Supplementary Information

**Differentiation of cultivation areas and crop years of milled rice using single grain mass spectrometry**

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Table of Contents

Experiments, Figures and Tables | Page
---|---
1. Samples and chemical reagents for the experiments | S-3
2. Experimental procedures of ESI-MS for rice powder sample | S-3
3. Experimental procedures of ESI-MS for single rice sample | S-4
4. Mass spectrometric analysis | S-4
Fig. S1 Optimization of experimental conditions by SG-ESI-MS. | S-6
Fig. S2 Calibration curve of heptanoic acid in the spiked samples. | S-7
Fig. S3 Calibration curve of pesticides in the spiked samples. | S-8
Fig. S4 Mass spectral patterns recorded from single rice sample and rice powder sample. | S-9
Fig. S5 Mass spectral patterns recorded from single rice sample. | S-10
Table S1 The rice samples investigated in this study | S-11
Table S2 The SG-ESI-MS/MS results of rice sample | S-12
Table S3 Analytes and their SG-ESI/Q-Orbitrap MS acquisition results | S-14
Table S4 Comparison of the proposed SG-ESI-MS with other methods for the determination of fatty acids and pesticides in rice | S-15
1. Samples and chemical reagents for the experiments

1.1 Reagents and Materials

Methanol and acetic acid, both of HPLC grade, were bought from ROE Scientific Inc. (Newark, NJ, USA). Ammonium hydroxide was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Heptanoic acid, palmitic acid, palmitoleic acid, behenic acid, γ-linolenic acid and tridecanoid acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stearic acid was purchased from Xiya Chemical Industry Co., Ltd. (Shandong, China). Oleic acid was purchased from Xilong Chemical Industry Co., Ltd. (Shantou, China). Lauric acid was purchased from Sea-salt Material Chemical Industry Co., Ltd. (Zhejiang, China). Linoleic acid, omethoate and dichlorvos were obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). All these standard chemicals were analytic grade. The deionized water used for the experiments was provided by the chemistry facilities at our laboratory.

The milled indica rice was provided from National Analysis Center for Iron and Steel, Beijing, China. The cultivation areas, crop years and quality of all the samples were guaranteed by the manufacturers. All rice samples were harvested in Sichuan, China during the fall of 2011. These rice samples were placed at -4 °C in sealed polyethylene packages before further analysis. The broken rice grains were hand-selected.

Single rice kernel was about 21.8 ± 1.2 mg (n=10). The newly harvested rice grains were used as the blank samples. The 1 mg/mL stock solutions of heptadecanoic acid were individually diluting into 10 μg/mL standard solutions with methanol/water (1:1, v/v) solutions. Methanol/water (1:1, v/v) solutions were used as blank solutions. For quantitative determination of typical FFAs in the single rice samples, a series of heptadecanoic acid working solutions were prepared by serially diluting 10 μg/mL standard solutions with methanol/water (1:1, v/v) solutions. Nicotine (100 ng/mL) was added into the spiked solutions and blank solutions as an internal standard to monitor the stability of the ionization source.

2. Experimental procedures of ESI-MS for rice powder sample
The single powdered rice was also conducted with electrospray ionization mass spectrometry (ESI-MS). The experimental method of ESI-MS was referred to the previous literature\(^1\) with modifications. The ESI-MS was constructed with syringe (Hongda Company, Nanchang, China), organic syringe filter (aperture size of 0.22 \(\mu m\), Tianjin Navigator Lab Instrument Co., Ltd, Tianjin, China), capillary (fused silica, \(i.d., 0.10 \text{ mm}, o.d., 0.15 \text{ mm}\), Agilent Technologies Co., Ltd., USA), ESI source (made in our laboratory) and ion trap mass spectrometer (Thermo Scientific, CA, USA). Before analysis, the experimental parameters was set, including capillary voltage (50 V), tune lens voltage (100 V), spray voltage (+6 kV), temperature (150 \(^\circ\text{C}\)) and the solution rate (6 \(\mu\text{L/min}\)). A single rice was powdered with an agate mortar. The organic syringe filter contained the rice powder with solutions (methanol/water/acetic acid, 40:40:20, \(v/v/v\)) extracting the analytes. All the samples were measured with three replications.

3. **Experimental procedures of ESI-MS for single rice sample**

A single grain was taken into a 1.5 mL-centrifuge tube with 100 \(\mu\text{L}\) of the extracting solution (methanol/water/acetic acid, 40:40:20, \(v/v/v\)) and kept for 1.5 min at the room temperature. Then, the rice sample was removed and the remaining solution was vortexed vigorously into a blender. All the samples were measured with three replications.

4. **Mass spectrometric analysis**

All mass spectra were recorded in the positive detection mode using a commercial linear ion trap mass spectrometer (Thermo Scientific, CA, USA).

To facilitate and standardize the sample manipulation, an extraction solvent (e.g., methanol/water/acetic acid, 40:40:20, \(v/v/v\)) biased with high voltage was fed at a flow rate of 6 \(\mu\text{L/min}\) by a syringe pump (150 \(\mu\text{L},\) Hamilton, USA). The analytes were extracted by the solvent while the solvents were running through the tissue section, producing a spray of charged droplets carrying endogenous chemicals toward the adjacent mass spectrometer inlet. The optimized temperature of the heated capillary was set at 150\(^\circ\text{C}\). Other parameters were set as default values of the instrument.
For ESI-MS, the voltage was set at +6 kV in the ESI experiments under the positive ion detection mode with 100 μL methanol/water/acetic acid (40:40:20, v/v/v) after soaking a single rice kernel for 1.5 min. Methanol/water/acetic acid mixed with sample was infused at a flow rate of 6 μL/min and nebulized by a nitrogen sheath gas (1.2 MPa). As to ESI-MS in rice powder samples, the voltage was set at +6 kV in the detection of single powdered rice contained in organic syringe filter (aperture size of 0.22 μm, Tianjin Navigator Lab Instrument Co., Ltd, Tianjin, China). Methanol/water/acetic acid as extract solvent was infused at a flow rate of 6 μL/min and nebulized by a nitrogen sheath gas (1.2 MPa).

The full scan mass spectra were recorded under an average time of 1.5 min with subtracted background. For tandem mass spectrometry, the precursor ions were isolated with a mass window width of 1.5 Da and the collision-induced dissociation (CID) was performed under collision energy of 15~30%.

Fig. S1 Optimization of experimental conditions by SG-ESI-MS. a) acid ratios (v/v/v, CH$_3$OH/H$_2$O/CH$_3$COOH); b) capillary temperatures; c) spray voltages; d) solvent injection rates.
Fig. S2 Calibration curve of heptanoic acid in the spiked samples. The obtained curve (9.19-459.56 ng/g, y=0.0312x+13.48, R^2=0.995) was based on the signal intensities of the characteristic fragment at m/z 85.
Fig. S3 Calibration curve of pesticides in the spiked samples. a) Omethoate (4.60-459.56 ng/g, y=0.0224x+5.0033, R²=0.998); b) Dichlorvos (2.30-459.56 ng/g, y=0.0258x+5.7922, R²=0.997). The two linear calibration curves were dependent on the corresponding secondary fragment ions, m/z 183 and m/z 145, respectively.
Fig. S4 Mass spectral patterns recorded from single rice sample and rice powder sample. a) the solid grain detected by SG-ESI-MS; b) the rice powder sample detected by ESI-MS.
**Fig. S5** Mass spectral patterns recorded from single rice sample. a) single rice directly detected by SG-ESI-MS; b) the CH$_3$OH/H$_2$O/CH$_3$COOH (40:40:20, v/v/v) solution soaking single rice for 1.5 min and then detected by ESI-MS.
**Table S1** The rice samples investigated in this study

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Rice type</th>
<th>Sampling numbers</th>
<th>Crop year</th>
<th>Cultivation area</th>
<th>Source</th>
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<tbody>
<tr>
<td>1</td>
<td><em>Indica</em></td>
<td>24</td>
<td>2016</td>
<td>Hubei</td>
<td>Local supermarket</td>
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<tr>
<td>2</td>
<td><em>Indica</em></td>
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<td>2016</td>
<td>Hunan</td>
<td>Online retailer</td>
</tr>
<tr>
<td>3</td>
<td><em>Indica</em></td>
<td>24</td>
<td>2016</td>
<td>Guangxi</td>
<td>Online retailer</td>
</tr>
<tr>
<td>4</td>
<td><em>Indica</em></td>
<td>24</td>
<td>2016</td>
<td>Shanxi</td>
<td>Online retailer</td>
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<tr>
<td>5</td>
<td><em>Indica</em></td>
<td>24</td>
<td>2016</td>
<td>Anhui</td>
<td>Online retailer</td>
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<tr>
<td>6</td>
<td><em>Indica</em></td>
<td>24</td>
<td>2016</td>
<td>Heilongjiang</td>
<td>Online retailer</td>
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<tr>
<td>7</td>
<td><em>Indica</em></td>
<td>16</td>
<td>2011</td>
<td>Sichuan</td>
<td>National Analysis Center for Iron and Steel, Beijing, China</td>
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<tr>
<td>8</td>
<td><em>Indica</em></td>
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<td>2012</td>
<td>Sichuan</td>
<td>National Analysis Center for Iron and Steel, Beijing, China</td>
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<tr>
<td>9</td>
<td><em>Indica</em></td>
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<td>2013</td>
<td>Sichuan</td>
<td>National Analysis Center for Iron and Steel, Beijing, China</td>
</tr>
<tr>
<td>10</td>
<td><em>Indica</em></td>
<td>16</td>
<td>2014</td>
<td>Sichuan</td>
<td>National Analysis Center for Iron and Steel, Beijing, China</td>
</tr>
</tbody>
</table>

* The rice samples were used for differentiation analysis of cultivation area.
* The rice samples were used for differentiation analysis of storage time.
Table S2 The SG-ESI-MS/MS results of rice samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Structural formula</th>
<th>Charge form</th>
<th>Precursor ions (m/z)</th>
<th>Product ions (m/z)</th>
<th>Collision energy (%)</th>
</tr>
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<tbody>
<tr>
<td>Nicotine (Internal standard)</td>
<td>![Nicotine structure]</td>
<td>[M+H]^+</td>
<td>163</td>
<td>132, 130, 84</td>
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<td>Acetic acid</td>
<td>![Acetic acid structure]</td>
<td>[M+K]^+</td>
<td>99</td>
<td>81, 71, 57</td>
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<tr>
<td>Hexanoic acid</td>
<td>![Hexanoic acid structure]</td>
<td>[M+H]^+</td>
<td>117</td>
<td>99, 71, 57</td>
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<td>Heptanoic acid</td>
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<td>[M+H]^+</td>
<td>131</td>
<td>113, 101, 85, 71</td>
<td>19</td>
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<tr>
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<td>![Nonanoic acid structure]</td>
<td>[M+H]^+</td>
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<td>131, 121, 99</td>
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<td>[M+K]^+</td>
<td>197</td>
<td>179, 161, 138</td>
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<td>Lauric acid</td>
<td>![Lauric acid structure]</td>
<td>[M+H]^+</td>
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<td></td>
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<td>[M+H2O+H]^+</td>
<td>219</td>
<td>201, 171</td>
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<td>Tridecanoic acid</td>
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<td>[M+Na]^+</td>
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<td>[M+H]^+</td>
<td>257</td>
<td>215, 197, 277, 259</td>
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<td>[M+K]^+</td>
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<td>[M+Na]^+</td>
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<td>[M+Na]^+</td>
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<td>Compound</td>
<td>Structure</td>
<td>Mass Form</td>
<td>M/Z</td>
<td>Retention Time (min)</td>
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<td>Linoleic acid</td>
<td><img src="image" alt="Linoleic acid" /></td>
<td>[M+H]$^+$</td>
<td>281</td>
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<tr>
<td>Oleic acid</td>
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<td>[M+H]$^+$</td>
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<td><img src="image" alt="Stearic acid" /></td>
<td>[M+H]$^+$</td>
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<td>Nonadecanoic acid</td>
<td><img src="image" alt="Nonadecanoic acid" /></td>
<td>[M+Na+H$_2$O]$^+$</td>
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<td>[M+H]$^+$</td>
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<td>[M+Na+H$_2$O]$^+$</td>
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<td>Dichlorvos</td>
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<td>Omethoate</td>
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<tr>
<td>Compound</td>
<td>Molecular formula</td>
<td>Charge form</td>
<td>Calculated value (m/z)</td>
<td>Experimental value (m/z)</td>
<td>Mass error (ppm)</td>
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<td>[M+Na]+</td>
<td>235.18250</td>
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<td>[M+H]+</td>
<td>255.23186</td>
<td>255.23179</td>
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<td>[M+H]+</td>
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<tr>
<td>Nonadecanoic acid</td>
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<td>[M+Na+H₂O]⁺</td>
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<td>Dichlorvos</td>
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<td>214.02974</td>
<td>214.02928</td>
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<td>C₄H₁₀C₁₂O₄P</td>
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<td>220.95318</td>
<td>220.95330</td>
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<tr>
<td>Analytes</td>
<td>Method</td>
<td>Sample</td>
<td>Sample treatment</td>
<td>Extraction procedure (Extractive solvent)</td>
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<tr>
<td>Fatty acids</td>
<td>FTIR</td>
<td>10 g rice</td>
<td>crushing</td>
<td>Solvent extraction (20 mL toluene)</td>
<td>&lt;30 min</td>
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<tr>
<td></td>
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<td>2 g milled rice and 0.1 g bran</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TLC-FID</td>
<td>2 g milled rice and 0.1 g bran</td>
<td>grinding</td>
<td>Solvent extraction (5 mL n-hexane)</td>
<td>&lt;26 min</td>
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<tr>
<td></td>
<td></td>
<td>10 g milled rice</td>
<td>/</td>
<td>Soxhlet lipid extraction (petroleum-ether) rapid lipid extraction (8 mL isopropanol)</td>
<td>/</td>
</tr>
<tr>
<td>Pesticides</td>
<td>GC</td>
<td>93 polished rice kernels</td>
<td>powdering</td>
<td>Solvent extraction/SPE (50 mL petroleum ether)</td>
<td>&lt;60 min</td>
</tr>
<tr>
<td></td>
<td>LC-MS/MS</td>
<td>5-10 g polished rice</td>
<td>/</td>
<td>QuEChERS (15 mL MeCN) /citrate buffered QuEChERS (15 mL MeCN) /citrate buffered QuEChERS without PSA and C-18 clean-up (15 mL MeCN) /acetate buffered QuEChERS without PSA clean-up (15 mL MeCN)</td>
<td>&lt;62 min</td>
</tr>
<tr>
<td>Method</td>
<td>Sample Type</td>
<td>Quantity</td>
<td>Co-Solvent</td>
<td>Method Details</td>
<td>Run Time</td>
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<td>---------------------</td>
<td>----------</td>
<td>----------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>----------</td>
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<tr>
<td>GC-MS/MS</td>
<td>5 g rice</td>
<td>/</td>
<td>mL MeCN with 1% HAC</td>
<td>QuEChERS (10 mL water, 10 mL MeCN, 100 μL MeCN with 1% acetic acid )</td>
<td>73 min</td>
</tr>
<tr>
<td>Fatty acids and pesticides</td>
<td>1 milled rice kernel</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>2 min</td>
</tr>
</tbody>
</table>

This study