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ORIGINAL ARTICLE

Influence of the preparation method on the bioactive compounds of olive tree leaf tea

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Abstract

The general objective of the research was to investigate whether the preparation methods interfere with the polyphenols availability and the antioxidant capacity of olive leaf teas prepared from dry leaves. Leaves of the cultivar Arbequina were dried in an oven with air circulation and renewal at 40 °C/48 hours. The teas were prepared with 7% dry leaves through infusion and boiling for 10 minutes of process. Part of the liquid teas were freeze-dried. The samples of liquid tea and freeze-dried teas were evaluated for: total phenolic compounds (TPC) (using the Folin-Ciocalteu reagent), polyphenol profile (High Performance Liquid Chromatography - HPLC), and antioxidant capacity by the ABTS assay. It was observed that the boiling method released more polyphenols than the infusion and a greater antioxidant capacity in liquid tea and freeze-dried tea. Oleuropein concentration was favored by the boiling method regardless of whether the tea was freeze-dried or not. Although boiled tea presented a higher total polyphenol content, there was a reduction in the flavonoid levels. Additionally, freeze-dried tea prepared by infusion also showed the highest levels of flavonoids and phenolic acids. In conclusion, the consumption of dehydrated olive leaf tea prepared by boiling offers higher levels of polyphenols and antioxidant capacity than tea prepared by infusion.

Keywords: Oleuropein; *Olea europaea* L.; Polyphenols; Phenolic acids; Leaf dehydration; Freeze-dried tea; Antioxidant; Infusion; Boiling.

Highlights

- Flavonoids are better preserved than phenylpropanoids in olive leaf tea infusion
- Boiling increases the availability of phenolic acids and oleuropein, but reduces the content of flavonoids Boiling provides higher levels of total phenolic compounds across different extractors
- The antioxidant capacity of olive leaf tea is enhanced through the boiling process

1 Introduction

Teas have been used for nutraceutical purposes in various societies and supported by several scientific studies (Badr et al., 2020; Basuny & Arafat, 2018; Boss et al., 2016; Burja et al., 2019; Cheurfa et al., 2019; Ferdousi et al., 2019; Liu et al., 2019). Olive leaves are among the most important herbal dietary products, with Mediterranean countries being the main producers and consumers (Scognamiglio et al., 2012). Olive tree products have spread globally among consumers interested in natural and preventive health products, including caffeine-free options (Basuny & Arafat, 2018).

Olive tree derivatives consumption such as tea and olive oil, is reported to mitigate coronary diseases and cancer incidence (Boss et al., 2016; Covas, 2007; Zeriouh et al., 2017), as well as to lower blood pressure in cases of hypertension (Marika et al., 2020; Psaltopoulou et al., 2004), have hematological health benefits (Ferdousi et al., 2019), and attenuate inflammatory activation (Burja et al., 2019). There is strong evidence that polyphenols play a crucial role in the benefits provided by the consumption of olive tree derivatives. Polyphenols act as antioxidants, preventing tissue damage caused by oxidative stress, which can lead to degenerative and inflammatory diseases (Zeriouh et al., 2017; Lockyer et al., 2017).

The main polyphenols found in olive leaves are ferulic acid, coumaric acid, vanillic acid, caffeic acid, apigenin, diosmetin, quercetin, luteolin, verbascoside, and oleuropein (Benavente-García et al., 2000; Pereira et al., 2007). Olive leaves contain oleuropein, the main polyphenol, whose levels vary by variety and harvest time (Xie et al., 2015). This compound is a secoiridoid, unique to the Oleaceae family. Oleuropein is an ester of hydroxytyrosol (3,4-dihydroxyphenylethanol) to elenolic acid (Özcan & Matthäus, 2017; Shamshoum et al., 2017). The derivation of the hydroxytyrosol and elenolic acid moieties is attributed to distinct pathways, specifically the phenolic and terpenoid routes, respectively (Alagna et al., 2016). Its high capacity for radical scavenging and health benefits are well documented due to its structural characteristics (Benavente-García et al., 2000; Liu et al., 2019). In addition, oleuropein has shown promising antidepressant activity by conserving biogenic amine levels (Badr et al., 2020) and providing protection against Alzheimer's disease (Rigacci & Stefani, 2016).

Even though the tea preparation methods might modify the polyphenols profile and, consequently, the antioxidant activity of the olive leaf tea, the high temperature is the best way to recover these compounds from leaves without using substances hazardous to health (Ahmad-Qasem et al., 2016). Olive leaves dehydrated at room temperature were favorable for the conservation of high concentrations of oleuropein when compared to leaves dehydrated under high temperatures, at 50 °C. However, the natural drying time is much longer and makes kiln drying more viable. In addition, thermal processing deactivates the oxidative enzymes to avoid the loss of polyphenols and allows a 10-fold greater recovery of oleuropein than in fresh leaves (Afaneh et al., 2015; Silva et al., 2006). Thus, tea preparation requires heat processing to access product benefits.

The research of Cagliari et al. (2022) demonstrated that the Brazilian olive leaves had the capacity to supply compounds with bioactive potential even after drying, which can be used as a resource and should not be discarded as a residue, thus reinforcing the questions about sustainability in olive growing. The drying Brazilian olive leaf represents a good way of preserving leaves and exploiting their bioactive potential, so future uses of the leaves include the production of extracts with potential use in the food and pharmaceutical industries.

The drying temperature of the leaves interferes with the availability of dry-leaf polyphenols (Ahmad-Qasem et al., 2016); however, the effects of tea preparation methods from dried leaves are still unknown. Likewise, sample preparation studies using freeze-drying usually address fresh or dried leaves (Afaneh et al., 2015; Silva et al., 2006) and not the tea itself. The liquid tea analysis would estimate the compounds extracted during tea preparation and are available during its ingestion and not only the potential concentrations that leaves have.

From this point of view, the intensity of heat processing (temperature) applied during domestic cooking is the factor that will determine the levels of recovered polyphenols in tea. Few studies assess the influence of infusion and boiling tea methods on olive leaf bioactive compounds availability (Casazza et al., 2017). The light of our knowledge, in the context of both tea preparation methods, the impact of freeze-drying on its influence has not yet been thoroughly studied. The freeze-drying utilization of concentrate compounds, including additives and

antioxidants, has become a prominent process in various industrial applications. This method has proven to be highly efficient in enhancing the stability and shelf-life of the product, while also reducing the overall weight and volume. Coppa et al. (2017) developed research with freeze-dried olive extract and proved that the addition of an extract rich in oleuropein can be positive even for increasing the oxidative stability of olive oil. This technique is widely used in industries, such as pharmaceuticals, food processing, and biotechnology, owing to its ability to preserve the quality and efficacy of the compounds being concentrated (Zoric et al., 2014).

Taking into account the topics addressed, in particular, the fact that olive leaves are an important source of polyphenols, it is suggested (1) the study of the constitutions (quality and quantity) of homemade teas obtained by different methods, since the extraction of such polyphenols from the leaves is facilitated by the use of heat, and also (2) the study of the use of freeze-drying, which is an interesting and viable technique to promote the concentration of these antioxidant compounds in the drink.

The specific goals for this research were to examine the methods and conditions used in tea-making as follows: i) to compare the polyphenols contents in olive leaf tea prepared by infusion and boiling methods; ii) to investigate the polyphenols content after tea concentration by freeze-drying and evaluated its bioactive compounds potential; iii) to assess olive leaf tea total phenolic compounds (TPC); and, iv) to evaluate how processing and freeze-drying interfered in the antioxidant capacity of olive leaf tea.

2 Material and methods

2.1 Materials

2.1.1 Chemicals

Methanol, phosphoric acid, acetonitrile, acetic acid, ethanol, acetone, sodium carbonate, Folin-Ciocalteu, ABTS (2, 2'-azino-bis [3-ethyl benzothiazoline-6-sulphonic acid]), Trolox (6-Hydroxy-2, 5, 7,8-tetramethylchromane-2-carboxylic acid), *polyphenols standards [*3, 3′, 4′, 5,6-pentahydroxyflavone (quercetin); 3′, 4′, 5,7-tetrahydroxyflavone (luteolin); 3′,5,7-Trihydroxy-4′-methoxyflavone (diosmetin); 4-Hydroxy-3-methoxybenzoic acid (vanillic acid); t-4-hydroxycinnamic acid (p-coumaric acid); and t-4-hydroxy-3-methoxycinnamic acid (t-ferulic acid); (2S,3E,4S)-3-Ethylidene-2-(β-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-pyran-4-acetic acid 2-(3,4-dihydroxyphenyl)ethyl est*er (oleuropein)]*.

2.1.2 Plant material

Sample collection was carried out by cutting branches with olive leaves (*Olea europaea* L. vr Arbequina) farmed in São Pedro - SP (L 22º32'55" S L 47º54'50" W). Immediately, the samples were taken to the Post-harvest and Processing laboratory at Sao Paulo Agribusiness Technology Agency (Regional APTA) in Piracicaba/SP (Brazil). These leaves were washed in water and shaken to remove the excess moisture. Subsequently, the leaves were manually separated from the branches.

2.2 Methods

2.2.1 Sample preparation and treatments

The leaves were packed in Kraft paper bags, containing 100 g of sample each, for drying. The leaves were placed in a cabinet tray dryer, with air circulation and renew-al, at 40 °C for 48 hours (Silva et al., 2006). From the dried leaves, treatments consisted of: 1) 7% of dried leaves immersed in previously heated water (80 °C) for 10 minutes (infusion); 2) 7% of dry leaves immersed in boiling water (100 ± 2 °C), for 10 minutes (boiling). Part of the teas, treatments boiled and infused, were strained and cooled to room temperature, and subsequently stored at -20 °C in the dark (Liquid tea treatments). Other parts of the tea samples boiled and infused were subjected to freeze-drying (Freeze-dried tea treatments) (Liotop L108, São Paulo, Brazil), under temperature of -55 °C ± 5 °C and pressure from 1 x 10-2 to 3 x 10-2 mbar (1 to 3 Pa).

2.2.2 Polyphenols profile by high performance liquid chromatography analysis (HPLC)

The polyphenols profile was analyzed for tea freeze-dried and for liquid tea samples prepared by infusion and boiling. Extraction of polyphenols was carried out with lyophilized tea samples prepared by infusion and boiling, in which methanol was used as an extractor. The methanol was preferred because it favors the recovery of both phenolic acids and oleuropein as well as flavonoids (Meirinhos et al., 2005; Pereira et al., 2007). The addition of 0.06 g kg^{-1} of a sample with 2 mL de methanol 100% was done in a glass falcon tube, followed by centrifugation at 3220 x g (Jouan BR4i, Paris, France) for 5 min and collection of the supernatant (polyphenols extract). Before the analysis, the extract was filtered in PVDF (poly-vinylidene difluoride) micropore membrane 0.45 µm pore size and kept in the dark. In both liquid tea treatments, 1.5 mL was filtered (PVDF micropore membrane 0.45 µm) to obtain the extract.

The polyphenols analysis was performed according to Colombo et al. (2006) with modifications. Polyphenols separation was performed through the injection of 10 µL of the samples in an HPLC (Shimadzu, Japan) equipment coupled with a DAD (Diode Array Detector). A C18 column (5 µm, 4.6 x 250 mm) (Shim-pack Velox, Shimadzu, Japan) was used at a temperature of 40°C, and the flow rate was set to 1.0 mL per minute. The mobile phase consisted of phosphoric acid 0.2% (solvent A) and acetonitrile 100% (solvent B). The gradients used were the following: 0-8 min, 10-13% B; 8-25 min, 13-20% B; 25-40 min, 20-40% B; 40-45 min, 40-60% B; 45-50 min, 60-100% B; 50-60 min, 100% B. Polyphenols were quantified at 270 nm and 350 nm, representing the absorption lines of phenolic acids and flavonoids, respectively, by determining peak areas under the curve in the HPLC calibrate against known amounts of standards. The procedure was conducted in triplicate, and the results were expressed in µg mL^{-1}.

2.2.3 Total phenolic compounds analysis

To understand the nature of the TPC present in the leaves of olive tea and how the tea preparation influenced the concentration of these compounds, the freeze-dried teas and the dry leaves were submitted to different extractors: 1) distilled water; 2) 80% aqueous methanol; 3) 90% methanol acidified to a ratio of 10/90 with 8% acetic acid (90: 8: 2, v: v: v); 4) 60% aqueous ethanol; 5) 80% aqueous acetone, and; 6) 50% aqueous acetone. TPC analysis was also performed with liquid tea from infusion and boiling techniques without the addition of extractors.

Freeze-dried tea, dry leaves, and liquid tea samples were used to analyze the content of TPC. After adding 10 mL of correspondent extract solution to 0.0025 g of freeze-dried and dry samples (except in the liquid tea), the extracts were stored at room temperature and in the dark for 5 h, according to Mylonaki et al. (2008). Then, the extract was centrifuged (Jouan BR4i, Paris, France), and the supernatant was collected. The Folin-Ciocalteu reagent was used according to Singleton and Rossi (1965), with modifications. From this stage on, boiled and infused liquid tea samples were also analyzed. The reaction contained 1.5 mL distilled water, 0.1 mL Folin-Ciocalteu reagent, and 0.2 mL sample extract. After 5 min, 0.2 mL sodium carbonate (200 g L^{-1}) was added. The reaction was incubated for 2 h in the dark according to Mylonaki et al. (2008), and spectrophotometric readings were taken at 765 nm (Biochrom Libra S22 UV-Vis, Cambridge, UK). A standard curve was generated with gallic acid, and the results were expressed in gallic acid equivalents on a dry weight basis (g GAE kg^{-1}).

2.2.4 Antioxidant capacity by ABTS assay

The antioxidant capacity was quantified according to Re et al. (1999) using an ABTS assay. To assess the antioxidant capacity, it was performed analysis in freeze-dried tea and liquid tea treatments, boiled and infused. The extract from freeze-dried tea was obtained by adding 10 mL of methanol (80%) to 0.06 g of freeze-dried tea sample. After that, the solution was centrifuged at 6440 × g for 10 min at 4 °C (Jouan BR4i, Paris, France). The reaction with ABTS radical was performed by adding 2 mL of ABTS·+ radical in 20 µL of extract during 6 min, and spectrophotometric readings were taken at 734 nm (Biochrom Libra S22 UV–Vis, Cambridge, UK). The analysis of the liquid tea treatments were carried out with samples of the unmixed

liquid tea. The results for both were expressed in µM TEAC mg^{-1} or mL^{-1}. A standard curve of 2 mM Trolox (0-20 µM) was used to calculate the antioxidant capacity.

2.3 Experimental design and statistical analysis

The experimental design was entirely randomized with six samples from the total phenolic content test and four samples from the polyphenols profile test with three repetitions for both. The analyses were performed in triplicate. Data were analyzed by Analysis of Variance (ANOVA) (*F*-test), and means were compared by Tukey's test ($p < 0.05$) using the R Studio (version 3. 2. 5) statistical software. The results were expressed as the mean values ± standard error (SE).

3 Results and discussion

3.1 Polyphenols profile by high performance liquid chromatography analysis (HPLC)

The contents of individual compounds of phenolic acids, flavonoids, and oleuropein in freeze-dried tea and liquid tea were analyzed. (Figure 1). There were differences between the freeze-dried tea (Figure 1A; 1C) and liquid tea (Figure 1B; 1D) for the individual polyphenols content analyzed in 350 and 270 wavelengths. Seven compounds were identified: hydroxycinnamic acids (coumaric acid and ferulic acid), hydroxybenzoic acids (vanillic acid), flavonols (quercetin), flavones (luteolin and diosmetin), and secoiridoid (oleuropein).

In freeze-dried tea, both phenolic acids (Figure 2A-B), *i.e.*, coumaric acid and vanillic acid, and flavonoids (Figure 2E-G), *i.e.*, quercetin, luteolin, and diosmetin, were present in higher levels when prepared by infusion. On the other hand, ferulic acid showed no differences between preparation methods (Figure 2C), and oleuropein had a higher concentration in freeze-dried tea boiling (Figure 2D). In liquid tea, the concentration of individual compounds was significantly lower than in freeze-dried tea. Furthermore, there were differences between levels found in liquid tea and freeze-dried tea, depending on the infusion or boiling preparation methods used. Liquid tea boiling showed higher levels of phenolic acids and oleuropein (Figure 2D). On the other hand, it reduced the content of flavonoids compared to tea infusion (Figure 2F-G). Regardless of the preparation method, olive-leaf liquid tea did not demonstrate quantifiable concentrations of quercetin (Figure 2E). Although the boiling method resulted in a 12.55% reduction in luteolin and diosmetin content, it caused a significant increase in the levels of oleuropein, coumaric acid, vanillic acid, and ferulic acid by 73.25%, 71.08%, 48.87%, and 47.95%, respectively (Figure 2).

Regardless of the type of tea analyzed, infusion favored the flavonoids group (Table 1). In addition, the proportions of each group of compounds were closer in liquid tea infusion (Figure 3). After a thorough comparison, it has been observed that the concentration of total polyphenols in liquid tea boiling is twice as high as that in liquid tea infusion. Concentrating the samples reduced these differences. The freeze-dried tea infusion had a higher concentration of total phenolic acids (Table 1).

The primary polyphenol present in olive tea is oleuropein, regardless of the sample type or treatment (Figure 3). Oleuropein contributed the highest percentage of total polyphenols when tea was boiled, exceeding infusion treatments by more than 22%. Flavonoids were the main compounds compromised by boiling. The compounds in freeze-dried tea infusion were ranked in order of contribution to the total polyphenols as follows: oleuropein> luteolin > quercetin > vanillic acid > ferulic acid > diosmetin > coumaric acid. In freeze-dried tea-boiled samples, the order was: oleuropein > luteolin > ferulic acid > vanillic acid > quercetin > coumaric > diosmetin. The order of contribution of the polyphenols in liquid tea infusion was oleuropein > luteolin > ferulic acid > vanillic acid > diosmetin > coumaric acid; and in liquid tea boiling the order was oleuropein > ferulic acid > vanillic acid > luteolin > diosmetin > coumaric acid (Figure 3).

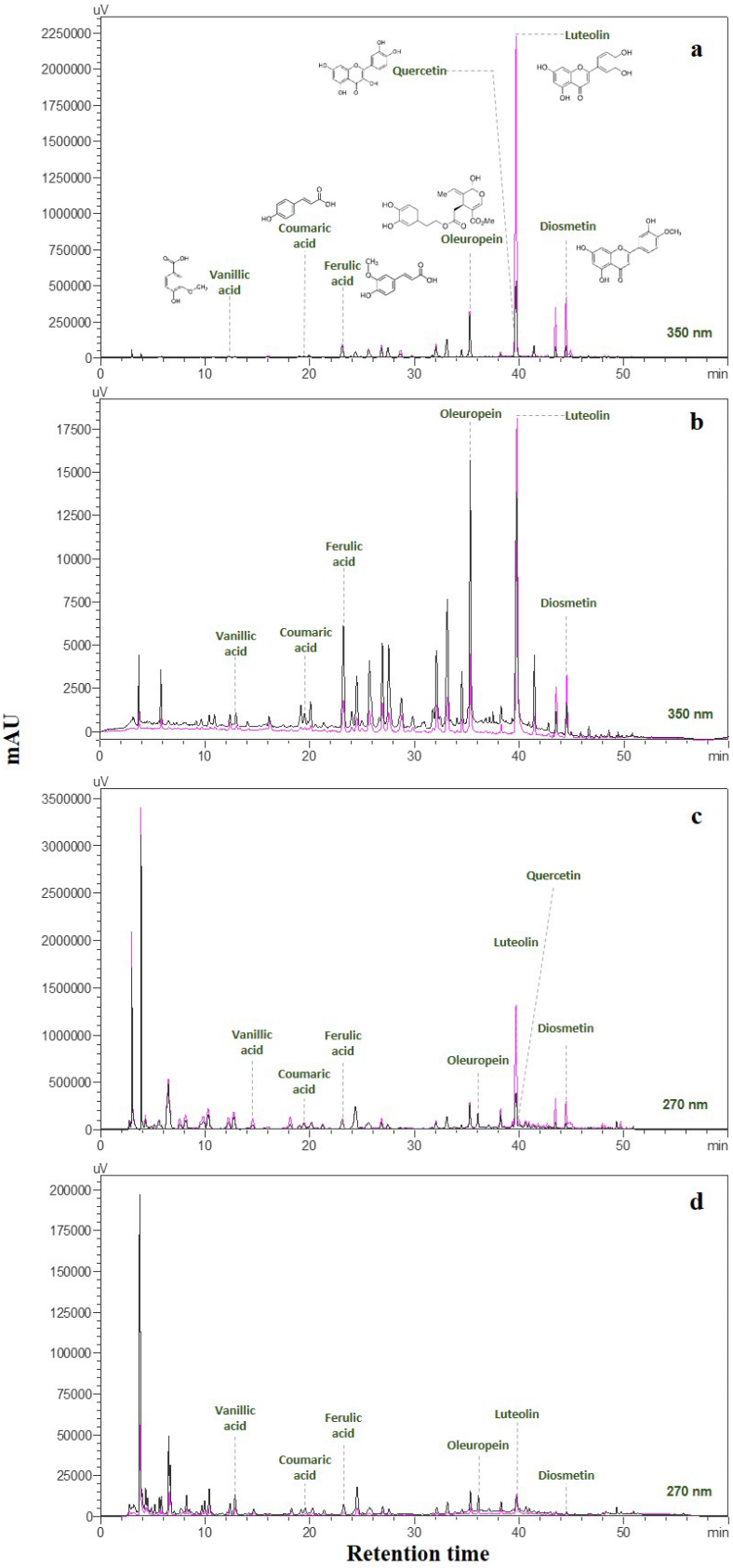

Figure 1. High performance liquid chromatography (HPLC) chromatogram of the polyphenols extracts of olive leaf tea. A) Olive leaf freeze-dried tea at 350 nm; B) Olive leaf liquid tea at 350 nm; C) Olive leaf freeze-dried tea at 270 nm; D) Olive leaf liquid tea at 270 nm. Black line = Boiling method; Pink line = Infusion method.

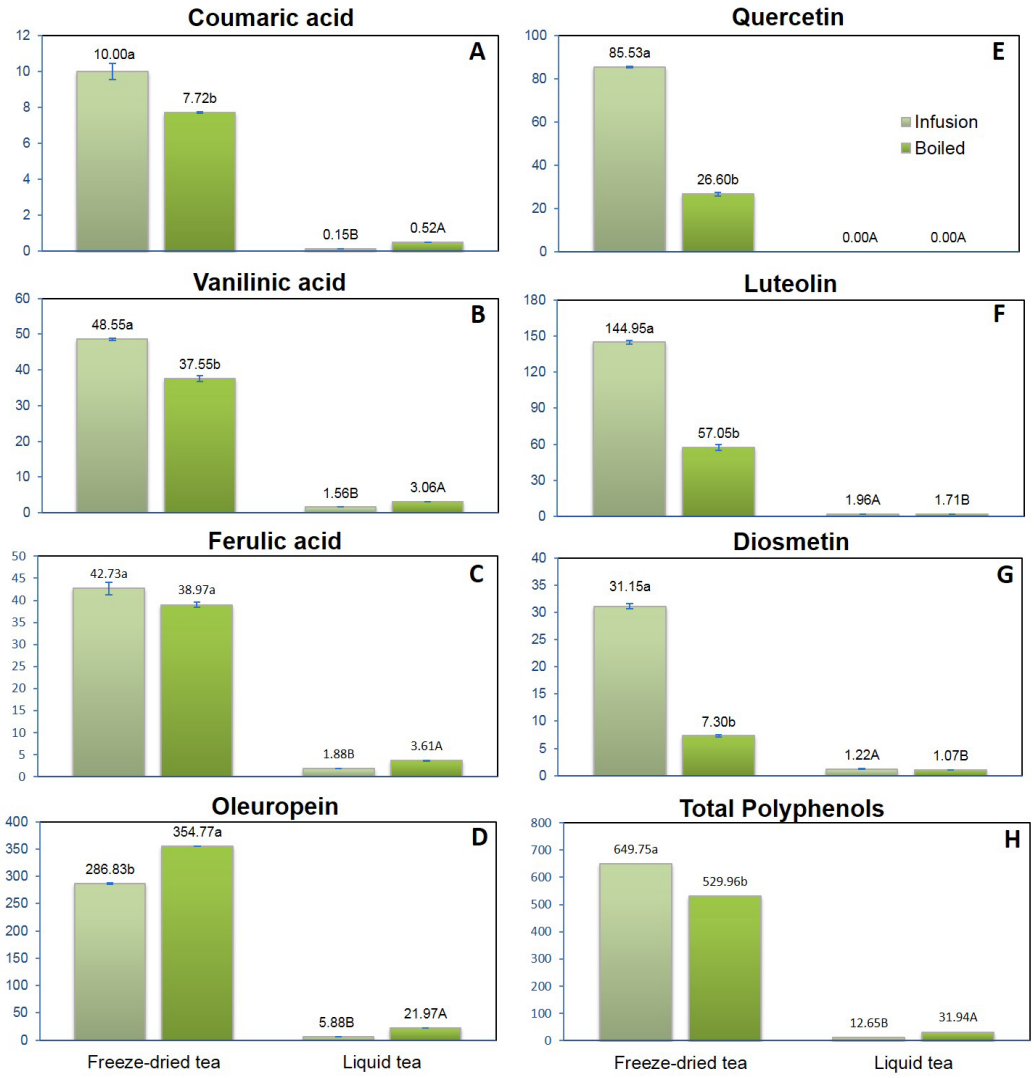

Figure 2. Effect of boiling and infusion on the phenolic acids (A-C), oleuropein (D), flavonoids (E-G), and total polyphenols (H) contents in the freeze-dried tea and the liquid tea. Different lowercase letters represent a significant difference at $p < 0.05$ (n = 3) between treatments of freeze-dried tea, and different uppercase letters represent a significant difference at $p < 0.05$ (n = 3) between treatments of liquid tea. Vertical bars represent the standard error of the mean (n = 3). The results were expressed as dry weight basis.

Table 1. Effect of boiling and infusion on the polyphenols compounds group content, directly in the liquid tea and in the freeze-dried tea*.

Treatments		Compounds group (μg mL^{-1})			Total polyphenols (μg mL^{-1})
		Phenolic acids	Secoiridoids**	Flavonoids	
Freeze-dried tea	Infusion	101.28 ± 2.67^{a}	286.83 ± 2.45^{b}	261.64 ± 1.67^{a}	649.75 ± 3.45^{a}
	Boiling	84.24 ± 2.57^{b}	354.77 ± 0.51^{a}	90.95 ± 3.30^{b}	529.96 ± 5.38^{b}
Liquid tea	Infusion	3.59 ± 0.01^{B}	5.88 ± 0.04^{B}	3.18 ± 0.02^{A}	12.65 ± 0.03^{B}
	Boiling	7.19 ± 0.11^{A}	21.97 ± 0.23^{A}	2.78 ± 0.11^{B}	31.94 ± 0.3^{A}

*Different lowercase letters in the liquid tea treatment columns and uppercase in the Freeze-dried tea treatment columns represent significant differences at $p < 0.05$ (n=3). **Oleuropein group.

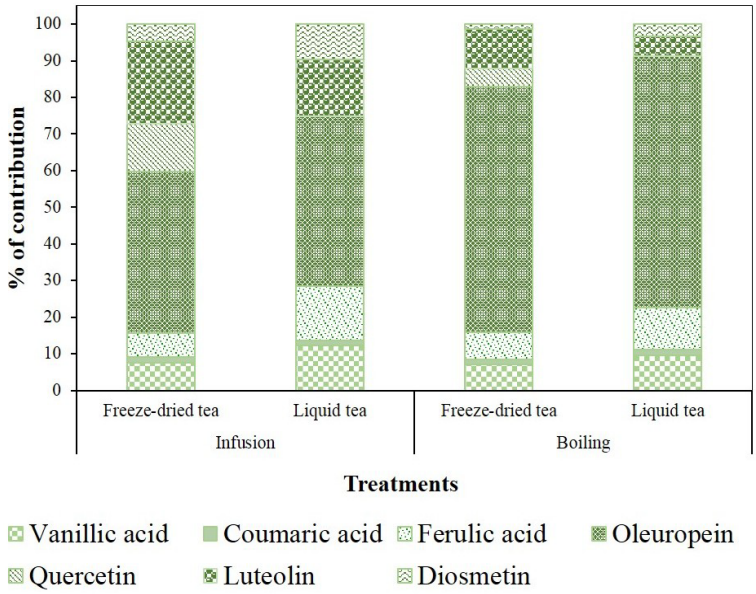

Figure 3. The relative contribution of each compound to total polyphenols content in the treatments boiling and infusion, performed directly in the freeze-dried tea and the liquid tea.

The preparation of tea by boiling yielded the highest concentrations of TPC, oleuropein, and phenolic acids. Prolonged exposure to temperatures exceeding 100 °C induced structural modifications in polyphenols (Zoric et al., 2014). These polyphenols are primarily stored in a conjugated form, often complexed with sugars, and subjecting them to elevated temperatures promoted the hydrolysis of these compounds, resulting in their conversion into aglycones (Deng et al., 2011). Furthermore, the application of thermal processing can disrupt the ester and ether bonds within insoluble-bound phenolics, leading to the release of phenolic acids from the cell walls and an increase in the concentration of free phenolics (Dewanto et al., 2002).

The primary free phenolic acids identified in our investigation include coumaric, ferulic, and vanillic acids. Notably, ferulic acid is a prominent free phenolic acid known for its capacity to establish cross-links with macromolecules within the cell wall (Yu et al., 2001). Of significant interest, our findings demonstrate that liquid tea prepared through boiling exhibited an approximate 47% higher concentration of ferulic acid compared to liquid tea prepared through infusion. This discrepancy, however, was ameliorated through the freeze-drying process, resulting in the production of freeze-dried tea (Dewanto et al., 2002; Yao et al., 2014). The complete liberation of phenolic acids in their free form necessitates exposure to temperatures exceeding 160 °C, as evidenced by previous research (Saulnier et al., 2001). However, elevated temperatures can trigger a self-hydrolysis reaction, a process that may lead to significant degradation of certain phenolic and flavonoid compounds (Lee & Choi, 2012; Malik & Bradford, 2008). Furthermore, the glycosylated and ester-linked forms of ferulic acid hold particular significance in the context of health benefits. Research indicates that these forms exhibit superior suppressive effects on lactate dehydrogenase compared to their free ferulic acid counterparts (Yao et al., 2014).

The most pronounced variations between freeze-dried tea prepared by boiling and freeze-dried tea prepared through infusion were observed in their flavonoid content. The freeze-dried tea derived from boiling exhibited a reduction of more than 50% in its flavonoid content. Elevated temperatures notably impacted quercetin, rendering it undetectable in the liquid tea, regardless of the treatment applied. However, upon concentration through freeze-drying, the disparities in polyphenol content between the infusion and boiling treatments were mitigated.

Unlike the outcomes derived from the direct analysis of liquid tea, the freeze-dried tea infusion samples exhibited the highest concentrations of flavonoids, including quercetin, luteolin, and diosmetin, as well as the highest levels of p-coumaric, ferulic, and vanillic acids. Despite the expected concentration and preservation of compounds through freeze-drying, the substantial difference in phenolic acid levels between

liquid tea and freeze-dried tea is especially intriguing. The observed difference may be attributed to the dilution of compounds in the liquid-tea samples. Consequently, how the tea samples were collected might have influenced these concentrations, underscoring the significance of freeze-drying for tea concentration in subsequent analyses.

If the objective is the consumption of teas from dried leaves, tea prepared by infusion emerges as a richer source of flavonoids. In contrast, the boiling method results in greater availability of oleuropein, and the ferulic, vanillic, and coumaric acids, consequently leading to a higher antioxidant capacity due to the elevated concentrations of total polyphenols it provides. However, for those seeking to utilize olive tree tea as a source of polyphenols, the process of freeze-drying offers distinct advantages in terms of compound recovery. Freeze-drying offers several benefits, including the preservation of a high content of bioactive compounds and an extended storage life.

In general, prolonged exposure and higher temperatures led to the most significant degradation of flavonoids. The degradation of polyphenols is associated with the activation energy (kJ mol-1) required for structural modifications (Zoric et al., 2014). The stability of these compounds is closely linked to their structure; glycosylated compounds exhibit greater stability than their corresponding aglycones (Chaaban et al., 2017). Flavonoids exhibit susceptibility to temperature and duration of heat exposure. As reported by Chaaban et al. (2017), a temperature of 90 °C resulted in more than 50% breakdown, while at 100 °C, an intensified loss of compounds was observed.

3.2 Total phenolic compounds analysis

To analyze the content of TPC, the extractor was investigated to identify the nature and recovery efficiency of the compounds. In freeze-dried tea, boiled preparation provided a higher content of phenolic compounds, regardless of the extractor (Table 2). Among the extraction solutions, water and acetone showed higher total phenolic content in freeze-dried tea boiling. Infusion treatment showed the best results with acetone 50%, followed by water, acetone 80%, and acidified methanol. On the other hand, the ethanol solution was not efficient in extracting the phenolics by the Folin-Ciocalteu method. Our results by the Folin-Ciocalteu method showed that regardless of the extractor, except for ethanol, there is good recovery of phenolic compounds.

Table 2. Total phenolic compound content (g kg^{-1}) performed with different extractors in the olive leaf tea treatments, by infusion and boiling, and in the dried leaves of olive*.

Extractors	*Treatments*		
	Infusion	**Boiling**	**Dry leaves**
Water	176.90 ± 3.38^{Bb}	283.70 ± 9.18^{Aa}	37.37 ± 1.58^{Cbc}
Methanol 80%	112.07 ± 3.97^{Bc}	186.19 ± 66.99^{Ac}	54.02 ± 1.02^{Cab}
Acidified methanol	138.96 ± 8.12^{Bbc}	210.41 ± 5.58^{Abc}	29.52 ± 3.86^{Cbc}
Ethanol 60%	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ad}	0.07 ± 0.00^{Ac}
Acetone 50%	263.80 ± 0.50^{Aa}	294.49 ± 4.05^{Aa}	90.44 ± 8.63^{Ba}
Acetone 80%	167.07 ± 6.11^{Bb}	231.46 ± 6.95^{Ab}	82.50 ± 2.62^{Ca}

*Different uppercase letters in the rows and lowercase in the columns represent significant differences at $p < 0.05$ (n=3).

The recovery of phenolic compounds from olive leaves after dehydration was lower than with additional heat treatment (boiling or infusion) and concentration by freeze-drying (Table 2). The extractors 50% acetone, 80% acetone, and 80% methanol were the most effective in the phenolic compound's extraction, and 60% ethanol was the least effective. The temperature was responsible for the increase of up to 7-fold in the content of phenolic compounds in the preparation by boiling when compared to water at room temperature

(~ 25°C). The methanol acidification did not influence the increase in phenolic extraction. The proanthocyanidin levels within the freeze-dried tea infusion were found to surpass those observed in the freeze-dried tea samples prepared through boiling (Figure 4A). Upon analyzing the liquid tea, the values were approximately eighteen times lower than those found in the freeze-dried tea samples and displayed no significant differences based on the preparation method.

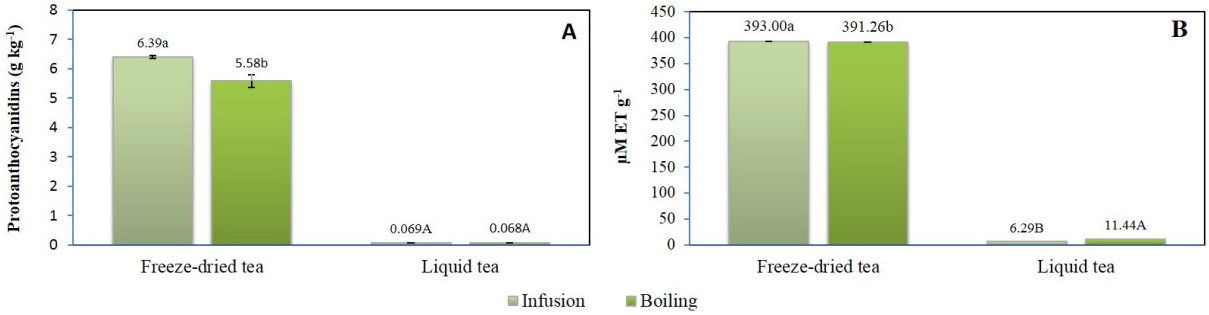

Figure 4. Effect of boiling and infusion methods on proanthocyanidin content (A) and antioxidant capacity (B), performed in the liquid tea and the freeze-drying tea. Different lowercase letter represents significant difference at $p < 0.05$ (n = 3) between treatments of freeze-dried, and different uppercase letters represent significant differences at $p < 0.05$ (n = 3) between treatments of liquid tea. Vertical bars represent the standard error of the mean (n=3).

The levels of phenolic compounds in olive tree teas and their availability in comparison to dry olive leaves were investigated. It was observed that the application of heat, infusion, and boiling treatments, significantly enhanced the availability of these compounds, even when employing acid extraction or less polar solutions. This enhancement can be primarily attributed to the release of bound compounds, a process for which aqueous and methanolic extraction methods may not provide sufficient energy to disrupt these chemical bonds. Specifically, aqueous extraction methods, without heat processing, exhibited a propensity for the extraction of phenolic acids and oleuropein, whereas methanolic solutions exhibited an increased efficiency in extracting flavonoids (Pereira et al., 2007).

In both freeze-dried tea infusion and boiling, 50% acetone proved to be one of the most effective polyphenol extractors. Notably, in the boiling treatment, the water exhibited a TPC concentration equivalent to that of 50% acetone. This result holds significant promise for the food industry, particularly because an inert solvent like water does not compromise product safety (Moure et al., 2001). Furthermore, it ensures that consumers who choose to consume either boiled liquid tea or freeze-dried tea can fully benefit from the product's bioactive potential. However, temperature variations had no discernible effect on tannin levels within the olive leaves, resulting in an absence of significant differences in proanthocyanidin content, which plays a role in shaping the flavor of the tea. It is noteworthy that the proanthocyanidin content in olive leaves naturally registers at lower levels when compared to the leaves of other plants commonly used for medicinal or culinary purposes (Škerget et al., 2005).

The infusion and boiling methods used for preparing olive leaf tea not only influenced the overall polyphenol content but also played a role in shaping the composition of these compounds. When dry olive leaves were ground and analyzed in water at a temperature of 20 °C, it was observed lower concentrations of phenolic compounds. This result underscores the fact that products derived from dried olive leaves are less amenable to the extraction of bioactive compounds, and thus thermal processing becomes imperative to enhance the yield of these compounds. Furthermore, our analysis of olive leaf by-products, encompassing teas prepared through infusion and boiling, as well as their respective freeze-dried iterations, revealed that the chosen preparation methods and concentration levels can also introduce variations in the recovery of these compounds.

3.3 Antioxidant capacity by ABTS assay

The radical scavenging capacity in the freeze-dried tea treatments was stronger than that present in the liquid tea samples, regardless of the treatment (Figure 4B). Among liquid tea treatments, boiling showed a

greater capacity to inhibit radicals compared to the infusion method. However, when the tea was freeze-dried, samples by the infusion method showed a greater radical scavenging capacity than samples prepared by the boiling method.

The hydrolysis of glycosylated compounds enhances the antioxidant capacity, a parameter intrinsically associated with the presence of polyphenols in olive leaves (Škerget et al., 2005; Podsedek, 2007). Therefore, increased levels of TPC and oleuropein played a crucial role in significantly enhancing the antioxidant capacity of boiled liquid tea.

The antioxidant capacity of aqueous or methanolic extracts following heating is influenced not only by the presence of intact compounds but also by the presence of degradation by-products (Özcan & Matthäus, 2017). The hydroxytyrosol moiety of oleuropein has significant radical scavenging potential (Benavente-García et al., 2000). Although its anti-radical capacity, along with that of phenolic acids, is not as high as flavonoids, its presence in abundance and synergistic interactions can modulate the overall antioxidant capacity (Xie et al., 2015; Özcan & Matthäus, 2017). In this way, the process of boiling liquid tea enhances bioactive compound content and radical scavenging potential.

4 Conclusion

The consumption of dehydrated olive leaf teas submitted to boiling offers higher levels of bioactive compounds and antioxidant potential than those prepared by infusion. The tea preparation by boiling the leaves for 10 min does not compromise the oleuropein concentration but reduces the flavonoid levels. The tea concentration by freeze-drying is a way to preserve the flavonoids, phenolic acids, and oleuropein, maintaining the high antioxidant activity of the extract. Thereby, our results suggest that tea freeze-drying is an effective way of recovering polyphenols, which in turn can be used in studies regarding its bioavailability, bioaccessibility, nanoparticle components, as natural additives for the industry, food supplements, time, and product stability.

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References

Afaneh, I., Yateem, H., & Al-Rimawi, F. (2015). Effect of olive leaves drying on the content of oleuropein. *American Journal of Analytical Chemistry*, *6*(3), 246-252. http://doi.org/10.4236/ajac.2015.63023

Ahmad-Qasem, M. H., Ahmad-Qasem, B. H., Barrajón-Catalán, E., Micol, V., Cárcel, J. A., & García-Pérez, J. V. (2016). Drying and storage of olive leaf extracts. Influence on polyphenols stability. *Industrial Crops and Products*, *79*, 232-239. https://doi.org/10.1016/j.indcrop.2015.11.006

Alagna, F., Geu-Flores, F., Kries, H., Panara, F., Baldoni, L., O’Connor, S. E., & Osbourn, A. (2016). Identification and characterization of the iridoid synthase involved in oleuropein biosynthesis in olive (*Olea Europaea*) fruits. *The Journal of Biological Chemistry*, *291*(11), 5542-5554. PMid:26709230. http://doi.org/10.1074/jbc.M115.701276

Badr, A. M., Attia, H. A., & Al-Rasheed, N. (2020). Oleuropein reverses repeated corticosterone-induced depressive-like behavior in mice: Evidence of modulating effect on biogenic amines. *Scientific Reports*, *10*(1), 3336. PMid:32094406. http://doi.org/10.1038/s41598-020-60026-1

Basuny, M. A., & Arafat, S. N. (2018). Olive leaves healthy alternative for green tea. *Current Trends in Biomedical Engineering & Biosciences*, *15*(4), http://doi.org/10.19080/CTBEB.2018.15.555919

Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. Leaves. *Food Chemistry*, *68*(4), 457-462. http://doi.org/10.1016/S0308-8146(99)00221-6

Boss, A., Bishop, K. S., Marlow, G., Barnett, M. P. G., & Ferguson, L. R. (2016). Evidence to support the anti-cancer effect of olive leaf extract and future directions. *Nutrients*, *8*(8), 513. PMid:27548217. http://doi.org/10.3390/nu8080513

Burja, B., Kuret, T., Janko, T., Topalović, D., Živković, L., Mrak-Poljšak, K., Spremo-Potparević, B., Žigon, P., Distler, O., Čučnik, S., Sodin-Semrl, S., Lakota, K., & Frank-Bertoncelj, M. (2019). Olive leaf extract attenuates inflammatory activation and DNA damage in human arterial endothelial cells. *Frontiers in Cardiovascular Medicine*, *6*, 1-11. PMid:31157238. http://doi.org/10.3389/fcvm.2019.00056

Cagliari, A., Martiny, T. R., Nascimento, R., Morais, M. M., & Rosa, G. S. (2022). Effects of different drying conditions on bioactive potential of brazilian olive leaf. *Brazilian Journal of Food Technology*, *25*, 1-16. http://doi.org/10.1590/1981-6723.14721

Casazza, A., Aliakbarian, B., Comotto, M., Souza, P. M., & Perego, P. (2017). Olive leaves infuse and decoct production: Influence of leaves drying conditions and particle size. *Chemical Engineering Transactions*, *57*, 1807-1812. http://doi.org/10.3303/CET1757302

Chaaban, H., Ioannou, I., Chebil, L., Slimane, M., Gérardin, C., Paris, C., Charbonnel, C., Chekir, L., & Ghoul, M. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation*, *41*(5), 1-12. http://doi.org/10.1111/jfpp.13203

Cheurfa, M., Abdallah, H. H., Allem, R., Noui, A., Picot-Allain, C. M. N., & Mahomoodally, F. (2019). Hypocholesterolaemic and antioxidant properties of *Olea europaea* L. leaves from chlef province, algeria using in vitro, in vivo and in silico approaches. *Food and Chemical Toxicology*, *123*, 98-105. PMid:30292622. http://doi.org/10.1016/j.fct.2018.10.002

Colombo, R., Lanças, F. M., & Yariwake, J. H. (2006). Determination of flavonoids in cultivated sugarcane leaves, bagasse, juice and in transgenic sugarcane by liquid chromatography-uv detection. *Journal of Chromatography. A*, *1103*(1), 118-124. PMid:16310199. http://doi.org/10.1016/j.chroma.2005.11.007

Coppa, C. F. S. C., Rosim, R. E., Oliveira, C. A. F., Rodrigues, C. E. C., & Gonçalves, C. B. (2017). Extração de oleuropeína a partir de folhas de oliveira utilizando solvente hidroalcoólico. *Brazilian Journal of Food Technology*, *20*(0), 1-9. http://doi.org/10.1590/1981-6723.16916

Covas, M. I. (2007). Olive oil and the cardiovascular system. *Pharmacological Research*, *55*(3), 175-186. PMid:17321749. http://doi.org/10.1016/j.phrs.2007.01.010

Deng, S., West, B. J., & Jensen, C. J. (2011). Thermal degradation of flavonol glycosides in noni leaves during roasting. *Advance Journal of Food Science and Technology: AJFST*, *3*(2), 155-159.

Dewanto, V., Wu, X., & Liu, R. H. (2002). Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*(17), 4959-4964. PMid:12166989. http://doi.org/10.1021/jf0255937

Ferdousi, F., Araki, R., Hashimoto, K., & Isoda, H. (2019). Olive leaf tea may have hematological health benefit over green Tea. *Clinical Nutrition (Edinburgh, Lothian)*, *38*(6), 2952-2955. PMid:30501915. http://doi.org/10.1016/j.clnu.2018.11.009

Lee, W. J., & Choi, S. W. (2012). Quantitative changes of polyphenolic compounds in mulberry (*Morus alba* L.) leaves in relation to varieties, harvest period, and heat processing. *Preventive Nutrition and Food Science*, *17*(4), 280-285. PMid:24471097. http://doi.org/10.3746/pnf.2012.17.4.280

Liu, L., Ahn, K. S., Shanmugam, M. K., Wang, H., Shen, H., Arfuso, F., Chinnathambi, A., Alharbi, S. A., Chang, Y., Sethi, G., & Tang, F. R. (2019). Oleuropein induces apoptosis via abrogating NF-KB activation cascade in estrogen receptor–negative breast cancer cells. *Journal of Cellular Biochemistry*, *120*(3), 4504-4513. PMid:30260018. http://doi.org/10.1002/jcb.27738

Lockyer, S., Rowland, I., Spencer, J. P. E., Yaqoob, P., & Stonehouse, W. (2017). Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: A randomised controlled trial. *European Journal of Nutrition*, *56*(4), 1421-1432. PMid:26951205. http://doi.org/10.1007/s00394-016-1188-y

Malik, N. S. A., & Bradford, J. M. (2008). Recovery and stability of oleuropein and other phenolic compounds during extraction and processing of olive (*Olea europaea* L.) leaves. *Journal of Food Agriculture and Environment*, *6*(2), 8-13.

Marika, M., Egeria, S., Maria Annunziata, C., Giuseppe, S., Tiziano, V., Raffaele, D. C., & Nadia, C. (2020). Effects of olive oil on blood pressure: Epidemiological, clinical, and mechanistic evidence. *Nutrients*, *12*(6), 1548. PMid:32466599. http://doi.org/10.3390/nu12061548

Meirinhos, J., Silva, B. M., Valentão, P., Seabra, R. M., Pereira, J. A., Dias, A., Andrade, P. B., & Ferreres, F. (2005). Analysis and quantification of flavonoidic compounds from portuguese olive (*Olea europaea* L.) leaf cultivars. *Natural Product Research*, *19*(2), 189-195. PMid:15715265. http://doi.org/10.1080/14786410410001704886

Moure, A., Cruz, J. M., Franco, D., Manuel Domínguez, J., Sineiro, J., Domínguez, H., Núñez, M. J., & Carlos Parajó, J. (2001). Natural antioxidants from residual sources. *Food Chemistry*, *72*(2), 145-171. http://doi.org/10.1016/S0308-8146(00)00223-5

Mylonaki, S., Kiassos, E., Makris, D. P., & Kefalas, P. (2008). Optimisation of the extraction of olive (*Olea europaea*) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. *Analytical and Bioanalytical Chemistry*, *392*(5), 977-985. PMid:18762919. http://doi.org/10.1007/s00216-008-2353-9

Özcan, M. M., & Matthäus, B. (2017). A review: Benefit and bioactive properties of olive (*Olea europaea* L.) leaves. *European Food Research and Technology*, *243*(1), 89-99. http://doi.org/10.1007/s00217-016-2726-9

Pereira, A. P., Ferreira, I. C. F. R., Marcelino, F., Valentão, P., Andrade, P. B., Seabra, R., Estevinho, L., Bento, A., & Pereira, J. A. (2007). Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. *Molecules*, *12*(5), 1153-1162. PMid:17873849. http://doi.org/10.3390/12051153

Podsedek, A. (2007). Natural Antioxidants and antioxidant capacity of brassica vegetables: A review. *Lebensmittel-Wissenschaft + Technologie*, *40*(1), 1-11. http://doi.org/10.1016/j.lwt.2005.07.023

Psaltopoulou, T., Naska, A., Orfanos, P., Trichopoulos, D., Mountokalakis, T., & Trichopoulou, A. (2004). Olive oil, the mediterranean diet, and arterial blood pressure: The greek european prospective investigation into cancer and nutrition (EPIC) study. *The American Journal of Clinical Nutrition*, *80*(4), 1012-1018. PMid:15447913. http://doi.org/10.1093/ajcn/80.4.1012

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9-10), 1231-1237. PMid:10381194. http://doi.org/10.1016/S0891-5849(98)00315-3

Rigacci, S., & Stefani, M. (2016). Nutraceutical properties of olive oil polyphenols: An itinerary from cultured cells through animal models to humans. *International Journal of Molecular Sciences*, *17*(6), 1-28. PMid:27258251. http://doi.org/10.3390/ijms17060843

Saulnier, L., Marot, C., Elgorriaga, M., Bonnin, E., & Thibault, J. F. (2001). Thermal and enzymatic treatments for the release of free ferulic acid from maize bran. *Carbohydrate Polymers*, *45*(3), 269-275. http://doi.org/10.1016/S0144-8617(00)00259-9

Scognamiglio, M., D'Abrosca, B., Pacifico, S., Fiumano, V., De Luca, P. F., Monaco, P., & Fiorentino, A. (2012). Polyphenol characterization and antioxidant evaluation of *Olea europaea* varieties cultivated in Cilento National Park (Italy). *Food Research International*, *46*(1), 294-303. http://doi.org/10.1016/j.foodres.2011.12.022

Shamshoum, H., Vlavcheski, F., & Tsiani, E. (2017). Anticancer effects of oleuropein. *BioFactors*, *43*(4), 517-528. PMid:28612982. http://doi.org/10.1002/biof.1366

Silva, S., Gomes, L., Leitão, F., Coelho, A. V., & Boas, L. V. (2006). Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. *Food Science & Technology International*, *12*(5), 385-396. http://doi.org/10.1177/1082013206070166

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.

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., & Knez, Ž. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry*, *89*(2), 191-198. http://doi.org/10.1016/j.foodchem.2004.02.025

Xie, P. J., Huang, L. X., Zhang, C. H., & Zhang, Y. L. (2015). Phenolic compositions, and antioxidant performance of olive leaf and fruit (*Olea europaea* L.) extracts and their structure-activity relationships. *Journal of Functional Foods*, *16*, 460-471. http://doi.org/10.1016/j.jff.2015.05.005

Yao, S. W., Wen, X. X., Huang, R. Q., He, R. R., Ou, S. Y., Shen, W. Z., Huang, C. H., & Peng, X. C. (2014). Protection of feruloylated oligosaccharides from corn bran against oxidative stress in PC 12 cells. *Journal of Agricultural and Food Chemistry*, *62*(3), 668-674. PMid:24397832. http://doi.org/10.1021/jf404841c

Yu, J., Vasanthan, T., & Temelli, F. (2001). Analysis of phenolic acids in barley by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *49*(9), 4352-4358. https://doi.org/10.1021/jf0013407

Zeriouh, W., Nani, A., Belarbi, M., Dumont, A., De Rosny, C., Aboura, I., Ghanemi, F. Z., Murtaza, B., Patoli, D., Thomas, C., Apetoh, L., Rébé, C., Delmas, D., Khan, N. A., Ghiringhelli, F., Rialland, M., & Hichami, A. (2017). Phenolic extract from oleaster (*Olea europaea* Var. sylvestris) leaves reduces colon cancer growth and induces caspase-dependent apoptosis in colon cancer cells via the mitochondrial apoptotic pathway. *PLoS One*, *12*(4), e0176574. PMid:28426813. http://doi.org/10.1371/journal.pone.0170823

Zoric, Z., Dragovi, V., Pedisi, S., & Garofuli, I. E. (2014). Kinetics of the degradation of anthocyanins, phenolic acids and flavonols during heat treatments of freeze-dried sour cherry marasca paste. *Food Technology and Biotechnology*, *52*(1), 101-108.

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