

Supplementary Information

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

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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 μ g/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 μ g/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 literature¹ with modifications. The ESI-MS was constructed with syringe (Hongda Company, Nanchang, China), organic syringe filter (aperture size of 0.22 μm , Tianjin Navigator Lab Instrument Co., Ltd, Tianjin, China), capillary (fused silica, *i.d.*, 0.10 mm, *o.d.*, 0.15 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 °C) and the solution rate (6 $\mu\text{L}/\text{min}$). 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 μL 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 $\mu\text{L}/\text{min}$ by a syringe pump (150 μL , 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°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 $\mu\text{L}/\text{min}$ 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 $\mu\text{L}/\text{min}$ 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%.

- 1 L. Song, J. Xu, K. Chingin, T. Zhu, Y. Zhang, Y. Tian, H. Chen and X. Chen, *J. Agric. Food Chem.*, 2017, **65**, 7006-7011.

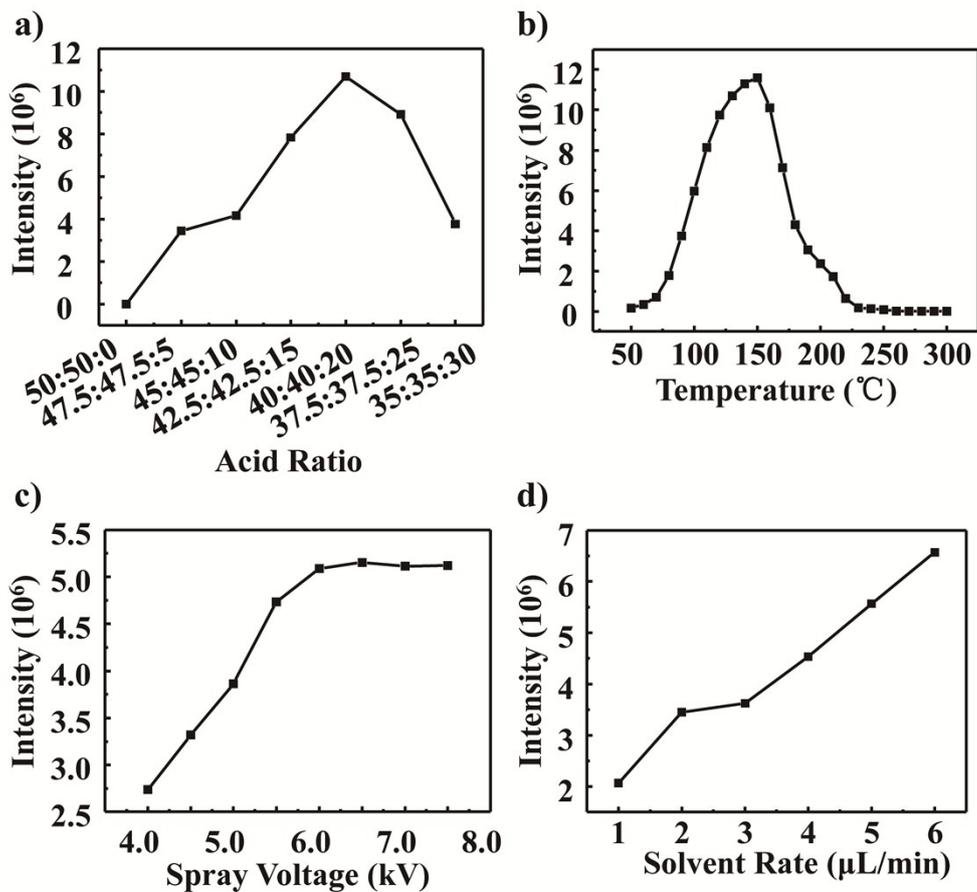


Fig. S1 Optimization of experimental conditions by SG-ESI-MS. a) acid ratios (v/v/v, CH₃OH/H₂O/CH₃COOH); b). capillary temperatures; c) spray voltages; d) solvent injection rates.

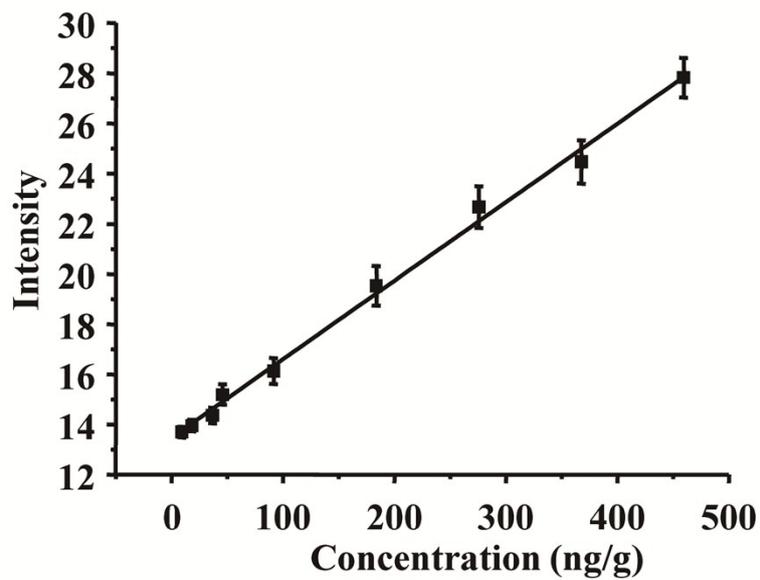


Fig. S2 Calibration curve of heptanoic acid in the spiked samples. The obtained curve (9.19-459.56 ng/g, $y=0.0312x+13.48$, $R^2=0.995$) was based on the signal intensities of the characteristic fragment at m/z 85.

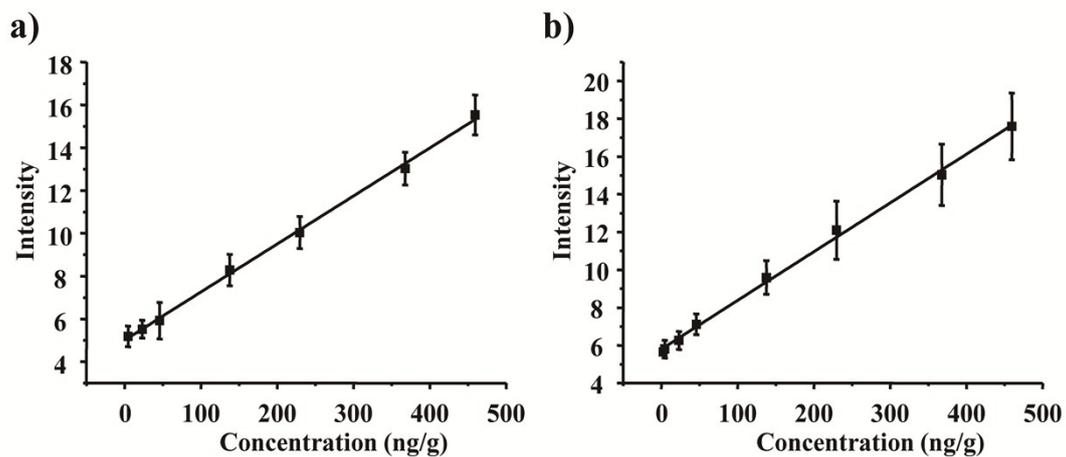


Fig. S3 Calibration curve of pesticides in the spiked samples. a) Omethoate (4.60-459.56 ng/g, $y=0.0224x+5.0033$, $R^2=0.998$); b) Dichlorvos (2.30-459.56 ng/g, $y=0.0258x+5.7922$, $R^2=0.997$). The two linear calibration curves were dependent on the corresponding secondary fragment ions, m/z 183 and m/z 145, respectively.

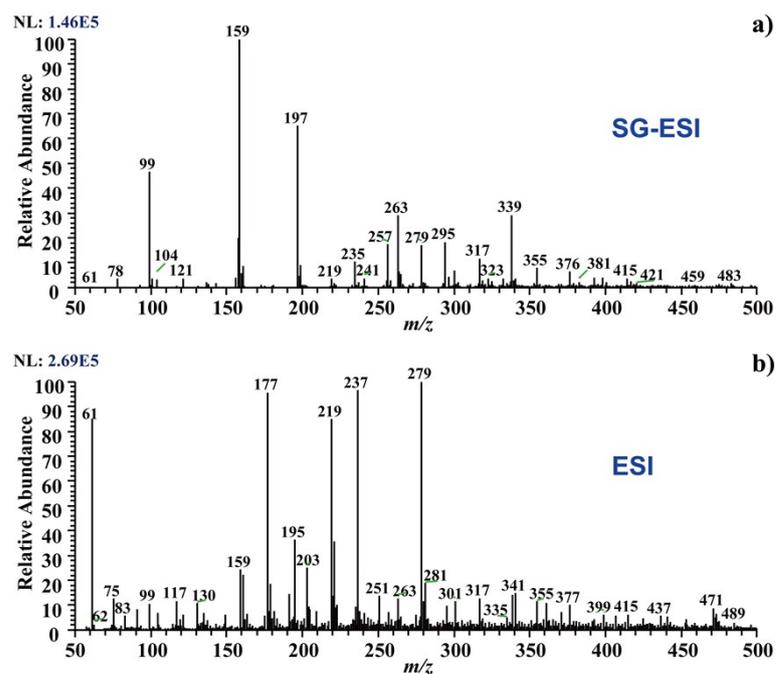


Fig. S4 Mass spectral patterns recorded from single rice sample and rice powder sample. a) the solid grain detected by SG-ESI-MS; b) the rice powder sample detected by ESI-MS.

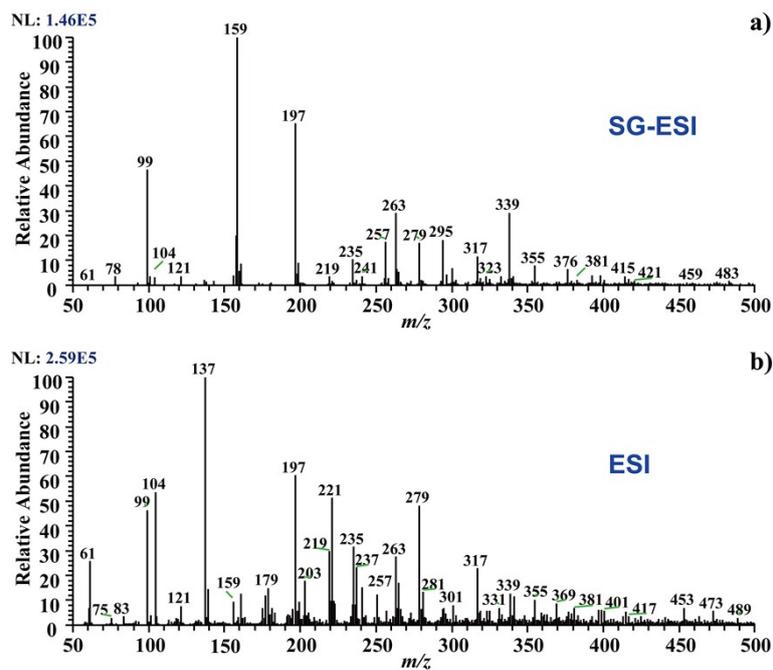


Fig. S5 Mass spectral patterns recorded from single rice sample. a) single rice directly detected by SG-ESI-MS; b) the $\text{CH}_3\text{OH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ (40:40:20, $v/v/v$) solution soaking single rice for 1.5 min and then detected by ESI-MS.

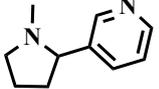
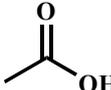
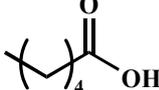
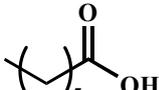
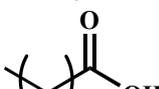
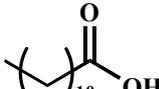
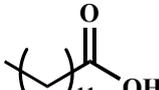
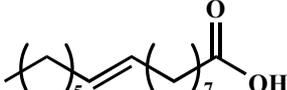
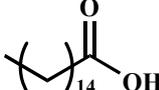
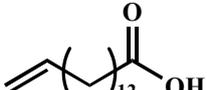
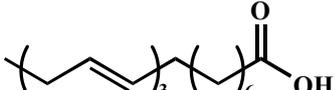
Table S1 The rice samples investigated in this study

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

^a. The rice samples were used for differentiation analysis of cultivation area.

^b. The rice samples were used for differentiation analysis of storage time.

Table S2 The SG-ESI-MS/MS results of rice samples

Analyte	Structural formula	Charge form	Precursor ions (<i>m/z</i>)	Product ions (<i>m/z</i>)	Collision energy (%)
Nicotine (Internal standard)		[M+H] ⁺	163	132, 130, 84	29
Acetic acid		[M+K] ⁺	99	81, 71, 57	29
Hexanoic acid		[M+H] ⁺	117	99, 71, 57	30
Heptanoic acid		[M+H] ⁺	131	113, 101, 85, 71	19
Nonanoic acid		[M+H] ⁺	159	131, 121, 99	29
Lauric acid		[M+K] ⁺	197	179, 161, 138	11
		[M+H] ⁺	201	183, 171, 85	22
Tridecanoic acid		[M+H ₂ O+H] ⁺	219	201, 171	20
		[M+Na] ⁺	235	180, 176, 93	18
Palmitoleic acid		[M+H] ⁺	255	237, 213, 195	15
Palmitic acid		[M+H] ⁺	257	239, 215, 197	27
		[M+K] ⁺	295	277, 259	20
Pentadecenoic acid		[M+Na] ⁺	263	257, 221, 203	12
Linolenic acid		[M+H] ⁺	279	261, 249, 219	17
		[M+Na] ⁺	301	273, 265, 255, 245	22
		[M+K] ⁺	317	289, 281,	20

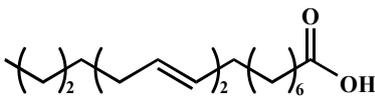
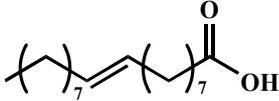
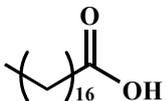
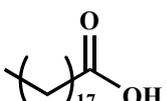
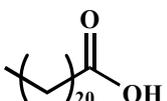
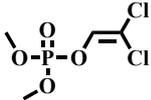
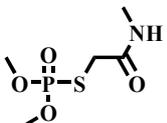
Linoleic acid		[M+H] ⁺	281	257, 263, 239, 221	16
Oleic acid		[M+H] ⁺	283	265, 253, 223	18
Stearic acid		[M+H] ⁺	285	243, 241, 225, 213	20
Nonadecanoic acid		[M+Na+H ₂ O] ⁺	339	321, 304, 247	15
Behenic acid		[M+H] ⁺	341	323, 305, 281	20
		[M+Na+H ₂ O] ⁺	381	363, 339, 321	18
Dichlorvos		[M+H] ⁺	214	109, 125, 183, 196	25
Omethoate		[M+H] ⁺	221	109, 127, 145	25

Table S3 Analytes and their SG-ESI/Q-Orbitrap MS acquisition results

Compound	Molecular formula	Charge form	Calculated value (<i>m/z</i>)	Experimental value (<i>m/z</i>)	Mass error (ppm)
Acetic acid	C ₂ H ₄ O ₂	[M+K] ⁺	98.98429	98.98433	0.4
Hexanoic acid	C ₆ H ₁₂ O ₂	[M+H] ⁺	117.09101	117.09090	0.9
Heptanoic acid	C ₇ H ₁₄ O ₂	[M+H] ⁺	131.10666	131.10668	0.2
Nonanoic acid	C ₉ H ₁₈ O ₂	[M+H] ⁺	159.13796	159.13804	0.5
		[M+K] ⁺	197.09384	197.09383	0.0
Lauric acid	C ₁₂ H ₂₄ O ₂	[M+H] ⁺	201.18491	201.18489	0.1
		[M+H ₂ O+H] ⁺	219.19547	219.19541	0.3
Tridecanoic acid	C ₁₃ H ₂₆ O ₂	[M+Na] ⁺	235.18250	235.18253	0.1
Palmitoleic acid	C ₁₆ H ₃₀ O ₂	[M+H] ⁺	255.23186	255.23179	0.3
Palmitic acid	C ₁₆ H ₃₂ O ₂	[M+H] ⁺	257.24751	257.24746	0.2
		[M+K] ⁺	295.20339	295.20348	0.3
Pentadecenoic acid	C ₁₅ H ₂₈ O ₂	[M+Na] ⁺	263.19815	263.19794	0.8
Linolenic acid	C ₁₈ H ₃₀ O ₂	[M+H] ⁺	279.23186	279.23180	0.2
		[M+Na] ⁺	301.21380	301.21351	1.0
		[M+K] ⁺	317.18774	317.18778	0.1
Linoleic acid	C ₁₈ H ₃₂ O ₂	[M+H] ⁺	281.24751	281.24721	1.1
Oleic acid	C ₁₈ H ₃₄ O ₂	[M+H] ⁺	283.26316	283.26341	0.9
Stearic acid	C ₁₈ H ₃₆ O ₂	[M+H] ⁺	285.27881	285.27882	0.0
Nonadecanoic acid	C ₁₉ H ₃₈ O ₂	[M+Na+H ₂ O] ⁺	339.28697	339.28684	0.4
Behenic acid	C ₂₂ H ₄₄ O ₂	[M+H] ⁺	341.34141	341.34148	0.2
		[M+Na+H ₂ O] ⁺	381.33392	381.33418	0.7
Dichlorvos	C ₅ H ₁₂ NO ₄ PS	[M+H] ⁺	214.02974	214.02928	2.1
Omethoate	C ₄ H ₇ C ₁₂ O ₄ P	[M+H] ⁺	220.95318	220.95330	0.5

Table S4 Comparison of the proposed SG-ESI-MS with other methods for the determination of fatty acids and pesticides in rice

Analytes	Method	Sample	Sample treatment	Extraction procedure (Extractive solvent)	Time consumed	Recovery	LOD/LOQ	References
Fatty acids	FTIR	10 g rice	crushing	Solvent extraction (20 mL toluene)	□30 min	/	/	9
	TLC-FID	2 g milled rice and 0.1 g bran	grinding	Solvent extraction (5 mL n-hexane)	□26 min	/	/	47
	Colorimetric and acid-base titration method	10 g milled rice	/	Soxhlet lipid extraction (petroleum-ether) rapid lipid extraction (8 mL isopropanol)	/	/	/	48
Pesticides	GC	93 polished rice kernels	powdering	Solvent extraction/SPE (50 mL petroleum ether)	□60 min	81.4-90.4%	/	49
	LC-MS/MS	5-10 g polished rice	/	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	□62 min	70-120%	0.07-10 ng/g	50

				mL MeCN with 1% HAC)				
	GC-MS/MS	5 g rice	/	QuEChERS (10 mL water, 10 mL MeCN, 100 μ L MeCN with 1% acetic acid)	73 min	70-122.7%	0.1-7.9 n/g	51
Fatty acids and pesticides	SG-ESI-MS	1 milled rice kernel	/	/	□2 min	77-91%	0.11-1.30 ng/g	This study
