

Differentiating Organic and Conventional Sage by Chromatographic and Mass Spectrometry Flow Injection Fingerprints Combined with Principal Component Analysis

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ABSTRACT: High-performance liquid chromatography (HPLC) and flow injection electrospray ionization with ion trap mass spectrometry (FIMS) fingerprints combined with principal component analysis (PCA) were examined for their potential in differentiating commercial organic and conventional sage samples. The individual components in the sage samples were also characterized with an ultraperformance liquid chromatograph with a quadrupole-time-of-flight mass spectrometer (UPLC Q-TOF MS). The results suggested that both HPLC and FIMS fingerprints combined with PCA could differentiate organic and conventional sage samples effectively. FIMS may serve as a quick test capable of distinguishing organic and conventional sages in 1 min and could potentially be developed for high-throughput applications, whereas HPLC fingerprints could provide more chemical composition information with a longer analytical time.

KEYWORDS: UPLC fingerprint, flow injection, mass spectral fingerprint, principal component analysis, sage (*Salvia officinalis*), rosmarinic acid

■ INTRODUCTION

The organic food market has been rapidly growing in recent years in the United States. A survey from the Organic Trade Association illustrated that the total amount of the U.S. organic industry was over \$29 billion in 2010, with more than 10% average annual growth in the past eight years.¹ Organic foods are generally marketed at much higher prices than their conventional counterparts due to consumer preference. A few previous studies also indicated that botanicals grown under organic and conventional conditions may differ in their chemical compositions and their nutritional values or health properties.^{2,3} For instance, the urinary concentration of quercetin and kaempferol was greater in human subjects after consuming organically produced foods for 22 days than in those who had consumed conventionally produced products.² Novel analytical tools are needed to differentiate organic and conventional foods to prevent intentional adulterations.

Sage (*Salvia officinalis*) is a common spice and food flavoring agent. Sage extracts have been reported to possess several health beneficial effects, including antioxidant capacity,^{4–6} antitumorigenic activity,⁵ improvement of Alzheimer's disease conditions,^{7–9} and anti-inflammatory properties.⁷ Carnosol, carnosic acid, and rosmanol were considered as the important bioactive substances in sage samples.⁵ In addition to the primary diterpene phenols, other phenolics such as flavonoids and phenolic acid derivatives might also play important roles in its health properties such as the antioxidant and antitumorigenic activities.⁵ Differentiation of conventional and organic sages is important, as both are commercially available and differ significantly in their prices.

Recently, our laboratory has evaluated potential analytical approaches for adulteration detection.^{3,10,11} In 2011, a high-performance liquid chromatography (HPLC) fingerprinting technique was successfully applied in differentiating di- and tetraploid Jiaogulan (*Gynostemma pentaphyllum*) botanical samples.¹⁰ In 2012, conventional and organic grown peppermints were differentiated using both HPLC and flow injection electrospray ionization with ion trap mass spectrometry (FIMS) fingerprinting techniques combined with principal component analysis (PCA).³ The result indicated that FIMS fingerprints rapidly differentiated organic and conventional peppermints in a shorter time, whereas HPLC fingerprints provided more detailed information about the chemical composition. This study was conducted to further test the hypothesis that HPLC-UV chromatographic and FIMS fingerprints combined with PCA could potentially differentiate commercial organic and conventional sages.

■ MATERIALS AND METHODS

Standard Compounds and Other Chemicals. LC-MS grade water and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA). LC-MS grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Materials and Sample Preparation. Ten commercial USDA certified organic and 10 conventional sage leaf samples were gifts from Frontier Natural Products Co-op (Norway, IA, USA). Dry

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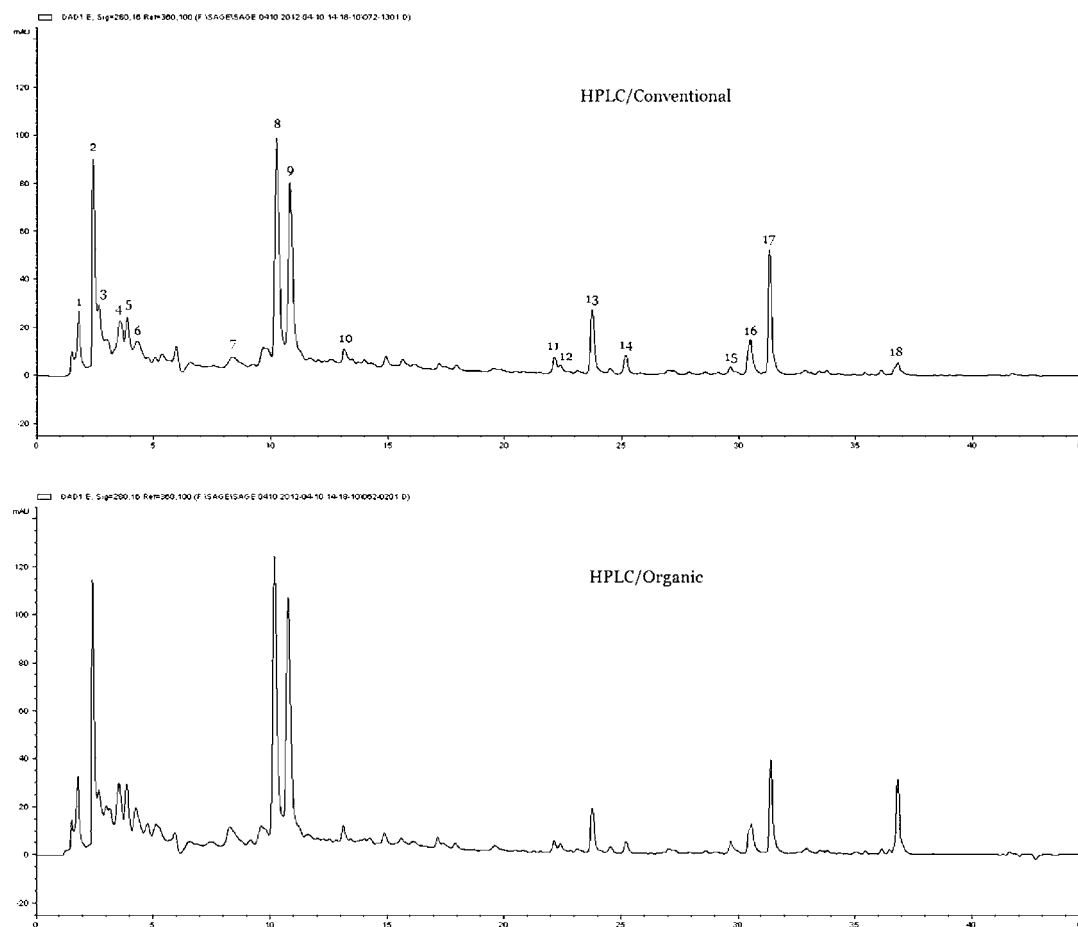


Figure 1. Representative HPLC fingerprints for conventionally and organically grown sage samples.

sage samples were ground to 20 mesh particle sizes using an IKA A11 analytical grinder (IKA, Staufen, Baden-Württemberg, Germany) and stored at -20°C before analysis. One hundred milligrams of each sage powder was accurately weighed and extracted with 10 mL of $\text{H}_2\text{O}/\text{MeOH}$ (1:1, v/v) with ultrasonication at ambient temperature for 30 min. The extracts were filtered through a $0.45\ \mu\text{m}$ nylon syringe filter (Alltech Associates, Deerfield, IL, USA) and subjected to HPLC and FIMS fingerprinting analyses. Each sample was analyzed in duplicate for HPLC and in triplicate for FIMS fingerprints.

HPLC-UV and IT-MS Conditions. Both HPLC-UV and IT-MS conditions were selected according to our previous study.³ Briefly, an Agilent 1100 HPLC system was used for HPLC fingerprinting (Agilent Technologies, Palo Alto, CA, USA) and connected with an LCQ Deca ion-trap mass spectrometer for FIMS (Agilent Technologies). Electrospray ionization (ESI) in negative ion mode was utilized.

A Waters Symmetry C18 column (2.1 mm i.d. \times 150 mm, $3.5\ \mu\text{m}$) (Waters, Milford, MA, USA) was used for HPLC-UV fingerprinting; mobile phase A consisted of 0.1% formic acid in H_2O , and mobile phase B consisted of 0.1% formic acid in acetonitrile. The elution began with 20% phase B, changed linearly to 50% B in 20 min, increased linearly to 95% B at 35 min, and washed at this ratio for 5 min. After washing, the mobile phase was returned to its initial conditions for 5 min to re-equilibrate the column for the next injection. The flow rate was 0.2 mL/min, with an injection volume of 10 μL and a detection wavelength of 280 nm.

For FIMS analysis, no analytical column but a two-way valve was used to minimize potential contamination in the MS system. Mobile phase A had 0.1% formic acid in H_2O (v/v), and mobile phase B contained 0.1% formic acid in acetonitrile (v/v) with an isocratic elution at a ratio of phase A to phase B at 60:40 (v/v) and a flow rate of 0.5 mL/min. Sage extractions were diluted 10 times with water, and

the injection volume was 2 μL . MS spectra were collected from 0.05 to 0.55 min, and the mass range was from m/z 150 to 1000, with a sheath gas flow rate of 80 L/min, auxiliary gas flow rate of 10 L/min, and spray voltage at 4.5 kV. Heated capillary temperature was 250°C , capillary voltage was $-4.0\ \text{V}$, and tube lens offset was 20 V. Triplicate analyses of 20 different sage samples provided 60 MS spectra.

UPLC Q-TOF MS Condition. A UPLC-Q-TOF MS system (Waters) was used for identification of major peaks in the sage extraction. The accurate mass weight was used combined with refs 5 and 12 and a chemical database to identify the components in sage. UPLC-Q-TOF MS condition was selected according to a previous study except the negative ion mode in ESI source.³ In brief, a BEH C18 column (2.1 mm i.d. \times 100 mm, $1.7\ \mu\text{m}$) (Waters) was used at 40°C . UPLC conditions were the same as for the HPLC fingerprint. The MS conditions were as follows: capillary voltage, 3.00 kV; sampling cone voltage, 30 V; extraction cone voltage, 4.0 V; source temperature, 120°C ; and desolvation temperature, 450°C . The cone gas flow rate was 50 L/h, and the desolvation gas flow was 800 L/h. A MS^E method (a scan model to get full information for both parent ion and daughter ion in one injection using tandem mass spectrometry) was used with mass range from m/z 100 to 1000, and the ramp collision energy was selected from 25 to 35 V. MassLynx 4.1 software (Waters) was used for alignment of peaks and accurate mass weight identification.

Data Processing. Eighteen major chromatographic peaks were selected in the UPLC fingerprints in both conventional and organic sages. Their absolute and relative peak areas were both selected for PCA. For absolute peak area, 18 peak areas in all of the chromatograms were analyzed with PCA directly. For relative peak area analyses, the largest peak area (peak 8) in every chromatogram was selected as the reference peak, and other peak areas were divided to reference peak in each chromatogram to become the relative peak areas. Peaks with areas $>5\%$ of the reference peak area were included.

For FIMS fingerprints, one-dimensional spectra ranging from m/z 150 to 1000 were collected for analysis. Data were imported to Excel (Microsoft, Inc., Bellevue, WA, USA) for data preprocessing. Sixty spectra (triplicate analyses for 20 samples) were sorted by sample names and filling every missing m/z with zero in the mass list so that the data points of each mass spectrum were aligned and then for PCA. PCA was performed using SIMCA-P software (Umetrics, Malmö, Skånelän, Sweden) to analyze the absolute areas of the 18 peaks in all 40 chromatograms (20 samples with duplicate analyses each) collected at 280 nm.³ PCA was also performed for relative peak areas, which was calculated using the largest peak area (peak 8) as the reference peak. One-dimensional FIMS fingerprints obtained at m/z 150–1000 were reorganized in Excel (Microsoft, Inc.) and analyzed using the SIMCA-P software.

RESULTS AND DISCUSSION

Chromatographic and MS Fingerprints. As shown in Figure 1, the organic sage had similar peak numbers and areas as in their conventional counterparts under the experimental conditions, except that the relative area of peak 18 seemed

Table 1. Identification of Major Peaks in the Sage Samples^a

peak	compound	RT	mass weight [M - H] ⁻	
			measured	calculated
1	unknown	1.79	nd	
2	luteolin-diglucuronide isomer I	2.39	637.0942	637.1041
3	luteolin-diglucuronide isomer II	2.67	637.0942	637.1041
4	saponarin	3.52	593.1507	593.1507
5	ericiocitrin	3.85	595.1663	595.1663
6	luteolin-7-O-glucuronide	4.26	461.0751	461.0720
7	unknown	8.27	nd	
8	rosmarinic acid	10.17	359.0844	359.0767
9	salvianolic acid K	10.75	555.1100	555.1139
10	luteolin-rutinoside	13.10	593.1507	593.1507
11	salvianolic F	22.11	313.0743	313.0712
12	rosmanol isomer I	22.37	345.1720	345.1702
13	rosmanol	23.75	345.1718	345.1702
14	rosmanol isomer II	25.19	345.1718	345.1702
15	carosolic acid	29.67	331.1945	331.1909
16	rosmarinic acid isomer	30.57	359.0844	359.0767
17	carosol	31.38	329.2020	329.2053
18	epirosmanol	36.82	345.1760	345.1702

^aDate were collected using a Waters UPLC-Q-TOF MS system.

larger in the organic samples than in the conventional ones. These results indicated that organic and conventional sage samples were similar and could not be effectively differentiated by direct visual judgment of the HPLC-UV fingerprints. A further UPLC-Q-TOF high-resolution mass spectrum analysis was able to characterize 16 individual compounds in sage, including luteolin-diglucuronide isomer I, luteolin-diglucuronide isomer II, saponarin, ericiocitrin, luteolin-7-O-glucuronide, rosmarinic acid, salvianolic acid K, luteolin-rutinoside, salvianolic F, rosmanol isomer I, rosmanol, rosmanol isomer II, carosolic acid, rosmarinic acid isomer, carosol, and epirosmanol, which corresponded to the peaks 2, 3, 4, 5, 6, 8, and 9–18, respectively (Table 1). The compounds were identified by comparing the accurate mass of each peak to that in the chemical database and references.

Typical FIMS fingerprints for the conventional and organic sages are shown in Figure 2. Visual difference may be noticed between the conventional and organic sage mass spectra. In the spectrum of the conventional sage, m/z 329 for carosol was the most abundant peak, followed by m/z 374 and 331 (carosolic acid). In contrast, m/z 364 and 345 (rosmanol) were the second biggest peaks in the organic sage, and the peak at m/z 329 (carosol) had the biggest ion count.

PCA of Chromatographic Fingerprints. The PCA scores plot for the absolute chromatographic peak areas is provided in Figure 3, and the corresponding loading plot is shown in Figure 4. The conventional sage samples are all on the left of the PCA scores plot, whereas all organic samples are located on the right side (Figure 3). PCA is a mathematical approach that transforms a large number of related variables into a small group of unrelated variables, the principal components (PCs). PCA of fingerprints visualized the results and made the comparison of the chromatographic fingerprints easier without subjective decisions.

In the loading plot of the UPLC absolute peak areas (Figure 4), peaks 9 (salvianolic acid K), 8 (rosmarinic acid), and 18 (epirosmanol) contributed significantly to the separation of organic samples from their conventional counterparts, whereas peaks 17 (carosol), 13 (rosmanol), and 16 (rosmarinic acid isomer) contributed significantly to the separation of conventional sage samples. With the HPLC chromatographic fingerprint (Figure 1) and PCA loading plot (Figure 4) taken into account, the organic sage samples contained greater levels of salvianolic acid K, rosmarinic acid, and epirosmanol, whereas

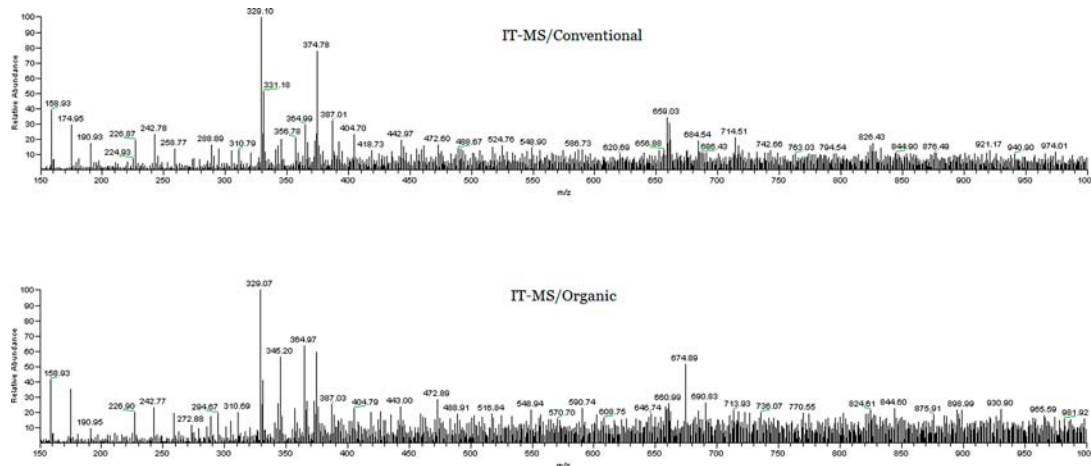


Figure 2. MS fingerprinting for IT-MS for conventionally and organically grown sage samples.

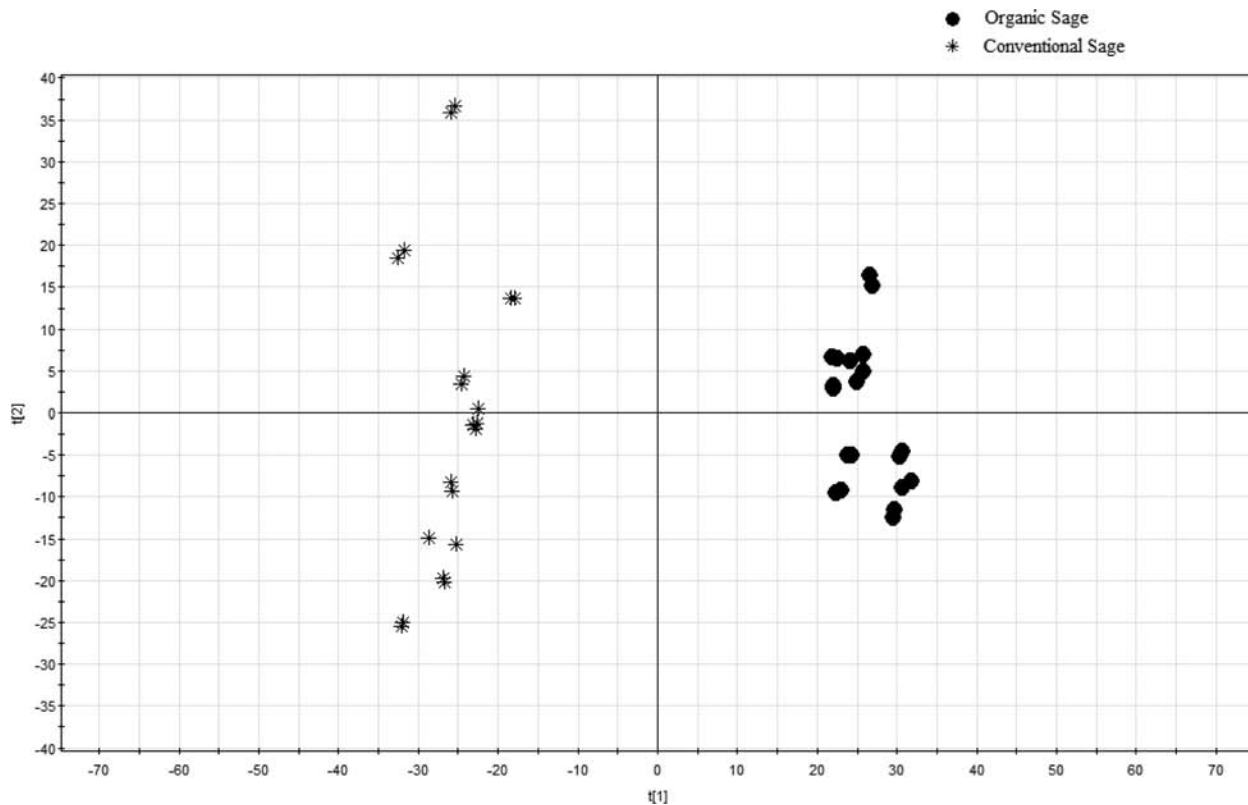


Figure 3. PCA scores plot for HPLC absolute peak areas of the organic and conventional sage samples.

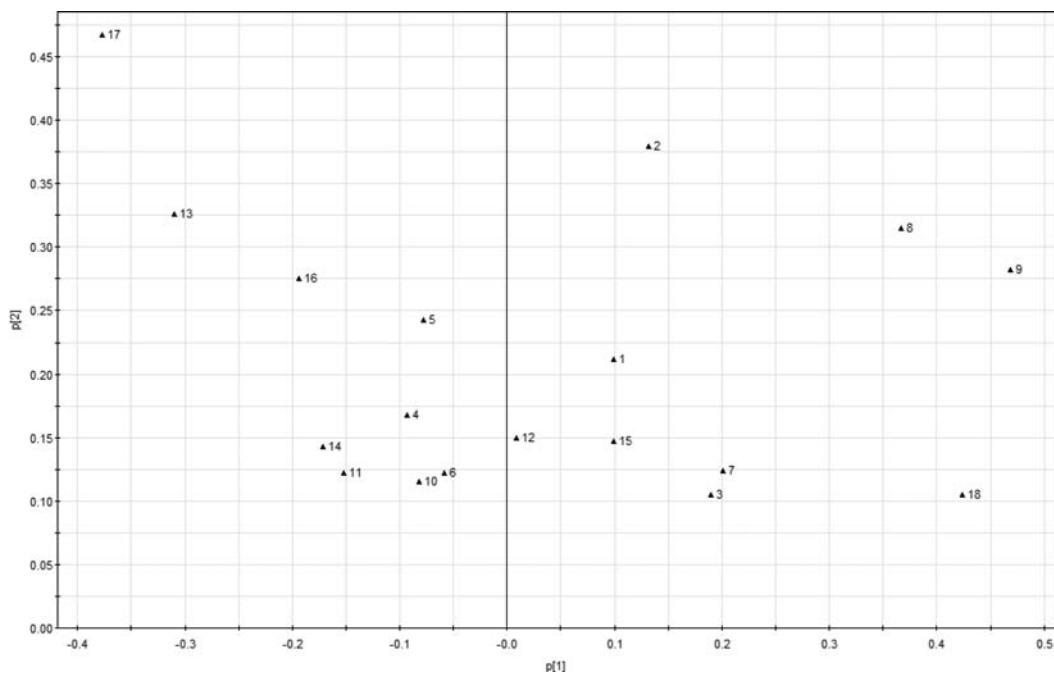


Figure 4. PCA loading plot for HPLC absolute peak areas of the organic and conventional sage samples.

the conventional sage samples contained more carnosol, rosmarone, and rosmarinic acid isomer.

Figures 5 and 6 show the PCA scores plot and loading plot of the relative chromatographic peak areas for the organic and the conventional sages. In all 18 peaks, peak 8 was the biggest peak and was selected as the reference peak (RP) for calculating the relative peak areas. Other major peaks were defined as at least 5% of the area of RP. In Figure 5, the organic sage samples were

clustered tightly on the left side of the scores plot, whereas the conventional sage samples were all on the right side of the scores plot. The similar trend of scatter plot between the organic and conventional sage samples indicated that sage samples had relatively uniform chemical profiles regardless of their farming practice. In the loading plot of the HPLC relative peak areas (Figure 6), peaks 18 and 9 were on the left side of the loading scatter plot, indicating that the relative areas of

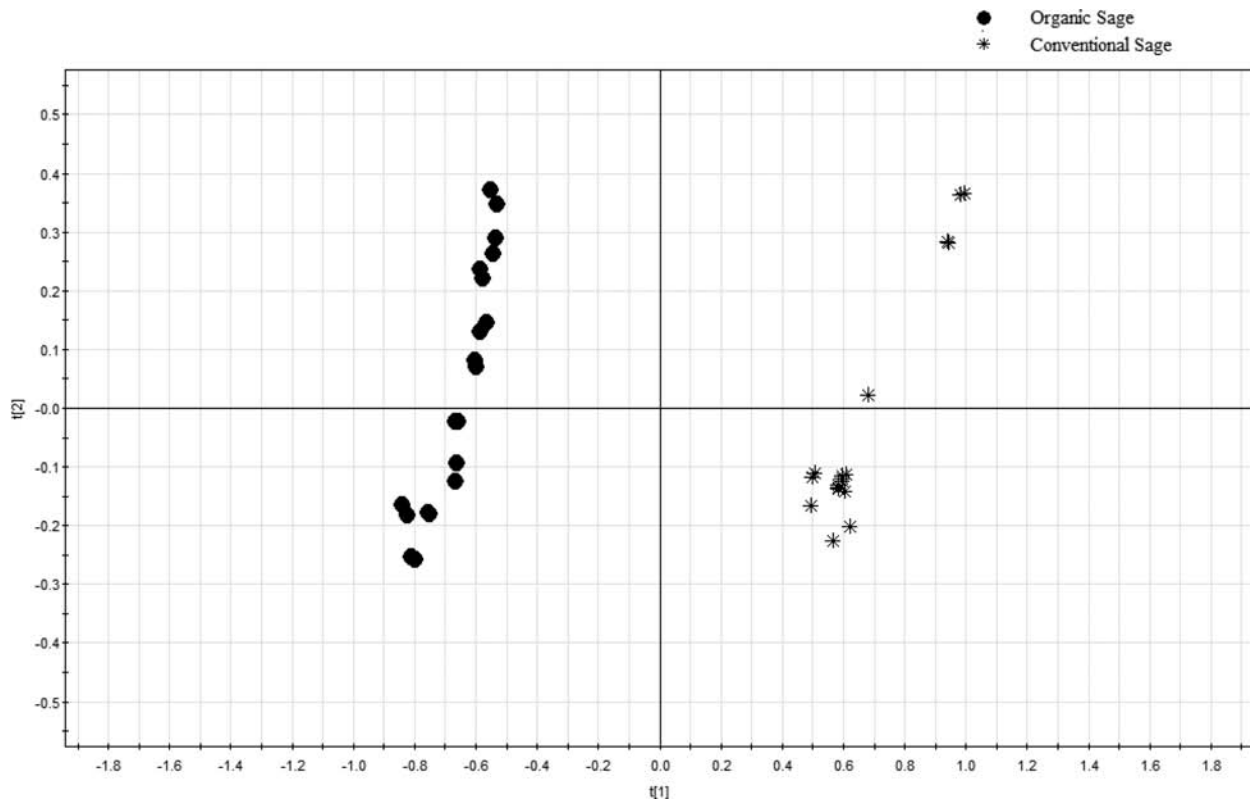


Figure 5. PCA scores plot for HPLC relative peak areas of the organic and conventional sage samples. All peak areas were divided by the area of peak 8 in the sample, and the results were used for PCA.

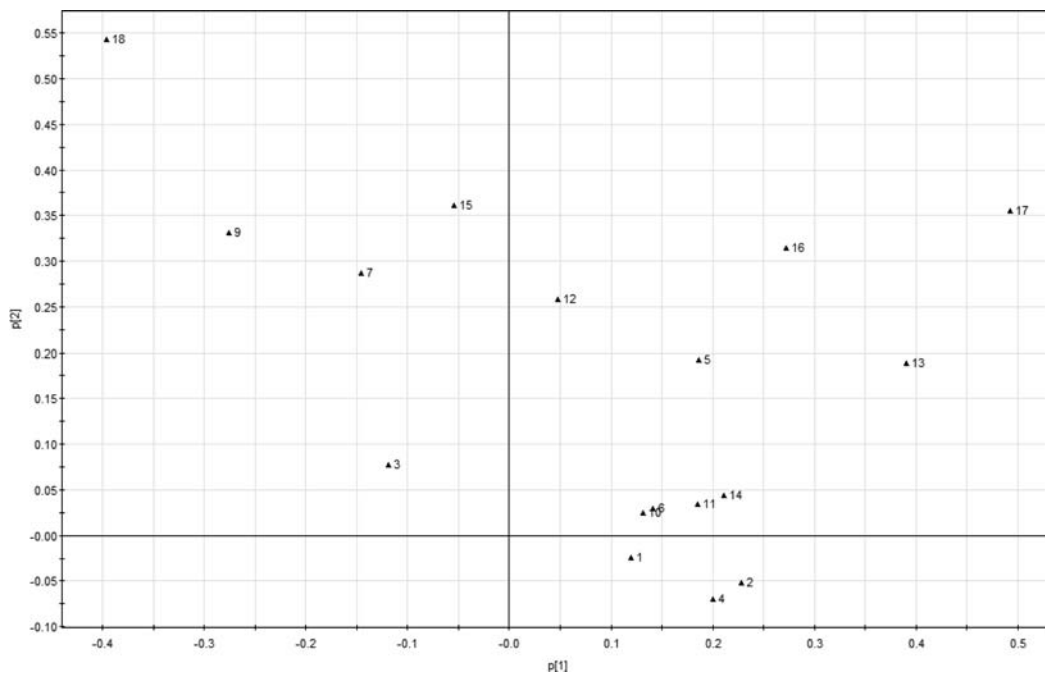


Figure 6. PCA loading plot for relative peak areas of the organic and conventional sage samples. All peak areas were divided by the area of peak 8 in each sample, and the results were used for PCA.

these two peaks were greater in the organic sage samples, and peaks 17, 13, and 16 were greater in the conventional sage samples. Because peak 8 was the RP, the results indicated that the peak areas of RP were greater in the organic samples, suggesting that the level of rosmarinic acid, salvianolic acid K, rosmanol, and carnosol may be important for determining if a

sage was organically or conventionally produced according to its HPLC chromatographic fingerprints.

PCA of FIMS Fingerprints. The FIMS PCA scores and loading plots are shown in Figures 7 and 8, respectively. In the PCA scores plot, all of the organic sage samples were on the left side, whereas the conventional sage samples were on the right

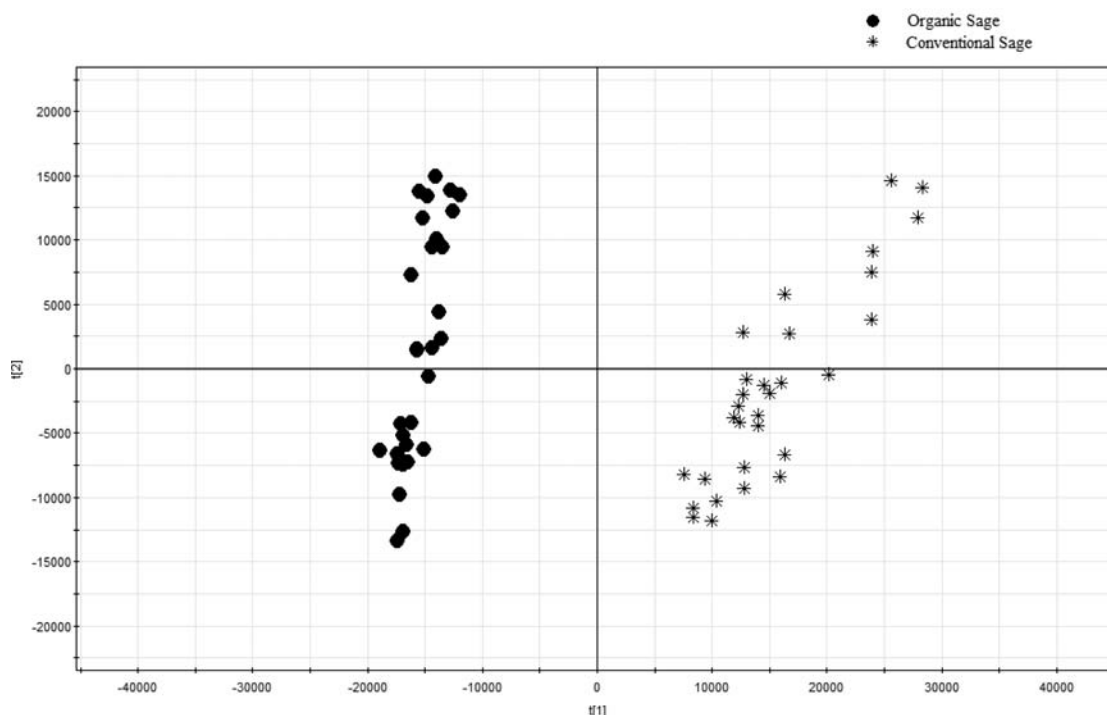


Figure 7. PCA scores plot for IT-MS fingerprints of the organic and conventional sage samples.

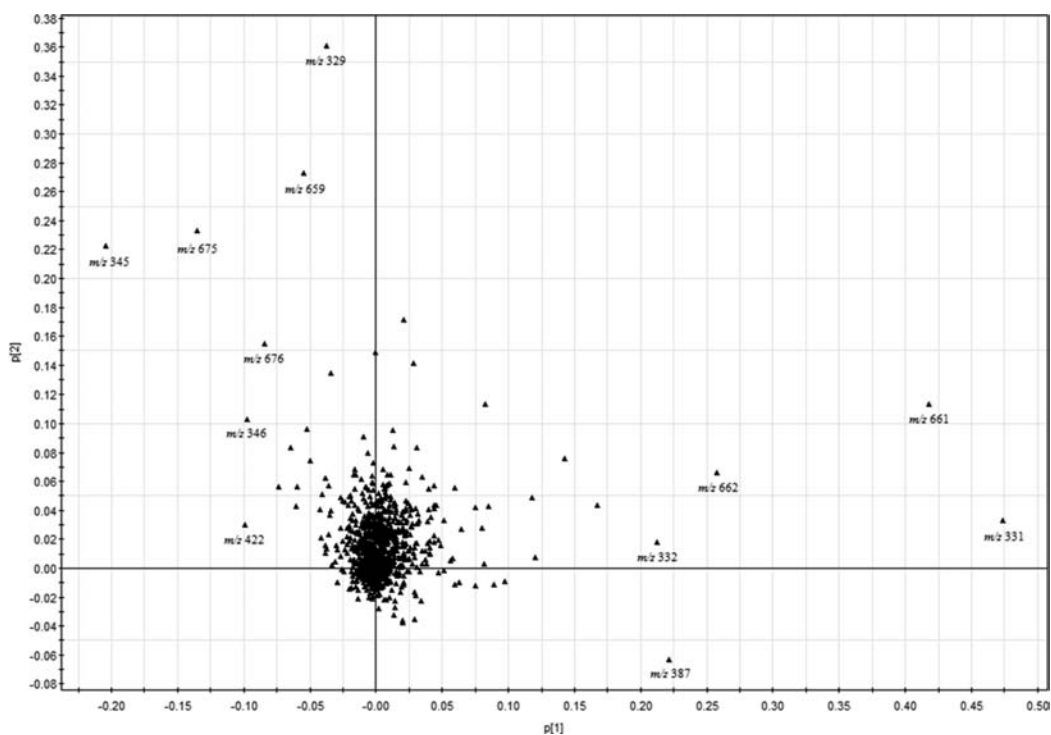


Figure 8. PCA loading plot for IT-MS fingerprints of the organic and conventional sage samples.

side (Figure 7). The organic and conventional sages were separated completely by PC1. The scores plot indicated that the FIMS fingerprinting technique could effectively differentiate commercial conventional and organic sages in 1 min.

In the loading plot of FIMS fingerprints, most of the ions were clustered near the origin (Figure 8). However, a few ions dispersed far away from the center, and these ions and their levels weighed more in determining the farming practices of

sage. In the upper left corner, ions m/z 329, 659, 675, and 345 together with their isomer peaks m/z 676 and 346 yielded a higher score and would lead to positions to the left side of PCA scores plot. The most intensive ion contributing to high scores was m/z 345 $[M - H]^-$, the molecular ion for rosmanol with a formula weight of 346. Another high-score ion was m/z 329 $[M - H]^-$, the molecular ion for carnosol with a molecular weight of 330.

The most noticeable ions that contributed positively to conventional sage were ions at m/z 331 and 661 together with their isomer peaks at m/z 662 and 332, respectively. The ion at m/z 331 $[M - H]^-$ was identified as the molecular ion for carnosic acid with a molecular weight of 332. The other ion that also contributed positively to conventional sage samples was the ion at m/z 387. The scores of these ions indicated that these compounds contributed most in differentiating organic and conventional sage samples. PCA of the FIMS fingerprints indicated that the three major diterpene phenols, including rosmanol, carnosol, and carnosic acid, were important in distinguishing organic and conventional sage samples. Rosmanol ($r = -0.854$, $P < 0.05$) and carnosol ($r = 0.911$, $P < 0.05$) contributed more to organically grown sage, whereas carnosic acid ($r = 0.819$, $P < 0.05$) contributed more to conventionally grown sage. Carnosol, rosmanol, and carnosic acid are also considered to be the most important bioactive substances in sage.⁵ During our study, carnosol and rosmanol contributed more to conventional sage samples in the LC fingerprint. In the MS flow injection fingerprint, however, carnosic acid contributed more to conventional sage, whereas carnosol and rosmanol contributed more to organic sage samples. These three major substances could be monitored to predict sage growing conditions.

In summary, PCA of either HPLC or FIMS fingerprint has potential in differentiating commercial organic and conventional botanical samples. FIMS fingerprinting may provide a rapid answer and has potential for high-throughput applications, whereas HPLC fingerprints may obtain more chemical composition information about the samples and take a longer analytical time.

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Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Organic Trade Association. The organic trade association's 2011 organic industry survey. July 17, 2012; website <http://www.ota.com/pics/documents/2011OrganicIndustrySurvey.pdf>.
- (2) Grønder-Pedersen, L.; Rasmussen, S. E.; Bugel, S.; Jørgensen, L. V.; Dragsted, L. O.; Gundersen, V.; Sandström, B. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *J. Agric. Food Chem.* **2003**, *51*, 5671–5676.
- (3) Gao, B.; Lu, Y.; Qin, F.; Chen, P.; Shi, H.; Charles, D.; Yu, L. Differentiating organic from conventional peppermints using chromatographic and flow-injection mass spectrometric (FIMS) fingerprints. *J. Agric. Food Chem.* **2012**, *60*, 11987–11994.
- (4) Lu, Y.; Foo, L. Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chem.* **2001**, *75*, 197–202.
- (5) Ho, C.; Wang, M.; Wei, G.; Huang, T.; Huang, M. Chemistry and antioxidative factors in rosemary and sage. *Bio Factors* **2000**, *13*, 161–166.
- (6) Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidant activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soc.* **1996**, *73*, 645–652.
- (7) Howes, M. J. R.; Perry, N. S. L.; Houghton, P. J. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother. Res.* **2003**, *17*, 1–18.
- (8) Perry, E. K.; Pickering, A. T.; Wang, W.; Houghton, P.; Perry, N. S. L. Medicinal plants and Alzheimer's disease: integrating ethnobotanical and contemporary scientific evidence. *J. Altern. Complem. Med.* **1998**, *4*, 419–428.
- (9) Perry, N. S. L.; Houghton, P. J.; Sampson, J.; Theobald, A. E.; Hart, S.; Lis-Balchin, M.; Hoult, J. R. S.; Evans, P.; Jenner, P.; Milligan, S.; Perry, E. K. In-vitro activity of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease. *J. Pharm. Pharmacol.* **2001**, *53*, 1347–1356.
- (10) Xie, Z.; Zhao, Y.; Chen, P.; Jing, P.; Yue, J.; Yu, L. Chromatographic fingerprint analysis and rutin and quercetin compositions in the leaf and whole-plant samples of di- and tetraploid *Gynostemma pentaphyllum*. *J. Agric. Food Chem.* **2011**, *59*, 3042–3049.
- (11) Zhao, Y.; Xie, Z.; Niu, Y.; Shi, H.; Chen, P.; Yu, L. Chemical compositions, UPLC/MS fingerprinting profiles and radical scavenging properties of commercial *Gynostemma pentaphyllum* (Thunb.) Makino samples. *Food Chem.* **2012**, *134*, 180–188.
- (12) Benno, F. Z.; Stephan, G. W.; Laura, N. T.; Wolf, S.; Drik, W. L. Rapid UHPLC determination of polyphenols in aqueous infusions of *Salvia officinalis* L. (sage tea). *J. Chromatogr., B* **2011**, *879*, 2459–2464.