Fractioning of Industrial Tannin Extract in Different Organic Solvents

Thomas Sepperer[1]* and Gianluca Tondi[1]

[1] Forest Products Technology and Timber Construction Department, Salzburg University of Applied Sciences, Markt 136a, 5431 Kuchl, Austria,
*thomas.sepperer@fh-salzburg.ac.at

Abstract. The industrial tannin extracts are products consisting mainly of polyphenols containing also 20-25 % of hydrocolloids and easy sugars. These powders are a completely natural product that is obtained by water extraction of bark and it is easily available in high quantities. Tannin is widely used in the leather tannery row, as wood adhesive as well as flocculant in the treatment of polluted water. During the last years, this bio-resource was also applied for innovative solutions like the wood preservative and the natural tannin-furanic rigid foams. Nowadays, the tannin-foams need a more defined chemistry of the polyphenolic building-block so that foams with increased properties can be synthetized. Goal of this study was to purify the raw material to get a chemically more defined and consistent material through selective liquid extraction. To purify the tannin, 1 g of the extract was solubilized in 100 ml of the organic solvent and magnetically stirred for 1 hour at ambient temperature. Afterwards the solutions were gravity filtrated and both, soluble and insoluble fractions were dried and pulverized again. RP-HPLC DAD analysis was performed on all fractions to identify changes in the composition or concentration of the main components. A separation into two fractions was achieved for most of the solvents. More promising separations were recorded for acetone, acetonitrile, ethanol and methanol. Although HPLC profiles were very similar for all fractions, higher concentration of gallic acid were registered for the soluble fractions in methanol, ethanol and acetone, while higher concentration of catechin was observed for the fraction soluble in acetone. Further analysis like GC-MS and GPC will be necessary for better qualifying the extracts.

Keywords: Condensed tannin, separation, solubility, HPLC-analysis.

1 Introduction

Condensed tannins are synthesized by various plants to chemically protect themselves against fungi, parasites [1] and animals [2]. They are mostly found in the bark of different species like pine, quebracho and wattle [3]. Condensed tannins (sometimes referred to as polyflavonoids or proanthocyadines) are highly complex phenolic compounds [4] which are constituted by repeating flavonoid units [5] (see Fig. 1.).

Today the most commonly used industrial tannin extract is obtained from black wattle through hot water extraction. Condensed tannins are a cheap source of polyphenols mainly used in the leatherworking industry and adhesive production [4].

Since several years, another use of condensed tannins was found to be the synthesis of aerogels [6] and formaldehyde-free rigid foams that consist of 100 % natural raw materials in their structure [7]. These foams have been studied in depth shown that they consist mainly of randomly organized oligomeric tannin branches and hydroxymethylated furanic chains [8]. A number of physical and mechanical properties (compression resistance, water uptake, thermal conductivity, etc.) of these foams have been studied [9], [10]. These properties are highly dependent on the density of the foams. Production of tannin foams with tailored structures of the flavonoid will allow to produce more chemically defined materials with improved properties. The objective of this study was to purify the industrial tannin extract through liquid-solid room temperature extraction. The obtained fractions were characterized by means of RP-HPLC DAD.

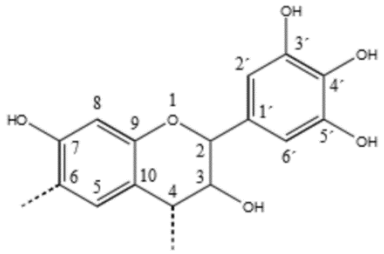

Fig. 1. Prorobinetinidin structure: Resorcinolic A-ring and pyrogallic B-ring[9]

2 Experimental

2.1 Materials

Industrial Tannin extract Weibull AQ was provided by Tanac S.A. (Brazil). According to the manufacturer, Weibull AQ is a natural extract, rich in tannin colloidal structure that is rich in phenols that is obtained by aqueous extraction of black wattle (Acacia mearnsii) bark.

Chemicals used for the analysis were HPLC super gradient grade water (VWR Chemicals), HPLC ultra methanol (VWR chemicals), HPLC super gradient grade acetonitrile (VWR chemicals), phosphoric acid 85 % (Roth), acetone > 99.7 % (Roth), Ethanol 96 % (Roth), 2-propanol (Roth), dichloromethane (Roth), (+)-catechin hydrate (Cayman Chemicals) and gallic acid (Alfa Aesar).

2.2 Methods

Determination of solubility. To determine the solubility of the tannin extract in organic solvents (acetone, acetonitrile, ethanol, methanol, dichloromethane, 2-propanol

and water) 1 g of absolutely dry tannin was dissolved in 100 ml of the solvent at ambient temperature and magnetically stirred for 1 hour. Afterwards the solution was poured through dry filter paper. The filter paper was then dried in the oven at 103 °C for two days and weight was measured to determine the percentage of the insoluble portion of the tannin extract (eq. 1). The liquid was evaporated at 30 °C under a 100 mbar vacuum using a Heidolph Laboratora 4000 efficient rotary evaporator (Heidolph Indstruments) and then dried at 103 °C in a laboratory oven (Binder). HPLC analysis was performed for both, soluble and insoluble, fraction for each solvent.

$$\left(\frac{m_{TF}-m_F}{m_T}\right) \cdot 100 = insol[\%] \quad (1)$$

m_{TF} is the weight of the dried filer and insoluble Tannin
m_F is the weight of the dried filter
m_T is the weight of the Tannin
insol [%] is the percentage of the insoluble Tannin

Separation of higher quantities. To purify more of the original tannin for foam production, 50 g of tannin were dissolved in 200 ml of acetone, ethanol, methanol and acetonitrile as these solvents have shown the highest degree of separation. After stirring for 1 hour at ambient temperature, the solutions were processed as described above. After drying, the tannin was pulverized using pestle and mortar.

HPLC analysis. HPLC analysis of the tannin extract was performed to understand the composition of the substance. The analysis was carried out using a HPLC setup consisting of a degassing unit DGU-20A, liquid chromatograph LC-20AT, column oven CTO-10AS with a C18 5 µm column (250 x 4.6 mm I. D.), refractive index detector RID-20A and photodiode array detector SPD-M20A all manufactured by Shimadzu, Japan. Solvent A was a 0.075 wt% mixture of HPLC water and phosphoric acid, solvent B was acetonitrile [12], [13], [1]. An isocratic elution was performed with 85 % A and 15 % B at 40 °C for 30 minutes as it was evaluated to be the most suitable elution condition for analyzing the tannin extract present, determined in a previous study (Sepperer, unpublished). The aqueous tannin solutions for analysis were prepared using HPLC water and a concentration of the extract of 1.25 g L^{-1}. Injection volume was 20 µL for each analysis. The chromatogram was registered at 280 nm, and the UV spectra were recorded between 190 and 800 nm.

3 Results

3.1 Determination of solubility

Table 1 shows the calculated solubility of the tannin extract in different solvents. The more significant separation was achieved by using acetone with 35.9 % soluble and 64.1 % insoluble part followed by acetonitrile and ethanol with roughly 75 % soluble and 25 % insoluble fraction. 15.6 % of the original tannin extract were soluble in meth-

anol. As expected, in the non-polar dichloromethane only 2.9 % were soluble, but surprisingly also only 1.6 % were soluble in 2-propanol. Almost all of the tannin (97.3 %) was soluble in deionized water.

Table 1. Solubility of industrial tannin extract in organic solvents

Solvent	polarity index	% soluble	% insoluble
Dichloromethane	3.1	2.9	97.1
2-propanol	3.9	1.6	98.4
Methanol	5.1	84.4	15.6
Acetone	5.1	35.9	64.1
Ethanol	5.2	73.2	26.8
Acetonitrile	5.8	75.9	24.1
Water	10.2	97.3	2.7

3.2 Separation of higher quantities

Solubilizing higher quantities of tannin extract in ethanol, methanol and acetonitrile worked fine. Only when dissolving 50 g of tannin in 200 ml of acetone, it appeared that just 5 g are soluble which is equal to only 10 % and far less than the 35.9 % calculated during the pre-test. After filtering the tannin acetone solution, the insoluble portion of the tannin was mixed with fresh acetone and again stirred for 1 hour. This step was repeated several times and the final amount of tannin completely insoluble in acetone was determined with 62.3 % which is comparable to the amount obtained during the pre-study (64.1 %). This means that acetone is saturated with tannin extract at a level of roughly 3.2 wt%. Solubility of the tannin in acetonitrile, ethanol and methanol was also identical as the one in the pre-study. When evaporating the ethanol, it was observed that the solution became gelatinous as soon as most of the ethanol was evaporated. When drying in the oven, the part soluble in ethanol did not become a powder as all others did, it was a shiny dark disk, which had a solid surface. For this fraction, it was not possible to be pulverized after drying. Conversely, for the three other solvents, it was possible to pulverize the soluble and the insoluble fractions after drying.

3.3 HPLC analysis

Fig. 2. shows the chromatogram of selected fractions (all from the pre-test) recorded with the PDA at 280 nm as well as the chromatographic profile of the unmodified industrial tannin extract. Seven peaks have been marked as they are of interest. At roughly 2 minutes after the start of the analysis the first noise appears on the PDA which can be explained with the death time of the used column, that is around 2.1 minutes according to the manufacturers data sheet.

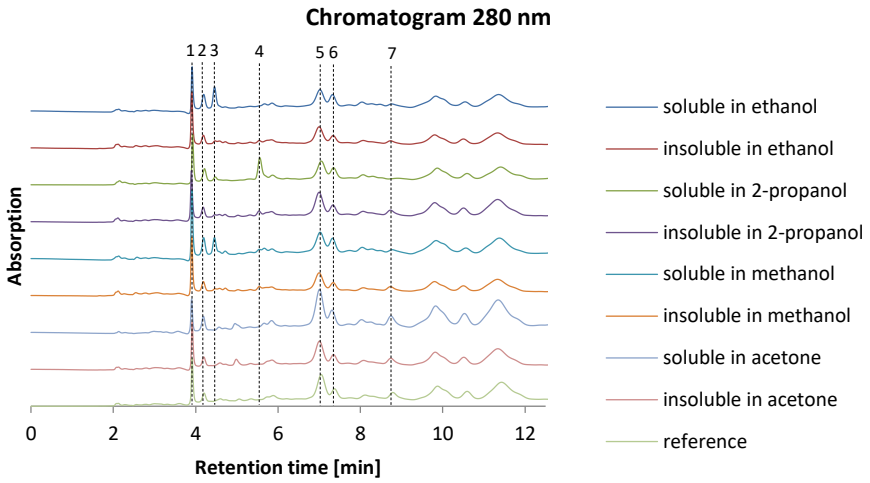

Fig. 2. Chromatogram of selected fractions at 280 nm

An overview of the marked peaks in terms of retention time, UV/vis spectra and identification of the peak composition is listed in Table 2. It was only possible to identify two peaks (2 as gallic acid and 7 as catechin). For a more detailed peak assignment, a mass spectrometer analysis would be necessary. After roughly 4 minutes, the first peak appeared for each fraction. This peak had a maximum in absorption for 276 nm. As the mobile phase of the analysis consisted of a relatively polar solvent it is expected that the substance eluted in the first peak has a highly hydrophilic character.

The second peak marked had a retention time of 4.2 minutes and was identified as gallic acid (3,4,5-trihydroxybenzoic acid), thanks to the UV/vis spectra matching with roughly 96 % for all fractions and a similar retention time of ± 0.05 minutes. The spectra showed a λ_{max} of 270 nm. The highest concentration of gallic acid was found for the fraction soluble in methanol with (0.29 %) followed by soluble in acetone and ethanol with roughly 0.27 %.

Peak 3 was detected after 4.46 minutes with the highest concentration found in the fraction that was soluble in ethanol, followed by the fraction soluble in methanol. Also in the fraction soluble in 2-propanol a small peak was detected. The detector showed no peak at this retention time for all other fractions or for the original tannin extract. It is possible that a chemical adduct takes place between the tannin extract and the simple alcohols. A maximum in the UV/vis spectra was observed at 296 nm.

The fourth peak with a retention time of 5.56 minutes was observed only in the fraction that was soluble in 2-propanol. It is thought that the compound is formed between 2-propanol and a polyphenol present in the tannin. Overall, tannin extract and alcohol present new peaks in the chromatogram, which suggest a particular affinity between them, possibly due to the establishment of several H-bonds. The UV/vis spectrum showed a maximum at 291 nm.

Peak 5 and 6 are a double peak present in all fractions. The highest concentration of peak 5 was recorded for acetone soluble followed by the original extract and 2-propanol insoluble. Peak 5 appeared after 7 minutes with a λ_{max} 277 nm and a shoulder at 230 nm. Peak 6 was detected after 7.35 minutes with a shoulder at 224 nm and a maximum of 278 nm in the UV/vis spectra. As both peaks had a very similar UV/vis spectra and a very close retention time it is estimated that the compounds are very similar to each other and only differ slightly in molecular mass or polarity.

The last marked peak was detected after 8.74 minutes for all fractions The peak was identified as catechin using retention time and comparison of the UV/vis spectra that matched between 98.9 and 99.6 % for each fraction. The highest concentration of catechin was detected for acetone soluble with 1.2 % followed by the original tannin and insoluble in isopropanol with 1.1 % and 1 %, respectively. Almost no catechin was detected for soluble in ethanol (0.6 %) and soluble in isopropanol (0.7 %).

All peaks that were detected afterwards are expected to be aromatic compounds of either higher molecular mass and/or lower polarity compared to catechin. But to get a more detailed idea of the molecular mass and composition of the fractions, analysis by means of GC-MS and GPC would be required.

Table 2. Peak assignment and λ_{max}

Peak	Retention time [min]	identification	UV/vis max/shoulder
1	3.915		276
2	4.198	gallic acid	270
3	4.458		296
4	5.558		281
5	7.006		230^{sh}/277
6	7.347		224^{sh}/278
7	8.739	catechin	229^{sh}/278

4 Conclusions

In the present study, it was shown that it is possible to separate industrial tannin extract using various organic solvents of different polarity. More significant separation was achieved by acetone followed by ethanol, acetonitrile and methanol. The fractions soluble in ethanol and methanol show additional peaks and lower concentration of gallic acid and catechin when analyzed with by HPLC compared with the original tannin extract. The highest concentration of catechin was found in the fraction soluble in acetone.

The study also showed that it is possible to separate higher quantities of tannin with acetone and methanol. Although it required more solvent or additional washing steps with fresh solvent each time. The separation of higher amounts with ethanol is critical as the soluble fraction forms a rigid conglomerate that cannot be pulverized easily but is still soluble in water.

Using the knowledge gained in the present study, higher quantities of industrial tannin extract will be separated in acetone and methanol. These fractions will be used to produce rigid foams.

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