Přílohy

Příloha 1: Fytokomory na Fakultě lesnické a dřevařské v Praze. Foto: David Musiolek

Příloha 2: Sazenice ve fytokomoře. Foto: David Musiolek

Příloha 3 – Metodika chemické analýzy asimilačních orgánů sazenic

LC-qTOF-MS/MS analysis of polyphenolic compounds

LC-MS-qTOF metabolomic analysis was carried out using on Agilent 1290 Infinity II coupled with Agilent 6546 LC/MS QTOF system (Agilent, USA) and Zorbax Eclipse Plus C18 column (2.1x50 mm, 1.8 µm), (Agilent, USA). Mobile phase A contained 0.05 % formic acid and mobile phase B was consisted of acetonitrile. The gradient elution was: 0–1 min, 100% A; 1–7 min, 35% A; 7–8 min, 100% A; 8–8.01, 100% A, 8.01–10 min, 100% A. The flow rate of mobile phase was set at 1.1 mL min-1 (Huang, 2020). The column temperature was set at 35 °C. The injection volume was 1 µL. The system was operated at negative ionization mode. The optimization of qTOF parameters were previously optimized using the standards. The qTOF parameters was as follows: scan range 100–1000 m/z; drying gas temperature, 350 °C; sheath gas flow rate, 12.0 L/min; sheath gas temperature, 400 °C; capillary voltage, 5.0 kV; nozzle voltage 0.9 kV; fragmentor, 140 V; collision energy at 10, 20 and 40 eV. MS/MS data were acquired at scan range was 50–800 m/z, 0.5 min retention time window, isolation window 1.3 amu and an aquisition rate of 2 spectra s-1 . The list of target compounds is presented in Table 16. During the analysis two reference masses: 112.9855 m/z and 966.0007 m/z were continuously measured to mass correction. The data collection was carried out using Agilent Mass Hunter Acquisition software. The data analysis was performed using Qualitative Analysis 10.0 and Q-TOF Quantitative analysis.

Table 2 List of compounds monitoring by LC-MS-qTOF in negative ionization mode.

Compound	**Formula**	**Mass**	**Retention time, min**	**Fragments**
Rutin	$C_{27}H_{30}O_{16}$	610.1607	2.93	609.1459; 300.0273
Taxifolin	$C_{15}H_{12}O_7$	304.0596	2.90	303.0499 285.0404; 177.0195; 125.0245
Catechin	$C_{15}H_{14}O_6$	290.0783	2.21	289.0715; 245.0819; 203.0716; 151.0402;

				125.0244; 109.0296
Quercetin	$C_{15}H_{10}O_7$	302.0420	3.75	301.0336; 273.0389; 178.9981; 151.0037; 121.0295
Gallic acid	$C_7H_6O_5$	170.0216	0.56	169.0141; 125.0245; 79.0189;
Procyanidin B1	$C_{30}H_{26}O_{12}$	578.1411	2.15	577.1343; 451.1019; 425.0876; 289.0713; 161.0243; 125.0242
Myricetin	$C_{15}H_{10}O_8$	318.0367	3.29	317.0305; 178.9982; 151.0039. 137.0241; 109.0298
Kaempferol	$C_{15}H_{10}O_6$	286.0476	4.13	285.0404; 171.0451; 107.0138
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0941	2.23	353.0873; 191.0560; 85.0392

Extraction procedure for determination of polyphenolic compounds

20 mg of freeze-dried and homogenized samples were accurately weighted and 500 µL of methanol:water (70:30 v/v) was added. Then, the sample were well-mixed using vortex. After, the sample were placed into ultrasonic bath with ice for 10 minutes. Then, the tubes were centrifuged at 13000 rpm for 10 min at 4 °C. The supernatant was collected and filtered through PTFE syringe filter (0.22 µm) prior to LC-qTOF-MS/MS analysis.

Determination of total phenolic content (TPC)

TCT was performed according to Makkar at al. 1993. Briefly, 20 µL of extract was placed to the test tube and make up the volume to 0.5 mL of water. Then, 250 µL of the Folin0Ciocalteu regent was added. After 3 min, 1 mL of 20% sodium carbonate was added. The test tube was well mixed and kept in the dark place for 40 min. The absorbance was recorded at 725 nm using spectrophotometer. The concentration of TPC was expressed in tannin acid equivalent.

Determination of non-tannins phenols content (TnTPC)

The 50 mg of polyvinylpyrrolidone was weighted into 2 mL test tube. Then, 0.5 mL of water and 0.5 mL of extract was added. The solution was vortexing and kept at 4 °C for 15 min. After, the centrifugation for 10 min at 13000 rpm, the supernatant was transferred to the new test tube. The procedure of determination of non-tannins phenols content was similar to TCP, but the taken aliquot was in two times higher

Determination of total flavonoid content (TFC)

The determination TFC was carried out slightly modified according to Baba 2015. Briefly, 75 µL of sample extract was placed into the 2 mL test tube and 465 µL of water was added . Then, 5 % solution of 5 % $NaNO_2$ was added. After 5 minutes, 1 mL of 10 % aluminium trichloride was added. After, 15 minutes the absorbance was measures at 510 nm, against blank. The concentration of flavonoid content was expressed as mg quercetin equivalent per g dry weight.

Determination of total condensed tannin content (TCT)

The total flavonoid content was determined following the method described by Porter, 1986. 75 µL of sample and 225 µL of 70% acetone was placed to 2-mL test tube. Then, 1.5 mL of butal-HCL reagent was added (butanol-HCL reagent was prepared by mixing of 95 ml of butanol and 5 mL of concentrated HCL (37%)). After, 50 µLof ferric reagent was added (ferric

feagent was prepared as 2 % ferric ammonium sulfate in 2N HCl). The solution was well mixed and placed to the thermoshaker () for 1h at 95 °C and 1000 rpm. When the solution was cooled, the absorbance was recordered at 550 nm. The concentration of TCT was expressed in cyanidin equivalent (mg g-1).