. *J Sci Food Agric* 97: 1725–1732. https://doi.org/10.1002/jsfa.7920
14. Lama-Muñoz A, Contreras MM, Espínola F, et al. (2020) Content of phenolic compounds and mannitol in olive leaves extracts from six Spanish cultivars: Extraction with the Soxhlet method and pressurized liquids. *Food Chem* 320: 126626. https://doi.org/10.1016/j.foodchem.2020.126626
15. Papoti VT, Papageorgiou M, Dervisi K, et al. (2018) Screening olive leaves from unexploited traditional Greek cultivars for their phenolic antioxidant dynamic. *Foods* 7: 197. https://doi.org/10.3390/foods7120197
16. De Leonardis A, Macciola V, Cuomo F, et al. (2015) Evidence of oleuropein degradation by olive leaf protein extract. *Food Chem* 175: 568–574. https://doi.org/10.1016/j.foodchem.2014.12.016
17. Afaneh I, Yateem H, Al-Rimawi F (2015) Effect of olive leaves drying on the content of oleuropein. *Am J Anal Chem* 6: 246–252. http://dx.doi.org/10.4236/ajac.2015.63023
18. Kamran M, Hamlin AS, Scott CJ, et al. (2015) Drying at high temperature for a short time maximizes the recovery of olive leaf biophenols. *Ind Crop Prod* 78: 29–38. https://doi.org/10.1016/j.indcrop.2015.10.031
19. Attya M, Benabdelkamel H, Perri E, et al. (2010) Effects of conventional heating on the stability of major olive oil phenolic compounds by tandem mass spectrometry and isotope dilution assay. *Molecules* 15: 8734. https://doi.org/10.3390/molecules15128734
20. Şahin S, Elhussein E, Bilgin M, et al. (2018) Effect of drying method on oleuropein, total phenolic content, flavonoid content, and antioxidant activity of olive (*Olea europaea*) leaf. *J Food Process Preserv* 42: e13604. https://doi.org/10.1111/jfpp.13604
21. Ahmad-Qasem MH, Ahmad-Qasem BH, Barrajón-Catalán E, et al. (2016) Drying and storage of olive leaf extracts. Influence on polyphenols stability. *Ind Crop Prod* 79: 232–239. http://dx.doi.org/10.1016/j.indcrop.2015.11.006
22. Ali Elhussein EA, Şahin S (2018) Drying behaviour, effective diffusivity and energy of activation of olive leaves dried by microwave, vacuum and oven drying methods. *Heat Mass Transf* 54: 1901–1911. https://doi.org/10.1007/s00231-018-2278-6
23. Obied HK, Allen MS, Bedgood DR, et al. (2005) Investigation of Australian olive mill waste for recovery of biophenols. *J Agric Food Chem* 53: 9911–9920. https://doi.org/10.1021/jf0518352
24. Brand-Williams W, Cuvelier M., Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* 28: 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
25. Suárez M, Macià A, Romero MP, et al. (2008) Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. *J Chromatogr A* 1214: 90–99. https://doi.org/10.1016/j.chroma.2008.10.098
26. Abaza L, Ben-Youssef N, Manai H, et al. (2011) Chétoui olive leaf extracts: influence of the solvent type on phenolics and antioxidant activities. *Grasas y aceites* 62: 96–104. https://doi.org/10.3989/gya.044710

27. López-Yerena A, Lozano-Castellón J, Olmo-Cunillera A, et al. (2019) Effects of organic and conventional growing systems on the phenolic profile of extra-virgin olive oil. *Molecules* 24: 1986. https://doi.org/10.3390/molecules24101986
28. Taamalli A, Lozano-Sánchez J, Jebabli H, et al. (2019) Monitoring the bioactive compounds status in *Olea europaea* according to collecting period and drying conditions. *Energies* 12: 947. https://doi.org/10.3390/en12050947
29. Babu AK, Kumaresan G, Aroul-Raj VA, et al. (2018) Review of leaf drying: Mechanism and influencing parameters, drying methods, nutrient preservation, and mathematical models. *Renew Sustain Energy Rev* 90: 536–556. https://doi.org/10.1016/j.rser.2018.04.002

AIMS Press