Full Paper

Journal of the Brazilian Chemical Society

https://dx.doi.org/10.21577/0103-5053.20260057

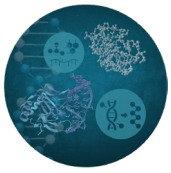
J. Braz. Chem. Soc. **2026**, *37-Spe1*, e-20260057, 1-14

Comparative Metabolomic Profiling of Soil-Based and Hydroponic Lettuce Varieties Using Paper Spray Ionization Mass Spectrometry and Chemometric Approaches

Lucas S. Machado,[#,a] Lanaia Í. L. Maciel,[#,a] Almir C. Batista Junior,[a] Yuri A. Rocha,[a] Boniek G. Vaz,[a] Andrea R. Chaves[,a] and Jussara V. Roque[*,a]*

[a]*Centro de Excelência em Estudos Moleculares, Energia e Petróleo (CEMEP), Instituto de Química, Universidade Federal de Goiás, 74690-900 Goiânia-GO, Brazil*

This study explores the application of paper spray ionization-mass spectrometry (PSI-MS/MS) in evaluating the metabolomic profiles of lettuce varieties cultivated under soil-based and hydroponic conditions. Compared to electrospray ionization tandem mass spectrometry (ESI-MS/MS), the PSI-MS/MS technique demonstrated improved sensitivity and robust ion signal intensity, allowing effective discrimination between the chemical constituents of the lettuce samples. Significant metabolic differences were identified between the cultivation methods, particularly in the negative ionization mode. PSI-MS/MS provided detailed insights into the chemical composition due to its high sensitivity and minimal sample preparation requirements. The study further analyzed three lettuce varieties (loose leaf, iceberg, and oak leaf) using PSI-MS/MS, revealing distinct ion profiles and variation in ion abundances. Chemometric techniques, including parallel factor analysis (PARAFAC) and partial least squares for discriminant analysis (PLS-DA) with variable selection were utilized to differentiate between lettuce varieties and cultivation methods. This approach highlighted the impact of growing conditions on the metabolic composition of lettuce, identifying key ions such as choline, mannose, and malic acid as significant markers. Overall, the integration of PSI-MS/MS with chemometric tools provides a comprehensive approach for metabolomic profiling, offering valuable insights into the effects of cultivation methods on the nutritional and chemical properties of lettuce. This study underscores the potential of PSI-MS/MS in agricultural and food science research, contributing to the optimization of cultivation practices and the enhancement of crop quality, while also adhering to the principles of green analytical chemistry.



Keywords: paper spray ionization, PARAFAC, PLS-DA, lettuce hydroponic and soil-based agriculture, iceberg lettuce, loose leaf lettuce, oak leaf lettuce

Introduction

Lettuce (*Lactuca sativa* L.) is among the most widely consumed leafy vegetables globally. The iceberg, loose leaf, and oak leaf varieties are extensively documented as rich sources of polyphenols and antioxidant compounds with significant health benefits.[1] Lettuce cultivation is typically performed using two primary methods: soil-based and hydroponic agriculture.[2] In the soil-based approach, plants are cultivated in soil, which supplies the essential nutrients necessary for their growth and development.[3] Despite the long-standing use of soil-based agriculture, it is increasingly challenged by soil degradation and reduced fertility of agricultural lands worldwide, necessitating alternative cultivation strategies. In this sense, hydroponic agriculture has seen widespread adoption over the past few decades.[4] In hydroponic systems, plants receive nutrients through a carefully managed aqueous medium, ensuring optimal development.[3]

Although lettuce varieties share similar physical characteristics, the literature reports[1,5] differences in their chemical profiles, highlighting the unique chemical composition of each variety, especially regarding bioactive compounds. Most studies on growth strategies focus on the growth rates of lettuces cultivated using soil-based and hydroponic methods, primarily considering physical aspects.[6,7] Traditional evaluation methods often exclude the chemical profile of lettuce samples, which could provide a better understanding of the availability of compounds based on the cultivation method used.[6,7] Furthermore, analyzing

*e-mail: andrea_chaves@ufg.br; jussara_roque@ufg.br
#Equal contribution to this work.
Editor handled this article: Alessandra Sussulini (Guest Editor)
This publication is part of the special issue "Omics Sciences"

the chemical profiles of lettuce varieties in conjunction with growth strategies could yield essential information to distinguish between lettuce samples, potentially linking these differences to the nutritional value of the vegetable.[8,9]

Metabolomic approaches are commonly applied to evaluate the chemical fingerprint of a single or a group of compounds in a living organism.[10] One of the most used techniques for metabolomics analysis is mass spectrometry (MS).[11,12] The use of MS tools is described as an outstanding strategy in food science for being able to provide a deeper understanding of the differences in the chemical profile as well as the presence of important food nutrients present in human diets according to their sources.[13,14] Therefore, leading to the acquisition of important information, such as the differences in the growing medium for different food matrices.[15] The application of analytical methods based on MS can provide a chemical ion profile of the sample which possibilities the creation of its fingerprint, allowing the differentiation of the plant according to the condition of growth submitted or based on its variety.[16]

Most metabolomic studies employing MS are coupled with chromatography. This integration ensures efficient separation of the compounds present in the sample, drastically reducing ion suppression effects, and consequently improving detectability and increasing the number of compounds that can be identified.[17] Even though these methods efficiently unveil metabolomic profiles, they normally require a preliminary sample preparation step to preserve the analytical system.[17] Furthermore, these analyses are time-consuming, demand high solvent consumption, and rely on expensive chromatographic columns.[17] In this context, paper spray ionization mass spectrometry (PSI-MS) is one of the most straightforward and cost-effective methods within ambient mass spectrometry (AMS), enabling the acquisition of specific molecular profiles in just a few minutes.[18,19] PSI-MS stands out within AMS for its high sensitivity and selectivity, allowing for *in situ* analyses and eliminating the need for sample preparation steps.[20,21] In traditional PSI-MS, the sample is placed on a triangular piece of paper, and ionization occurs upon applying a voltage of 2 to 5 kV.[20,21] Adding a basic or acidic solution to the surface of the paper facilitates analyte desorption, generating a Taylor cone spray that is directed toward the MS inlet.[22,23] PSI has been used to fingerprint food sources, enabling differentiation and discrimination based on the chemical profiles of samples using established metabolomic databases.[24,25]

Metabolomics datasets can encompass thousands of metabolites, rendering manual analysis both labor-intensive and susceptible to errors.[12,26,27] Chemometric approaches offer more precise data processing methods, such as normalization and scaling, which mitigate systematic variations and enhance sample comparability.[12,26,27] Considering the numerous advantages of PSI-MS over traditional techniques, such as reduced solvent usage, high sensitivity, and high analytical throughput, its application in metabolomic protocols has been encouraged.[24,28] Moreover, the integration of data provided by MS with chemometric approaches opens new possibilities regarding the separation and classification of samples based on their metabolomic profiles.[24,28] Therefore, this study is committed to evaluating the metabolomic profile of lettuce in relation to different cultivations and varieties, and to integrating PSI-MS data with chemometric analyses for high-accuracy sample classification. This approach could encourage the application of PSI-MS in metabolomic studies, contributing to the identification and characterization of compounds with high analytical throughput and low solvent consumption, aligning with the principles of green chemistry.

Experimental

Chemicals and reagents

High-performance liquid chromatography (HPLC)-grade methanol was purchased from J. T. Baker (Phillipsburg, U.S.A.). Whatman #1 paper (11 μm pores) was purchased from Whatman International Ltd. (Maidstone, England). Ultra-purified water was obtained from MS2000 WFI equipment (Gehaka, São Paulo, SP, Brazil).

Soil-based and hydroponic lettuce extracts obtention

Soil-based and hydroponic lettuce samples were obtained in the local markets (Goiânia-GO, Brazil). The study evaluated three varieties of lettuce (*Lactuca sativa* L.): iceberg lettuce, loose leaf lettuce, and oak leaf lettuce. The samples were carefully stored at a controlled temperature (8 ºC) for posterior analysis. For the extract obtention, 300 mg of crushed lettuce were placed in a 15 mL Falcon tube and macerated using a glass rod. Subsequently, 10 mL of a methanol/water (80:20, v/v) mixture were added, and the sample was sonicated for 30 min at room temperature (25 °C). The extract was centrifugated at 10000 rpm for 10 min (Thermo Scientific Sorvall Legend X1R). Lastly, the supernatant was collected for further PSI-Orbtrap-MS and ESI-Orbtrap-MS analysis. Figure 1 illustrates the analytical protocol from sample preparation to data processing. It showcases the key phases of the study: sample preparation, PSI-MS/MS analysis, and data processing.

The figure provides a schematic workflow of the entire study, detailing each step and highlighting the integration of various techniques and analyses.

Mass spectra analysis

A Q Exactive Quadrupole-Orbitrap hybrid mass spectrometer (Thermo Scientific, San Jose, USA) was used for the electrospray ionization tandem mass spectrometry (ESI-MS/MS) and PSI-MS/MS analyses. The ESI-MS/MS analyses were obtained using a sample solution containing 500 µL of lettuce extract and 500 µL of methanol/water solution (80:20 v/v). ESI-MS/MS was performed both in positive and negative ionization modes. The optimized ESI-MS/MS parameters were: capillary temperature: 275 °C; capillary voltage: 20 V; spray voltage: 4.5 kV; S-lens voltage: 60 V; nebulizer gas: N_2; source temperature: 50 °C; sheath gas: 2 Arb (arbitrary units); auxiliary gas: 5 Arb; sweep gas: 0 Arb; injection flow: 2 µL min^{-1}, resolution at 140000, three micro scans, and collision energy for MS/MS (higher-energy collisional dissociation - HCD) ranging from 20 to 30 eV.

PSI-MS/MS analysis were performed using 20 µL of the extract onto the surface of a triangular paper (1 cm on each side). The tip of the paper was positioned approximately 4 mm from the mass spectrometer inlet. A 10 µL of methanol/water (80:20 v/v) were used as a spray solvent immediately after applying the lettuce extract solution to the paper. The experiment was carried out in triplicate measurements, and the mass spectra were collected for 1 min (54 scans). All analyses were performed in positive (spray voltage: 4.5 kV) and negative (spray voltage: 3.0 kV) ionization modes, with the capillary temperature maintained at 275 °C, S-lens: 60 V, resolution at 140000, three micro scans, and collision energy for MS/MS HCD ranging from 20 to 30 eV. The order of sample injections by PSI-MS/MS and ESI-MS were randomized, with spectra acquired in a *m/z* range of 100 to 1000. The resulting spectra was processed using the Xcalibur Analysis software package (version 2.0, Service Release 2, Thermo Electron Corporation, 2011) and exported as .raw files for further chemometric analysis.

Chemometric analysis

Forty-three lettuce samples were used in data analysis, comprising twenty-three samples from soil-based agriculture and twenty from hydroponic culture. These samples were categorized into three different varieties: loose leaf, iceberg, and oak leaf lettuce. Specifically, the loose leaf variety included 15 samples, with 8 from hydroponic and 7 from soil-based systems. The iceberg variety had 16 samples, consisting of 4 hydroponic and 12 soil-based. Finally, the oak leaf variety consisted of 12 samples, with 8 from hydroponic and 4 from soil-based systems. The obtained .raw files were converted into .cdf format using Xcalibur software (Thermo Scientific, San Jose, USA, 2011) and subsequently imported to MATLAB R2024a (Math Works, Natick, MA, USA, 2024) for further chemometric analysis.

To explore the complex metabolomic profiles of the lettuce samples and differentiate between the cultivation methods and varieties, a series of advanced chemometric analyses were applied. These analyses not only aid in

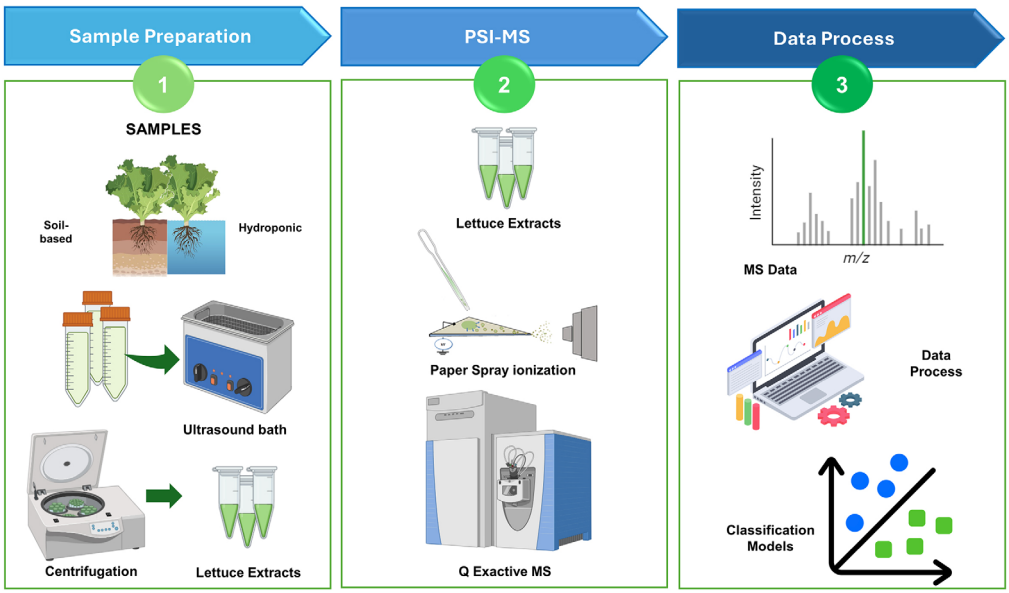


Figure 1. Workflow of metabolomic analysis of lettuce using PSI-MS and chemometric approaches.

decomposing and interpreting the multidimensional data obtained from PSI-MS/MS but also enhanced the accuracy of classification and variable selection. The chemometric methods were crucial in uncovering the subtle metabolomic differences influenced by agriculture and the specific lettuce varieties.

Parallel Factor Analysis (PARAFAC) was employed to explore complex metabolomic profiles and facilitate the interpretation of metabolomic data. This multivariate technique is widely used for decomposing data into independent components, thus being particularly effective for analyzing datasets with three-dimensional or higher structures, such as those obtained in metabolomics.[29,30] In this study, PSI-MS/MS data were organized into a three-dimensional tensor, where the three dimensions represented lettuce samples, time acquisition, and mass spectra (*m/z*). The tensor originally included the mass spectra from 43 lettuce samples, with 54-time acquisition scans, and 499345 and 372679 variables (*m/z* 100 to 1000) from positive and negative ionization modes, respectively. To reduce the dimensionality of the *m/z* axis, principal component analysis (PCA) was applied in normalized data, retaining five principal components that accounted for over 99% of the variance in both ionization modes. This dimensionality reduction facilitated the PARAFAC analysis with non-negativity constraints applied to sample and time acquisition modes to ensure meaningful and interpretable results.

Partial Least Squares Discriminant Analysis (PLS-DA) was applied to model the separation of different cultivations and varieties in positive and negative ionization modes of datasets. This supervised pattern recognition method, based on PLS regression, is frequently used for classifying samples into predefined categories.[31] In PLS-DA, a categorical dependent variable containing zeros and ones to indicate if a sample belongs or not to the class. In this study, PLS-DA was applied separately in two datasets: $\mathbf{X}_{pos}$ and $\mathbf{X}_{neg}$, corresponding to the data from the positive and negative ionization modes, respectively. Each dataset was analyzed with two dependent variables: the $\mathbf{Y}_1$ with two categories of soil-based and hydroponic agriculture lettuce samples classification and $\mathbf{Y}_2$ with three classes related to lettuce varieties. This approach resulted in the construction of four distinct models, two for each ionization mode, one set of models distinguishing between cultivation methods and the other between lettuce varieties. To ensure the robustness of the models and prevent overfitting, random cross-validation was employed to determine the optimal number of latent variables (nlv). In each iteration, the dataset was randomly partitioned into training and validation sets, with 10% of the samples reserved for validation. The number of latent variables was selected based on the cross-validation results, ensuring an optimal balance between model complexity and predictive performance.

A variable selection method was also applied to improve classification and to find the most important variables related to the differentiation of cultivation and lettuce varieties. Ordered predictor selection for discrimination analysis (OPSDA) is an adaptation from the OPS method, a variable selection method for regression.[32] In this study, OPSDA was applied in combination with PLS-DA as the underlying supervised classification method. OPSDA is based on an informative vector that contains information about the location of the best response variables for differentiation. Our study used the variable importance on projection (VIP) as an informative vector. The original variables (**X** matrix columns) were differentiated according to the corresponding absolute values obtained by VIP scores. Then, the differentiated variables were sorted in descending order, and a window-increment (10-5) strategy were applied until the best set of variables were found. Classification models were evaluated using sensitivity, specificity, and classification error.[33] VIP scores were used to rank the most important variables for distinguishing between different cultivation methods and lettuce varieties.

Compounds characterization

The most significant variables (*m/z*) selected by chemometric tools were submitted to the Global Natural Products Social Molecular Networking (GNPS), the Human Metabolome Database (HMDB), and MassBank Europe database to identify and elucidate their structures. The MS/MS spectra associated with each variable were then employed for chemical structure elucidation and identification.

Results and Discussion

Comparison between the direct infusion and PSI-MS approaches for soil based and hydroponic lettuce agriculture analysis

PSI-MS/MS and direct infusion of extracted samples using ESI-MS/MS were employed to assess the ion profiles of lettuce cultivated under soil-based agriculture. ESI-MS/MS analyses were conducted in both positive and negative ionization modes to comprehensively evaluate the ion profiles of the samples. According to Figures 2a and 2d, distinct visual differences between the average mass spectra obtained by PSI-MS/MS and direct infusion ESI-MS/MS were observed within the mass range of *m/z* 100 to 1000.

The mass spectra generated by ESI-MS/MS exhibited lower sensitivity compared to those obtained by PSI-MS/MS. The enhanced sensitivity of PSI-MS/MS is evident from the higher ion intensity signals, indicating its superior performance in detecting and characterizing the chemical constituents of the lettuce samples. Additionally, Figures 2a and 2d illustrate differences in ion intensity signals between the two analytical methods, demonstrating the superior sensitivity of PSI-MS/MS in analyzing the extracted lettuce samples. Moreover, the observed differences in ion profiles between the two methods suggest that PSI-MS/MS may be more suitable for applications requiring high sensitivity and detailed chemical characterization.

The literature[34,35] documents the use of both direct infusion ESI-MS/MS and PSI-MS/MS methods for analyzing the ionic profiles of various samples. Although both techniques are effective, PSI-MS/MS demonstrates superior analytical performance compared to direct infusion ESI-MS/MS.[36] This enhanced effectiveness is primarily due to the chromatographic paper used as a substrate in PSI-MS/MS, which aids in the ionization of analytes. The substrate, composed of cellulosic fibers, features amorphous porous channels that allow for solvent absorption and transport by capillarity, akin to traditional chromatographic separations. These characteristics facilitate the resolubilization of samples on chromatographic paper, thereby significantly increasing the sensitivity of PSI-MS/MS experiments.[37] As a result, PSI-MS/MS provides a more robust and sensitive analysis of the chemical constituents within the samples compared with ESI-MS, highlighting its value for comprehensive metabolomic studies.

Metabolic differences in leafy vegetable samples are well-documented in the literature. Hameed *et al.*[38] investigated these differences in lettuce samples grown under various conditions (soil, substrate, and hydroponic) with the application of essential nitrogenous compounds for plant development. Their study utilized separation methods such as gas chromatography mass spectrometry (GC-MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) for metabolic evaluation. However, these methods usually involve more laborious sample preparation, higher solvent consumption, and longer analysis times.

Nevertheless, the introduction of AMS methods addresses several limitations of conventional techniques. In this study, we demonstrated the effectiveness of PSI-MS/MS in eliminating the extensive sample preparation required by traditional metabolic profiling methods. The comparison between ESI-MS/MS and PSI-MS/MS reinforces the utility of PSI-MS/MS in metabolomic studies, particularly within agricultural and food sciences, where precise chemical profiling is essential for understanding the impact of different cultivation methods on the nutritional and chemical composition of production. Furthermore, low solvent consumption and rapid ionization process of the PSI-MS/MS method make it a straightforward and

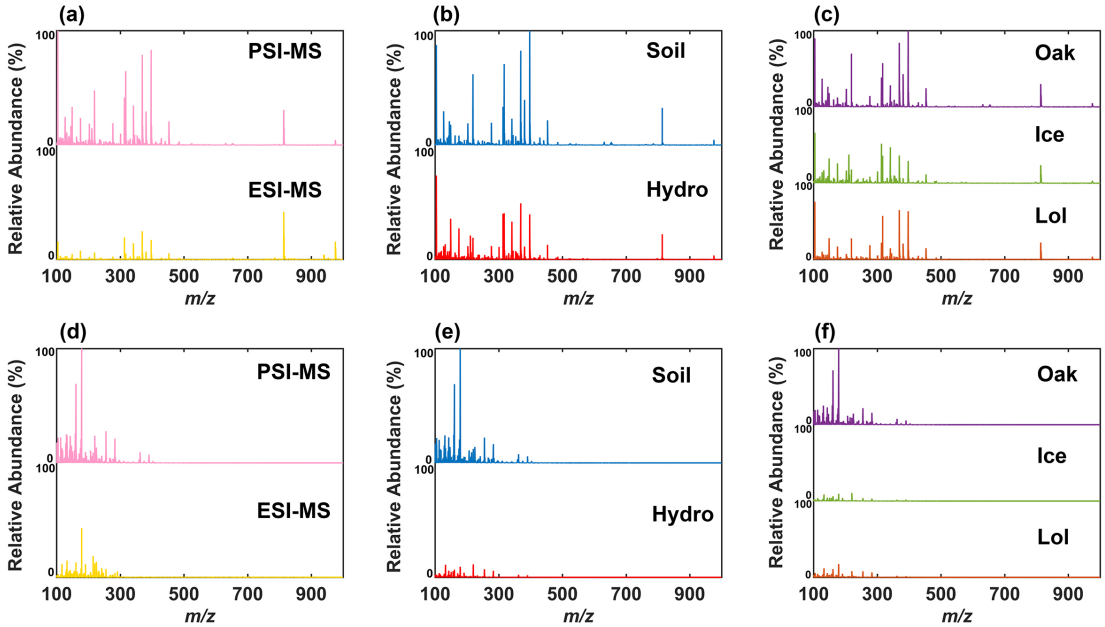


Figure 2. Average mass spectra (relative abundance) obtained from (a) lettuce samples by PSI-MS/MS or ESI-MS/MS positive ionization mode, (b) hydroponic agriculture or soil-based lettuce samples by PSI-MS/MS positive ionization mode, (c) oak leaf (Oak), iceberg (Ice), or loose leaf (Lol) variety lettuce samples by PSI-MS/MS positive ionization mode, (d) lettuce samples by PSI-MS/MS or ESI-MS/MS negative ionization mode, (e) hydroponic agriculture or soil-based lettuce samples by PSI-MS/MS negative ionization mode (f) oak leaf (Oak), iceberg (Ice), or loose leaf (Lol) variety lettuce samples by PSI-MS/MS negative ionization mode.

efficient technique, aligning with green analytical chemistry principles.[39]

PSI-MS/MS analysis of soil-based and hydroponic agriculture lettuce samples

Lettuce extracts of soil-based and hydroponic agriculture were analyzed using PSI-MS/MS. Figure 2b shows the mass spectra of lettuce extracts grown in soil and hydroponic conditions analyzed in positive ionization mode, while Figure 2e shows the mass spectra in negative ionization mode. Positive PSI-MS/MS spectra exhibited similar mass profiles with some differences in signal intensity between the two cultivation methods. On the other hand, distinct differences were observed in the negative ionization mode. According to the findings, the metabolic differences between lettuce extracts grown in conventional soil and hydroponic conditions were evidenced most prominently in the negative ionization mode PSI-MS/MS.

These variations in the chemical profile of the samples are due to its cultivation, once plants synthesize a variety of essential substances in response to their growing conditions. Thus, comparing the nutrients of plants grown in soil-based *versus* hydroponic agriculture involves numerous factors, such as specific cultivation practices, soil type, nutrients in the hydroponic solution, and management of growing conditions. Environmental factors, including temperature variations, can also influence vapor pressure deficit, affect evaporative stress and potentially alter differences in water usage.[40,41] Consequently, the metabolic composition and the bioactive compounds are directly impacted. These bioactive compounds play crucial roles in the defense mechanisms of the plant against biotic (pathogens, pests) and abiotic (temperature, light, water availability) stress. These substances include sugars and amino acids, phenolics, proteins, starches, and cell wall components, which are important bioactive compounds that improve the quality of agricultural products.[42] The bioactive compounds collectively enhance the quality and nutritional profile of agricultural products, making them more beneficial for human consumption and more resilient to environmental challenges. Therefore, the ion profiles of hydroponically grown lettuce extracts (Figure 2e) showed lower intensities compared to those grown in soil, likely due to differences in cultivation conditions.

PSI-MS/MS analysis of different lettuce varieties

After validating the performance of PSI-MS/MS on lettuce extracts grown in soil and hydroponic conditions across positive and negative ionization modes, the method was extended to analyze extracts from different lettuce varieties. Figure 2c presents the mass spectra of extracts from loose leaf, iceberg, and oak leaf lettuce in positive mode, while Figure 2f displays the corresponding spectra in negative mode. Visual inspection of the mass spectra revealed similar ion profiles for all lettuce varieties in positive mode, though with varying ion abundances among loose leaf, iceberg, and oak leaf (Figure 2c). In negative mode, distinct mass spectra profiles and ion abundances were observed for each lettuce variety. Notably, the oak leaf variety exhibited higher sensitivity compared to loose leaf and iceberg (Figure 2f).

While the PSI-MS/MS method provides valuable visual differentiation of ion profiles for distinguishing between lettuce varieties, this approach alone does not offer a thorough analysis of the ion profiles. To achieve a more comprehensive understanding of the metabolic differences among lettuce varieties, it is essential to apply chemometric tools. These tools facilitate advanced data analysis and interpretation, enabling a deeper insight into the nuanced variations between the varieties.

Chemometrics differentiation and compounds characterization

Following the application of the chemometrics tool for data interpretation, PARAFAC analysis was conducted on a **T** tensor constructed for each ionization mode. The PARAFAC model for the positive ionization mode converged after 42 iterations, achieving a core consistency of 93% with four components. The loading plots for the samples mode, presented in Figure 3, provide a detailed visualization of the differentiation achieved. The first component effectively discriminates between lettuce varieties. As shown in Figures 3a-3c, the loose leaf, iceberg, and oak leaf varieties form distinct clusters along this component. This separation indicates unique metabolic profiles for each variety, demonstrating the ability of the PARAFAC model to identify differences in metabolic composition. Subsequent components highlight the separation of cultivation methods within each variety. Figure 3d shows the distinction between hydroponic and soil-based agriculture for iceberg samples along component 2. Similarly, Figures 3e and 3f illustrate the separation of loose leaf and oak leaf samples along components 3 and 4, respectively. These separations underscore the significant metabolic differences induced by the growing conditions.

The PARAFAC model for the negative ionization mode converged after 16 iterations, achieving a core consistency of 99% with two components. The loading

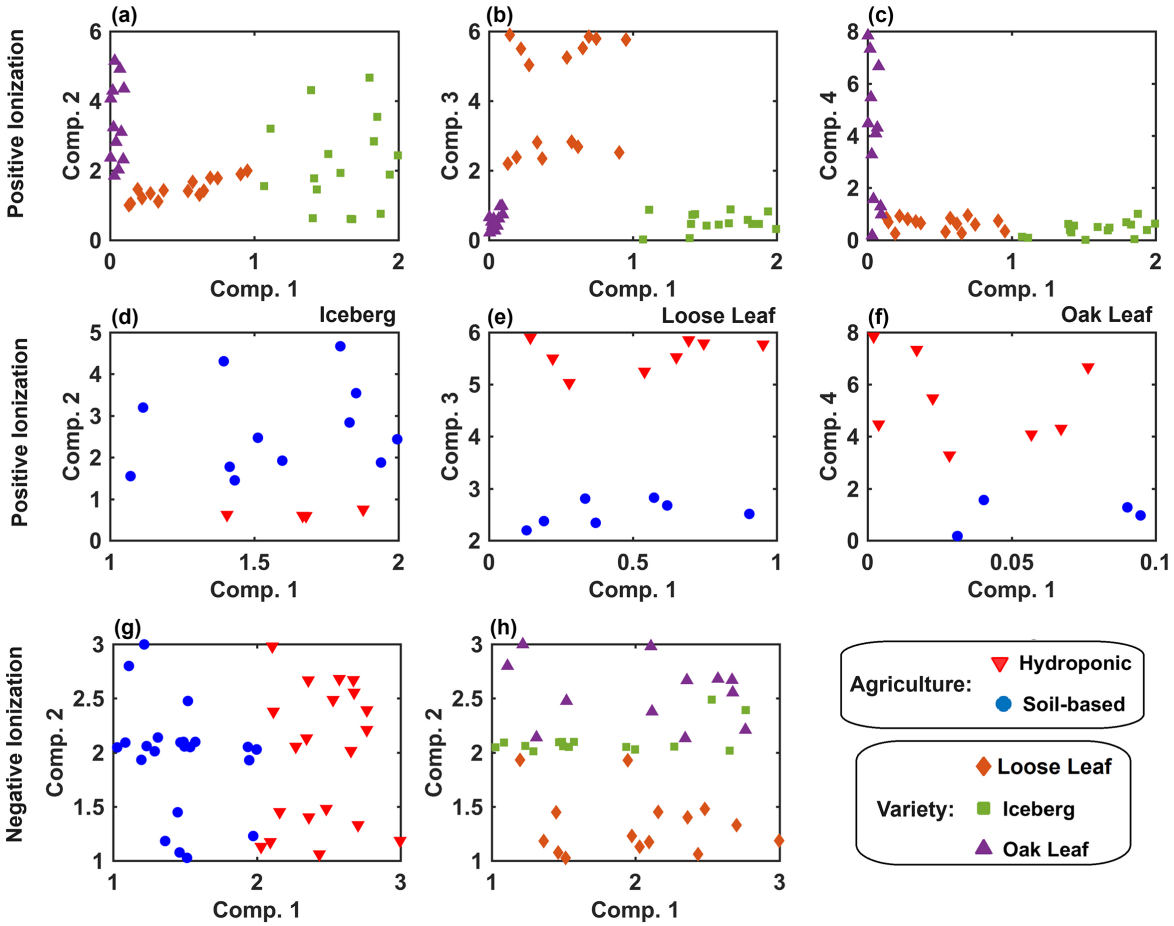


Figure 3. Loading plots from samples for PARAFAC models applied to **T** tensor with 43 lettuce samples. For the positive ionization mode, four independent components were identified, showing differentiation between lettuce varieties (a-c) and cultivation methods (d-f). For the negative ionization mode, two independent components were identified, distinguishing cultivation methods (g) and lettuce varieties (h).

plots for the samples mode, presented in Figures 3g and 3h, provide a detailed visualization of the differentiation achieved. The first component effectively separates the samples based on the cultivation method. As illustrated in Figure 3g, hydroponic and soil-based samples form distinct groups along the axis of component 1. This clear separation indicates a strong influence of the cultivation method on the metabolic profile of the lettuce samples, reflecting significant differences in the metabolic pathways activated under different growing conditions. The second component further distinguishes the samples by lettuce variety. As shown in Figure 3h, the loose leaf, iceberg, and oak leaf varieties are separated along the axis of component 2. This differentiation highlights the inherent metabolic differences among the lettuce varieties, demonstrating unique metabolic signatures specific to each variety. The clear separations achieved in components 1 and 2 emphasize the significant impact of growing conditions on the metabolic composition of lettuce, providing valuable insights for optimizing cultivation practices and improving crop quality.

The acquisition of mass spectra over time during solvent application to form the spray in positive ionization mode is visualized on a surface response graph (Figure 4a). This plot illustrates the variation in ion intensities over time and between different *m/z* values, providing a dynamic view of the mass spectral data. The surface response graph reveals how specific ions appear, intensify, and decrease throughout the spray application, highlighting the temporal behavior of the metabolites. This visualization is crucial for understanding the kinetics of ion formation and their stability during the experiment. The PARAFAC model was applied to decompose the complex mass spectral data into four distinct components, with the resulting profiles shown in Figures 4b-4e. Before PARAFAC analysis, PCA was used to decompose the *m/z* mode, reducing the dimensionality of the data. The components obtained from the PARAFAC analysis were then reconstructed using the PCA scores, allowing us to remap these components back to the original *m/z* variables. This approach enabled the interpretation of the decomposed data in the context of the full *m/z* range, preserving the original spectral information.

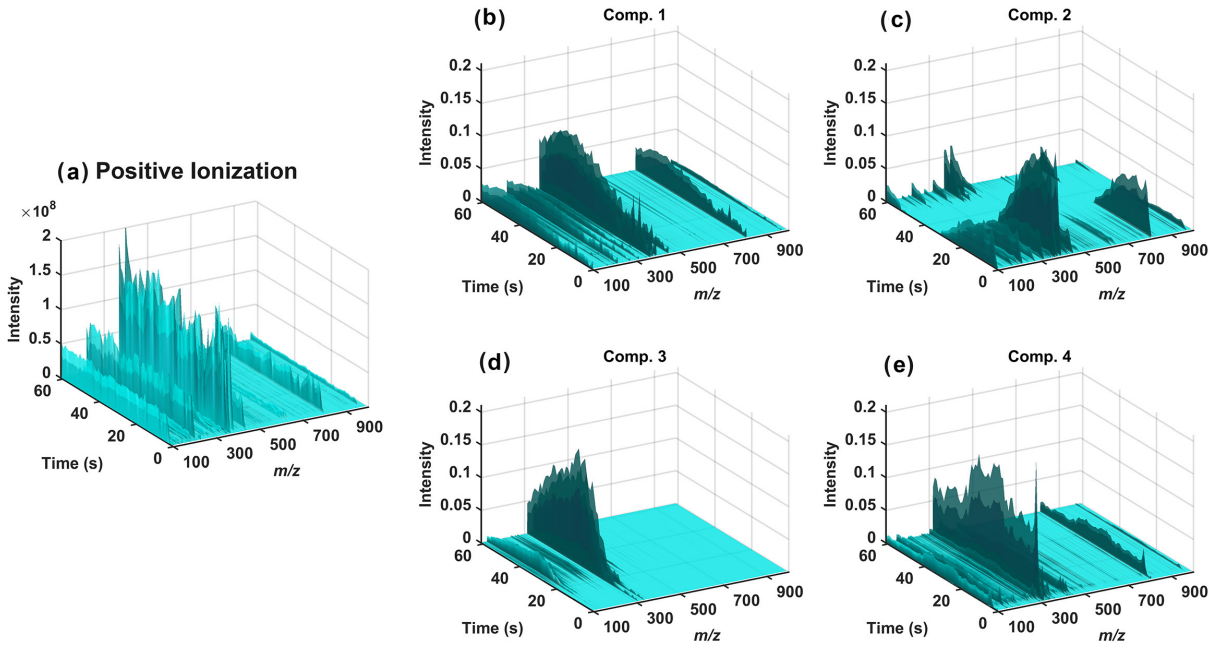


Figure 4. (a) Surface response plot illustrating the acquisition of mass spectra over time during solvent application to form the spray in positive ionization mode. (b-e) Combined loadings profiles of the four components across time acquisition and *m/z* modes, as identified by the PARAFAC model. This representation illustrates how the components are distributed and contribute to the overall variance in these two modes.

Each component profile represents a different pattern in the mass spectra, identifying key *m/z* values and their associated intensities. The component profiles provide deeper insights into the underlying sources of variation in the data. By analyzing these profiles, we can identify specific metabolites that contribute significantly to the overall mass spectra and understand their behavior. The distinct patterns observed in each component highlight the diversity in the metabolic composition of the samples.

Figure 5a shows a response surface representing the acquisition of mass spectra over time during the application of pulverization solvent in negative ionization mode. Similar to the positive ionization mode, this visualization allows for observing the variation in ion intensity over time and across different *m/z* values. This approach provides a dynamic view of the mass signal intensities, facilitating the identification of temporal patterns and significant variations in the spectra. Figures 5b and 5c show the profiles of the two components resulting from the PARAFAC model after reconstruction using PCA scores. These profiles represent the characteristic spectral signatures associated with each component identified in the model, allowing for a more detailed interpretation of the principal sources of variation in the mass spectrometric data. The results from the PARAFAC analysis provided a foundational understanding of the data's multi-dimensional structure and helped identify initial patterns related to the cultivation methods and lettuce varieties. Based on these findings, PLS-DA and OPSDA were subsequently applied to refine the classification and enhance the differentiation between the crop types and lettuce varieties. The following results present the detailed findings of these analyses, highlighting the classification accuracy and the significant variables identified through these advanced chemometric techniques.

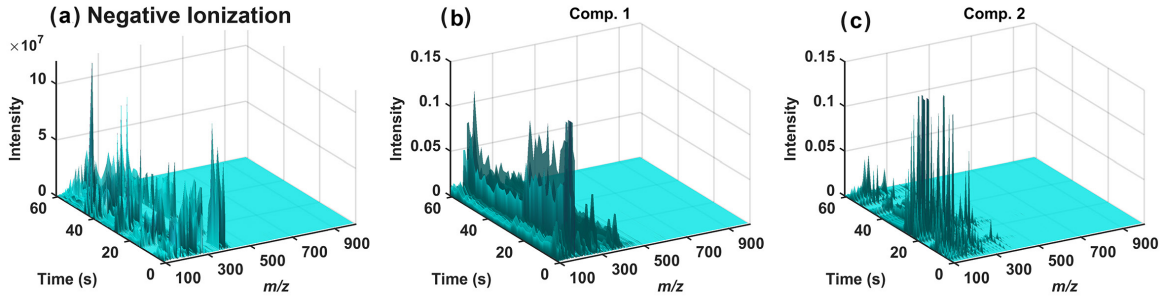


Figure 5. (a) Surface response plot illustrating the acquisition of mass spectra over time during solvent application to form the spray in negative ionization mode. (b-c) Combined loadings profiles of the two components across time acquisition and *m/z* modes, as identified by the PARAFAC model. This representation illustrates how the components are distributed and contribute to the overall variance in these two modes.

Figure 6 presents the PLS-DA results for classifying lettuce grown using conventional hydroponic and soil-based methods, as well as different lettuce varieties, in both positive and negative ionization modes with the selected variables identified by OPSDA. In Figure 6a, the separation of hydroponic and soil-based lettuce in positive ionization mode is illustrated, showing a threshold of 0.049 and an accuracy of 95.35%, with two false positives (two soil-based samples classified as hydroponic). The model was built using three latent variables and achieved an accuracy of 90% in cross-validation. The sensitivity for the hydroponic class, defined as the proportion of correctly classified hydroponic samples, was 100%, as all 20 hydroponic samples were correctly identified. The specificity for the soil-based class, representing the proportion of correctly classified soil-based samples, was calculated based on 21 correctly identified soil-based samples out of 23, resulting in a specificity of approximately 91.3%.

In negative ionization mode, depicted in Figure 6e, the separation of hydroponic and soil-based lettuce achieved a threshold of –0.059 with 100% accuracy, without any misclassifications. The model was built using seven latent variables and achieved an accuracy of 96% in cross-validation. The sensitivity was 100%, as all 20 hydroponic samples were correctly identified. Similarly, the specificity was also 100%, with all 23 soil-based samples accurately classified.

As illustrated in Figures 6b-6d and 6f-6h, the PLS-DA evaluation achieved an accuracy of 100%, successfully distinguishing between the lettuce varieties loose leaf, iceberg leaf, and oak leaf in both ionization modes. The sensitivity for each variety of classification was 100%, and the specificity was also 100%, demonstrating the versatility of the chemometric tool in differentiating the ion profiles of complex samples. In positive ionization mode, the models were built using nine latent variables and achieved a cross-validation accuracy of 95%, whereas in negative ionization mode, thirteen latent variables were used, resulting in a cross-validation accuracy of 90%.

A Venn diagram illustrating the number of variables selected by OPSDA from the PLS-DA evaluation of conventional hydroponic and soil-based agriculture, as well as different lettuce varieties, is shown in Figure 7. Figure 7a depicts the selected variables for hydroponic and soil cultivation in positive ionization mode. The analysis identified 65 important variables for hydroponic agriculture and 15 for soil cultivation, with 12 variables common to both classes. For the lettuce varieties, Figure 7b illustrates the variables found in positive ionization mode: 80 variables were selected for loose leaf, 65 for iceberg, and 25 for oak leaf, with 9 variables shared among the varieties. The negative ionization mode for cultivation types and lettuce varieties is represented in Figures 7c and 7d, respectively. Figure 7c highlights the variables in hydroponic and soil cultivation, with 220 variables selected for hydroponic, 185 for soil-based cultivation, and 144 variables common to both classes. Figure 7d details 54 variables for loose leaf, 330 for iceberg, and

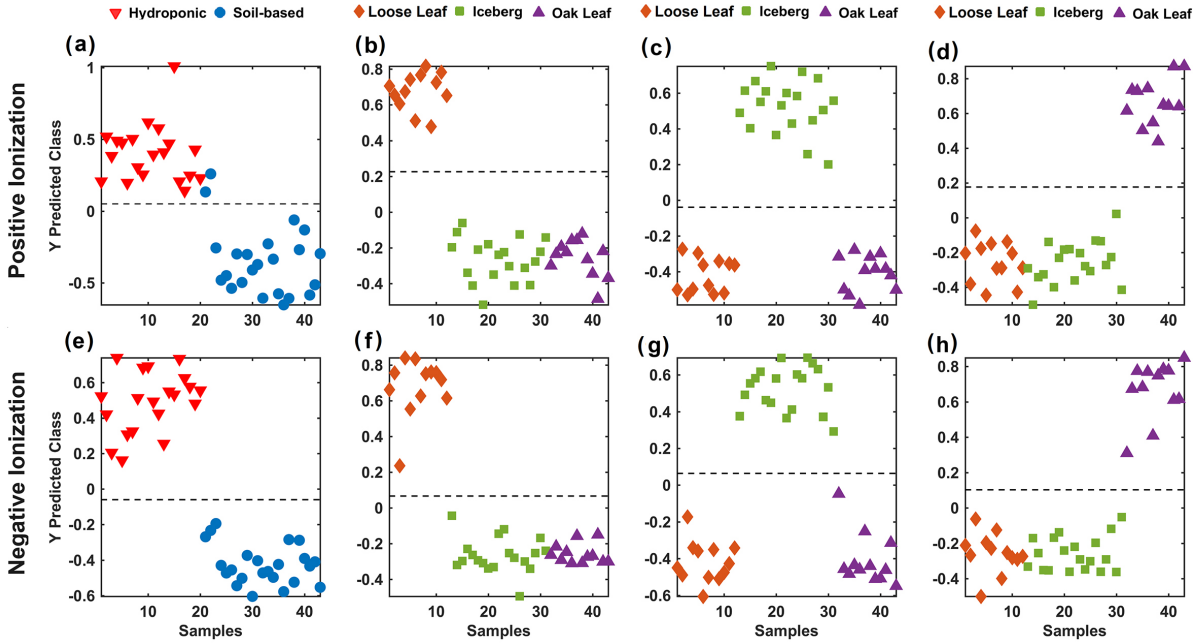


Figure 6. Classification by PLS-DA model with selected variables by OPSDA for the prediction of lettuce samples in positive ionization mode (a-d) and negative ionization mode (e-h). Lettuce hydroponic and soil-based agriculture in positive ionization mode (a); lettuce hydroponic and soil-based agriculture in negative ionization mode (e); lettuce varieties in positive ionization mode (b-d); lettuce varieties in negative ionization mode (f-h).

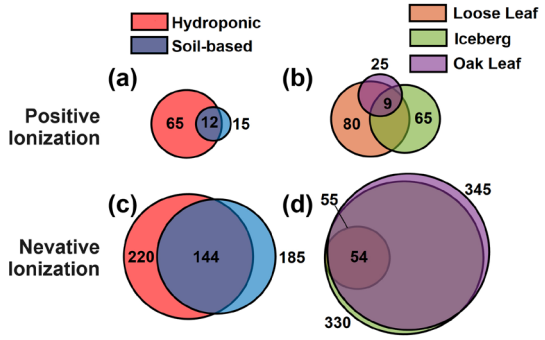


Figure 7. Venn diagram for (a) variables found in hydroponic and soil cultivation in positive ionization mode, (b) variables found for the lettuce varieties in positive ionization mode, (c) variables found in hydroponic and soil cultivation in negative ionization mode, and (d) variables found for the lettuce varieties in negative ionization mode.

345 for oak leaf, with 55 variables shared among the lettuce varieties.

To illustrate the ions that significantly influenced the distinction between cultivation methods (conventional soil and hydroponic agriculture) and lettuce varieties (iceberg, loose leaf, and oak leaf), an informative vector of OPSDA, the VIP scores, is presented in Figure 8. The VIP scores of the 20 most important variables for classification were selected. Figures 8a and 8c display the VIP scores for growing methods in positive and negative ionization modes, respectively. Figures 8b and 8d show the VIP scores for lettuce varieties in positive and negative ionization modes, respectively. According to the main findings, most of the 20 ions highlighted in the VIP scores are listed in Table 1.

Table 1. Tentative metabolite annotation of lettuce extracts analyzed PSI-MS/MS in positive and negative ionization modes

Compound	Molecular formula	*m/z* theoretical	*m/z* observed	Adduct	Chemical class
Succinic acid semialdehyde	$C_4H_5O_3^-$	101.02386	101.02416	$[M - H]^-$	fatty acid
Choline	$C_5H_{14}NO^+$	104.10753	104.10736	$[M + H]^+$	amino acid
D-Glyceric acid	$C_3H_5O_4^-$	105.01933	105.01910	$[M - H]^-$	sugar
Proline	$C_5H_{10}NO_2^+$	116.07115	116.07092	$[M + H]^+$	amino acid
Valine	$C_5H_{12}NO_2^+$	118.08625	118.08657	$[M + H]^+$	amino acid
Phloroglucinol	$C_6H_7O_3^+$	127.03951	127.03911	$[M + H]^+$	phenolic compounds
3-Methyl-2-oxovaleric acid	$C_6H_9O_3^-$	129.05516	129.05566	$[M - H]^-$	amino acid
Glutaric acid	$C_5H_7O_4^-$	131.03443	131.03490	$[M - H]^-$	fatty acid
Isoleucine	$C_6H_{14}NO_2^+$	132.10190	132.10207	$[M + H]^+$	amino acid
Malic acid	$C_4H_5O_5^-$	133.01369	133.01402	$[M - H]^-$	carboxylic acid
Octanoic acid	$C_8H_{15}O_2^-$	143.10720	143.10767	$[M - H]^-$	fatty acid
Levoglucosan	$C_6H_9O_4^+$	145.05008	145.05050	$[M + H - H_2O]^+$	carbohydrate
Lysine	$C_6H_{15}N_2O_2^+$	147.11280	147.11288	$[M + H]^+$	amino acid
Phthalic anhydride	$C_8H_5O_3^+$	149.02332	149.02343	$[M+H]^+$	benzofurans
Vanillin	$C_8H_7O_3^-$	151.03951	151.03979	$[M - H]^-$	benzaldehyde
Pelargonic acid	$C_9H_{17}O_2^-$	157.12285	157.12322	$[M - H]^-$	fatty acid
2-Oxoadipic acid	$C_6H_7O_5^-$	159.02934	159.02976	$[M - H]^-$	fatty acid
1,6-Anhydro-beta-D-glucopyranose	$C_6H_{10}O_5^+$	163.06010	163.06017	$[M + H]^+$	sugar
Phenylalanine	$C_9H_{12}NO_2^+$	166.08625	166.08641	$[M + H]^+$	amino acid
Decanoic acid	$C_{10}H_{19}O_2^-$	171.13850	171.13894	$[M - H]^-$	fatty acid
Arginine	$C_6H_{14}O_2N_4^+$	175.11895	175.11911	$[M + H]^+$	amino acid
D-Gulono-1,4-lactone	$C_6H_9O_6^-$	177.03991	177.04019	$[M - H]^-$	sugar
Mannose	$C_6H_{11}O_6^-$	179.05611	179.05597	$[M - H]^-$	sugar
Citric acid	$C_6H_7O_7^-$	191.01973	191.01956	$[M - H]^-$	carboxylic acid
Tryptophan	$C_{11}H_{11}N_2O_2^+$	205.09715	205.09732	$[M + H]^+$	amino acid
Palmitic acid	$C_{16}H_{31}O_2^-$	255.23240	255.23292	$[M - H]^-$	fatty acid
Stearic acid	$C_{18}H_{35}O_2^-$	283.26370	283.26447	$[M - H]^-$	fatty acid
2,4-Dihydroxyheptadecyl acetate	$C_{19}H_{37}O_3^+$	313.27372	313.27360	$[M + H - H_2O]^+$	fatty acid
Monopalmitin	$C_{19}H_{39}O_4^+$	331.28428	331.28433	$[M + H]^+$	fatty acid
Erucamide	$C_{22}H_{44}NO^+$	338.34174	338.34192	$[M + H]^+$	fatty amide
Chlorogenic acid	$C_{16}H_{17}O_9^-$	353.08670	353.08792	$[M - H]^-$	phenolic acid
1-Monostearin	$C_{21}H_{43}O_4^+$	359.31558	359.31545	$[M + H]^+$	fatty acid
Sucrose	$C_{12}H_{22}O_{11}K^+$	381.07937	381.07949	$[M + K]^+$	sugar

The ions corresponding to choline (*m/z* 104.107) and *m/z* 397.272 were the most significant in differentiating between growing conditions in the positive mode. Additionally, in the positive mode, Figure 8b shows that the ion with the most influence on the differentiation of lettuce varieties was choline (*m/z* 104.107). It is also observed that the ions *m/z* 381.079 significantly contributed to this differentiation; however, the authors could not identify these ions in the mass databases. In the negative mode, Figure 8c revealed that the sugar mannose (*m/z* 179.056) had the most influence on the ion profile differentiation between conventional soil and hydroponic agriculture. Differentiation of lettuce varieties in the negative mode, as shown in Figure 8d, indicated that the ions malic acid (*m/z* 133.014) and mannose (*m/z* 179.056) were the most important for separating iceberg, loose leaf, and oak leaf lettuce samples.

Evaluating the important ions for the separation between growing methods and lettuce varieties, both in positive and negative modes, it is possible to observe that choline, mannose, and malic acid were important for both separations. Choline (*m/z* 104.107) is essential for plant growth and development. It can be oxidized to produce glycine betaine, an osmolyte that accumulates in plants to mitigate various abiotic stresses.[43] Additionally, an increase in choline concentration may be associated with responses to drought, salt, and oxidative stress. Consequently, variations in choline concentration can indicate differences in cultivation environments and variations among varieties depending on their stress resistance.[43] Mannose (*m/z* 179.056) is a simple sugar derived from mannitol and, like some primary metabolites, it plays a central role in plant growth, cell replacement, resource allocation, and differentiation. In addition, there are reports of its concentration varying in response to drought and different nutritional environments, with mannose exerting an osmo-protective function.[44] Malic acid (*m/z* 133.014) is the predominant organic acid in lettuce. As a primary metabolite, it plays a crucial role in plant physiology by inhibiting microorganisms through mechanisms associated with pH, the proportion of related organic acid forms, chain length, cellular physiology, and metabolism. Furthermore, studies indicate that malic acid content can vary depending on factors such as light exposure, water availability, and lettuce variety.[45]

Considering aspects related to lettuce consumption, Table 1 shows that among the 20 most important ions for distinguishing between cultivation methods and varieties, amino acids, fatty acids, and phenolic compounds are included. Amino acids are fundamental biomolecules that act as substrates for protein synthesis and are critical for cellular growth, differentiation, and function.[46] Fatty acids

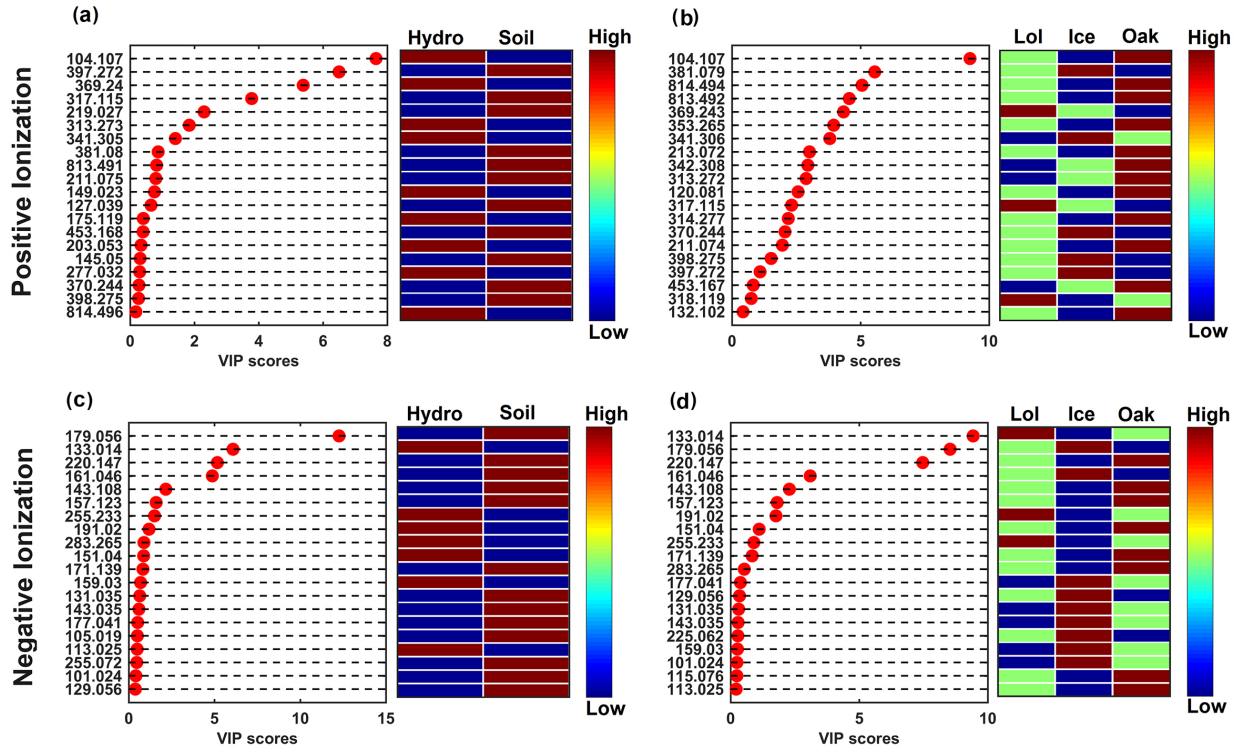


Figure 8. VIP scores for ion profiles of (a) lettuce cultivation in hydroponic and soil-based agriculture in positive ionization mode, (b) lettuce varieties differentiation in positive ionization mode, (c) lettuce cultivation in hydroponic and soil-based agriculture in negative ionization mode, and (d) lettuce varieties differentiation in negative mode.

are highly efficient energy reservoirs that are incorporated into triglycerides, phospholipids, and other complex lipids.[47] Beyond their role as energy substrates, they regulate cellular signaling pathways and exert a critical influence on the structural integrity and functional dynamics of tissues and organs.[48] Phenolic compounds are secondary metabolites widely distributed in plant tissues, known for their diverse biological activities, including antioxidant, anti-inflammatory, antitumor, and antimicrobial effects.[49] According to the literature,[50-52] the composition of amino acids, fatty acids, and phenolic compounds in lettuce is influenced by both variety and growing conditions, which is consistent with the results of this study.

Therefore, analyses using PSI-MS/MS in both ionization modes, together with chemometric tools, enabled the identification and separation of lettuce samples according to variety and cultivation methods. These analyses also allowed the identification of the ions that contributed most to this separation. This study contributes to the application of PSI-MS/MS in metabolomics by providing a simple approach that requires minimal acquisition time.

Conclusions

In this study, the use of PSI-MS/MS and direct infusion ESI-MS/MS were compared to evaluate the ion profiles of lettuce samples cultivated under soil-based and hydroponic conditions. PSI-MS/MS demonstrated superior sensitivity and more robust ion signal intensity compared to ESI-MS/MS, highlighting its effectiveness in detecting and characterizing the chemical constituents of lettuce samples. The study revealed significant metabolic differences between lettuce grown in soil and hydroponic conditions, especially in the negative ionization mode, with PSI-MS/MS proving to be a valuable tool for detailed metabolomic profiling due to its high sensitivity and minimal sample preparation requirements.

The investigation extended to analyzing various lettuce varieties (loose leaf, iceberg, and oak leaf) using PSI-MS/MS, which revealed distinct ion profiles and differences in ion abundances among the varieties. Chemometric analysis using PARAFAC and PLS-DA facilitated the differentiation of lettuce varieties and cultivation methods, underscoring the significant impact of growing conditions on the metabolic composition of lettuce. Key ions, including choline, mannose, and malic acid, were identified as crucial for differentiating between growing methods and lettuce varieties. Additionally, the contribution of amino acids, fatty acids, and phenolic compounds to this differentiation was observed, which may lead to nutritional differences among these plants.

In summary, PSI-MS/MS, combined with chemometric tools, proved to be a powerful approach for exploratory metabolomic studies, offering detailed chemical profiling and valuable insights into the effects of different cultivation methods on the nutritional and chemical composition of lettuce. The discrimination achieved using PARAFAC and OPSDA should be regarded as preliminary yet promising, demonstrating the potential of these models to differentiate lettuce varieties and growing conditions. Further studies employing independent validation sets will be required to fully assess their predictive capability. Overall, the findings support the utility of PSI-MS/MS in agricultural and food science research, providing a robust and efficient method for metabolomic analysis with potential applications in optimizing cultivation practices, improving crop quality, and aligning with the principles of green analytical chemistry.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author. The original data supporting this study are publicly available in the Mendeley Data repository: http://dx.doi.org/10.17632/h5zs29psnk

Acknowledgments

This study was financed in part by the CAPES - Finance Code 001. The authors are grateful to FAPEG and CNPq for scholarships. ChatGPT (OpenAI, GPT-4º) was used solely to assist with grammar and language correction under human supervision. The authors reviewed and approved all content to ensure accuracy and intended meaning.

Author Contributions

L. S. M. and L. Í. L. M. were responsible for conceptualization, investigation, methodology, and writing original draft. A. C. B. J. and Y. A. R. for investigation, methodology and writing original draft. B. G. V. for funding acquisition and resources. A. R. C. for funding acquisition, project administration, resources, supervision and writing - review and editing. J. V. R. for formal analysis, supervision, validation and writing - review and editing.

References

1. Llorach, R.; Martínez-Sánchez, A.; Tomás-Barberán, F. A.; Gil, M. I.; Ferreres, F.; *Food Chem.* **2008**, *108*, 1028. [Crossref]
2. Wang, L.; Ning, S.; Zheng, W.; Guo, J.; Li, Y.; Li, Y.; Chen, X.; Ben-Gal, A.; Wei, X.; *Front Plant Sci.* **2023**, *14*, 1165856. [Crossref]

3. Mohammad, Z. H.; do Prado, I.; Sirsat, S. A.; *Heliyon* **2022**, *8*, e11050. [Crossref]
4. Rajendran, S.; Domalachenpa, T.; Arora, H.; Li, P.; Sharma, A.; Rajauria, G.; *Heliyon* **2024**, *10*, e26823. [Crossref]
5. Otify, A. M.; ElBanna, S. A.; Eltanany, B. M.; Pont, L.; Benavente, F.; Ibrahim, R. M.; *Food Res. Int.* **2023**, *172*, 113178. [Crossref]
6. Lei, C.; Engeseth, N. J.; *LWT* **2021**, *150*, 111931. [Crossref]
7. Majid, M.; Khan, J. N.; Ahmad Shah, Q. M.; Masoodi, K. Z.; Afroza, B.; Parvaze, S.; *Agric. Water Manage.* **2021**, *245*, 106572. [Crossref]
8. Collado-González, J.; Piñero, M. C.; Otalora, G.; Lopez-Marín, J.; del Amor, F. M.; *Food Chem.* **2022**, *384*, 132506. [Crossref]
9. Medina-Lozano, I.; Bertolín, J. R.; Díaz, A.; *Food Chem.* **2021**, *359*, 129864. [Crossref]
10. Wu, W.; Zhang, L.; Zheng, X.; Huang, Q.; Farag, M. A.; Zhu, R.; Zhao, C.; *Food Chem. X* **2022**, *16*, 100500. [Crossref]
11. Leygeber, S.; Grossmann, J. L.; Diez-Simon, C.; Karu, N.; Dubbelman, A.-C.; Harms, A. C.; Westerhuis, J. A.; Jacobs, D. M.; Lindenburg, P. W.; Hendriks, M. M. W. B.; Ammerlaan, B. C. H.; van den Berg, M. A.; van Doorn, R.; Mumm, R.; Hall, R. D.; Smilde, A. K.; Hankemeier, T.; *Metabolites* **2022**, *12*, 1194. [Crossref]
12. Lima, G. S.; Lima, N. M.; Roque, J. V.; de Aguiar, D. V. A.; Oliveira, J. V. A.; dos Santos, G. F.; Chaves, A. R.; Vaz, B. G.; *Molecules* **2022**, *27*, 7937. [Crossref]
13. Fu, J.; Zhang, L.-L.; Li, W.; Zhang, Y.; Zhang, Y.; Liu, F.; Zou, L.; *Food Chem.* **2022**, *367*, 130697. [Crossref]
14. Khanijou, J. K.; Kulyk, H.; Bergès, C.; Khoo, L. W.; Ng, P.; Yeo, H. C.; Helmy, M.; Bellvert, F.; Chew, W.; Selvarajoo, K.; *Metab. Eng. Commun.* **2022**, *15*, e00209. [Crossref]
15. Mialon, N.; Roig, B.; Capodanno, E.; Cadiere, A.; *Food Chem.* **2023**, *398*, 133856. [Crossref]
16. Zhang, R.; Realini, C. E.; Middlewood, P.; Pavan, E.; Ross, A. B.; *Food Chem.* **2022**, *386*, 132758. [Crossref]
17. Maciel, L. Í. L.; Ramalho, R. R. F.; Ribeiro, R. I.; Pinto, M. C. X.; Pereira, I.; Vaz, B. G.; *J. Am. Soc. Mass Spectrom.* **2021**, *32*, 2513. [Crossref]
18. Chaves, A. R.; Martins, R. O.; Maciel, L. I. L.; Silva, A. R.; Gondim, D. V.; Fortalo, J. M.; Santos, S. S.; Roque, J. V.; Vaz, B. G.; *Braz. J. Anal. Chem.* **2022**, *9*, 52. [Crossref]
19. Erkok, S. D.; Gallois, R.; Leegwater, L.; Gonzalez, P. C.; van Asten, A.; McCord, B.; *Talanta* **2024**, *278*, 126414. [Crossref]
20. Maciel, L. I. L.; Carvalho, T. C.; Pereira, I.; Vaz, B. V.; *Quim. Nova* **2019**, *42*, 676. [Crossref]
21. Talarico, I. R.; Bartella, L.; Rocio-Bautista, P.; Di Donna, L.; Molina-Diaz, A.; Garcia-Reyes, J. F.; *Talanta* **2024**, *267*, 125152. [Crossref]
22. Xiong, L.; Sun, S.; Lu, X.; Wang, X.; Yu, Q.; Qian, X.; *Talanta* **2024**, *278*, 126476. [Crossref]
23. Martins, R. O.; de Araújo, G. L.; de Freitas, C. S.; Silva, A. R.; Simas, R. C.; Vaz, B. G.; Chaves, A. R.; *J. Chromatogr. A* **2021**, *1640*, 461949. [Crossref]
24. Ramos, A. L. C. C.; Silva, M. R.; Mendonça, H. O. P.; Mazzinghy, A. C. C.; Silva, V. D. M.; Botelho, B. G.; Augusti, R.; Ferreira, R. M. S. B.; de Sousa, I. M. N.; Batista-Santos, P.; de Araújo, R. L. B.; Melo, J. O. F.; *Food Res. Int.* **2022**, *160*, 111687. [Crossref]
25. Luo, W.; Qin, Y.; van Beek, T. A.; Chen, B.; Zuilhof, H.; Salentijn, G. I. J.; *Food Chem.* **2024**, 140481. [Crossref]
26. Kalogiouri, N. P.; Ferracane, A.; Manousi, N.; Zachariadis, G.; Tranchida, P. Q.; Mondello, L.; Samanidou, V. F.; Rosenberg, E.; *Talanta* **2024**, *266*, 125027. [Crossref]
27. Qin, Z.; Wang, J.; Wang, D.; Xiao, H.; Lv, X.; Chen, H.; Wei, F.; *Trends Food Sci. Technol.* **2024**, *143*, 104298. [Crossref]
28. Santos, A. M. S.; Faria, R. C.; Pereira, I.; de Lima, L. A. S.; Leal, C. M.; Nascimento, A. R.; Simas, R. C.; Lima, N. M.; Vaz, B. G.; *J. Food Compos. Anal.* **2022**, *109*, 104467. [Crossref]
29. Rubio, L.; Sarabia, L. A.; Ortiz, M. C.; *Talanta* **2015**, *138*, 86. [Crossref]
30. Sinha, A. E.; Fraga, C. G.; Prazen, B. J.; Synovec, R. E.; *J. Chromatogr. A* **2004**, *1027*, 269. [Crossref]
31. Barker, M.; Rayens, W.; *J. Chemom.* **2003**, *17*, 166. [Crossref]
32. Roque, J. V.; Cardoso, W.; Peternelli, L. A.; Teófilo, R. F.; *Anal. Chim. Acta* **2019**, *1075*, 57. [Crossref]
33. Wartha, C. A.; Porto, N. A.; Tomaz, A. C.; Roque, J. V.; Diniz, M. B. T.; de Queiroz, M. E. L. R.; Teófilo, R. F.; Barbosa, M. H. P.; *Phytochemistry* **2022**, *199*, 113175. [Crossref]
34. Rankin-Turner, S.; Sears, P.; Heaney, L. M.; *Anal. Sci. Adv.* **2023**, *4*, 133. [Crossref]
35. Chen, J.; Nichols, K. K.; Wilson, L.; Barnes, S.; Nichols, J. J.; *Ocul. Surf.* **2019**, *17*, 347. [Crossref]
36. Duarte, L. C.; Pereira, I.; Maciel, L. I. L.; Vaz, B. G.; Coltro, W. K. T.; *Anal. Chim. Acta* **2022**, *1190*, 339252. [Crossref]
37. Smith, B. L.; Boisdon, C.; Romero-Perez, D.; Sham, T.-T.; Bastani, B.; Zhou, Y.; McWilliam, S.; Badu-Tawiah, A. K.; Maher, S.; *Int. J. Mass Spectrom.* **2022**, *471*, 116737. [Crossref]
38. Hameed, M. K.; Umar, W.; Razzaq, A.; Aziz, T.; Maqsood, M. A.; Wei, S.; Niu, Q.; Huang, D.; Chang, L.; *Metabolites* **2022**, *12*, 444. [Crossref]
39. Ramalho, R. R. F.; Pereira, I.; Lima, G. S.; dos Santos, G. F.; Maciel, L. I. L.; Simas, R. C.; Vaz, B. G.; *J. Food Compos. Analysis* **2022**, *107*, 104362. [Crossref]
40. Verdoliva, S. G.; Gwyn-Jones, D.; Detheridge, A.; Robson, P.; *Sci. Hortic.* **2021**, *279*, 109896. [Crossref]
41. Fussy, A.; Papenbrock, J.; *Plants* **2022**, *11*, 1153. [Crossref]
42. Kusano, M.; Yang, Z.; Okazaki, Y.; Nakabayashi, R.; Fukushima, A.; Saito, K.; *Mol. Plant* **2015**, *8*, 58. [Crossref]
43. Gao, H.; Mao, H.; Ullah, I.; *Agriculture* **2020**, *10*, 406. [Crossref]

44. Gent, M. P. N.; *HortScience* **2014**, *49*, 173. [Crossref]
45. Gent, M. P. N.; *J. Sci. Food Agric.* **2012**, *92*, 3007. [Crossref]
46. Ling, Z.-N.; Jiang, Y.-F.; Ru, J.-N.; Lu, J.-H.; Ding, B.; Wu, J.; *Signal Transduction Targeted Ther.* **2023**, *8*, 345. [Crossref]
47. Günenc, A. N.; Graf, B.; Stark, H.; Chari, A. In *Macromolecular Protein Complexes IV*, vol. 99, 1st ed.; Harris, J. R.; Marles-Wright, J., eds.; Springer: Cham, CH, 2022, p. 1. [Crossref]
48. Olejnik, A.; Gornowicz-Porowska, J.; Jenerowicz, D.; Polańska, A.; Dobrzyńska, M.; Przysławski, J.; Sansone, A.; Ferreri, C.; *Nutrients* **2023**, *15*, 3857. [Crossref]
49. Rocchetti, G.; Gregorio, R. P.; Lorenzo, J. M.; Barba, F. J.; Oliveira, P. G.; Prieto, M. A.; Simal-Gandara, J.; Mosele, J. I.; Motilva, M.; Tomas, M.; Patrone, V.; Capanoglu, E.; Lucini, L.; *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 811. [Crossref]
50. Tadevosyan, A.; Martirosyan, G.; Tovmasyan, G.; Terteryan, H.; Gasparyan, N.; *Bioact. Compd. Health Dis.* **2025**, *8*, 104. [Crossref]
51. Santos, F. T.; Goufo, P.; Santos, C.; Botelho, D.; Fonseca, J.; Queirós, A.; Costa, M. S. S. M.; Trindade, H.; *Food Chem.* **2016**, *209*, 293. [Crossref]
52. Riga, P.; Benedicto, L.; Gil-Izquierdo, Á.; Collado-González, J.; Ferreres, F.; Medina, S.; *Food Chem.* **2019**, *272*, 227. [Crossref]

Submitted: November 5, 2025
Accepted Article online: March 27, 2026
Final version online: April 16, 2026