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# Extraction Methods and Their Influence on Yield When Extracting Thermo-Vacuum-Modified Chestnut Wood

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**Abstract:** Improvements in the yield and solubility of chestnut wood extractives, by using different extraction methods and molybdenum catalysts as support, have rarely been reported in literature. Many studies focus on the different parts of trees, except for the chemical characteristics of the remaining extractives achieved from thermally modified (THM) chestnut (*Castanea sativa* Mill) wood. This research seeks to better understand the effects of extraction techniques and catalysts on the yield and solubility of extractives. GC-MS analysis of the chloroform soluble and insoluble fractions was also used. Accelerated Solvent Extraction (ASE) 110 °C, Soxhlet, and autoclave extraction techniques were used to obtain extractives from untreated and thermally modified (THM) chestnut wood (170 °C for 3 h). Ethanol/$H_2O$, ethanol/toluene, and water were the solvents used for each technique. A polyoxometalate compound ($H_3PMo_{12}O_{40}$) and $MoO_3$ supported on silica were used as catalysts. The THM induced a change in the wood's surface color ($\Delta E = 21.5$) and an increase in mass loss (5.9%), while the equilibrium moisture content (EMC) was reduced by 17.4% compared to the control wood. The yields of the extractives and their solubility were always higher in THM and mainly used ASE as the technique. GC-MS analysis of the extractives, without catalyst support, showed different results for each extraction technique and type of wood (untreated and THM). Ultimately, the amount of extractive compound dissolved in each solvent will differ, and the choice of extraction technique will depend on the intended final application of the extracted chemical product.

**Keywords:** *Castanea sativa*; thermo-modified wood; extractives; ASE; phosphomolybdic acid; silica-supported molybdenum trioxide



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## 1. Introduction

The European bioeconomy strategy addresses the production of renewable biological resources and their conversion into vital products and bioenergy. According to this recent program, chemical compounds derived from natural sources will be more available in regions where these compounds can be obtained, in a more economical and environmentally-friendly way than more expensive synthetic chemicals. In this respect, biorefineries are becoming important aspects of green chemistry development aimed at ensuring the ability to achieve the best objectives, as readily as possible, using restricted natural resources, such as forest biomass. One of the main goals of this program is to generate diversified, innovative, and renewable products using locally available bioresources, such as wood and tree residues.

The scientific literature has widely disseminated the technical advantages and disadvantages of wood modification [1–4]. Based on the data reported in Jones et al. [5], the annual commercial production volumes of modified wood depend on the process technology applied to the wood material.

The possible reuse of wood waste to obtain valuable substances is, on the other hand, an attractive research field. Moreover, the obtained extractives depend on the

thermal degradation of wood cell wall components, which is strongly correlated to the treatment conditions.

The industrial potential of the chemical compounds derived from the remaining modified wood, however, has not yet been adequately addressed. One of the interests of this study is to define the properties of chemical compounds formed and extracted in the wood after thermal modification, particularly the secondary metabolites derived from wood. This study also proposes additional utilization possibilities for such chemical compounds.

Extracts are considered nonstructural secondary metabolites of wood and can be obtained with different types of solvents and methods. However, several methodologies used for extraction and concentration may restrict the use of nonstructural components [6].

Furthermore, the antioxidant activities of phenolic compounds extracted from different types of wood have been described. In comparison with the other wood species, it has been noted that the chestnut (*Castanea sativa* Mill) samples gallic acid content was higher than the content of ellagic acid and high concentrations of 4-hydroxybenzoic acid and p-coumaric acid. Chestnut was the species with the highest concentrations of vescalagin, roburin E, and castalagin [7]. For example, the extraction of phenolic compounds of chestnut leaves showed how extraction time and temperature affected the yields of soluble solids and phenolics strongly. The authors stated that the extractives (9.48–13.5/100 g of dry biomass) depend on the extractive solvent used. The major compounds identified in the leaf extracts were gallic acid, protocatechuic acid, 4-hydroxyben-zoic acid, vanillic acid, rutin, quercetin, and apigenin [8]. The extractives of chestnut wood have been characterized in HPLC studies using different extraction techniques. Sanz et al. [9], have demonstrated the composition of phenolic and tannic of heartwood extracts from chestnut, before and after toasting in cooperage, and some low molecular weight phenolic compounds and hydrolyzable tannins were found. The low molecular weight phenolic compounds were lignin constituents as the acids gallic, protocatechuic, vanillic, syringic, ferulic, and ellagic, the aldehydes protocatechuic, vanillic, syringic, coniferylic, and sinapic, and the coumarin scopoletin. In research by Canas et al. [10], heat treatment showed a very significant influence on the majority of low molecular weight extractable. As the temperature increases, the increment of syringyl-type compounds (sinapaldehyde and syringaldehyde) is much higher than those of guaiacyl-type compounds (coniferyl aldehyde and vanillin).

Chestnut trees occupy a significant share of forested areas, but their wood is often used mainly for the production of poles for fencing. Besides, wood is also used for the production of finished goods, such as furniture and construction elements. The chestnut tree is one of the most representative wood species in Europe, which makes it very interesting to study new possibilities for the wood's utilization and identify the products of greater quality and market value that can be derived from this species. Among other physical and mechanical changes introduced by thermal modification [11], the nature and performance of extractives derived from thermo-modified wood can significantly differ from that of native wood materials. Extractives of modified wood can be altered via thermal modification, but new compounds of extractives can also form during this process, and represent important natural resources in the wood industry [12].

Chestnut wood resources, mainly those derived from processing waste of wood industry, can be recovered by obtaining extractives that represent a source of natural compounds worthy of attention. Recovery of this remaining wood material, before energetic use, could provide a sustainable and environmentally friendly means for obtaining natural chemical components suitable for various industrial applications. On the other hand, European forest and wood sectors are currently going through a process of changes in approach and general tendencies, as far as the use and reuse of natural wood material are concerned.

The availability and high quality of natural extractives in trees make these natural substances a special resource that will continue to be investigated for other potential industrial uses, as these extractives have multiple applications in food, cosmetics, and pharmaceutics [13]. Understanding how these novel compounds from thermally modified chestnut wood can produce desirable effects on health is critical.

To the best of our knowledge, no detailed studies have yet reported on the extracts derived from thermally modified chestnut wood (THM) or on the effects of different extraction techniques and solvents for extractive characterization, even in the presence of molybdenum catalysts.

As reported by D'Auria et al. [14], the possible use of oxidant catalysts [15] to induce the oxidative depolymerization of wood, thereby increasing the amount of low molecular mass chemical compounds from that wood, has received little attention to date.

Previous studies have shown that the water extraction procedure made it possible to obtain mainly water-soluble compounds while non-polar or less polar lipophilic compounds cannot be extracted using this procedure [16].

Furthermore, if the extraction is performed in the presence of a microcrystalline polyoxomethalate compound, the amount of extractives increases significantly. Based on the oxidizing properties of the compound used, it seems to increase the degradation processes involving the lignin and the cellulosic fraction of the wood [17].

However, molybdenum compounds were first used by Mecca et al. [16] and Mecca et al. [17].

These studies show that heat modification and the use of molybdenum catalysts increase fatty acid esters (which have possible use in biodiesel synthesis) and that an increased amount of carbohydrates allows obtaining valuable amounts of biologically active compounds [16,17].

Compared to the previous study, we hypothesize that the Accelerated Solvent Extraction (ASE) technique allows an increase of both extraction yield and chemical compounds with industrial application.

Thus, to determine and evaluate the natural products from native and thermally modified chestnut wood, the main focus of this study is to evaluate the effects of THM along with the relevant extraction techniques, solvents, and catalysts on (i) the extraction yield and (ii) solubility in $CHCl_3$. We also perform an (iii) extractive characterization using CG-MS and (iv) determine the effect of molybdenum catalysts on the extractive characterization derived from THM wood.

## 2. Materials and Methods

### 2.1. Materials

The chestnut timber in this study originated from a high forest stand located in Southern Italy (Basilicata Region). For this study, 5 trees with an age of almost 100 years were felled. Randomly selected chestnut boards of 30 × 250 × 2500 $mm^3$ (thickness × width × length respectively) (*Castanea sativa* Mill.) were then utilized as the experimental samples. A total of 100 boards equally distributed between unmodified and modified materials were used. From those sets of wood boards, we then used little quantity of randomized powders for the subsequent experiments.

### 2.2. Thermo-Vacuum Treatment

Each board was split into two parts to generate twin samples, corresponding to the reference (no treated) and thermo-modified sample sets. The second set of boards was exposed to a thermally modified (THM) process in a thermo-vacuum plant produced by WDE Maspell srl (Terni, Italy). Both wood drying and effective thermal treatment were performed in the same processor without removing the samples.

Wood was dried to 0% moisture content under vacuum plant system (200 mbar) at 90°C for 12h. From an initial (pre-drying) temperature of 30 °C, the temperature was increased 5 °C each hour to 90 °C. At the end of the drying cycle, the kiln was momentary open and the wood samples were quickly weighted.

The thermal treatment itself started after wood drying by further increasing the ambient air temperature to 170 °C over 10h. The duration of the treatment on the core of the wood was 3 h. Cooling of the timber to ~100 °C was conducted over ~5 h after

the thermo-vacuum modification. Details regarding the thermo-vacuum process and its technical aspects are described in the literature [18–20].

The mass loss (ML) was determined by weighing each heat-treated board immediately after the drying process (when the wood moisture content (MC) was 0%) and at the end of the thermal modification.

The wood samples were then conditioned at 20 ± 2 °C and 65 ± 5% relative humidity to reach the equilibrium moisture content (EMC).

The color variation of the wood surface was obtained by a Minolta CM2002 Spectrophotometer (Minolta Corp, Osaka, Japan) with a spot probe 8 mm in diameter. The instrument measured the L* a* b* (lightness, red/green, and yellow/blue, respectively) chromaticity coordinates [21]. Fifty measurements were taken for each treatment.

### 2.3. Extraction Procedures

#### 2.3.1. Accelerated Solvent Extraction (ASE)

A portion of the samples was reduced to small size using a mill, and then 10 g particles of similar sizes were subjected to Accelerated Solvent Extraction (ASE at 110 °C) [22].

The extraction was carried out with a mixture of ethanol and distilled water (70:30, $v/v$) using a Dionex extractor monitored by an extracting system ASE 100 at a pressure of 10 bars over 3 cycles at 110 °C. The static time was 5min.

All extracts were then filtered, and the solvent was removed by a rotary evaporator at 37 °C. The dried extracts were kept in the dark at room temperature until use, and the yield was calculated as follows: (extract (g)/dried sample (g)) × 100.

#### 2.3.2. Soxhlet

The sample of wood was dried at 105 °C overnight. Then, it was grinded through a 40-mesh screen using a Wiley Mill. The obtained material (1.0 g) was put into an extraction thimble placed into a Soxhlet extraction apparatus. The sample was then extracted with 300 mL of 1:2 ethanol/toluene mixtures ($v/v$) for 7 h. After this period, the solvent was evaporated in a vacuum.

The extraction apparatus consisted of a 500-mL flask, Soxhlet tube, and 300-mm Allihn condenser.

Samples were put in cellulose thimbles (33 × 80 mm) of medium porosity [23].

#### 2.3.3. Autoclave Extraction

A sample of wood (10 g) was put into an airtight glass jar with 50 mL of distilled water and placed in the autoclave (Vapor Matic 770 Asal srl, Cernusco, Italy) for 20 min at a temperature of 120 °C and a pressure of 1 bar for the extraction. The autoclave had a maximum capacity of 23 L. The instruments were normally used as sterilizing equipment with vertical charging. The cycle was completely automatic, thermoregulated, and controlled by an HMOS microprocessor that enables programming different times and temperatures (from 100 to 130 °C).

The sample was filtered and frozen at a temperature of −28 °C. Then, it was freeze-dried to remove the water. The obtained extract was fractionated by treating the extract with chloroform (20 mL) and then filtering it, thereby obtaining two fractions: chloroform soluble and chloroform insoluble. The solvent was evaporated and the residue was chromatographed using thin-layer chromatography on silica gel in the presence of a fluorescent indicator. The different zones revealed by UV irradiation were separated and eluted with ethyl acetate. The solvent was evaporated, and the residue was analyzed as described below. The chloroform insoluble fraction was also treated as described below.

### 2.4. Solubility in $CHCl_3$

The extracts were treated with chloroform (5 mL) and the mixture was stirred for 10 min. The mixture was filtered and the solvent was evaporated.

### 2.5. Derivatization

We added 1 mL of pyridine to about 100 mg of the chloroform insoluble fraction of the extractives followed by 1 mL of acetic anhydride. The sample was allowed to sit at room temperature for 48 h, and then the solvent was changed with ethanol under reduced pressure followed by drying in a vacuum. The residue was chromatographed using thin-layer chromatography on silica gel in the presence of a fluorescent indicator. The different zones revealed by UV irradiation were then separated and eluted with ethyl acetate. The solvent was evaporated, and the residue was analyzed (see below).

### 2.6. Catalysts

The extractions were also performed in the presence of phosphomolybdic acid, which acted as an oxidant to favor the decomposition of lignin and cellulose. The experiments were performed only on THM wood to increase the extractive yield from this material. We did not report the results obtained while performing ASE extraction in the presence of phosphomolybdic acid because this procedure did not provide relevant results.

#### 2.6.1. Synthesis of the $H_3PMo_{12}O_{40}$ Catalyst

Heteropolyoxometalate $H_3PMo_{12}O_{40}$ was prepared according to the procedures in the literature [24]. We successively added 3.4 mL of $H_3PO_4$ (85% m/m) and 142 mL of 12 M $HClO_4$ to 210 mL of a 2.85 M solution of $Na_2MoO_4$, and the mixture was then cooled to room temperature. The warm solution took on a yellow color, and the disodium salt $Na_2H(PMo_{12}O_{40})$ was precipitated. After cooling to room temperature, the microcrystalline powder was filtered and air-dried. The salt was then recrystallized from a 20/100 mL $Et_2O/H_2O$ mixture, thereby obtaining about 50 g of a greenish precipitate. $H_3PMo_{12}O_{40}$ was obtained from a solution of 50 g of $Na_2HPMo_{12}O_{40}$, just recrystallized, in 100 mL of $H_2O$, acidified by 25 mL of 12 M HCl and extracted with 150 mL of $Et_2O$. When the water was added, yellow crystals of $H_3PMo_{12}O_{40}$ precipitated. The solid so obtained was filtered and dried at 70–80 °C [25]. The FTIR spectrum of the catalyst showed peaks at $\nu_{max}$ 750, 830, 952, and 1064 $cm^{-1}$, in agreement with the reported data [26]. Infrared spectra were obtained utilizing a Bruker Alpha FT-IR spectrophotometer (Bruker Photonics, Billerica, MA) configured for attenuated total reflectance (ATR) at ambient temperature.

#### 2.6.2. Synthesis of $MoO_3$ Supported on the Silica Catalyst

The calculated amount of 5 g aqueous ammonium heptamolybdate solution (81 wt% as $MoO_3$ (4.05 g)) was added into a suspension of 25 g of fumed silica (Aerosil 380) in distilled water (16% $MoO_3$/fumed Silica). The solution was then evaporated to dryness at 100 °C in a rotary evaporator. The solid obtained was dried at 120 °C overnight and calcined at 650 °C over 5 h in a muffle furnace. We obtained about 25 g of $MoO_3/SiO_2$. XPS analysis of the catalyst showed a Mo 3d5/2/Si 2p ratio of 2.97 in agreement with the value of 3.06 reported in the literature [27]. The signal of Mo 3*d* was found at 233.3 eV, while that of Si 2*p* was detected at 103.8 eV. The XPS spectra were acquired with an LH X1 Leybold (Germany) instrument using a dual achromatic Mg Kα (1,2) (1253.6 eV) and Al Kα (1,2) (1486.6 eV) source operating at a constant power of 260 W. Wide and detailed spectra were collected using the fixed analyzer transmission (FAT) mode of operation with channel widths of 1.0 or 0.1 eV and pass energies of 50 eV. Under these conditions, the instrumental contribution to the line width was kept constant. The measured full width at half maximum (FWHMs) of the Au 4f7/2 (84.0 eV) and Cu 2p3/2 (932.7 eV) signals used for calibration purposes were 1.3 and 1.6 eV, respectively.

#### 2.6.3. Extraction in Soxhlet in the Presence of Mo Catalysts

A sample (1.0 g) and 0.1 g of the catalysts ($MoO_3/SiO_2$ or $H_3PMo_{12}O_{40}$) were put into an extraction thimble, which was placed in a Soxhlet extraction apparatus. The sample was extracted with 300 mL of 1:2 ethanol/toluene mixture (*v*/*v*) for 7 h. After this period, the solvent was evaporated in a vacuum and the residue was dissolved in chloroform. The

soluble fraction was chromatographed using thin-layer chromatography on silica gel in the presence of a fluorescent indicator. The different zones revealed by UV irradiation were separated and eluted with ethyl acetate. The solvent was then evaporated, and the residue was analyzed (see below). The insoluble fraction was derivatized as described above.

#### 2.6.4. Extraction in an Autoclave in the Presence of Mo Catalysts

A sample (10.0 g) was put into an airtight glass jar with 50 mL of distilled water and 1 g of the catalysts $MoO_3/SiO_2$ or $H_3PMo_{12}O_{40}$. This mixture was put into the autoclave (Vapor Matic 770) for 20 min at a temperature of 120 °C and a pressure of 1 bar. Then, the sample was filtered, frozen at a temperature of −28 °C, and lyophilized to remove water.

### *2.7. Gas Chromatographic-Mass Spectrometric Analyses*

Analyses of all the extractives obtained using the procedures described above were accomplished with an 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 a mass spectra and NITS 11 library comparison.

## 3. Results and Discussion

### *3.1. Wood Change*

Although determining the wood properties were not the main goal of this research, the changes in some parameters can certainly help understand the chemical results obtained in this study. Mass loss, for instance, is considered an important indicator of the severity of the thermal process since it affects the degradation of the chemical components of wood, mainly hemicellulose, with a direct effect on extractives [28].

In this research, the value of mass loss compared to unmodified wood samples was 5.9%. In terms of EMC, the THM chestnut wood showed a reduction value of 17.4% compared to the untreated wood (11.7). The color change due to the thermo-modification showed, from a statistical perspective, a significant darkening of 21.0 (ΔL), while the Δa and Δb coordinates resulted in a value of 3.7 and 0.2, respectively, both indicating a slight change. The total color change of the modified wood samples (ΔE) was 21.5.

In our study, the slight reduction in mass loss depended on the low-temperature regime used during the process (170 °C). Indeed, as stated by Candelier et al. [29], thermo-degradation reactions begin at higher temperatures, around 200 °C. However, looking at the scientific literature, no studies were found on the effects of the vacuum process on EMC, color, and mass loss changes for chestnut wood material. Akyildiz and Ates [30] showed similar results in terms of EMC variation for chestnut wood thermo-treated for 2 h at 180 °C. Correal-Mòdol et al. [31] reported similar mass loss values, but the process was performed under the different conditions and at a temperature of 200 °C, and the color variation was lower in our study. In another study by Lo Monaco et al. [11], wood samples of chestnut were thermally treated for 6 h in a conventional oven at different temperatures. In this study the color parameter changes showed similar results at 170 °C, but even in this case, the process was not comparable. As stated by Candelier at al. [32], color change is a consequence of the production of degradation compounds from hemicelluloses and extractives, and this evidence strongly depends on the thermal process.

### *3.2. Yield and Solubility*

Extractives were quantified for the unmodified and modified wood samples (Table 1).

**Table 1.** Extraction yield and solubility of chestnut wood based on different extraction techniques and solvents. THM: thermally modified chestnut wood.

| Wood | Technique | Solvent | Yield (%) | Solubility |
|---|---|---|---|---|
| Control | ASE 110 °C | EtOH/$H_2O$ | 12.5 | 0.8 |
| | Soxhlet | EtOH/toluene | 7.4 | 1.7 |
| | Autoclave | $H_2O$ | 4.2 | 0.5 |
| THM | ASE 110 °C | EtOH/$H_2O$ | 27.3 | 3.7 |
| | Soxhlet | EtOH/toluene | 15.7 | 3.2 |
| | Autoclave | $H_2O$ | 7.6 | 2.7 |
| THM | Soxhlet | EtOH/toluene + $H_3PMo_{12}O_{40}$ | 19.1 | 12.4 |
| | Soxhlet | EtOH/toluene + $MoO_3$ | 14.8 | 15.5 |
| | Autoclave | $H_2O$ + $H_3PMo_{12}O_{40}$ | 15.8 | 1.2 |
| | Autoclave | $H_2O$ + $MoO_3$ | 8.8 | 0.3 |

For all techniques, the results showed the positive effects of THM on the extractive yield. Moreover, the yield extracted by ASE was the highest compared to other techniques for both the control and THM wood. In this case, these compounds were extracted using a method where the temperature has a relevant effect on yield extraction.

The chloroform solubility of the extracts was strictly related to the composition of the extracts. When phenolic compounds were the main components of the extracts, the solubility in chloroform was high. On the other hand, the solubility was very low when the fraction in the extract derived from carbohydrates was significant.

The ASE technique showed that temperature has a relevant effect on yield extraction. The different extraction yields could be explained by the different acting mechanisms. The diffusion process observed on chestnut wood by Gironi and Piemonte [33] plays a key role in these highlighted differences. Thus, the ASE technique, due to the use of temperature as a parameter [34], is more efficient than other extraction methods.

The subsequent chemical identification of extractives from thermally-modified chestnut wood by GC-MS indicated that the basic wood constituents degraded during thermal modification and produced additional new species of extractives. The most volatile extractives can expire from the wood or become degraded principally due through processes of lignin and cellulose degradation [29]. This study clearly showed the different influences that the extraction technique and solvent may have on the yield of extractives. Concerning the yield of extractives, it cannot be generally stated that the thermo-vacuum modifications caused an increase in the yield of extractives. This increase depended on the species in question, the solvent used, and the extraction technique applied.

### *3.3. ASE Technique*

The samples obtained using ethanol/water mixture as a solvent, and ASE at 110 °C as the extraction technique, are characterized by GC-MS in Table S1.

In the case of the untreated wood sample, 212 peaks were observed, but only two were identified by comparison with the National Institute of Standards and Technology (NIST) library. However, these compounds were the main components of the extracts (i.e., 2,3′-dimethyl-1,1′-biphenyl (0.60%) and 1,2,3-benzenetriol (13.55%)).

For THM chestnut wood, the GC-MS analysis of the extracts also allowed us to identify a few peaks. We identified a peak at a retention time of 4.32 min corresponding to 5-methyl-2-furan carboxy aldehyde (0.34%), a peak at 8.54 min corresponding to vanillin (2.94%, the main peak on the chromatogram), and one at retention time 9.15 min corresponding to 1,2,3-benzentriol (1.84%). In the chloroform-insoluble fraction of both untreated and THM chestnut, inositol was the main component.

### *3.4. Soxhlet Technique*

Extraction using an ethanol/toluene mixture on the untreated wood provided 7.4% extracts, only 1.7% of which were soluble in chloroform (Table 1). The GC-MS analysis of

this fraction is reported in Table S1. The main components were methyl (E)-9-octadecenoic acid (11.15%), (Z,Z)-9,12-octadecadienoic acid (6.31%), hexadecanoic acid (4.60%), and behenic alcohol (4.29%), an antiviral agent [35]. However, the main fraction is insoluble in the organic solvent. After derivatization, the GC-MS analysis of this fraction highlighted the results reported in Table S1.

The main component was inositol, a biologically active compound known as vitamin B7, which is active in the control of insulin release and polycystic ovary syndrome in women [36,37].

Under the same condition (ethanol/toluene), treatment of THM chestnut wood highlighted an impressive increase in the extractive amounts (15.7%). Furthermore, the yields of the chloroform soluble fraction were 3.2% (1.7% in untreated wood) (Table 1).

The GC-MS composition of the chloroform soluble fraction is reported in Table S1. The main component found was methyl (Z)-13-octadecanoate (8.65%).

The composition of the insoluble fraction is reported in Table S2. In this case, the main component was also inositol.

### 3.5. Autoclave

When extraction was performed in water in an autoclave on untreated wood using a procedure recently described in [14], fewer extracts were obtained in comparison with the ASE or Soxhlet extraction (Table 1). Furthermore, for untreated wood, only 0.50% of the extracts were soluble in chloroform (Table 1). The GC-MS characterization of this fraction is reported in Table S1.

The composition of the extracts was completely different, and the main component was coniferyl (4.73%) and 4-hydroxy-3,5-dimethoxybenzaldehyde (4.06%). Moreover, in this case, the main fraction was insoluble in chloroform. The GC-MS characterization of this insoluble fraction is reported in Table S2. The main component was inositol.

The same type of treatment was performed on the THM chestnut wood, and the amount of extractive increased compared to that obtained using untreated wood (7.6%). Furthermore, an increase in the chloroform soluble fraction was observed (2.7% from 0.5%) (Table 1). The composition of the chloroform soluble fraction, determined via GC-MS analysis, did not show the presence of other compounds besides linear long-chain hydrocarbons.

The GC-MS analysis of the insoluble fraction is reported in Table S2. The main component was inositol.

### 3.6. Catalysts

Recently, a new procedure was reported for increasing the extractive yields during both ethanol/toluene Soxhlet extraction and under water extraction in an autoclave. This new procedure uses $MoO_3$ supported on silica, and $H_3PMo_{12}O_{40}$, a polyoxometalate compound, as a catalyst [14,38].

The effect of the oxidants seems to induce mainly the oxidative fission of cellular membranes. The use of a molybdenum catalyst depended on the evidence that molybdenum can act as a catalyst in Fenton-like reactions [17].

By using $H_3PMo_{12}O_{40}$ as a catalyst in the Soxhlet technique, THM wood provided 19.1% extractives (Table 1). Furthermore, 12.4% of these extractives were soluble in chloroform (Table 1). Unfortunately, GC-MS analysis of this fraction did not allow the components of the mixture to be identified. The insoluble fraction showed allo-, myo-, and muco-inositol as the only components (not shown in Table S1). When the extraction was performed in the presence of silica-supported $MoO_3$, we observed a slight reduction in the extractive yield (14.8%). However, the yield of the chloroform soluble fraction increased to 15.5% (Table 1). In this case, the soluble fraction was analyzed by GC-MS, and the results are presented in Table S1. The main components are phenolic compounds, including 3-(4-hydroxy-3-methoxyphenyl)-2-propenal. The results of the GC-MS analysis of the insoluble fraction are reported in Table S2, confirming the presence of inositol as the main component.

The possible effects of Mo catalysts on the extractives were also studied for the autoclaving technique and for THM wood. The presence of the polyoxometalate compound induced an increase in the extractives (15.8%) and a decrease in the amount soluble in chloroform (from 2.7% to 1.2%) (Table 1), while in the presence of silica-supported $MoO_3$, we observed a strong reduction in the extractive yield (8.8%) and solubility (0.3%).

Table S1 presents the results of the GC-MS analysis of the chloroform soluble fraction only for $H_3PMo_{12}O_{40}$. This fraction contained mainly phenolic compounds, where the main components were 3-hydroxy-4-methoxy benzoic acid, 4-hydroxy-3,5-dimethoxybenzaldehyde, and vanillin.

In Table 2 are the compounds present in Table S1 that can find practical application in various industrial sectors.

**Table 2.** Compounds with the industrial application found in GC-MS analysis of the chloroform soluble fraction of chestnut wood extractives. ASE: Accelerated solvent extraction.

| | | | Untreated (Chloroform Soluble Fraction) | | | THM (Chloroform Soluble Fraction) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Extraction Technique | | | Extraction Techniques | | | | |
| | | | ASE | E/T [a] | Water | ASE | E/T [a] | Water | E/T/M [b] | W/POM [c] |
| **Compound** | **r.t. [min]** | **KI** | **Area [%]** | | | **Area [%]** | | | | |
| 2-Furancarboxylic Acid | 4.28 | 836 | | | 0.36 | | | 0.36 | 0.12 | 2.18 |
| Benzaldehyde | 4.31 | 961 | | 0.29 | | | 1.34 | | 1.49 | |
| Levulinic Acid | 5.34 | 1063 | | | 0.10 | | | 0.10 | | 9.91 |
| Vanillin | 8.95 | 1440 | 0.34 | 0.16 | 1.56 | 0.26 | | 1.56 | 0.93 | 6.82 |
| Apocynin | 9.14 | 1498 | | | 0.21 | | | 0.21 | 0.16 | 1.21 |
| 4-Hydroxy-3,5-Dimethoxybenzaldehyde | 10.22 | 1652 | | 0.30 | 4.06 | 0.22 | 0.30 | 4.06 | 2.56 | 8.07 |
| 4-Hydroxy-2-Methoxycinnamaldehyde | 10.67 | 1720 | | | 1.84 | | 1.40 | 1.84 | 4.27 | |
| Hexadecanoic Acid | 12.05 | 1968 | 4.05 | 4.60 | 2.12 | 3.96 | 4.01 | 2.12 | 1.78 | 1.20 |
| Octadecanoic Acid | 13.33 | 2140 | 1.55 | 3.73 | 1.82 | 4.57 | 2.85 | 1.82 | 1.36 | 1.02 |

[a] E/T = Ethanol/toluene in Soxhlet; [b] E/T/M = Ethanol/toluene in Soxhlet in the presence of silica-supported $MoO_3$; [c] W/POM = water extraction in an autoclave in the presence of $H_3PMo_{12}O_{40}$.

The 2-furancarboxylic acid is an organic compound most widely found in food products as a preservative and a flavoring agent. Other uses for 2-furoic acid include nylon preparation and optic technologies [39]. Benzaldehyde is commonly employed to confer almond flavor to foods and scented products. It is sometimes used in cosmetics products [40]; commonly employed to confer almond flavor to foods and scented products. It is also used as a flavor chemical in JUUL e-cigarette pods [41]. Levulinic Acid is used as a precursor for pharmaceuticals, plasticizers, and various other additives [42]. Apocynin is also known as acetovanillone, is an inhibitor of NADPH oxidase activity and thus is effective in preventing the production of the superoxide in human white blood cells or neutrophilic granulocytes; Apocynin has also anti-arthritic and anti-asthmatic properties [43,44]. Vanillin commonly employed in the biological and biotechnological industries as a flavoring agent in foods and pharmaceuticals products and as a fragrance in perfumes. It has the potential to be a key intermediate for the synthesis of biopolymers [45]. The 4-Hydroxy-3,5-Dimethoxybenzaldehyde, known as Syringaldehyde, has antioxidant, antioncogenic, antimicrobial, and antifungal activities, which make the compound important commercially [46]. The 4-Hydroxy-2-methoxycinnamaldehyde related to ferulic acid, an important precursor in the manufacture of aromatic compounds [47].

Octadecanoic acid deriving from the oxidative fission of cellular membranes of the wood. In a few cases, some compounds can be originated from the waxes hexadecanoic acid, deriving from the oxidative fission of cellular membranes of the wood. In a few cases, some compounds can be originated from [14,38].

The insoluble fraction analyzed using GC-MS is reported in Table S2. Inositol was the main component in this case. Moreover, in this analysis, it was found that the molecular compound was interesting for the practical application. Table 3 presents the compounds, such as allo-inositol hexaacetate, myo-inositol hexaacetate and muco-inositol hexaacetate, with a biologically active compound known as B7 vitamin, active in the control of insulin release and polycystic ovary syndrome in women [36]. Sitosterol is most commonly used for lowering cholesterol levels and improving symptoms of an enlarged prostate (benign prostatic hyperplasia or BPH) [48,49].

**Table 3.** Compounds with the industrial application found in GC-MS analysis of the chloroform insoluble fraction of chestnut wood extractives.

| | | | **Untreated (Chloroform Insoluble Fraction)** | | | **THM (Chloroform Insoluble Fraction** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Extraction Technique** | | | **Extraction Technique** | | | | |
| | | | **ASE** | **E/T** [a] | **Water** | **ASE** | **E/T** [a] | **Water** | **E/T/M** [b] | **W/POM** [c] |
| **Compound** | **r.t. [min]** | **KI** | **Area [%]** | | | **Area [%]** | | | | |
| Allo-inositol hexaacetate | 12.64 | | 21.05 | 16.92 | 17.52 | 12.07 | 19.38 | 8.25 | 13.13 | 5.37 |
| Myo-inositol hexaacetate | 12.87 | | 12.74 | 17.20 | 11.05 | 24.29 | 4.05 | 21.94 | 29.55 | 12.24 |
| Muco-inositol hexaacetate | 13.00 | | 4.89 | | 3.68 | 7.30 | | 4.78 | 6.77 | 2.17 |
| Sitosterol | 30.39 | 3290 | | | | | | | 0.59 | |

[a] E/T = Ethanol/toluene in Soxhlet; [b] E/T/M = Ethanol/toluene in Soxhlet in the presence of silica-supported $MoO_3$; [c] W/POM = water extraction in an autoclave in the presence of $H_3PMo_{12}O_{40}$.

## 4. Conclusions

Chestnut wood is difficult to treat at high temperatures, and undesired collapses are frequent. Considering the investigated wood parameters, our results confirm that using moderate temperatures during the thermal processing of this species can change some aesthetical surface characteristics, reduce the moisture uptake, and constrain the loss of mass within reasonable limits.

This research showed that extractives of modified wood are altered during thermal modification, with new compounds of extractives formed during the process.

Chestnut biomass resources can be recovered to obtain extractives that represent a source of natural compounds worthy of attention. Extraction from wood can apply different approaches, and specific methods have been applied to influence the yields and types of the extracted compounds. The amount of extractive compound dissolved in each solvent will differ, and the choice of appropriate extraction techniques will depend on the intended final application of the extracted chemical product.

As a general statement, the results confirm the positive effects of THM on the extractive yield. Moreover, the yield extracted by the ASE technique was the highest compared to other techniques for both the control and THM wood.

To obtain the most abundant mixture of phenolic compounds, among which vanillin is an important component, the extraction of THM chestnut wood with water in an autoclave in the presence of $H_3PMo_{12}O_{40}$ offers the best method among those tested in this study.

Furthermore, the same extraction conditions allowed us to obtain 5.88% 2-methoxyphenol, a flavor agent of many substances, such as whiskey, and a radical scavenger compound; 4.16% 2,6-methoxyphenyl, 8.07% 4-hydroxy-3,5-dimethoxybenzaldehyde, and 9.07% 4-hydroxy-2-methoxycinnamaldehyde, which are flavoring agents, and 9.19% 3-hydroxy-4-methoxybenzoic acid, a therapeutic agent for circulating disease. All of these compounds were obtained only from the THM wood.

The isolation of inositol in the insoluble fraction is an interesting target considering the important biological properties of this compound. The best procedure seems to be that on

unmodified wood material using an ethanol/toluene extraction in an ASE apparatus, where we can obtain the highest percentage of allo-inositolo hexaacetate. ASE technique also produced the highest percentage of muco-inositolo hexaacetate from extractive fractions derived from both unmodified and THM wood material.

This study presents the findings of a preliminary result that aims to explore the effect of techniques and solvents on the yield and solubility of extractives. More research is needed to understand and confirm the relevance of the thermal modification on the obtained results, as well as to understand the exact mechanism operating during the different extraction procedures.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/1999-4907/12/1/73/s1, Table S1: GC-MS analysis of the chloroform soluble fraction of chestnut wood extractives, Table S2: GC-MS analysis of the chloroform insoluble fraction of chestnut wood extractives.

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