GC-MS and SPME Techniques Highlighted Contrasting Chemical Behaviour in the Water Extractives of Modified Castanea sativa Mill. and Fagus sylvatica L. Wood

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Abstract: The sweet chestnut (Castanea sativa Mill.) and European beech (Fagus sylvatica L.) are wood species largely present in the European forest area. The composition and relative variation of the secondary metabolites of chestnut and European beech wood under thermal effect is a little-explored area. The wood material was thermally modified at 170 °C for 3 h using a thermo-vacuum technology. Raw and modified wood extracts were obtained with aqueous extraction techniques in an autoclave, subsequently lyophilized, solubilized in ethyl acetate, and determined by Gas Chromatographic-Mass Spectrometric Analyses (GC-MS). In addition, the volatile compounds were determined by Solid-Phase Micro Extraction (SPME) analyses. As a general statement, the extraction in an autoclave produced a higher number of compounds in the modified chestnut and beech wood compared to unmodified wood material. Beech wood showed low degradation in the compounds after modification. Notably, squalene and ar-tumerone were the main bioactive compounds present in beech wood extractives. Chestnut, conversely, showed a greater degradation after thermo-modification. However, a reduction in chemical compounds in the modified samples was also observed. In this case, the main biologically active compounds detected only in the chestnut control samples were apocynin and ar-tumerone. The recovery of this residual wood material, before energy consumption, could provide a sustainable and environmentally friendly means of obtaining natural chemicals suitable for various industrial applications.

Keywords: Castanea sativa; Fagus sylvatica; wood; thermal modification; autoclave extraction; extractives; GC-MS; SPME

1. Introduction

In the last decades, with the aid of various investigation techniques, the study of the chemical composition of wood and in particular of its extractives has gained noticeable importance. Wood represents a natural resource, largely used for energy purposes, in the construction, furniture, pulp and paper industries, and for the creation of chemical products and compounds of great industrial use, widely used in the cosmetics, biomedical, pharmaceutical and food sectors, and even in biodiesel production [1,2]. The field of wood chemistry and wood-derived products covers various aspects of chemical technology applied to the wood sector: from the amounts of lignin, cellulose and hemicelluloses, analysis of extractives, up to the characterization of adhesives and studies on the interphase between wood and adhesive formulations or finishing products. Many research groups have implemented the wood modification processes to make the wood material more suitable for any type of application [3].

Thermal modification is a technique, based on the principle according to which wood, subjected to high temperatures, modifies its chemical–physical structure, increasing its
durability, dimensional stability and altering its colour; this technique is very effective in improving some wood properties without resorting to the use of chemical additives [4]. However, it can reduce its mechanical properties [5]. The use of different heating techniques over a wide range of time and temperatures allows a more detailed analysis of the wood concerning its heterogeneous thermal response.

Although numerous studies have been conducted on improving the overall properties of wood, there has recently been a growing interest in characterizing the thermal degradation of modified wood products [6,7], which could be related to both the increase in demand for wood and the new concept of environmental management policies [8,9].

Recently, the European research program has been placing a lot of importance on biorefinery and green chemistry; one of the main purposes is to guarantee and produce innovative and renewable products using local bioresources such as wood and residual biomass. Although several studies have reported the profile of secondary metabolites and bioactivity of extractives obtained from the bark or other parts of the tree [10], the profiles of natural compounds have not yet been well studied and defined, obtained from heat-treated wood, even that is of growing interest. Some authors have shown the influence of heat treatment on extractives and related antioxidant properties of poplar [11], beech and spruce wood [12,13], highlighting potential alternative applications of biomass beyond energy or industrial uses. Other researchers have also studied the change in yield of extractives following heat treatment of beech and found that it was particularly relevant at 160 °C, possibly due to hemicellulose decomposition [14]. On the contrary, it has been shown that the heat treatment of beech (Fagus sylvatica Linnaeus, 1753) and spruce (Picea abies Karsten, 1881) at 220 °C produces structural alterations both of thermally labile carbohydrates and of some lignin fractions [15].

The thermal decomposition process of wood and wood products is based on a series of complex reactions influenced by many factors, such as heating rate, temperature, exposure time, pressure, humidity, biomass composition and size of the particles. Most of the changes in the properties of wood are related to changes that occur at a chemical level, i.e., the microstructure of wood is altered due to the action of heat. During the heat treatment process, volatile extractives are formed by the alteration and degradation of polysaccharides and lignin [16] and are usually removed first from the wood, followed by new products and compounds resulting from various chemical reactions. The chemical changes occur in the degradation of its structural polymers. The first chemical component that changes is hemicellulose as it undergoes deacetylation reactions which ultimately lead to the formation of low-molecular-weight compounds possibly extractable from wood [17–19]. As for the non-structural compounds, that is, the extractives, during the heat treatment the most volatile ones are released from the wood, while others are completely degraded [20] or transformed into new compounds [21]. These new compounds can be extracted and used in different market sectors because they have important properties, also acquiring a certain value through the development of new products [22]. A better understanding of the differences between extractives during heat treatment is needed to provide their use in different fields such as pharmaceutical and cosmeceutical. For these reasons, the enhancement of secondary wood products remains an open challenge of great interest.

The extraction composition is highly variable both within and between species [23] and depends on the age, seasonality and position of the tree [24].

Extractives can be extracted from wood with different extraction techniques that affect the yield and the type of compound extracted. Currently, the most commonly used extraction technique requires the Soxhlet apparatus with solvents or the autoclave with hot water as a simple ecological method. Extractives are generally classified as water-soluble, ethanol-toluene soluble or ether soluble, depending on the solvent used [22].

The type and quantity of extractive compound dissolved in each solvent is different and the choice of the most appropriate solvent depends on the application. Organic solvents are capable of extracting resin compounds, while water is useful for extracting phenolic compounds, glycosides, carbohydrates and soluble salts [25].
Therefore, since extractives are the key constituents that guarantee the durability of wood, the aim of this research work was to evaluate the effect of thermal modification on extraction yield, chemical composition and quality of the extractives, and demonstrate that new green chemistry procedures available could be used to increase the quantity of recoverable extractives.

For this purpose, unmodified and thermally modified Castanea sativa and Fagus sylvatica wood was used.

The total Italian forestry area is 10.5 Mha, of which the area covered by beech trees amounts to 1.035 Mha, about 10.7% of the total, while that covered by chestnut trees amounts to 0.788 Mha, 8.1% of the total [26]. Castanea sativa, or sweet chestnut, is a plant species of the Fagaceae family, native to Europe and Asia Minor, and is one of the most durable wood species in Europe. Due to its good durability, wood is often used for poles and other outdoor applications, both in and above ground and for the manufacture of furniture, barrels and constructions, particularly in southern Europe [27]. One of the reasons for the good duration is the presence of extractives, mainly tannins, used in the past for the production of some medicines, for the production of leather, as well as playing a role in the flavouring of various types of alcoholic beverages [28]. Among other physical and mechanical changes introduced by thermal modification [29], the nature and performance of extractives derived from thermo-modified wood can differ significantly from that of native wood materials. Modified wood extractives can be altered by thermal modification, but new extractives compounds can also form during this process, which are important natural resources in the wood industry [30].

Fagus sylvatica, whose colour ranges from blond to reddish, is a wood of medium hardness and rather heavy, widespread in the Italian Alps and Apennines. Easy to work, it is used in carpentry to make rustic furniture and furnishing accessories; a compact wood, but easily attacked by woodworms and fungi if not properly treated [31]. Beech wood is used almost only as firewood; therefore, it is interesting to pay particular attention to the recovery of this wood and its compounds.

The woody resources of Castanea s. and Fagus s., mainly those derived from processing waste from the wood industry, can be recovered by obtaining extracts that represent a source of valuable natural compounds. The recovery of this residual wood material, before energy consumption, could provide a sustainable and environmentally friendly means of obtaining natural chemicals suitable for various industrial applications, as measured dietary supplements, nutraceuticals and natural health-promoting compounds.

2. Materials and Methods

2.1. Sample Preparation and Thermal Treatment

The sweet chestnut and beech timber used in this study originated from a high forest stand located in Southern Italy (Basilicata Region). For this study, five trees with an age of almost 100 years were felled. Randomly selected chestnut and beech boards of $30 \times 250 \times 2500$ mm$^3$ (thickness $\times$ width $\times$ length, respectively) were then utilized as the experimental samples.

The wood was dried at 0% humidity under vacuum (200 mbar) at 90 °C for 12 h. From an initial (pre-drying) temperature of 30 °C, the temperature was increased by 5 °C every hour to 90 °C. At the end of the drying cycle, the oven was shortly opened and the wood samples were quickly weighed [32].

The thermal modification itself began after the drying of the wood by further increasing the ambient air temperature to 170 °C in 10 h. The duration of the treatment on the wood core was 3 h. The cooling of the wood to 100 °C was carried out in about 5 h after the modification of the thermo-vacuum [33].

2.2. Autoclave Extraction

A wood sample (10 g) was placed in an airtight glass jar with 50 mL of distilled water and placed in an autoclave (Vapor Matic 770 Asal srl, Cernusco, Italy) for 20 min, at a
temperature of 120 °C and a pressure of 1 bar for extraction. The sample was filtered and frozen at a temperature of −28 °C and subsequently lyophilized to remove the water [34]. The resulting mixture was solubilized in ethyl acetate and analysed (see below).

2.3. Gas Chromatographic-Mass Spectrometric Analyses

Analyses of all the extractives obtained using the procedures described above were accomplished with an Hewlett-Packard (HP) 6890 Plus gas chromatography equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm FT) (Agilent, Milan, Italy). An HP 5973 mass selective detector (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. A split injector was maintained at 250 °C and the detector was maintained at 230 °C. The oven was held at 80 °C for 2 min, gradually warmed at 8 °C/min, up to 250 °C, and held for 10 min. The tentative identification of aroma components was based on mass spectra and NIST (National Institute of Standards and Technology) 11 library comparison [32].

2.4. Solid-Phase Micro Extraction Analyses

In order to determine the chemical composition of the volatiles, we used SPME sampling; 20 mL of water extractives were placed in Headspace vials and incubated at 50 °C for 15 min (incubation time) to promote volatile compounds in the headspace. Extractions were realized by immersing the fiber in the headspace for 30 min (extraction time) and then withdrawn into the needle and transferred to a GC/MS system [35]. A 50/30-μm DVB/CAR/PDMS module (57328-U, Supelco, Milan, Italy) was employed to determine volatile organic compounds (VOCs).

Analyses were accomplished with an HP 6890 Plus gas chromatograph (Agilent) equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm FT) (Agilent, Milan, Italy). An HP 5973 mass selective detector (Agilent) was utilized with helium at 0.8 mL/min as the carrier gas. A splitless injector was maintained at 250 °C and the detector at 230 °C. The oven was held at 40 °C for 2 min, then gradually warmed, 8 °C/min, up to 250 °C and held for 10 min. Tentatively, identification of aroma components was based on mass spectra and NIST 11 library comparison. Single VOC peak was considered as identified when its experimental spectrum matched with a score over 90% that was present in the library and if the retention time was in agreement with the reported retention index.

3. Results and Discussion

Few literature data have reported the impact of the modification on the extraction potential of volatile and non-volatile compounds for chestnut and beech wood following aqueous extraction, and discrepancies can often be easily found in them. It is known that wood subjected to heat treatment undergoes a greater cross-linking of the chemical bonds that constitute it, compared to non-thermo-treated wood [36]. It is also true that with heat treatment, the hemicellulose undergoes such reactions as to produce polar compounds that are easily extractable with polar solvents such as water.

Treatment at temperatures above 150 °C can change the colour, improve resistance to biodegradation and enhance dimensional stability [37]. However, losses in the mechanical strength of wood may also occur, and this drawback is a limitation for the use of modified wood in a broad range of products. Indeed, according to the work conducted by Sundqvist [38], cellulose degradation can contribute to the loss of mechanical strength in wood under high-temperature treatment. Heat modification changes the properties of wood by changing its composition, which varies widely between species. These chemical changes due to heating depend on the temperature and duration of the treatment [11]. In treatments carried out at low temperatures (20 °C–150 °C), when the wood dries, there is first a loss of free water and subsequently a loss of bound water. On the other hand, during moderate heating temperatures (180 °C–250 °C), in the typical range of heat treatment, the
wood undergoes important chemical changes, and finally, at high temperatures (>250 °C) the carbonization processes begin [4].

The following Tables 1 and 2 show the total percentage areas of chestnut and beech wood extractives, both for unmodified and modified wood material, obtained with the GC-MS and SPME analytical techniques. All the compounds reported were tentatively identified by comparison of the registered mass spectrum with those collected in NIST 11 library. The differences in the chemical composition of the extracts can be attributed, in large part, to the thermal process that modifies the chemical composition of the wood as it causes the degradation of the biopolymers through pyrolysis and thermolysis [38].

Table 1. Compounds found in GC-MS and SPME analysis of (a) unmodified and (b) modified (HT) beech wood water extractives.

| Compound                                      | GC-MS | SPME | Compound                                      | GC-MS | SPME |
|-----------------------------------------------|-------|------|-----------------------------------------------|-------|------|
| (a) Fagus s.                                   |       |      | (b) Fagus s. HT                               |       |      |
| 1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl | 1.42  |      | 1(2H)-Naphthalenone, 7-(1,1-dimethylethyl)-3,4-dihydro | 0.22  |      |
| 2,6-Diisopropynaphthalene                      | 0.79  |      | 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester | 0.32  |      |
| 2-Bromo dodecane                               | 0.29  |      | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester | 0.10  |      |
| 2-Hepten-1-one, 1-(2-hydroxy-5-methylphenyl)   | 2.67  |      | 1-Decanol, 2-hexyl                               | 2.25  |      |
| 2-methyloctacosane                             | 0.35  |      | 1H-Benzimidazol-2-amine                         | 1.11  |      |
| 3-Acetylphenanthrene                           | 1.77  |      | 1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl | 0.34  |      |
| 3-Methoxyanphemamine                           | 4.48  |      | 1H-Indole, 2-methyl                             | 9.76  |      |
| 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol  | 1.15  |      | 3-Isobutyl-4,5-dimethyl-3H-isobenzofuran-1-one | 0.35  |      |
| 4,7-Dimethoxy-2-methyl-1H-indene               | 0.37  |      | 4-(3,5-Dimethyl-2-benzofuranyl)-2-butanone      | 0.32  |      |
| 4-Acetyl-1-methylcyclohexene                   | 0.49  |      | 4-Acetyl-1-methylcyclohexene                    | 0.29  |      |
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 1.72  |      | Acetic acid                                    | 5.10  |      |
| Acetic acid                                    | 3.82  |      | Ar-tumerone                                    | 1.48  |      |
| Ar-tumerone                                    | 0.77  |      | Cresol                                         | 0.22  |      |
| Azulen-2-ol, 1,4-dimethyl-7-(1-methylethyl)    | 1.07  |      | Decane, 5,6-dipropyl                           | 4.65  |      |
| Benzene, (1,1-dimethyl-2-propenyl)             | 0.19  |      | Eicosane                                       | 2.74  |      |
| cis(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene | 0.15  |      | Ethanone, 1-(2-methylphenyl)                   | 0.19  |      |
| Decanal                                        | 0.16  |      | Ethanone, 1-(9-anthracenyl)                    | 1.20  |      |
| Dodecane                                       | 0.10  |      | Ethyl Acetate                                  | 1.21  |      |
| Eicosane                                       | 4.02  |      | Furfural                                       | 0.56  |      |
| Ethanone, 1-(4-methylphenyl)                   | 0.43  |      | Hentriacontane                                 | 1.23  |      |
| Ethyl Acetate                                  | 0.44  |      | Hentriacontane                                 | 0.85  |      |
| Ethyltetramethylcyclopentadiene                | 10.20 |      | Heptacosane                                    | 1.00  |      |
| Heptacosane                                    | 1.01  |      | Hexacosane                                     | 1.07  |      |
| Heptadecane                                    | 1.48  |      | Hexadecane                                     | 0.66  |      |
### Table 1. Cont.

| (a) *Fagus s.* | (b) *Fagus s. HT* |
|----------------|------------------|
| **Compound**   | **GC-MS** | **SPME** | **Compound** | **GC-MS** | **SPME** |
| Heptadecanoic acid, 16-methyl, methyl ester | 1.11 | Mequinol | 0.23 |
| Hexacosane | 0.16 | Methyl stearate | 1.15 |
| Hexadecane | 0.45 | Naphthalene, 1,2,3,4-tetrahydro-1-methoxy | 3.54 |
| Hexadecane | 0.87 | Nonadecane | 0.21 |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 6.31 | Octadecane | 0.63 |
| Hexane, 2,3,4-trimethyl | 0.02 | Octadecanoic acid | 2.38 |
| Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) | 3.46 | Squalene | 3.45 |
| Naphthalene, 1,2,3,4-tetrahydro-6,7-dimethyl | 0.93 | Tetratetracontane | 0.65 |
| Naphthalene, 6-ethyl-1,2,3,4-tetrahydro | 0.61 | Triacontane | 1.06 |
| n-Hexadecanoic acid | 1.38 |
| Nonacosane | 0.84 |
| Octacosane | 0.10 |
| Octadecanoic acid | 0.84 |
| Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 6.92 |
| Octane, 2,3,3-trimethyl | 0.07 |
| Octicizer | 0.27 |
| Oxalamide, N-(2-hydroxyethyl)-N'-(2-hydroxyethyl)-N'-isochroman-1-ylmethyl | 5.66 |
| Pentacosane | 2.49 |
| Pentadecane | 0.48 |
| Pentadecane, 2,6,10-trimethyl | 0.53 |
| Phenol, 2,4-bis(1,1-dimethylethyl) | 0.07 |
| Phenol, 3,4,5-trimethoxy | 0.22 |
| Supraene/Squalene | 3.05 |
| Tetracosane | 1.40 |
| Tetratetracontane | 1.63 |
| Triacontane | 1.13 |
| Tributyl acetylcitrate | 1.09 |
| Undecane, 2,7-dimethyl | 0.12 |
Table 2. Compounds found in GC-MS and SPME analysis of (a) *Castanea s.* and (b) *Castanea s.* heat-treated (HT) wood water extractives.

| Compound | GC-MS | SPME | Compound | GC-MS | SPME |
|----------|-------|------|----------|-------|------|
| 1H-Benzocycloheptene, 2,4a,5,6,7,8,9a-octahydro-3,5,5-trimethyl-9-methylene-,(4aS-cis) | 2.40 | 1.36 | 10-Methylnonadecane | 0.75 |
| 1H-Inden-1-one, 2,3-dihydro-3,3,5,6-tetramethyl | 0.64 | 1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl | 0.50 |
| 1H-Indole-2,3-dione, 1-(tert-butyldimethylsilyl)-6-[tert-butyldimethylsilyl]oxy]-3-(O-ethylxime) | 1.61 | 1H-Inden-1-one, 2,3-dihydro-3,3,5,7-tetramethyl | 0.67 |
| 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethyl) | 0.16 | 1H-Indole, 2,3-dihydro-4-methyl | 0.31 |
| 2-Ethylhexyl salicylate | 0.08 | 1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene | 0.21 |
| 2-Furancarboxylic acid | 0.36 | 2,6-Bis(1,1-dimethyl)-4-(1-oxopropyl)phenol | 0.25 |
| 2-Nonenal, (E) | 0.19 | 2-Benzoxazolamine,N-(1,1-dimethyl) | 0.42 |
| 3,5-Dimethoxy-4-hydroxycinnamaldehyde | 2.14 | 2-Furancarboxaldehyde, 5-methyl | 1.16 |
| 3-Allyl-6-methoxyphenol | 0.13 | 2-Nonenal, (E) | 1.51 |
| 3-Furancarboxylic acid, methyl ester | 0.10 | 6,7-Dimethoxyquinoline | 0.42 |
| 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol | 4.73 | Acetic acid | 0.90 |
| 4-Hydroxy-2-methoxycinnamaldehyde | 1.84 | Acetic acid, 2-phenylethyl ester | 0.73 |
| 4-Methoxy-4',5'-methylenedioxybiphenyl-2-carboxylic acid | 3.20 | Acetic acid, octyl ester | 1.16 |
| 5-Hydroxymethylfurfural | 3.60 | alpha-Amino-3'-hydroxy-4'-methoxyacetophenone | 0.15 |
| Acetic acid | 8.16 | Benzaldehyde | 0.10 |
| alpha-Amino-3'-hydroxy-4'-methoxyacetophenone | 0.28 | Benzene, 1-methyl-3-(1-methylethyl) | 0.50 |
| Apocynin | 0.21 | Creosol | 0.21 |
| Ar-tumerone | 0.48 | Decanal | 2.82 |
| Benzaldehyde | 0.10 | Eicosane | 3.71 |
| Benzaldehyde, 2-hydroxy-,(2,4-dinitrophenyl) hydrazone | 0.19 | Ethanon, 1-(2-hydroxyphenyl) | 2.73 |
| Benzene, (3-methyl-2-butenyl) | 0.19 | Eugenol | 10.53 |
| Benzene, 1-(1-buten-3-yl)-4-pentyl | 0.76 | Furfural | 1.89 |
| Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl) | 0.44 | Hexacosane/Heneicosane | 2.12 |
| Benzene, 2-(2-butenyl)-1,3,5-trimethyl | 0.74 | Hexadecane/Heptadecane | 0.21 |
| Benzoic acid, 2,3-dihydro-2-methyl | 0.11 | Indan, 1,1,6,7-tetramethyl | 0.16 |
| Benzoic acid, 4-hydroxy-3,5-dimethoxy | 6.32 | Isochroman | 12, 986 | 7 of 12 |
Autoclave extraction resulted in a large number of compounds in the thermo-modified chestnut and beech, with a greater amount of alkyl compounds only found in modified wood of both wood species.

Derivatives of fatty acids have been extracted from both modified wood species, in particular, octadecanoic acid. This is a saturated fatty acid obtained from hydrolysis or the degradation of fatty acids during the heat process. Octadecanoic acid is used in the production of candles, soaps, plastics, oil pastels, and cosmetics [39].

As can be seen from Tables 1 and 2, sweet chestnut and beech showed a different response to thermal treatment, although both were processed at a temperature of 170 °C. At temperatures of 150–200 °C, the degradation rate during thermal modification was four times higher for hemicellulose than for cellulose and lignin, and VOCs, formed due to heating of wood, were trapped in the process and promote the degradation rate [37]. These

Table 2. Cont.

| Compound                                                                 | GC-MS | SPME   | Compound                                    | GC-MS | SPME   |
|--------------------------------------------------------------------------|-------|--------|---------------------------------------------|-------|--------|
| Benzoic acid, 4-hydroxy-3-methoxy                                        | 3.83  | Naphthalene, 1,4,6-trimethyl                 | 0.60  |
| Benzoazole, 2-methyl                                                     | 2.59  | Naphthalene, 1,6-dimethyl-4-1-methylethyl   | 0.16  |
| Decanal                                                                  | 0.13  | n-Hexadecanoic acid                         | 1.06  |
| Eicosane                                                                 | 0.32  | Octadecanoic acid                           | 1.76  |
| Eugenol                                                                  | 3.28  | Pentacosane                                  | 1.74  |
| Furfural                                                                 | 0.33  | Tetradecanal                                 | 0.30  |
| Heptacosane                                                              | 0.41  | Tetrahydro-1,3-oxazine-2-thione              | 3.22  |
| Homovanillic acid                                                        | 0.65  | Vanillin                                     | 0.83  |
| Ledene oxide-(I)                                                         |       | 0.08                                          |
| Maltol                                                                   | 0.11  |
| Mandelic acid, 3,4-dimethoxy-, methyl ester                              | 1.23  |
| Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl                          | 0.04  |
| Naphthalene, 1,6,7-trimethyl                                              | 0.21  |
| Naphthalene, 1,6-dimethyl-4-(1-methyl)                                   | 0.19  |
| n-Hexadecanoic acid                                                      | 2.12  |
| Nonadecane, 9-methyl                                                     | 0.37  |
| Octacosane                                                               | 0.22  |
| Octadecane                                                               | 0.55  |
| Octadecanoic acid                                                        | 1.82  |
| Pantolactone                                                             | 0.06  |
| Pentanoic acid, 4-oxo                                                    | 0.10  |
| Phenol                                                                   | 0.05  |
| Phenol, 2,6-dimethoxy                                                    | 0.50  |
| Phenol, 2,6-dimethoxy-4-(2-propenyl)                                     | 0.49  |
| Phenol, 2-methoxy-4-propyl                                               | 0.19  |
| Tetradecane                                                              | 0.25  |
| Toluene                                                                  | 0.02  |
| Tridecanal                                                               | 0.10  |
| Vanillin                                                                 | 1.56  |
modifications, however, also strongly depend on the species used and their microstructural characteristics. Chestnut and beech wood showed several differences in terms of the morphological anatomical parameters and this evidence can be one of the explanations of the different effects of thermal modification on the obtained results.

3.1. Fagus sylvatica

In beech wood, there were no substantial changes in the compounds, therefore results showed less degradation due to thermal modification. Among the main bioactive compounds found in beech, squalene (detected by GC-MS) and ar-tumerone (detected by SPME) are worthy of attention (Table 1).

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a triterpene that acts as an intermediate in the biosynthesis of phytosterol or cholesterol in plants, animals and humans. In its pure form, it is a colourless, odourless, tasteless, and highly stable hydrocarbon oil that is biocompatible with skin, non-toxic and non-irritant, and prevents moisture loss, restoring skin’s suppleness and flexibility [40]. It presents remarkable antioxidant and antibiotic activities derived from its chemical structure of six double bonds. Squalene is used to make other chemicals such as drugs and rubber chemicals. It is used in cosmetics including sunscreen lotions, lipstick foundations, nail products, hair conditioners and moisturizers, and it is also used as a dietary supplement and in traditional medicine for disease management and therapy [41]. It offers a wide range of benefits such as emollient, hydrating, high spreadability, light consistency, non-greasy texture, rapid transdermal absorption, and reduction of skin damage by UV radiation; moreover, it has been used in healing eczema and the protection against ageing and wrinkles. It is also used in the preparation of stable emulsion as an adjuvant for vaccine delivery stimulating the immune response and increasing the patient’s response to the vaccine. Although squalene by itself is a weak inhibitor, it has been shown to prevent or arrest tumour growth in conjunction with anticancer drugs in various experimental tumour models [42,43]. The liver oil of sharks from the deep sea represents the richest natural source of squalene. Important reasons are limiting the use of this source, such as the presence of persistent organic pollutants in the sea, which can still be found in the purified squalene; the regulations against the overfishing of sharks; and the increasing trend among the consumers towards the use of plant-based products. Squalene has multiple applications due to its unique properties.

Considering the abovementioned factors, there is a great interest in finding new, natural, mainly vegetable, sources for squalene. The most relevant plant sources of squalene are oils extracted from amaranth, olive, palm, rice, wheat germ, grape seed, peanut, and soybean [44]. Most vegetal oils are obtained by mechanical pressing or extraction with solvents like hexane; therefore, water extraction can offer a valid alternative of green chemistry for obtaining these oils. The growth in the cosmetics and pharmaceutical industry, along with the increasing research in the oncology segment, are the major factors driving the growth of the squalene market.

The terpene ar-Tumerone is a compound found mainly in curcumin, a perennial plant cultivated throughout tropical Asia, India, and China. This compound has been investigated in clinical trials for its potential therapeutic properties, including anticancer activity [45]. Ar-Tumerone acts through various mechanisms, including the induction of apoptosis, inhibition of angiogenesis, and modulation of tumour suppressor genes [46]. A new study, analyzing the effect that this substance brings to brain cells both in vitro and in vivo, showed that when brain cells were exposed to Ar-Tumerone, neural stem cells increased in number through greater proliferation. Furthermore, these newly formed neural stem cells also increased the number of fully differentiated neuronal cells, indicating that they immediately initiated a healing effect [47].

3.2. Castanea sativa

On the other hand, sweet chestnut showed greater degradation following thermal modification at 170 °C, with a reduction of chemical compounds in the modified sam-
samples compared to the unmodified sample. The main biologically active compounds detected only in the unmodified chestnut samples are apocynin (detected by GC-MS) and ar-tumerone (detected by SPME), previously described in Table 2.

Apocynin, also known as acetovanillone, is a natural organic compound structurally related to vanillin [33]. Apocynin is an inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and thus is effective in preventing the production of the superoxide in human white blood cells or neutrophilic granulocytes. Due to the selectivity of its inhibition, apocynin can be widely used as an inhibitor of NADPH oxidase without interfering in other aspects of the immune system. Apocynin has also anti-arthritic and anti-asthmatic properties [48–50].

As is known, the different yields of the extraction technique could be explained by the different mechanisms of action such as the rupture of the plant tissue [51], or by a diffusion process as previously observed for the chestnut [28]. These results showed that the intermediate treatment of 180 °C, regardless of the extraction technique, did not significantly affect the extraction yield which showed values similar to those of untreated wood.

4. Conclusions

One of the most important aspects to be considered in the biorefinery is finding green solutions in the extraction techniques and at the same time preserving the bioactivity of the extracts avoiding their degradation during extraction processes.

Extraction in an autoclave produced a high number of compounds in the modified chestnut and beech, with a greater quantity of alkyl compounds only in the heat-treated wood of both wood species. Derivatives of fatty acids have been extracted from both thermo-modified wood species, in particular octadecanoic acid, but chestnut and beech showed a different response to the heat process.

Beech did not show substantial changes in the compounds due to less degradation after the heating process. Among others, squalene and ar-tumerone represented the main bioactive compounds.

In recent years, the recovery of bioactive compounds from plant biomass has increased. Beech wood is recently used mainly as firewood, so it is interesting to underline the recovery of this wood and its biologically active compounds before burning material.

In contrast, sweet chestnut showed a greater degradation after modification, with a reduction in compounds in the modified samples compared to the unmodified ones. The main biologically active compounds detected only in the unmodified chestnut samples were apocynin and ar-tumerone.

The contrasting results observed in our study can be attributed to the species used and their different microstructural characteristics.

In both species, the importance of the Solid-Phase Micro Extraction technique (SPME), complementary to Gas Chromatographic-Mass Spectrometric analyses (GC-MS), is evident to obtain a greater overview of the available biologically active volatile and non-volatile compounds with potential use in the biological, pharmaceutical and chemical fields.

The application of natural wood extractives could be an important opportunity for the economic development of mountain areas due to the enormous quantity of biomass that can be used as a biorefinery. Additionally, further investigation should be conducted on such extracts to determine their potential biological activities and evaluate their applications as measured dietary supplements, nutraceuticals and natural health-promoting compounds.

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