


Tracing the origins of red cabbage moss using a QTOF instrument for rapid nutrient identification

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ABSTRACT

Red cabbage moss is one of the main vegetables grown in winter and spring in Hubei Province in China. It has high nutritional value as a source of iron, phosphorus, calcium, carotene and vitamins. In this experiment，a QTOF instrument was used to analyze the nutrient components in the stems, flowers, skins and leaves of red cabbage moss from 4 different regions in Hubei Province. This approach takes advantage of the scanning speed of the QTOF instrument in data-dependent acquisition (DDA) mode. In addition, improved MS/MS library matching and compound identification were shown through enhanced fragment ion detection with the Zeno trap and generation of unique fragments with electron activated dissociation (EAD).

INTRODUCTION

Red cabbage moss is one of the main vegetables grown in winter and spring in Hubei Province in China. It has high nutritional value as a source of iron, phosphorus, calcium, carotene and vitamins. In this experiment, a QTOF instument was used to analyze the nutrient components in the stems, flowers, skins and leaves of red cabbage moss from 4 different regions in Hubei Province. This approach takes advantage of the scanning speed of the QTOF instrument in DDA mode. In addition, the fragment ion sensitivity of the Zeno trap along with EAD fragmentation can improve MS/MS library matches and compound identification.

MATERIALS AND METHODS

Sample preparation:
The sample was ground into a fine powder and a 0.5 g aliquot was added to a 15 mL centrifuge tube, followed by 2 mL of 50:50 (v/v) methanol/water. The samples were extracted with ultrasonication for 10 min and then centrifuged at 4℃ at 15,000 rpm. The supernatant was filtered using a 0.22 µM nylon membrane filter and transferred to an autosampler vial for analysis.

HPLC:
A Shimadzu Prominence LC system was used for chromatographic separation with Waters ACQUITY HSS T3 (2.1 x 100 mm, 1.8 µm) column at 40 ℃. Mobile phase A was water (2 mM ammonium acetate and 0.05% formic acid) and mobile phase B 7:3 (v/v) methanol/acetonitrile. The flow rate was 300 µL/min and the injection volume was set to 1 µL (Figure 1,2).
MS/MS conditions:
The mass spectrometer was the ZenoTOF 7600 system from SCIEX with an electrospray ionization (ESI) probe. Data were acquired using DDA. The collection method was TOF MS and MS/MS using DDA.. Precursor ion scans ranged had an m/z range of 50–1,200 Da. Fragment ion scans had an m/z range of 30–1,200 Da and used a declustering voltage of 60 V for TOF MS and MS/MS and a collision energy of 40±20 V.

When using EAD with TOF MS and MS/MS, precursor ion scans had an m/z range of 50–1,000 Da. Fragment ions were acquired with an m/z range of 50–1,000 Da. With the EAD method, the electronic beam current was 5,000 Na, the electron KE was 12 eV, reaction time was 30 ms, the Zeno threshold was 20,000 cps, the declustering voltage was 60 V and the collision energy was 10 V.

The source and gas conditions were 35 psi for curtain air (CUR), 8 psi for collision gas (CAD), 5,500/-4,500 V for spray voltage (IS), 500° C for temperature (TEM), 50 psi for GS1 and 55 psi for GS2.

Using SCIEX OS software in combination with a high-resolution natural product secondary standard spectrum library enabled fast and accurate identification results. EAD fragmentation can obtain more secondary fragments and was used to improve the accuracy of the library matching and the qualitative analysis results and to increase the number of unknown objects identified. A total of 183 nutritional components were discovered (Figure 3,6,7,8).

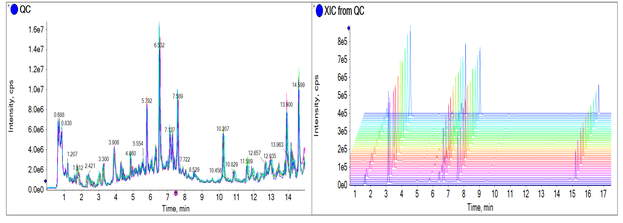

Figure 1. QC total ion chromatogram (TIC) chart reproducibility.

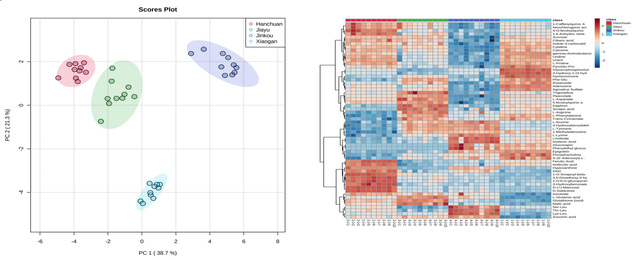

Figure 2. Reproducibility of compounds at different time points

Figure 4. Principal component analysis (PCA) of compounds in red cabbage moss stems from 4 producing areas.

Figure 5. Heat maps of compounds in the stems of red cabbage moss from 4 production areas (60 before t-test).

RESULTS

In this study, the stems, flowers, skins and leaves of red cabbage moss from 4 different regions in Hubei Province in China—Xiaogan, Hanchuan, Jiayu and Jinkou— were analyzed. Using a QTOF instrument in combination with a natural products and metabolite database, 183 nutrient components were quickly detected. The contents of the nutrients were significantly different depending on the region in which the red cabbage moss was grown.

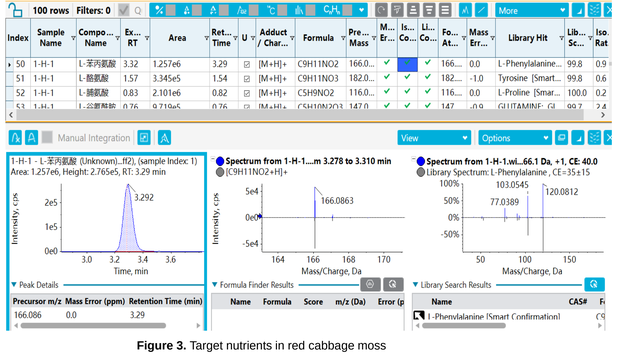

Figure 3. Target nutrients in red cabbage moss

The analysis also showed that the number of nutrients differed depending on the part being analyzed. While about 140 types of nutrients were found in the stems of red cabbage moss from Xiaogan,this was less than the number of nutrients identified in its flowers and leaves. Some flavonoid glycosides were barely detected in stems. The nutrient levels were shown to be higher in flowers and leaves (Figure 4,5).

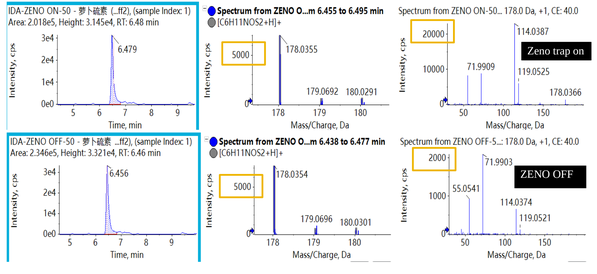

Figure 6. Comparison of collection methods for radish sulfur when the Zeno trap is on (top) and when the Zeno trap is off (bottom).

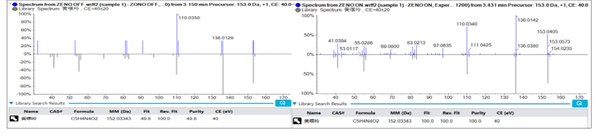

Figure 7. Comparison of the matching results of xanthine secondary spectra when the Zeno trap is off and when the Zeno trap is on (right).

In addition, the unique EAD fragmentation technology provides more secondary fragments that are different from collision-induced dissociation (CID), which is more conducive to structural analysis and confirmation **(Figure 6,7,8)**.

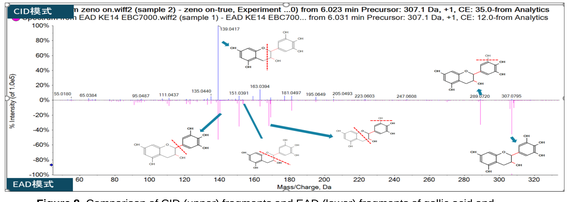

Figure 8. Comparison of CID (upper) fragments and EAD (lower) fragments of gallic acid and catechins in red cabbage moss.

CONCLUSIONS

In this study, the stems, flowers, skins and leaves of red cabbage moss from 4 different regions in Hubei Province in China were analyzed. A total of 183 nutrient components were quickly detected. The contents of the nutrients were significantly different depending on the region in which the red cabbage moss was grown.

The stems of red cabbage moss from Xiaogan and Jinkou were found to have a much higher content of 2 kinds of glucosinolates than the stems of red cabbage moss from Hanchuan and Jiayu. The stems of red cabbage moss in Hanchuan were found to have a higher content of phenolic acid and sugar. The analysis also showed that the number of nutrients differed depending on the part being analyzed.

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