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Effects of thermo-vacuum treatment on secondary metabolite content and antioxidant activity of poplar (*Populus nigra* L.) wood extracts

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ABSTRACT

A renewed interest in producing thermo-treated wood extracts and the potential applications of these extracts was observed in chemical, pharmaceutical, and food industries. Poplar (Populus spp.), belonging to the Salicaceae family, is one of the most cultivated woody plant for industrial purposes, one of the least expensive hardwoods, rarely used in the production of fine furniture, but extensively used for pulp and panel productions and have therefore an important economic impact worldwide. The aim of this study was to verify the influence of thermo-vacuum treatment (at 180, 200, and 220 °C) on wood extracts obtained via three different extraction techniques: maceration, ultrasound-assisted extraction and accelerated-solvent extraction. Effect of temperature on extraction was verified by measuring the total contents of polyphenols, tannins and flavonoids. Secondary metabolites are often related to antioxidant activity measured by several *in vitro* tests, including the 2,2-diphenyl-1-picrylhydrazyl radical-scavenging method, the ferric-reducing-ability power test, and the beta-carotene bleaching assay. Our results showed the presence and the effect of heat treatments and extraction techniques on polyphenol and flavonoid contents. Extracts obtained from wood heated at 200 and 220 °C showed the highest flavonoid and polyphenol contents, and, we observed a relationship with the shown antioxidant activity levels. Our study clearly showed the differential effects of temperature and extraction technique on both antioxidant activity and secondary metabolite contents. The detailed knowledge about the extractives from poplar wood can contribute, on the one hand, to better understand the effect of temperature during thermo treatment, and, on the other demonstrates the potential of this species as a source of bioactive compounds for nutraceutical or pharmaceutical applications by identifying appropriate extraction techniques.

1. Introduction

Wood has been treated thermally since the beginning of the last century (Poncsak et al., 2009), when several studies demonstrated that high-temperature treatment reduced the equilibrium moisture content and dimensional shrinkage of wood (Bekhta and Niemz, 2003; Esteves and Pereira, 2008). Heat treatment is one of several alternative treatments for protecting wood. Use of heat treatment reduces the need for toxic chemical applications, which are normally required to increase wood durability, and enhances dimensional stability by reducing wood hygroscopicity (Pétrissans et al., 2003). On the other hand many changes of wood properties are related to modifying wood's physical and chemical compositions by altering mainly the wood microstructure and cell-wall components. During heat treatment, the most volatile extractives may leave the wood or be degraded (Esteves and Pereira, 2008), with new chemical compounds being obtained. However, incomplete information is available on the potential use of such extractives as value-added chemical products.

According to recent bioeconomy programs, chemical compounds derived from natural sources will be more available in regions where these compounds can be obtained economically than more expensive synthetic chemicals. Shimizu et al. (2002) highlighted that, in commercial applications, a large proportion of wood is used as timber, while the rest is most often used as a source for fuel. Several researchers showed how different biomass sources could provide interesting results to exploit the economic and industrial potential of the biomass refinery. In this sense several recent studies were focused on the characterization of bark extractives (Hofmann et al., 2015; Rosdiana et al., 2017) and the antioxidant properties of the bioactive compounds from Eucalyptus (Luís et al., 2016; Santos et al., 2017) or Cameroonian woods (Saha et al., 2013).

In addition the biorefinery is becoming an important aspect for green chemistry development aimed at ensuring the necessity to achieve the best objectives as favourable as possible from restricted

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natural resources such as forest biomass. One of the main scope is to generate diversified, innovative and renewable products using on-site bioresources such as wood and tree residues. The possible scenarios and the future direction of such transformation system was deeply explained by Fernando et al., since 2006. The importance of biomass as a source for biopesticide active substances was recently highlighted by Villaverde et al. (2016). Poplar tree species, including all their huge varieties, are largely cultivated in the word as a fast growing bioenergy crop. However the enormous potential of this trees in the field of bio-based chemicals is still under-evaluated. The potential commercial opportunities, the limitations and challanges of poplar phytochemicals as value-added co-products was recently discussed by Devappa et al. (2015). The authors suggested that poplar species could have a relevant importance in the biorefineries by enabling the development of the bioeconomy. In this respect, the various implications of this evidence and the gained attention could play an important future role by increasing the attention toward the poplar plantations as a potential source for use in biorefinery processes.

Black poplar (*Populus nigra* L.), a member of the Salicaceae family, grows in Europe from the British Isles to the Mediterranean coast. The rapid growth and spread of this tree account for its widespread cultivation and naturalization throughout the world. The wood is commonly used as fuel, but is also used for furniture, panelling and plywood production. Due to its high cellulose and relatively low lignin content, black poplar is suitable for pulp and paper production (Balatinecz et al., 2001). In their study of thermo-treatment of poplar wood, Ayadi et al. (2003) showed that the total phenol and sugar contents of poplar increased after heat treatment, due to degradation of the wood's chemical components, such as hemicelluloses, lignin and extracted compounds. Some studies have aimed to understand other uses for wood components of *Populus* spp., such as the use of extracted components for biomass energy (Demirbaş, 2005). However, few studies have focused on the extractive yield or have evaluated the wood after thermal treatment (Kamdem et al., 2000; Chaouch et al., 2013).

Poplar wood, due to its lightness, availability and homogeneity, may provide a useful material for several industrial applications; however, more knowledge of its potential is essential. Although black poplar belongs to a group of woody plants mainly used for bio-energy production, it is possible that useful secondary metabolites could be derived from the wood. Secondary metabolites are chemical compounds produced by several plant tissues (e.g. leaves, bark, roots, buds, wood) that provide different medicinal applications, including antioxidant, anticancer, anti-inflammatory, antifungal and other properties (Rowe, 1989; Milella et al., 2014; Armentano et al., 2015; Gambacorta et al., 2014; Dekdouk et al., 2015; Bhuvaneshwari et al., 2016). Previous studies on the potential of *Poplar* spp. extracts are particularly related to the medicinal properties of buds, leaves and bark (Palo, 1984).

Extractives can be drawn from wood by different approaches, and specific methods have been applied to influence the yield and type of extracted compound. The amount of extractive compound dissolved in each solvent will differ, and the choice of the appropriate extraction techniques will depend on the final application. It is also widely know as the presence of lipophilic compounds is a critical issue in pulping and bleaching processes. As reported by Kang et al. (2007) several methods have been used to improve the pulping and refining efficiency of the wood chips.

To the best of our knowledge, only Ahajji et al. (2009) reported some interesting results on the influence of heat treatment of beech and spruce woods on their antioxidant properties, and no detailed studies have been reported about the antioxidant capacities of extracts from poplar wood treated at different temperature and/or the effects of various extraction techniques.

The focus of this work is to study the effects of thermo-vacuum treatment on the polyphenolic and flavonoid contents and antioxidant activity of native black poplar wood extracts. Specifically, this research sought to address whether: *i*) the temperature of treatment affects the above mentioned parameters, and *ii*) the different extraction techniques (maceration, ultrasound and accelerated solvent extraction) affect the extraction yield, secondary metabolite content and their biological activity.

2. Material and methods

2.1. Wood treatment

This study used free defect poplar boards (1 m^3; dimensions: 30 × 200 × 2300 mm) from the Apennine Mountains of Italy (Basilicata Region, collected in December 2015). Boards were cut from logs and thermally modified at a vacuum plant, developed by WDE Maspell srl (Terni, Italy), located at the University of Basilicata. A dual function machine was used, which can dry wood under vacuum conditions and treat wood at a high temperature (up to 250 °C). The laboratory kiln (4 × 1 m) can hold two layers of boards, which are loaded manually. Boards lie between two metal plates, which contain diathermic hot oil that provides conductive heat transfer to the boards. Pressure in the kiln can be regulated in the range 60–1000 mbar. Vacuum is maintained through a water ring-type pump equipped with a heat exchanger. Under pressure, the plates provide a force on the boards that prevents potential deformation of the wood (Ferrari et al., 2013).

Drying and thermal treatment were applied in the same plant. Before treatment, each board was cut in two. One board was thermally modified, while the other was used as a reference. Wood was dried to 0% moisture content under vacuum (185 mbar) at 85 °C for 12 h. From an initial (predrying) temperature of 30 °C, the temperature was increased 5 °C each hour to 85 °C (Table 1). Next, the wood was thermally treated. Table 2, exemplified for 200 °C, describes the process parameters (pressure, temperature, and time) adopted during thermal treatment, all samples were conditioned in a climatic chamber (T = 20 °C and relative humidity 65%) until they reached a constant weight, before the extraction procedures.

2.2. Extraction of secondary metabolites

All samples, including untreated controls (C) and wood heated at 180 °C (T180), 200 °C (T200) and 220 °C (T220), were reduced to a small size with a mill saw. Aliquots of similarly sized particles of each sample were prepared and subjected to three different solid/liquid extraction techniques: maceration extraction (ME), ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE).

ME was carried out at room temperature by stirring the sample (10 g) for 1 h in solvent at a sample-to-solvent ratio of 1:12 (w/v). First, samples were treated with the solvent *n*-hexane to remove lipophilic components. A recent paper demonstrated as *Populus* × *euramericana* stem *n*-hexane extract contains glycerides, mainly triglycerides, as the largest component group of the lipophilic extractives (Xu et al., 2010). These compounds are largely known to do not possess antioxidant activity (Table 3). Then, the solid phase was extracted with an ethanol: water solution (70:30 v/v) (Ahjji et al., 2009). UAE was performed for 1 h in an ultrasonic bath (Branson 1800). Each sample (10 g) was pretreated with *n*-hexane and extracted in an ethanol: water (70:30 v/v) solution at a sample-to-solvent ratio of 1:12 (w/v) (Table 3). For both

Table 1
Parameters of pre-drying, drying and cooling phases.

Phase	Time (h)	Pressure (mbar)	T (°C)
Predrying	4	800	85
Drying	12	185	85
Cooling	0.5	185	–

Table 2
Thermo-treatment phases for poplar wood heated at 200 °C.

Variable	Preheating	Phase 1	Phase 2	Phase 3	Cooling
Time (h)	3	3	3	3	10
Pressure (mbar)	260	290	320	350	350
Temperature (°C)	160	173	187	200	–

Table 3
Extraction techniques and procedures.

	ME	UAE	ASE
Lipophilic extraction	120 mL hexane × 3 times	120 mL hexane × 3 times	hexane 90 °C; static time 5 min; 3 cycles × 3 times
Polar extraction	120 mL EtOH/H_2O (70:30) × 3 times	120 mL EtOH/H_2O (70:30) × 3 times	EtOH/H_2O (70:30) 100 °C × 3; static time 5 min; 3 cycles × 3 times

Maceration extraction (ME); ultrasound-assisted extraction (UAE); accelerated-solvent extraction (ASE). For each extraction procedure, 10 g of wood material was used and repeated for 3 times.

extraction procedures (ME and UAE) and for each solvent (hexane and ethanol: water mixture), the extraction process was repeated three times (Milella et al., 2016).

Sequential extraction was carried out with an ASE system (Dionex Corp.). Plant woody material (10 g) was introduced in a 22 mL cell and pretreated with *n*-hexane at 90 °C three times until the resulting solid phase was extracted. The material was again treated three times in an ethanol/water (70:30 v/v) solution at 100 °C (Table 3). In all cases, assays were performed at 1500 psi for three cycles of 5 min each. All extracts were filtered through a paper filter. 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 yields were calculated.

2.3. Total polyphenol content (TPC)

TPC was evaluated by the Folin–Ciocalteu reagent method, as reported by Russo et al. (2012). A 75 µL aliquot of extract was mixed with 425 µL of water and 500 µL of Folin–Ciocalteu phenol reagent and allowed to react for 5 min. Then, 500 µL of Na_2CO_3 solution (10 ×) was added. The mixture was incubated for 1 h, and the absorbance of the reaction mixture was read at 723 nm. The TPC of the extract was expressed as milligrams of Gallic acid equivalent (GAE) per gram of dried extract.

2.4. Total flavonoid content (TFC)

TFC was determined by using aluminium chloride ($AlCl_3$), according to the method reported by Armentano et al. (2015), using quercetin as a standard. Plant extract (500 µL) was added to 15 µL of $NaNO_3$ (5 ×). After 5 min, 30 µL of $AlCl_3$ (10 ×) was added. After 1 min, the reaction mixture was treated with 100 µL of 1 mM NaOH. Finally, the reaction mixture was diluted with 1 mL of water, and the absorbance was measured at 510 nm. Results were expressed as milligrams of quercetin equivalents (QE) per gram of dried extract.

2.5. Total tannin content (TTC)

TTC was evaluated by protein precipitation, as reported by Armentano et al. (2015). To each 250 µL of extract, 500 µL of bovine serum albumin solution in 0.2 M acetic buffer (pH 5.0 with 0.17 M NaCl) was carefully added. The mixture was incubated for 15 min. Samples were centrifuged at 5000 rounds for 15 min. Supernatant was removed, and remaining pellet was dissolved in 1 mL of aqueous solution containing SDS (1%) and triethanolamine (4%). Then, 250 µL of 0.01 M $FeCl_3$ in 0.01 M HCl was added. After 30 min, the absorbance at 510 nm was recorded. TTC was expressed as milligrams of tannic acid equivalent (TAE) per gram of dried extract. Tannic acid was used to construct a regression curve.

2.6. Radical-scavenging activity

Antioxidant activity was measured in terms of hydrogen-donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Experiments followed the method reported by Russo et al. (2015a). Reduction of the radical was followed by a decrease in absorbance at 515 nm. A 50 µL aliquot of methanol extract at different concentrations and 200 µL of DPPH methanol solution were added in a 96 well plate. Tubes were kept in the dark for 30 min. Absorbance at 515 nm was measured by using Trolox as a standard. Results were expressed as milligrams of Trolox equivalent (TE) per gram of dried sample. Each assay was carried out in triplicate (Russo et al., 2015b).

2.7. Ferric reducing antioxidant power (FRAP)

Extracts were submitted to a previously described FRAP assay (Dekdouk et al., 2015) with some modifications. An appropriately diluted sample of 25 µL of methanol (for the blank) was added to 225 µL of FRAP reagent and incubated at 37 °C for 40 min in the dark. FRAP reagent was prepared fresh before each experiment by mixing 300 mM acetate buffer in distilled water at pH 3.6, 20 mM $FeCl_3$ $6H_2O$ in distilled water and 10 mM TPTZ in 40 mM HCl in a ratio of 10:1:1. Reduction of a colourless ferric complex (Fe^{3+} tripyridyltriazine) to a blue coloured ferrous complex (Fe^{2+} tripyridyltriazine) was determined at 593 nm by the reaction of electron-donating antioxidants. Trolox was used as a standard. FRAP values were expressed as milligrams of TE per gram of dried extract.

2.8. Inhibition of lipid peroxidation

Ability of extracts to prevent inhibition of lipid peroxidation was tested by using a β-carotene bleaching assay (BCB), as reported by Dekdouk et al. (2015). A stock solution of β-carotene/linoleic acid was made by dissolving 0.2 mg of β-carotene in 0.2 mL of chloroform, linoleic acid (20 mg) and Tween 20 (200 mg). Chloroform was completely removed by using a rotary evaporator, and distilled water (50 mL) was added with oxygen. The resulting emulsion was vigorously stirred. Aliquots (950 µL) of the mixture were transferred to test tubes containing 50 µL of sample (final concentration for all tested samples was 0.1 mg/mL) or methanol (as the blank). BHT was used as a positive standard. This solution (250 µL) was transferred to a 96 well plate and left to absorb the solution for 3 h at 50 °C. Absorbance was monitored at 470 nm and measured every 30 min. Results were expressed as the percentage of antioxidant activity (AA), measured on the basis of β-carotene bleaching inhibition, calculated as follows:

$$\%\ AA = [1 - (A\ sample\ T_{0'} - A\ sample\ T_{180'})/(A\ blank\ T_{0'} - A\ blank\ T_{180'})] * 100$$

2.9. Statistical analysis

Results are expressed as the mean ± standard deviation (SD) of three independent experiments. Each experiment, thermal treatment and extraction technique, was performed for each test in a different week. To verify the correlations among used methods, Pearson coefficient was determined and p values of 0.05 or less were considered statistically significant. Statistical analyses were performed using GraphPad Prism 5 Software (San Diego, CA, USA) (Lamoral-Theys et al.,

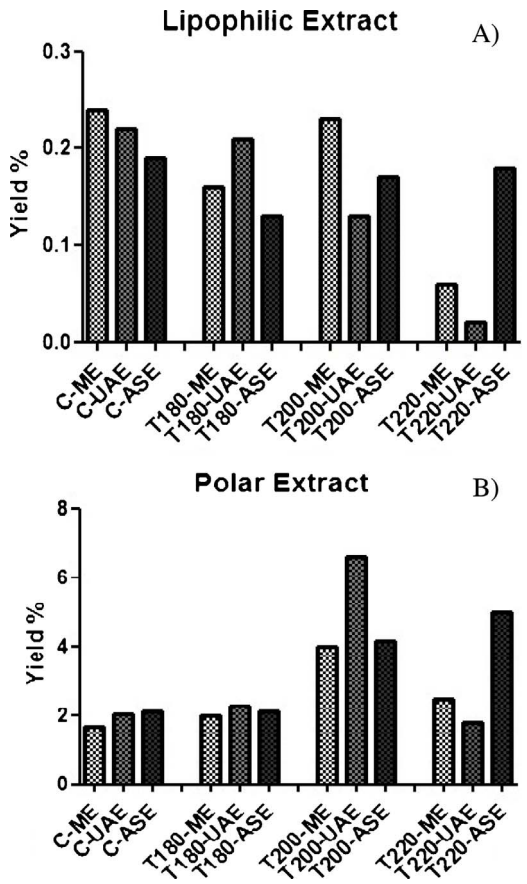

Fig. 1. Yields (%) of lipophilic (a) and polar (b) components of untreated and thermo-treated poplar wood obtained using various extraction techniques. Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

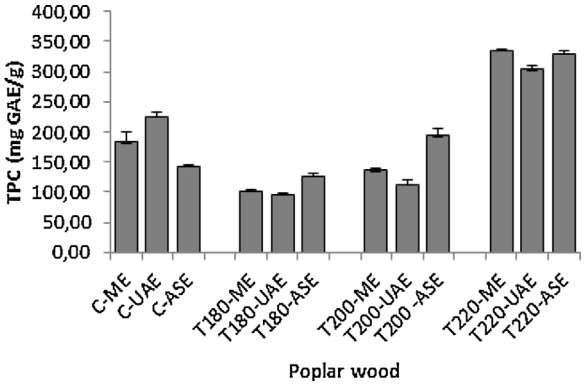

Fig. 2. Total polyphenolic contents (TPCs) of untreated and thermo-treated extracts of poplar wood. mgGAE/g = milligrams of Gallic Acid Equivalent per gram of dried extract. Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

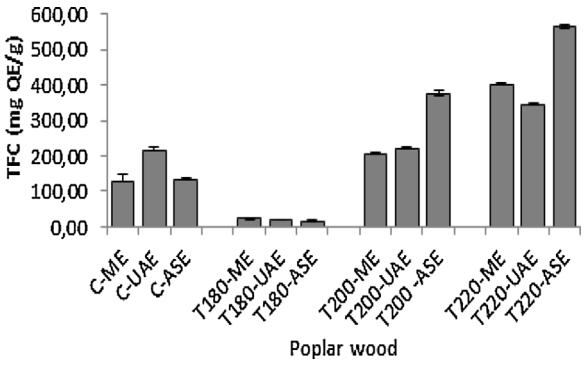

Fig. 3. Total flavonoid contents (TFCs) of untreated and thermo-treated extracts of poplar wood. mgQE/g = milligrams of Quercetin Equivalent per gram of dried extract. Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

2011).

3. Results

3.1. Extracts

Different extractive yields of lipophilic (*n*-hexane extracts) and polar components (EtOH-H_2O extracts) were obtained by using different extraction techniques (Fig. 1A and B). When ME and UAE were used, the extractive yield showed very low amounts of lipophilic components, and yields decreased at temperatures above 200 °C, while extractive yield remained almost unchanged with ASE. The thermo-treatment process mainly produced polar compounds. An important increase in polar compounds in poplar wood was observed between 200 and 220 °C, particularly by using ASE at 220 °C and using ME or UAE at 200 °C.

3.2. TPC, TTC and TFC

Polar extracts, obtained with EtOH-H_2O (70:30), were tested to evaluate the content and antioxidant activity of polyphenols, tannins and flavonoids. TPC was evaluated by using Folin-Ciocalteu reagent (Fig. 2). TPC significantly increased after treatment at 220 °C, with similar contents being obtained by UAE (303.25 ± 6.51 mg GAE/g) and ME (334.87 ± 3.91 mg GAE/g). The lowest TPC values were observed at 180 °C (96.69 ± 0.43 mg GAE/g for UAE, 101.90 ± 2.30 mg GAE/g for ME, and 125.69 ± 6.73 mg GAE/g for ASE). These values were lower than the TPC values of untreated wood. Flavonoid content was quantified spectrophotometrically before and after thermal treatment (Fig. 3) by using the $AlCl_3$ method. In this method, $AlCl_3$ forms acid-stable complexes with the C-4 keto group and the C-3 or C-5 hydroxyl group of flavones and flavonols. $AlCl_3$ forms acid-labile complexes with the *ortho*-dihydroxyl groups in the A or B rings of flavonoids (Armentano et al., 2015). Except for the 180 °C treatment, our technique of using progressively high temperatures and our modification of the extraction technique itself allowed us to extract high amounts of flavonoids from poplar wood. The highest TFC (563.44 ± 10.64 mg QE/g) was observed after the 200 °C treatment with ASE; this extraction method greatly enhanced the extraction of flavonoids from poplar wood treated at 200–220 °C. The lowest TFC values were obtained when extraction was performed by any technique at 180 °C (average: 18.89 mg QE/g).

Tannin content was measured by using the protein precipitation assay. However, in all extracts, tannins were found in only trace amounts (data not shown).

3.3. Antioxidant activities

All extracts obtained from untreated and thermo-treated poplar wood were tested to evaluate antioxidant activity (Fig. 4). No single assay can represent total antioxidant capacity; for this reason, three complementary assays were used to evaluate antioxidant activities of extracts (Dekdouk et al., 2015).

Extracts showed DPPH scavenging activity, with the highest values occurring at 220 °C (Fig. 4D; 675.35 ± 24.45 mg TE/g with ME, 513.56 ± 15.19 mg TE/g with UAE and 505.93 ± 7.52 mg TE/g with ASE), on the other hand we obtained lower DPPH scavenging activity levels for wood treated at 180 or 200 °C (Fig. 4 B-C) than untreated poplar wood (Fig. 4A).

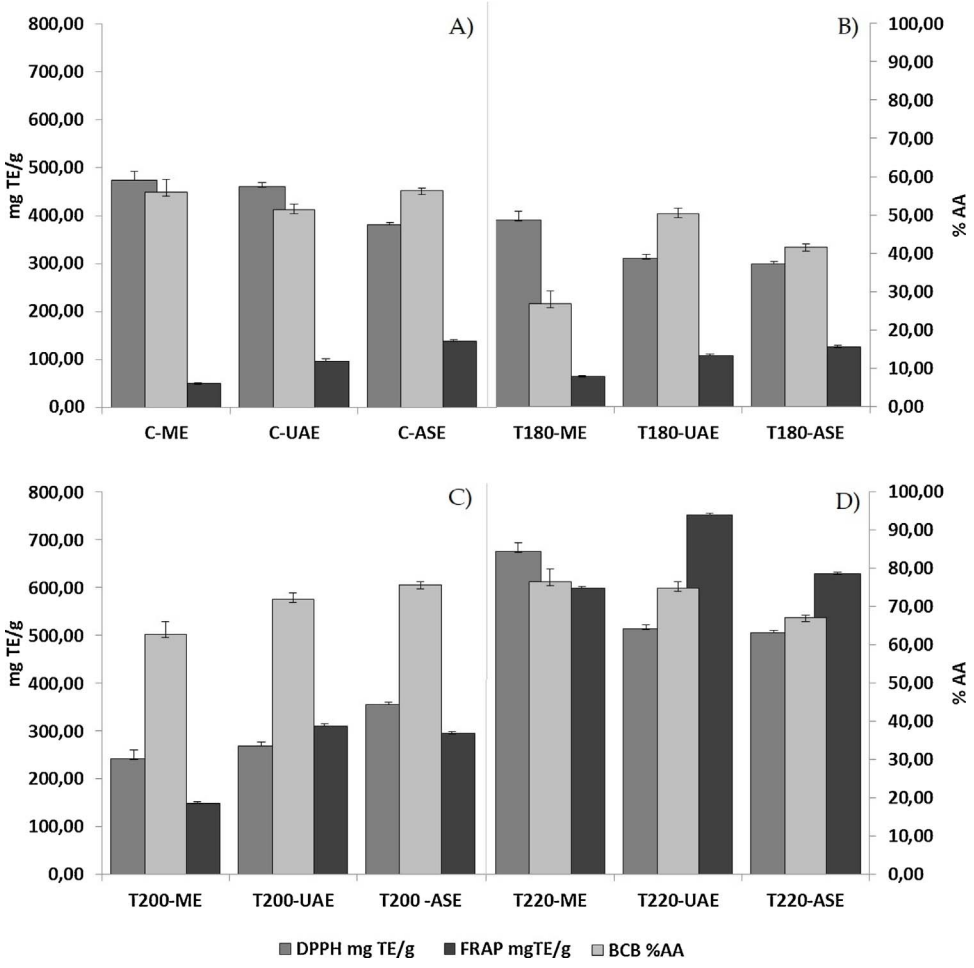

Fig. 4. Antioxidant activity levels of wood samples after various extraction techniques. Antioxidant activity of control − C (A), treated at 180 °C − T180 (B), at 200 °C − T200 (C) and at 220 °C − T220 (D) poplar extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl)-scavenging activity, ferric reducing ability power (FRAP), and beta carotene bleaching (BCB) assay. Results of DPPH and FRAP were expressed as mg of Trolox equivalent per gram of dried extract (mg TE/g). BCB was expressed as percentage of antioxidant activity (% AA). Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

Thermo-treatment had a positive effect on FRAP values, which showed also a significant increase at 220 °C (Fig. 4D). At this temperature, extracts obtained by UAE had the highest FRAP value (752.29 ± 5.81 mg TE/g), followed by extracts obtained by ASE (628.03 ± 9.50 mg TE/g) and ME (599.33 ± 3.62 mg TE/g). Extracts obtained after 180 °C treatment showed slightly higher FRAP values than untreated wood. Extracts obtained by heat treatment at 200 °C generated FRAP values that were twice those of the pretreatment condition.

We evaluated the ability of extracts to prevent inhibition of lipid peroxidation by measuring the bleaching of β-carotene spectrophotometrically. As reported in Fig. 4, untreated wood showed similar values for all extractive techniques at about 50% AA. Temperature only slightly affected the inhibition of lipid peroxidation. Highest values were observed after the 200 and 220 °C treatments, ranging from 62.84 ± 2.30% (T200 ME) to 76.47 ± 3.93% (T220 ME).

To get a complete picture of the antioxidant capacity, relative antioxidant capacity index (RACI), a hypothetical concept, is used. This index was calculated comparing the antioxidant activity values obtained from different chemical assays, TPC was included in this calculation as previously suggested (Armentano et al., 2015). As shown in the histogram (Fig. 5), poplar extracts obtained after heat treatment at 220 °C showed the highest RACI value. This result confirms that high-temperature treatment increases the antioxidant activity. At last, we have evaluated the potential correlation among secondary metabolite quantification and the biological activities demonstrated by our samples using the Pearson correlation coefficient (Table 4). It showed a positive correlation among all assays and secondary metabolite quantification, but the highest correlations were found between TPC and DPPH (0.84), TPC and FRAP (0.85), and TFC and FRAP (0.88). On the other hand, BCB resulted mostly related to TFC than to TPC (0.64 *vs* 0.33). This result is congruent to the nature of BCB chemical assay. In fact, it has been previously demonstrated, as due to its lipophilic nature, there can be less correlation with TPC than with other constituents (Dekdouk et al., 2015).

4. Discussions

Thermal treatment of wood provides many benefits, such as better dimensional stability and attractive dark colours, without the need of toxic chemicals (Poncsak et al., 2009). Thermal treatment alters wood properties by modifying the wood's composition, which varies widely among species. Chemical changes due to heating depend on the duration and temperature of treatment. For low temperature treatments (20–150 °C), loss of free water occurs as the wood dries, followed by loss of bound water. At moderate temperatures of heating (180–250 °C) in the typical range of heat treatment, wood undergoes important chemical transformations while at high temperatures (> 250 °C), carbonization processes begin (Esteves and Pereira, 2008). Most extracted components disappear during heat treatment, but additional compounds can be extracted from wood by degrading structural components of the cell wall (Todaro et al., 2013). Extracts are non-structural secondary metabolites of wood that can be obtained by using various solvents and methods. Several methodologies currently used for extraction and concentration may restrict the use of non-structural components. As stated by Jablonský et al. (2015), the valorization, amount and nature of the compounds generated greatly depend on the used isolation methods.

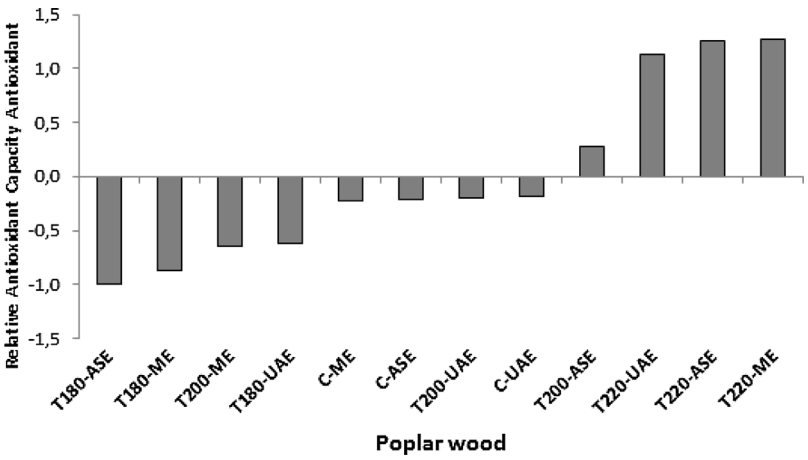

Fig. 5. Relative antioxidant capacity index (RACI) values of wood extracts after extraction by different methods. Maceration Extraction (ME), Ultrasound Assisted Extraction (UAE), Accelerated Solvent Extraction (ASE).

Table 4
Pearson correlation coefficient calculation.

Assay	TPC	TFC
DPPH	0.84	0.48
FRAP	0.85	0.88
BCB	0.33	0.64

Pearson correlation was calculated among Total Polyphenol Content (TPC), Total Flavonoid Content (TFC) and antioxidant activity assays (DPPH, FRAP and BCB).

An interesting finding of the present research is that the untreated poplar wood seems to contain the greatest portion of lipophilic compounds. As a matter of fact, by using ME and UAE, the yield of the extractives decreases gradually until a minimum is reached around 220 °C. Specifically, excluding poplar 200 °C, non-polar component obtained by ME decreased as thermo-treatment increase while yield extracted by UAE strongly decreased at the highest temperature.

On the contrary, non-polar component yield extracted by ASE showed more independence from the thermal treatment and these results may depend on the change of the heat-resistant compounds induced by heat treatment. In this case, these compounds are extracted using ASE technique where, evidently, the temperature plays a relevant effect on yield extraction. As it well known, it is important to note that the different extraction yield technique performances could be explained by the different acting mechanisms such as breakup of plant tissue (Aspé and Fernández, 2011), or by a diffusion process as observed by Gironi and Piemonte (2011) for chestnut wood.

In terms of polar component, these results showed that the intermediate treatment of 180 °C, regardless of extraction technique, did not affect, sensibly, the extractive yield that showed similar values to those of untreated wood. However, above 180 °C, as general terms, a progressive increase of the extractives was depicted while for strongest treated samples (220 °C) the ASE technique provided constant increase of extractives yield.

From this information, it is clearly possible to conclude that in case of high temperature in the thermo-treatment of poplar, the ASE technique, due to the temperature as parameter (Jablonský et al., 2015) was more efficient than other extraction methods.

In accord to a previous study on Jack pine extracts (Poncsak et al., 2009), most extracted compounds are forced from wood at temperatures under 200 °C. Most of the subsequent products appear only at temperatures above 200 °C. Extracted compounds of untreated Jack pine are dominated by nonpolar components, whereas thermo-transformation seems to generate mainly polar compounds. During the thermal treatment process, volatile extracts are generally removed from the wood first, followed later by new products and bioproducts resulting from various chemical reactions.

Our results are in agreement with a previous study, by Ahajji et al. (2009); these authors reported the effects of temperature on the DPPH scavenging activity and the polyphenol content of heat-treated wood extracts of beech and spruce. As antioxidant activity increased with temperature from 210 to 235 to 250 °C, extracts were more active for beech wood than for spruce. The authors suggested that because wood composition varies from species to species, antioxidant activity might vary as well, and our results confirm this hypothesis.

In this study, we observed that a high temperature treatment of wood, produced extracts with higher polyphenol content and higher antioxidant activity. As general statement, we observed that the TPC increased at the highest temperature without relevant influence of the extraction technique. On the contrary, the TFC was clearly affected by the ASE technique.

As stated by Chemat et al. (2012), is important to make further efforts in finding green solution in the extraction techniques trying to minimize the use of solvents. On the other hand, it is also true that, as confirmed by Aspé and Fernández (2011), a high extraction yield, although required for an efficient process, not necessarily ensure a high concentration of bioactive components. Our results are in agreement to this statement. In fact, as also suggested by Aspé and Fernández (2011), one of the most important aspects to be considered is to preserve the bioactivity of the extracts while avoiding their degradation during extraction processes. Therefore, these evidences increase the hypothesis that strengthens the link between the extraction yield and the preservation of bioactive components should be considered when an extraction method is selected, even more for the wood material both untreated and thermo-treated.

5. Conclusions

Recovery of wood material and liquid waste produced during or after thermo-treatment could provide a sustainable and environmentally friendly means for obtaining natural chemical components suitable for various industrial applications. Few systematic studies regarding the effect of thermo treatment combined with extraction technique are reported in literature. Although the potential industrial role of wood extracts could have a relevant application also in the pharmaceutical field, it has not been sufficiently evaluated. Using various techniques for isolating wood extracts from poplar, we evaluated the content and antioxidant activity of phenolic compounds (including tannins and flavonoids) extracted from thermo-treated and untreated wood. Heat treatment modified the extracts, and temperatures over 180 °C clearly increased the extractive yield for poplar wood. The thermo-process produced mainly polar compounds, particularly for poplar wood subjected to temperatures between 200 and 220 °C. Extracts obtained after the 200 °C treatment showed the highest contents of secondary metabolites, such as flavonoids and polyphenols, and

the highest antioxidant activity levels. Extracts of poplar wood obtained at the highest temperature reported the highest RACI values.

The present study can be considered the first report on the reducing antioxidant power (FRAP) and the inhibition of lipid peroxidation (BCB) of extracts derived from thermo-vacuum treated wood, together with the evidence about the influence of differential thermal-treatment *vs* extraction techniques. Moreover, we have demonstrated as statistical significant correlation among chemical constituents and the biological activity demonstrated by 3 different chemical assays.

Further investigations should be performed on poplar extracts to determine their potential applications as food supplements, nutraceuticals and health promoting natural compounds. More studies are needed to understand wich compounds are the main responsible of the measured biological activities, through their isolation, identification and observe their potential structural changes in response to thermal treatment.

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