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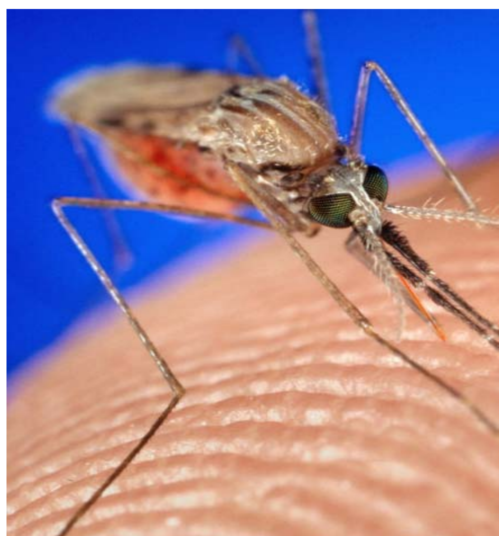

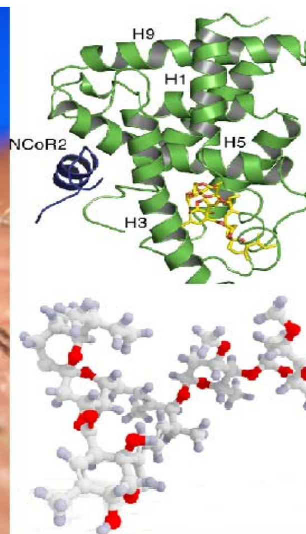

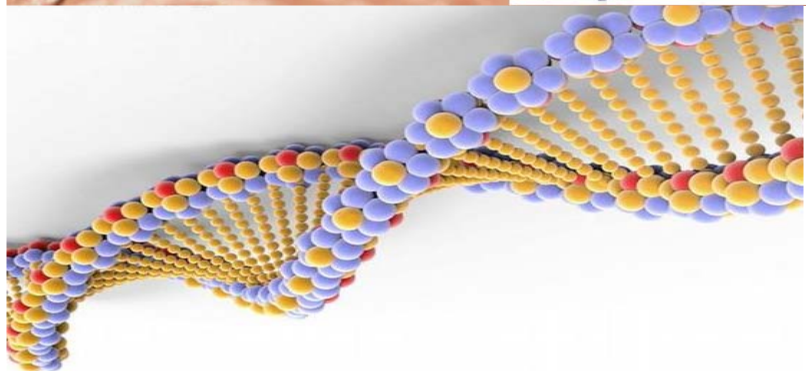

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Antioxidant Capacity and Phenolic Content in Olive Leaf Tisane as Affected by Boiling Treatment

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Abstract *This paper investigated the effect of preparation method on the quality of olive leaf tisane. Secondly, it aimed at evaluating and understanding the effect of boiling treatment on phenolic compounds and antioxidant capacity of an aqueous extract of olive leaves. The Phenolic content was determined by Folin-Ciocalteu method. The antioxidant capacity was assessed by ABTS^{+} method. The Phenolic content and antioxidant capacity depended on extraction procedure of olive leaf tisane. It was found that boiling leads to a decrease in the phenolic content and a rise of antioxidant capacity of aqueous extract from olive leaves. The mass molecular distribution of the polymeric aromatic fraction was analyzed by gel filtration chromatography on Sephadex G50. Results suggested the hydrolysis of phenolic polymers following boiling. Moreover, HPLC analyses showed an increase in rutin, oleuropein and caffeic acid levels in treated sample.*
As a conclusion, thermal processing could be useful for enhancing the antioxidant capacity and the extractability of phenolic compounds in olive leaf tisane.

Key Words: *Olive leaf; Tisane; Antioxidant capacity (TEAC); Total phenolic content (TP); Thermal treatment.*

1. Introduction

In recent years, researchers and consumers have been interesting in biologically active compounds, notably polyphenols, in foods and beverages. These compounds are known by their beneficent effects for human health. Only small amounts of food and beverages are consumed in their raw state. However, most of them need to be processed for safety, quality and economic reason. Processing methods could affect chemical constituents of treated foods and beverages. Thus, they could modify the biological activities of functional compounds [5, 11, 12, 19, 26]. The evaluation of the influence of food processing is a key factor while establishing technological conditions that enable to preserve or improve original activity and bioavailability of naturally occurring functional compounds. To obtain food and beverages of consumption quality, it is important to understand the effect of processing on the biologically active components. Moreover, understanding the consequences of food processing on food composition is one of the important steps to a correct interpretation of study results regarding dietary habits, nutrition and human health [18].

Olive leaves from *Olea europaea* tree, native Mediterranean, have been reported to contain biologically active constituents, especially, phenolic compounds. The main active phenolic compounds in olive leaves are oleuropein, verbascoside, luteolin-7-glucoside and rutin, among others [2, 13]. Phenolic compounds in olive leaves are well known by their antioxidative [2, 13], antimicrobial [21], antiviral [16], anti-inflammatory [17], hypolipidemic [9] and hypotensive properties [22]. These properties make olive leaves a neutraceutical material which benefits human health. Historically, olive leaves have been used as a folk medicine for fever and malaria. Nowadays, this vegetable material is proved and recommended for treating many diseases, such as diabetes, cardiovascular diseases, viral and microbial infections [16, 21, 22]. Therefore, several forms and preparations of olive leaves (powder, intact leaves…) and their extracts are commercialized as phytotherapeutic medicines for human uses. Tisane is the most known and widespread form of preparation from olive leaves for human use.
The objective of the present research was to determine the effect of the preparation method of olive leaf tisane on phenolic compounds and antioxidant capacity. Secondly, this work aimed at evaluating and understanding the effect of boiling treatment on phenolic compounds and on the antioxidant capacity of an aqueous extract of olive leaves.

2. Material and methods

2.1. Chemical reagents and standards

ABTS [2, 2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt] and gallic acid were purchased from Sigma. Oleuropein and hydroxytyrosol were obtained from Extrasynthèse (Geney). Sodium carbonate was purchased from Riedel-de Haën. Folin-Ciocalteu phenol reagent, Trolox, potassium persulfate and syringic acid were obtained from Fluka. Acetonitrile and methanol were of HPLC grade. All other reagents were of analytical grade.

2.2. Sample preparation and experimental process

Olive leaves (*Olea europaea L.* var *Chemlali North*) were randomly hand harvested in August. Collected leaves were washed with tap water and dried at 30°C for three days in the dark under ventilation. Dried leaves were milled and homogenized. Olive leaf powder was passed through a sieve with a mesh size of 18-35 (1-0.5 mm), then it was employed to prepare tisanes using an aqueous extraction procedure. The extraction procedure was carried out in 150 mL glass bottles hermetically sealed. The volume of the extraction was 100 mL.
A complete experimental design (Table 1) was developed to evaluate the influence of preparation method of an olive leaf tisane on Total Phenolic content (TP) and on Trolox Equivalent Antioxidant Capacity (TEAC). The applied design investigated three independent variables. The experimental design included a set of 8 variable combinations. Analytical parameters (TP content and TEAC) were measured in supernatants obtained by centrifuging samples at 3000 rpm during 5 min. Multiple linear regressions of data were done in order to evaluate individual and interactive effects of the tested independent variables. Coefficients of estimated effects were determined using STATGRAPHICS PLUS Software Version 1.4 for Windows and subjected to the analysis of variance (ANOVA) for detecting significance at 95 % level (p-value < 0.05).

A second experiment was carried out in order to investigate the effect of heating time on phenolic compounds and TEAC of an aqueous extract of olive leaves. In this frame, olive leaf powder (1 g) was macerated in 100 mL of distilled water for 5 min. The aqueous extract was separated from the solid phase by means of centrifugation at 3000 rpm for 5 min. The extract was heated until boiling, which was maintained for a desired period of time (0, 5, 30, 60 and 120 min). Independent samples were used for each test.

2.3. Colorimetric determination of Total Phenolic content (TP)

TP content in the samples was determined by UV spectrophotometry, based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin–Ciocalteu reagent [23]. Appropriately diluted centrifuged samples (3.6 mL) were mixed with 0.2 mL of Folin-Ciocalteu reagent and 3 min later, 0.8 mL of sodium carbonate (20 % w/v) was added. The mixture was heated at 100 °C for 1 min. After cooling, absorbance at 750 nm was measured against distilled water. Gallic acid was used as an external standard to prepare a calibration curve. All determinations were performed in triplicate for each sample.

2.4. Determination of Total antioxidant capacity

Trolox Equivalent Antioxidant Capacity (TEAC) assessment of the samples was determined in terms of radical scavenging ability by using improved $ABTS^+$ radical cation decolorization assay [20]. A stock solution of $ABTS^+$ radical cation was prepared by mixing 7 mM ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) Diammonium salt] and 2.45 mM potassium persulfate. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30 °C. Ten microliters of sample were added to 1 mL $ABTS^+$ solution and absorbance decrease at 734 nm was recorded after 6 min. Percentage inhibition of oxidation (PI) was calculated for each sample according to the following equation (Eq. 1):

$$\text{PI (\%)} = \frac{(OD_0 - OD_{6\,\text{min}}) \times 100}{OD_0} \qquad \text{(Eq. 1)}$$

Where: OD_0 denotes the initial absorbance of diluted $ABTS^+$,
$OD_{6\ min}$ denotes the absorbance of the sample after 6 min of reaction.

Series of dilutions were prepared such that they produced between 20 % and 80 % inhibition of the blank absorbance. TEAC calculation was performed as follows (Eq. 2):

$$\text{TEAC (mg L}^{-1}\text{)} = \frac{PI \times dilution}{43.21} \times MM \qquad \text{(Eq. 2)}$$

Where: MM denotes the mass molecular of Trolox (MM= 250 g mol^{-1}).

Trolox was used as an external standard to prepare a calibration curve. The results were expressed as milligrams Trolox equivalent antioxidant capacity per 1L (mg L^{-1} TEAC) in the aqueous extract. All determinations were performed in triplicate for each sample.

2.5. Molecular mass distribution of polyphenols

Chromatography gel filtration on Sephadex G-50 was used to analyze the polymeric aromatic fraction present in an aqueous extract from olive leaf. Three milliliters of the extract were filtered and placed on a Sephadex coarse G-50 column (2.5 - 60 cm) previously equilibrated with $NaNO_3$ 0.05 M containing 0.02 % sodium azide at a flow rate of 0.6 mL min^{-1}. The sample was collected on the basis of 3 ml per tube. The optic density of these fractions was measured spectrometrically at 280 nm. The column was calibrated with syringic acid (MM = 198 Da), lysozyme (MM = 15 kDa) and blue dextran (MM = 200 kDa) [1].

2.6. HPLC analyzes of phenolic compounds

HPLC analyzes were performed with an analytical HPLC unit (Agilent technologies 1200 series), equipped with a diode array detector. The stationary phase was an Atlantis® Waters dC18 column (5 µm particle size; 250 mm; 4.6 mm). The mobile phases were formic acid (19:1) (A) and methanol (B), starting with 5% B and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 35% B at 25 min, 45% B at 35 min, 50% B at 40 min, 100% B at 45 min, 5% B at 46 min, finally, re-equilibration in 4 min to initial composition. The flow rate was 0.9 mL min^{-1} with elution at room temperature. The injection volume was 20 µL and chromatograms were recorded at 280

nm. The data were processed by the ChemStation Agilent technologies software. Phenolic compounds in samples were identified by matching the retention time and the UV spectra of a peak in the sample chromatogram with the peak of a known standard compound. Identifications were confirmed by analyzing a sample supplemented by the corresponding standard.

2.7. Statistical analysis

All measurements were carried out at least in duplicate. The mean values and standard deviations were determined by Excel Software Version 2003 for Windows. The ANOVA analyses, Simple and Multiple Linear Regression were performed using STATGRAPHICS PLUS Software Version 1.4 for Windows. For all analyses, parameters were considered significant at 95 % level when *p-value* < 0.05.

3. Results

3.1. Effect of preparation method of an olive leaf tisane on TP content and TEAC

Various extraction conditions could be used in order to make a tisane. A complete experimental design (Table 1) was developed in order to study simultaneously the effects and interactions of three variables conditions - 1) extraction mode (maceration- decoction), 2) extraction time (5- 60 min) and 3) powder dose (1 %- 10 %)- on TP content and TEAC of prepared olive leaf tisanes. This experimental design was also used to find optimized conditions to obtain olive leaf tisane with the highest nutraceutical value.

Table 1. Influence of preparation method of an olive leaf tisane on total phenolic content (TP) and Trolox Equivalent Antioxidant Capacity (TEAC): Results collected from tests planned according to the complete experimental design 2^3 with three variables.

Tests	Variables			Analytical parameters	
	EM[a]	ET[b] (min)	PD[c] (%)	TP[d] (mg Gallic Acid equivalent. g^{-1} Dry Powder)	TEAC[e] (mg Trolox equivalent. mg^{-1} TP)
Tests performed according to the 2^3 experimental design					
1	Maceration	5	1	5.04 ± 0.32	8.89 ± 0.51
2	Decoction	5	1	5.15 ± 0.34	5.91 ± 0.64
3	Maceration	60	1	3.97 ± 0.60	11.31 ± 1.62
4	Decoction	60	1	4.69 ± 0.63	14.52 ± 2.75
5	Maceration	5	10	2.59 ± 0.16	12.13 ± 1.22
6	Decoction	5	10	2.70 ± 0.10	12.58 ± 0.53
7	Maceration	60	10	2.70 ± 0.17	12.08 ± 0.46
8	Decoction	60	10	3.70 ± 0.17	12.15 ± 0.71

[a] EM: Extraction method; [b] ET: Extraction time; [c] PD: Powder dose (g of powder per 100 mL of water); [d] TP: total phenolic content; [e] TEAC: Trolox Equivalent Antioxidant Capacity.

Results are reported as the Mean ± Standard Deviation of analysis carried out in triplicate.
Results collected from tests planned according to the complete experimental design were presented in table 1. The most active and richest olive leaf tisane in terms of TP content (370.62 ± 17.98 mg L^{-1} GA eq) and in terms of TEAC (4496.66 ± 40.41 mg L^{-1} Trolox eq) was obtained by decoction during 60 min of 10 g powder in 100 mL water. On the other hand, 1 g powder decocted during 5 min in 100 mL water was shown sufficient to get the most efficient extractability of TP content with a yield of 5.15 ± 0.34 mg GA eq. g^{-1} Dry Powder. When considering the antioxidant capacity per mg of TP, the most active phenolic composition (14.52 ± 2.75 mg Trolox eq. mg^{-1} TP) was found in

tisane prepared by decoction during 60 min of 1 g powder in 100 mL water. In all cases, optimal results of TP content and TEAC were obtained by decoction (i.e. thermal treatment at 100 °C).
Statistical analyzes (Table 2) of results obtained with the experimental design proved a significant effect of powder dose on TP content. All other estimated effects were not statistically significant, and neither was the combined effect (i.e. interaction) of the studied variables. The coefficient for TP content was negative, indicating that increasing the powder level decreased the yield of extractability of TP.

Table 2. Influence of preparation method of an olive leaves tisane on total phenolic content (TP) and antioxidant capacity (TEAC): Multiple linear regression results for the estimated effects of three variables and interactions between them.

Variables and Interactions (x)	Analytical parameters					
	TP[d] (mg Gallic Acid equivalent. g^{-1} Dry Powder)			TEAC[e] (mg Trolox equivalent. g^{-1} TP)		
	Coefficient	± error	*p-value*	Coefficient	± error	*p-value*
EM[a]	0,48	0,07	*0,09*	0,18	1,64	*0,92*
ET[b]	-0,10	0,07	*0,36*	2,63	1,64	*0,34*
PD[c]	-1,79 *	0,07	*0,02*	2,07	1,64	*0,41*
EM x IT	0,37	0,07	*0,11*	1,45	1,64	*0,54*
EM x PD	0,07	0,07	*0,50*	0,07	1,64	*0,97*
IT x PD	0,66	0,07	*0,06*	-2,87	1,64	*0,32*
Constant	3,81	0,03		11,19	0,82	

[a] EM : Extraction method; [b] ET : Extraction time; [c] PD : Powder dose; [d] TP : total phenolic content; [e]TEAC : Trolox Equivalent Antioxidant Capacity.
* indicates significance at 95% level (*p-value* < 0.05).

3.2. Effect of boiling time on thermostability of TP and TEAC in an aqueous extract of olive leaves

In this section, the authors aimed at evaluating and understanding the effect of thermal treatment on phenolic compounds and antioxidant capacity of an aqueous extract of olive leaves. Within this framework, an aqueous extract from olive leaves, freshly prepared by maceration, was heated until boiling, which was maintained for a desired period of time (0, 5, 30, 60 and 120 min). TEAC, TP content, and OD at 280 nm were determined for the control and the treated samples. All results are shown graphically in Fig. 1.

A significant increase in TEAC ($p < 0.05$) was observed after boiling, in comparison with the control (322.75 ± 3.92 mg L^{-1} Trolox eq). The thermal treatment leads to an 11 % gain in TEAC, which rises up to 377.16 ± 14.79 mg L^{-1} Trolox eq. A significant difference between the control and treated samples was observed for TP content, as well as for TEAC. However, unlike TEAC, the boiling of an aqueous extract from olive leaves resulted in a loss of TP content when compared to the control (52.8 ± 2.59 mg L^{-1} GA eq). The significant decrease in TP content was noted beyond 5 min of boiling time. TP content was maintained at 46.66 ± 0.74 - 48.2 ± 2.71 mg L^{-1} GA eq (non-significant difference) within 5 to 60 min of boiling time. The loss rate of TP content was 9 %, by comparing to the control. However, the decrease was more pronounced after 120 min of boiling, with a loss rate of 21 %, leading to a TP content of 41.29 ± 3.87 mg L^{-1} GA eq. On the other hand, OD 280nm increased significantly ($p < 0.05$) with increasing the time of boiling. It raised from 7 ± 0.1 to 7.49 ± 0.06.
During thermal treatment in the present studies, the increase of TEAC was correlated with an increase in OD280.

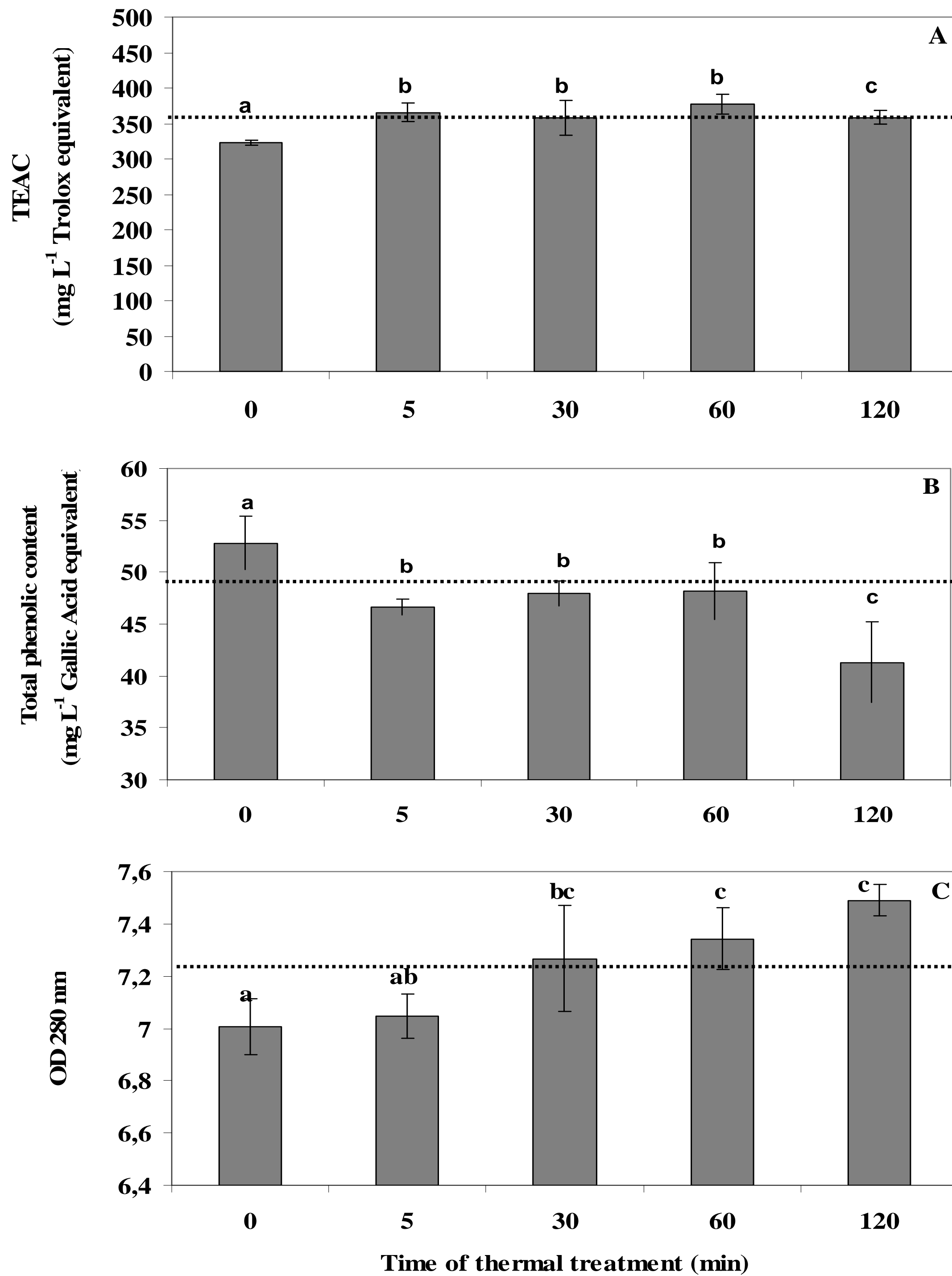

Fig. 1. Thermal treatment at 100 °C of an aqueous extract from olive leaves (obtained by maceration of powder 1 % in water for 5 min): Effect of heating time on Trolox Equivalent Antioxidant Capacity (TEAC) (A), total phenolic content (TP) (B) and color (OD at 280 nm) (C).
Data are reported as the averages of results carried out in triplicate and error bars indicate standard deviations. Significant difference at 95 % level (p-value < 0.05) proved by ANOVA for TEAC (p-value = 0.0136), for TP (p-value = 0.0034) and for OD 280 nm (p-value = 0.0037). The threshold of significance (┅┅), determined by Duncan's multiple range tests, are 350.21 mg L^{-1} Trolox equivalent for TEAC, 48.26 mg L^{-1} Gallic Acid equivalent for TP and 7.23 for OD 280 nm.

3.3. Effect of thermal treatment on mass molecular distribution of polyphenols and on phenolic composition of olive leaf aqueous extract

The present section aimed at evaluating the qualitative changes of phenolic compounds in olive leaf aqueous extract, which could occur following boiling treatment. The mass molecular distribution of the polymeric aromatic fraction was analyzed by gel filtration chromatography on Sephadex G50. The results of the control and heated samples for 120 min are shown in Fig. 2.

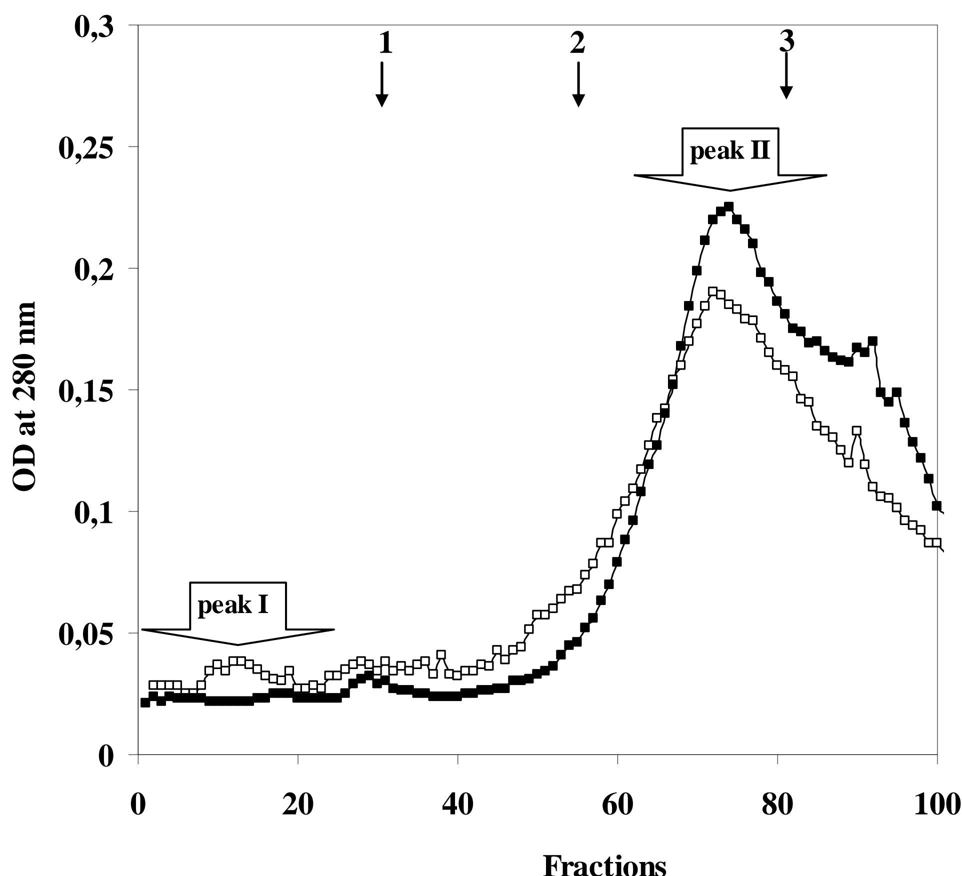

Fig. 2. Effect of thermal treatment of an aqueous extract from olive leaves (obtained by maceration of powder 1 % in water for 5 min) on molecular mass distribution of polyphenolic: The elution pattern of untreated sample (─□─) and heated sample at 100 °C for 120 min (─■─), obtained by Gel filtration chromatography on Sephadex G50.
Standards: 1: Blue dextran (MM = 200 kDa), 2: Lysozym (MM = 15 kDa), 3: Syringic acid (MM = 198 Da).

The elution pattern of the control sample showed two peaks. A small peak (peak I) corresponds to a family of aromatics with molecular mass higher than 200 kDa. A large peak (peak II) represents a family of aromatics with lowest molecular mass (< 200 kDa). Some modifications were observed in the elution pattern of heated sample, in comparison with the control. Peak I was disappeared. It has been also observed the increase in the intensity of peak II, indicating the rise of the fraction with lowest molecular mass.

Results of HPLC analyzes of the main phenolic compounds in olive leaf aqueous extract, as affected by boiling treatment for different durations, are shown in Table 3. Six phenolic compounds, namely, hydroxytyrosol, catechin, caffeic acid, verbascoside, oleuropein, and rutin,

were identified and quantified in olive leaf aqueous extract. The most abundant compound is oleuropein. This results showed that the boiling treatment affected the content of some phenolic compounds. In fact, caffeic acid, oleuropein and rutin levels in treated samples were significantly higher than those in the control. This effect was noted at 5 min of boiling time. However, hydroxytyrosol, catechin, and verbascoside contents were maintained during thermal treatment. The observed variations of the contents of these compounds were not significant.

Table 3. Abundance (% peak area) of the main phenolic compounds present in an aqueous extract from olive leaves (obtained by maceration of powder 1 % in water for 5 min) as affected by thermal treatment at 100°C for different durations.

Compounds	RT*	Time of thermal treatment					*p-value*
		0	5	30	60	120	
Hydroxytyrosol	8.65	0.46 ± 0.05 [a]	0.63 ± 0.04 [a]	0.61 ± 0.12 [a]	0.64 ± 0.07 [a]	0.64 ± 0.03 [a]	*0.1910*
Cathechin	11.32	0.09 ± 0.01 [a]	0.12 ± 0.00 [a]	0.11 ± 0.00 [a]	0.09 ± 0.02 [a]	0.25 ± 0.00 [b]	*0.0020*
Caffeic acid	15.84	0.11 ± 0.01 [a]	0.20 ± 0.02 [b]	0.20 ± 0.05 [b]	0.22 ± 0.01 [b]	0.15 ± 0.04 [ab]	*0.0404*
Verbascoside	26.50	0.38 ± 0.02 [a]	0.51 ± 0.02 [a]	0.51 ± 0.39 [a]	0.49 ± 0.09 [a]	0.46 ± 0.07 [a]	*0.9410*
Oleuropein	32.60	13.84 ± 1.32 [a]	18.88 ± 1.10 [b]	18.49 ± 0.50 [b]	18.08 ± 0.83 [b]	15.71 ± 0.20 [a]	*0.0095*
Rutin	33.90	0.48 ± 0.14 [a]	1.10 ± 0.01 [b]	1.06 ± 0.00 [b]	0.58 ± 0.01 [b]	0.53 ± 0.09 [a]	*0.0027*

*RT: Retention time (min); [ab] Values in each row with different letters differ significantly ($p < 0.05$). Results are reported as the Mean ± Standard Deviation of analysis carried out in duplicate

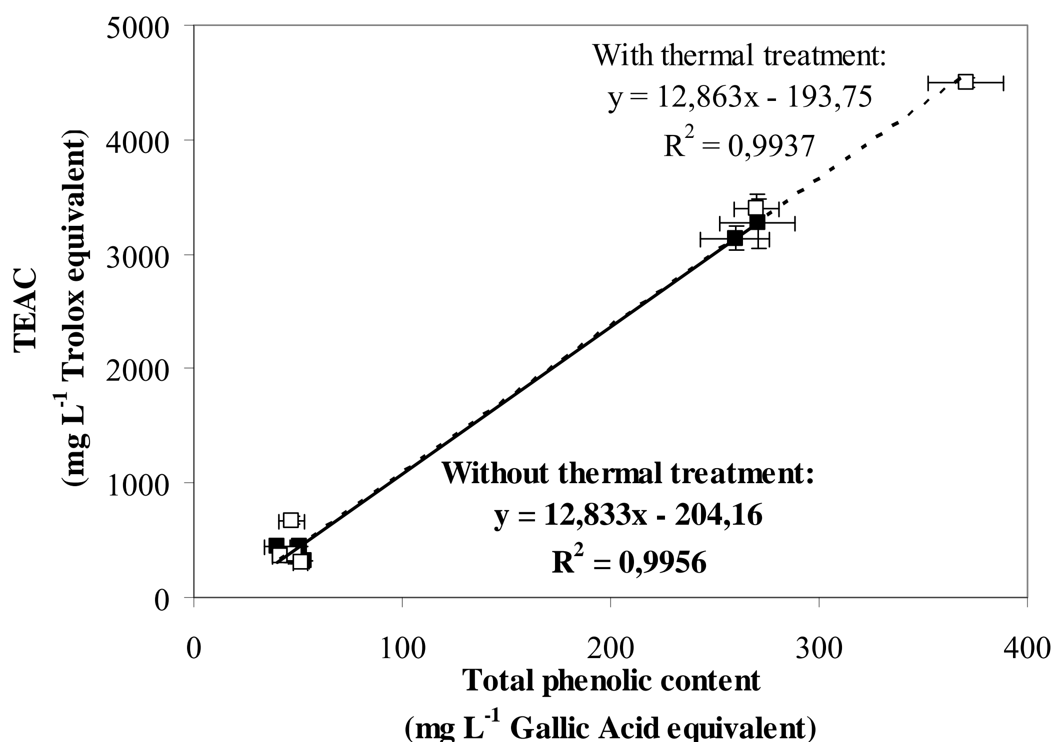

Fig. 3. Effect of thermal processing on the correlation between total phenolic content (TP) and Trolox Equivalent Antioxidant Capacity (TEAC) in an olive leaves tisane: a curve with (□ - - -) and without (■—) thermal treatment. Data are reported as the averages of results carried out in triplicate and error bars indicate standard deviations for TP and TEAC.

3.4. Effect of thermal processing on the correlation between TP content and TEAC in an aqueous extract of olive leaves

Fig. 3 shows the correlation between TP content and TEAC in an aqueous extract from olive leaves before and after boiling treatment. A strong linear positive correlation (Pearson coefficient of 0.995) was observed with a significance level ($p < 0.05$) for untreated extract. After thermal treatment, the correlation remained good with a Pearson coefficient of 0.993 ($p < 0.05$). Although the complexity of the qualitative and quantitative changes occurring during boiling, it appeared that the correlation between TP content and TEAC in an aqueous extract of olive leaves was not affected.

4. Discussion

4.1. Effect of preparation method of an olive leaf tisane on TP content and TEAC

Tisane is a beverage with medicinal effects. It is obtained using aqueous extraction procedure, which consists of a solid-liquid extraction of hydrosoluble compounds. A previous study showed that powder was the best form for obtaining the most active and richest tisane in terms of TP content and TEAC, in comparison with intact leaves. Hence, an increase of specific area seems to significantly influence the extraction of antioxidants and phenolic compounds, because of the much higher specific area of the former that is available for mass transfer [7]. Therefore, it was decided in the present work to use powder form for the preparation of olive leaf tisane.

Heat treatment was observed to increase the extractability of phenolic compounds from olive leaves, as previously noted in various products such as tea [8], citrus peels [10], dictamnus, olive and orange tree leaves [24]. The disruption of the plant cell wall following thermal treatment is one among many mechanisms that could explain the rise of TP content. The cellular disruption leads to the release of polyphenolic compounds which were bound to cellular structures such as lignin and polysaccharides [3]. Besides, exposure to higher temperatures could alter the chemical composition of polyphenolic compounds, causing them to be more extractable [5]. Heat treatment could also deactivate endogenous oxidative enzymes [15]. Therefore, another reason for increased TP contents could be explained by preventing enzymatic oxidation, which causes loss of the TP compound in the raw plant materials [18, 4].

Complicated changes in the qualitative and quantitative chemical composition, notably phenolic compounds, could be occurring during the extraction procedure. Phenolic constituents may interact to produce synergistic or antagonistic antioxidant effects with each other and with other compounds [6]. Thus, the presence of various phenolic compounds could cause diversity in the antioxidant behavior of the extract [8].

4.2. Effect of boiling time on thermostability of TP and TEAC in an aqueous extract of olive leaves

The present study showed a decrease of TP content and an improve of TEAC in an aqueous extract of olive leaves. These findings are in agreement with the findings of Faller and Fialho [5], who reported that domestic cooking of broccoli led to a reduction in TP content and an increase in antioxidant capacity. The changes in the TP content and the overall antioxidant properties as a consequence of heat treatment were depending on the treated materials, among other factors. Rakic et al. [19] reported that thermally treated samples of *Quercus robur* and *Quercus cerris* acorn kernels possess the higher antioxidant capacity and higher TP content than do the native ones. Like *Quercus robur* and *Quercus cerris* acorn kernels, the TP contents and antioxidant activities in the extracts from Shiitake (*Lentinus edodes*) mushroom increased as heating temperature and time increased [4]. While a decrease in the antioxidant potential of sauerkraut juice was found for short heat treatments, a partial recovery of these properties was observed by prolonging heating periods [12]. On another side, the study of Zhang et al. [26] showed that thermal treatment of whole-meal flour from Tartary buckwheat (*Fagopyrum tataricum Gaertn.*) caused a decrease in TP content and

antioxidant capacity. Similarly, domestic cooking of carrot and white cabbage were also found to lead to reductions in TP content and antioxidant capacity [5]. Li et al. [14] noted that the heat treatment did not markedly change the antioxidant capacity of purple wheat bran. However, there was a significant reduction in TP contents. Furthermore, Gawlik-Dziki [6] reported that boiling significantly decreased the amounts of phenolic compounds and the antiradical capacity in fresh broccoli (*Brassica oleracea var. botrytis italica*) florets. In the case of frozen broccoli, boiling increased the TP content and retained the antiradical capacity [6]. Besides, domestic cooking of onion was found to raise TP content. However, it decreases the antioxidant capacity of the treated material [5].
The decrease in TP content in olive leaf extract following boiling could be explained by a modification of chemical structures and decomposition of polymers of phenolic compounds [11, 19], leading to the formation of compounds with the lowest reactivity with the Folin–Ciocalteu reagent. Qualitative changes in chemical composition could explain the alteration of TEAC in treated olive leaf extract since different chemical structures have distinct radical scavenging properties. Besides, synergistic or antagonistic antioxidant effects could occur in a mixture of antioxidant compounds.
During thermal treatment in the present studies, the increase of TEAC was correlated with an increase in OD280. This result suggests the formation of novel antioxidant compounds with maximum absorbance at 280 nm. Positive correlation between the intensity of the color and antioxidant properties has been found in the case of the development of Maillard reactions in heated food products, such as honey [25], white cabbage [12] and Tartary buckwheat flour [26]. In the present study, the rise of TEAC could be explained by the formation of Maillard products frequently occurring in the thermal processing. The formation of these products would mask the real decrease of TP content.

4.3. Effect of thermal treatment on mass molecular distribution of polyphenols and on phenolic composition of olive leaf aqueous extract

The present study noted a modification in the mass molecular distribution of polyphenols following the thermal treatment of the olive leaf aqueous extract. It could suggest that heating of olive leaf extract causes hydrolyses of polyphenolic compounds with high molecular mass, leading to the formation of compounds with lowest molecular mass. Within this frame, Faller & Fialho [5] noted that the conversion of insoluble phenolic compounds into more soluble forms could occur after domestic cooking of vegetables. Heating could release phenols from a polymer such as lignin and polysaccharide as explained by Choi et al. [4] and Faller & Fialho [5]. As for Rakic et al. [19], they observed that hydrolysable tannins were degraded during thermal treatment of oak acorns. As the result of this degradation, it was noted an increase of non-tannin phenolic content, especially gallic acid, after thermal processing. Thus, thermally treated samples possess higher antioxidant capacity than do the native ones. Accordingly, Rakic et al. [19] believe that presence of gallic acid and its low molecular mass derivatives caused the potent antioxidant capacity of the investigated samples. Similarly, Kim et al. [11] showed that thermally processed tannic acid for 15 min had about 67% higher antioxidant capacity. Kim et al. [11] explained the enhanced antioxidant capacity of processed tannic acid by hydrolyzed gallic acid and the hydroxyl groups newly formed on the galloyl group as a result of thermal hydrolysis.

5. Conclusion

This is the first time where the effect of thermal processing on phenolic content and antioxidant capacity was investigated on an aqueous extract from olive leaves. This processing decreases phenolic content while it increases antioxidant capacity. The novelty of this work concerns also his contribution to explaining the effect of thermal processing on antioxidant compounds and capacity in a natural matrix. Using a combination of HPLC and gel

chromatography analysis, we proved the hydrolysis of phenolic polymers and the increases of rutin, oleuropein, and caffeic acid levels, which could explain the antioxidant capacity increase and the phenolic content decrease, following thermal processing of an olive leaves aqueous extract.

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Conflict of interest
The authors declare no conflict of interest.

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