Identification of peach species using the X500R QTOF system

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INTRODUCTION

Peaches are an important stone fruit crop grown all over the world, and they are both nutritionally and economically important. Peaches are rich in fructose, dietary fiber, polyphenols, trace elements and other nutrients. Understanding the differences in metabolites between cultivars is critical for nutritional studies of peaches. Accurate mass spectrometry systems have proven valuable for qualitative analysis. In this study, we collected four kinds of peaches: honey peach, flat peach, nectarine and yellow peach, to evaluate their metabolite differences by liquid chromatography-high resolution mass spectrometry.

MATERIALS AND METHODS

Sample preparation: Homogenized peaches were placed in a centrifuge tube, acetonitrile was added for extraction, then anhydrous sodium sulfate was added. After mixing, the acetonitrile layer was removed and the sample was dried, then resuspended in 50% methanol in water. The sample was injected onto the SCIEX X500R QTOF system coupled with an ExionLC system.

HPLC conditions: A 20-minute gradient on Phenomenex Kinetex C18 column (2.6 µm, 2.1 × 100 mm) was employed to ensure high sensitivity and good separation. Composition of mobile phases was 2 mM ammonium acetate in water/methanol. The flow rate of 300 µL/min. The injection volume was set to 5 µL.

MS/MS conditions: TOF MS data and MS/MS data were acquired in one injection using the information-dependent acquisition (IDA) scan mode with dynamic background subtraction (DBS) enabled. The MS source conditions were optimized as follows: curtain gas (CUR), 30 psi; collision gas (CAD), medium; nebulizing gas (GS1), 55 psi; heater gas (GS2), 55 psi; ion spray voltage (IS), 5500V/-4500V in positive mode /negative mode; and source temperature, 550°C. Other mass spectrometry parameters are in Table 1. Data processing was performed using SCIEX OS software 2.1, and principal component analysis (PCA) in MarkerView software.

Table 1. Mass spectrometry parameters.

Mass spectrometry parameters	Settings
TOF MS (Da)	*100 - 1000*
TOF MS/MS (Da)	*50 - 1000*
Dynamic Background Substraction	*On*
Product of Candidate MS/MS per cycle	*12*

RESULTS

The high-speed and high-sensitivity IDA-DBS data acquisition approach, in both positive and negative mode, effectively triggered MS/MS of compounds of interest in peaches. TOF MS data and MS/MS data were acquired in one injection (for each polarity).

Using the SCIEX OS software and the high-resolution natural products and metabolite libraries, the main components of peaches were quickly identified, and the target could be confirmed automatically according to mass error, isotope distribution, and MS/MS spectrum, to ensure the accuracy and reliability of identification results. The identification results showed that the mass deviation of most compounds was within 1 ppm (Figure 1). The matching of isotopes and MS/MS spectra was high, showing the excellent stability and accuracy of the X500R QTOF system. Some 81 compounds in peaches, including phenols, vitamins and organic acids were quickly identified from the positive and negative ion data.

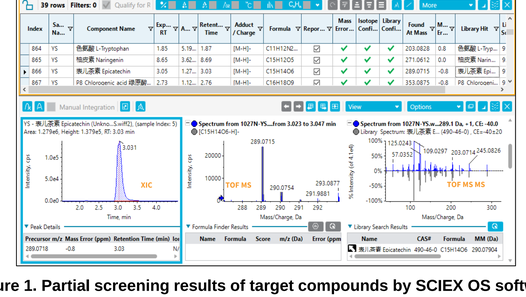

Figure 1. Partial screening results of target compounds by SCIEX OS software.

Using MarkerView software, PCA analysis and t-test analysis were performed on the identified 81 compounds in peaches to find differential compounds. The PCA analysis results showed that the 4 different kinds of peaches could be effectively distinguished (Figure 2). Through t-test analysis, 12 compounds in honey peach were significantly different from those of other species (Figure 3). Honey peach is rich in organic acids, chlorogenic acid, catechins, and proanthocyanidins. In addition, 16 different markers were found in flat peach, nectarine and yellow peach – mainly polyphenols and organic acids.

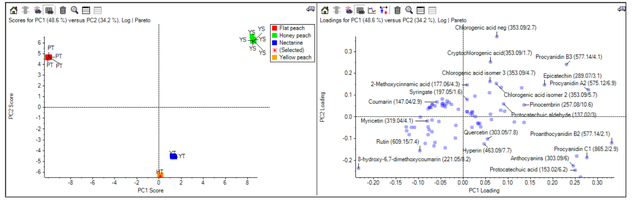

Figure 2. Statistical analysis of the four kinds of peaches using MarkerView software. Principal Component Analysis (PCA) was performed using the quantitative results from the identified compounds. The scores plot on the left shows that in both cases the samples from the different types of peaches clearly separate, with replicates clustering tightly together. Using the Loadings plot on the right, the compounds responsible for the separation can be determined.

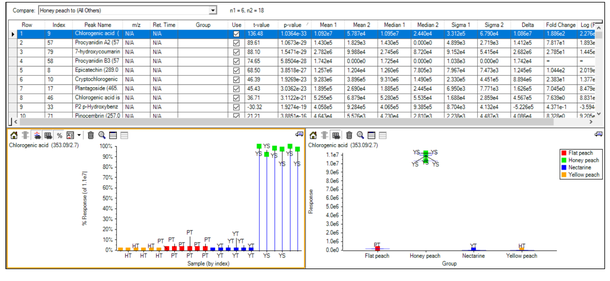

Figure 3. T-test. A t-test can also be performed which provides an additional way to determine specific compounds that differ significantly between the different peaches. Shown is chlorogenic acid, significant higher in the honey peach samples.

CONCLUSIONS

A high efficiency and sensitive method for evaluation of metabolite differences in four kinds of peaches was successfully achieved.

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