



Article

Vis/NIR Absorbance and Multivariate Analysis for Identifying Infusions of Herbal Teas Cultivated Organically

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Abstract: Ready-to-drink herbal teas are increasingly popular due to their pleasant aroma and taste, with plants cultivated organically showing improved quality properties. Vis/NIR absorbance and multivariate analysis were used for classifying infused herbal teas cultivated under organic systems, in addition to testing various spectral pretreatments to assess the identification accuracy improvement. A total of 150 herbal tea infusions (boldo, carqueja, chamomile, fennel, and lemon grass) were evaluated, and six spectral pretreatments (centering, standard normal variation, object-wise standardization, first derivative, second derivative, and detrending) were applied to the spectra. Principal component analysis (PCA) and the partial least squares discriminant analysis (PLS-DA) were used to distinguish the infused herbal teas. Clustering patterns were affected by the pretreatments, and the PCA was capable of separating the infused herbal teas. The PLS-DA was efficient in identifying the infusions, reaching kappa values from 0.97 to 1.00 with optimal latent variable numbers from two to five. Detrending and object-wise standardization pretreatments led to better results and required fewer latent variables. The proposed methodology presents the potential to be used in a fast, safe, environmentally friendly (without chemical reagents), and nondestructive way, appearing as essential for meeting the technological development of the agrifood industry.

Keywords: tea classification; absorbance; spectroscopy



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1. Introduction

The widespread usage and popularity of ready-to-drink herbal teas are justified due to their pleasant aroma and taste. Additionally, their benefits on human health are well documented in the pharmacopoeias of different countries, as well as in the scientific literature [1–4]. Tea is rich in a diverse range of compounds, including alkaloids, polyphenols, vitamins, proteins, catechins, and free amino acids [5]. There are many herbal teas, and their infusions have been identified by color, odor, and flavor, depending on their chemical compounds and processing methods. The cultivation system also affects tea properties, with a growing number of producing countries adopting organic farming techniques to enhance quality and preserve the health benefits of tea [6].

There is a rich cultural and medicinal diversity in herbal tea consumption around the world, with more than 700 species cataloged. Although different species of herbal teas are native to specific regions, their cultivation has expanded as they have become more popular. Additionally, the rise in demand for ready-to-drink herbal teas and soft drinks has contributed to the development and promotion of new products based on traditional herbal teas. In this study, five herbal teas were analyzed, chosen primarily for their popularity,

with a focus on selecting species that are adapted and cultivated worldwide. These plants were fennel (*Foeniculum vulgare* Mill., *Apiaceae*), chamomile (*Matricaria recutita* L., *Asteraceae*), lemon grass (*Cymbopogon citratus* Stapf., *Poaceae*), boldo (*Peumus boldus* Mol., *Monimiaceae*), and carqueja (*Baccharis genistelloides* (Lamarck) Persoon, *Asteraceae*).

Fennel is a popular aromatic plant native to the Mediterranean basin and cultivated worldwide, with the infusions from its fruits being valued for nutritional and health benefits [4]. Chamomile is an annual herb native to Europe but well-adapted to different environmental conditions. The tea brewed from its flowers has been consumed by humans for centuries for medicinal purposes [3]. Lemon grass is a perennial fragrant herb from South India and Sri Lanka, but also grown throughout tropical America and Asia. The tea composed of its dried leaves is used medicinally [7]. Boldo is an indigenous plant, originating in the Central Chilean forests but also found in other Latin American regions, such as Brazil and Argentina. The tea composed of its dried leaves has a strong and slightly bitter flavor, with a camphor-like aroma and medicinal properties [1]. Carqueja is a plant that grows natively in Latin America, especially in the South of Brazil, Paraguay, Argentina, and Uruguay, but it can also be cultivated in a variety of climates. The therapeutic use of its tea, composed of dried leaves, is widespread in folk medicine for the treatment and prevention of diverse disorders [8].

The market for ready-to-drink beverages (polyethylene terephthalate packaged, bottled, and canned) and soft drinks has been growing globally [9,10]. As the demand for discriminating between various infused herbal teas is rising for sensory evaluations, quality control, and grading procedures, it is important to develop safe, fast, environmentally friendly, and nondestructive methods for this task [11]. Organoleptic and instrumental analyses, especially high-performance liquid chromatography (HPLC) and gas chromatography (GC), are commonly used when evaluating and classifying infused herbal teas [12]. However, these are subjective, destructive, and costly techniques [13]. A promising alternative lies in the identification through Vis/NIR spectroscopy, which aligns with the technological advancements in the tea industry and responds effectively to market demands [14].

Aside from being a noninvasive and rapid method, spectroscopy has many advantages, such as high efficiency, accuracy, quick data analysis, and better repeatability [15]. The basic procedures involved in spectrometry application encompass acquiring spectral signatures, conducting chemometric processing (calibration and validation), and applying the resultant models in industry or field. The spectra can be obtained in a variety of wavelength ranges, quantifying the amount of light transmitted, reflected or absorbed by the samples [16]. The chemometrics, or multivariate analysis, relates the spectral measurements to the product features obtained from conventional techniques using mathematical and statistical methods [17]. The resultant models can then be used to estimate many product features from the spectral signatures, reducing the spectroscopic data dimensions and speeding the analyses.

Generally, the original or raw spectra contain background information on measuring environments as well as chemical and physical properties of samples. This requires that spectral pretreatments be applied to remove irrelevant information which cannot be handled by the chemometric pattern recognition methods [18]. Several spectral pretreatments (from averaging over spectra to derivative transformations) have been developed with this aim [19].

Many scientific articles have discriminated against teas or evaluated their quality based on fresh, powdered, or bagged samples of the raw material used for brewing them [14,20,21]. Few studies considered the infused teas in the analyses, focusing on *Camellia sinensis* L. and performing comparisons by using color models, such as the $L^*a^*b^*$ or the RGB [22,23], or chemometric methods [11,24,25]. The classification of infused herbal teas based on spectral

data and multivariate analyses is scarce [5,26]. Discriminating infused teas by computer vision or organoleptic techniques is more complex than for powdered samples since it requires additional parameters to the visual image, such as taste or aroma. Furthermore, given that computer vision is heavily impacted by the image quality and the consistency of ambient lighting, the error probability increases when employing this methodology for infused samples.

Tea infusions are generally yellowish due to the presence of compounds like polyphenols and flavonoids, which are water-soluble and released during the brewing process. These compounds can vary in color but most of them impart yellow or golden hues to the liquid. Additionally, the brewing time and water temperature can influence the intensity of the color. The yellowish color typical of the great diversity of herbal tea infusions makes identification more difficult compared to powdered samples.

The objective and innovation of this research lie in the utilization of Vis/NIR absorbance coupled with multivariate analysis to differentiate and identify five infused herbal teas cultivated under organic systems. Furthermore, various spectral pretreatment methods were assessed to enhance discrimination accuracy. This is important, particularly in the context of the agrifood industry and consumer experience, since it ensures consistency in quality analyses and eliminates variations caused by human subjectivity. Automatic identification of infused herbal teas also results in cost savings by minimizing the need for manual labor and chemical reagents in industrial processes. Furthermore, the results of the automated discrimination can help to understand better the differences among herbal teas, contributing to new product development and marketing strategies, such as the creation of customized blends and products that cater to diverse consumer preferences.

2. Materials and Methods

2.1. Organic Cultivation and Tea Infusion Preparation

Five herbal tea species (boldo, carqueja, chamomile, fennel, and lemon grass) were grown organically on a certified farm situated in Capim Branco, Minas Gerais, Brazil ($19^{\circ}34' \text{ S}$, $44^{\circ}10' \text{ W}$, 816 m a.s.l.) (Figure 1). According to Köppen's classification, the region's climate is Cwa (humid subtropical with dry winter and hot summer) [27].



Figure 1. Location of the organic growing farm of herbal plants in the Minas Gerais State, Brazil (Map data ©2025 Google—Imagery ©2025 TerraMetrics).

Figure 2 summarizes the steps from organic cultivation to the preparation of tea infusions. The herbal plants were cultivated without applying synthetic pesticides, chemical fertilizers, or growth regulators. A mixture of poultry and cattle manure was used to enrich the soil, while the maintenance of the experimental area consisted of manual weeding. The experimental areas were equipped with drip irrigation pipes, which were operated depending on microclimatic conditions [4,7].

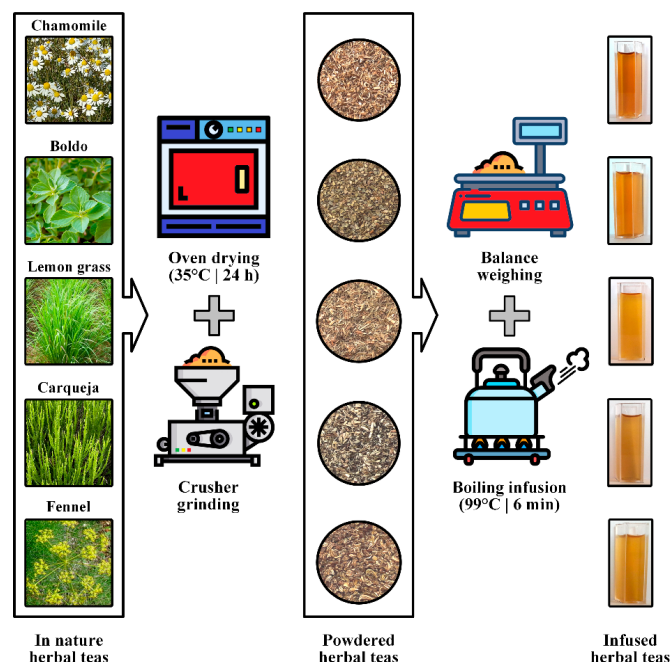


Figure 2. Schematic diagram of the steps from organic cultivation to the preparation of herbal tea infusions.

Fennel and chamomile seeds were manually sown in two different areas of the open field, with a spacing of 0.5 m between rows and 0.3 m between plants [3,4]. The lemon grass slips (0.25 m in height) were transplanted by hand in another area of the field, with a spacing of 0.5 m between plants and 0.6 m between rows [28]. During the transplanting of boldo and carqueja seedlings to two different experimental areas in the field, a spacing of 0.5 m was maintained between plants and 1.0 m between rows [29,30].

The fennel fruits, also known as seeds, were harvested approximately 80 days after planting when the flowers had turned brown and were fully formed, but before they began to release their fruits to avoid the bitterness of the herb [31]. The cut was made 2.5 cm above the ground to encourage regrowth. Harvesting of chamomile flowers was performed when more than 50% of flowers had their petals open at 180° [32]. Aboveground lemongrass leaves were cut to a length of 0.5 m, with grass showing spots or browning on the edges discarded [7]. The adult boldo leaves were harvested before flowering, with the woody shoots being cut at the base of the plant [1,30]. The carqueja leaves were harvested by removing the entire aerial part of the plant, leaving 0.3 m of stem for regrowth [29].

The fennel fruits, chamomile flowers, as well as the lemon grass, boldo, and carqueja leaves were washed with clean water to remove any foreign matter and dried at 35 °C for 24 h in a drying oven (LUCA-80/64, Lucadema, São José do Rio Preto, SP, Brazil) to preserve the essential oils, flavor, and medicinal properties, without the degradation of volatile compounds [33]. The dried products were then separately ground in an automatic grinder (KJB22A, KitchenAid, São Paulo, SP, Brazil) and passed through a 60-mesh sieve.

Prior to the infusion process, the powdered herbal teas were stored separately in airtight plastics, away from sunlight, at an ambient temperature of 25 °C and a controlled

relative humidity of 65%. Powdered samples of 2 g (30 of each herbal tea) were individually weighted from a precision analytical balance (UX620H, Shimadzu, Barueri, SP, Brazil) and infused with 100 mL of boiling pure water (99 °C) during 6 min. After the infusion time, the extracts were filtered, and the volumes homogenized. From each infusion, an aliquot of 3.5 mL was taken to fill the quartz cuvette with a light pass length of 10 mm. A total of 150 infused herbal tea samples were prepared (Figure 2).

2.2. Absorbance Measurements and Multivariate Analysis

The absorbance spectra were measured using a miniature spectrometer (JAZ-EL350, Ocean Optics, Orlando, FL, USA), which was preconfigured via OceanView™ software (version 1.6.7) to capture and save data within the 480 to 1000 nm range, with a resolution of 1.3 nm. A light source module (tungsten-halogen) integrated into the spectrometer was connected to the input of a cuvette holder with a light attenuator (FHSA—TTL, Ocean Optics) by a premium fiber (600 µm). The spectrometer captured the beam of light that passed through the infusion contained in the cuvette (Figure 3).

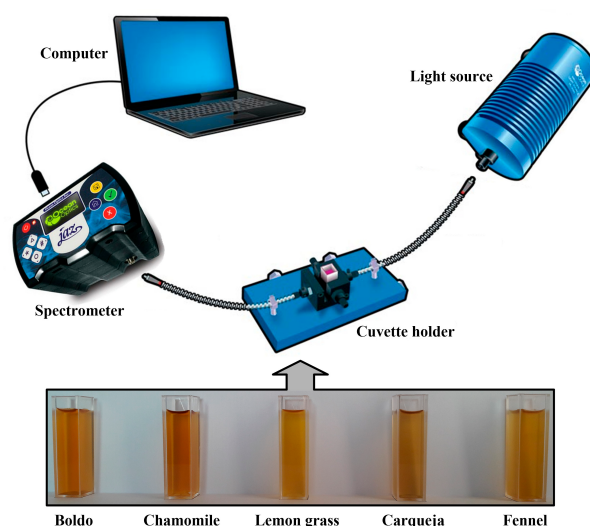


Figure 3. Experimental arrangement for absorbance measurements in infused herbal teas.

Before conducting absorbance measurements on the herbal tea infusions, the light source was allowed to reach thermal equilibrium by waiting for the warm-up period (20 min). Additionally, two reference spectra were acquired to ensure consistent baseline values for all measurements. A quartz cuvette with pure water was used as a reference standard to measure the absorbance, while the reference for light absence was obtained by manually adjusting the attenuator of the cuvette holder to block light.

Multivariate analysis was applied to the pretreated and original absorbance spectra to assess the variability within the data, also detecting outliers, random noises, and any potential undesirable components present in the data. These components could potentially compromise the accuracy and precision of the models developed. In this study, the pretreatments evaluated were centering, first derivative, second derivative, detrending, and normalization by object-wise standardization (OWS) and standard normal variate (SNV).

The mean centering corresponded to the subtraction of the average absorbance spectrum of each herbal tea from the original spectra [16]. Spectra derivatives were obtained using the first and second-order Savitzky–Golay filters with a 10-point window and optimally fitting the data to least-squares polynomials [20]. Detrending involved fitting a polynomial using the Vandermonde matrix and subtracting it from each original spectrum [34]. The SNV pretreatment entailed subtracting each spectrum by the spectra average

and then dividing the result by the spectra standard deviation, while the OWS pretreatment involved dividing each tea spectrum by the spectra standard deviation directly [14].

An initial principal component analysis (PCA) was conducted to uncover inherent similarities within the data and to detect outliers and patterns [12,35]. The PCA utilized the spectral absorbance values of infused samples.

The partial least squares discriminant analysis (PLS-DA) was employed for distinguishing the infused herbal teas. For this, each herbal tea was treated as a separate class. For each pretreated and original spectra, the calibration dataset consisted of 2/3 of the samples (100 infusions, 20 from each class), which were used to establish a decision boundary in the response pattern space during the development of the discrimination models. The remaining 1/3 of the samples (50 infusions, 10 from each class) were utilized as an external validation dataset for assessing the capacities of the discrimination models to identify unknown samples and guarantee dataset representativeness [20,36].

The calibration with cross-validation was applied during the development of the models [37], dividing each original calibration dataset (original and pretreated spectra) into five groups and leaving one group (20 samples, four of each herbal tea) out from the model fit. This remaining group was then used for distinguishing the tea infusions according to the calibrated model. The prediction residuals were computed for each iteration, repeating the process with different subgroups of the calibration dataset, until every subgroup was left out once. The models with the smallest prediction residuals were applied to the external validation dataset, performing independent identifications. The PLS-DA modelling transformed the spectral data into latent variables (LVs), each comprising a projection of the original variables onto the multivariate subspace. This procedure reduced the data dimensionality and properly described the underlying phenomena. The optimal LV number for each PLS-DA model was determined by minimizing the root mean square errors (RMSE) during the cross-validation process [20].

The loadings and the variable importance for projection (VIP) were obtained for each PLS-DA model to identify the wavelengths that most contributed to the predictions and represented greater variability inside the datasets [38]. The loadings were computed from the least-squares regressions of the original and pretreated spectra, while VIP corresponded to the weighted sum of squares of the loadings. Wavelengths with VIP exceeding 1.0 highly influenced the identification of the herbal tea infusions. The VIP values from 0.8 to 1.0 moderately affected the PLS-DA models, appearing as useful predictors but with less impact compared to the higher values. Wavelengths with VIP below 0.8 were less influential in distinguishing the herbal tea infusions [39].

The accuracy of the developed PLS-DA models was assessed using confusion matrices and Kappa coefficients based on the external validation data [14,38,40]. The Kappa coefficient is useful for classifiers with both imbalanced and balanced datasets, providing a robust measure of agreement [37]. Its value ranges from -1 to 1 , and is divided into six benchmarks for interpretation purposes: poor (<0.00), slight (0.00 – 0.20), fair (0.21 – 0.40), moderate (0.41 – 0.60), substantial (0.61 – 0.80), and almost perfect (0.81 – 1.00) [41]. The pretreatments and multivariate methods were implemented in the SCILAB software version 6.0.1 (Scilab Enterprises, Versailles, France).

3. Results

The average absorbance spectra of the infused herbal teas, considering original and pretreated data, are presented in Figure 4. Similar spectral patterns were verified for each individual pretreatment, while both absorbance magnitudes and shapes of the spectral signatures differ greatly among the distinct pretreatments.

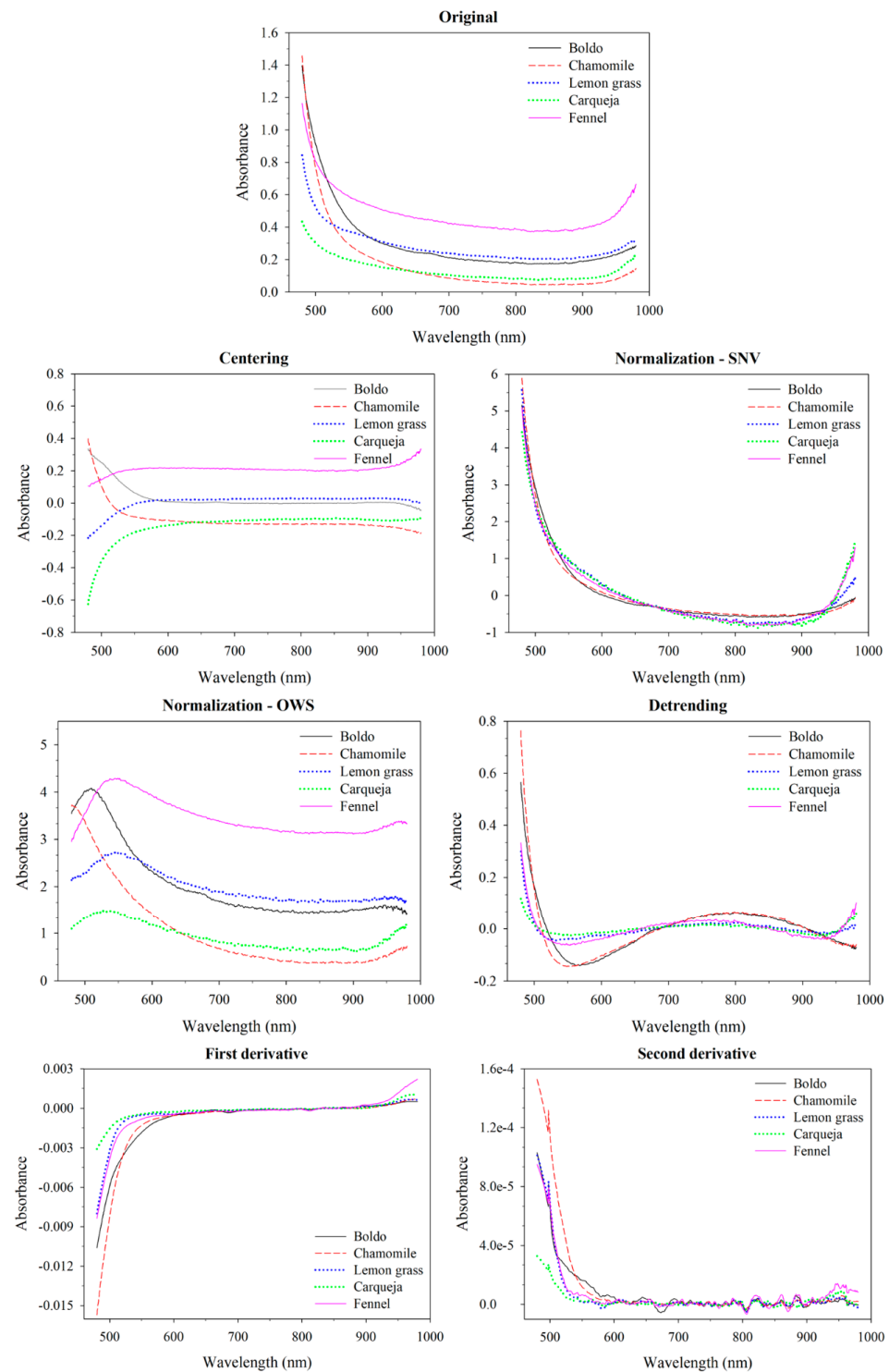


Figure 4. Average original and pretreated spectral signatures of the herbal tea infusions.

The original, centered, and OWS average spectra demonstrated a clear visual separation of the infusions for almost the entire wavelength range, with marked differences from 480 to 650 nm and between 930 and 1000 nm. These bands were also sensitive for distinguishing the herbal tea samples when using the other pretreatments, which resulted in more overlapping wavelengths.

The score plots of PCA (Figure 5) indicated the potential for separating the infused herbal teas, simplifying the discrimination analysis, reducing the multidimensional dataset, and helping to recognize outliers. The first principal component (PC1) represented 79.5%

(centering) to 97.7% (second derivative) of data variance, while the second principal component (PC2) varied between 1.1% (second derivative) and 19.5% (centering). Scattered clusters were observed when applying OWS and centering, as well as for original data, for fennel, boldo, and carqueja. These pretreatments separated all the herbal tea classes that had been studied. Lemon grass infusions formed a PCA cluster close to the fennel class when using the derivatives and the detrending pretreatments, probably requiring data of an additional principal component to improve their separation. Chamomile and boldo also formed closer groups for the first derivative spectra. The SNV pretreatment did not separate the tea infusions well using two principal components. For the other datasets, carqueja samples presented the best grouping pattern, tending to be easily identified by classifiers based on Vis/NIR absorbance and multivariate analysis.

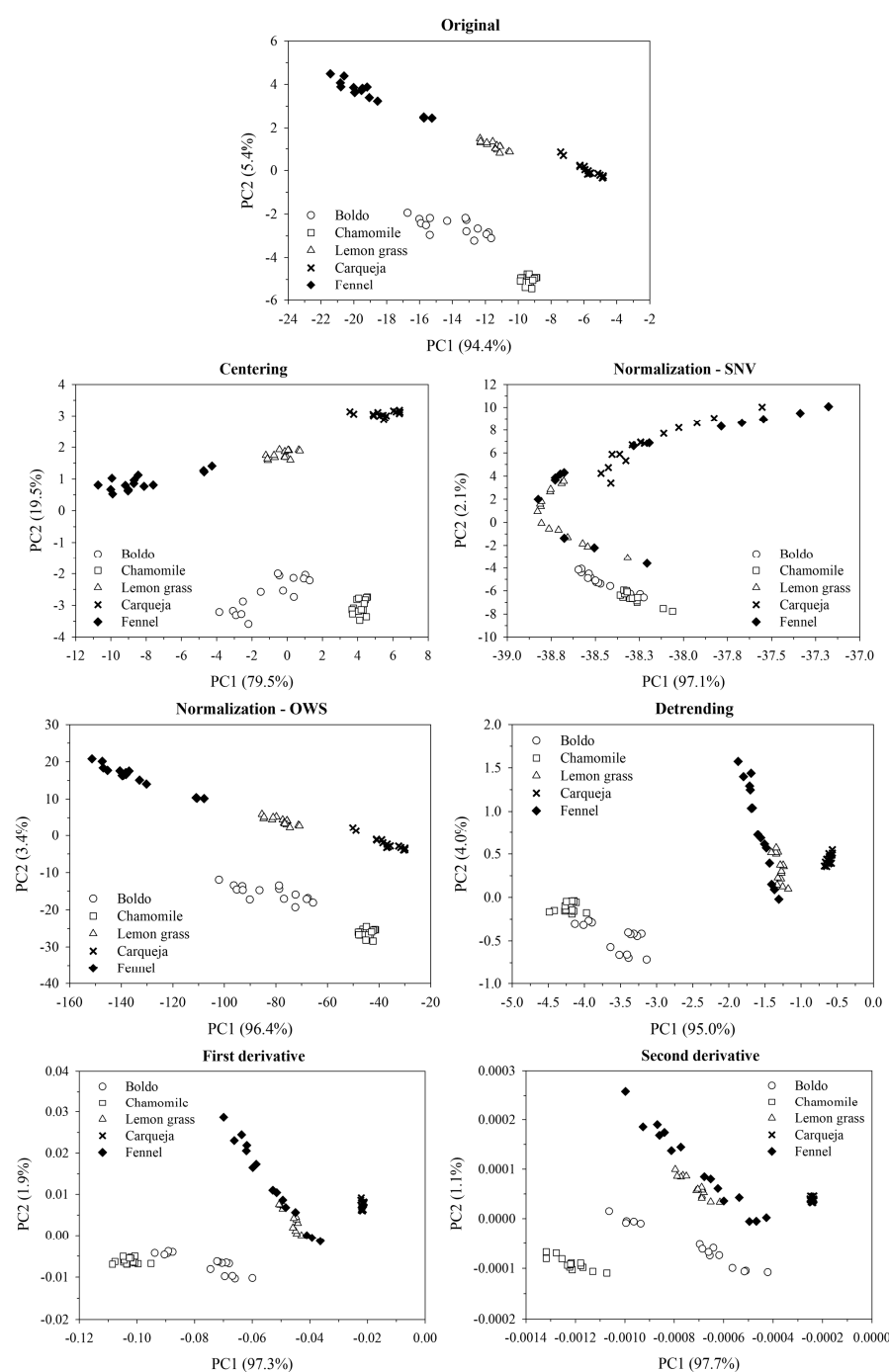


Figure 5. PCA scores of original and pretreated spectral signatures of the herbal tea infusions.

The Kappa coefficients obtained when utilizing the PLS-DA with different spectral pretreatments, and LVs are presented in Figure 6. This index measured the overall accuracy of the model predictions, considering the probability of chance agreement. The lowest Kappa values, classified as moderate, were verified when using LV equal to one with original, centering, detrending, and OWS pretreatments. The other Kappa values ranged from 0.736 to 1.000, indicating substantial or almost perfect predictions. Following the PCA trends, OWS spectra required only two LVs to reach the maximum overall accuracy (Kappa coefficient) of 1.00, while the detrending pretreatment required LV equal to four. The centered and original spectra reached maximum overall accuracies of 0.995, requiring two LVs. The maximum overall accuracies of derivatives (first and second) and SNV were 0.995, 0.970, and 0.993, requiring LVs equal to three, four, and five, respectively.

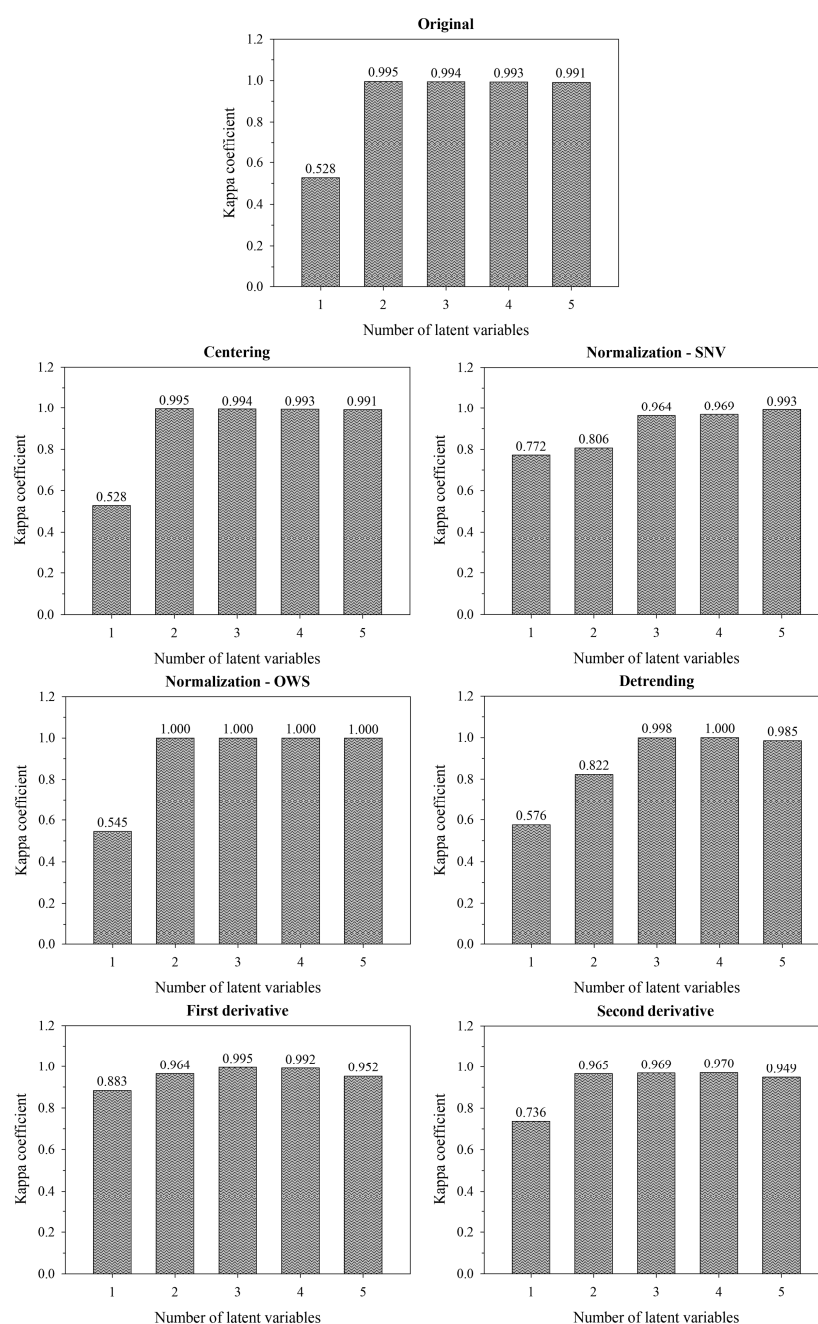


Figure 6. Classification performance of PLS-DA models, represented by the Kappa coefficients, varying the LVs, and using different pretreatments for discriminating the herbal tea infusions.

The loadings and VIP, considering the optimal number of LVs for original and pretreated spectra, are presented in Figures 7 and 8, respectively. The loadings patterns (Figure 7) showed considerable variation, mainly for the PLS-DA models that required more LVs. However, well-defined peaks and valleys could be detected for all pretreatments, evidencing wavelengths that carry valuable information for discriminating the infused herbal teas. The SNV was the pretreatment that required more LVs, while the second derivative presented a loading profile where distinguishing accentuated peaks and valleys was more challenging. The VIP patterns were similar for all pretreatments (Figure 8), with original, centered, and OWS spectra showing higher values from 480 to 550 nm. Negative VIP values were not observed, indicating that all wavelengths contributed to the predictions. Few wavelengths resulted in VIP values below 0.8, confirming that most spectral regions were moderately or highly influential in effectively discriminating the infused herbal teas.

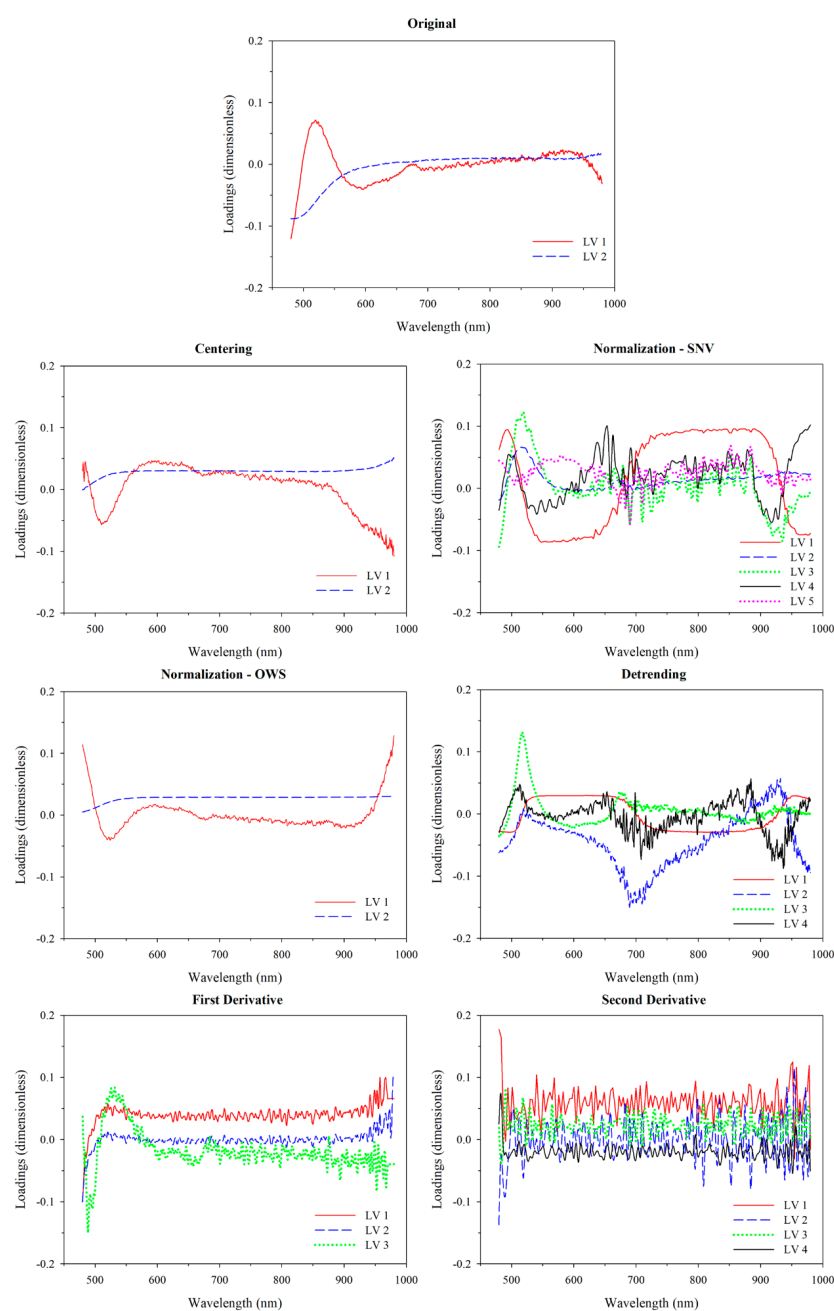


Figure 7. PLS-DA loadings of original and pretreated spectral signatures of the herbal tea infusions.

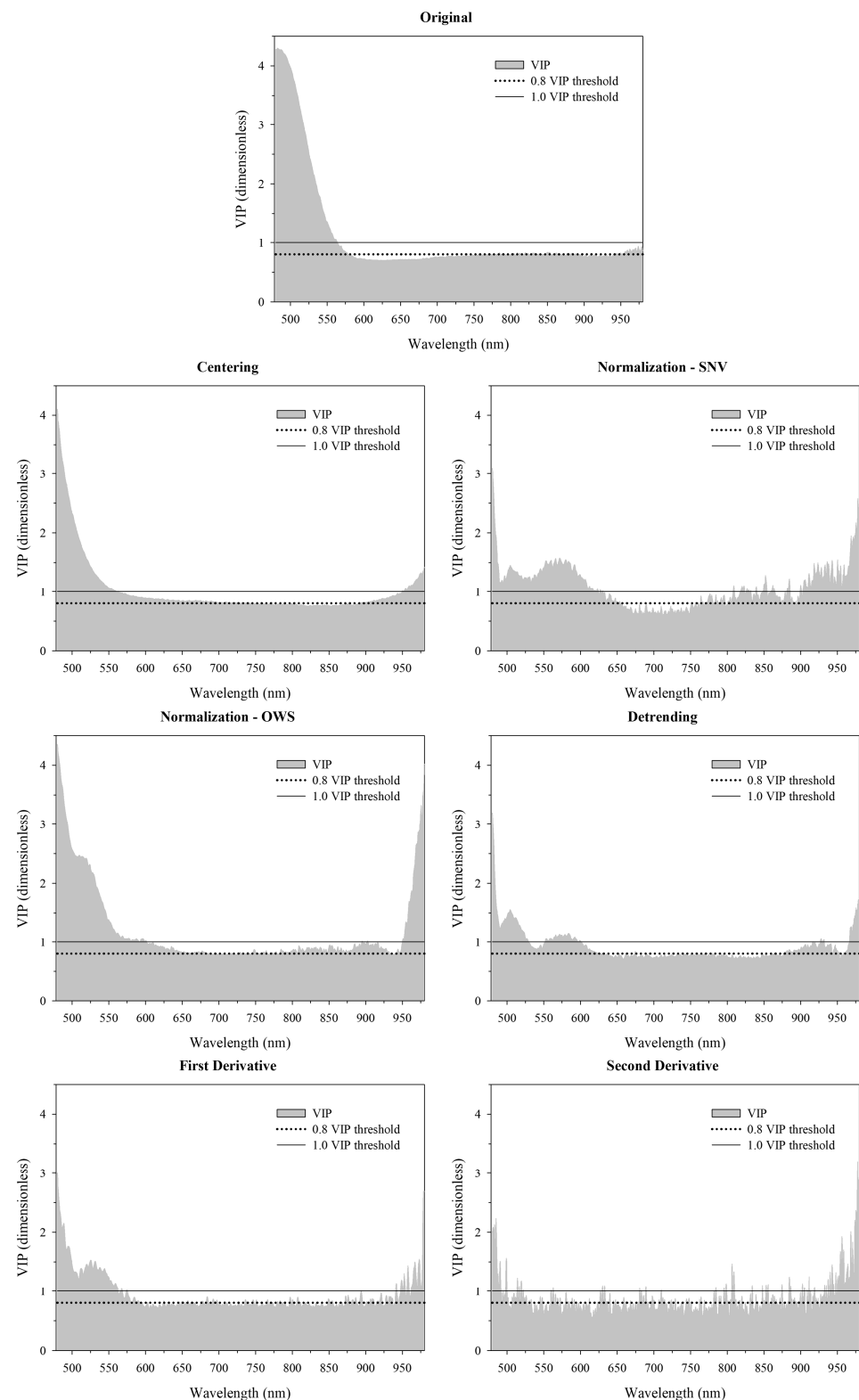


Figure 8. VIP of the PLS-DA models for original and pretreated spectral signatures of the herbal tea infusions.

4. Discussion

The average absorbance spectra (Figure 4) indicated that the herbal tea infusions were sensitive to the visible and NIR bands, agreeing with previous studies. The wavelengths 490, 498, and 554 nm have already been selected as significant for measuring the soluble solid

content of tea soft drinks [42], while the wavelengths of 506, 554, and 642 nm were pointed as sensitive for pH determination of tea beverage [43]. Additionally, herbal tea infusions are colored products formed by the oxidation of polyphenolic compounds (theaflavins, thearubigins, flavonols, pheophorbides, pheophytins, and carotenes). The theaflavins and the flavonols (as their glycosides) strongly contribute to the yellow color with a trace of red or brown in tea infusions [44]. The high absorption of radiation energy in the visible spectral region was already associated with these pigments [16,18,45]. Other studies also registered the NIR wavelengths (700–2500 nm) as efficient in analyzing tea polyphenols, caffeine, and free amino acid [46], to predict the concentrations of tea adulterants [47], and to identify teas of different geographical origins, varieties, and fermentation degrees [18].

Both SNV and OWS do not involve the least square fitting in their algorithms, which increases their sensitivity to noisy entries in the spectra. However, these pretreatments were capable of removing the variability between samples due to scatter. The SNV decreased the within-class variance for the infused herbal teas, which was also verified for identifying oolong, black, and green powdered teas [48]. The detrending and derivative pretreatments removed baseline offsets of the herbal tea samples despite leading to more overlapping bands compared with other pretreatments. The OWS was more efficient in overcoming the scatter and extracting meaningful information from the infused tea spectra. Similar results were observed when discriminating between injured and healthy coffee beans after roasting and grinding based on NIR spectroscopy [49]. The mean centering is considered a resolution enhancement pretreatment [16] and was capable of eliminating baseline drifts and improving subtle spectral differences for the infused herbal spectral signatures. The same behavior was observed when applying the NIR spectroscopy for classifying white teas brewed from fresh leaves with distinct maturity levels [25]. However, in this study centering pretreatment caused a minimal effect on the results, leading to identical Kappa values and subtle differences in the PCA scores, VIP, and loading profiles. This probably occurred because the original spectra already had approximately zero mean due to the spectrometer baseline correction. Additionally, it seems that the spectral features of interest for discriminating the herbal tea infusions are already dominant in the original data, meaning that centering did not considerably alter the results.

The PCA was already successfully used for extracting variables which were correlated to color, taste, and volatile compounds of infused green tea [22], as well as for discriminating white teas produced from fresh leaves with distinct maturity levels [25]. In this study, the fact that some infusion spectra were visually distinct and that PCA effectively distinguished all herbal teas (Figure 5) made it easier to explore the high-dimensional data. However, a method capable of automating quantitative discrimination is required for practical applications. PLS-DA is indicated in these cases, especially when class information is available and important for the analysis. Thus, the PLS-DA was used to provide models capable of grouping the herbal tea infusions into preset classes, allowing for evaluation of the accuracy of the discriminations and considering automated tea classifiers in industrial applications. The PLS-DA is a well-established and efficient technique for classifying tasks, and its benefits were proven by previous studies with tea and other agricultural and food products [11,16,36].

The Kappa values (Figure 6) obtained when utilizing the PLS-DA for identifying the infused herbal teas agreed with those obtained when classifying five kinds of Chinese tea (Black, Lung Ching, Tikuan Yin, Yunnan, and Jasmine) using visible spectroscopy [38]. Kappa values of 1.00 were also verified when discriminating green tea with different grades and varieties using NIR spectroscopy and supervised orthogonal locality preserving projections [45]. The second derivative, SNV, and detrending pretreatments required more LVs to correctly discriminate the herbal tea infusions, resulting in more complex final

models. The PLS-DA models with fewer LVs and higher Kappa coefficients are indicated to provide fast, nondestructive, chemical-free, and safe automated classifiers for herbal tea infusions. The LVs found in this research agree with those verified when discriminating organic teas from conventional ones using PLS-DA [50]. LVs from two to seven were also obtained in performance tests of distinct methods for discriminating green teas with different varieties and geographical origins [45].

The VIP profile showed that most of the spectral regions were moderately or highly influential in effectively discriminating the infused herbal teas, tending to enhance class separation and supporting the high Kappa values. The joint analysis of VIP and Kappa results also confirmed that cross-validation and external validation were effective in ensuring model stability. As expected and noted in Figure 6, using more than the optimal number of LVs tends to cause model overfitting, which reduces Kappa values. On the other hand, using fewer LVs than optimal increases the influence of random errors, leading to lower Kappa values.

In this study, as in many chemometric analyses, spectral signatures were typically smooth (Figure 4), while loading profiles from PLS-DA appeared irregular (Figure 7). The herbal tea spectra followed broad, continuous absorption patterns due to molecular vibrations, electronic transitions, and scattering effects. Instrumental response functions and the natural bandwidth of spectral features also contributed to smooth spectral signatures. On the other hand, the loadings represented the contribution of each wavelength to an LV being derived from a decomposition technique (PLS-DA). This method emphasized variance, capturing subtle differences and noise that may not be as visible in spectra. As expected, the decomposition algorithm distributed the noise into higher order LVs, making them look more irregular. This was more evident in SNV and detrending pretreatments, which required more LVs and exhibited smoother curves for LV1 and LV2, whereas the loadings for LV3, LV4, and LV5 were more irregular.

Despite these differences, the spectra of the studied herbal tea infusions (Figure 4) were consistent with loadings and VIP profiles (Figures 7 and 8), with the sensitivity of the spectral region around 500 nm showing that pigments, such as chlorophyll and carotenoids, play an important role in discriminating herbal tea infusions by spectroscopy. Except for original spectra, wavelengths between 950 and 980 nm were also evidenced by the loading profiles, indicating that bioactive chemical components of infusions, including polyphenols, can also influence the spectral discrimination of herbal teas.

These findings were expected, as the bending vibrations and stretching of various chemical bonds have a significant impact on the visible and NIR regions, with different tea infusions exhibiting subtle differences in their chemical composition, content, and proportion [46]. These chemical bonds include O-H, N-H, C-N, and C-H, which are associated with pigments, proteins, water, alkaloids, and other spectrally active components [11,47]. In general, bonds that link nearly identical parts of a molecule, such as C=C, are less reactive than weakly polarizable bonds like those mentioned above [49]. As a result, they typically appear only in higher spectral ranges, between 5000 and 7042 nm [18].

The absorbance pattern of different herbal tea infusions is associated with their chemical and physical compositions [2], evidencing differences in color, taste, and aroma. This variability is also affected by distinct infusion times, water temperatures, tea-processing methods, and the origin of the same species or variety, influencing the developed PLS-DA models regarding optimal LV numbers, accuracies, loading profiles, and VIP values.

The spectral sensitivity and the pretreatment effects on the tea spectra agreed with those verified when analyzing powdered samples for the same herbal teas [14]. For powdered samples, PC1 and PC2 in the PCA accounted for over 95% of the variance in the data as well. Additionally, employing the OWS and detrending pretreatments resulted

in enhanced prediction accuracy for both powdered and infused teas, utilizing PLS-DA models with comparable LVs. But, boldo powder was easily discriminated against other teas by utilizing two PCs, while carqueja reached better results for infusions.

Although powdered samples present more visual differences than the infusions (color, shape, and size of particles), the spectral separation among the different herbal teas was more discernible for infusions. This is probably due to the oxidation and chemical reactions that occur during the infusion process, which extracts various compounds from the tea powder, such as polyphenols, caffeine, catechins, and theanine, and affects the absorbance of the samples [2]. The compound amount extracted depends on the tea-processing methods, temperature, and time of infusion, but it also differs according to the tea type.

Both for powdered and infused herbal teas, the PLS-DA accuracy is affected by the measurement procedures and the spectrometer features. The accuracy of PLS-DA models depends on the use of adequate spectrometer accessories, proper sample preparation, and correct calibration procedures. Equipment with appropriate spectral resolution and high signal-to-noise are additional factors that contribute to acquiring good-quality spectra, thus enhancing the predictive capabilities of the models. Additionally, classifying herbal tea infusions using spectroscopy and multivariate analysis requires quality control of raw materials and proper tea brewing standardization. The geographical location of tea cultivation, soil type, use of fertilizers, climate, pest and disease control, and water availability can influence the chemical profile of tea leaves, the infusions, and, consequently, the tea spectral signatures. Differences in brewing methods, like cleaning of raw material, as well as temperatures and times of drying and infusions, are other factors that may alter the spectral data, making it harder for the classifier to identify patterns. Future research should expand the proposed methodology to include other herbal tea species and infusion blends, also evaluating different growing and processing factors. Furthermore, when applied to large-scale production, models may be adjusted by calibration procedures due to variability in sample collection, infusion techniques, and equipment. This process does not require relevant modifications to the proposed algorithm but is capable of improving the consistency of the results and the reliability of the classifiers in practical applications.

The Vis/NIR absorbance and the PLS-DA modelling proved effective in identifying and distinguishing infused herbal teas, presenting a compelling opportunity to enhance the agrifood industry processes. It also demonstrates adaptability to various spectral datasets. Compared to the traditional discrimination procedures, classifiers based on spectroscopy and multivariate analysis offer significant advantages in terms of speed, accuracy, and cost savings. The proposed method comprises an objective and standardized approach that eliminates dependence on human senses, ensuring greater consistency in results. This technique is nondestructive and requires minimal labor, maintaining product integrity and allowing for further analysis or use. Its rapid processing facilitates swift decision-making without the need for extensive human training or time investment. Moreover, spectroscopy proves to be a cost-efficient alternative, removing the necessity for reagents or sensory panels while also producing digital data that can be stored, analyzed, and compared over time. However, the practical implementation of this technology depends on technical, financial, and regulatory factors. The agrifood industries must invest in modern equipment and system integration to ensure the successful application of new methods. As innovative techniques advance and costs decrease, spectroscopy-based classifiers are likely to become more accessible and widely adopted across various industries, providing real-time information and being integrated into production lines.

5. Conclusions

This work assessed the effectiveness of six spectral preprocessing techniques (centering, standard normal variation, object-wise standardization, first derivative, second derivative, and detrending) combined with multivariate analysis to differentiate between five infused herbal teas cultivated organically (boldo, carqueja, chamomile, fennel, and lemon grass) using Vis/NIR absorbance. The PCA indicated the potential for separating the herbal tea infusions, with samples being clustered into different group patterns depending on the spectral pretreatment applied. For distinct spectral pretreatments, the PLS-DA modelling reached Kappa values that were almost perfect (0.97–1.00) by using optimal latent variable numbers from two to five. Original and OWS spectra led to accurate predictions with fewer latent variables. The Vis/NIR spectroscopy and the PLS-DA modelling were efficient in identifying and discriminating the infused herbal teas, presenting the potential to be utilized in the sensory evaluations, quality control, and grading procedures of agrifood industries, and resulting in a safe, nondestructive, chemical-free, and fast option.

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