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Spectroscopic Profiling of Hydroponic versus Soil-Grown Red Spinach (*Amaranthus tricolor* L.): Ultraviolet Visible Spectrophotometric and Chemometric Approaches for Optimizing Sustainable Cultivation Strategies

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Abstract. The metabolic profile of red spinach (*Amaranthus tricolor* L.) is distinctly modulated by growing environments. Rapid analytical techniques are required for quality control and market authenticity. This study investigated spectral differences between hydroponically and soil-cultivated red spinach using UV-Vis spectrophotometry with advanced chemometric analysis. Ten samples, with five biological replicates per cultivation method, were analyzed. The data were subjected to Savitzky–Golay smoothing and standard normal variate normalization to enhance signal quality. Principal component analysis (PCA) revealed a clear separation between groups, explaining 94.84% of the total variance (PC1 = 87.93%, PC2 = 6.91%). Spectral patterns were visually similar, but intensity differences were pronounced. Hydroponic samples showed higher absorbance at 408, 470.5, and 665 nm, indicating elevated chlorophyll a and b accumulation. This was likely due to optimized nitrogen uptake. Soil-grown samples showed peak intensity at 267 nm, representing phenolic compounds, potentially driven by natural environmental stress responses. Statistical validation using t-tests with false discovery rate (FDR) correction confirmed significant variations ($p < 0.05$) at wavelengths of approximately 267 and 408 nm, with PLS-DA feature selection aligned with statistical findings. Furthermore, the classification model assessment of partial least squares discriminant analysis (PLS-DA), support vector machine (SVM), and k-nearest neighbors (k-NN) using leave-one-out cross-validation (LOOCV) achieved 100% accuracy in discriminating cultivation methods. Permutation analysis confirmed the statistical robustness of PLS-DA ($p = 0.049$) and k-NN ($p = 0.019$) models, whereas the SVM model ($p = 0.792$) showed overfitting due to a small sample dataset. This study demonstrates that UV-Vis spectroscopy is a rapid and cost-effective tool for profiling the impact of cultivation. These findings establish a baseline for precision crop management, ultimately supporting sustainable agricultural practices by enabling growers to optimize production systems for targeted nutritional quality.

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1. Introduction

The implementation of sustainable agricultural practices is increasingly important in natural resource management and conservation. The global population is projected to reach 9-10 billion by 2050. This is expected to raise the demand for agricultural foodstuffs [1]. Consequently, challenges related to water and food scarcity are likely to intensify. Conventional soil-based agriculture is resource-intensive and depletes large amounts of land and water. It also causes serious environmental problems, such as soil degradation, deforestation, and groundwater pollution, due to excessive fertilizer and pesticide use [2,3]. Climate change is making conventional land use more challenging and endangering food security, thereby intensifying pressure on natural ecosystems [4].

Conversely, hydroponic technology offers an innovative solution for sustainable agriculture, especially in urban areas with limited land [5]. Research has demonstrated that hydroponic systems can substantially enhance agricultural yields. Studies have reported a lettuce yield increase of over 134% compared to soil-based systems [6] and an acceleration in plant growth of between 30% and 500%, resulting in yields that exceed those achieved through conventional methods [5]. Furthermore, hydroponics are distinguished by their remarkable water resource efficiency. This system has been demonstrated to significantly reduce water usage, with reports indicating that irrigation water savings of up to 95% can be achieved compared with conventional agricultural practices [7]. Hydroponic systems have been shown to reduce water consumption for lettuce production by 20% to over 90% through the implementation of a closed-loop hydroponic system in greenhouse settings [8]. Therefore, sustainable agricultural practices are urgently required to address resource constraints, reduce negative environmental impacts, and ensure efficient and stable future food production.

Red spinach (*Amaranthus tricolor* L.) has emerged as a food product with significant potential to address global food security issues owing to its highly nutritious profile, which provides high amounts of protein, carbohydrates, dietary fiber, and a variety of essential vitamins and minerals, such as potassium, calcium, iron, and vitamin C [9–12]. This plant is also notable for its remarkable resistance to various abiotic stresses, including drought and salinity, making it a resilient and adaptive crop for cultivation in areas vulnerable to climate change and infertile soils [12–15]. Its rich phytochemical and antioxidant contents, such as betacyanins, carotenoids, and flavonoids [9,13,16], not only provide significant health benefits but also support plant resistance to environmental stress, making it a nutritious and affordable commodity for improving food security in communities worldwide, thus offering an efficient and sustainable food solution.

The application of integrated UV-Visible spectroscopy and chemometric techniques to red spinach can provide insights into how different cultivation methods, including hydroponics and conventional, affect the biochemical composition and nutritional quality of plants in real time and non-destructively [17]. Research conducted on lettuce leaves has demonstrated the efficacy of UV-Vis spectroscopy in the rapid and precise detection of alterations in photosynthetic pigment content (chlorophyll and carotenoids) and secondary metabolite compounds (anthocyanins and phenolics) [18]. These changes have been shown to directly correlate with nutritional quality and stress resistance. Research on red spinach cultivated under diverse LED light spectra has demonstrated that specific cultivation conditions can significantly alter metabolite profiles, sugar, and nitrate, and polyphenol content. The study found that blue light enhanced polyphenol content, while red light increased nitrate content [19]. Nevertheless, further research on red spinach using different growing methods and employing UV-Vis spectroscopy and chemometrics is limited.

In the present study, we employed a UV-Vis spectroscopy approach in conjunction with chemometric analysis to identify spectral changes in red spinach (*Amaranthus tricolor* L.) with different cultivation methods and identify specific biomarkers without damaging the samples. This approach can serve as the basis for developing a spectroscopy-based monitoring system that can simultaneously improve the production efficiency and nutritional quality of red spinach, contributing to sustainable agriculture and food security.

2. Materials and Methods

To systematically investigate the spectral and metabolic distinctions between hydroponic and conventional cultivation systems, this study utilized an experimental protocol that involved sample preparation, spectral data acquisition, and chemometric analysis.

2.1 Plant preparation

Red spinach (*Amaranthus tricolor* L.) was cultivated in soil and hydroponic systems for 25 days from June to July 2025 in Padang, Indonesia. Fresh plant samples were obtained and cleaned before further sample preparation. Sample powder was obtained after a 72-hour freeze-dry process, followed by grinding, before further analysis. In subsequent analyses, the hydroponic samples were designated as H and the conventional samples as C.

2.2 Spectroscopy data acquisition

A 10 mg sample of each red spinach powder was diluted in 10 mL of methanol (70%; Merck, Germany) and sonicated for 10 min. The spectral data of the methanolic extract were recorded at intervals of 0.5 nm within the 200–800 nm wavelength range using a UV-1800 series spectrophotometer (Shimadzu, Japan). Five technical replicates were used for each sample. The recorded data were exported to a txt file and rearranged in a comma-separated value (CSV) format.

2.3 Chemometric data processing

The obtained spectral data were preprocessed and analyzed using the open-source R statistical software (2025.05.0 ver.). Savitzky–Golay smoothing was applied with a filter width of 15 data points, a third-order polynomial, and a zero-order derivative. The smoothed spectra were normalized using the standard normal variate (SNV) transformation to reduce multiplicative scatter. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), k-nearest neighbors (kNN), and support vector machine (SVM) were used in the multivariate analysis for feature extraction and supervised classification. Due to the limited number of spectra, supervised classification models were evaluated using leave-one-out cross-validation (LOOCV) and a permutation test (100 iterations). A volcano plot (t-test and false discovery rate (FDR) correction) and VIP score were used to identify biomarkers specific to each class.

3. Results and Discussion

In this study, each cultivation system was represented by five technical replicate spectra acquired from the same batch of red spinach (*Amaranthus tricolor* L.), resulting in a total of ten spectra. Technical replicates were used to evaluate the feasibility of combining UV-Vis spectroscopy with chemometric analysis for discriminating cultivation environments at the spectral level.

3.1 Spectroscopy spectral analysis

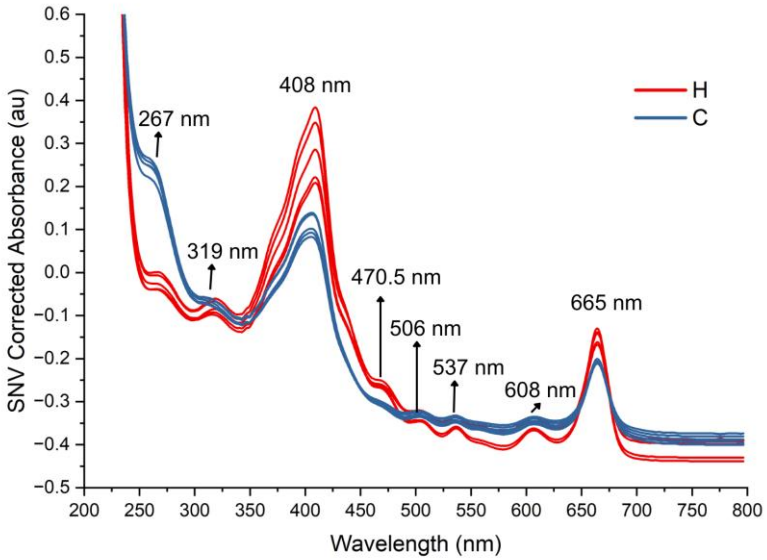


Figure 1. UV-Visible spectra of red spinach in hydroponic and conventional-grown method.

Ten samples from two different cultivation methods for red spinach (*Amaranthus tricolor* L.) were evaluated using UV-Vis spectroscopy to identify biochemical changes owing to different growing conditions. **Figure 1** shows the spectral differences at several wavelengths that represent the pigments and metabolites of the hydroponic and conventional soil-based cultivation methods. The key absorption peaks were identified at 267 and 319 nm, which represented polyphenols and flavonoids in the UV region, whereas 408 nm, 470.5 nm, 506 nm, and 665 nm indicated chlorophylls, carotenoids, polyphenol conjugates, and betalains [20] in the visible region. This result corroborated a previous study that specifically characterized betacyanins (amaranthin and iso-amaranthin) and anthocyanins in *Amaranthus tricolor* and drought-tolerant Amaranthus species [16,21].

The chemical differences reflected the physiological and metabolic responses of plants to their respective cultivation environments, with implications for photosynthetic efficiency and secondary metabolite accumulation. A previous study reported that cultivation conditions significantly affected the metabolite composition of leaf lettuce, with the hydroponically grown lettuce exhibiting increased levels of chlorophyll a, which serves as an indicator of photosynthetic capacity and efficiency. Meanwhile, phenolics and flavonoids are stress-response metabolites that accumulated differentially depending on cultivation conditions [22,23]. A recent study indicated that the levels of phenolic compounds in hydroponically cultivated Amaranthus specimens were significantly lower than those in conventionally grown plants. This observation is explained by the production of anti-nutrient metabolic processes in the hydroponic environment under conditions of stress. Additionally, the hydroponic system exhibited a threefold increase in flavonoid accumulation [24]. Furthermore, the controlled hydroponic environments with optimized light spectra enhanced pigment accumulation, such as carotenoids, compared to variable soil conditions [25].

3.2 Chemometric analysis

Principal Component Analysis (PCA) was instrumental in reducing complex spectral data and revealing a clear separation between the two cultivation methods (**Figure 2a**). The first (PC1) and second (PC2) principal components captured 87.93% and 6.91% of the variance, respectively,

forming two distinct clusters that successfully differentiated hydroponic from soil-grown plant samples. This indicates that cultivation methods significantly impact plant metabolic profiles. This is consistent with previous research on some species of cove crop species, which reported that PCA analysis showed strong distinctions between plant species with clear clustering patterns, supporting the principle that spectral fingerprints contain sufficient biological information to drive robust classification when reduced through PCA [26]. Li, et al. (2022) analyzed 289 Amaranthus accessions across nine species using principal component analysis on comprehensive phytochemical and antioxidant datasets, demonstrating distinctive aggregation patterns across species with clear clustering into informative groups [27], establishing that PCA is an effective tool for parsing complex multivariate phytochemical data from Amaranthus species.

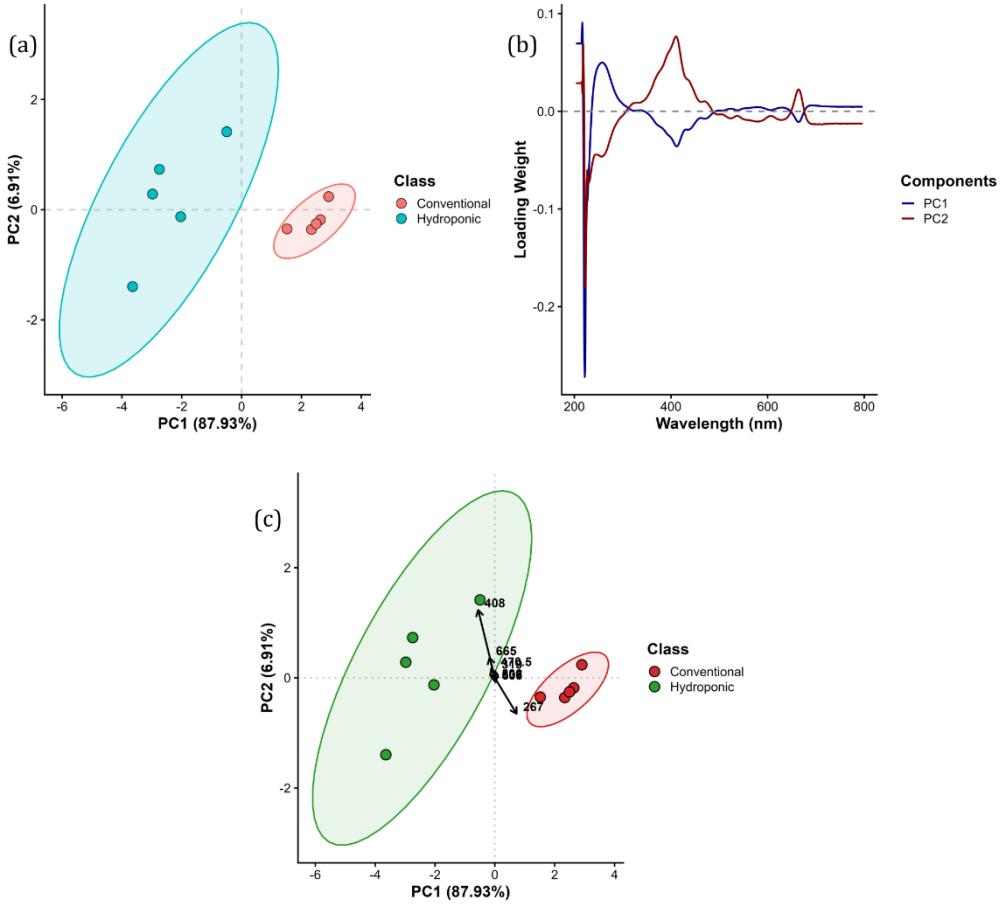


Figure 2. Principal component analysis (PCA): (a) score plot, (b) loading plot, and (c) biplot of the red spinach UV-Vis data.

The high variation captured in PC1 (87.93%) indicated that the differences in hydroponically and conventionally grown red spinach resulted from different plant responses to the environment, whereas the 6.91% PC2 variance indicated that there were small but measurable differences in the metabolism of each cultivation system. The loading plot (**Figure 2b**) shows that PC1 is characterized by strong positive loadings in the visible region at approximately 405 and 665 nm, whereas negative loadings occur in the UV region around 263 nm. This pattern is consistent with previous spectral analyses. **Figure 2c** further confirmed that hydroponic observations are oriented in the direction of the positive loading vectors at 405 and 665 nm, whereas conventional observations are shifted toward the negative loading vector at 263 nm. This

confirms that PC1 primarily reflects a biological trade-off between pigment-dominated visible absorption and phenolic-dominated UV absorption under different growing conditions and is consistent with previous spectroscopic studies of plant leaves, in which the first few principal components, driven by pigment-related features, explain the vast majority of variance and provide effective discrimination or prediction [28,29].

Three classification models, partial least squares discriminant analysis (PLS-DA), k-nearest neighbors (k-NN), and support vector machine (SVM), were employed, which achieved high accuracy in discriminating between the two groups of red spinach (**Figure 3**). PLS-DA is instrumental in identifying key wavelengths, k-NN performs optimally with well-defined data clusters, and SVM adeptly manages complex datasets. Sohn, et al. (2021) demonstrated that Vis-NIR spectroscopy combined with machine learning methods, specifically SVM with Savitzky-Golay preprocessing, achieved a maximum mean classification accuracy of 99.7% for the discrimination of Amaranthus species, corroborating the SVM's superior performance in high-dimensional spectral datasets by maximizing the decision boundary margin between classes [30]. Collectively, these models demonstrate that employing UV-visible spectroscopy alongside these classification techniques provides a dependable method for distinguishing plant growth conditions, comparable to more advanced methodologies.

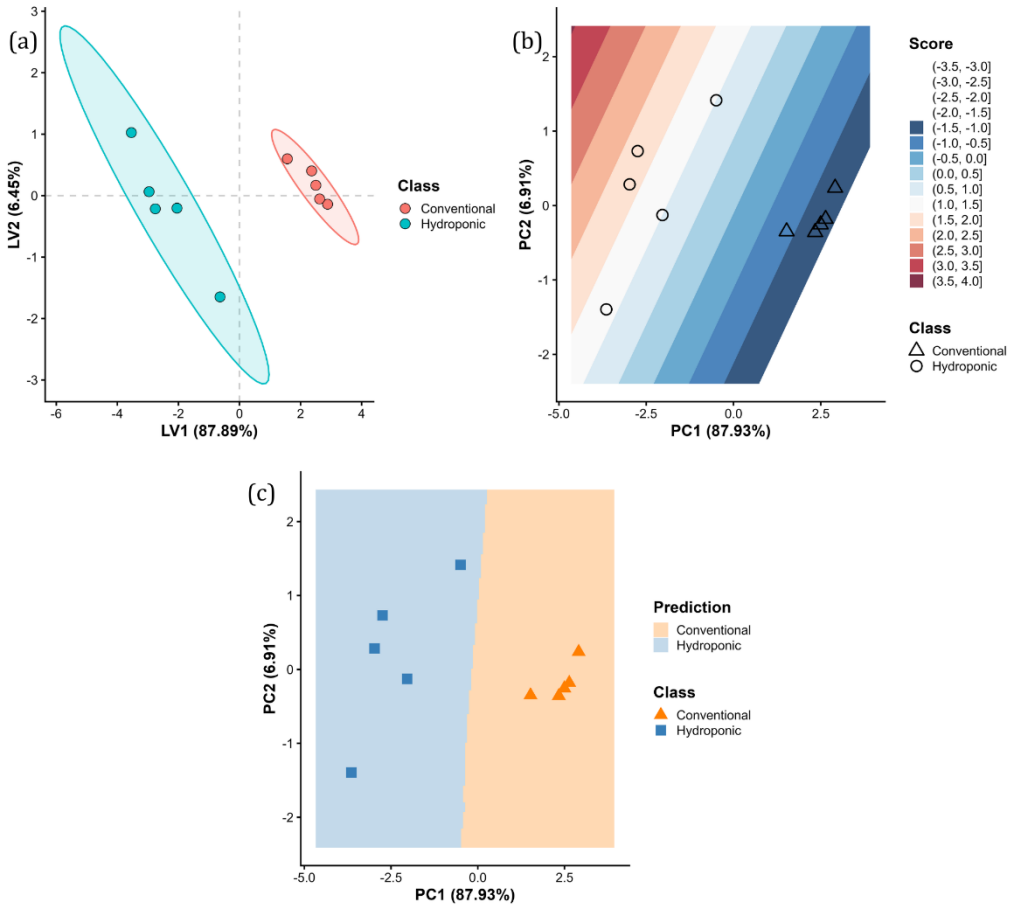


Figure 3. Comparison of (a) partial least squares discriminant analysis (PLS-DA), (b) support vector machine (SVM), and (c) k-nearest neighbor (kNN) classification models of red spinach UV-visible data.

The achievement of 100% accuracy by the three classification models—PLS-DA, k-NN, and SVM— has been achieved under the leave-one-out cross-validation (LOOCV) method (**Figure 4**). However, permutation testing revealed key differences in statistical robustness, with PLS-DA showing a permutation p-value of 0.049, kNN a stronger signal (p=0.019), and SVM a non-significant result (p= 0.792), indicating that only PLS-DA and kNN models are unlikely to have achieved perfect discrimination by chance. This suggests that the latent variable exploited by PLS-DA and the local distance-based decision rule by k-NN capture spectral differences between hydroponic and soil-grown samples, whereas SVM is more consistent with overfitting in the small dataset. A combination of cross-validation and permutation testing has been recommended as a critical safeguard against classification in small number datasets [31]. This is consistent with a previous study on other high-dimensional biological datasets, such as cancer microarrays, where k-NN and related classifiers have achieved equal or lower misclassification than alternative algorithms, including SVM under small number and large feature conditions [32,33]. Nearest-neighbor-based algorithms and latent dimensionality reduction may be more stable than margin-based classifiers, such as SVM.

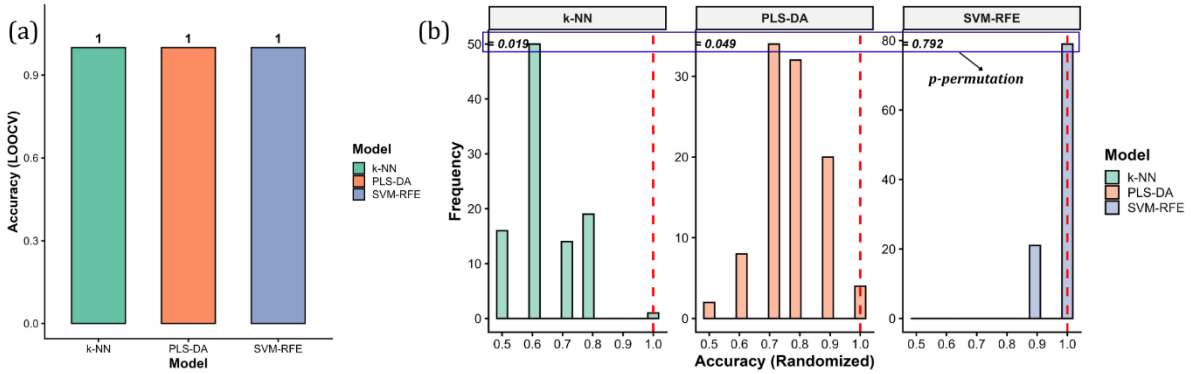


Figure 4. Comparison of (a) model accuracy and (b) permutation test analysis of supervised models

The biomarker of the specific wavelength was identified using statistical univariate (volcano plot) and variable importance of projection (VIP) scores derived from PLS-DA, as shown in **Figure 5**. The volcano plot analysis provides robust statistical validation for the VIP-identified critical wavelengths, confirming distinct metabolic profiles between hydroponic and soil-grown plants. Hydroponic plants show elevated absorbance at 665 nm (chlorophyll a), 470.5 nm, and 506 nm (chlorophylls/carotenoids), appearing in the upper-right quadrant of the volcano plot (positive fold-change, $p<0.05$), indicative of enhanced photosynthesis. Conversely, soil-grown plants exhibit higher absorbance at 267 nm and 319 nm (aromatic amino acids/phenolics), clustering in the upper-left quadrant (negative fold-change, $p<0.05$), reflecting increased stress-related secondary metabolites. Further analysis through VIP scores highlighted 267 and 408 nm as the most critical wavelengths, indicating key absorption representing chlorophylls a and phenolics. This dual validation, where wavelengths are significant in both VIP and volcano plots, strengthens their biological relevance and ensures robust biomarker identification across cultivation methods, consistent with similar studies using UV-vis spectroscopy and chemometrics [34].

The spectral differentiation between hydroponic and soil-grown plants reveals contrasting metabolic strategies with significant implications for sustainable agriculture and resource conservation. Hydroponically grown plants prioritize photosynthetic efficiency, evidenced by elevated chlorophyll absorption at 665, 506, and 470.5 nm, indicating optimized light capture and

energy conversion in controlled environments. This metabolic profile is directly correlated with improved water use efficiency (WUE) and fertilizer productivity, as confirmed by similar studies demonstrating that hydroponic systems achieve higher nutrient and water utilization rates than conventional soil cultivation [35].

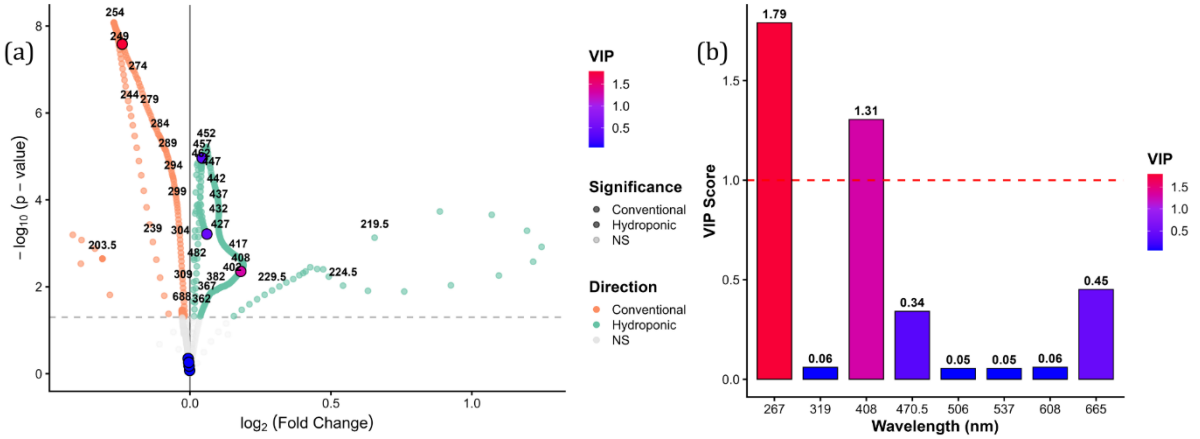


Figure 5. (a) Volcano plot and (b) variable importance in projection (VIP) scores of red spinach's UV-visible spectral data.

Soil-grown plants produce more secondary metabolites at 267 nm and 319 nm, which are phenolics and aromatic compounds. These are stress responses to changing soil conditions, moisture levels, and interactions with microbes. These stress-induced compounds improve the nutritional value and availability of plant-based medicines for human health [36]. However, they also indicate that plants are not using resources efficiently and need more energy and water to adapt to their environment [37]. UV-visible spectroscopy identifies dual metabolic pathways, providing insights into sustainable agriculture. This enables farmers to optimize inputs while reducing environmental impact. UV-visible spectroscopy showed that spectral fingerprinting can screen plants for stress tolerance and bioactive compounds, supporting the preservation of adapted genetic resources. Although this study did not directly quantify resource use efficiency or environmental impact, the results indicate that UV-visible spectroscopy combined with chemometrics can capture cultivated-related shifts in pigment and phenolic profiles in red spinach. Coupling spectroscopic analysis with machine learning enables the rapid identification of efficient cultivars without multi-year trials. However, we note that this design does not include independent biological replicates; therefore, the present findings should be interpreted as a proof-of-concept rather than as a population-level validation. In addition, perfect classification suggests potential overfitting, as spectral signatures vary with environmental conditions.

4. Conclusion

This study demonstrated that UV-visible spectral fingerprinting combined with multivariate analysis (PCA, PLS-DA, k-NN, and SVM) shows strong potential to differentiate red spinach (*Amaranthus tricolor* L.) grown in hydroponic versus soil-based systems and identify metabolic biomarkers that reflect differences in photosynthetic efficiency and secondary metabolite production. Dual validation, integrating VIP scores with volcano plot analysis, strengthens biomarker identification. This non-destructive approach provides a cost-effective tool for precision agriculture that supports sustainable resource management. However, given the small

number of datasets and lack of biological replication and external validation, these findings should be regarded as preliminary and hypothesis-generating. Future research should incorporate longitudinal spectroscopic monitoring, integrate spectroscopic data with yield and nutritional quality metrics, validate findings across multiple crop species and geographic regions, and conduct cost-benefit analyses to enhance the applicability of this methodology for sustainable agricultural decision-making.

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