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Chemometric analysis of derivative spectrophotometric fingerprinting for shallot authentication

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ABSTRACT

High-value and high-volume products, like the Bima Brebes shallots from Indonesia, are particularly vulnerable to such fraud. Derivative spectrophotometric fingerprinting combined with chemometrics was used to distinguish between authentic and adulterated shallot varieties. The aim of this study was to identify the original spectra and their derivative spectrophotometric fingerprints, as well as classify and differentiate between shallot varieties using chemometrics. UV-Visible (UV-Vis) spectrophotometry was used to test essential oil (EO) samples from three shallot varieties and their mixtures, followed by spectral derivatization. The spectral data revealed unique patterns for each sample, encompassing individual varieties and mixtures. Subsequently, this data underwent analysis using Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA). The original spectra and their derivatives showed similarities across the samples. The results of the PCA and PLS-DA analyses demonstrate that the second-order derivative data produced the most distinguishable separation. Principal Component 1 (PC1) and Principal Component 2 (PC2) yielded values of 62.2% and 60.1%, respectively. Additionally, the wavelength with the highest Variable Importance in the Projection (VIP) score was determined to be 225 nm. The PLS-DA results were validated to ensure that the model was not overfit, as evidenced by a satisfactory cross-validation quality (Q2/R2) value of 0.693 and a significant permutation test. The combination of derivative spectrophotometry fingerprinting and a chemometric approach effectively classified different samples, allowing for the determination of the authenticity of a specific shallot variety.

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1. INTRODUCTION

Food products are often adulterated by substituting cheaper ingredients or misleading customers about their value [1]. High-value and high-volume products, like the Bima Brebes shallots from Indonesia, are particularly vulnerable to such fraud. This highlights the necessity for authentication methods. Although visually similar, different varieties of shallot bulbs have distinct secondary metabolites, such as essential oils (EOs), that affect aroma and flavor quality [2]. Organoleptically, it is difficult to distinguish these mixed varieties, which is why fingerprinting technology provides a reliable, quick, and reproducible authentication method [3]. Gas Chromatography-Mass Spectrometry

(GC-MS) is a widely used technique for profiling EO samples. However, it has limitations, such as long analysis durations and high expenses[4].

Spectrophotometry can be a cost-effective alternative for developing authentication methods, as it requires easy sample preparation and does not damage the sample [5]. Ultraviolet-visible (UV-Vis) spectrophotometry can detect cycloartenol compounds in various shallot varieties, such as Bima Brebes, through their EOs [2, 6]. However, UV-Vis spectrophotometric fingerprinting produces complex spectra with overlapping spectral interference due to limited selectivity. A spectral pre-processing algorithm called statistical spectra derivatization can address this issue [7]. Derivative UV-Vis spectrophotometry enhances spectrum fingerprints, reduces noise, and improves clustering between samples by derivatizing zero-order spectra to first or higher derivatives [7, 8, 9]. However, sample fingerprint spectra generate a large amount of data and may exhibit similarities between different shallot variety samples, which requires the use of chemometrics for multivariate analysis [10].

Chemometric models, such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), are valuable tools for comparing sample datasets, identifying similarities, detecting clustering patterns among varieties, and developing reliable models for classifying unknown objects [1]. These models have been combined with UV-Vis spectrophotometric fingerprinting techniques for plant sample authentication [11, 12]. For instance, UV-Vis spectroscopy with PCA-based chemometrics identified counterfeit Arabica and Robusta coffee, resulting in robust pure and mixed models [11]. Another study was conducted to identify counterfeit paprika using high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) fingerprinting instrument. This study employed partial least squares (PLS) and n-way partial least squares (N-PLS), showing that N-PLS with second-order data provided more accurate results than PLS with first-order data [12]. Additionally, a study on classifying medicinal plants using derivative spectra from UV-Vis spectrophotometry and chemometrics demonstrated better sample grouping with first derivative spectra compared to zero-order spectra [7].

Previous studies have focused on other agricultural products, but there has been no specific research on using this approach for shallots. In this study, we aimed to fill this gap by using UV-Vis spectrophotometric techniques and chemometrics to authenticate shallots. By analyzing the grouping profile of both original and derivatized fingerprinting spectra, we determined the classification of each shallot variety and confirmed their authenticity.

2. RESEARCH METHOD

This study employed an experimental design to analyze and classify shallot varieties based on their EO profiles using UV-Vis spectrophotometry and chemometric analysis, following these steps:

2.1. Collection and Preparation of Shallot Samples

Samples of three shallot varieties—Bima Brebes, Bauji, and Maja Cipanas—were obtained from farmers in Brebes, Lembang, and Nganjuk, respectively. Each variety had blue label certification. A total of 5 kg of shallots were collected per variety, resulting in 15 kg in total. The shallots were randomly selected from different farms to ensure a representative sample. They were washed under running water for 5 minutes to remove soil and impurities, then drained for 15 minutes to remove excess water. Each sample was finely sliced to increase surface area and facilitate the release of EO.

2.2. Essential Oil Extraction and Fractionation

The Stahl distillation method, which is a modified version of the original method developed by other researchers, was used for the extraction process [13]. First, 3 kg of each shallot variety were added to a distillation flask. After distillation, the distillate was separated by liquid-liquid fractionation to isolate the EO, ensuring efficient extraction from each shallot variety.

2.3. Organoleptic Observation of Shallot Essential Oil

Organoleptic test of shallot EO was conducted by observing the color, aroma, and consistency of EO in each shallot variety [14]. This qualitative assessment provided initial insights into the sensory characteristics of the oils.

2.4. Preparation of Shallot Essential Oil Samples

The samples used in this study were divided into two categories: EO samples without any mixture and mixed EO samples created by combining the oils from different varieties. Both types of samples were diluted 50,000 times to achieve optimal absorbance through multiple stages of dilution. Mixed samples were prepared by combining oils from two varieties in a 1:1 ratio and following the same dilution method as the unmixed samples.

2.5. Analysis by UV-Vis Spectrophotometry Derivative Fingerprinting

This study modified the methodology of Johnson et al. [4]. Samples of EO were prepared, and their UV-Vis spectra were obtained using a Shimadzu UV-1780 UV-Vis spectrophotometer from Japan. The spectra were collected at 1 nm intervals between 200 and 800 nm using a quartz cuvette. Before the measurements, the instrument was calibrated using 100% n-hexane p.a. as a blank [4]. The data obtained is the absorbance profile and wavelength of the sample. The data collected included the absorbance profile and wavelength. The UV-Vis spectra were then pre-processed through derivatization, from first to third orders, using UV-Probe 2.70 software.

2.6. Chemometric Analysis

The UV-Vis data will be analyzed using MetaboAnalyst 6.0 software. This analysis will involve entering absorbance values from UV-Vis spectrophotometric fingerprinting derivatives, with at least three replicates per sample. PCA and PLS-DA will be used to classify the shallot varieties based on their spectral data [15].

3. RESULTS AND DISCUSSIONS

3.1. Extraction of Essential Oil from Shallots

Extraction is conducted to isolate the EO found in shallots. The Stahl water distillation method was utilized for extraction in this study. The EO extraction results presented in Figure 1 show uniform EO color, which is a clear yellowish hue, the aroma is typical of shallots, and slightly thick liquid consistency. However, the EO of shallots from the bima brebes variety has a stronger aroma than the bauji and maja cipanas varieties.

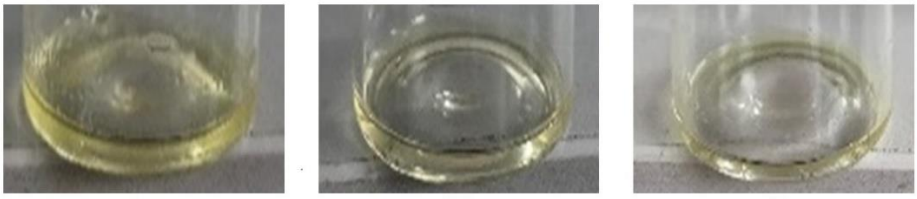

Figure 1. Color of EO from three shallot varieties. (a) bima brebes variety, (b) bauji variety, (c) maja cipanas variety.

The EO yields for the three shallot varieties were: bima brebes with 0.156 ml from 3,000 grams of bulbs (0.005% v/b), bauji with 0.200 ml (0.006% v/b), and maja cipanas with 0.100 mL (0.003% v/b). The bulb size and weight correlate with water and EO content, as larger bulbs generally produce more EO [16]. Specifically, bima brebes has an average bulb diameter of 20.89 cm, bauji 22.16 cm, and maja cipanas 19.46 cm [17]. Consequently, bauji, with the largest diameter, yields the most EO, while maja cipanas, with the smallest diameter, yields the least. Thus, bauji produces the highest EO yield among the varieties studied.

3.2. Derivative Fingerprinting UV-Vis Spectrophotometric Analysis.

Fingerprinting is an analytical method used to obtain information from a sample based on its chemical components [18]. UV-Vis spectrophotometry, operating across 200-800 nm, generates fingerprinting profiles to analyze various shallot EOs, distinguishing between mixed and unmixed samples. In this study, the entire 200-800 nm range was analyzed to capture the complete absorbance spectrum of the samples. Figures were cropped to focus on regions with significant spectral features, ensuring clarity

and high resolution. Figures 2a-2h display the most critical wavelength ranges, highlighting differences between shallot varieties. The results, shown as wavelength vs. absorbance spectra, cover both unmixed and mixed EOs. Second derivative UV–Vis spectrophotometry (SDUVS) improves resolution, reduces interference, and provides detailed analysis of overlapping bands in organic mixtures [19]. This technique is ideal for the precise analysis of complex essential oils. Figure 2 includes the zero, first, second, and third-order derivatized spectra.

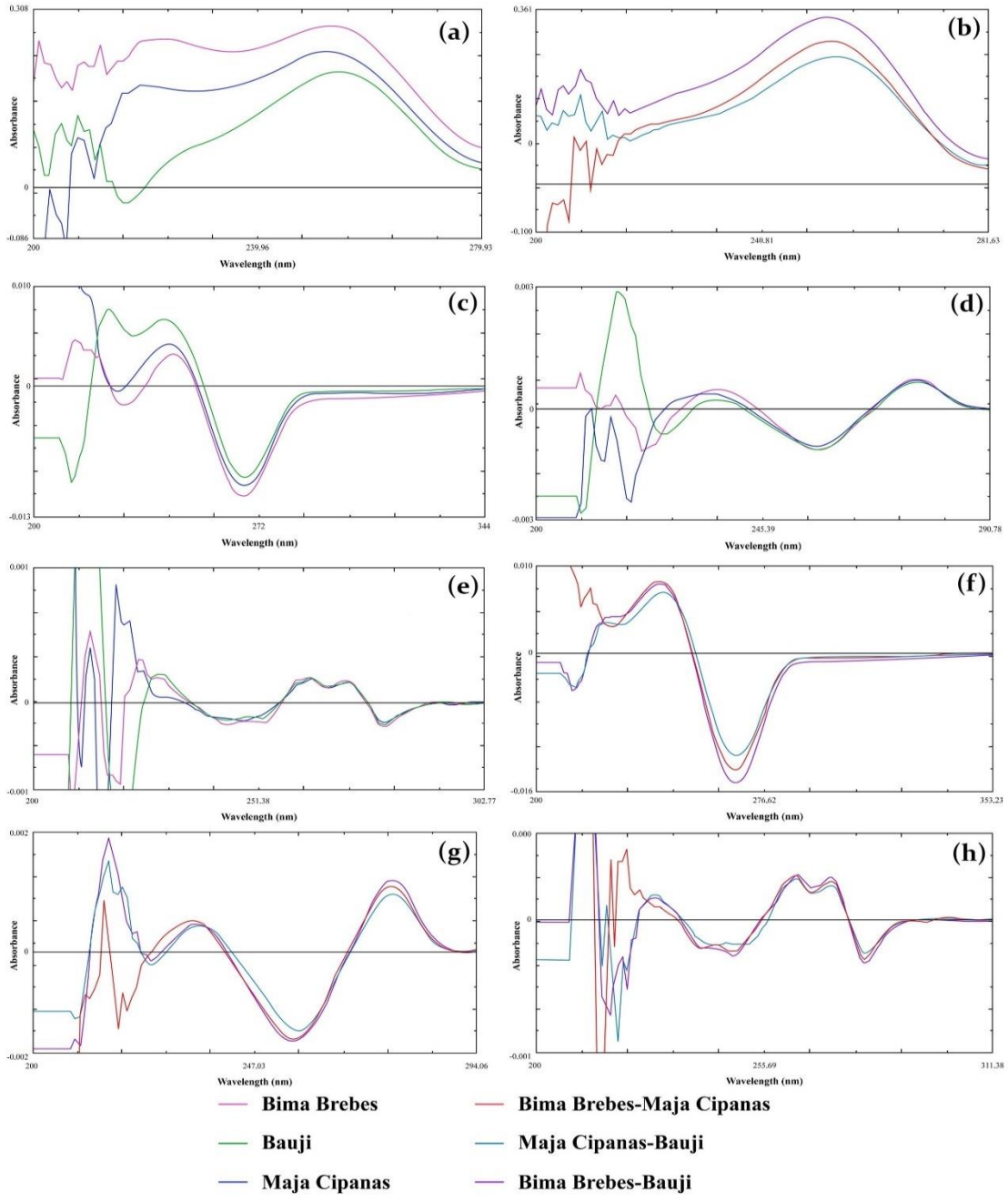

Figure 2. Spectra of EOs from various shallot varieties and their mixtures (1:1 v/v). (a) Zero-order spectra of EO without mixture, (b) Zero-order spectra of EO mixture, (c) First-order spectra of EO without mixture, (d) Second-order spectra of EO without mixture, (e) Third-order spectra of EO without mixture, (f) First-order spectra of EO mixture, (g) Second-order spectra of EO mixture, (h) Third-order spectra of EO mixture.

The zero-order spectra of EOs from unmixed shallot varieties in Figure 2a shows similar maximum wavelengths. Bima brebes and maja cipanas both peak at 253 nm, while bauji peaks at 254 nm. The spectra shapes of bima brebes and maja cipanas are also similar, with dominant peaks at 223 nm and 254 nm for bima brebes, and 220 nm and 254 nm for maja cipanas. This aligns with findings by Johnson et al. who observed predominant peaks in tea tree oil's EOs and eucalyptus EOs between 204 and 221 nm, attributed to $\pi \rightarrow \pi$ and $n \rightarrow \pi$ electron transfers [4]. The absorbance values associated with these wavelengths indicate that higher absorbance corresponds to a higher concentration of cycloartenol or other compounds present at these specific wavelengths. Cycloartenol, a triterpenoid compound, is present in the EOs of bima brebes, bauji, and maja cipanas varieties [2].

Triterpenoids, such as cycloartenol in shallot essential oil, absorb UV light within the 220-275 nm range, with peaks typically around 225 nm and 272.5 nm [6]. This indicates that triterpenoid compounds play a role in the absorbance observed in the 220-254 nm region, along with other compounds present in shallot EO. However, bauji EO spectra differ from the other varieties in the 220-223 nm range, likely due to the presence of 16,28-secosolanid-5-en-3-ol, (3.beta.), which has a predicted maximum wavelength of 228 nm using the Woodward-Fieser rule [2], [20]. Differences between predicted and observed wavelengths may result from chromophore distortion and compound complexity [20]. Previous studies, such as Kucharska-Ambrożej et al. [21], have noted similar spectral patterns for EOs of mint species, where bands at 220 nm and 240-270 nm correspond to $\pi \rightarrow \pi$ and $n \rightarrow \pi$ electron transfers, though the specific band locations can vary depending on the presence of chromophores and their interactions.

Furthermore, it is known that the zero-order spectrum of mixed EO between different shallot varieties in Figure 2b exhibit similar overall spectrum shape and maxima at the maximum wavelength, resulting in visual similarity. Specifically, the maximum wavelength of the mixed sample between bima brebes and bauji varieties is 253 nm. The EO of the mixed maja cipanas-bima brebes varieties also has a maximum wavelength of 253 nm. However, the EO of the mixed maja cipanas-bauji varieties has a slightly different maximum wavelength of 254 nm.

The mixed bima brebes-maja cipanas spectra show only one prominent maxima, while both unmixed varieties have spectra with two dominant peaks. This phenomenon can be attributed to spectral interference, which occurs when the spectra of two overlapping or adjacent peaks interact with each other [22], which can be minimized by derivatization to enhance resolution [7]. The similar spectrum shapes among the three mixture samples make it difficult to determine the authenticity of "bima brebes" in the shallot EO mixture, necessitating further chemometric analysis.

The first-order derivatization results show that the absorbance peak at the maximum wavelength is reduced to zero, with the first order starting and ending at zero, forming one maxima and one minima [23]. When examining the first-order spectra of unmixed EOs in Figure 2c, distinct spectral peaks are observed among varieties. The zero-order spectra of the three shallot varieties exhibit noise in the 200-213 nm region, which is resolved in the first-order spectra. The reduction of the absorbance peak at the highest wavelength to zero is a key characteristic of first-order derivatization, leading to the formation of one maxima and one minima from the zero-order spectra.

The first-order spectra of mixed EOs from different shallot varieties in Figure 2d appear similar when visually analyzed. However, when compared to the zero-order spectra of individual EO in Figure 2a from the same varieties, the mixed varieties spectra show some differences. The zero-order spectra of unmixed bima brebes and maja cipanas varieties are nearly identical, and when mixed with the bauji variety, the first-order spectra become similar. Initially, the mixed spectra show noise at 200-217 nm, but this is resolved by first-order derivatization. The three mixtures between varieties form a dominant maximum at 242 nm and a minimum at 267 nm, making it difficult to visually distinguish between them. Therefore, further chemometric analysis is needed to verify the authenticity of a particular shallot variety.

The second-order derivatization results show a maximum absorbance peak transitioning into a minima at the maximum wavelength, along with the formation of two dominant maxima, or satellite bands [24]. When visually examining the second-order spectra of unmixed EOs in Figure 2e, distinct

peak shapes are observed between shallot varieties, featuring two dominant maxima and one minima at the maximum wavelength. The second-order spectra of bima brebes-bauji and maja cipanas-bauji EOs are similar, but the bima brebes-maja cipanas spectra differ from the others in Figure 2f.

The spectra of the mixed EO of bima brebes-bauji and maja cipanas-bauji varieties display a minimum at a wavelength of 256 nm, while the mixture of bima brebes-maja cipanas varieties is located at a wavelength of 255 nm. The maximum wavelength in the second-order spectra of each sample slightly differs from the maximum wavelength at zero order due to the impact of the solvent used on the spectral shift towards shorter or longer wavelengths in the second-order spectra [25]. Furthermore, the second-order spectra of the unmixed bauji variety EO exhibit one prominent maxima and an additional minimum at wavelengths of 217 and 226 nm, respectively. This suggests that derivatization of the spectra can enhance the resolution of the obtained peaks, as the bauji variety only has one clear dominant peak at zero order.

The third order also has two dominant maxima and two minima, with absorbance at the maximum wavelength being zero, similar to the first order [26]. Unmixed EOs spectra in Figure 2g showed third-order characteristics and distinct spectral shapes among varieties. The mixed EOs of bima brebes-bauji and maja cipanas-bauji varieties are almost identical, while the bima brebes-maja cipanas blend shows noticeable differences in Figure 2h. Despite conforming to ideal third-order characteristics, all samples exhibit noise at 200-220 nm in the third-order spectra. As the derivatization order increases, the signal-to-noise ratio decreases, making further derivatization unnecessary if good results have already been achieved [25].

3.3. Chemometric Analysis

The authenticity of shallots can be determined through chemometric analysis techniques such as PCA and PLS-DA. These methods utilize zero, first, second, and third-order spectra within the wavelength range of 200-800 nm. PCA is a multivariate statistical method that synthesizes information from multiple variables observed in the same subject into principal components (PCs) [27]. By identifying genuine differences in samples, PCA ensures that PLS-DA functions based on the actual pattern rather than known sample groups [28]. Additionally, PLS-DA generates scores plots similar to PCA [29]. However, the sample points in PLS-DA tend to be more tightly grouped as it is a supervised approach that takes into account the category of each sample prior to analysis.

The scores plots in Figure 3a-3d show the results of PCA analysis. PC1 and PC2 are responsible for the majority of variation in the analyzed samples for each order. These components form a plot that visually represents the proximity between different sample types. The close clustering of points confirms the successful classification of shallot samples based on spectral data, addressing our objective of determining grouping profile of shallot varieties. Points clustered together indicate similar chemical profile, while those far apart reflect differences in chemical profiles between samples [29].

When considering the values of PC1 and PC2, it becomes clear that the first order in Figure 3b is the most optimal. This is because the combination of the first two PCs is able to account for a substantial 95.8% of the total variability, with PC1 being particularly influential. This implies that a significant amount of spectral data can be accurately represented using just these two components. Furthermore, the scores plot in PLS-DA (Figures 3e-3h) also illustrates successful classification by showing Components 1 and 2 for each order, confirming the grouping of the shallot samples and supporting the authenticity determination. In PLS-DA, the first order has the highest values for Components 1 and 2, enabling these two components to explain 95.6% of the total variability.

The second-order spectra in particular (Figure 3g) show the most distinct separation of samples, making it the most effective for differentiating shallot varieties. Despite some overlap between the bauji variety and the Bauji-Bima Brebes mixture due to their similar chemical profiles, the classification still aligns with the study's objective of establishing authenticity among the shallot varieties. Overall, the PCA and PLS-DA scores plots indicate the second order derivatization achieves the best separation/classification compared to the zero, first, and third orders.

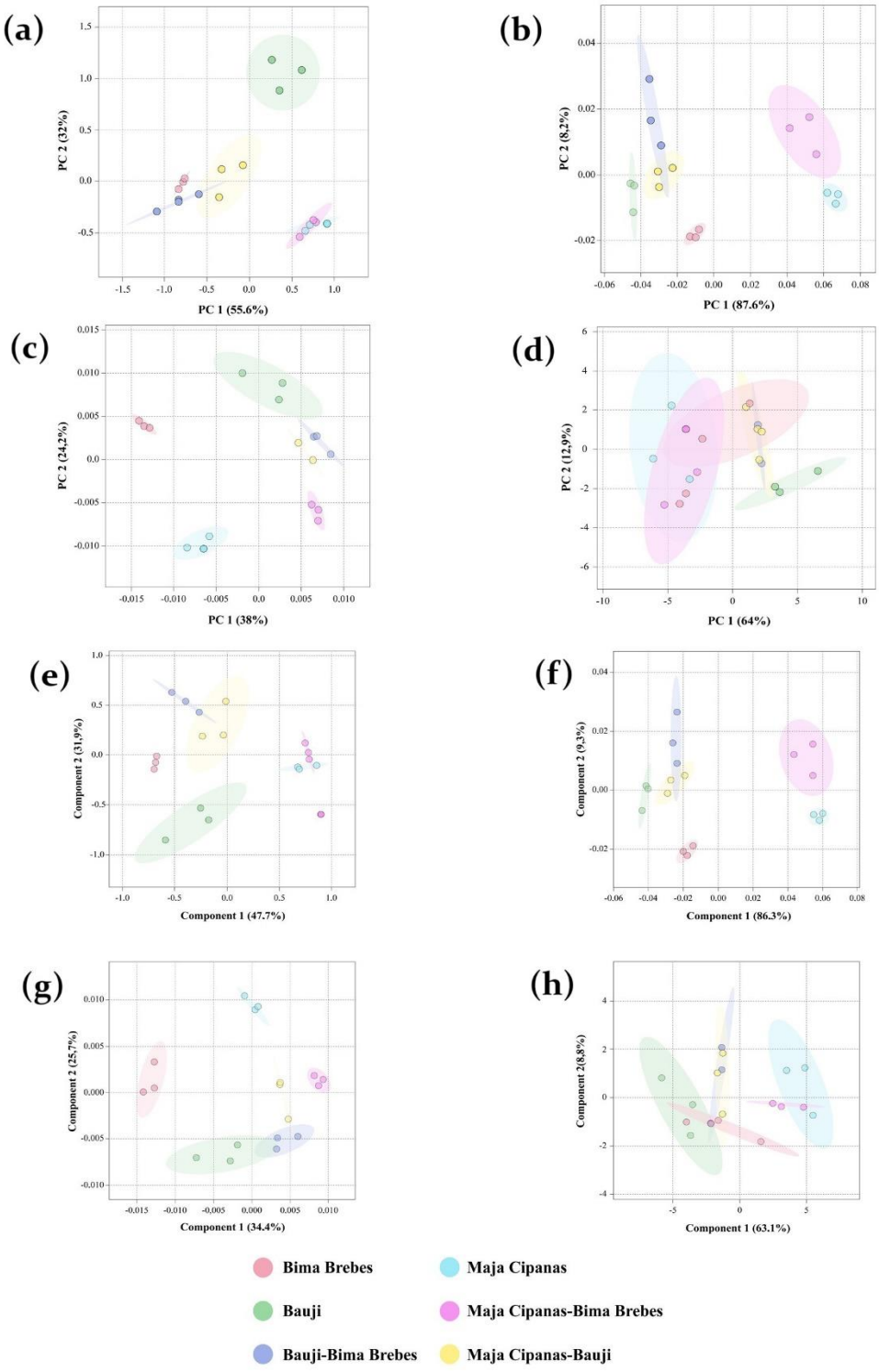

Figure 3. The scores plots of PCA (a-d) and PLS-DA (e-h) from chemometric fingerprinting analysis. (a,e) Zero order, (b,f) First order, (c,g) Second order, (d,h) Third order.

Comparing our findings with previous research, Barbosa et al. applied PCA and PLS-DA to authenticate paprika using Ultra High-Performance Liquid Chromatography Coupled with High-Resolution Mass Spectrometry (UHPLC-HRMS) [30]. In their study, distinct clustering of paprika samples indicated clear groupings related to authenticity. Similarly, our study applied PCA and PLS-DA to UV-Vis spectrophotometry data, successfully clustering shallot samples based on their essential oil profiles. Unlike the chromatographic data used in Barbosa's study, UV-Vis spectrophotometry is simpler and more cost-effective, yet still effective for authentication.

PLS-DA also includes a Variable Importance in the Projection (VIP) score for each variable. The VIP score signifies the significance of each variable in the projections used in the PLS-DA model [31]. VIP scores greater than 1 are considered to have a significant impact on the PLS-DA model [32]. In this study, wavelengths with VIP scores above 1 play a crucial role in clustering samples. The second order, which exhibits the best results in chemometric analysis, also has the highest VIP score. This is because it corresponds to a wavelength that does not include noise in the overall spectra of each sample, and it is known to contain triterpenoid compounds in the form of cycloartenol. The highest VIP score value, observed at a wavelength of 225 nm with a score of 3.8388, was derived from the PLS-DA chemometric analysis of the second order. This wavelength value indicates the significance of this wavelength in distinguishing between shallot samples, emphasizing its importance in the chemometric model.

PLS-DA models can be validated using two different approaches, internal validation and external validation. Internal validation, specifically cross-validation (CV), is employed when the dataset or sample size is limited (<50) [33]. The validation test is conducted on the second order, which produces the best separation. In addition to CV, a permutation test is also performed to validate the PLS-DA model. This test determines whether the differences found between groups are significant or not. A permutation of 20,000 times is carried out on the second-order dataset.

The results of the CV with Leave-one-out cross-validation (LOOCV) method for the second order are as follows: R2 value of 0.85517, Q2 value of 0.59265, and a Q2/R2 ratio of 0.69302. The Q2 value indicates that the variation shown by the 2 components provides the best accuracy for the prediction model. A Q2/R2 ratio greater than 0.5 signifies significant correlations [34]. Since the second order has the highest R2, Q2, and Q2/R2 ratio values, we can conclude that the resulting separation is not overfit and is valid for predicting the authenticity of shallots. Furthermore, the validation of the PLS-DA model through CV and permutation tests confirms the robustness of the second-order model. The p-value for the second order is <0.0005. This result indicates that the observed separation is statistically significant and unlikely to be due to random chance [15].

Overall, our study demonstrates that chemometric analysis using PCA and PLS-DA is a reliable and effective approach for authenticating shallot varieties. The second-order spectra provided the most accurate classification, successfully addressing the research gap related to the authentication of shallot varieties using UV-Vis spectrophotometry. This methodology holds potential for broader application in agricultural product authentication, contributing to quality control and economic protection.

4. CONCLUSION

This study shows that zero-order UV-Vis spectra and their derivatives exhibit consistent patterns across different shallot EO varieties. Using fingerprinting derivative UV-Vis spectrophotometry combined with PCA and PLS-DA, we accurately classified each variety, with second-order techniques proving most effective. This research enhances spectrophotometric analysis, providing robust methods for shallot authentication and quality control in the food industry. The findings address our research questions, confirming the generation of spectra patterns and successful classification through these techniques. However, the focus on specific shallot varieties under controlled conditions limits the study. Future research should explore broader shallot varieties and additional chemometric methods.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest that could potentially influence the findings reported in this paper.

REFERENCES

[1] M. Esteki, Z. Shahsavari, and J. Simal-Gandara, "Gas Chromatographic Fingerprinting Coupled to Chemometrics for Food Authentication," *Food Rev. Int.*, vol. 36, no. 4, pp. 384–427, May 2020, doi: 10.1080/87559129.2019.1649691.

[2] R. Y. Galingging, Sobir, S. Aisyah, and A. Maharijaya, "GC-MS Profiling of Volatile Compounds from Fifteen Different Varieties of Indonesian Shallot Grown in Tidal Swampland," *Rasayan J. Chem.*, vol. 11, no. 2, pp. 575–581, 2018, doi: 10.31788/RJC.2018.1123001.

[3] V. García-Seval, C. Martínez-Alfaro, J. Saurina, O. Núñez, and S. Sentellas, "Characterization, Classification and Authentication of Spanish Blossom and Honeydew Honeys by Non-Targeted HPLC-UV and Off-Line SPE HPLC-UV Polyphenolic Fingerprinting Strategies," *Foods*, vol. 11, no. 15, p. 2345, Aug. 2022, doi: 10.3390/foods11152345.

[4] J. B. Johnson, P. R. Thani, and M. Naiker, "Detection of eucalyptus oil adulteration in Australian tea tree oil using UV–Vis and fluorescence spectroscopy," *Talanta Open*, vol. 6, pp. 1–6, 2022, doi: 10.1016/j.talo.2022.100169.

[5] I. Batubara, S. SaadatulHusna, M. Rafi, T. Sumaryada, S. Uchiyama, B. Juliandi, S. P. Putri, and E. Fukusaki, "A Combination of UV-Vis Spectroscopy and Chemometrics for Detection of Sappanwood (Caesalpinia sappan) Adulteration from Three Dyes," *Sains Malays.*, vol. 51, no. 3, pp. 775–781, Mar. 2022, doi: 10.17576/jsm-2022-5103-11.

[6] M. D. Astuti, E. M. Kuntorini, and F. E. P. Wisuda, "Isolasi dan Identifikasi Terpenoid dari Fraksi n-Butanol Herba Lampasau (Diplazium esculentum Swartz)," *J. Kim. Val.*, vol. 4, no. 1, pp. 20–24, 2014, doi: 10.15408/jkv.v4i1.1048.

[7] I. M. Simion and C. Sârbu, "The impact of the order of derivative spectra on the performance of pattern recognition methods. Classification of medicinal plants according to the phylum," *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, vol. 219, pp. 91–95, Aug. 2019, doi: 10.1016/j.saa.2019.04.038.

[8] G. S. Bumbrah, R. K. Sarin, and R. M. Sharma, "Derivative Ultraviolet Spectrophotometric Studies on Ignitable Liquids," *J. Forensic Chem. Toxicol.*, vol. 4, no. 1, pp. 11–21, 2018. doi: http://dx.doi.org/10.21088/jfct.2454.9363.4118.2

[9] A. Rohman, I. Irnawati, and F. D. O. Riswanto, *Kemometrika*. Yogyakarta: Gadjah Mada University Press, 2021.

[10] G. P. Danezis, A. S. Tsagkaris, F. Camin, V. Brusic, and C. A. Georgiou, "Food authentication: Techniques, trends & emerging approaches," *TrAC Trends Anal. Chem.*, vol. 85, pp. 123–132, Dec. 2016, doi: 10.1016/j.trac.2016.02.026.

[11] M. Yulia, R. Iriani, D. Suhandy, S. Waluyo, and C. Sugianti, "Study on The Use of UV-Vis Spectroscopy and Chemometrics to Quickly Identify The Falsification of Arabica And Robusta Coffees," *J. Tek. Pertan. Lampung*, vol. 6, no. 1, pp. 43–52, 2016. doi: https://dx.doi.org/10.23960/jtep-l.v6i1.%p

[12] X. Sun, M. Zhang, P. Wang, J. Chen, S. Yang, P. Luo, and X. Gao, "Detection and Quantitation of Adulterated Paprika Samples Using Second-Order HPLC-FLD Fingerprints and Chemometrics," *Foods*, vol. 11, no. 15, p. 2376, Aug. 2022, doi: 10.3390/foods11152376.

[13] E. A. El-Wakil, M. M. El-Sayed, and E. E.-S. Abdel-Lateef, "GC-MS Investigation of Essential oil and antioxidant activity of Egyptian White Onion (Allium cepa L.)," vol. 6, 2015, [Online]. Available: https://www.ijpsr.info/docs/IJPSR15-06-03-014.pdf

[14] J. Schaduw, J. A. Pojoh, and T. O. Djabar, "Isolasi dan Identifikasi Minyak Atsiri pada Daun Nilam," *J. Ilm. Farm.*, vol. 3, no. 2, pp. 61–63, 2012, [Online]. Available: https://ejurnal.poltekkes-manado.ac.id/index.php/jif/article/view/280

[15] B. Biocrates, *MetaboAnalyst Tutorial*. Innsbruck, Austria: Biocrates Life Sciences, 2020, [Online]. Available: https://labplan.ie/content/uploads/2018/01/Biocrates-Tutorial-MetaboAnalyst-V01.pdf

[16] R. Adisonda, B. N. Rochman, and B. Handoko, "Upaya Peningkatan Kandungan Minyak Atsiri pada Bawang Merah Varietas Tuk-Tuk dengan Perlakuan Berbagai Macam Dosis Pupuk Kimia Pabrikan," *Sci. Timeline*, vol. 2, no. 2, pp. 75–86, 2022, [Online]. Available: https://jurnal.unupurwokerto.ac.id/index.php/sciline/article/view/65

[17] A. S. Harahap, D. A. Luta, and S. M. B. Sitepu, "Karakteristik Agronomi Beberapa Varietas Bawang Merah (*Allium ascalonicum* L.) Dataran Rendah," *Prosiding Seminar Nasional UNIBA Surakarta*, pp. 287–296, 2022, [Online]. Available:

https://download.garuda.kemdikbud.go.id/article.php?article=2950508&val=26176&title=KARAKTERISTIK%20AGRONOMI%20BEBERAPA%20VARIETAS%20BAWANG%20MERAH%20Allium%20ascalonicum%20L%20DATARAN%20RENDAH

[18] L. Cuadros-Rodríguez, C. Ruiz-Samblás, L. Valverde-Som, E. Pérez-Castaño, and A. González-Casado, "Chromatographic fingerprinting: An innovative approach for food 'identitation' and food authentication – A tutorial," *Anal. Chim. Acta*, vol. 909, pp. 9–23, Feb. 2016, doi: 10.1016/j.aca.2015.12.042.

[19] W. Xu, H. Yu, F. Yang, F. Yang, D. Liu, K. Lu, H. Gao, and Y. Song, "Second derivative UV–visible spectroscopy characterizing structural components of dissolved and particulate organic matter in an urbanized river," *Environ. Sci. Eur.*, vol. 34, no. 1, p. 29, Dec. 2022, doi: 10.1186/s12302-022-00609-z.

[20] T. Suhartati, *Dasar-dasar Spektrofotometri UV-Vis dan Spektrometri Massa untuk Penentuan Struktur Senyawa Organik*. Bandar Lampung: Anugrah Utama Raharja, 2017, [Online]. Available: http://repository.lppm.unila.ac.id/2700/1/buku%20dasar-dasar%20spektrofometri__upload.pdf

[21] K. Kucharska-Ambrożej, A. Martyna, J. Karpińska, A. Kiełtyka-Dadasiewicz, and A. Kubat-Sikorska, "Quality control of mint species based on UV-VIS and FTIR spectral data supported by chemometric tools," *Food Control*, vol. 129, pp. 1–12, 2021, doi: 10.1016/j.foodcont.2021.108228.

[22] L. Antonov and D. Nedeltcheva, "Resolution of Overlapping UV–Vis Absorption Bands and Quantitative Analysis," *Chem. Soc. Rev.*, vol. 29, no. 3, pp. 217–227, 2000, doi: 10.1039/a900007k.

[23] S. I. Hiremath, N. Gawas, S. Mulla, S. P. Kulkarni, S. Patil, and M. S. Palled, "First Order, Second Order, And Third Order Derivative UV-Spectrophotometric Method Development And Validation: A Review," *Eur Chem Bull*, vol. 12, no. 10, pp. 597–614, 2023.

[24] K. Sahini and D. C. N. Nalini, "A Review On Derivative Spectroscopy Aand Its Benefits in Drug Analysis," *Int. J. Creat. Res. Thoughts*, vol. 8, no. 12, pp. 1459–1464, 2020, [Online]. Available: https://www.ijcrt.org/papers/IJCRT2012176.pdf

[25] V. K. Redasani, P. R. Patel, D. Y. Marathe, S. R. Chaudhari, A. A. Shirkhedkar, and S. J. Surana, "A Review on Derivative UV-Spectrophotometry Analysis of Drugs in Pharmaceutical Formulations and Biological Samples Review," *J. Chil. Chem. Soc.*, vol. 63, no. 3, pp. 4126–4134, 2018, doi: 10.4067/s0717-97072018000304126.

[26] A. I. Vogel, G. H. Jeffery, J. Bassett, J. Mendham, and R. C. Denney, *Vogel's Textbook of Quantitative Chemical Analysis*, 5th ed. rev. Harlow (GB) New York: Longman scientific & technical J. Wiley & Sons, 1989.

[27] M. Greenacre, P. J. F. Groenen, T. Hastie, A. I. D'Enza, A. Markos, and E. Tuzhilina, "Principal Component Analysis," *Nat. Rev. Methods Primer*, vol. 2, no. 1, p. 100, Dec. 2022, doi: 10.1038/s43586-022-00184-w.

[28] S. Syafri, I. Jaswir, F. Yusof, A. Rohman, M. Ahda, and D. Hamidi, "The use of instrumental technique and chemometrics for essential oil authentication: A review," *Results Chem.*, vol. 4, p. 100622, Jan. 2022, doi: 10.1016/j.rechem.2022.100622.

[29] E. J. Abraham and J. J. Kellogg, "Chemometric-Guided Approaches for Profiling and Authenticating Botanical Materials," *Front. Nutr.*, vol. 8, pp. 1–24, 2021, doi: 10.3389/fnut.2021.780228.

[30] S. Barbosa, J. Saurina, L. Puignou, and O. Núñez, "Classification and Authentication of Paprika by UHPLC-HRMS Fingerprinting and Multivariate Calibration Methods (PCA and PLS-DA)," *Foods*, vol. 9, no. 4, p. 486, Apr. 2020, doi: 10.3390/foods9040486.

[31] P. Banerjee, S. Ghosh, M. Dutta, E. Subramani, J. Khalpada, S. RoyChoudhury, B. Chakravarty, K. Chaudhury, and K.-H. Baek, "Identification of Key Contributory Factors Responsible for Vascular Dysfunction in Idiopathic Recurrent Spontaneous Miscarriage," *PLoS ONE*, vol. 8, no. 11, p. e80940, Nov. 2013, doi: 10.1371/journal.pone.0080940.

[32] D. Yue, Y. Zhang, L. Cheng, J. Ma, Y. Xi, L. Yang, C. Su, B. Shao, A. Huang, R. Xiang, and P. Cheng, "Hepatitis B virus X protein (HBx)-induced abnormalities of nucleic acid metabolism revealed by 1H-NMR-based metabonomics," *Sci. Rep.*, vol. 6, no. 1, p. 24430, Apr. 2016, doi: 10.1038/srep24430.

[33] E. Noviana, G. Indrayanto, and A. Rohman, "Advances in Fingerprint Analysis for Standardization and Quality Control of Herbal Medicines," *Front. Pharmacol.*, vol. 13, p. 853023, Jun. 2022, doi: 10.3389/fphar.2022.853023.

[34] S.-C. Cheng, K. Chen, C.-Y. Chiu, K.-Y. Lu, H.-Y. Lu, M.-H. Chiang, C.-K. Tsai, C.-J. Lo, M.-L. Cheng, T.-C. Chang, and G. Lin, "Metabolomic biomarkers in cervicovaginal fluid for detecting endometrial cancer through nuclear magnetic resonance spectroscopy," *Metabolomics*, vol. 15, no. 11, p. 146, 2019, doi: 10.1007/s11306-019-1609-z.