

# 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°C 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 °C. 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  $1 \mu L$  (Figure 1,2).

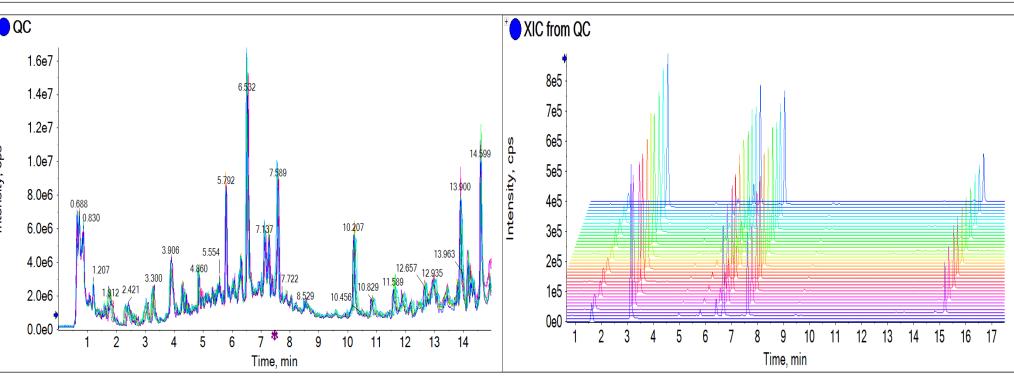
#### **MS/MS** conditions:

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  $40 \pm 20$  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.

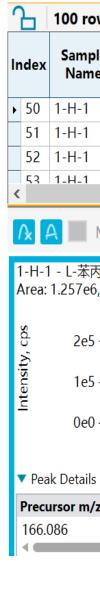
The source and gas conditions were 35 psi for curtain air (CUR), 8 psi for collision gas (CAD), 5,500/-4,500 V for spray voltage (IS), 500° C for temperature (TEM), 50 psi for GS1 and 55 psi for GS2.

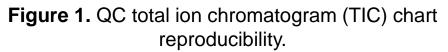
Using SCIEX OS software in combination with a high-resolution natural product secondary standard spectrum library enabled fast and accurate identification results. EAD fragmentation can obtain more secondary fragments and was used to improve the accuracy of the library matching and the qualitative analysis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

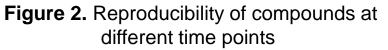


# RESULTS

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.



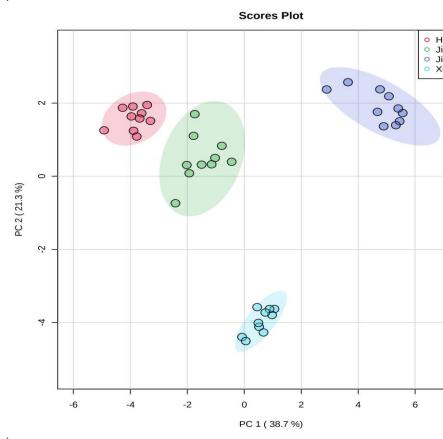




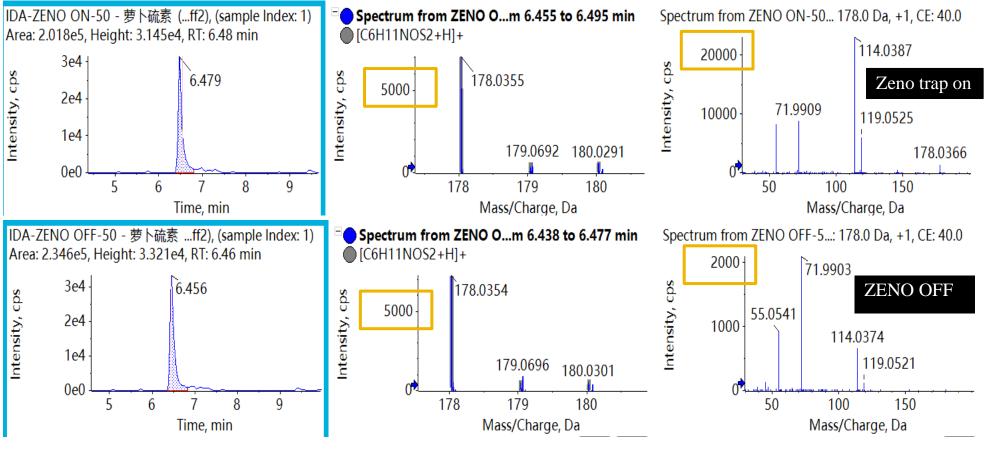
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	L-苯丙氨酸	3.32	1.257e6	3.29		[M+H]+	C9H11NO2	166.0	~	<b>V</b>	~	166	0.0	L-Phenylalan	nine 99.8	0.9
	L-酪氨酸	1.57	3.345e5	1.54	$\checkmark$	[M+H]+	C9H11NO3	182.0	~	~	~	182	-1.0	Tyrosine [Sm	nart 99.8	0.6
	L- <mark>脯氨酸</mark>	0.83	2.101e6	0.82		[M+H]+	C5H9NO2	116.0	~	~	~	116	0.0	L-Proline [Sm	nar 100.0	0.2
	Ⅰ-公氢酰胺	076	0 710 <b>⊳</b> ۲	0.76		[M+H]+	C5H10NI2O3	147 0	~	~	~	147	-0.0	GUITAMINE	GI 997	24
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大天天 26, He	ight: 2.765e5, 	RT: 3.29	min 2	Intensity, cps	H11N 5e4 0e( -5e4	NO2+H]+ 4 1 4	166.08 166 1 Mass/Charge	63			Ŭ Lib	rary Spe 100% 50% 0%	ctrum: L 7 	-Phenylalanine 103.0545 77.0389	e, CE=35±1 120.0812 150	
丙氨酮 5- 5- 0	ight: 2.765e5, 3.0 3.2 Ti	RT: 3.29 3.29 3.4 me, min	min 2	• Lots Intensity, cps Lots Form	H11N 5e4 0e( -5e4	NO2+H]+ 4 4 164	166.08 166 1 Mass/Charge	63	170		Librar	rary Spe 100% 50% 0% -50%	ctrum: L 7 	-Phenylalanine 103.0545 77.0389	e, CE=35±1 120.0812 150	

**Figure 3.** Target nutrients in red cabbage moss

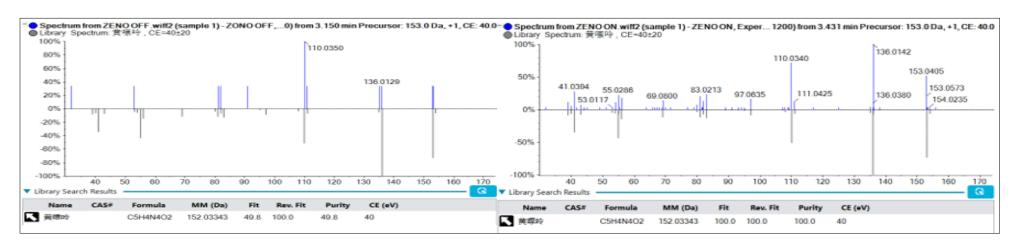
The analysis also showed that the number of nutrients differed depending on the part being analyzed. While about 140 types of nutrients were found in the stems of red cabbage moss from Xiaogan, this was less than the number of nutrients identified in its flowers and leaves. Some flavonoid glycosides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

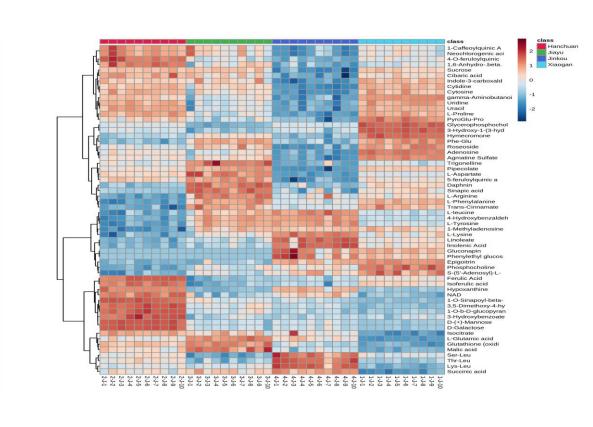


**Figure 4.** Principal component analysis (PCA) of compounds in red cabbage moss stems from 4 producing areas.







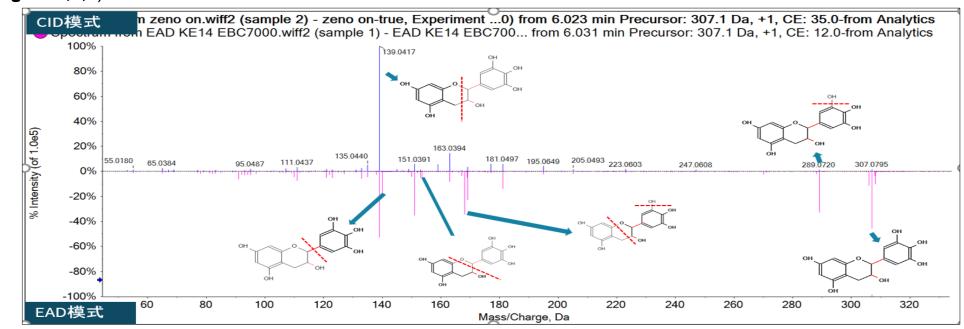


**Figure 5.** Heat maps of compounds in the stems of red cabbage moss from 4 production areas (60 before t-test).

**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).

(Figure 6,7,8).



## 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.

## REFERENCES

1. Optimization of Extraction Process of Polyphenols from Brassica campestris L. var. purpurea Bailey by Response Surface Analysis (RSA). Food Research and Development, April 2013, Issue 8, 5-8. 2. Comparative Study on the Antioxidant Activity of Ethanol Extracts from Red Cabbage Moss and Chinese Moss. Food Science and Technology, 2014, 39 (12).3 Syoku-An No. 0124001, 2005. 3. Genetic Diversity Analysis of Red Vegetable Moss Using SSR. Journal of Plant Genetic Resources, 2013, 13 (6), 1088-1092.

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

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