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Thermo-treatment affects *Quercus cerris* L. wood properties and the antioxidant activity and chemical composition of its by-product extracts

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ABSTRACT

Nowadays, there is an increasing interest on thermo-treatment and its effects on wood structure and extraction processes, connected to the wood use for industrial application and for its use as biorefinery. The present investigation aimed to provide the main changes on wood properties (mass loss, color variation and modulus of elasticity) and a comparative analysis of the antioxidant properties and GC–MS profile of the extracts from Turkey oak (*Quercus cerris* L.) wood. Untreated and thermo-treated wood (170 °C x 3 h) samples were compared. Thermo-treatment induced a mass loss (5.1%) in wood, a darkening of color surface (ΔE = 7.6) and a decrease of MOE (4.1%). Moreover samples were extracted using different techniques: maceration (ME), ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE). Extracts were tested to evaluate the content of polyphenols and flavonoids along with the *in vitro* antioxidant activity. Results showed that extracts obtained from thermo-treated wood reported the highest Relative Antioxidant Capacity Index and extraction techniques affected the value in the following rating: UAE > ME > ASE. Qualitative and quantitative measurements of chemical compounds were carried out by GC–MS system. Taking into account the thermo-treatment and extraction techniques, principal component analysis (PCA) was performed also in order to evaluate the relationships among principal chemical compounds. According to results obtained, thermo-treatment and extraction technique had a determinant role in the antioxidant efficiency and, consequently, on the potential application of extracts.

1. Introduction

Turkey oak (*Quercus cerris* L.) is an important forest species largely present in all the South-Eastern Mediterranean countries (Lavisci et al., 1991). In this respect, Turkey oak covers a huge part of European forest area with high relevance from the economic point of view for people who live in marginal Mountain zone.

From a qualitative point of view and technological performances (*e.g.* low dimensional stability, prone to crack, different technological properties between heartwood and sapwood, *etc.*), its wood is poorly appreciated for industrial application but widely used for energetic purposes (Bernetti et al., 1998). The great potential of this wood species and its industrial utilization is strongly linked to the need of pre-treatment in which heat and water play a crucial role. The lack of technical references on the technological properties is certainly connected with the fact that most of the attention has been focused on its defects: less dimensional stability, elevated internal tensions, and low durability. However, the need to provide further information on the characteristics and performance of such wood material is arising because it is among the most widely distributed species in South-East Europe. Wood modification is referred to a process used to improve the physical, mechanical, or aesthetic properties of sawn timber, veneer or wood particles and it is performed by different methods (Sandberg et al., 2017). During the recent years, a thermo-vacuum process has been tested on Turkey oak wood with promising results. In fact the wood material after modification might be used in a wide range of industrial applications such as flooring and garden furniture (Todaro, 2012; Todaro et al., 2012a,b; Ferrari et al., 2013; Todaro et al., 2013, 2015).

Recently biorefinery is becoming an important focus in the European research program, and one of the main scope of green chemistry development is to ensure and generate, innovative and

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renewable products using on-site bioresources such as wood and residual biomass. Moreover, several studies reported the secondary metabolite profile and bioactivity of extracts obtained from bark or other parts of tree (Tálos-Nebehaj et al., 2017). Otherwise, the natural compound profiles achieved from thermo-treated wood is not yet well investigated, even that of increasing interest. Some authors showed the influence of heat treatment on poplar antioxidant properties (Todaro et al., 2017), beech and spruce wood extracts (Ahajji et al., 2009) pointing out biomass potential alternative application beside the en-ergetic or industrial uses.

Generally the thermo-treatment process cause a strong change at the chemical level (Esteves et al., 2008), a better understanding of the differences among extracts during thermo-process is required to provide their use in different fields such as pharmaceutical and/or cosmeceutical. For the reasons described above the valorization of secondary wood products remains an open challenge of a great interest. To the best of our knowledge the influence of different extraction techniques on the antioxidant activity on both, untreated and thermo-treated Turkey oak wood, has not yet been reported. The aim of this study focused on the influence of thermo-treatment on *Quercus cerris* L. wood properties for its valorisation. *Quercus cerris*, despite its wide distribution, is commonly used as firewood, so an increase of its industrial application together with the possible use of its by-products as source of antioxidants could add economic value to this crop species. The thermo-treatment has been combined with different extraction techniques, because it has been demonstrated as the extraction process could impact on yield, phytochemical composition and biological properties.

2. Material and methods

2.1. Thermo-vacuum treatment

A total of 6 m^3 of Turkey oak wood from the Apennine Mountains, collected in the 2016 (Basilicata Region, Italy, long. 16°08′33 lat. 40°32′31), were used for the experiment. Half part of total boards, with a singular dimensions of 30 × 200 × 2300 mm (Radial x Tangential x Longitudinal), were left as control (Ctrl) while the other half material were thermo-treated (TH) with a vacuum plant system, developed by WDE Maspell srl (Terni, Italy) according to the following process:

1 Wood was dried to 0% moisture content under vacuum plant system (185 mbar) at 85 °C for 12 h. From an initial (pre-drying) temperature of 30 °C, the temperature was increased 5 °C each hour to 85 °C. At the end of drying cycle, the kiln was momentary open and the wood samples were quickly weighted. The measurement of the oven dried mass condition was evaluated on ten wood samples previously prepared and included in the vacuum plant system. Moisture content measurements was done according to UNI ISO (1985).
2 Under the same vacuum plant system wood samples were then thermally treated at 170 °C for 3 h. At the beginning of the thermo treatment the air temperature of the vacuum plant system was increased until reaching the set value of 170 °C after 10 h; then the temperature was kept constant for 3 h (1 h for each cm tick). At the end a cooling phase of 6 was conducted until the temperature inside the system reached 60 °C.
3 All the wood boards were at the end conditioned at the controlled ambient of 20 °C and 65 of relative humidity until the equilibrium moisture content was reached.

2.2. Wood properties

The mass loss (ML) was determined by weighting each treated board immediately after the drying process and at the end of the thermal treatment, as previously detailed by Ferrari et al. (2013). However, the development of a numerical tool to predict the mass loss of a wood board during heat treatment as a marker of the process advancement has been recently published (Silveira et al., 2018).

Color analysis was obtained by a Minolta CM-2002 Spectrophotometer (Minolta Corp, Osaka, Japan) with a spot probe of 8 mm diameter. According to CIE $L^*a^*b^*$, which is a color space organized in a cube form and defined by the International Commission on Illumination (French *Commission Internationale de l'Eclairage*) the chromaticity coordinates as L^*, a^* and b^* were measured. The CIE $L^*a^*b^*$ system is made up of three coordinates. The L axis from top to bottom and the value maximum is 100, which would be a perfect reflecting diffuser (lightness), while the minimum for L^* would be 0, which would be black. The a and b axes have no specific numerical limits. Positive a^* is red; negative a^* is green. Positive b^* is yellow and negative b^* is blue. Forty measurements were recorded for each treatment covering both side of the boards. A total of 40 boards have been used between control and thermo-treated material. The total color change ΔE* was calculated according to Todaro et al. (2013). The total color change ΔE* was calculated to evaluate the color variation, measured in a same point of a board, due to the thermal treatment. In this case the coordinates L^*, a^* and b^* refer to the same point and are measured at different moments (before and after the thermal treatment).

The modulus of elasticity (MOE) of the boards, a wood stiffness measurement, was evaluated by the Microsecond Timer (Fakopp Enterprise, Agfalva, Hungary), with a resonance frequency of 23 kHz. This device measures the stress wave velocity in the fiber direction of wood samples through a transducer pin that was placed at a distance of 2 m with an angle of 45°. At least 5 measurements were performed on each board sample, for a total of 40 boards equally distributed between control and thermo-treated material (Cetera et al., 2016).

2.3. Preparation of wood extracts and their UV–vis spectra

Untreated (Ctrl) and thermo-treated (TH) wood samples after the above aforementioned conditioning were randomly selected, splitted and milled in powder trough a 40 sieve-mesh by cutting mill machine (Retsch GmbH, Germany). Drive power 1.5 kW and the rotor speed at 1.500 min^{-1} was used for size reduction. Both untreated and thermo-treated wood have been used for the extraction. For the extraction three different techniques have been used: maceration extraction (ME), ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE). For all extraction techniques, 10 g of small size wood were extracted by using a mixture of ethanol:water (70:30 v/v) as solvent, after a pretreatment with *n*-hexane in the same conditions of ethanol:water extraction (shown below) to remove lipophilic components largely known to do not possess antioxidant activity (Todaro et al., 2017; Ahajji et al., 2009). ME was carried out at room temperature by stirring the sample for 1 h in solvent at a sample-to-solvent ratio of 1:5 (w/v), whereas UAE by using an ultrasonic bath (Branson 1800) at the same conditions of ME. Solvent has been replaced three times. Extraction by ASE system (ASE 150, Dionex Corporation, Sunnyvale, CA) was carried out at 100 °C at 1500 psi for 3 cycles of 5 min each. After the extraction, the solutions were filtered and solvent was removed with a rotary evaporator at 37 °C. Dried extracts were kept in the dark at room temperature until their use (48 h) and extraction yields were calculated according the following formula (Eq. (1)):

$$\% = \frac{dried\ extracts\ (g)}{milled\ wood\ (g)} \times 100 \quad (1)$$

Obtained extracts were used to compare their UV–vis spectra and spectrophotometric analysis were acquired using a SPECTROstar Nano (BMG Labtech, Germany).

2.4. Total phenol content (TPC)

The total polyphenol content (TPC) was determined by the Folin–Ciocalteu reagent method (Mezrag et al., 2017). An aliquot of

extract (75 µL) was mixed with, 500 µL of Folin–Ciocalteu and 500 µL of Na_2CO_3 solution (10X) and finally, water was added to reach the final volume of 1.5 mL. After the incubation for 1 h in the dark at room temperature, the absorbance of the mixture was read at 723 nm. The TPC of the extracts was expressed as mg of Gallic acid equivalent (GAE) per gram of dried extract ± standard deviation (SD).

2.5. Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined by using aluminum chloride (Todaro et al., 2017). Quercetin was used as standard reference. An aliquot of extracts (500 µL) was added to 15 µL of $NaNO_3$ (5X). After 5 min, 30 µL of $AlCl_3$ (10X) was added, after 1 min, the reaction mixture was treated with 100 µL of 1 mM NaOH and finally, was diluted with 1 mL of water. The absorbance was measured at 510 nm. Three independent experiments were carried out and results were expressed as mg of quercetin equivalents (QE) per gram of dried extract ± standard deviation (SD).

2.6. Radical-scavenging activity

The radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured (Russo et al., 2015). DPPH methanol solution (200 µL) at 100 µM were added to 50 µL of extract at different concentrations in 96-well plate and kept in the dark for 30 min. Absorbance at 515 nm was recorded. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity (Shah and Modi, 2015). Three independent experiments performed in triplicate and results of radical scavenging of each samples were expressed as milligrams of Trolox equivalent (TE) per gram of dried extract ± standard deviation (SD).

2.7. Ferric reducing antioxidant power (FRAP)

As reported by Todaro et al. (2017), FRAP reagent was prepared daily with 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ (in 40 mM hydrochloric acid) in 10:1:1. ratio. FRAP reagent (225 µL) was added to 25 µL of extract or methanol (for the blank) in the 96-well plate and incubated at 37 °C for 40 min. Trolox was used as standard. Three independent experiments performed in triplicate and results were expressed as mg of Trolox equivalent (TE) per gram of dried extract ± standard deviation (SD) (Russo et al., 2015).

2.8. β-carotene bleaching assay (BCB)

The inhibition of lipid peroxidation of wood extracts was assayed by the β-carotene bleaching (BCB) method (Dekdouk et al., 2015). BHT was used as the standard. β-Carotene (0.2 mg in 0.1 mL chloroform), linoleic acid (0.02 mL) and Tween 20 (0.2 mL) were transferred into a round flask. Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator and 50 ml of distilled water was added. Extract (50 µL) at 1 mg/ml was added to 950 µL of emulsion, and then the solution (250 µL) was transferred into a 96 well plate. The absorbance was read every 30 min (0, 30, 60, 90, 120, 150 and 180 min) at 470 nm. Three independent experiments performed in triplicate and result of lipid peroxidation inhibition was expressed as percentage of antioxidant activity (% A.A.) ± standard deviation (SD) using the following formula (Eq. (2)):

$$\%A.\,A. = \left[1 - \left(\frac{A\ sample\ T0' - A\ sample\ T180'}{A\ blank\ T0' - A\ blank\ T180'}\right)\right] \times 100 \quad (2)$$

2.9. Gas chromatography-mass spectrometry (GC–MS)

Gas Chromatography-Mass Spectrometry (GC–MS) was used for qualitative and quantitative determination of organic compounds in Turkey oak extracts. The extracts were injected into a gas chromatographic system consists of a capillary column into a thermostat oven, crossed by a stream of helium. The various chemical species that compose the sample were separated into the column and detected by mass spectrometer. Separation and detection of the analytes was achieved using an HP 6890 GC system equipped with an HP 5963 MS selective detector and a capillary column (HP-5MS, 30 m × 0.25 mm I.D., 0.25-µm film thickness; J&W Scientific, CA, USA). Samples were injected directly into the column at a temperature of 80 °C. After injection, the temperature was held at 80 °C for 3 min, and then heated to 250 °C at a rate of 20 °C min^{-1} and held for 20 min, according to Lovaglio et al. (2017). The identification of the compounds was based on computer matching of the mass spectra with the NIST11 library.

2.10. Statistical analysis

Results are expressed as mean ± standard deviation (SD) of three independent experiments performed in triplicate. Principal Component Analysis (PCA), an unsupervised multivariate statistical tool that analyses data sets consisting of a large number of variables, has been also used. It is able to develop a new and easier model with a smaller number of artificial variables that accounts for most of the variance in the normalized data set. To verify the correlations among antioxidant methods and chemical compounds, Pearson correlation coefficient was determined. It has been considered the relationship between compounds (at least present in 3 or more samples) and antioxidant activity results obtained from each test. PCA and Pearson coefficient were computed using the statistical package Statistica for Windows (ver. 5.1., 1997, Statsoft Inc., Tulsa, OK, USA).

Relative Antioxidant Capacity Index (RACI), as new concept has been used to easily understand and compare results obtained from different assays (Sun and Tanumihardjo, 2007). RACI is an adimensional index, that has been demonstrated to be an useful tool for comparison of results coming from different assays. In fact, RACI has been used to have a clear comparison among the antioxidant activity results obtained from DPPH, FRAP and BCB assays, and it has been calculated using Excel software (Microsoft, Washington, USA). The values of antioxidant capacity in each data set are transformed into standard scores, derived by subtracting the mean from the raw data divided by the standard deviation (Sun and Tanumihardjo, 2007). The mean of standard scores was used for the RACI calculation.

3. Results and discussion

3.1. Wood properties

Mass loss (ML) is considered an important indicator of the severity of the thermal process (Ferrari et al., 2013). It is affected by the degradation of the chemical components of wood, mainly hemicellulose, with direct effect on extract composition. The current study reported that the value of ML for TH T. oak was found to be 5.1%. This result was higher to that reported by Ferrari et al. (2013) for oak treated in thermo-vacuum conditions (1.5%) by using similar parameters. This difference could be due to the dissimilar drying processes of wood between the two experiments. However, literature describes that mass loss increases with increasing the process temperature, duration of the treatment and relative humidity in the heating atmosphere (Esteves et al., 2007). The mass loss is mainly due to the degradation of carbohydrates such as hemicelluloses and cellulose (Hakkou et al., 2005).

In term of color the lightness (L*) decreased significantly with heat treatment, suggesting that the surface of thermally modified wood darkened significantly. The a* value showed a slight rise, b* remained unchanged, whereas the total color variation (ΔE*) was 7.6 ± 0.9 (Table 1). This indicates a moderate red shifting of wood color, according to Ferrari et al. (2013). It is largely known that during thermal

Table 1
Mass loss, color variation and MOE for untreated (Ctrl) and thermo-treated (TH) *Quercus cerris* wood.

	ML (%)	L^*	a^*	b^*	ΔE^*	MOE (N/mm^2)
Ctrl		61.0 (*1.1*)	7.7 (*0.5*)	21.4 (*0.8*)		16,171.3 (*1443.4*)
TH	5.1 (*0.4*)	53.4 (*1.3*)	8.5 (*0.3*)	21.6 (*0.7*)	7.6 (*0.9*)	15,530.3 (*686.0*)

Results were reported as mean (standard deviation); ML (%), mass loss expressed as percentage; L^*, lightness; a^* (red/green chromaticity); b^* (yellow/blue chromaticity); ΔE^*, Total color change; MOE (N/mm^2), modulus of elasticity expressed as Newton/millimeter^2).

treatment of wood the changes in color are principally due to the presence of extracts (Varga and van der Zee, 2008). Generally, the changes in wood color due to the effect of thermal treatment was mainly attributed to formation of color compounds after chemical reactions such as aldehydes and phenols. The darker tonality is often the results of colored degradation products from hemicelluloses (Sehlstedt-Persson, 2003; Esteves and Pereira, 2008) whereas the yellowing of wood surfaces indicate modification of lignin (Pandey, 2005). However, darker treated wood is often appreciated as substitute for some tropical hardwoods. The drawback is that the brownish color is not stable against light exposure (Tolvaj and Mitsui, 2005). In fact if the wood surface is exposure outdoors, the color pigments are degraded and washed out leaving a bleaching and greyish appearance. In addition, Rowe (1979) studied the extractives in hardwoods and affirmed that catechin and gallocatechin in oak wood are undoubtedly involved because they are easily oxidized to deeply colored quinones.

MOE provides substantial insights into the mechanical properties of T. oak wood. The temperature (170 °C) used for the treatment highlighted a slight decrease of MOE value in TH wood, providing important information for the industry with regard to the mechanical behavior of this wood. As already demonstrated (Cetera et al., 2016) the low differences in MOE between Ctrl and TH could be attributed to the absence of steaming process and the moderate temperature utilized during thermal process. However, Volkmer et al. (2014) highlighted the negligible influence of heat pressure steaming on MOE for Common oak (*Quercus robur* L.).

On the other hand the temperature and time of thermal treatment depends on wood species. High temperature could cause an increase of mass loss and dark colour, but a decrease of equilibrium moisture content. As reported by Ferrari et al., 2013, these changes are more pronounced in oak wood. The thermo treatment at the temperature of 170 °C was set for industrial application, taking in account that higher temperature were recognized to be the cause of pronounced defects on oak wood samples *i.e.* cracks and deformations.

3.2. Extraction yield and UV–vis spectra

Generally, the properties and yield of secondary metabolites from natural sources are strongly associated with various parameters, such as extraction technique, solvent, and temperature. To obtain more information on the chemical profile and antioxidant activity of Turkey oak wood, in this study, three different techniques as ME, UAE and ASE, were applied to Ctrl and TH wood by using 70% ethanol solution for the extraction, as previously reported (Ahajji et al., 2009; Todaro et al., 2017). The extract yield was calculated and results are reported in Table 2. The highest extraction yields were reported by using ASE technique, and it is also evident as the TH affected positively the extraction (Table 2) with an increase of the yield in TH-ASE. The TH did not affect the yield when ME and UAE were used showing similar value of Ctrl wood extracts. Ultrasound waves have been shown to aid the extraction in a number of plant materials by significantly reducing extraction times and increasing maximum extraction yields compared with maceration (Aspé and Fernández, 2011; Paniwnyk et al., 2001; Toma et al., 2001), but in our case the increase was not significant. While, the use of high temperature of the extract technique ASE (100 °C) on TH and Ctrl T. oak wood, allowed to obtain an extraction yield almost thrice than those obtained by ME and UAE.

Table 2
Extractive yield (%) of wood extracts from *Quercus cerris*.

	Ctrl	TH
ME	1.01 ± 0.12^a	1.09 ± 0.98^a
UAE	1.32 ± 0.19^a	1.20 ± 0.15^a
ASE	2.55 ± 0.31^b	2.90 ± 0.25^c

Untreated (Ctrl) and Thermo-treated (TH) *Quercus cerris* wood, Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE). Different letters indicate statistically significant differences ($p < 0.05$). Values represent means ± SD (N = 3).

A preliminary screening of the different composition of wood extracts has been made evaluating the UV–vis spectrum of the extracts (Fig. 1). All spectra, acquired at the same concentration (0.2 mg/mL), demonstrated evident differences. Extracts showed UV absorbance at 280 nm (typical of phenolic compounds), and a shoulder at 330–350 nm; this latest appeared more pronounced in thermo-treated extracts, in particular in TH-UAE and TH-ME.

3.3. Total polyphenol (TPC) and flavonoid (TFC) content and antioxidant activity

According to Luís et al. (2012) the mixture of water and ethanol is the solvent more appropriate to extract chemical compounds for plant materials with biological activity. All extracts were tested to evaluate the total content of polyphenols (TPC) and flavonoids (TFC).

Results reported that quantitative differences of TPC were observed for both Ctrl and TH taking into account the extraction techniques (Fig. 2A). The thermo-treatment affected positively the content of polyphenols in UAE (350.28 ± 33.91 mg GAE/g) and ME (270.41 ± 14.91 mg GAE/g) showing content twice than that obtained from Ctrl. Instead no difference in TPC was observed in Ctrl and TH extracts obtained by ASE, but this technique allowed to recover higher amount of polyphenols than Ctrl-ME and Ctrl-UAE.

Previous study was conducted on T. oak heartwood from different site in Kosovo, showing that the TPC ranged from 284.2 to 338.8 mg GAE/g extract by using ultrasonic bath at 40 °C and 50% ethanol/water solution as solvent (Bajraktari et al., 2018). This result is congruent with what we have reported here, and it confirmed that the solvent and the extraction technique substantially affect the content of secondary metabolites. Bajraktari et al., 2018 also investigated the presence of flavonoids in *Q. cerris* wood extract showing results as mg of catechin equivalent (mg CE) per gram of dry extract (range from 61.2 to 67.4 mg CE/g of dry extract).

In this study the flavonoid content of wood extracts ranged from 85.0 ± 3.0 to 263.4 ± 4.6 mg QE/g. It is evident as the thermo-treatment increased the content of flavonoids in wood extracts (Fig. 2B) in the following order UAE > ME > ASE. Thus, the ultrasound waves allowed to obtain a higher content of flavonoids from TH-UAE, but not from Ctrl-UAE. These results are congruent with what has been shown above, in the preliminary UV–vis spectra analysis, taking into account that flavonoids absorb in 330–360 nm range. It is interesting to observe as the high extraction yield obtained with ASE was not always related with an increase of content of polyphenols and flavonoids, suggesting as other not phenolic compounds have been extracted.

3.4. Antioxidant activity and Relative Antioxidant Capacity Index (RACI)

Previous reports demonstrated as different chemical methods must be used for the determination of the antioxidant activity (Luís et al.,

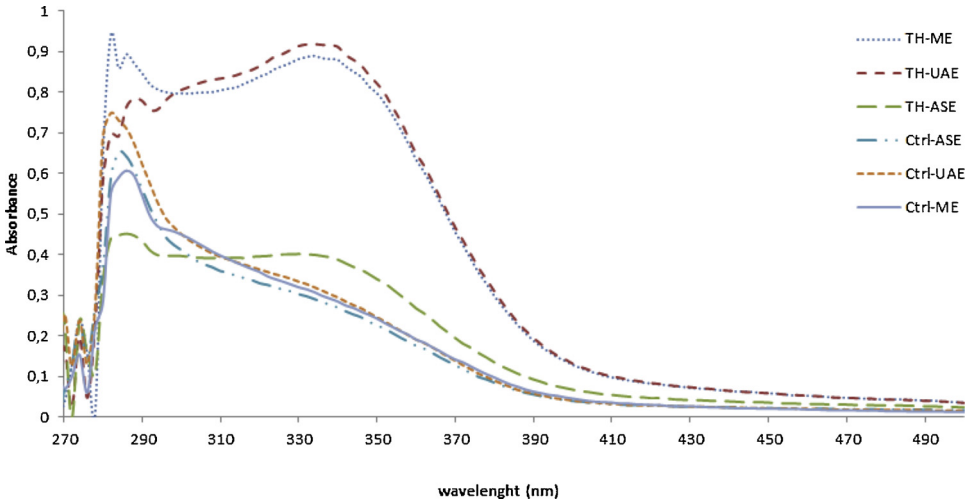

Fig. 1. Absorbance spectrum of control and thermo-treated Turkey oak samples.
Untreated (Ctrl) and Thermo-treated (TH) *Quercus cerris* wood, Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

2012; Fidelis et al., 2018). For this reasons, three different complementary *in vitro* assays were used to evaluate the radical scavenging activity (DPPH), reducing power (FRAP), antilipoperoxidative effect (BCB).

All extracts demonstrated a different behavior with the *in vitro* antioxidant activity, probably due to their chemical composition after the thermo-treatment and the extraction techniques.

TH-UAE reported the highest radical scavenging activity (101.1 ± 13.5 mg TE/g) and reducing power (450.4 ± 13.9 mg TE/g), followed by TH-ME in both assays (Fig. 3A). Also TH-ASE showed higher reducing power (413.7 ± 18.1 mg TE/g) than its Ctrl extract (161.9 ± 6.4 mg TE/g) (Fig. 3B). On the other hand Ctrl-ASE was more active *vs*. the DPPH radical than TH. Lipid peroxidation inhibition was measured by BCB test. All extracts reported a percentage of inhibition higher than 50% and they ranged from 54.3 ± 2.7 to 64.7 ± 0.5, in Ctrl-ASE and TH-ASE, respectively (Fig. 3C). No differences were observed between Ctrl and TH extracts.

To get a complete picture of antioxidant capacity, results obtained from different chemical assays for evaluating of antioxidant activity, along with TPC, were used to calculate Relative Antioxidant Capacity Index (RACI). According to RACI results the heat treatment affected positively the antioxidant activity. In fact, all the extracts obtained from TH wood reported the highest RACI values (Fig. 3D). Among extract techniques, UAE assisted extraction allowed to obtained extracts with the highest relative antioxidant activity (RACI of 1.17). Although the ASE showed the highest extract yield, the extracts reported lowest RACI. Thus, the high temperatures used for the extraction affected positively the yield, but not the antioxidant activity.

The ultrasound waves can penetrate the matrix material, rupturing the cell walls and driving the solvent into the matrix to extract the targeted components (Sun et al., 2011; Romdhane and Gourdon, 2002). It seems that UAE could preserve the bioactivity of the extracts, whereas the high temperature of the ASE could have negatively affected the activity of the extracts, although it increased the extraction yield, confirming the presence of compounds other than phenolics.

3.5. Gas chromatography–mass spectrometry (GC–MS)

Gas chromatography-mass spectrometry (GC–MS) is generally used for the identification and quantification of chemical compounds in several plant extracts (Pisoschi and Negulescu, 2011; De Falco et al., 2018).

Table 3 summarizes the characteristics of the GC–MS peaks of Ctrl and TH extracts subjected to different extraction techniques.

Qualitative analysis demonstrated that several aromatic and phenolic compounds were found in all extracts either TH or Ctrl, such as 2,6-dimethoxy- Phenol, 3–4,5-trimethoxy-Phenol, 4-hydroxy-3,5-dimethoxy- Benzoic acid, 4-Methoxy-4′,5′-methylenedioxybiphenyl-2-carboxylic acid and 3,5-dimethoxy-4-hydroxycinnamaldeide. The area (%) of this latest, called also sinapaldehyde, increased with the thermal treatment, in fact was found to be abundant in TH samples in the following order TH-UAE (12.34%), TH-ME (7.14%) TH-ASE (6.11%). Instead the presence of coniferaldehyde [3-(4- hydroxy-3-methoxyphenyl)-2-Propenal] was reported only in TH extracts with the highest percentage area value in TH-UAE. Some compounds were found exclusively in TH-UAE as 1-(2,4,6-trihydroxy-3-1-Butanone methylphenyl)-, 14-Pentadecenoic acid and bis(2-ethylhexyl) ester Hexanedioic acid. Results of GC–MS are consistent with *in vitro* assay,

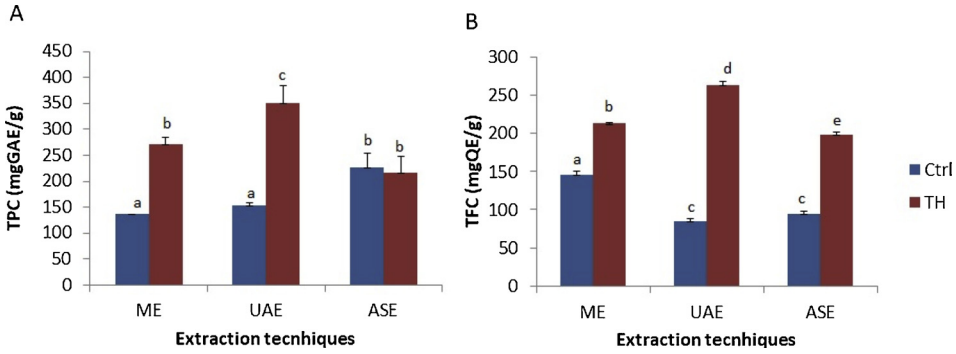

Fig. 2. Total polyphenol content (A) and total flavonoid content (B) of Turkey oak extracts. Bars with different letters indicated mean values significantly different ($P < 0.05$) according to the Duncan test. Total polyphenol content (TPC) expressed as milligrams of Gallic acid Equivalent per gram of dry extract (mgGAE/g), total flavonoid content (TFC) expresses as milligrams of Quercetin Equivalent per gram of dry extract (mgQE/g), Untreated (Ctrl) and Thermo-treated (TH) *Quercus cerris* wood, Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

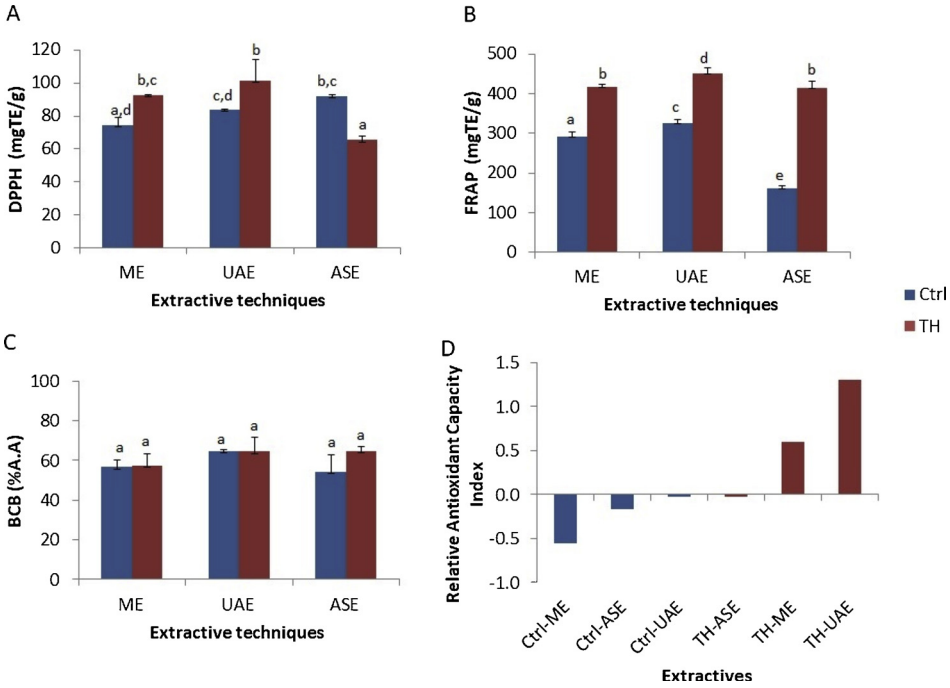

Fig. 3. Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl-DPPH (A), Ferric Reducing Antioxidant Power-FRAP (B), β-carotene bleaching assay-BCB (C) assays and Relative antioxidant capacity index (D). Bars with different letters indicated mean values significantly different ($P < 0.05$) according to the Duncan test. Untreated (Ctrl) and Thermo-treated (TH) *Quercus cerris* wood, Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

where TH-UAE showed the highest TPC value. Compounds as [3-(4-hydroxy-3-methoxyphenyl)-2-Propenal] coniferaldehyde (17.04%) and (3,5-dimethoxy-4-hydroxycinnamaldeide) sinapaldehyde (12.34%), showed a major area. Among monomer compounds derived from lignin, the presence of homovanillic and 4-Hydroxy-3,5-dimethoxy-benzoic acid, vanillin, (4-hydroxy-3,5-dimethoxy-Benzaldehyde) syringaldehyde, coniferaldehyde and sinapaldehyde were also depicted.

3.6. PCA and correlation analysis

The metabolomic profiles got from the GC—MS analysis has been used for PCA statistical approach. It is a widely used method able to depict the influence of analyzed variables on two dimensions using the two principal components calculated interpolating data. The first three components accounted more than 75% of the variance. More in detail PC 1 expressed 31.6% while PC 2 27.3% (Fig. 4). It was possible to observe as all Ctrl samples clustered separately, underling the influence of the extraction procedure on secondary metabolites contents and distribution. On the other hand, TH—ME and TH-UAE samples grouped closer, indicating a lower influence of these two extraction procedures when applied on TH samples. This fact can be explained by the alteration of wood microstructure exposed to thermo-treatment. In fact these extraction procedures, mainly ME and UAE, do not increase the temperature during extraction as ASE does. The cavitation phenomena imposed by ultrasound, demonstrated to be able to slightly increase extract yield. In both cases Ctrl-UAE and TH-UAE showed a higher effectiveness of bioactive secondary metabolites. PCA evidenced as well, that the most active samples (Ctrl-UAE and TH-UAE), among groups of Ctrl and TH samples, were strongly and positively related to PC 1.

3-Hydroxy-4-methoxybenzoic acid, Vanillin, Mandelic acid, 3,4-dimethoxy-, methyl ester, Benzoic acid, Syringic acid, *n*-hexadecanoic acid, Octadecanoic acid, 5-Hydroxymethylfurfural, 4-Amino-2,3-xylenol, trans-Isoeugenol, 2-Propenal, Coniferaldehyde, 2, 4′ –Dihydroxy-3′- methoxyacetophenone, Homovanillic acid, cis-13-Octadecenoic acid, Benzaldehyde, Syringaldehyde and phenol were the compounds that showed an Eigenvalue higher than 0.1 on the PC 1.

Pearson coefficient was used to determine the correlation between identified compounds and measured antioxidant activity (Table 4). Moreover, *n*-Hexadecanoic acid (Palmitic acid), Benzaldehyde, 4-hydroxy-3,5-dimethoxy-Benzaldehyde (Syringaldehyde), Octadecanoic acid (Stearic acid) Vanillin, 3-(4- hydroxy-3-methoxyphenyl)-2-Propenal (Coniferaldehyde), trans-Isoeugenol were also the compounds with the highest Pearson correlation with antioxidant assay demonstrating again their contribution as previously evidenced.

Positive correlation have been also found between DPPH and Vanillin or 3-Hydroxy-4-methoxybenzoic acid (Isovanillic acid) or Benzoic acid, 4-hydroxy-3,5-dimethoxy- (Syringic acid) evidencing that these compounds could act as radical scavenger. Santosh Kumar et al. (2002) observed that vanillin and *o*-vanillin were able to scavenge free radicals. The vanillin has phenolic −OH groups and after reacting with DPPH form phenoxyl radicals.

On the other hand FRAP and BCB were found to be related to *n*-Hexadecanoic acid (Palmitic acid) Vanillin, 2-Propenal, 3-(4- hydroxy-3-methoxyphenyl)- (Coniferaldehyde), 3,5-dimethoxy-4-hydroxycinnamaldehyde (Sinapaldehyde).

Suhaj (2006) confirmed that vanillin exhibited the highest antioxidant activity in the peroxidase-based assay. No correlation have been found with Benzoic acid, 4-hydroxy-3,5-dimethoxy- (Syringic acid) showing a neutral Pearson value. Moreover the Pearson values calculated between TPC or TFC and single antioxidant assays demonstrated as TPC were the most probable responsible of the antioxidant activity measured with DPPH and FRAP assays, and as TFC also contributed mostly in the reducing power of extracts (FRAP). On the other hand, BCB was not significantly related to TPC or TFC showing lower values, 0.19 and 0.33 respectively. This result was congruent with previous findings in which it has been hypothesized that, due to the lipophilic nature of this assay, obtained results could not be in agreement with other water based assays. In fact the presence of lipophilic compounds that act synergistically with phenolics can enhance the biological activity in BCB test. Moreover, BCB results evidenced also as the responsible of this activity, that cannot be ascribed to TPC or TFC, were less dependent from the extraction procedure used. Dekdouk et al (2015) obtained similar results ascribing this difference to the indication of the levels of both lipophilic and hydrophilic compounds that came from TPC whether BCB mainly gave an indication of the levels of lipophilic compounds in a phytocomplex. *N*-hexadecanoic acid reported the highest Pearson correlation with lipid peroxidation inhibition.

Table 3
Chemical compounds identified by GC–MS in extracts from untreated and thermo-treated Turkey oak wood.

ID	Compound	Formula	Rt (min)	Untreated			Thermo-treated		
				Extraction techniques			Extraction techniques		
				ME Area (%)	UAE	ASE	ME Area (%)	UAE	ASE
1	Furfural	$C_5H_4O_2$	3.46	0.16	0.27	0.08			
2	Phenol	C_6H_6O	5.08					0.25	
3	2,3-dihydro-3,5-dihydroxy-6-methyl4H-Pyran-4-one	$C_6H_8O_4$	6.79			0.56			
4	5-Hydroxymethylfurfural	$C_6H_6O_3$	7.81		0.84				
5	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	8.38	1.24		0.47		0.20	
6	2,6-dimethoxy Phenol	$C_8H_{10}O_3$	8.62	1.73	0.75	1.04	0.52	1.33	1.13
7	Vanillin	$C_8H_8O_3$	8.92		1.74		1.18	1.64	
8	3-hydroxy-4-methoxy Benzaldehyde	$C_8H_8O_3$	9.00	2.07		1.63			1.09
9	trans-Isoeugenol	$C_{10}H_{12}O_2$	9.26		1.42	1.19	0.42	1.06	0.93
10	2-methoxy-4- (1-propenyl) Phenol	$C_{10}H_{12}O_2$	9.27	1.26					
11	4-Amino-2,3-xylenol	$C_8H_{11}NO$	9.34		1.16				
12	2-methoxy-4-propyl- Phenol	$C_{10}H_{14}O_2$	9.36	1.29			0.70		
13	3-Hydroxy-4-methoxybenzoic acid (Isovanillic acid)	$C_8H_8O_4$	9.97		2.16	1.76	2.13	2.16	
14	3,5-dimethoxy-, methyl ester benzoic acid	$C_{10}H_{12}O_4$	10.03	1.53					0.57
15	2, 4' –Dihydroxy- 3'- methoxyacetophenone	$C_9H_{10}O_4$	10.12				0.63		
16	3-4,5-trimethoxy- Phenol	$C_{17}H_{18}O_4$	10.20	3.72	2.78	1.99	1.81	1.73	1.36
17	Homovanillic acid	$C_9H_{10}O_4$	10.44				0.85		
18	,4-hydroxy-3,5-dimethoxy-Benzaldehyde (Syringaldehyde)	$C_9H_{10}O_4$	10.56		4.27	3.49		4.43	3.32
19	2,6-dimethoxy-4-Phenol		10.75	2.36	3.30	4.00			3.40
20	3-(4- hydroxy-3-methoxyphenyl)- 2-Propenal (Coniferaldehyde)	$C_{10}H_{10}O_3$	11.01				13.61	17.04	16.44
21	4-((1E)-3- hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	11.05			9.92			
22	1-(2,4,6-trihydroxy-3-1-Butanone methylphenyl)-	$C_{11}H_{14}O_4$	11.15					4.40	
23	1-(2,4,6-trihydroxyphenyl) 2-Pentanone	$C_{11}H_{14}O_4$	11.16						3.54
24	Desaspinidol	$C_{11}H_{14}O_4$	11.17		4.78	3.93			
25	4-hydroxy-3,5-dimethoxy- Benzoic acid	$C_9H_{10}O_5$	11.41	1.25	2.36	1.91	2.33	1.61	1.56
26	*n*-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	12.06		2.46	1.47	2.77	2.71	3.04
27	3,4-dimethoxy-,methyl ester mandelic acid,	$C_{11}H_{14}O_5$	12.30		1.63		1.92		
28	alpha.-phenyl-, methyl ester Benzeneacetic acid	$C_{15}H_{14}O_2$	12.35	1.58		1.59			1.54
29	3,5-dimethoxy-4-hydroxycinnamaldehyde (Sinapaldehyde)	$C_{11}H_{12}O_4$	12.38	3.55	1.99	2.02	7.14	12.34	6.11
30	Oleic acid	$C_{18}H_{34}O_2$	13.20						1.74
31	14-Pentadecenoic acid	$C_{15}H_{28}O_2$	13.20					2.04	
32	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	13.35		3.13		1.02		1.18
33	9-Octadecanoic acid, (E)	$C_{18}H_{34}O_2$	13.22		2.02	1.52			
34	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	13.22				1.79		
35	11-Tetradecen-1-ol, acetate, (Z)-	$C_{16}H_{30}O_2$	13.62			1.26			
36	bis(2-ethylhexyl) ester Hexanedioic acid	$C_{22}H_{42}O_4$	15.68					1.49	
37	Cyclotetradecane	$C_{14}H_{28}$	17.18			1.27			
38	4-Methoxy-4', 5'-methylenedioxybiphenyl-2-carboxylic acid	$C_{15}H_{12}O_5$	20.55	4.67	3.78	3.76	3.72	4.12	4.72
39	7,9 Diethylbenz[a]anthracene	$C_{22}H_{20}$	25.96		1.26	1.23	0.16	0.31	

Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE), Retention time (Rt) in minutes (min), Identification number of identified compounds (ID).

4. Conclusions

Turkey oak (*Quercus cerris* L.) is widespread in several Mediterranean countries and the improvement of the knowledge on wood technological properties and the biological characteristics of extracts may be of high interest from the economic point of view. T. oak wood shows high density, and is difficult to be treated at high temperatures (undesired collapses are frequent), and considering the investigated wood parameters, our results confirm that moderate temperature during thermal-process for this species are able to change some aesthetical characteristics dealing moderate decrease of mechanical performance. Furthermore thermal treatment of the wood leads an increase of extraction yield by using ME and ASE extraction techniques and an increment of the content of polyphenols and flavonoids, mainly observed when UAE was used for the extraction. The thermal treatment produced extracts with the highest antioxidant activity as reported by Relative Antioxidant Capacity Index (TH-UAE > TH-ME > TH-ASE).

Further work is in progress in our laboratory to confirm the possible use of these extracts evaluating their effects on cell model assays, to confirm their antioxidant activity. The application of natural antioxidants from wood, in our opinion, could be an important opportunity for the economic development of mountain areas for the enormous amount of biomass usable as biorefinery. Moreover, it will be necessary to study their potential more in details for other reasons as the increasing demand of natural products and the demonstrated effect that natural extracts already demonstrated on human health.

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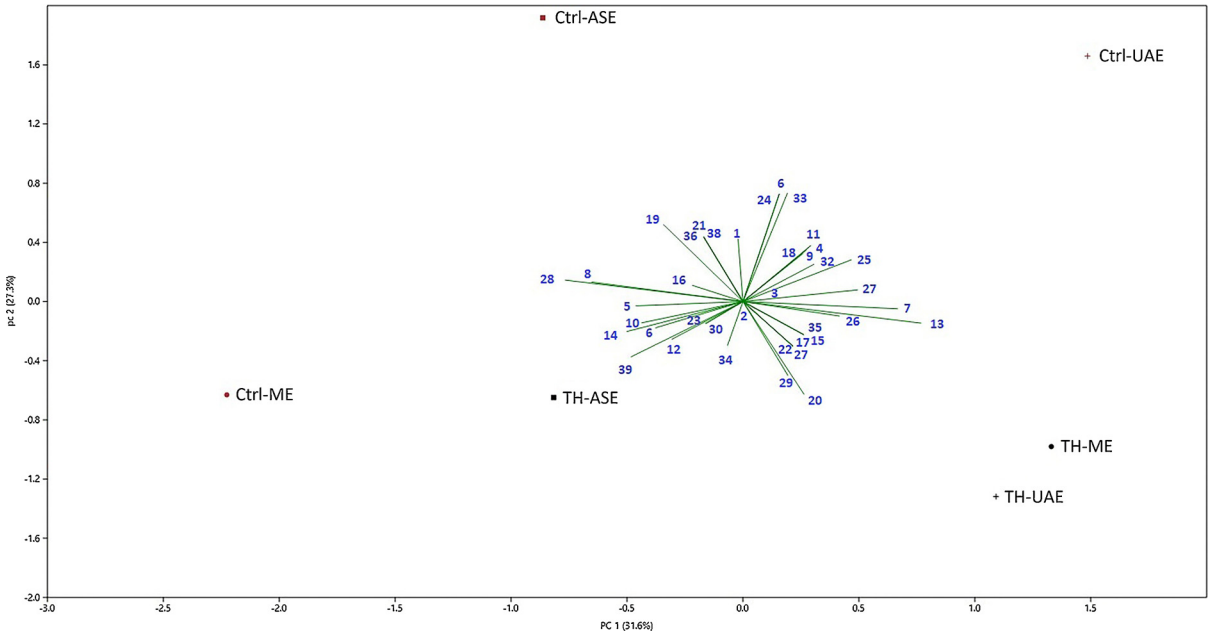

Fig. 4. Principal component analysis (PCA) of chemical compounds referred to column ID identified by GC–MS as reported in Table 3. Untreated (Ctrl) and Thermo-treated (TH) *Quercus cerris* wood, Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE). Numbers correspond to compound ID code reported in Table 3.

Table 4
Pearson correlation coefficient calculated among measured antioxidant activities and quantified chemical compounds.

COMPOUND	DPPH	FRAP	BCB
n-hexadecanoic acid	0.18	0.64	0.64
Vanillin	0.60	0.51	0.53
3-(4- hydroxy-3-methoxyphenyl) 2-propenaL	0.10	0.85	0.48
3-hydroxy-4-methoxybenzoic acid	0.63	0.55	0.42
3,5-dimethoxy-4-hydroxycinnamaldehyde	0.42	0.80	0.41
N-hexadecanoic acid	−0.65	0.47	0.24
4-hydroxy-3,5-dimethoxy- benzoic acid	0.40	0.00	0.03
TFC	0.24	0.84	0.33
TPC	0.69	0.52	0.19

DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = Ferric Reducing Antioxidant Power; BCB = β-carotene bleaching assay; TFC = Total flavonoid content; TPC = Total polyphenol content.

References

Ahajji, A., Diouf, P.N., Aloui, F., Elbakali, I., Perrin, D., Merlin, A., George, B., 2009. Influence of heat treatment on antioxidant properties and colour stability of beech and spruce wood and their extracts. Wood Sci. Technol. 43 (1-2), 69.

Aspé, E., Fernández, K., 2011. The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from *Pinus radiata* Bark. Ind. Crop. Prod. 34 (1), 838–844. https://doi.org/10.1016/j.indcrop.2011.02.002.

Bajraktari, A., Nunes, L., Knapic, S., Pimenta, R., Pinto, T., Duarte, S., Miranda, I., Pereira, H., 2018. Chemical characterization, hardness and termite resistance of *Quercus cerris* heartwood from Kosovo. Maderas-Cienc. Tecnol. 20 (3).

Bernetti, I., Fagarazzi, C., Romano, S., 1998. Biomass production as an energy source in coppices of the province of Florence, Italy. Sixth Conference on Food, Agriculture and the Environment. Minneapolis.

Cetera, P., Todaro, L., Lovaglio, T., Moretti, N., Rita, A., 2016. Steaming treatment decreases MOE and compression strength of Turkey oak wood. Tech. Rep. CRDLR US Army Chem. Res. Dev. Lab. 61 (2), 255–264.

de Falco, B., Fiore, A., Bochicchio, R., Amato, M., Lanzotti, V., 2018. Metabolomic analysis by UAE-GC MS and antioxidant activity of *Salvia hispanica* (L.) seeds grown under different irrigation regimes. Ind. Crop. Prod 112, 584–592.

Dekdouk, N., Malafronte, N., Russo, D., Faraone, I., De Tommasi, N., Ameddah, S., Severino, L., Milella, L., 2015. Phenolic compounds from *Olea europaea* L. Possess antioxidant activity and inhibit carbohydrate metabolizing enzymes in vitro. *Evid. Based Complement.* Alternat. Med. https://doi.org/10.1155/2015/684925. Volume 2015.

Esteves, B., Pereira, H., 2008. Wood modification by heat treatment: a review. BioRes 4 (1), 370–404.

Esteves, B., Marques, A.V., Domingos, I., Pereira, H., 2007. Influence of steam heating on the properties of pine (*Pinus pinaster*) and eucalypt (*Eucalyptus globulus*) wood. Wood Sci. Technol. 41 (3), 193.

Esteves, B., Graca, J., Pereira, H., 2008. Extract composition and summative chemical analysis of thermally treated eucalypt wood. Holzforschung 62 (3), 344–351. https://doi.org/10.1515/HF.2008.057.

Ferrari, S., Allegretti, O., Cuccui, I., Moretti, N., Marra, M., Todaro, L., 2013. A re-valuation of Turkey oak wood (*Quercus cerris* L.) through combined steaming and thermo-vacuum treatments. BioResources. 8 (4), 5051–5066. https://doi.org/10.15376/biores.8.4.5051-5066.

Fidelis, Q.C., Faraone, I., Russo, D., Aragão Catunda-Jr, F.E., Vignola, L., de Carvalho, M.G., De Tommasi, N., Milella, L., 2018. Chemical and Biological insights of *Ouratea hexasperma* (A. St.-Hil.) Baill.: a source of bioactive compounds with multifunctional properties. Nat. Prod. Res. 1–4.

Hakkou, M., Pétrissans, M., Zoulalian, A., Gérardin, P., 2005. Investigation of wood wettability changes during heat treatment on the basis of chemical analysis. Polym. Degrad. Stabil. 89 (1), 1–5.

Lavisci, P., Scalbert, A., Masson, D., Janin, G., 1991. Quality of Turkey oak (*Quercus cerris* L.) wood. I. Soluble and insoluble proanthocyanidins. Holzforschung 45 (4), 291–296. https://doi.org/10.1515/hfsg.1991.45.4.291.

Lovaglio, T., D'Auria, M., Rita, A., Todaro, L., 2017. Compositions of compounds extracted from thermo-treated wood using solvents of different polarities. iForest 10 (5), 824.

Luís, Â., Gil, N., Amaral, M.E., Domingues, F., Duarte, A.P.C., 2012. Ailanthus altissima (Miller) Swingle: A source of bioactive compounds with antioxidant activity. BioRes. 7 (2), 2105–2120.

Mezrag, A., Malafronte, N., Bouheroum, M., Travaglino, C., Russo, D., Milella, L., Dal Piaz, F., 2017. Phytochemical and antioxidant activity studies on *Ononis angustissima* L. Aerial parts: isolation of two new flavonoids. Nat. Prod. Res. 31 (5), 507–514.

Pandey, K.K., 2005. Study of the effect of photo-irradiation on the surface chemistry of wood. Polym. Degrad. Stab. 90 (1), 9–20. https://doi.org/10.1016/j.polymdegradstab.2005.02.009.

Paniwnyk, L., Beaufoy, E., Lorimer, J.P., Mason, T.J., 2001. The extraction of rutin from flower buds of Sophora japonica. Ultrason. Sonochem. 8 (3), 299–301. https://doi.org/10.1016/S1350-4177(00)00075-4.

Pisoschi, A.M., Negulescu, G.P., 2011. Methods for total antioxidant activity determination: a review. Anal. Biochem. 1 (1), 1–10.

Romdhane, M., Gourdon, C., 2002. Investigation in solid–liquid extraction: influence of ultrasound. Chem. Eng. J. 87 (1), 11–19. https://doi.org/10.1016/S1385-8947(01)00206-6.

Rowe, J.W., 1979. Extractives in eastern hardwoods: a review. Gen. Tech. Rep. FPL-18. Madison, WI: US department of agriculture, forest service. Forest Products Laboratory 67, 18.

Russo, D., Malafronte, N., Frescura, D., Imbrenda, G., Faraone, I., Milella, L., Fernandez, E., De Tommasi, N., 2015. Antioxidant activities and quali-quantitative analysis of different*Smallanthus sonchifolius* [(Poepp. and Endl.) H. Robinson] landrace extracts. Nat. Prod. Res. 29 (17), 1673–1677.

Sandberg, D., Kutnar, A., Mantanis, G., 2017. Wood modification technologies-a review. iForest 10 (6), 895.

Santosh Kumar, S., Priyadarsini, K.I., Sainis, K.B., 2002. Free radical scavenging activity

of vanillin and o-vanillin using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Redox Rep. 7 (1), 35–40.
Sehlstedt-Persson, M., 2003. Colour responses to heat-treatment of extractives and sap from pine and spruce. Int. IUFRO Wood Drying Conf. 459–464.
Shah, P., Modi, H.A., 2015. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. Int. J. Res. Appl. Sci. Eng. Technol 3 (June (VI)).
Silveira, E.A., Lin, B.J., Colin, B., Chaouch, M., Pétrissans, A., Rousset, P., Chen, W.H., Pétrissans, M., 2018. Heat treatment kinetics using three-stage approach for sustainable wood material production. Ind. Crop. Prod. 124, 563–571.
Suhaj, M., 2006. Spice antioxidants isolation and their antiradical activity: a review. J. Food Anal. 19 (6-7), 531–537.
Sun, T., Tanumihardjo, S.A., 2007. An integrated approach to evaluate food antioxidant capacity. J. Food Sci. 72 (9). https://doi.org/10.1111/j.1750-3841.2007.00552.x.
Sun, Y., Liu, D., Chen, J., Ye, X., Yu, D., 2011. Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-β-carotene from citrus peels. Ultrason. Sonochem. 18 (1), 243–249. https://doi.org/10.1016/j.ultsonch.2010.05.014.
Tálos-Nebehaj, E., Hofmann, T., Albert, L., 2017. Seasonal changes of natural antioxidant content in the leaves of Hungarian forest trees. Ind. Crop. Prod. 98, 53–59.
Todaro, L., 2012. Effect of steaming treatment on resistance to footprints in Turkey oak wood for flooring. Eur. J. Wood Wood Prod. 70 (1-3), 209–214. https://doi.org/10.1007/s00107-011-0542-2.
Todaro, L., Zanuttini, R., Scopa, A., Moretti, N., 2012a. Influence of combined hydrothermal treatments on selected properties of Turkey oak (*Quercus cerris* L.) wood. Wood Sci. Tech. 46 (1-3), 563–578. https://doi.org/10.1007/s00226-011-0430-2.
Todaro, L., Zuccaro, L., Marra, M., Basso, B., Scopa, A., 2012b. Steaming effects on selected wood properties of Turkey oak by spectral analysis. Wood Sci. Tech. 4 (1-3), 89–100. https://doi.org/10.1007/s00226-010-0377-8. 46.
Todaro, L., Dichicco, P., Moretti, N., D'Auria, M., 2013. Effect of combined steam and heat treatments on extractives and lignin in sapwood and heartwood of turkey oak (*Quercus cerris* L.) wood. BioResources 8 (2), 1718–1730. https://doi.org/10.15376/biores.8.2.1718-1730.
Todaro, L., Rita, A., Moretti, N., Cuccui, I., Pellerano, A., 2015. Assessment of thermo-treated bonded wood performance: comparisons among Norway spruce, common ash, and Turkey oak. BioResources 10 (1), 772–781.
Todaro, L., Russo, D., Cetera, P., Milella, L., 2017. Effects of thermo-vacuum treatment on secondary metabolite content and antioxidant activity of poplar (*Populus nigra* L.) wood extracts. Ind. Crop. Prod 109, 384–390. https://doi.org/10.1016/j.indcrop.2017.08.052.
Tolvaj, L., Mitsui, K., 2005. Light source dependence of the photodegradation of wood. J. Korean Wood Sci. Technol. 51 (5), 468–473. https://doi.org/10.1007/s10086-004-0693-4.
Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T.J., 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason. Sonochem. 8 (2), 137–142. https://doi.org/10.1016/S1350-4177(00)00033-X.
UNI ISO, 1985. Wood-Determination of Moisture Content for Physical and Mechanical Tests". 3130. .
Varga, D., Van der Zee, M.E., 2008. Influence of steaming on selected wood properties of four hardwood species. Eur. J. Wood Prod. 66 (1), 11.
Volkmer, T., Lorenz, T., Hass, P., Niemz, P., 2014. Influence of heat pressure steaming (HPS) on the mechanical and physical properties of common oak wood. Eur. J. Wood Prod. 72 (2), 249–259. https://doi.org/10.1007/s00107-014-0777-9.