Analytical characterization of purified mimosa (*Acacia mearnsii*) industrial tannin extract: Single and sequential fractionation

André L. Missio [a], Bruna Tischer [b,c], Patricia S.B. dos Santos [a], Cristiane Codevilla [b], Cristiano R. de Menezes [b], Juliano S. Barin [b], Clovis R. Haselein [a], Jalel Labidi [d], Darci A. Gatto [a], Alexander Petutschnigg [e], Gianluca Tondi [e,*]

[a] *Laboratório de Produtos Florestais (PPGEF), Centro de Ciências Rurais, Universidade Federal de Santa Maria, P.O. Box 221, 97105-900 Santa Maria, Brazil*
[b] *Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria, Santa Maria 97105-900, RS, Brazil*
[c] *Programa de Pós-Graduação em Sistemas e Processos Industriais, Universidade de Santa Cruz do Sul, 96815-900 Santa Cruz do Sul, Brazil*
[d] *Chemical and Environmental Engineering Department, University of the Basque Country, Escuela Politécnica de San Sebastián, Plaza Europa, 1, 20018 Donostia-San Sebastián, Guipuzcoa, Spain*
[e] *Salzburg University of Applied Sciences, Forest Product Technology & Timber Construction Department, Marktstraße 136a, 5431 Kuchl, Austria*

ABSTRACT

Mimosa (Black wattle) tannin extract is one of the few industrially available natural sources of polyphenols. Even if its composition is rather known, limited attempts for its purification were done until now. A Soxhlet extraction, using various solvents, with single and sequential processes was performed, and the separated fractions were analytically investigated. Yield, molecular mass, phenolic and condensed tannin contents, antioxidant activity and FT-IR spectroscopy have shown that the ethyl acetate fraction strongly contains antioxidants and low molecular mass tannins (also hydrolyzable), while the alcoholic fractions contain purified flavonoids with higher molecular mass; the final residues resulted enriched in carbohydrates and ashes. Sequential extractions optimize the class of compounds extracted according to the specific application. Conforming to statistical analysis, significant correlations have been observed between phenolic content and the antioxidant activity, as well as between molecular masses and ashes content.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

In a world where alternative and more sustainable resources than crude-oil are required, plants derivatives are the principal feedstock for bio-refineries and green chemistry processes [1,2]. In this context, the plant extracts are a very promising opportunity for supplying phenolic substances [3]. For some species, such as chestnut, oak (for hydrolyzable tannins), mimosa and quebracho (for condensed tannins), the extracts are particularly rich in polyhydroxyphenols. The high yields found in the extracts justify their industrial distillation [4].

Tannins have been used for centuries for leather tanning [4–6] and decades for the production of adhesives [7,8], oenology [9], as well as flocculants for water treatment [10]. More recently, the use of these phenolic substances was introduced also for pharmaceutical and advanced material purposes [4,11–14].

With about 200 thousand tons/year, corresponding to around 90% of the worldwide production, the condensed tannins are the most abundantly extracted natural substances [4]. This particular family of tannins, known as polyflavonoids and proanthocyanidins [5,15], is very interesting because they couple their strong antioxidant and antifungal activities [16] with a good chemical reactivity towards aldehydes, protein and other reagents [4]. Due to the latter feature, the condensed tannins can be considered as a suitable bio-resource for the synthesis of adhesives [17], coatings [18], wood preservatives [19,20] and even porous materials like foams [13,14,21] and aerogels [22]. The industrial extract of Acacia mimosa (*Acacia mearnsii*) is the most convenient for the synthesis of new materials because (i) it is the most sustainable – the Acacia mimosa is a fast growing tree, abundantly available, and its bark contains up to 45% of tannins [23] – and (ii) it is more performing: the mimosa-based polymers are less brittle and more easily manipulated than the other condensed tannins [7].

However, the mimosa extract is not completely constituted of phenolic substances [14]: easy sugars, organic acids and hydrocolloids are also contained in the extract [24]. A purification process is

---

therefore required to refine the chemical composition, especially when applied for advanced purposes.

The concept of purification of biomasses is the same being developed in the bio-refineries to obtain "green" chemicals [25]: it starts from easily available bio-resources in order to retrieve pure chemicals or specific families of chemicals. The actual market trend indicates an increasing interest for the use of natural & natural-derived materials for several applications, like food antioxidant and pharmaceuticals [26–29]. Indeed, the tannin extracts can be still used in large volumes for leather tanning and flocculation purposes; however, purified substances can be applied for higher added value purposes, such as pharmaceutical, food and cosmetic, as well as for more technologically advanced materials, such as thin films, wood-plastic composites, foams and aerogels [13].

Currently, the main tannin fractionation and purification methods use countercurrent chromatography (CCC) [30], usually with the use of Sephadex LH-20 [31,32]. These methods require complex analytic techniques and expensive equipment, therefore, an easier approach for the fractionation could be considered. Not many studies have been presented on this subject [33,34], although the "Soxhlet" method has already been proven to efficiently purify other hydroxy-aromatic organic compounds, such as lignin [35].

In this context, the present study proposes a fractionation and purification method of tannin industrial extracts applying different organic solvents. Antioxidant activities, total phenolic and condensed tannins contents, as well as the fractions molecular masses, were measured to observe the properties of the extract and to evaluate if this purification method could be considered for specific applications. Single and sequential extractions with hexane, ethyl acetate, pentanol, propanol and methanol were applied in order to purify tannin industrial extracts.

2. Materials and methods

2.1. Raw material

The tannin industrial extract was kindly supplied by SETA® industry, located in the town of Estancia Velha – RS (Brazil). The extract was obtained from the bark of the *Acacia mearnsii* – black wattle through an industrial process. The extraction of tannin was obtained by a simple water extraction with specific temperatures (75-85 °C), pressure and time, in a series of autoclaves performing a countercurrent process. The extracted liquor was concentrated and air-dried to fine powder before packing. According to the industry data, the tannin extract is composed of condensed tannins, around 70–80%, and 20–30% in hydrocolloid gums, sugars and small molecules.

The solvents hexane, ethyl acetate, 1-pentanol, 1-propanol and methanol were all analytical grade and purchased by Sigma-Aldrich, Brazil.

The reactants were: Folin-Ciocalteu reactant (Merck, Germany), HCl (Diadema, Brazil), dimethylformamide (DMF), dimethylsulphoxide (DMSO), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, gallic acid and catechin (Sigma–Aldrich, USA), monobasic and dibasic potassium phosphate and sodium carbonate (Vetec, Brazil) and vanillin (Nuclear, Brazil).

2.2. Solvent fractionation

For each purification, 15 g of raw tannin industrial extract (M_T) were separated with five organic solvents with different relative polarity (Rp) and boiling temperature (Bt): Hexane; ethyl acetate;

1-pentanol; 1-propanol and methanol. The single fractionation process was carried out directly on the industrial extract, producing a soluble fraction called extract and an insoluble fraction called residue. Conversely, the sequential fractionation was carried out using the same solvents following the polarity sequence: hexane, ethyl acetate, 1-pentanol, 1-propanol and methanol, according to Fig. 1. In this technique, the residues of the previous separation were purified with the next solvent (e.g. the insoluble part of the hexane extraction was separated in ethyl acetate, and so on). Six replicates were performed for each purification.

The extractions were performed in a Soxhlet extractor for 6 hours at the boiling temperature of each solvent. The extracted fractions and the residues were both dried in an oven at 70 °C and the weight of the remained fraction (M_F) was registered.

The extract yield (EY) was calculated as in Eq. (1).

$$EY = \left(\frac{M_F}{M_T}\right) \cdot 100 \tag{1}$$

M_F = final mass after purification (g); M_T = initial mass of tannin extract (g).

2.3. Gel permeation chromatography (GPC)

The solid tannin fractions were dissolved in a dimethylformamide (DMF) forming a 3% (w/w) solution. They were left under magnetic stirring for 24 hours to ensure complete dissolution. The solution was then filtered and the filtrate was injected into the chromatograph. Size exclusion chromatography analysis were used to evaluate the average molecular weight (Mw) and number average molecular mass (Mn) of the fractions. N,N-Dimethylformamide (DMF) eluent was used as GPC mobile phase, at 35 °C and a flow rate of 0.7 mL/min, using a Jasco Inc. chromatograph provided with an LCNetII/ACD interface, a column oven CO-2065Plus and a RI-2031Plus Intelligent Refractive Index Detector. A guard column and two columns PolarGel-M (Varian Inc.) were employed. Calibration was made using polystyrene standards provided by Fluka, ranging from 250 to 70,000 Da [36,37].

2.4. Total phenolic content

The total phenolic content (TPC) was measured following a modified Folin-Ciocalteu method (Chandra and Mejia 2004). Briefly, 0.25 mL of 4 N Folin-Ciocalteu reagent were added to 1 mL of each sample (0.05 mg/mL); this mixture was kept reacting for 5 min before the addition of 2 mL of 20% sodium carbonate. The solution was stored for 10 min before measuring the absorption at 730 nm using an UV–visible spectrophotometer Agilent 8453 (Agilent Technologies, USA). Gallic acid was used as standard and the samples were measured in triplicate. The total phenolic content was expressed in milligram equivalents of gallic acid per gram of extract (mgGAE/g). For the quantification, it was used the equation obtained with the standard gallic acid (0.005–0.04 mg/mL): $y = 34.9368x − 0.0008$ ($r = 0.9999$). This analysis was performed in triplicate.

2.5. Total condensed tannin

Total condensed tannin content (TCT) was determined by the vanillin method, according to Morrison et al. [38]. In this methodology, to each sample at the concentration of 50 mg/mL (0.1 mL) were added methanol (0.9 mL), solution A (8.0 mL of concentrated HCl in 100.0 mL methanol, 2.5 mL) and solution B (1.0 g of vanillin in 100.0 mL methanol, 2.5 mL). After immersion in a water bath for 20 min., the absorbance at 500 nm was registered with the UV-spectrometer. Catechin was used to obtain the calibration curve
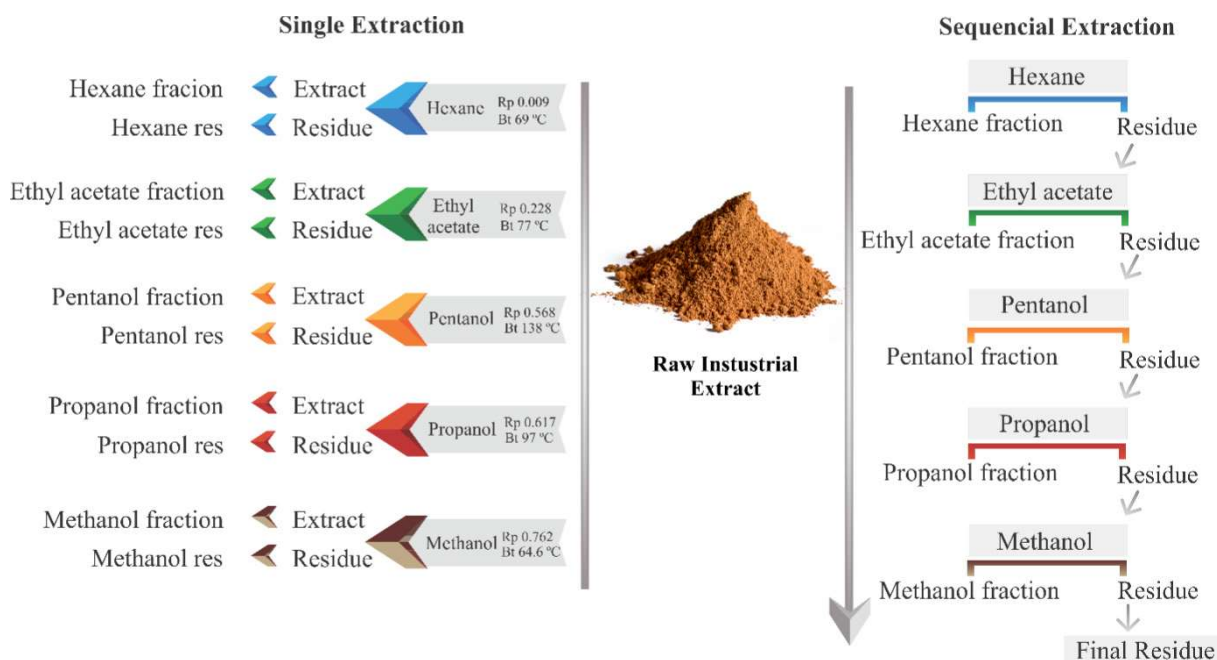
Fig. 1. Diagram of single and sequential extractions of Mimosa tannin raw industrial extract.

and the tests were performed in triplicate. The condensed tannins content was expressed in milligram equivalents of catechin per gram of extract (mgCE/g). For the quantification, it was used the equation obtained with the standard (5–60 mg/mL of catechin): y = 0.025x + 0.173, r = 0.9935. This analysis was performed in triplicate.

2.6. Antioxidant activity of tannin fractions

The antioxidant activities of all the tannin fractions were determined using the oxygen radical absorbance capacity (ORAC) method, described by Ou et al. [39]. The ORAC assay is used to measure the ability of an antioxidant to protect the disodium fluorescein from oxidation catalyzed by peroxyl radicals [40]. This kinetic assay is based on the measurement of radical scavenging activity of extracts against peroxyl radicals, produced by the addition of the AAPH (2,2′-azobis-2-methylpropanimidamide, dihydrochloride) radical inductor. For the determination of the optimal extract concentration for analyze, preliminary tests were made with known concentrations ranging from 5 to 300 mg L^{-1} (diluted in ethanol); the optimal concentration was determined to be 10 mg L^{-1}. A volume of 25 µL of sample (10 mg L^{-1}) or Trolox solution was added to a potassium phosphate buffer 75 mmol L^{-1} (pH 7.4) on a microplate, with incubation for 10 min at 37 °C. 150 µL of disodium fluorescein solution (81 nmol L^{-1}) were used as indicator and 25 µL of AAPH (152 mmol L^{-1}) were added as peroxyl radical generator. The fluorescence was then measured every minute (emission and excitation wavelengths were 530 ± 25 and 485 ± 20 nm, respectively) with SpectraMax M5 (Molecular Devices, Sunnyvale, CA, EUA) at 37 °C for 90 min. The ORAC values were calculated by a regression equation obtained with Trolox solutions (0–96 µmol L^{-1}) and area under curve (AUC) of the fluorescein decay. This analysis was performed in triplicate and were expressed as µmol of Trolox equivalents (TE) per g of extract in dry weight. The AUC was calculated according to Eq. (2):

$$AUC = 1 + \frac{f1}{f0} + \frac{f2}{f0} + \frac{f3}{f0} + \cdots \frac{fn}{f0} \tag{2}$$

fn = fluorescence in a read cycle (1 min); f0 = fluorescence at zero time.

2.7. Ash content of tannin fractions

Ash content of each fractions were measured following the methodology described in TAPPI standard (T 211 om-02) [41]. This analysis was performed in triplicate.

2.8. ATR FT-IR analysis

Vibrational spectroscopic measurements were performed directly in the ATR device by laying the different dried powders in an intimate contact with the surface of the diamond. The samples were scanned with a Nicolet Nexus 470 spectrometer in the spectral range between 4000 and 600 cm^{-1} with resolution of 4 cm^{-1}. Triplicates of the 32 scans for powder measurements were run and averaged. The spectra were, then, baseline corrected and area normalized and finally the fingerprints region between 1800 and 600 cm^{-1} of the spectra were compared as already described elsewhere [13].

2.9. Data analysis

Pearson correlation (simple correlation analysis) was applied to calculate the relation between variables. Significance levels were defined at p < 0.05, p < 0.01 and p < 0.001. From the correlation results, the regression models of the variables of interest were adjusted as a function of coefficient of determination (r^2) and mean absolute error (MAE). Moreover, a p-value of 0.001 (corresponding to 0.1% of significance) and Durbin-Watson statistic (p > 0.05 − no indication of a series residual autocorrelation) was considered.

3. Results and discussion

3.1. Extraction yield

Two major parameters have to be considered when the extraction process is evaluated: the polarity of the solvent and the

extraction temperature. Tannins can be considered relatively polar compounds, therefore, it would be expected that solvents with high polarities and high boiling temperature extracts the majority of the material. In Fig. 2 the extraction yields for single and sequential extractions are summarized.

For the single extractions, it can be observed that propanol and methanol are the two solvents which extract the higher amount of material (86.9% and 82.2% respectively). Far lower yields are registered for the less polar extracts: pentanol purifies 28.2% of the original material, ethyl acetate extracts only 6.1%, while hexane separates only traces (0.2%). For this reason, the extract by hexane will not be considered any further. When sequential extraction is applied, the original material is more homogeneously distributed between the different alcohol fractions. Pentanol extracts 30.1%, propanol 22.4% and methanol 30.9%, so more than 80% of the original material is collected in these fractions. Assuming that the yield of the single extraction of the polar solvent should register values comparable with the sum of the sequential extraction up to that solvent, the highest difference is observed for propanol, where the yield of the single extraction (86.9%) is around 20% higher than the sum of the sequential extracts of hexane (0.2%), ethyl acetate (6.0%), pentanol (30.1%) and propanol (22.4%). This difference can be due to extract modifications occurring during the heat-drying cycles.

3.2. Molecular mass distribution

The GPC analysis performed on the extracted fractions have shown that the molecular masses of the purified substances depend on the solvent used. In Table 1, Mw, Mn and the polydispersity index (Mw/Mn) for single and sequential extractions are reported.

Single and sequential extractions present similar results in terms of extracted molecular mass. The ethyl acetate fraction resulted to be the one having far lower molecular mass, while the alcoholic fractions present decreasing molecular masses with polarity increase. The higher molecular mass (Mw and Mn) polymers are purified when the extraction temperature is higher. In the sequential extraction, the average molecular masses for the alcoholic extracts result slightly higher because a significant amount of low molecular mass substances were previously "selectively" separated by ethyl acetate. The fractions with lower molecular masses have also lower polydispersity, which suggests that the bigger macromolecules are strongly included in the high molecular fractions (e.g. pentanol and propanol); on the other hand, smaller molecules can also be found in the fraction with higher average molecular mass, suggesting a higher selectivity for bigger molecules. The polydispersity index increase slightly
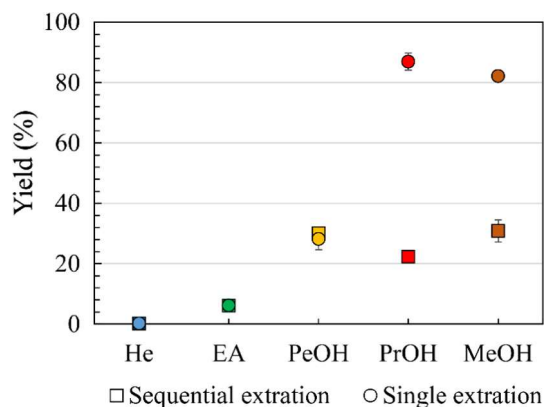
Table 1
Mw, Mn and Polydispersity (Mw/Mn) of the tannin fractions for single and sequential extraction.

	Mw	Mn	Mw/Mn
Single extraction			
Ethyl acetate	1478	648	2.28
Pentanol	4234	990	4.28
Propanol	3677	939	3.92
Methanol	2146	741	2.94
Sequential extraction			
Ethyl acetate	1575	652	2.41
Pentanol	4734	1040	4.55
Propanol	3840	970	3.95
Methanol	3281	957	3.42

for the sequential extraction, which means that the sequential extracts produce a broader distribution of molecular masses.

3.3. Total phenolic content (TPC) and total condensed tannin (TCT)

The determination of phenolic and condensed tannin content is fundamental to estimate the chemical properties of the fractions. In Fig. 3a and b the measurement of the two parameters are observed for the single and sequential extractions, respectively.

In the single extraction, it can be observed that TCP and TCT have completely opposite trends. Phenolic content increase with the polarity of the alcohols, while condensed tannins decrease; moreover, the ethyl acetate extract presents very high amount of phenolics and very low amount of condensed tannin. Being that condensed tannins are phenolics, it would be expected that the condensed tannin (TCT) and the phenolic (TPC) content correlate, but for analytical reasons this does not occur. The Folin-Ciolcateu method is relatively high selective for phenols [42], but the vanillin-method for condensed tannins presents two significant drawbacks: i) the fractions with lower molecular masses produce lower color intensity in the adduct with vanillin and ii) the lower reactivity of condensed tannin with resorcinolic A-ring reduce the response of these compounds. This means that the mimosa tannin extract, which is mainly robinetinidin (hence a 5-deossi proantocyanidin) results less sensitive to the vanillin assay [7,43]. In particular, we can state that the ethyl acetate fraction is the one containing lower molecular mass substances, while the pentanol fraction is the one which present the extract with higher molecular mass. According to this interpretation, in the sequential extraction we observe that the pentanol extract contains significantly less TCT than expected. This can be explained by the presence of higher molecular masses chains, which reduce significantly their reactivity against vanillin when exposed to sequential temperature/solvent stresses.

3.4. Antioxidant activity

The extracts were tested for their capacity to stabilize radicals and the results of the ORAC tests are reported in Fig. 4a for the single and Fig. 4b for the sequential extractions.

In both graphics it appears immediately that the antioxidant activity of the extracts is elevated and always higher than Trolox, a synthetic homologue of Vitamin E, which is suited as biological antioxidant for the high capacity of capturing ROS (reactive oxygen species) [44]. The extracts of the single extraction have a higher total antioxidant effect than the sequentially obtained extracts. This means that the low molecular mass extracts of ethyl acetate have a higher antioxidant effect than the other (total antioxidant activity, AUC = 4982 μmol TE/g). The lower antioxidant effect registered for the pentanolic fraction (total antioxidant activity,

Fig. 2. Extraction yield of the single and sequential Mimosa tannin extractions.
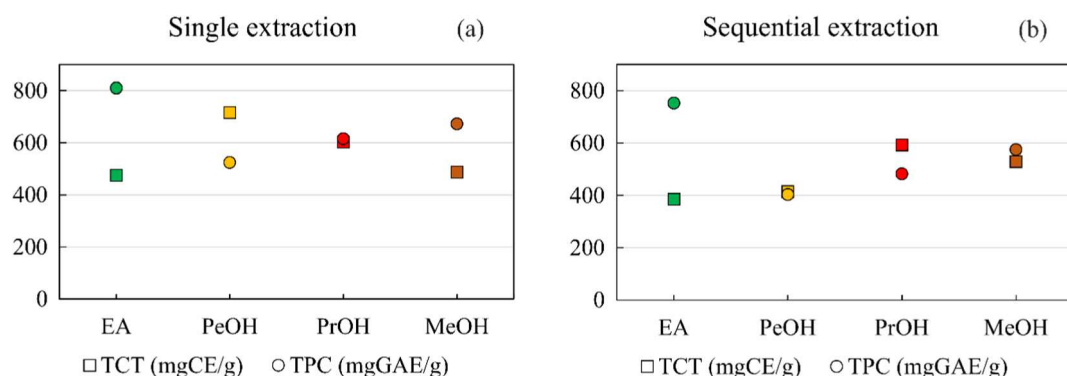
Fig. 3. Total phenolic (TPC) and condensed tannin (TCT) content for the single and sequential extracts.
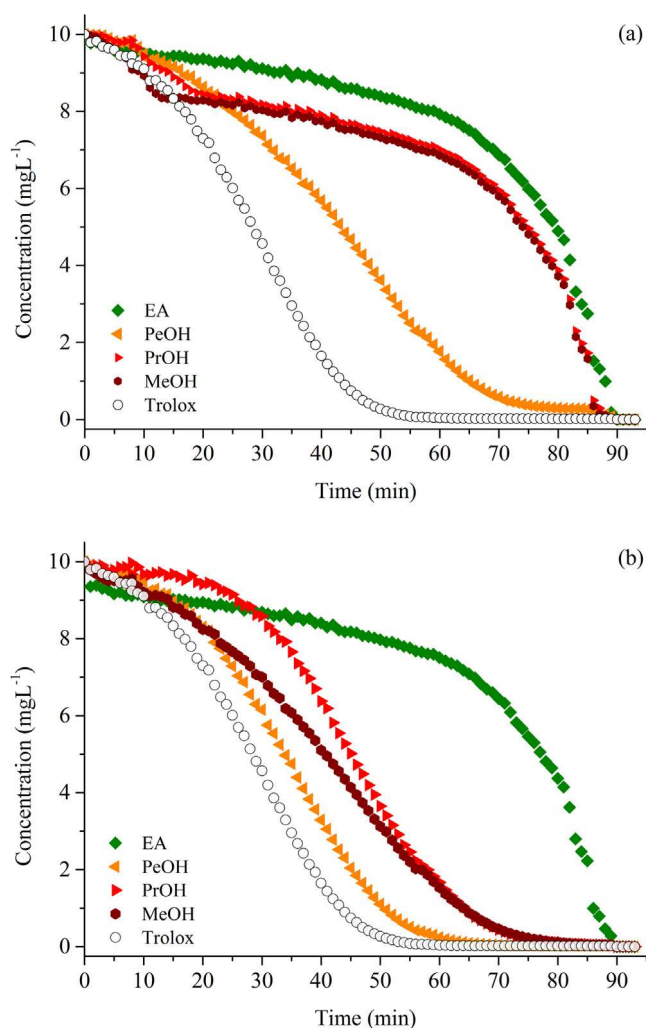

Fig. 4. Antioxidant capacity during time of the different tannin extract (a) single and (b) sequentially extracted in comparison with Trolox.

AUC = 3189 μmol TE/g) suggests that part of the hydrocolloids is solubilized in this fraction.

3.5. Residual fractions

The residual fractions were also analyzed and their properties are resumed in Table 2. The residual fractions present very different properties, which strongly depend on the extraction yield. It is interesting to observe that the residues with lower yields (higher extract yield), namely propanol, methanol and sequential residues, present: high molecular mass, high polydispersity index, low phenolic and condensed tannin content, low antioxidant activity and very high ash content. This means that high molecular mass carbohydrates and inorganic molecules accumulate in these fractions.

3.6. FT-IR analysis

More chemical information were obtained by fractions infrared spectroscopy, as shown in Fig. 5. The profile of the single extracted powders was found very similar for the alcoholic fractions, while the ethyl acetate fraction presented different peaks and intensities. In particular, the latter shows two new peaks at (i) $1705 \, cm^{-1}$ due to the carboxyl groups and (ii) $1260 \, cm^{-1}$ due to C—O stretching of the carboxyl group [45]; moreover, increased intensity were observed at $1510 \, cm^{-1}$ (C=C aromatic skeletal vibration), $1285 \, cm^{-1}$ (C—O stretching of pyrogallic ring), $1200 \, cm^{-1}$ (OH—plane deformation), $1100 \, cm^{-1}$ (CH bending phenolic in-plane) and $805 \, cm^{-1}$ (CH aromatic bending out-of-plane). On the other hand, the band at $1070 \, cm^{-1}$ (C—C bending of the B ring) decreases [46]. This information suggest that a small part (20–40%) of the ethyl acetate extract, which is only 6.1% of the entire extract, is constituted of hydrolizable tannins.

Much less difference can be observed between the alcoholic extracts: the spectra of the methanol extract present lower intensities at $1285 \, cm^{-1}$ and slightly higher absorption at $1100 \, cm^{-1}$. These evidences would suggest that the methanolic fraction contains slightly less pyrogallic B-ring (more fisetinidin) and higher freedom for the C—H aromatic bendings, which could be explained also with a lower molecular mass. Pentanol and propanol extracts are almost the same, the only small difference in the spectra is found at around $890 \, cm^{-1}$, where the pentanolic extract has a higher peak than the propanolic. This signal can be attributed to anomeric carbons in carbohydrates [47]. The spectrum of the total residue presents very high distance from the spectra of the extracted substances. New peaks can be observed at 1740 and $1150 \, cm^{-1}$, which can be assigned to C=O stretching and C—O—C asymmetric vibrations. The signal at $1740 \, cm^{-1}$ suggests the presence of esters (hydroxyaromatic acid with carbohydrates), while the peak at $1150 \, cm^{-1}$ is typical for di- to poly-saccharides. The very broad bands between $1450–1350 \, cm^{-1}$ and $1150–950 \, cm^{-1}$ have to be principally attributed to carbohydrates [48,49]: the broadness suggest their polymeric arrangement. These spectral patterns suggest that the insoluble residues are carbohydrates which can be also covalently combined with some hydroxyl-aromatic compound.

Table 2
Summary of the results for the residues found in single and sequential extractions.

|	Yield (%)	Mw (Da)	Mn (Da)	Mw/Mn	TPC (mgGAE/g)	TCT (mgCE/g)	ORAC (μmol TE/g)	Ashes (%)
Single extraction								
He	99.86 ± 0.08	2922	816	3.58	405 ± 11	161 ± 4	512 ± 18	5.10 ± 0.001
EA	94.86 ± 2.12	3306	945	3.50	320 ± 9	115 ± 12	454 ± 22	5.05 ± 0.02
PeOH	71.81 ± 2.97	3846	1169	3.29	380 ± 4	325 ± 1	1379 ± 19	7.9 ± 0.45
PrOH	13.03 ± 1.59	5743	1351	4.25	243 ± 7	107 ± 7	318 ± 6	14.5 ± 0.065
MeOH	17.83 ± 2.19	7063	1312	5.38	225 ± 11	6 ± 4	194 ± 6	12 ± 0.09
Sequential extraction								
Final residue	10.47 ± 1.70	4132	913	3.94	167 ± 8	204	6.7 ± 0.7	13.8 ± 0.07
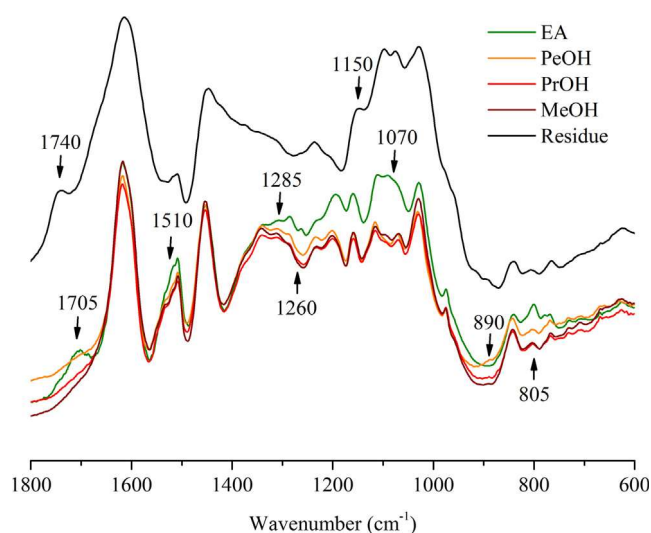
Fig. 5. FT-IR spectra of different tannin extracts and total residue.

3.7. Correlations

It is interesting to observe which variable correlate to others. In Table 3 the correlations are resumed.

The more significant correlations were observed for the phenolic content (TPC): particularly, extremely significant correlation occurred with the antioxidant activity, mass average molar mass and ashes content. The most important correlations highlighted through the Pearson analysis were further considered to estimate the regression models (Fig. 6).

This means that the Folin-Ciocalteu reaction on tannin extract can indirectly give very interesting information about the antioxidant activity and molecular mass of the polyflavonoid. These findings confirm the studies of Mokrani and Madani [50] and Price [43]. Furthermore, it is very interesting to see that the simple measurement of the ashes allows estimating (with a certain error) very

important chemical features of the extract such as phenolic content, antioxidant capacity and molecular mass. The values of the coefficient of determination (r^2) and the significance (p value) of the regression models are reported in Table 3 for the most interesting Pearson correlations previously observed.

4. Conclusions

The purification of the industrial extract of Mimosa was performed. It was observed that the Soxhlet extraction of the tannin powders has higher yield for polar solvents and for higher extraction temperatures. In particular, the propanolic extraction is the one which guarantees yields of around 87%. The ethyl acetate extract resulted richer in polyphenols with low molecular mass and very high antioxidant capacity. A small amount of hydrolysable tannin, 1–2% of the total extract, was also observed. The pentanolic extract is constituted of high molecular condensed tannins, where a small portion of carbohydrates is also observed. The propanolic and methanolic fractions differ only for the slightly lower molecular mass of the methanolic fraction, which contain slightly higher portion of fisetinidin. The residues are enriched of carbohydrates/hydrocolloids and ashes content. These findings suggest that the extraction with different organic solvents was satisfactory, increasing the performance and application spectrum of the extracts/those new materials. Lighter fractions can be more easily suited as antioxidant, while heavier fractions can be ideal as adhesive filler (requiring less amount of hardener to cure).

The technique of sequential extractions has shown interesting applications to purify the fractions and in particular, the pre-extraction with ethyl acetate ensure the removal of the hydrolizable tannins. Extremely significant correlations have been observed between phenolic content and i) antioxidant capacity and ii) mass average molecular mass.

Table 3
Pearson correlation coefficients between different assays (N = 13).

	Yield (%)	Mw (Da)	Mn (Da)	TPC (mgGAE/g)	TCT (mgCE/g)	ORAC (μmol TE/g)	Ashes (%)
Yield (%)	1						
Mw (Da)	−0.15[ns]	1					
Mn (Da)	−0.02[ns]	0.93[***]	1				
TPC (mgGAE/g)	0.03[ns]	−0.81[***]	−0.78[**]	1			
TCT (mgCE/g)	0.05[ns]	−0.48[ns]	−0.51[ns]	0.70[**]	1		
ORAC (μmol TE/g)	0.04[ns]	−0.77[**]	−0.79[**]	0.97[***]	0.75[**]	1	
Ashes (%)	−0.15[ns]	0.60[*]	0.65[*]	−0.78[**]	−0.74[**]	−0.78[**]	1

[ns] p > 0.05, no significant correlation
[*] p < 0.05, significant correlation.
[**] p < 0.01, very significant correlation.
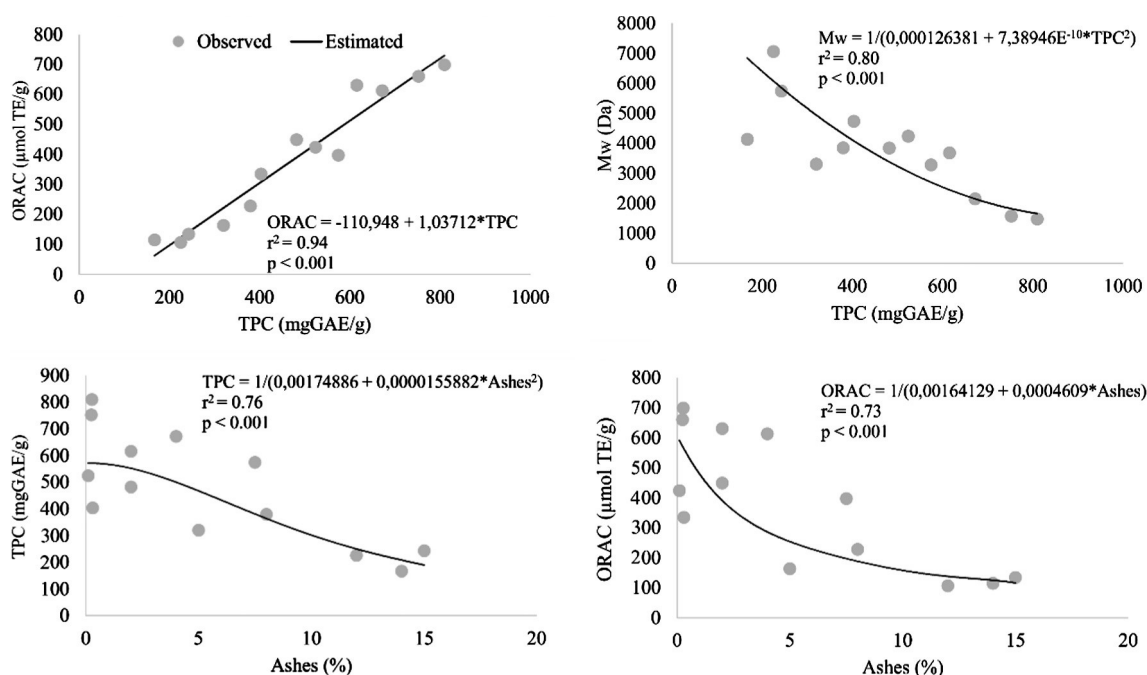[***] p < 0.001, extremely significant correlation.

Fig. 6. Regression models between chemical properties of the different tannin extracts and residues.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seppur.2017.06.010.

References

[1] H.-J. Huang, S. Ramaswamy, U.W. Tschirner, B.V. Ramarao, A review of separation technologies in current and future biorefineries, Sep. Purif. Technol. 62 (2008) 1–21, http://dx.doi.org/10.1016/j.seppur.2007.12.011.

[2] T.V. Lourençon, F.A. Hansel, T.A. da Silva, L.P. Ramos, G.I.B. de Muniz, W.L.E. Magalhães, Hardwood and softwood kraft lignins fractionation by simple sequential acid precipitation, Sep. Purif. Technol. 154 (2015) 82–88, http://dx.doi.org/10.1016/j.seppur.2015.09.015.

[3] E. Haslam, Plant Polyphenols: Vegetable Tannins Revisited, Cambridge University Press, 1989.

[4] A. Pizzi, Tannins: Major Sources, Properties and Applications, in: M.N. Belgacem, A. Gandini (Eds.), Monomers, Polymers and Composites from Renewable Resources, Elsevier, Amsterdam, 2008, p. 553.

[5] R.W. Hemingway, J.J. Karchesy, S.J. Branham, Chemistry and Significance of Condensed Tannins, Springer, 1989.

[6] A. Pizzi, C. Simon, B. George, D. Perrin, M.C. Triboulot, Tannin antioxidant characteristics in leather versus leather light stability: models, J. Appl. Polym. Sci. 91 (2004) 1030–1040, http://dx.doi.org/10.1002/app.13047.

[7] A. Pizzi, Advanced Wood Adhesives Technology, Marcel Dekker Inc, New York, 1994.

[8] A. Pizzi, Pine tannin adhesives for particleboard, Holz als Roh- und Werkstoff 40 (1982) 293–301, http://dx.doi.org/10.1007/BF02610623.

[9] A. Rinaldi, G. Blaiotta, M. Aponte, L. Moio, Effect of yeast strain and some nutritional factors on tannin composition and potential astringency of model wines, Food Microbiol 53 (Part B) (2016) 128–134, http://dx.doi.org/10.1016/j.fm.2015.09.013.

[10] J. Sánchez-Martín, J. Beltrán-Heredia, C. Solera-Hernández, Surface water and wastewater treatment using a new tannin-based coagulant. Pilot plant trials, J. Environ. Manage. 91 (2010) 2051–2058, http://dx.doi.org/10.1016/j.jenvman.2010.05.013.

[11] A. Aires, R. Carvalho, M.J. Saavedra, Valorization of solid wastes from chestnut industry processing: extraction and optimization of polyphenols, tannins and ellagitannins and its potential for adhesives, cosmetic and pharmaceutical industry, Waste Manage. 48 (2016) 457–464, http://dx.doi.org/10.1016/j.wasman.2015.11.019.

[12] G. Tondi, V. Fierro, A. Pizzi, A. Celzard, Tannin-based carbon foams, Carbon 47 (2009) 1480–1492, http://dx.doi.org/10.1016/j.carbon.2009.01.041.

[13] G. Tondi, M. Link, C.W. Oo, A. Petutschnigg, A simple approach to distinguish classic and formaldehyde-free tannin based rigid foams by ATR FT-IR, J. Spectrosc. (2015), http://dx.doi.org/10.1155/2015/902340.

[14] G. Tondi, A. Pizzi, Tannin-based rigid foams: characterization and modification, Ind. Crops Prod. 29 (2009) 356–363, http://dx.doi.org/10.1016/j.indcrop.2008.07.003.

[15] A.E. Hagerman, K.M. Riedl, G.A. Jones, K.N. Sovik, N.T. Ritchard, P.W. Hartzfeld, T.L. Riechel, High molecular weight plant polyphenolics (Tannins) as biological antioxidants, J. Agric. Food Chem. 46 (1998) 1887–1892, http://dx.doi.org/10.1021/jf970975b.

[16] M. Abu Zarin, H.Y. Wan, A. Isha, N. Armania, Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from Leucaena leucocephala hybrid-Rendang, Food Sci. Human Wellness 5 (2016) 65–75, http://dx.doi.org/10.1016/j.fshw.2016.02.001.

[17] F. Santiago-Medina, G. Foyer, A. Pizzi, S. Caillol, L. Delmotte, Lignin-derived non-toxic aldehydes for ecofriendly tannin adhesives for wood panels, Int. J. Adhes. Adhes. 70 (2016) 239–248, http://dx.doi.org/10.1016/j.ijadhadh.2016.07.002.

[18] L. Pan, H. Wang, C. Wu, C. Liao, L. Li, Tannic-acid-coated polypropylene membrane as a separator for lithium-ion batteries, ACS Appl. Mater. Interfaces 7 (2015) 16003–16010, http://dx.doi.org/10.1021/acsami.5b04245.

[19] G. Tondi, F.M. Thevenon, B. Mies, G. Standfest, A. Petutschnigg, S. Wieland, Impregnation of Scots pine and beech with tannin solutions: effect of viscosity and wood anatomy in wood infiltration, Wood Sci. Technol. 47 (2013) 615–626, http://dx.doi.org/10.1007/s00226-012-0524-5.

[20] G. Tondi, S. Wieland, T. Wimmer, M.F. Thevenon, A. Pizzi, A. Petutschnigg, Tannin-boron preservatives for wood buildings: mechanical and fire properties, Eur. J. Wood Wood Prod. 70 (2012) 689–696, http://dx.doi.org/10.1007/s00107-012-0603-1.

[21] G. Tondi, M. Link, C. Kolbitsch, R. Lesacher, A. Petutschnigg, Pilot plant up-scaling of tannin foams, Ind. Crops Prod. 79 (2016) 211–218, http://dx.doi.org/10.1016/j.indcrop.2015.11.013.

[22] G. Amaral-Labat, A. Szczurek, V. Fierro, A. Pizzi, A. Celzard, Systematic studies of tannin–formaldehyde aerogels: preparation and properties, Sci. Technol. Adv. Mater. 14 (2013) 015001, http://dx.doi.org/10.1088/1468-6996/14/1/015001.

[23] J.M. Monteiro, U.P.D. Albuquerque, E.D.L. Araújo, E.L.C.D. Amorim, Taninos: Uma abordagem da química à ecologia, Química Nova 28 (2005) 892–896.

[24] A. Arbenz, L. Averous, Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures, Green Chem. 17 (2015) 2626–2646, http://dx.doi.org/10.1039/C5GC00282F.

[25] EMBRAPA, Biorrefinarias, in: P.e.A. Ministério da Agricultura (Ed.), Brasília, 2011, pp. 6.

[26] J. Xi, L. Yan, Optimization of pressure-enhanced solid-liquid extraction of flavonoids from Flos Sophorae and evaluation of their antioxidant activity, Sep. Purif. Technol. 175 (2017) 170–176, http://dx.doi.org/10.1016/j.seppur.2016.10.013.

[27] P. Huang, Q. Zhang, H. Pan, L. Luan, X. Liu, Y. Wu, Optimization of integrated extraction-adsorption process for the extraction and purification of total flavonoids from Scutellariae barbatae herba, Sep. Purif. Technol. 175 (2017) 203–212, http://dx.doi.org/10.1016/j.seppur.2016.11.031.

[28] Y. Zhang, Z. Li, H. Wang, X. Xuan, J. Wang, Efficient separation of phenolic compounds from model oil by the formation of choline derivative-based deep eutectic solvents, Sep. Purif. Technol. 163 (2016) 310–318, http://dx.doi.org/10.1016/j.seppur.2016.03.014.

[29] X.-X. Chen, Y. Shi, W.-M. Chai, H.-L. Feng, J.-X. Zhuang, Q.-X. Chen, Condensed Tannins from Ficus virens as Tyrosinase inhibitors: structure inhibitory activity and molecular mechanism, PLOS One 9 (2014) e91809, http://dx.doi.org/10.1371/journal.pone.0091809.

[30] L.J. Putman, L.G. Butler, Fractionation of condensed tannins by counter-current chromatography, J. Chromatogr. A 318 (1985) 85–93, http://dx.doi.org/10.1016/S0021-9673(01)90666-1.

[31] H.-C. Zhou, Y.-M. Lin, S.-D. Wei, N.F.-y. Tam, Structural diversity and antioxidant activity of condensed tannins fractionated from mangosteen pericarp, Food Chem. 129 (2011) 1710–1720, http://dx.doi.org/10.1016/j.foodchem.2011.06.036.

[32] L.P. Meagher, G. Lane, S. Sivakumaran, M.H. Tavendale, K. Fraser, Characterization of condensed tannins from Lotus species by thiolytic degradation and electrospray mass spectrometry, Anim. Feed Sci. Technol. 117 (2004) 151–163, http://dx.doi.org/10.1016/j.anifeedsci.2004.08.007.

[33] B. Teng, T. Zhang, Y. Gong, W. Chen, Molecular weights and tanning properties of tannin fractions from the Acacia mangium bark, Afr. J. Agric. Res. 8 (2013) 5996–6001, http://dx.doi.org/10.5897/AJAR11.1911.

[34] B. Teng, X. Jian, Y. Wang, W. Chen, Characterization of Acacia mangium tannin fractions extracted with different organic solvents, Asian J. Chem. 27 (2015) 1847–1850, http://dx.doi.org/10.14233/ajchem.2015.18090.

[35] T.-Q. Yuan, J. He, F. Xu, R.-C. Sun, Fractionation and physico-chemical analysis of degraded lignins from the black liquor of Eucalyptus pellita KP-AQ pulping, Polym. Degrad. Stab. 94 (2009) 1142–1150, http://dx.doi.org/10.1016/j.polymdegradstab.2009.03.019.

[36] O. Gordobil, R. Herrera, R. Llano-Ponte, J. Labidi, Esterified organosolv lignin as hydrophobic agent for use on wood products, Prog. Org. Coat. 103 (2017) 143–151, http://dx.doi.org/10.1016/j.porgcoat.2016.10.030.

[37] O. Gordobil, I. Egüés, J. Labidi, Modification of Eucalyptus and Spruce organosolv lignins with fatty acids to use as filler in PLA, React. Funct. Polym. 104 (2016) 45–52, http://dx.doi.org/10.1016/j.reactfunctpolym.2016.05.002.

[38] I.M. Morrison, E.A. Asiedu, T. Stuchbury, A.A. Powell, Determination of lignin and tannin contents of cowpea seed coats, Ann. Bot. 76 (1995) 287–290, http://dx.doi.org/10.1006/anbo.1995.1097.

[39] B. Ou, M. Hampsch-Woodill, R.L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, J. Agric. Food Chem. 49 (2001) 4619–4626, http://dx.doi.org/10.1021/jf010586o.

[40] H. Metrouh-Amir, C.M.M. Duarte, F. Maiza, Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of Matricaria pubescens, Ind. Crops Prod. 67 (2015) 249–256, http://dx.doi.org/10.1016/j.indcrop.2015.01.049.

[41] TAPPI, Ash in wood, pulp, paper and paperboard: combustion at 525 ℃. T 211 om-93, Technical Association of the Pulp and Paper Industry TAPPI Press, Atlanta, USA, 1999.

[42] J.D. Everette, Q.M. Bryant, A.M. Green, Y.A. Abbey, G.W. Wangila, R.B. Walker, Thorough study of reactivity of various compound classes toward the Folin–Ciocalteu reagent, J. Agric. Food Chem. 58 (2010) 8139–8144, http://dx.doi.org/10.1021/jf1005935.

[43] M.L. Price, S. Van Scoyoc, L.G. Butler, A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain, J. Agric. Food Chem. 26 (1978) 1214–1218, http://dx.doi.org/10.1021/jf60219a031.

[44] E.M. Messier, K. Bahmed, R.M. Tuder, H.W. Chu, R.P. Bowler, B. Kosmider, Trolox contributes to Nrf2-mediated protection of human and murine primary alveolar type II cells from injury by cigarette smoke, Cell Death Dis. 4 (2013) e573. http://www.nature.com/cddis/journal/v4/n4/suppinfo/cddis201396s1.html.

[45] G. Tondi, A. Petutschnigg, Middle infrared (ATR FT-MIR) characterization of industrial tannin extracts, Ind. Crops Prod. 65 (2015) 422–428, http://dx.doi.org/10.1016/j.indcrop.2014.11.005.

[46] F.D.S. Grasel, M.F. Ferrão, C.R. Wolf, Development of methodology for identification the nature of the polyphenolic extracts by FTIR associated with multivariate analysis, Spectrochim. Acta Part A: Mol. Biomol. Spectrosc. 153 (2016) 94–101, http://dx.doi.org/10.1016/j.saa.2015.08.020.

[47] M. Schwanninger, J.C. Rodrigues, H. Pereira, B. Hinterstoisser, Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose, Vib. Spectrosc. 36 (2004) 23–40, http://dx.doi.org/10.1016/j.vibspec.2004.02.003.

[48] M.-L. Kuo, J.F. McClelland, S. Luo, P.-L. Chien, R.D. Walker, C.-Y. Hse, Applications of infrared photo-acoustic spectroscopy for wood samples, Wood Fiber Sci. 20 (1988) 132–145.

[49] A.L. Missio, B.D. Mattos, P.H.G. Cademartori, A. Pertuzzatti, B. Conte, D.A. Gatto, Thermochemical and physical properties of two fast-growing eucalypt woods subjected to two-step freeze–heat treatments, Thermochim. Acta 615 (2015) 15–22, http://dx.doi.org/10.1016/j.tca.2015.07.005.

[50] A. Mokrani, K. Madani, Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit, Sep. Purif. Technol. 162 (2016) 68–76, http://dx.doi.org/10.1016/j.seppur.2016.01.043.