Tracing the origin of Chinese lotus root varieties using high-resolution mass spectrometry

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ABSTRACT

Chinese lotus root is primarily grown in the Chinese provinces of Hubei, Hunan, Zhejiang and Guangdong. Lotus root is a thick rhizome of the genus *Nymphalidae* and is rich in carbohydrates, proteins, vitamins and other compounds. In addition to its edible value, it has been shown to stop bleeding, regulate endocrine function and calm the nerves. Lotus root samples from different origins have varying nutritional content due to differences in climate, soil mineral content and water source. In this study, we used a ZenoTOF 7600 system to analyze the nutritional components of lotus root from 3 different lotus root-producing areas in Hubei Province, including Chenhu Town of Hanchuan, Jiangxia District of Wuhan and Honghu City.

INTRODUCTION

Chinese lotus root is slightly sweet and crispy, can be eaten raw or cooked and has a high medicinal value. Lotus roots from different regions have different nutritional content due to varying regions, climates and water sources. Studying the differences in nutritional components of lotus roots in different regions is not only helps guide consumption and medicinal purposes, but also provides a reference for studying the quality control of lotus roots in different seasons and varieties. This experiment followed a non-targeted method that used data-dependent acquisition (DDA) and dynamic background subtraction on the ZenoTOF 7600 system with the Zeno trap activated. SCIEX OS software was used for both data acquisition and processing of the Chinese lotus root samples.

MATERIALS AND METHODS

Sample preparation:
Dried lotus roots were ground into a powder and 0.5 g was added to a 15 mL centrifuge tube. Then, 2 mL of 50:50 (v/v) methanol/water was added and sample mixtures were extracted by immersing tubes in an ultrasonic wave bath for 10 min. Extracts were centrifuged at 4 °C at 20,664 g, and the resulting supernatant was filtered through a 0.22 µm nylon membrane and transferred to a vial for UHPLC-MS/MS analysis.

HPLC conditions:
ExionLC AD system was used with a Waters T3 (100 x 2.1 mm, 1.8 µm) column and a Waters HILIC (100 x 2.1 mm, 1.7 µm) column at 40 °C. The T3 column-with a gradient of eluent A water + 0.05% formic acid and eluent B acetonitrile-was used at a flow rate of 400 µL/min. The HILIC column-with a gradient of eluent A water + 25 mM ammonium formate +25 mM ammonium hydroxide and eluent B acetonitrile-was used at a flow rate of 300 µL/min.

MS/MS conditions :
Samples were run on the ZenoTOF 7600 systems used data dependent acquisition (DDA). The TOF MS precursor ion scan was performed from m/z 60 to 1200, 12 candidates ions were selected for fragment ion scans ranging from m/z 40 to 1200. Dynamic background subtraction on and Zeno trap on. Each sample was injected in both positive and negative polarity. Data-dependent acquisition experiments were performed using collision-induced dissociation (CID) or electron-activated dissociation (EAD) fragmentation modes. The information-rich fragmentation spectra derived from CID- and EAD-based fragmentation provided robust data to support accurate identification.

Data processing:
Data of 21 samples were identified by SCIEX OS software with Natural Product Compound Library and Accurate Mass Metabolite Spectral Library and ChemSpider, and MetaboAnalyst software for statistical analysis.

RESULTS

Among the different samples, 191 nutrients were identified including flavonoids, polyphenols, organic acids, amino acids and derivatives (Figure 1). The quality of the MS/MS data was significantly improved by activating the Zeno trap during acquisition, which increased the instrument response between 5- and 20-fold compared to runs in which the Zeno trap was not activated.

Low concentration compounds can also obtain high-quality MS/MS data and high library matching scores. This function can more accurately identify low concentration compounds (Figures 2 and 3).

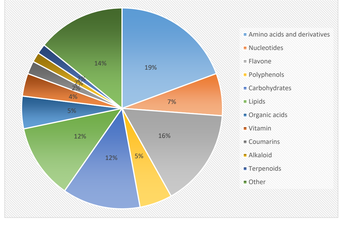

Figure 1. Nutritional composition of lotus root.

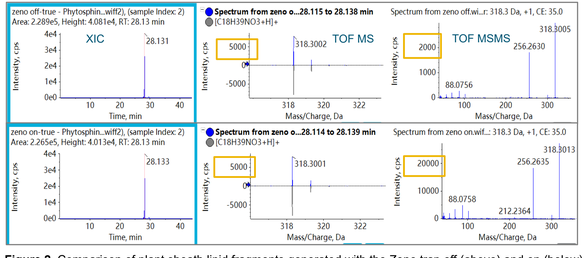

Figure 2. Comparison of plant sheath lipid fragments generated with the Zeno trap off (above) and on (below).

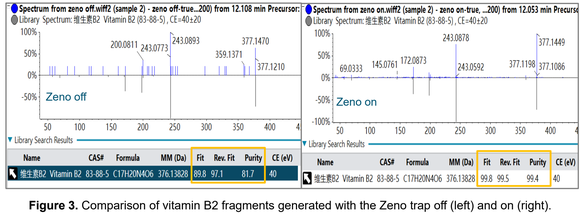

Figure 3. Comparison of vitamin B2 fragments generated with the Zeno trap off (left) and on (right).

For some compounds, CID produced too few or no diagnostic MS/MS fragments, which prevented correct compound identification via MS/MS analysis. To address this deficiency, samples were analyzed using EAD-based fragmentation, which generated additional fragments that were more structurally diagnostic as compared to those generated by CID (Figure 4).

For example, sphingosine only produced 4 primary fragment ions in CID mode while EAD fragmentation produced more than 15 fragments. The fragment-rich spectra obtained via EAD fragmentation provided the means to identify unknown compounds accurately.

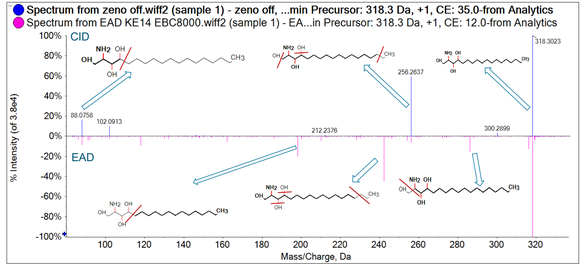

Figure 4. Comparison of plant sheath lipid fragments generated by CID (above) and EAD (below).

The results of the principal component analysis (PCA), which compared data from different lotus root samples, showed that compounds in the same variety of lotus root gathered from 3 places of origin could be effectively distinguished (Figures 5 and 6). According to the statistical analysis, 61 different compounds with p<0.05, FC>2 or <0.5 and VIP>1 were identified according to the t-test and OPLS-DA results. The relative portion of most identified compounds in lotus root from Honghu City was higher than that found in the samples from Chenhu Town, Hanchuan City and Jiangxia District, Wuhan City. For example, the levels of leucine, isoleucine, phenylalanine, proline and an additional 10 amino acids in samples from Honghu City were significantly higher than those found in the other regions.

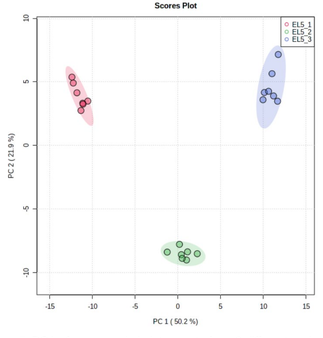

Figure 5. PCA of compounds in lotus roots from 3 different regions.

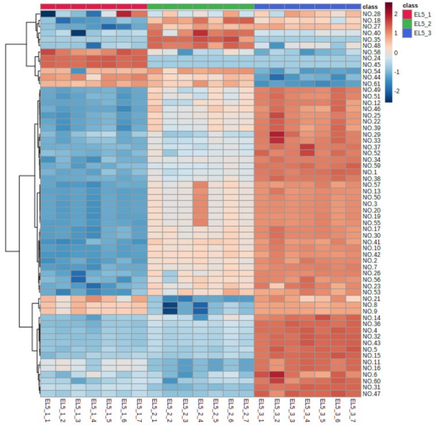

Figure 6. Heat maps of differential compounds in lotus roots from 3 different regions.

CONCLUSIONS

This experiment used the ZenoTOF 7600 system to study the metabolomics of lotus root. Activating the Zeno trap significantly enhanced the quality of the MS/MS data and improved the accuracy of qualitative results. EAD fragmentation generated MS/MS data that differs from CID technology, helping to confirm the compound structure. The data collected by the ZenoTOF 7600 system ensures comprehensive and rich compound identification and helps to identify the origin and variety of lotus roots.

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