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Assessment of the Amount and Quality of Bio-Chemicals Recoverable from Waste Wood from a Parquet Factory

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I. INTRODUCTION

Transition to a more circular economy is a central issue for future development both in the EU and in other areas of the world (Bocken et al., 2014; Haas et al., 2015; Su et al., 2013). According to the communication from the European Commission entitled "Closing the loop – An EU action plan for the Circular Economy" (EC, 2015), waste management, together with production and consumption, is indicated as a strategic and central activity for the full implementation of a circular economy. In the same document five priority areas were identified characterized by specific challenges in the context of circular economy due to their products, value chain, environmental footprint and/or dependency on material from outside the EU; they are: plastics; food waste; critical raw materials; construction and demolition; biomass and bio-based products. Concerning the last one, bio-based materials such as wood can be used in a wide range of products like furniture, construction, textiles, chemicals and energy (e.g. biofuels). In the USA the amount of wood waste generated has been estimated to be about 63 Tg/year, of which 31 Tg/year comes from municipal solid

waste (MSW) and the remaining amount from the Construction and Demolition (C&D) sector (USEPA, 2010; Falk and McKeever, 2012). Concerning the presence of wood in MSW, 35% was recovered for producing new products, 16% burned for energy production, 16% non-usable material and about 32% available for but not recovered (i.e. about 10 Tg). In 2012 about 57.5 Tg of wood waste was generated in the EU28 (Eurostat, 2015). In this case 6% was recycled, about 15% was used as biomass for energy recovery, and from 20% to 70% was incinerated, land filled and exported to other areas (Sander et al., 2004). In Italy the amount of wood waste collected separately was about 635 Gg/year in 2013, of which 210 Gg were from packaging and 425 Gg from other wood waste (ISPRA, 2014). Waste wood can be roughly separated into the following categories, depending on its origin: untreated waste wood (10-15%), non-hazardous waste wood (75-80%) and hazardous waste wood (5-15%) (Sander et al., 2004). This aspect plays a relevant role in the effective recycling of this material. According to EU legislation, hazardous waste must be managed, treated and disposed of in appropriately authorized facilities (e.g. incinerators, landfills). Effective recovery and recycling operations can be performed successfully only on non-hazardous and untreated wood waste. Ng et al. (2014) investigated the benefits of using recycled wood waste for the production of pallets instead of virgin soft wood and reported a reduction in greenhouse gas emissions of about 11.5%. They (Ng et al., 2011) also demonstrated some benefits in the production of doors from recycled instead of virgin wood. Buehlmann et al. (2009) assessed the recycling and industrial capacity of wooden pallets after they were banned from land filling in North Carolina. The results showed that 20% of the wooden pallets were reused, 45% refurbished, 19% recycled and 15% ground up. Recycling consisted mainly in using the waste wood as boiler fuel, mulch and animal litter. Asari et al. (2004) developed a bio-monitoring tool for assessing the contaminants in waste wood recycled as animal bedding in Japan. Other authors have investigated the exploitation of waste wood as fuel for energy production by co-combustion (Skodras et al., 2004) or as gas fuel production by gasification (Shet and Babu, 2009, 2010). The relatively

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low energy content, seasonality and isolated geographic availability of biomass feed stocks, in general, have been noted as limitations to their use for the large volume demand for energy and fuels (Fitz Patrick et al., 2010). In contrast, chemical production requires far lower volumes of biomass to satisfy demand. This is another important sector for the use of waste wood, i.e., for the production of bio-chemicals able to substitute fossil fuel and other raw materials in a bio-based chemistry. Conventional refinery industries exploiting crude oil and other raw materials for the production of fuels, materials and chemicals will be substituted in the near future by biorefineries. Conceptually a biorefinery should integrate biomass conversion processes, applying hybrid technologies from different fields, including polymer chemistry, bioengineering and agriculture (Ohara, 2003). The aim of the technological process in biorefining is depolymerizing and deoxygenating the biomass components. In order to convert biomass into valuable products several technological processes must be jointly applied (Cherubini, 2010): thermo chemical; biochemical; mechanical; chemical. Lignocellulosic biomass is one of the most relevant feed stocks available for biorefining (Cherubini, 2010) even if lignin makes cellulosic compounds difficult to hydrolyse. In this case pre-treatments are usually necessary which involve mechanical size reduction followed by chemical processes able to change the chemical structure of the molecules. The literature cited above indicates the importance of wood waste as feedstock for the full implementation of a bio-based economy, even if more attention has been focused on the production of renewable fuels and energy.

The aim of the present study was to assess the amount of biochemicals extractable from untreated waste wood arising from a parquet factory and then usable mainly in the pharmaceutical and food industry. The study was carried out by the aid of different experimental procedures for extracting the chemicals, based on lab-scale mechanical, physical and chemical treatments. The wood samples used in the study were *Myocarpus frondosus* and *Quercus petraea* largely used in the manufacture of parquet.

II. MATERIALS AND METHODS

a) Collection of the wood samples

The one-year naturally seasoned heartwood samples of *Quercus petraea* and *Myocarpus frondosus* came, respectively, from Fontaines forests (Chalon-sur-Saône, France) and from the Misiones province (northeastern Argentina), and were provided by an Italian parquet factory.

b) Standard Compounds and Reagents

All the reagents and standards used were of analytical grade. Reference compounds used as

standards were: caffeic acid, (E)-coniferyl alcohol, (Z)-coniferyl alcohol, (+)-catechin, dihydrosinapyl alcohol, (-)-epicatechin, ferulic acid, gallic acid, p-hydroxybenzoic acid, protocatechuic acid, (E)-sinapyl alcohol, (Z)-sinapyl alcohol syringic acid, syringic aldehyde, vanillic acid and vanillin (vanillic aldehyde). Coniferyl and sinapyl alcohol were synthesized according to the procedures reported below. The solvents used for fractionation were ethyl acetate and diethyl ether. Ethyl acetate (EtOAc), diethyl ether (Et₂O), tetrahydrofuran (THF), methanol (MeOH), hydrochloric acid (HCl, 37% w/w), sodium hydroxide (NaOH), anhydrous sodium sulphate (Na₂SO₄), pyridine (Py) and the silylation agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich. Water for sample extraction was purified with a New Human Power I Scholar water purification system (Human Corporation, Seoul, South Korea)

i. Synthesis of the standard coniferyl alcohol

NaBH₄ (4.5 equivalents) was added to a coniferyl aldehyde (200 mg) ethanol solution (10 mL). The solution was stirred for 30 minutes at room temperature, and then 10 mL of ethanol and 50 mL of water were added. After ethanol removal, the solution was extracted with ethyl acetate. The extraction procedure was repeated three times and finally the overall organic phase obtained was dried with anhydrous Na₂SO₄ and then concentrated to give coniferyl alcohol as a pale yellow oil, which was not purified further (Nascimen to et al., 2000). Identification was made on the basis of the mass spectrum (Fig. 1).

ii. Synthesis of standard sinapyl alcohol

LiAlH₄ (12 equivalents) was added to a tetrahydrofuran (5 mL) solution of sinapic acid (20 mg). After 5 hours of stirring at room temperature, the reaction product did not form (the reaction was monitored through TLC). Consequently, the reaction mixture was stirred and heated until the beginning of reflux, which was maintained for 5 hours. Following reduction, the excess hydride was quenched by drop wise addition of water. After tetrahydrofuran removal, the reaction mixture was extracted with ethyl acetate, then washed with water and the organic fraction was dried with anhydrous Na₂SO₄. The reaction mixture was not purified furthermore. According to the literature data, the GC/MS analysis revealed the formation of both isomers of sinapyl alcohol, together with the product of double bond reduction (Herbert et al., 1998).

c) Extractions

Two types of extraction protocols (EP) were performed on the heartwood samples: EP1 by means of a CH₃OH/H₂O (1:1, v/v) solution at neutral pH, and EP2 using a CH₃OH/NaOH 1.5 M (1:1, v/v) solution (measured pH=12.7). All the initial extractions of the wood samples were conducted in an Erlenmeyer flask

hermetically closed, in dark conditions, at a fixed temperature and under mechanical mixing. A Soxhlet apparatus was not used in order to simulate realistic industrial extraction conditions. Fig.7 shows a comparative flow chart of the two protocols.

i. *EP1 extraction protocol at neutral pH*

Ten grams of *Quercus petraea* and *Myrocarpus frondosus* heartwood were mechanically milled to sawdust, up to obtain particles with size below 3 mm. Afterwards, the samples were extracted using 300 ml of a CH₃OH/H₂O (1:1, v/v) solution for 24 hours at 25°C. The extracts were filtered on a Buchner funnel (Wathman filter paper n. 42). Methanol was then removed by a rotary evaporator at 35°C. The aqueous solutions were fractionated by liquid-liquid (L/L) extraction with diethyl ether and ethyl acetate by 4x30 ml portions of each solvent (Fernández de Simón et al., 1996a,b; Fernández de Simón et al., 1999b).

ii. *EP2 extraction - hydrolysis protocols of the Quercus petraea samples*

Three ten-grams samples of *Quercus petraea* (denoted as A, B and C) were milled mechanically to sawdust up to obtain particles with size below 3 mm). Afterwards, they were treated according to the procedures reported in sections 2.3.3, 2.3.4 and 2.3.5.

iii. *Protocol EP2-A*

Sample A was extracted and hydrolyzed for 72 hours at 50°C with a solution of 100 ml of CH₃OH and 100 ml of NaOH 1.5 M. The extracts were filtered on a Buchner funnel and centrifuged to remove wooden particles, then concentrated in a rotary evaporator at 35°C (methanol removal). The aqueous solution was neutralized (from an initial value of 12.7) with 60 ml of ethyl acetate. After filtration and centrifugation to remove sodium acetate, the pH was lowered up to 1 with 12 ml of HCl (37%, w/w). The acidic solution obtained was fractionated by L/L extractions by ethyl acetate (4x25 ml).

iv. *Protocol EP2-B*

sample B was extracted and hydrolyzed for 24 hours at 50°C with 200 ml of a CH₃OH/NaOH 1.5 M (1:1, v/v) solution. After the removal of wooden sawdust and methanol, the aqueous phase obtained was acidified with 10 ml of HCl (37%, w/w) up to pH 1, and fractionated by portions of ethyl acetate (4x25 ml).

v. *Protocol EP2-C*

sample C was extracted and hydrolyzed with 200 ml of a CH₃OH/NaOH 1.5 M (1:1, v/v) solution for 72 hours, at 50°C. After the removal of wooden particles and methanol, the aqueous solution was acidified with 10 ml of HCl (37 %, w/w) up to pH 1. This phase was fractionated by L/L extractions with portions of diethyl ether (4x25 ml).

d) *Sample preparation for analysis and compounds quantification*

The organic fractions obtained were dried under reduced pressure conditions, at 35°C, and then dissolved again in a known quantity of the fractioning solvent under ultrasonic stirring, in view of the quantitative analysis. One milliliter of each re-dissolved sample underwent a standard silylation protocol of the phenolic compounds (Zafra et al. 2006), to make the extracts to be determined compatible with the chromatographic column used in the analysis. A mixture of BSTFA and pyridine in ethyl acetate was used as silylation reagent. One ml of each extract sample (in ethyl acetate or diethyl ether) was evaporated to dryness and transferred to a micro-vial for GC/MS analysis. Moisture is a major competitor of phenolic hydroxyl groups during derivatization with the BSTFA-pyridine mixture and, consequently, may produce low recoveries. To avoid this problem, all reagents used in silylation were previously dried by adding anhydrous sodium sulfate. Therefore, the sample residue was re-dissolved in 200 µl of anhydrous ethyl acetate and the vial was stoppered. Fifty micro-liters of the deriving mixture BSTFA-pyridine-ethyl acetate (4:1:5, v/v/v) were added and the whole solution was mechanically shaken (using a vortex mixer) for 1 minute at room temperature. Before injecting, samples were further diluted with 250 µl ethyl acetate up to a total volume of 500 µl (Zafra et al., 2006).

For quantitative determination, chromatographic analysis was repeated three times by using three different one-milliliter quantities picked up from each re-dissolved solution of the extracted compounds. After correction for the dilution factors, the mean values obtained were used to calculate the global amount extracted from the initial 10 grams samples by the relation:

$$(1/3) (f.V.) \sum df_i [X]_i, i=1, 2, 3 \quad (I)$$

where (f.V.) is the final volume of the re-dissolved extracts, df_i and $[X]_i$ respectively are the dilution factors and the concentrations in mg/ml of the three one-milliliter portions undergone to the silylation procedure.

The quantification was performed using reference compounds by means of the external standard method, and exclusively for peaks indicated as pure by the "peak purity" matching function of the GC/MS apparatus.

e) *GC-MS analysis apparatus*

Separation and identification of the organic compounds was performed by a chromatograph coupled with a mass spectrometry detector based on electron impact ionization. Identification of the compounds and attribution of the chromatogram peaks were performed by a software library of the fragmentation patterns which was set up from a collection of pure standards and according to literature

data reported in Pecina et al. (1986), Kuroda et al. (2000), Zafra et al. (2006), Ribechini et al. (2012) and Proestos et al. (2013). The ion fragments of each standard were compared with the signals produced by the samples. Gas chromatographic analyses were performed using an Agilent 6850 Series Gas Chromatograph apparatus (Agilent Technologies, Santa Clara, CA, USA) fitted with a splitless injector for a low background HP-5MS fused silica capillary column (60m×0.25mm i.d.×0.25µm film thickness) supplied by Agilent. A silylated injector liner split/splitless (2 mm i.d.) was used. Detection was carried out with a 5975B Mass single quadrupole spectrometer (Agilent Technologies).

The GC-MS operation control and data processing were carried out by ChemStation software package (Agilent Technologies). The sample volume injected was 1 µl. The electron impact (EI) ionization energy was 70 eV; the mass range scanned was 140-465 m/z. The injector temperature was 250°C and the detector one 280°C. As gas carrier was used helium with a flow rate of 1.0 ml min⁻¹. The oven temperature conditions were: held at 90°C for 1 min, then increased to 220 °C at a heating rate of 6 °C min⁻¹, then to 290 °C at 10 °C min⁻¹, held for 1.23 min and finally to 310°C (held for 7.5 min) at 40°C min⁻¹. The total run time was 37.67 min. (Zafra et al., 2006).

III. RESULTS AND DISCUSSION

The goals of the extraction/hydrolysis experiments were: (a) to achieve and quantify useful by-products from neutral extraction (EP1) and from lignin hydrolysis protocols (EP2s); (b) to implement an analytical procedure for the determination of the lignin degradation degree in a basic environment. The two target compounds chosen for this determination were coniferyl and sinapyl alcohols, which are nominally the constituent monomers (monolignols) of the lignin structure in angiosperm species (Brauns et al., 1960; Fengel et al., 1989; Sjöström, 1993; Baeza et al., 2001; Sakakibara et al., 2001)

In EP2s first diethyl ether was used as fractionation solvent of the alkaline phase, but chromatograms so obtained showed no trace of both the two target alcohols. By acidifying with HCl the aqueous phase before fractionation, the two target compounds were detected, so extraction protocols in acidic environment were implemented. In fact, coniferyl and sinapyl alcohols are soluble in aqueous alkaline environment, but in an acidic one it is possible to extract them by current solvents used in liquid-liquid fractionations. Moreover, extractions carried out at neutral pH (EP1) of the two species using ethyl acetate as fractionation solvent, gave the best results, namely the highest number of compounds identified, with respect to those performed by diethyl ether. Hence, for EP1 compounds identification and quantification, only

the chromatograms obtained by the first type of separation protocol, namely EP1-L/L EtOAc, were used (Fig. 2, 3).

(E)-coniferyl alcohol, was not only unambiguously identified in all the extract solutions examined, but it was also present in amounts which allowed quantitative evaluations to be made (Tables 2,4).

Quantitative determination of (E)-sinapyl alcohol was not achieved, due to the low grade of purity of its standard, but qualitative identification was possible for the two species, following the protocol at neutral pH (EP1). Moreover, it was identified in the *Quercus petraea* extracts obtained by EP2-B and by EP2-C (Table 2).

The extractions and analyses described above allowed the identification of the following compounds (Table 1, in order of retention time): vanillin, p-hydroxy benzoic acid, syringaldehyde, vanillic acid, protocatechuic acid, (E)-coniferyl alcohol, gallic acid, dihydrosinapyl alcohol, (Z)-sinapyl alcohol, ferulic acid, caffeic acid, (E)-sinapyl alcohol.

Table 2 shows the percentage matching of the extracted compounds with the TMS (Tetra MethylSilane derivative)-standards, calculated by the data acquisition software. Table 3 reports the concentrations of the compounds determined in their liquid-liquid extraction solvent.

Table 4 shows the calculated yields in milligrams from the extraction/hydrolysis protocols of the ten grams samples, as described in Section 2.4. Table 4 data for syringaldehyde show that its yield increases by 8.5 times from EP1 to EP2-A protocol, and by 370 times performing EP2-C.

(E)-coniferyl alcohol was quantified by all the performed protocols and its EP2/ EP1 yield ratios are reported in Table 5. It is apparent that EP2-B, the protocol with the minor hydrolysis duration, presents the high (E)-coniferyl alcohol yield, not only as absolute value (Table 4), but also with respect to the initial naturally occurring level determined by EP1. Lower EP2-A/ EP1 and EP2-C/ EP1 yield ratios are due to the prolonged hydrolyzation time, which induces the oxidation of (E)-coniferyl alcohol to ferulic and vanillic acids (EP2-A) and to vanillin and vanillic acid EP2-C.

Absolute yields in Table 4 and yield ratios EP2_P/EP2_YCA in Table 5, where P= A, B, C indicates the three *Quercus petraea* hydrolysis protocols and YCA is the respective (E)-coniferyl alcohol yield, suggest that oxidation reaction towards acids is favored by the EtOAc neutralization and by the strong acidic treatment adopted in EP2-A, whereas aldehydes formation is the main reactive result by the milder reaction conditions of EP2-C. Since the (E)-coniferyl alcohol yields by EP2s protocols represent the amounts of this compound

which derive from the lignin degradation and which were not oxidized to byproducts, the EP2_P/EP2_YCA ratios in Table 5 provide an evaluation tool of the lignin hydrolysis. Another way to estimate the lignin degradation byproducts is shown in Table 6, where are reported the per cent yields of the lignin degradation-oxidation byproducts related to (E)-coniferyl alcohol with respect to their global amount. The higher per cent yield of (E)-coniferyl alcohol in EP2-C, confirms the lower grade of oxidation induced by that protocol, with respect to EP2-A.

Chromatograms in Figure 2 and 3 show the peaks of the compounds detected in the *Quercus petraea* and *Myrocarpus frondosus* extracts, following the neutral extraction protocol EP-1. *Quercus petraea* sample extracted by EP-1 is characterized by the massive presence of gallic acid (Table 4, Figure 2). In contrast, *Myrocarpus frondosus* shows no trace of it (Table 4, Figure 3). The compounds identified in the samples of *Quercus petraea* were: p-hydroxybenzoic acid, syringaldehyde, vanillic acid, protocatechuic acid, syringic acid, (E)-coniferyl alcohol, gallic acid and (E)-sinapyl alcohol (Table 2). These findings are consistent with literature data (Fernández de Simón et al., 1996a; Cadahía et al., 2001; Fernández de Simón et al., 2009; Sanz et al., 2012). Quantitative evaluations were obtained for (E)-coniferyl alcohol, gallic acid, and syringaldehyde; the values obtained for the second and the last of these three compounds are in very good agreement with the concentrations reported in (Fernández de Simón et al., 1996a), but vanillin and ferulic acid, quantified in that work, were not detected at all by EP-1. The absence of vanillin in the *Quercus petraea* sample extracted by neutral protocol can be in part related to the short seasoning period (12 months).

The *Quercus petraea* sample extracted using protocol EP1 (Figure 2, Table 4) and the three samples treated following protocols EP2-A, B, C (Figs. 4-6, Table 4) show a conspicuous amount of (E)-coniferyl alcohol, which can be attributed, respectively, to the length of ageing and to the induced process of lignin degradation (Brauns et al., 1960; Fengel et al., 1989; Sjöström, 1993).

The *Myrocarpus frondosus* sample is characterized by an unexpectedly low amount of (E)-coniferyl alcohol, which can be explained by the durability of this wood (i.e. by its resistance to degradation due to biotic agents, which depends on the presence of extractives with a strong antimicrobial activity). The presence of vanillic acid in the *Myrocarpus frondosus* sample is in agreement with (Ataide da Silva et al., 2009). For this species, two compounds were quantified: vanillic acid and (E)-coniferyl alcohol (Tables 3, 4). P-hydroxybenzoic, protocatechuic, syringic acids and (E)-sinapyl alcohol were also detected (Table 2).

Figures 4-6 show typical chromatograms obtained from the extraction-basic degradation process performed on *Quercus petraea* samples by protocols.

EP2-A, B, C. EP2-C, characterized by the use of diethyl ether as L/L fractionation solvent, was the only one which allowed the quantitative evaluation of vanillin, although (E)-coniferyl alcohol, syringaldehyde and vanillic acid were also determined in EP2-A, by means of ethyl acetate. EP2-A also allowed the evaluation of ferulic acid.

In conclusion, protocol EP2-C allows to obtain mainly syringaldehyde, vanillin and vanillic acid, whereas EP2-A reaction conditions are more suitable for the (E)-coniferyl alcohol, ferulic and vanillic acids quantitative achievement (Table 4).

The very mild physical reaction conditions (24 hours of extraction at 25°C) of EP1 were able to extract a noteworthy amount of gallic acid, whereas the stronger conditions (72 hours of extraction-hydrolysis at 50°C) applied in EP2-A and EP2-C induced the decarboxylation of that compound to pyrogallol, as suggested by its lack of detection in the hydrolysis protocols (Table 4). From the *Myrocarpus frondosus* sample, EP1 allowed the quantitative recovery of (E)-coniferyl alcohol and vanillic acid.

Vanillic acid is an effective antioxidant largely exploited in pharmaceutical and food applications, respectively (Brand-Williams et al., 1995; Itoh et al., 2009). Similar properties and uses have also been reported for ferulic acid (Kikuzaki et al., 2002; Ou and Kwok, 2004). Yen et al. (2002) reported that antioxidant and pro-oxidant properties of gallic acid were equivalent to those of ascorbic acid largely used as a preservative in the food industry and as a food supplement. Kim et al. (2006) reported pharmaceutical properties of gallic acid as an inhibitor of histamine release and pro-inflammatory cytokine production in mast cells. Similar antioxidant properties have also been reported for the syringaldehyde (Da Porto et al., 2000) in the agricultural and food industry.

IV. CONCLUSIONS

The urgent necessity of implementing the bio-based economy and industry able to substitute crude oil and other mineral raw materials with renewable ones requires the development of new processes and technologies based on the bio-refinery concept. The quite limited volume required for this aim makes the exploitation of lignocellulosic biomass sustainable compared, for example, to the energetic one. The waste sector can be an important source of bio-materials resulting from different industrial and civil activities and for this reason they are not in competition with food crops.

The results of this study demonstrate that the extraction/hydrolysis processes and the untreated wooden wastes from a parquet factory can contribute to achieving this goal. The mild extraction procedures based on alkali-induced lignin degradation, after wastes mechanical pre-treatment, result simple and rapid, and able to obtain useful compounds within 72 hours. Another relevant result achieved in this work was that the unambiguous GC/MS peak detection and quantification of (E)-coniferyl alcohol in all the samples analyzed. This can provide the basis for a reliable analytic method aimed to the quantitative evaluation of the natural and alkali-induced lignin degradation processes.

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Table 1: GC/MS peak assignments for the compounds TMS-derivatives identified.

Peak number	Retention time (min.)	MW	Fragments (m/z)	Compounds identified (+)
1	12.65	224	193, 194, 209, 224	Vanillin
2	14.33	282	193, 223, 267, 282	p-Hydroxybenzoic acid
3	15.91	254	195, 224, 239, 254	Syringaldehyde
4	16.84	312	149, 165, 223, 253, 267, 282, 297, 312	Vanillic Acid
5	17.87	370	193, 223, 267, 311, 355, 370	Protocatechuic acid
6	19.20	342	253, 297, 312, 327, 342	Syringic Acid
7	20.30	324	204, 235, 293, 309	(E)-Coniferyl Alcohol
8	20.22	458	147, 178, 281, 443, 444, 458, 459	Gallic acid
9	20.47	356	210, 240, 341, 356	Dihydrosinapyl alcohol
10	20.60	354	204, 323, 339, 354	(Z)-Sinapyl Alcohol
11	22.22	338	219, 249, 293, 279, 308, 323, 338	Ferulic Acid
12	22.85	396	179, 191, 219, 381, 396	Caffeic Acid
13	22.47	354	204, 323, 339, 354	(E)-Sinapyl Alcohol

(+) Identification was done according to Pecina et al. (1986), Kuroda et al. (2000), Zafra et al. (2006), Ribechini et al. (2012) and Proestos et al. (2013).

Table 2: Percentage (%) matching with the relative TMS-standards for the compounds identified in the wood extracts analyzed.

Compound	Myrocarpus frondosus ^x	Quercus petraea ^x	Quercus petraea ^a	Quercus petraea ^b	Quercus petraea ^c
Vanillin	-	-	-	99	99
p-Hydroxybenzoic acid	93	95	99	-	99
Syringaldehyde	-	98	98	98	99
Vanillic acid	99	99	99	-	99
Protocatechuic acid	99	92	99	-	-
Syringic acid	99	99	99	-	99
(E)-Coniferyl alcohol	99	99	99	99	99
Gallic acid	-	99	-	-	-
Dihydrosinapyl alcohol	-	-	99	-	-
Ferulic acid	-	-	99	-	-
Caffeic acid	-	-	-	-	-
(E)-Sinapyl alcohol	99	98	-	99	99

Legend:

(^x) EP1

(^a) EP2-A

(^b) EP2-B

(^c) EP2-C

Table 3: Concentrations (mg/ml, in the fractionation solvent) of some compounds identified in the extracts.

TMS-Derivatives	Myrcarpus frondosus ^x	Quercus petraea ^x	Quercus petraea ^a	Quercus petraea ^b	Quercus petraea ^c
vanillin	-	-	-	-	0.055
syringaldehyde	-	0.002	0.011	-	0.740
vanillic acid	0.015	-	0.220	-	0.170
(E)-coniferyl alcohol	0.006	0.020	0.028	0.099	0.013
gallic acid	-	0.110	-	-	-
ferulic acid	-	-	1.064	-	-

Legend:

(^x) EP1

(^a) EP2-A

(^b) EP2-B

(^c) EP2-C

Table 4: Global amount (milligrams) calculated with respect to the initial 10 g extracted sample and per cent Yields evaluated with respect to a theoretical heartwood lignin content (30%).

Amount in wood samples (mg/10g)					
Compound	Myrcarpus frondosus ^x	Quercus petraea ^x	Quercus petraea ^a	Quercus petraea ^b	Quercus petraea ^c
Vanillin	-	-	-	-	0.55 (0.018%)
Syringaldehyde	-	0.02 (0.00067%)	0.17 (0.0057%)	-	7.40 (0.25%)
Vanillic acid	0.15 (0.005%)	-	3.27 (0.11%)	-	1.70 (0.057%)
(E)-Coniferyl alcohol	0.06 (0.002%)	0.20 (0.0067%)	0.42 (0.014%)	0.99 (0.033%)	0.13 (0.0043%)
Gallic acid	-	1.14 (0.011%)*	-	-	-
Ferulic acid	-	-	15.96 (0.53%)	-	-

Legend:

(^x) EP1

(^a) EP2-A

(^b) EP2-B

(^c) EP2-C

(*) Calculated with respect the extracted mass (10 g)

Table 5: EP2_Y/EP2_YCA and EP2 /EP1 Yield Ratios values.

Protocol	EP2_Y/EP2_YCA Vanillin	EP2_Y/EP2_YCA Vanillic acid	EP2_Y/EP2_YCA Ferulic acid	EP2/EP1 Coniferyl alcohol
EP2-A	n.d.	7.8	38	2.10
EP2-B	d.	n.d.	n.d.	4.95
EP2-C	4.2	13	n.d.	0.64

Legend:

n.d.: not detected

d.: detected, but not quantified

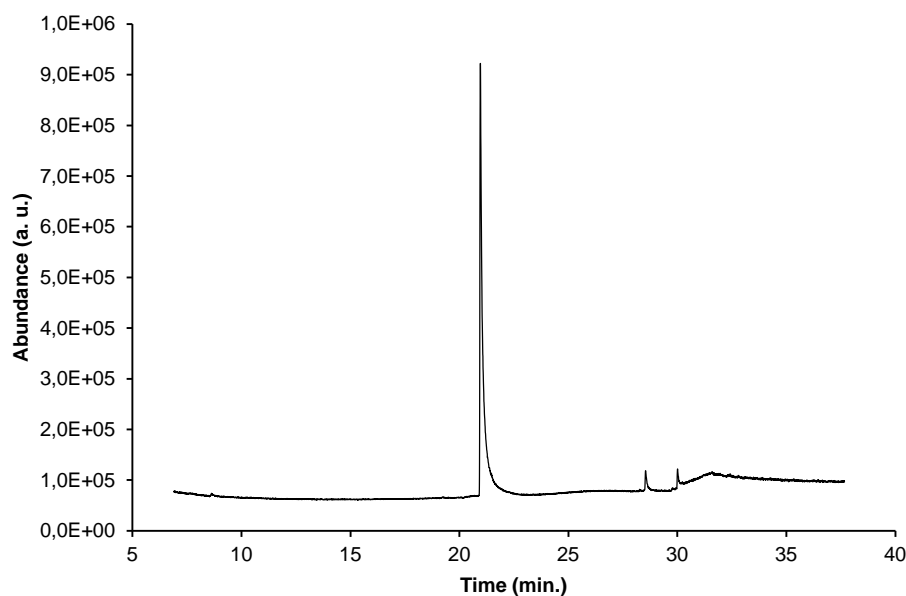
Table 6: Per cent yields of the lignin degradation-oxidation byproducts related to (E)-Coniferyl alcohol with respect to their global amount.

Protocol	Vanillin (%)	Vanillic acid (%)	Ferulic acid (%)	Coniferyl alcohol (%)
EP2-A	n.d.	17.54	85.62	2.25
EP2-B	d.	n.d.	n.d.	~100
EP2-C	23.11	71.43	n.d.	5.46

Legend:

n.d.: not detected

d.: detected, but not quantified



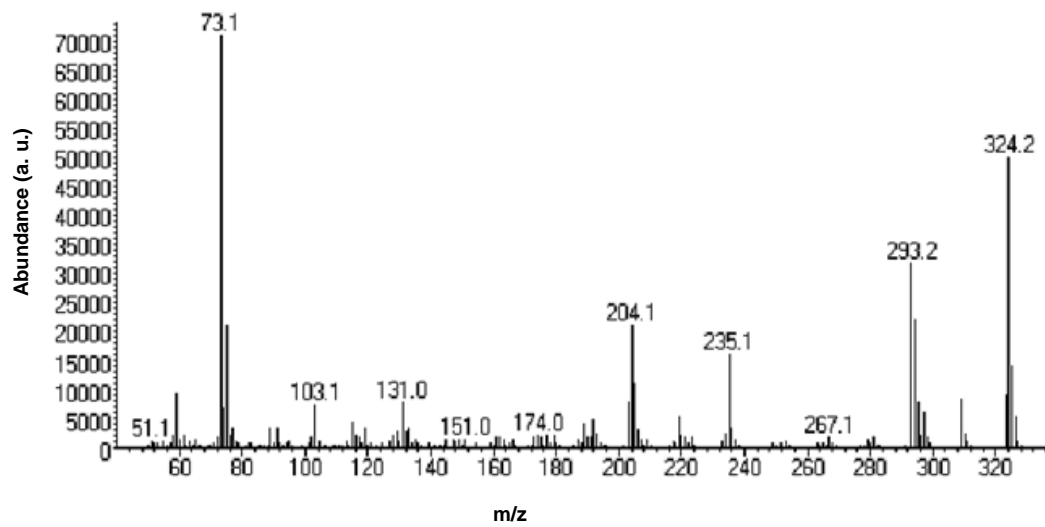


Fig. 1: Standard identification chromatogram (upper panel) and mass spectrum (lower panel) of coniferyl alcohol.

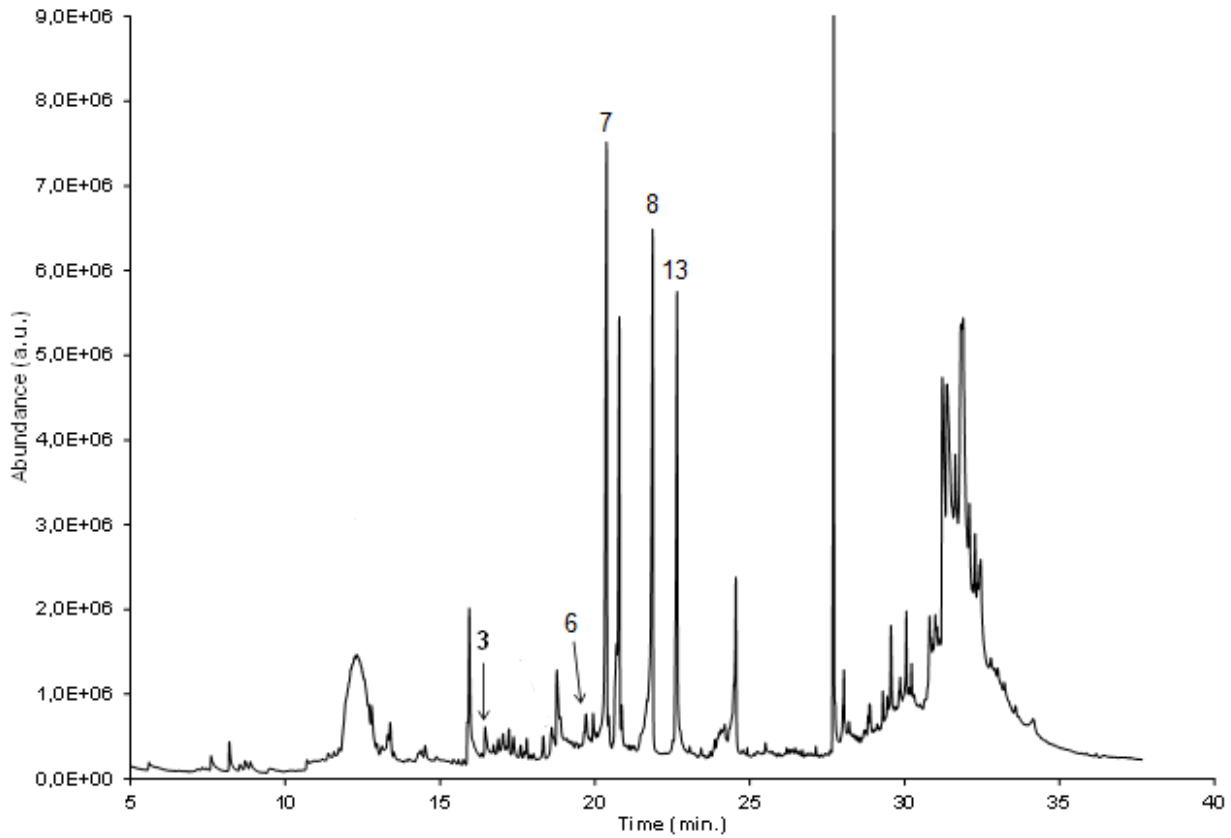


Fig. 2: *Quercus petraea* EP1 extracts chromatogram: neutral extraction, fractionation by ethyl acetate. Bold numbers identify the compounds in Table 1.

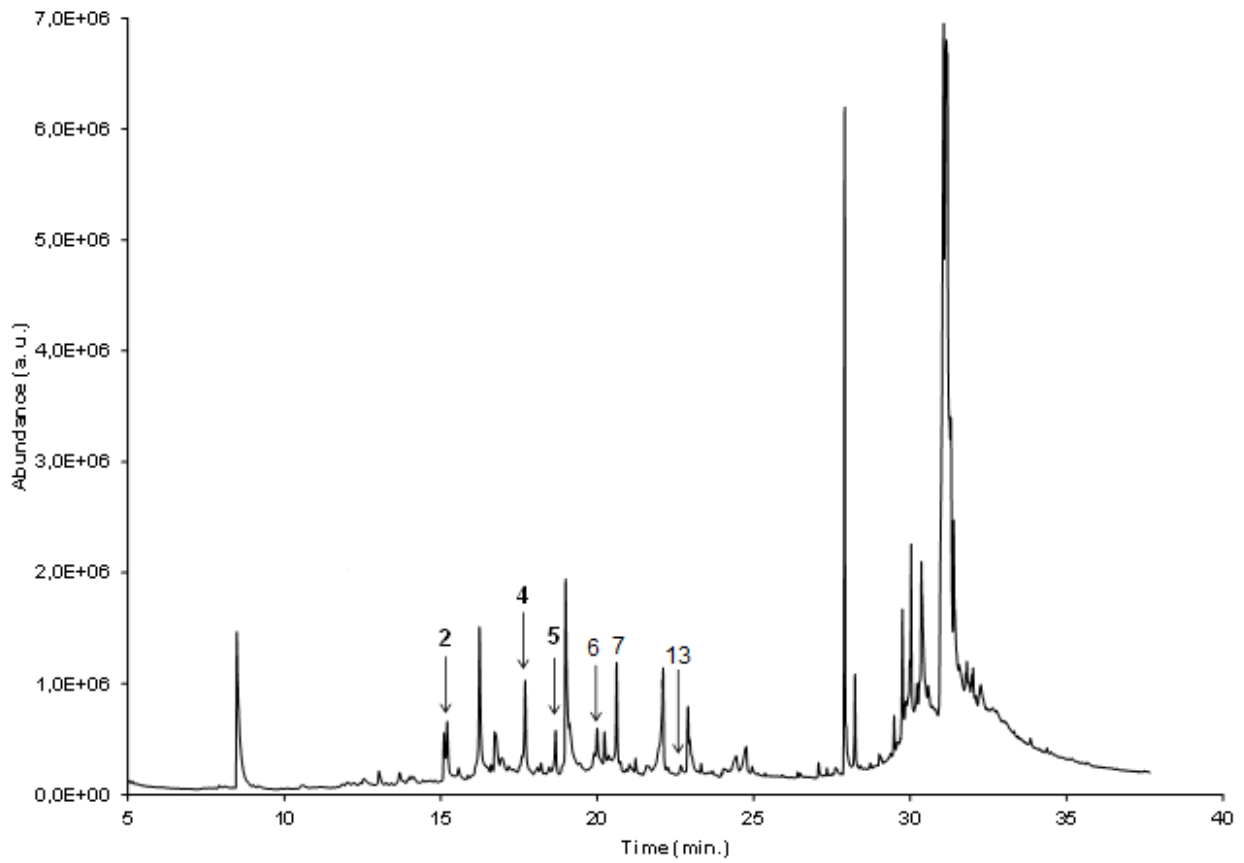


Fig. 3: Myrocarpus frondosus EP1 extracts chromatogram: neutral extraction, fractionation by ethyl acetate. Bold numbers identify the compounds in Table 1.

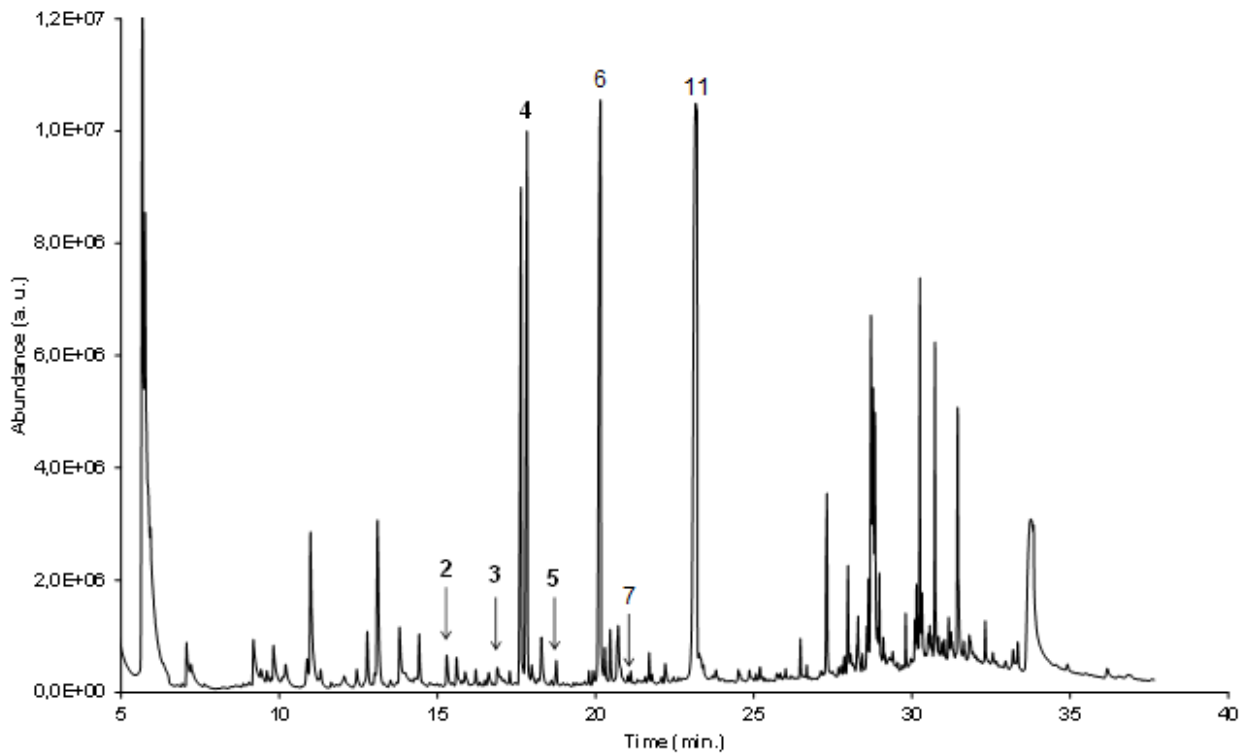


Fig. 4: Chromatogram of Quercus petraea extracts by EP2-A. Bold numbers identify compounds listed in Table 1.

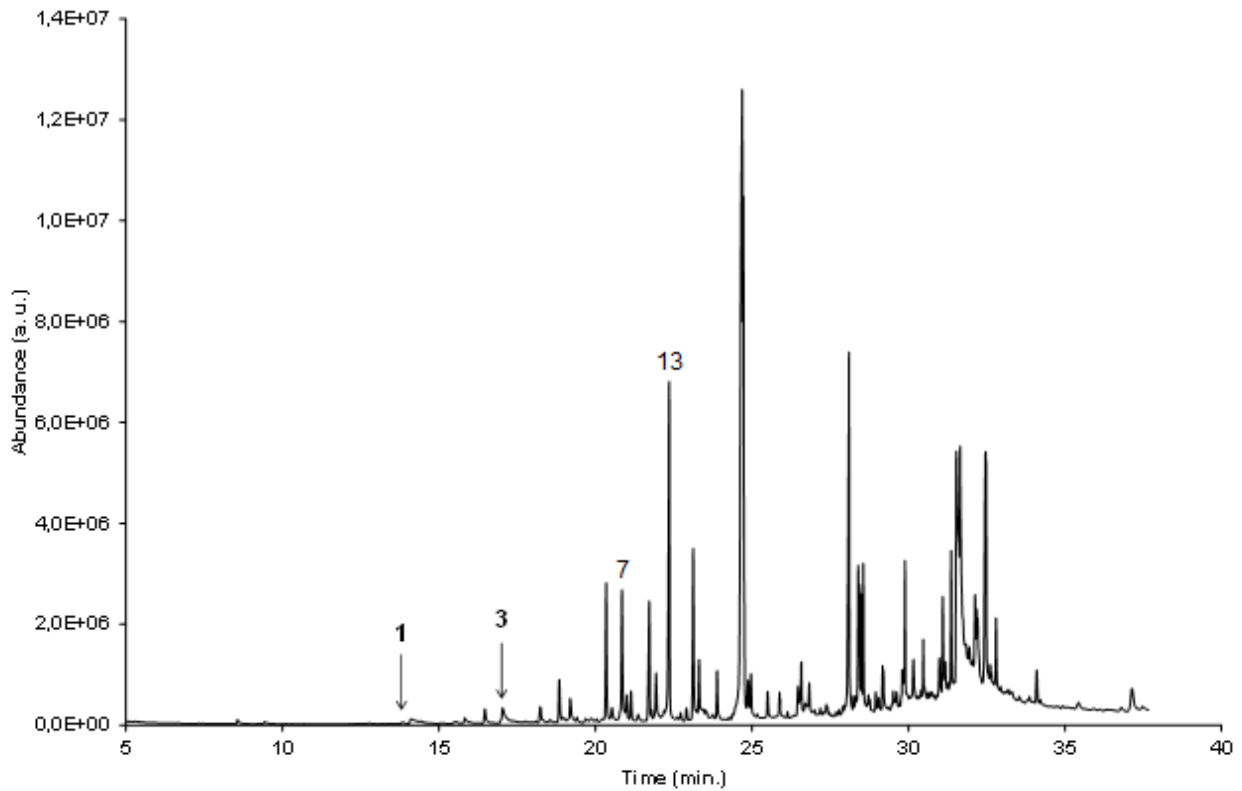


Fig. 5: Chromatogram of *Quercus petraea* extracts by EP2-B. Bold numbers identify compounds listed in Table 1.

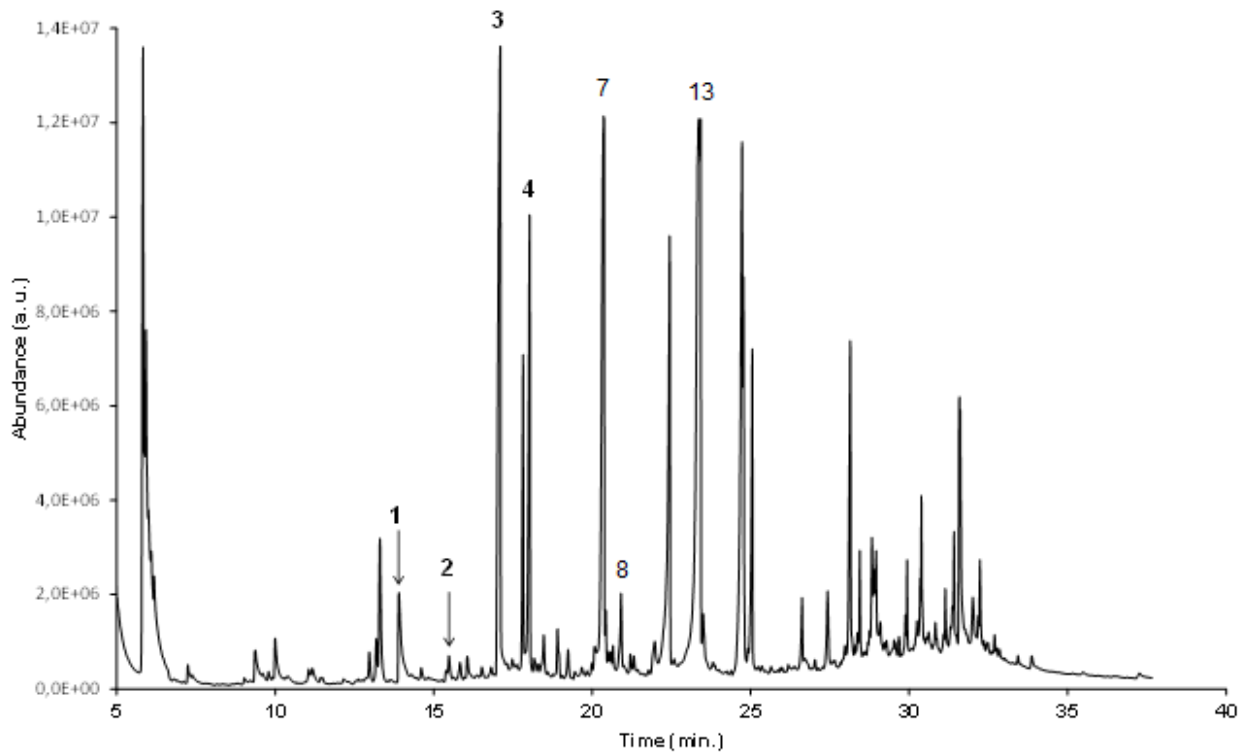


Fig. 6: Chromatogram of *Quercus petraea* extracts by EP2-C. Bold numbers identify compounds listed in Table 1.

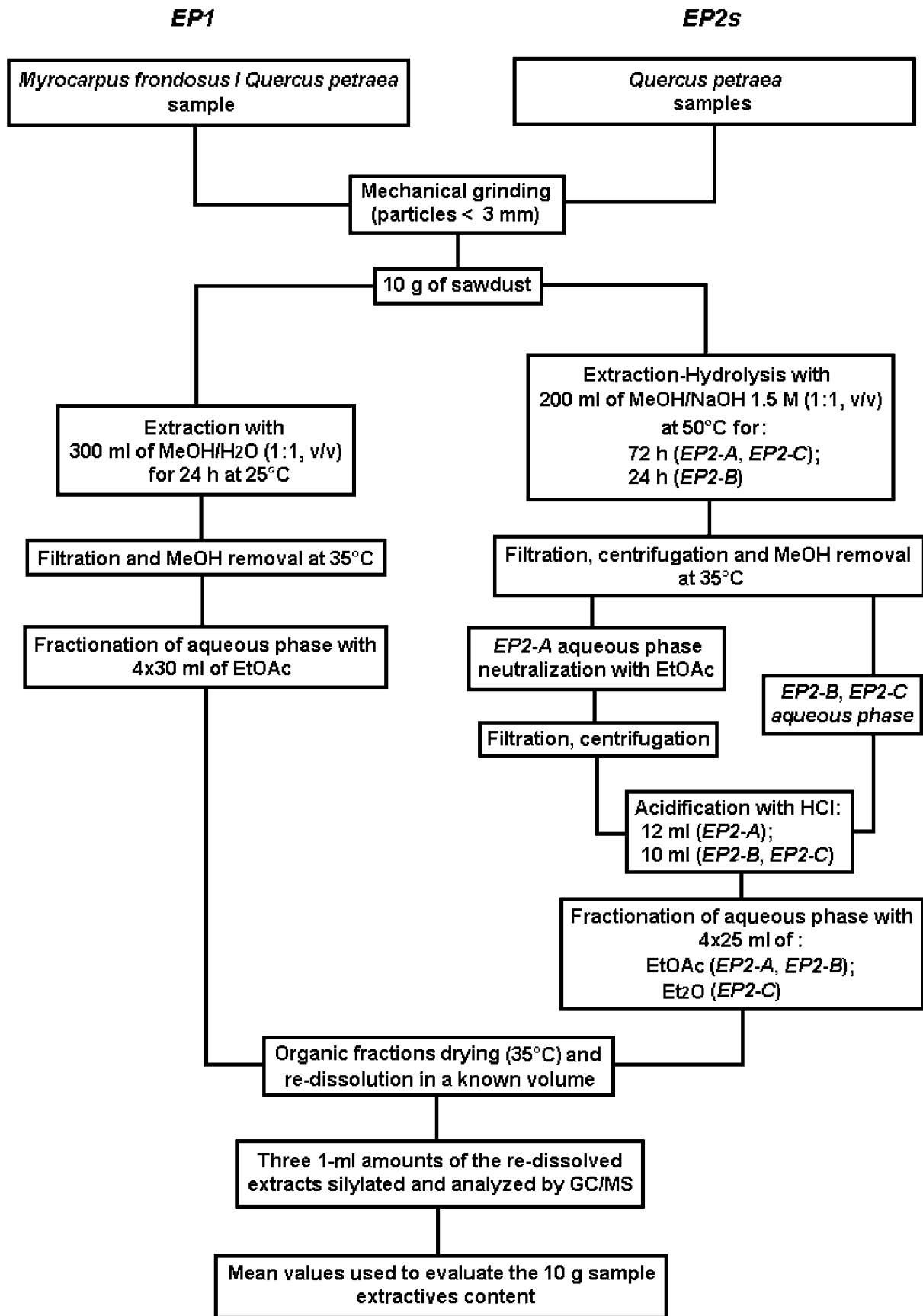


Fig. 7: Comparative flow chart of EP1 and EP2s.