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SUPPLEMENTARY MATERIAL TO

Hydrothermal hydrolysis of sweet chestnut (*Castanea sativa*) tannins

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ADDITIONAL DATA FOR EXPERIMENTAL

Total tannins

The total tannins content was determined by the Folin–Dennis method.[1] A water-soluble samples of tannin (1 mL) was mixed with 2.5 mL of Folin–Dennis reagent (diluted with water 1:10) and 2 mL of Na_2CO_3 solution (75 g L^{-1}). The mixture was left to stand at room temperature for 30 min. The absorbance of the mixture was measured at 760 nm using a UV–Vis spectrophotometer. The control sample was prepared in the same way, but instead of tannin extract solution, deionised water was used. The quantification was realised based on a calibration curve obtained with tannic acid.

Total phenols

The total phenols content was determined by the Folin–Ciocalteu method described by Ravber *et al.*[2]

Total carbohydrates

The total carbohydrates content was determined by a simple colorimetric phenol––sulphuric method as described in a previous work.[3]

Antioxidant activity

The antioxidant activity of water-soluble products obtained by hydrothermal treatment of a tannin extract was determined by the DPPH method described in a previous work.[4]

HPLC method

The analysis of water-soluble samples was performed on an Agilent 1100 Series HPLC system equipped with a binary solvent-delivery pump system, an autosampler, a column heater and a variable-wavelength detector. The separation of compounds was realised on a column C18 (4.0 mm×250 mm, 5 μm particle size) at room temperature. The method was adapted from the literature[5] with small corrections. The mobile phase consisted of two solvents: water–formic acid (99.5:0.5, solvent A) and acetonitrile (solvent B). The following gradient was set: 0 to 2 min 5 % B, from 2 to 10 min 5–20 % B, from 10 to 15 min 20–30 %

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B, from 15 to 20 min 30–35 % B, from 20 to 60 min 35–80 % B, from 60 to 65 min 80–85 %, from 65 to 70 min 85–5 % B. The flow rate through the system was 0.89 mL min^{-1}. The injector volume of the samples was 20 μL. The analysis was performed at wavelengths of 254 and 280 nm. The quantification of each compound was performed using calibration curves for gallic acid measured at 280 nm (r^2 = 0.9991) and ellagic acid measured at 254 nm (r^2 = = 0.9992). The tannins were identified by comparing the retention times and UV spectrum to literature data.[5] The quantification of tannins (vescalin, castalin, vescalagin, castalagin and 1-*O*-galloyl castalagin) was realised using an ellagic acid calibration curve obtained at 254 nm. The correlation factors were applied for the quantification: for vescalin and castalin (6322/302), for vescalagin and castalagin (934/302), for 1-*O*-galloyl castalagin (1086/302).[5]

ADDITIONAL DATA FOR RESULTS AND DISCUSSION

Typical chromatograms of A – initial material, B – water-soluble product obtained at a temperature of 150 °C and a reaction time of 30 min, C – water-soluble product obtained at a temperature of 250 °C and a reaction time of 30 min and D – sample obtained by acid hydrolysis are depicted in Fig. S-1.

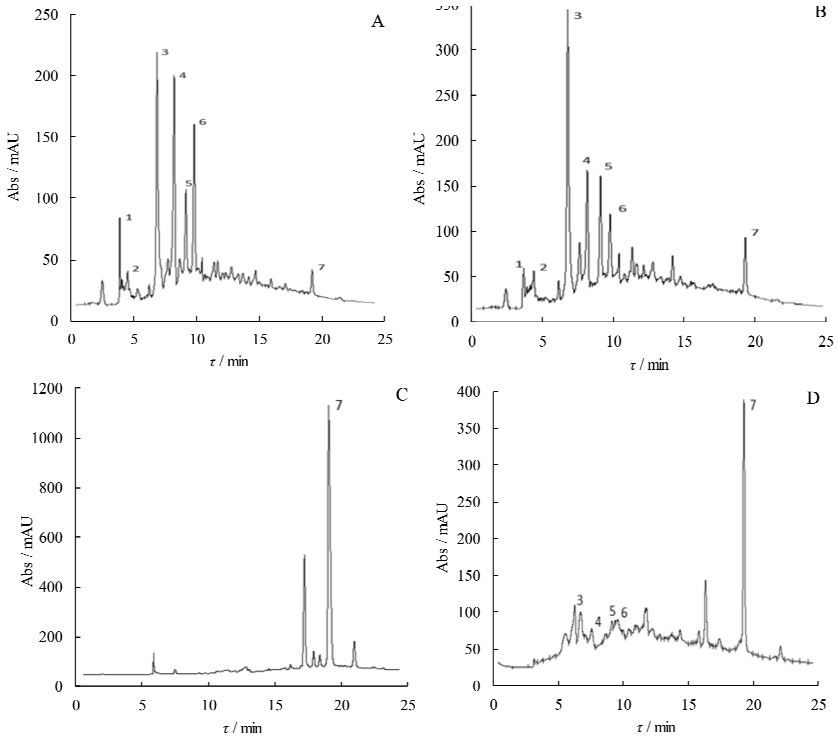

Fig. S-1. Typical chromatogram of A – initial tannin extract, B – chestnut tannin extract treated with water at a temperature of 150 °C and a reaction time of 30 min; C – chestnut tannin extract treated with water at a temperature of 250 °C and a reaction time of 30 min; D – acid hydrolysed chestnut tannin extract. Detected compounds: 1 - vescalin, 2 - castalin, 3 - gallic acid, 4 - vescalagin, 5 - 1-*O*-galloyl castalagin, 6 - castalagin and 7 - ellagic acid.

As can be observed from chromatograms, the detected peaks represent gallic and ellagic acids and ellagitannins, such as vescalin, castalin, vescalagin, castalagin and 1-*O*-galloyl-castalagin. The tannins were determined based on the data published by Comandini *et al.*[5] and the obtained UV spectra. Comparing chromatograms A and B from Fig. S-1, it could be noticed that using water under mild conditions (150 °C and 30 min) the content of ellagitannins was already decreased and the concentration of ellagic acid started to increase slightly, while gallic acid was still stable. Observing Fig. S-1C, it is obvious that there was no gallic acid or elagitannins in the product, but the concentration of ellagic acid was drastically increased. Figure S-1D shows that gallic acid and ellagitannins were not stable under the conditions of acid hydrolysis and hence, they were present in very small amount in the product. It could also be noticed that ellagic acid was the predominant compound in the product obtained by acid hydrolysis.

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