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Differentiating Organic from Conventional Peppermints Using Chromatographic and Flow Injection Mass Spectrometric (FIMS) Fingerprints

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ABSTRACT: High-performance liquid chromatography (HPLC) and flow injection mass spectrometric (FIMS) fingerprinting techniques were tested for their potential in differentiating organic and conventional peppermint samples. Ten organic and ten conventional peppermint samples were examined using HPLC-UV and FIMS methods. Principal component analysis (PCA) showed that both HPLC and FIMS fingerprints could determine the difference in the commercial organic and conventional peppermints. FIMS fingerprinting provided a rapid test to differentiate organic and conventional peppermints in 1 min of analysis and has potential for high-throughput applications. On the other hand, HPLC fingerprints provide more information about the chemical composition of the samples, but take a longer time to differentiate organic and conventional peppermint samples.

KEYWORDS: *HPLC fingerprint, flow injection, mass spectral fingerprint, principal component analysis, peppermint (Mentha piperita), kaempferol 7-O-rutinoside*

INTRODUCTION

Consumer demand for organic foods has continuously increased because of the perception that they might contain greater amounts of beneficial components than their conventionally produced counterparts. The organic industry grew to over $26 billion in 2010, increasing over 4 times from $6 billion in 2000.[1] Conflicting findings on the quality and nutritional values of organic foods compared to their conventional counterparts have been reported. For instance, a human crossover intervention study involving 16 subjects demonstrated that organic and conventional fruits and vegetables differed in their concentrations of five selected flavonoids and resulted in different urinary excretions of the major dietary flavonoids.[2] In contrast, a systematic review concluded that there was no evidence indicating the nutritional quality difference between organic and conventional foods.[3]

Peppermint, *Mentha piperita* L., is one of the most important and widely used flavoring agents and spices. Peppermint extract exhibits several health properties, including antioxidant, radioprotective, and antitumorgenic activities.[4−7] Peppermint oil and its primary component, menthol, might have an antiemtic effect by reacting on the 5-HT_3 receptor of the ion-channel complex[8] and induce Ca^{2+} influx in a subset of sensory neurons from dorsal root and trigeminal ganglia.[9] Both conventional and organic peppermints are commercially available and differ significantly in their prices.

Our recent study showed that organic peppermint extract was more effective than that of conventional peppermint in suppressing IL-1β, IL-6, and COX-2 mRNA expressions at a 10 μg botanical equivalent per milliliter concentration in the LPS-stimulated J774A.1 mouse macrophage cells, but there was no difference in inhibiting MCP-1 and TNF-α mRNA expressions.[10] The organic peppermint also had greater *p*-coumaric acid content than the conventional counterpart, and both had the same levels of gallic, caffeic, and syringic acids, as well as catechin and epigallocatechin gallate (EGCG) contents, suggesting potential differences between organic and conventional peppermints or other edible botanicals in their chemical compositions and biological activities. It will be interesting to find out how organic and conventional peppermints may differ from each other in their chemical compositions, and if organic and conventional peppermints could be differentiated by instrumental analysis.

Our recent study successfully differentiated the tetra- and diploid *Gynostemma pentaphyllum* and the different parts of the same *G. pentaphyllum* genotype plant using HPLC-UV fingerprints and principal component analysis (PCA).[11] Later in 2012, 11 commercial *G. pentaphyllum* samples were evaluated for their homogeneity and similarity using an online HPLC-UV-MS technique combined with PCA.[12] The 11 *G. pentaphyllum* samples were classified into three clusters or groups using the MS data for flavonoids and gypenosides

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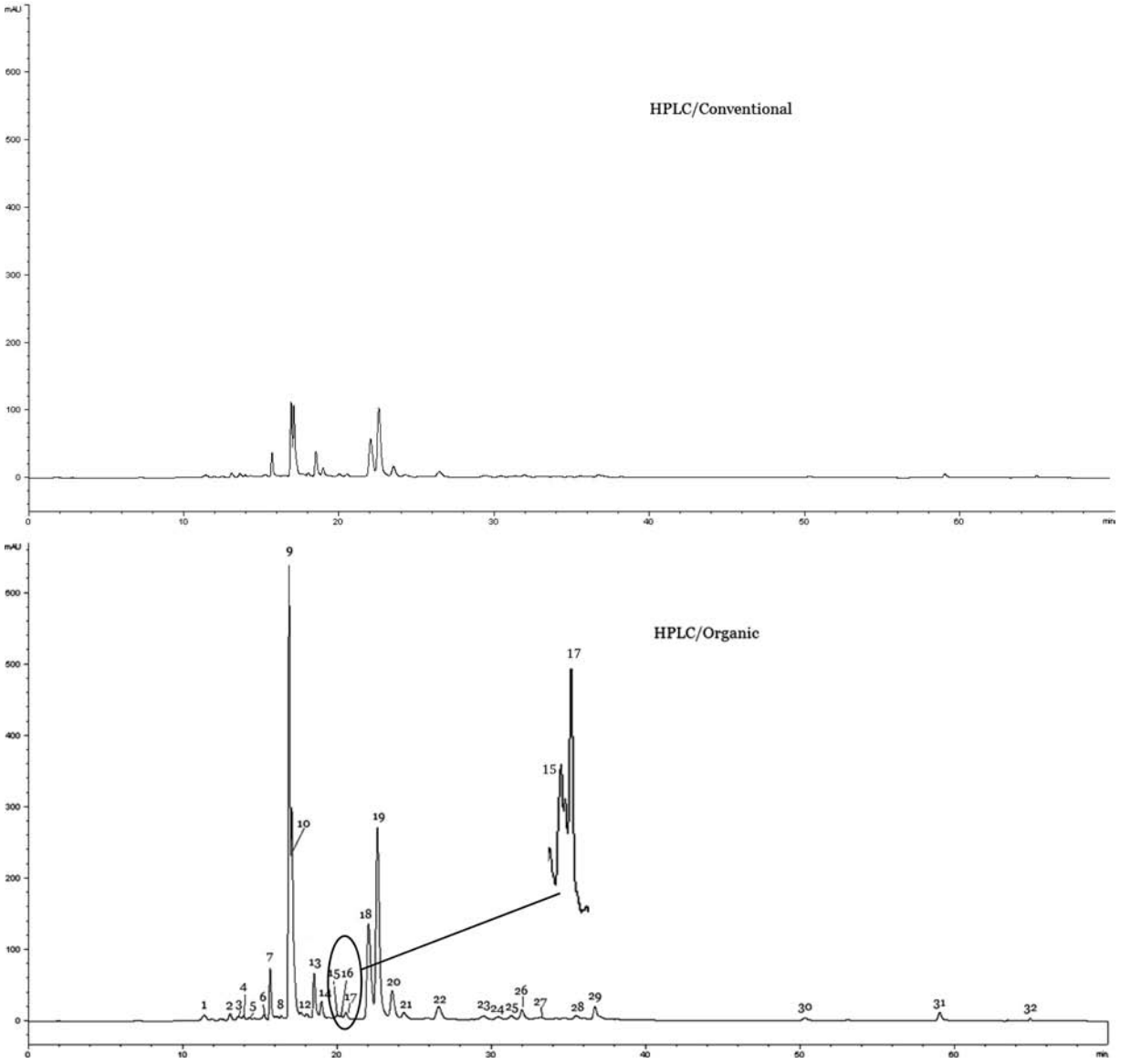

Figure 1. HPLC-DAD fingerprintings of conventional and organic peppermints. Peaks 1, 3, 7, 9, 10, 11, 13, 15, 17, 19, 26, 30, 31, and 32 were pebrellin, eriodictyol 7-*O*-rutinoiside, tricetin 3′-*O*-glucoside 5′-*O*-rhamnoside, eriocitrin, kaempferol 7-*O*-rutinoside, luteolin 7-*O*-neohesperidoside, narirutin, 4′-methoxykaempferol 7-*O*-rutinoside, hesperidin, isosafrole, cyclohexanecarboxylic acid, gardenin D, 5,6-dihydroxy-4′,7,8-trimethoxyflavone, and gardenin B, respectively.

collected under negative mode. In addition to chromatographic fingerprint analysis, flow injection mass spectrometric (FIMS) fingerprinting has also been used to differentiate botanicals from different growing conditions. In 2010, FIMS fingerprinting was used to differentiate between Rio Red grapefruits grown under organic and conventional farming practices, as well as from different years and times of harvest.[13] HPLC fingerprinting provides detailed information about every major chemical but requires more time for method development and HPLC analysis. FIMS fingerprinting does not supply the concentration information but quickly provides more information about the entire specific ions of the sample because of direct injection involving no chromatographic separation. These two methods examine different aspects of chemical information about the food samples because of their different chemical mechanisms. If used together, combined chromatographic and FIMS fingerprints may better determine the difference between conventional and organic foods.

In the present study, the chemical patterns represented by HPLC-UV chromatographic fingerprinting and FIMS fingerprints were analyzed using PCA to differentiate commercial organic and conventional peppermints.

MATERIALS AND METHODS

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

Plant Materials and Sample Preparation. Ten organic and ten conventional commercial dry peppermint samples were gifts from Frontier Natural Products Co-op (Norway, IA, USA). The organic peppermint samples were USDA certified. All peppermint samples were ground to 20 mesh particle size using an IKA A11 analytical grinding machine (IKA, Staufen, Baden-Württemberg, Germany) and stored at −20 °C before analysis. One hundred milligrams of each peppermint powder was weighed and extracted with 10 mL of H_2O/MeOH (1:1, v/v) by ultrasonication for 30 min using a Fisher brand

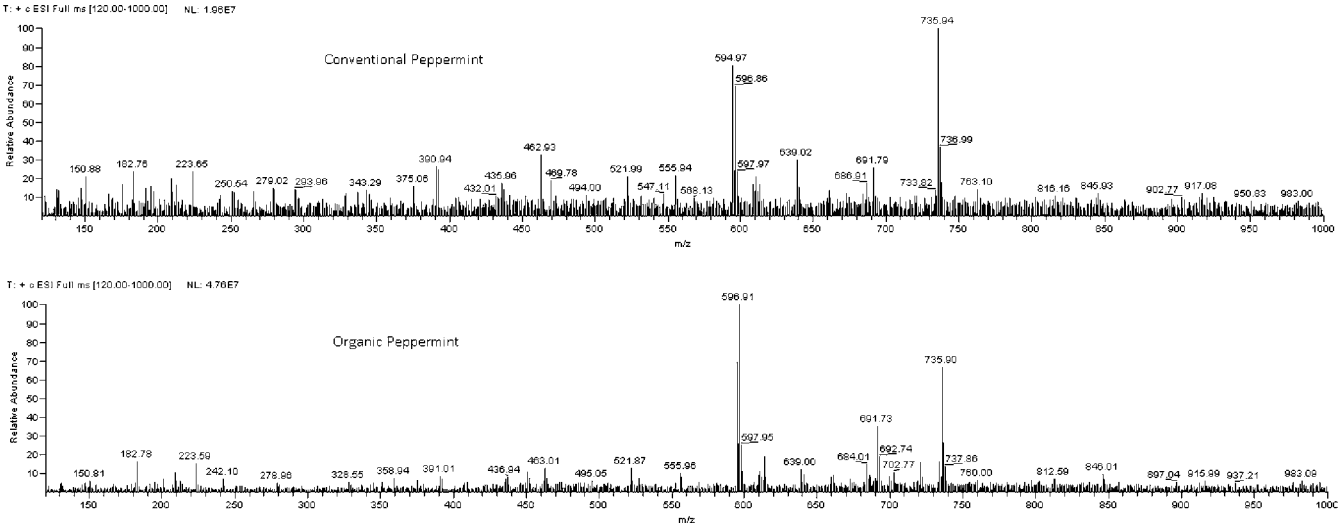

Figure 2. MS fingerprinting for IT-MS: conventional and organic peppermints.

ultrasonic machine (Fisher, Pittsburgh, PA, USA). The extracts were filtered through a 0.45 μm nylon syringe filter (Alltech Associates, Deerfield, IL, USA). Each extract was analyzed in triplicate.

HPLC-UV and IT-MS Analyses. An Agilent 1100 HPLC system was used with a binary pump, a vacuum degasser, a column oven, and an autosampler (Agilent Technologies, Palo Alto, CA, USA). FIMS system consisted of the same HPLC system in combination with an LCQ Decaion-trap mass spectrometer (IT-MS) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Electrospray ionization (ESI) in positive ion mode was used.

The HPLC conditions were as follows: A Symmetry C18 column (2.1 mm i.d. × 150 mm, 3.5 μm) (Waters, Milford, MA, USA) was used with a column temperature set to 40 °C. 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 initial ratio of B was 5%; this was changed linearly to 22% B in 10 min, kept at 22% B until 20 min, then increased linearly to 30% B in 30 min, held constant at 30% B to 35 min, increased to 35% B linearly at 45 min, kept at 35% B to 50 min, linearly raised to 95% B in 65 min, and then washed at this ratio for 5 min. After that, the ratio was returned to initial conditions for 5 min to re-equilibrate the column for the next injection. The flow rate was 0.2 mL/min, the injection volume was 10 μL, and the detection wavelength was set to 330 nm.

The conditions for IT-MS were as follows: No analytical column was used, but a guard column (Adsorbosphere All-Guard Cartridge, C18, 5 μm, 4.6 × 7.5 mm, Alltech Associates, Inc.) was used to minimize potential contamination of the MS system. Mobile phase A consisted of 0.1% formic acid in H_2O, and mobile phase B consisted of 0.1% formic acid in acetonitrile with isocratic elution at 60:40 (v/v) at the flow rate of 0.5 mL/min. Peppermint extractions were diluted 10 times with water, and the injection volume was 2 μL. MS Spectra were collected from 0.2 to 1 min, and the mass range was from m/z 120 to 1000. Sheath gas flow rate was 80 L/min, auxiliary gas flow rate was 10 L/min, spray voltage was 4.5 kV, heated capillary temperature was 250 °C, capillary voltage was −4.0 V, and tube lens offset was 20 V. Triplicate analyses of 20 different peppermint samples provided 60 MS spectra.

UPLC-Q-TOF MS Analysis. A Waters UPLC-Xevo G2 Q-TOF MS system (Milford, MA, USA) was used to further confirm the compound identification using the accurate mass weight. The conditions were as follows: peppermint extractions used an injection volume of 2 μL. A BEH C18 column (2.1 mm i.d. × 100 mm, 1.7 μm) (Waters) was used with a column temperature set to 40 °C. Flow rate was 0.2 mL/min. 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 initial ratio of B was 20%; this was changed linearly to 50% B in 20 min, then increased linearly to 95% B in 35 min, and washed at this ratio for another 5 min. The ratio was returned to initial conditions for 5 min to re-equilibrate the column for the next injection. The flow rate was 0.2 mL/min, the injection volume was 2 μL, and the detection wavelength was set at 330 nm. An ESI positive ion source was selected; capillary voltage was 3.00 kV, sampling cone voltage was 30 V, and extraction cone voltage was 4.0 V. Source temperature was set at 120 °C, with a desolvation temperature at 450 °C. Cone gas flow rate was 50 L/h, and the desolvation gas flow was 800 L/h. The mass range was from m/z 100 to 1000; ramp collision energy was from 25 to 35 V.

Data Processing. A total of 32 peaks were selected for identification and quantitation in the HPLC fingerprints of conventional and organic peppermints, and their absolute peak area and relative peak area were analyzed by PCA. For absolute peak area analyses, 32 peak areas in all 60 chromatograms (20 samples with triplicate analyses each) were analyzed with PCA directly. For relative peak area analyses, the largest peak area was selected as the reference peak, and other peak areas were converted to the relative peak areas against the reference peak in each chromatogram; the relative peak areas were analyzed for PCA using the SIMCA-P software (Umetrics, Malmo, Skåne län, Sweden) based on UV data.

FIMS fingerprints obtained as one-dimensional spectra (m/z 120−1000) were used for comparison. The data were imported into Excel (Microsoft, Inc., Belleview, WA, USA) for data preprocessing and then to SIMCA-P 10.5 (Umetrics) for PCA. The processing in Microsoft Excel was to combine the 60 spectra, sort the data by sample names, and fill the mass matrix with zero for each missing m/z in the mass list so that the data points of each mass spectrum were aligned at 881. The resulting two-dimensional matrix was 60 × 881 (60 samples and 881 masses for sample wash). The matrix was exported to SIMCA-P, and the mean centering was used before PCA.

RESULTS AND DISCUSSION

Chromatographic and MS Fingerprints. Fourteen components were identified using the accurate mass weight detected by UPLC-Q-TOF MS. These included pebrellin, eriodictyol 7-*O*-rutinoiside, tricetin 3′-*O*-glucoside 5′-*O*-rhamnoside, eriocitrin, kaempferol 7-*O*-rutinoside, luteolin 7-*O*-neohesperidoside, narirutin, 4′-methoxykaempferol 7-*O*-rutinoside, hesperidin, isosafrole, cyclohexanecarboxylic acid, gardenin D, 5,6-dihydroxy-4′,7,8-trimethoxyflavone, and gardenin, which correspond to peaks 1, 3, 7, 9, 10, 11,13, 15, 17, 19, 26, 30, 31, and 32 in a typical chromatographic fingerprint of peppermint (Figure 1), respectively. Typical chromatographic fingerprints for conventional and organic peppermints are shown in Figure 1. For each sample, a total of 32 major peaks

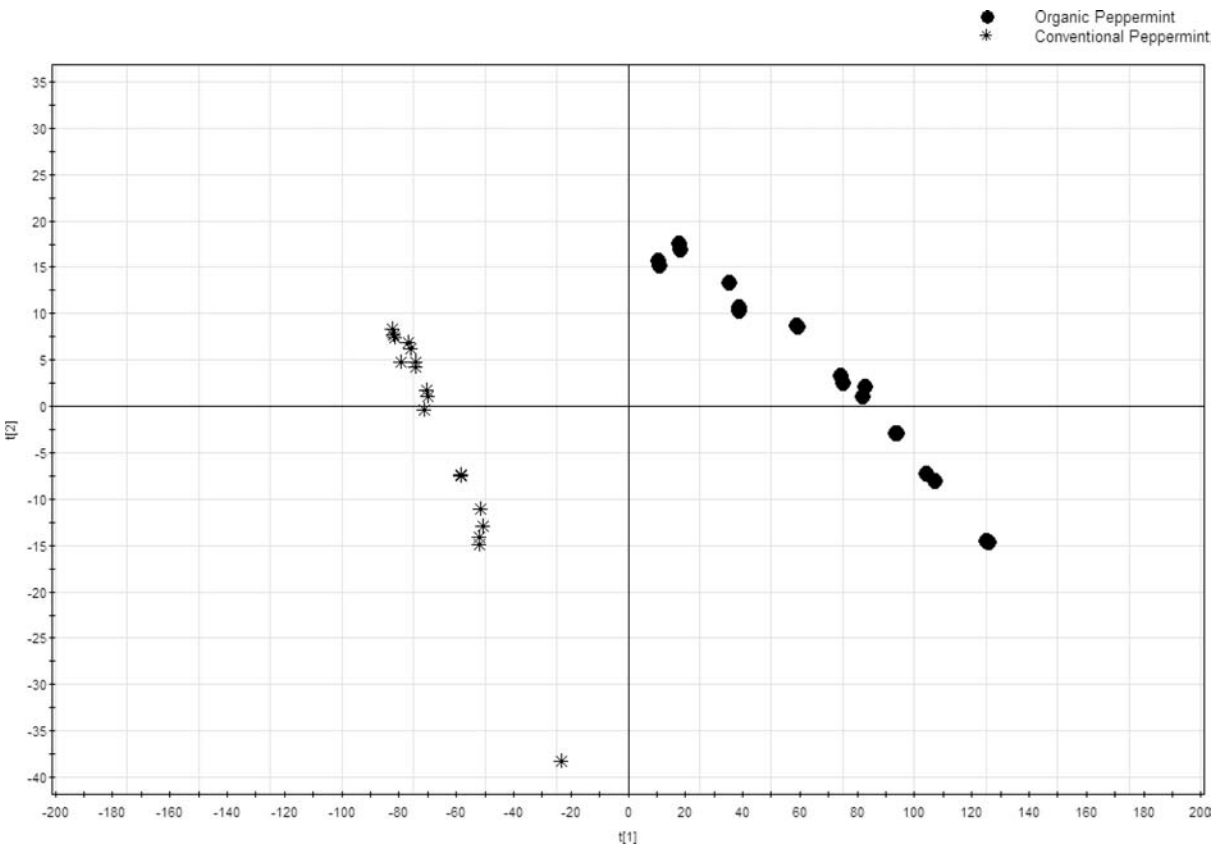

Figure 3. PCA scores plot for absolute peak area in different farming modes for HPLC fingerprinting.

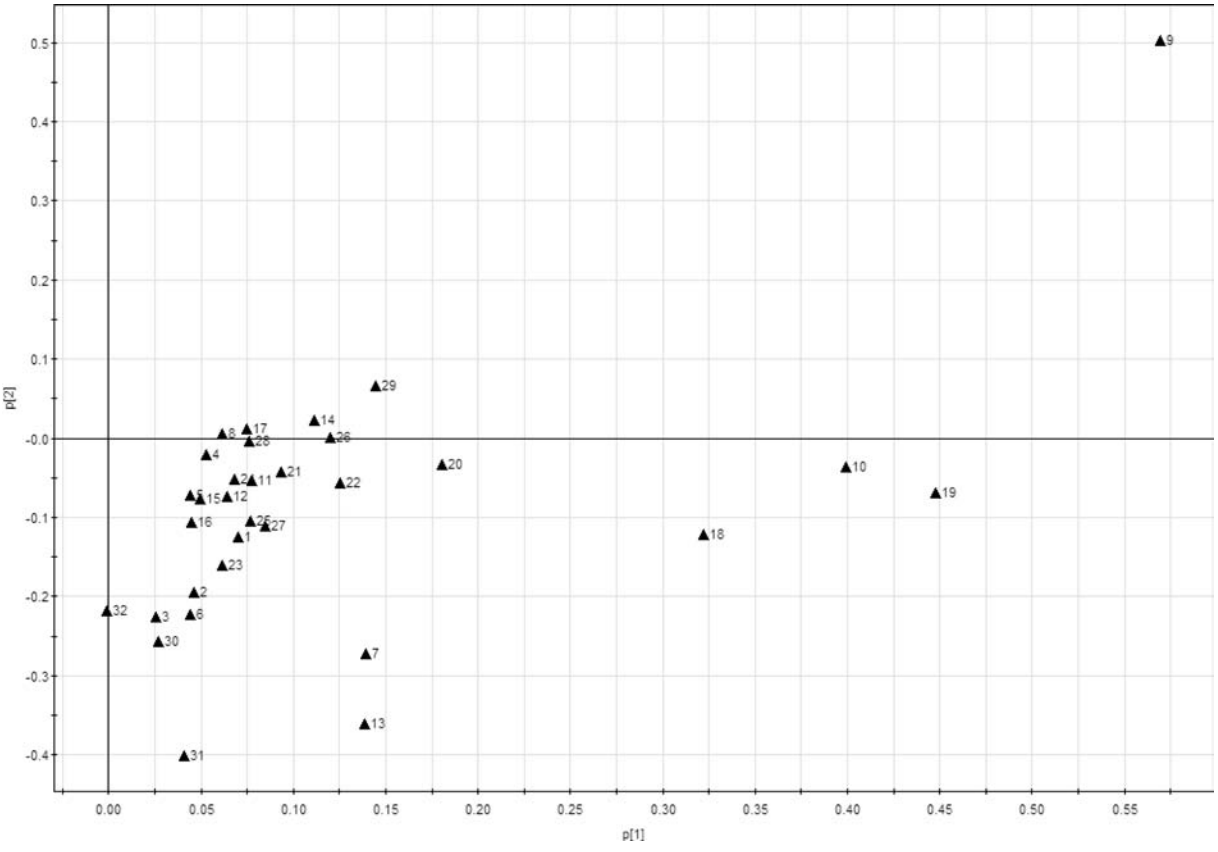

Figure 4. PCA loading plot for absolute peak area in different farming modes for HPLC fingerprinting.

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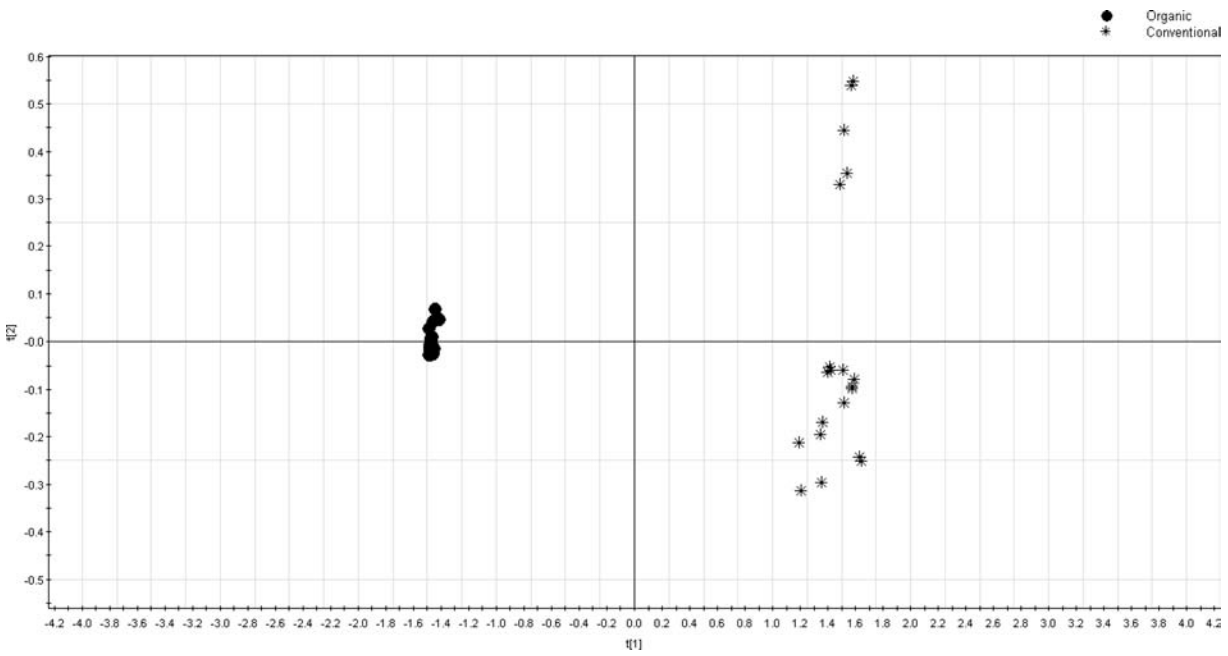

Figure 5. PCA scores plot for relative peak area in different farming modes. All peak areas were converted to their relative areas against that of peak 9 in the sample, and the relative peak areas were used for PCA.

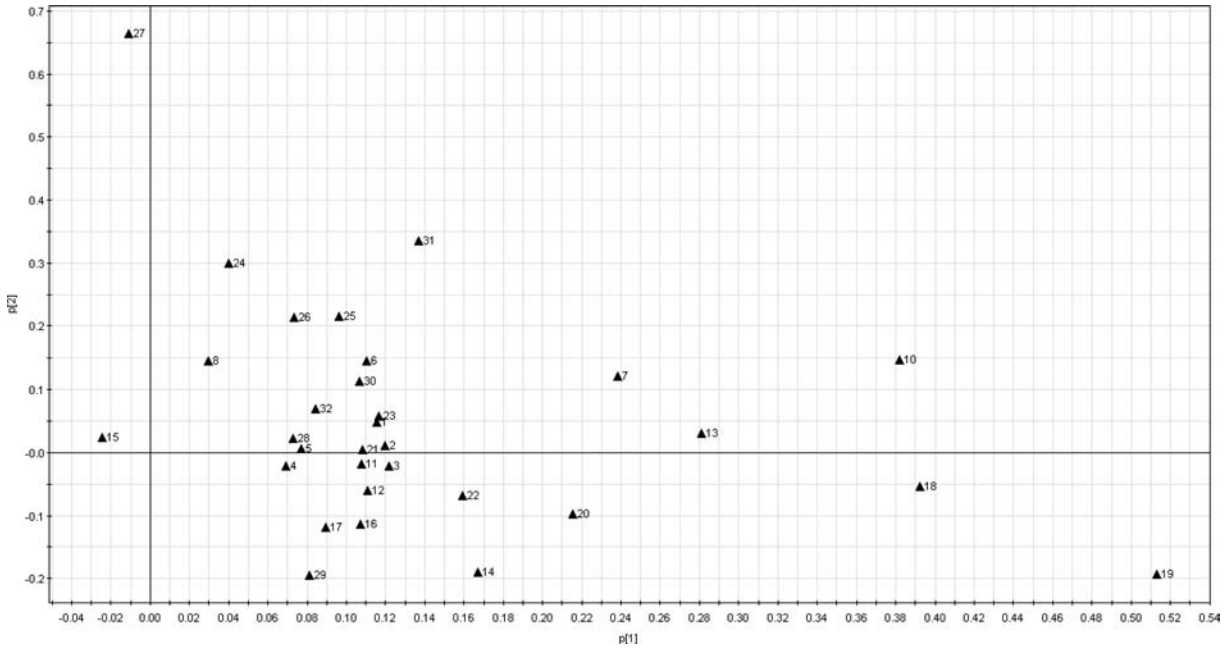

Figure 6. PCA loading plot for relative peak area in different farming modes. All peak areas were converted to their relative areas against that of peak 9 in the sample, and the relative peak areas were used for PCA.

were detected. Peak 9 was the greatest peak and was selected as the reference peak (RP) for calculating the relative peak area. Other major peaks were defined as at least 5% of the area of RP besides the last three peaks.

As shown in Figure 1, the peaks in the organic peppermint were generally greater than the corresponding peaks in the conventional counterpart sample on a same per botanical weight basis. Peak 15 was not detectable in any conventional peppermint samples. In addition, the average total area under the 32 peaks was 16581.26 ± 1322.06 for organic peppermint samples, which was 2 times more than that of 7388.28 ± 2741.41 for the 10 conventional peppermints.

Typical FIMS fingerprints for the conventional and organic peppermints are shown in Figure 2. Although the mass spectrum had almost the same significant peaks between conventional and organic peppermint extracts, the natural abundances of their major peaks were different. In the spectrum of conventional peppermint, m/z 735 was the greatest natural abundance peak, followed by m/z 594 (kaempferol 7-*O*-rutinoside), whereas for the organic peppermint, m/z 596 was generally the greatest peak and m/z 735 was the second. In addition, the average of total ion responses of FIMS spectra for organic samples was 4.75×10^7, more than 2 times greater than that of 1.96×10^7 for the conventional peppermint.

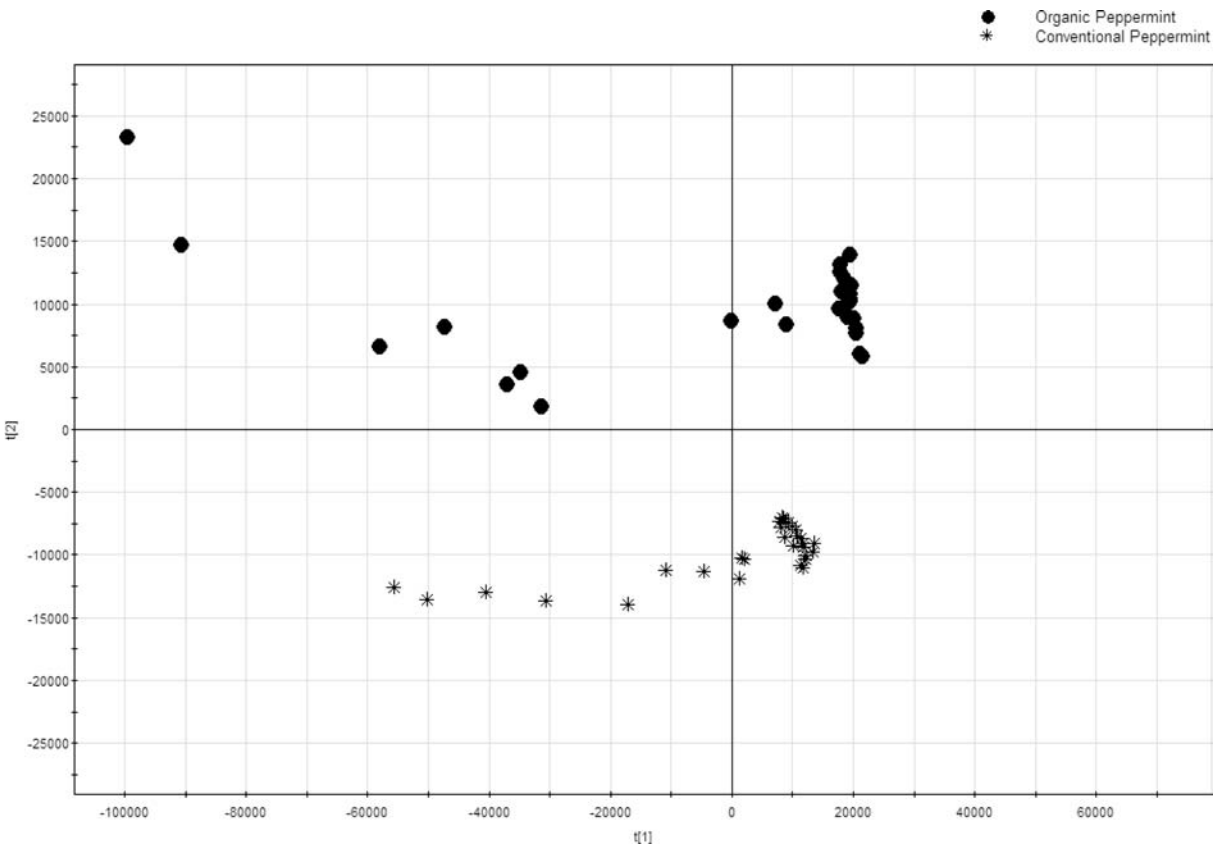

Figure 7. PCA scores plot for farming mode for IT-MS fingerprintings.

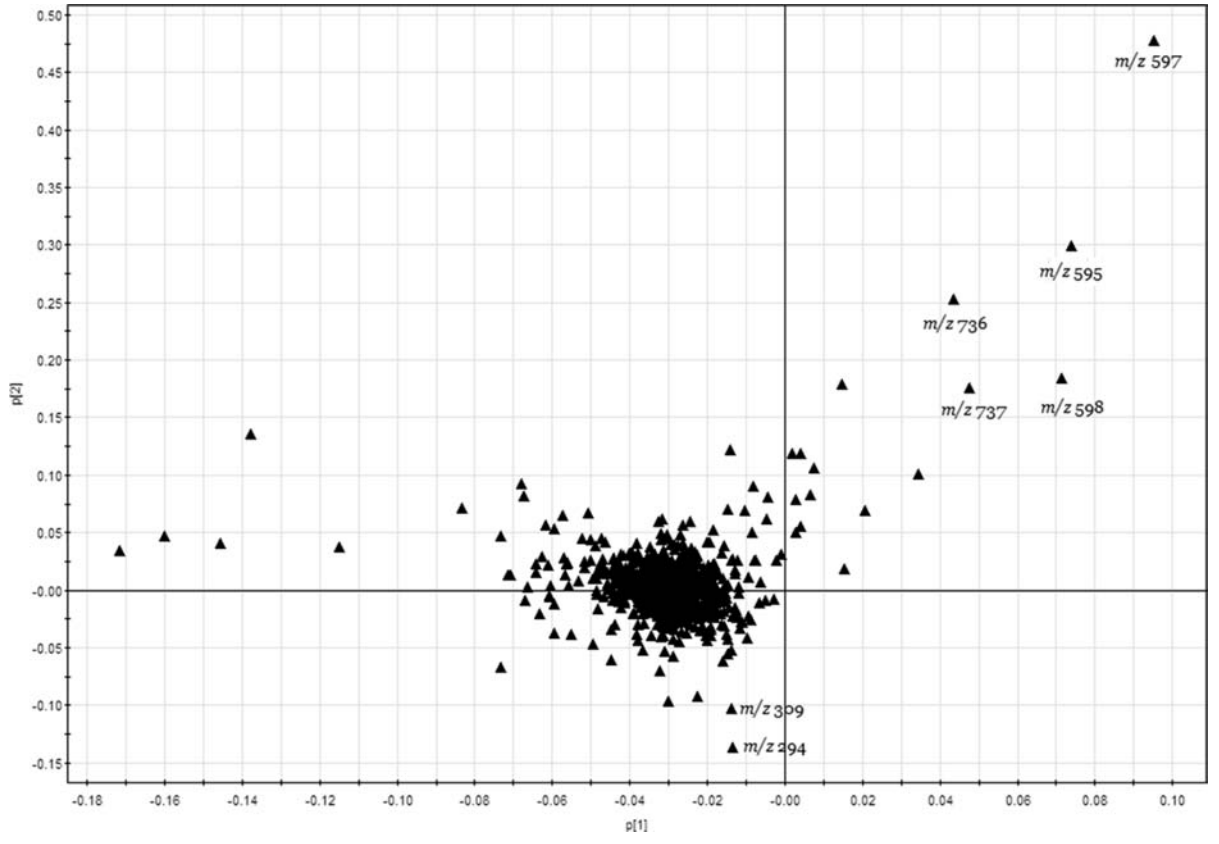

Figure 8. PCA loading plot for farming mode for IT-MS fingerprintings.

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PCA of Chromatographic Fingerprints. PCA uses a mathematical procedure to transform a number of correlated variables into a smaller group of uncorrelated variables, the principal components. PCA of fingerprints generally makes it easy to visually compare significant difference and avoids subjective decisions.

The PCA scores plot and loading plot of the chromatographic absolute peak areas are reported in Figures 3 and 4, respectively. In these two figures, the absolute peak areas of the 32 peaks were selected for PCA. As shown in Figure 3, the conventional peppermint samples were all on the left of the PCA scores plot, whereas all organic samples were located on the right side. In the loading plot of HPLC absolute peak areas (Figure 4), peaks 9, 19, 10, and 18 contributed significantly to the separation of organic samples from the conventional ones. With the HPLC chromatographic fingerprint (Figure 1) and PCA loading plot (Figure 4), the organic peppermint samples contained greater concentrations of almost all of the chemical compounds than its conventional counterpart.

Figures 5 and 6 are the PCA scores plot and loading plot of the relative peak areas for the organic and conventional peppermints, respectively. In Figure 5, all of the organic peppermint samples were clustered tightly in the left side of the scores plot, whereas conventional peppermint samples were all in the right side of the score plot. The tight cluster of the scores plot from relative peak areas indicated that all of the organic peppermint samples had relatively uniform chemical profiles, or the concentration of 31 major chemical components in all of the organic peppermint samples had a similar ratio relative to peak 9. In the loading plot of relative peak areas (Figure 6), only peaks 15 and 27 had negative PC1 values, indicating that the relative areas of these two peaks were greater in the organic peppermint samples, and the relative areas for the remaining peaks were greater in the conventional peppermint samples. Because peak 9 was selected as the RP and the areas of all the other peaks were normalized against RP, the results also indicated that the peak areas of RP were greater in organic samples. Furthermore, peaks 19, 10, and 18 contributed most to the organic peppermint in the loading plot, confirming that peaks 9, 10, 18, and 19 not only represented the four primary chemical components but also were the four most important peaks to determine if a peppermint was organically or conventionally produced using HPLC chromatographic fingerprinting.

PCA of MS Fingerprints. The FIMS PCA scores plot and loading plot are shown in Figures 7 and 8, respectively. In the scores plot of MS flow injection, all of the organic peppermint samples were in the upper side, whereas the conventional peppermint samples were in the lower side (Figure 7). The organic and conventional peppermints were separated completely by PC2. The scores plot indicated that the FIMS fingerprinting technique might effectively differentiate conventional and organic peppermints in 1 min.

In the loading plot of FIMS fingerprints, most of the ions were clustered near the central zero line (Figure 8). However, a few ions dispersed far away from the center, and these ions and their concentrations were most important to determine the farming practice of peppermint. In the upper right corner, ions m/z 595, 597, and 736 together with their isomer peaks m/z 596, 598, and 737 yield a higher score and would lead to positions in the upper side of the PCA scores plot. The most intense ion contributing to high scores was m/z 597 $[M + H]^+$, the molecular ion of 598 for eriodictyol 7-*O*-rutinoside. Another high score ion was m/z 736 $[M + H]^+$ for molecular ion 737.

The most noticeable ions that contributed negatively to PC2 were ions at m/z 294 and 309. The other ions that also contributed positively to conventional peppermint samples were ions at m/z 266 and 280. The scores of these ions indicated that these compounds contributed most in the differentiation of organic and conventional peppermint samples.

In summary, the results from this study indicated that the commercial organic and conventional peppermints may have significantly different chemical profiles and nutrient compositions. Both HPLC and FIMS fingerprints could effectively differentiate organic and conventional peppermints. FIMS may provide a rapid test and has potential for high-throughput applications. On the other hand, HPLC fingerprints provide more information about the chemical composition for the samples with a longer analysis time.

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Notes

The authors declare no competing financial interest.

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