

The application of a QTOF instrument to enable traceability of the origin of ginger

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ABSTRACT

Ginger root is widely used across the globe, often as an ingredient in a variety of food and drinks. In China and India, ginger has been used to treat headaches, nausea, rheumatism and colds for more than 2,500 years. However, the quality and flavor of ginger cultivated in China can vary depending on where it is grown as producing areas are affected by regional differences, climate environment and production conditions. In this study, we used a QTOF instrument to analyze the nutritional components of ginger from three different areas in China: Huaihua in Hunan Province, Xiangxi in Hunan Province and Shandong Province. The result showed that there were some differences in the nutritional components of ginger from different producing areas, which also indicated that the quality of ginger from different producing areas was different.

INTRODUCTION

Ginger is widely used in the world as a food additive and spice for its aroma and pungency. It is widely cultivated in tropical and subtropical countries, especially in China and India. Ginger has been reported to possess numerous significant pharmacological properties such as antioxidant, anti-inflammatory, anticarcinogenic and so on. The quality and flavor of ginger vary from region to region. In this study, we used a QTOF instrument to analyze the nutritional components of ginger from three different areas in China: Huaihua in Hunan Province, Xiangxi in Hunan Province and Shandong Province.

MATERIALS AND METHODS

Sample Preparation:

The ginger samples were ground into a fine powder, and 1.0 g of powder was weighed into a 15 mL centrifuge tube. A total of 5 mL of 50% methanol water was added, then sonicated for 10 min extracted and centrifuged at 4°C at 15,000 rpm. The supernatant was filtered through a 0.22 µm nylon membrane and transferred to a vial for UPLC-MS/MS analysis

HPLC Conditions

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. The gradient of eluent A water + 2mM ammonium acetate + 0.02% formic acid and eluent B water/methanol (10/90) + 5mM ammonium acetate+ 0.05% formic acid in Positive ion mode, a gradient of eluent A water + 2mM ammonium acetate and eluent B acetonitrile /methanol (50/50) in Negative ion mode. The flow rate were 300 μ L/min and the injection volume were set to 5 μ L.

MS/MS Conditions:

The X500R QTOF mass spectrometry system with TOF MS scanning mode, the m/z range is 100-1000 and TOF MS was followed by 10 product ion scan, the m/z range is 50-1000; Dynamic background subtraction was used. Every sample was injected twice in positive and negative polarity. Data-dependent acquisition experiments were performed using collision-induced dissociation (CID) fragmentation modes. The informationrich fragmentation spectra derived from CID based fragmentation provided robust data to support accurate identification.

RESULTS

In this study, sample preparation and LC-QTOF parameters were optimized to develop a final screening method. After acquisition, sample data and a compound screening list were imported into SCIEX OS software, which was used with a SCIEX-based MS/MS natural products and metabolites library to rapidly and accurately identify features in the ginger samples. The precursor ion mass error and isotopic abundance ratio of the suspected compounds were automatically matched by the software(Figure 1,2). A total of 147 compounds were identified, which were mainly amino acids and derivatives, flavonoids, phenolic acid and gingerol(Figure 3).



Figure 1. Target nutrients in ginger







Figure 3. Nutritional composition of ginger root

Using the MetaboAnalyst platform, PCA analysis was performed on ginger from Huaihua in Hunan Province, Xiangxi in Hunan Province and Shandong Province in China. The results showed that compounds of ginger such as gingerol, narcissin, 10-dehydroparadol and so on were cultivated in the three different producing areas and was of the same variety could be effectively distinguished. Statistical analysis was performed on the identification of ginger samples from the three producing areas, with p <0.01, FC >2 or <0.5 selected, according to the t-test results (Figure 4,5,6).





Figure 4. PCA and PLS-DA of compounds which were identified by positive and negative patterns in ginger from different regions

	✓ n1 = 9, n2 = 9														
	m/z	Ret. Time	Group	Use	t-value	p-value /	Mean 1	Mean 2	Median 1	Median 2	Sigma 1	Sigma 2	Delta	Fold Change	Log (Fold Change)
d (N/A	N/A		\checkmark	64.06	1.0191e-20	6.198e6	2.630e6	6.170e6	2.620e6	1.503e5	7.297e4	3.568e6	2.357e0	3.723e-1
 (N/A	N/A		\checkmark	53.93	1.5809e-19	1.240e5	4.838e4	1.250e5	4.850e4	3.708e3	1.987e3	7.562e4	2.563e0	4.088e-1
-n	N/A	N/A		\checkmark	53.73	1.6774e-19	7.723e3	4.258e2	7.670e3	4.470e2	3.906e2	1.161e2	7.298e3	1.814e1	1.259e0
.1/	N/A	N/A		\checkmark	51.98	2.8391e-19	3.676e5	4.064e4	3.680e5	4.120e4	1.868e4	2.635e3	3.269e5	9.043e0	9.563e-1
/1	N/A	N/A		\checkmark	51.53	3.2657e-19	2.528e4	1.829e3	2.530e4	1.730e3	1.352e3	1.924e2	2.345e4	1.382e1	1.141e0
(1	N/A	N/A		\checkmark	51.26	3.5440e-19	8.012e4	3.000e4	8.010e4	3.000e4	2.490e3	1.550e3	5.012e4	2.671e0	4.266e-1
n	N/A	N/A		\checkmark	50.12	5.0648e-19	2.677e5	9.132e4	2.730e5	9.270e4	1.000e4	3.376e3	1.763e5	2.931e0	4.670e-1
(1	N/A	N/A		\checkmark	50.12	5.0648e-19	2.677e5	9.132e4	2.730e5	9.270e4	1.000e4	3.376e3	1.763e5	2.931e0	4.670e-1
^	••••	••••	1	_											>
	1 44 A2 A5 A6 A3 A HN-HH	A9 A3 7 B1 B2 B3	HN-> B5 B B4 B6	C1	89 C2 C5 C7 C3 C4 C6 C			trans-Ferulic ac 1.2e5 1.1e5 1.0e5 9.0e4 8.0e4 7.0e4 6.0e4 4.0e4 3.0e4 2.0e4 1.0e5	id (193.05/5.2	4 4	B2 B1 B4 B1	as Be Th	ac ac ac ac ac ac	QC QC cing areas	C3 C7 C4 C5 C2
	A5	B1	B6 San	nple (by	C2 C7 /index)	QC	QC		HN-HH		HN-XX	Grou	QC P		SD

Figure 5 T-test data of ginger from different sources



CONCLUSIONS

The analysis found that the content of 31 compounds of ginger from Huaihua in Hunan Province was higher than the content of ginger from Shandong Province, and the content of 11 compounds was lower. Compared with the content of ginger from Shandong Province, the content of 13 compounds of ginger from Xiangxi in Hunan Province was higher, and the content of 3 compounds was lower. Compared with the content of ginger from Xiangxi in Hunan Province, the content of 17 compounds of ginger from Huaihua in Hunan Province was higher, and the content of 11 compounds was lower.

TRADEMARKS/LICENSING

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Figure 6. Heat maps of differential compounds in ginger from different regions

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