# Rapid detection of tea adulteration using FT-NIR spectroscopy combined with t-SNE analysis

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# Abstract

Tea is one of the most popular non-alcoholic beverages internationally, and it is not uncommon to find commercial tea preparations mixed with leaves and parts of other plants to increase profit and production volume, which constitutes fraud. The aim of this study was to perform Fourier transform-near-infrared spectroscopic characterization of leaves and pieces (petioles and stems) of three types of medicinal plants (Chamomile, Ginseng, and Quebra-pedras) used in the preparation of teas. Cluster analysis methods were used to evaluate the ability of Fourier transform-near-infrared to identify plant types, with t-SNE presenting the best discriminatory power. The deconvolution of the spectra showed that 15 vibration bands allow a good characterization of the samples, all with R<sup>2</sup> greater than 0.99.

Keywords: infrared; non-linear statistical method; fraud; medicinal plants.

**Practical Application:** There is an increasing appreciation for teas and infusions, valued not only for their sensory qualities but also for their therapeutic benefits, which are deeply intertwined with cultural and historical traditions. This growing interest underscores the critical issue of food authenticity, particularly in addressing adulteration and maintaining quality through advanced analytical techniques. Among these methods, FT-NIR spectroscopy stands out for its efficiency, precision, and environmentally friendly nature, receiving prominence to evaluate the quality of the tea. This study highlights the application of FT-NIR spectroscopy to analyze the chemical composition of three medicinal plants commonly used in teas, integrating vibrational spectroscopy with clustering methods to ensure precise characterization and identification.

# **1 INTRODUCTION**

Many beverages are made from extracts of fruits, flowers, stems, roots, seeds, and other parts of plants. Examples include coffee, fruit juices, and teas. Among these beverages, infusions and teas are appreciated not only for their pleasant taste and aroma but also for their therapeutic and medicinal effects. Calming teas for stress reduction and improving sleep quality are easily found in pharmacies, supermarkets, and health food stores, as well as those with pain relief, anti-inflammatory, diuretic, antidiabetic, antihypertensive, and functional properties (Alkufeidy et al., 2024; Ocieczek et al., 2023; Okafor et al., 2024; Zhao et al., 2024). The perception of the effects that teas have on the body has an important relationship with the culture and dietary intake of some ancient peoples (Amsaraj & Mutturi, 2024; Luo et al., 2024; Van Wyk & Gorelik, 2017). With the development of scientific methods, teas began to be studied in more detail, and their health benefits were confirmed over time, with proof of functional properties, identification of bioactive compounds, and biomolecules of pharmacological interest (Alkufeidy et al., 2024; Fang et al., 2024; Johnson et al., 2022, 2023).

Determining food authenticity and detecting adulteration are critical concerns for consumers, industries, and policymakers throughout the production process. A significant issue in food authenticity is the mislabeling of ingredients, where high-value components are replaced with cheaper alternatives without proper disclosure (Kunbhar et al., 2025; Li, Logan, et al., 2024; Lin et al., 2017). To combat this, many analytical techniques have been used to detect and quantify food adulteration, including teas and infusions. The most common methods are chromatography (liquid and gas), electrophoresis, mass spectrometry, fluorescence, X-ray, ultraviolet–visible, and infrared spectroscopy, as well as numerous other traditional techniques, such as thermogravimetric analysis, volumetric titration, refractometry, and analysis of the electrical properties of materials (Li, Elliot, et al., 2024).

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Fourier transform-near-infrared (FT-NIR) spectroscopy is renowned for its ability to simultaneously detect multiple analytes, offering key advantages, such as short analysis times, low operating costs, high repeatability, and accurate results. Moreover, FT-NIR is non-destructive, requires no preparatory steps, and avoids the use of chemical solvents or toxic compounds commonly associated with traditional analytical methods (Kong et al., 2024; Kumar et al., 2023; Li & Chase, 2010; Mendes et al., 2020). As with other food products, the raw materials used in the production of teas and infusions must be classified, identified, and thoroughly characterized to prevent fraud and adulteration (Li, Elliot, et al., 2024). FT-NIR spectroscopy has been extensively applied in tea quality assessment, encompassing applications such as locality identification (He et al., 2012), variety discrimination (He et al., 2007), and tea beverage quality evaluation (Li et al., 2007), as highlighted by Li et al. (2019).

This study aimed to use FT-NIR spectroscopy to characterize and identify the vibrational bands associated with the main chemical groups present in the leaf, petiole, and stem of three medicinal plants commonly used in tea production. The plants analyzed were Chamomile (Matricaria recutita L.), Ginseng (Panax ginseng), and Quebra-pedras (Phyllanthus niruri). In addition to graphical analyses such as deconvolutional and derivative spectroscopy, three clustering methods were explored. Among these, t-SNE, a non-linear statistical technique widely used for visualizing high-dimensional data, proved particularly effective in revealing local structures within complex datasets. Compared to linear methods like PCA, t-SNE often excels in identifying non-linear patterns. However, PCA retains an advantage in preserving global variance and offering interpretable dimensions. To complement these analyses, a dendrogram was also constructed to assess the capability of FT-NIR spectroscopy in classifying and grouping raw tea materials. Together, these methods provided valuable insights into the structural organization and classification potential of the data.

# 1.1 Relevance of the work

The growing appreciation for teas and infusions extends beyond taste to therapeutic benefits rooted in cultural traditions. This trend emphasizes food authenticity, particularly in preventing adulteration and ensuring quality. Fourier transform near-infrared spectroscopy (FT-NIR) spectroscopy stands out for its efficiency, precision, and eco-friendliness in tea analysis. This study applies FT-NIR spectroscopy to assess the chemical composition of three medicinal plants used in teas, integrating vibrational spectroscopy with clustering methods for accurate characterization and identification.

# 2 MATERIAL AND METHODS

### 2.1 Sample collection and preparation

Three dehydrated samples of each plant type (Chamomile, Ginseng, and Quebra-pedras) were collected, properly identified, and labeled as R1, R2, and R3, respectively. Additionally, a fraction consisting of petioles and leaf stems from each plant type was labeled as "piece" and included in the analysis. All samples were sieved using a coarse mesh sieve (mesh size 10), and a mass of  $0.200\pm0.005$  g from each sample was precisely measured and reserved for FT-NIR spectroscopy analysis. Each FT-NIR spectroscopy analysis was performed in triplicate, with measurements taken from different portions of the material to ensure accuracy.

### 2.2 Fourier transform-near-infrared spectroscopy analyses

The analyses were conducted using a Bruker Multi-Purpose FT-NIR Analyzer operating in reflectance mode, covering the wavenumber range of 4000–12000 cm<sup>-1</sup>. The instrument was equipped with a Te-InGaAs detector and operated at a resolution of 4 cm<sup>-1</sup>. Data acquisition was performed using OPUS<sup>®</sup> software version 5.5. Samples were loaded into 8-mm-thick borosilicate cuvettes. Each sample was analyzed in triplicate, with 64 scans recorded per replicate for both simulated and control samples.

### 2.3 Statistical analyses and treatment of experimental data

Initially, the FT-NIR spectra were normalized to the range of 0–1 using the SAS OnDemand statistical package. Fityk software, version 1.3.1, was used for baseline correction, derivative spectroscopy analyses, and deconvolution of the spectra.

The cluster analyses were performed in the Orange Data Mining software, version 3.37.0, using the PCA, t-SNE, and dendrogram modules.

LibreOffice Calc software, version 24.2.5, was used to tabulate the data. The graphs were created in the SciDAVis software, version 2.8, and edited in the Inkscape software, version 1.3.2, for color enhancement.

### **3 RESULTS AND DISCUSSION**

#### 3.1 Fourier transform-near-infrared derivative spectroscopy

Figure 1A presents the normalized FT-NIR reflectance spectra of all samples in the experiment. This figure shows that the FT-NIR spectra of the samples do not overlap throughout the analyzed frequency range, indicating that near-infrared spectroscopy is a potential technique to characterize and detect differences between the plants studied based on the vibrational characteristics of the chemical groups present in their composition. Figure 1B and C refers to the derivative analysis of the spectra and shows that for the same plant, its chemical composition is very similar among the different experimental replicates of the dehydrated leaves, as their reflectance bands occur practically at the same frequencies of the spectrum, and with approximately equal amplitudes. The same is not observed for the "piece," a material composed of leaf petioles and stems, whose vibration bands and intensities are different from those observed in the infrared spectra of the leaves. Thus, it is possible to identify commercial teas that have been intentionally tampered with by the addition of leaf petioles and stems to adulterate the product. The same pattern is observed in the other derivative spectra for the Ginseng and Quebra-pedras samples.



**Figure 1**. In (a), the normalized FT-NIR spectra for all samples are shown. In (b), the first derivative of the normalized FT-NIR spectroscopy signals for Chamomile (leaves and pieces) is presented. In (c), the first derivative of the normalized FT-NIR spectroscopy signals for Ginseng (leaves and pieces) is displayed, and in (d), the first derivative of the normalized FT-NIR spectroscopy signals for Quebra-pedras (leaves and pieces) is illustrated.

### 3.2. Deconvolutional spectroscopy

#### 3.2.1. Analysis of Chamomile spectra

Some chemical compounds present in Chamomile are chamazulene, beta-farnesene, alpha-bisabolol oxide A, alpha-bisabolol oxide B, alpha-muurolol, spathulenol, beta-eudesmol, E-spiroether, hexadecanoic acid, E,E-alpha-farnesene, and nonacosane. These compounds have functional groups that vibrate at different frequencies in the FT-NIR spectrum, as shown in Figure 2A-2C. The chemical composition observed among the different repetitions is quite similar, with bands that have approximately the same amplitude and the same wavenumbers. Some groups, however, do not follow this pattern, such as the band presented at 10340.70 cm<sup>-1</sup>, which does not appear in Figure 2A but is present in the other figures, including D, corresponding to the spectrum of leaf petioles and stems. This region of the spectrum (10340.70 cm<sup>-1</sup>) is associated with the vibration of functional groups that have C-H bonds, including C=C-H of alkene groups and aromatic rings, and C-C-H bonds, associated with cycloalkanes and aliphatic chains of organic compounds (Lin & Sun, 2020).

The band near 4703.34 cm<sup>-1</sup> that appears in Figure 2A and D is also not present in the other spectra, B and C, indicating that the chemical composition of tea may undergo small variations depending on the part of the plant used (leaves or petioles and stems). This band is typical of the stretching vibrations of

the O–H group, which are very common in the alcohol groups (ROH) present in essential oils (Haseli et al., 2023).

In Figure 2A–D, the deconvolution of the spectra shows high values of coefficient of determination (R2R^2R2) and low values of the weighted sum of squared residuals (WSSR), indicating an excellent representation of the original spectrum by means of Gaussians.

#### 3.2.2 Analysis of Ginseng spectra

Ginseng is a plant known as the "king of herbs," a title attributed to its wide variety of active compounds with medicinal effects and potential health benefits (He et al., 2021; Li et al., 2023; Qiao et al., 2024). Among the bioactive compounds reported in the literature are ginsenosides, polysaccharides, volatile oils (essential oils), alkaloids, and amino acids, which are associated with antioxidant, anticancer, anti-inflammatory, weight control, antistress, and anti-aging effects, among others (Chen et al., 2020; Liu et al., 2020; Qiao et al., 2024).

In terms of infrared reflectance, the analyzed samples do not present significant spectral discrepancies, with similarities in the positions and amplitudes of all vibration bands, except for the spectrum in Figure 3D, which refers to the petioles and stems of the leaves. The band amplitudes in this spectrum are visibly different from those observed for the leaf samples. The three bands with the largest area under the Gaussian are at 7524.23 cm<sup>-1</sup>, 8753.56 cm<sup>-1</sup>, and 10367.10 cm<sup>-1</sup>, respectively.



**Figure 2**. In (a)-(c), the normalized deconvolution spectra of Chamomile leaves for repetitions 1–3, respectively, are shown. In (d), the normalized deconvolution spectrum of the pieces (petiole+ stem) of Chamomile leaves is presented.



Figure 3. In (a)–(c), the normalized deconvolution spectra of Ginseng leaves for repetitions 1-3, respectively, are shown. In (d), the normalized deconvolution spectrum of the pieces (petiole+ stem) of Ginseng leaves is presented.

In the region close to 7524.23 cm<sup>-1</sup>, C–H bonds vibrate, associated with the presence of alkyne and alkene groups. Near 8753.56 cm<sup>-1</sup> is a region associated with the vibration of the carbonyl group (C=O), present in various organic compounds, such as esters, aldehydes, and ketones. The band near 10367.10 cm<sup>-1</sup> corresponds to vibrations of the C–H group, associated with alkanes, alkenes, cycloalkanes, and several other organic compounds (Kostina et al., 2023).

In Figure 3A–D, the deconvolution of the spectra shows high values of R2R^2R2 and low values of WSSR, indicating an excellent representation of the original spectrum by means of Gaussians.

#### 3.2.3 Analysis of Quebra-pedras spectra

Methyl salicylate, flavonoids, phyllanthin, phyllocrisin, filavin, cineole, cymol, linalool, securimine, filatidine, and salicylic acid are chemical compounds present in stone breaker, as reported by Jorge & Vaz (2007). Functional and bioactive attributes include hepatoprotective and antioxidant activities, as described by P E et al., 2023, while antidiabetic potential is reported by Bhushan et al., 2024.

The Quebra-pedras leaves did not present a homogeneous spectral pattern, as observed when comparing Figure 4A and B, particularly regarding the amplitude of the signals. The band in the region 12055.70 cm<sup>-1</sup> is present in Figure 4B and D but absent in the other samples. This region corresponds to the vibration of N–H groups, indicating the possible presence of compounds containing amine and amide groups, such as nirurin and phyllanthin present in the plant (Wolstenholme et al., 2021).

The bands with large areas under the Gaussian, in the region close to 9947.94 cm<sup>-1</sup>, in the spectra of all samples in Figure 4, refer to characteristic vibrations of C–H bonds, which are present in many organic compounds, especially short-chain al-kanes, cyclohexane, cycloalkanes, and alkenes (Lin & Sun, 2020). These bands have a proportionally larger area in the petiole and stem samples of the stone breaker compared to the leaf samples.

In each of the deconvolution graphs presented in Figures 2–4, a total of 15 Gaussians were fitted to represent the entire normalized spectrum. All fittings had an  $R^2$  value greater than 99%, with a low error, as indicated by the WSSR parameter.

#### 3.3 Cluster analysis of teas

The cluster analysis presented in Figure 5A and B shows that both techniques used, PCA and t-SNE, are effective in representing FT-NIR spectral differences in a two-dimensional space, with minimal loss of information. This conclusion is based on the percentage values of the variance representation observed in the axes of principal components (PCs) PC1 (89.86%) and PC2 (9.03%), which together account for 98.89% of the total variance for these first two PCs. t-SNE proved to be the most efficient in forming and identifying groups, possibly due to its non-linear clustering approach that considers spectral features not detected by PCA. Thus, it can be inferred that plant samples not fitting within the regions (ellipses) presented in Figure 5A and B may potentially be considered fraudulent, as their FT-NIR spectra do not match the patterns obtained for the leaves and pieces of Chamomile, Ginseng, and Quebra-pedras.



Figure 4. In (a)-(c), the normalized deconvolution spectra of Quebra-pedras leaves for repetitions 1–3, respectively, are shown. In (d), the normalized deconvolution spectrum of the "pieces" (petiole+ stem) of Quebra-pedras leaves is presented.

The dendrogram in Figure 6, obtained using a linear clustering method, demonstrates that it is possible to group samples based on their similarity using FT-NIR spectroscopy data. FT-NIR spectroscopy can detect the presence of key chemical groups, allowing for the classification of samples into different categories. Leaf and plant parts are also placed into distinct groups. By considering a cut line at 30 Euclidean distance units (vertical dashed line in Figure 6), the result allows for the grouping of plant parts (stems and petioles) into categories different from those formed exclusively by leaves.

### **4 CONCLUSIONS**

This study demonstrated that the combination of FT-NIR spectroscopy and multivariate analysis techniques constitutes an analytical methodology capable of forming groups within which the spectroscopic and vibrational characteristics that define the chemical markers of the medicinal plants under study are preserved. This is important because spectra obtained from complex samples or those that do not belong to these groups clearly indicate possible adulteration, suggesting fraud. It was possible to differentiate samples obtained from leaves from those obtained from leaf stems and petioles (pieces). Derivative spectroscopy was used as an exploratory investigation technique, allowing the identification of frequency bands with the greatest indications and evidence of differences between the samples studied. Among the grouping methods used, t-SNE showed the best separation capacity between the samples, as the samples formed graphically well-defined and distant groups, making their identification unequivocal. The deconvolution of the spectra showed that a total of 15 Gaussians were sufficient to characterize the main vibration frequencies of the chemical groups present in the samples. These frequencies represent a variety of vibrational modes, such as bond vibrations, combinations, and overtone bands. The study constitutes an approach that has great potential for quality control by industries and governmental agencies.

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**Figure 5**. In (a), the PCA of all Chamomile, Ginseng, and Quebra-pedras samples is shown. In (b), the t-SNE plot of all Chamomile, Ginseng, and Quebra-pedras samples is displayed. In the figures, points with "G" as the first letter refer to Ginseng samples, with "C" as the first letter refers to Chamomile samples, and with "Q" as the first letter refers to Quebra-pedras samples. The subscripts 1, 2, and 3 correspond to samples 1, 2, and 3, respectively, while the subscript "P" indicates piece.



Figure 6. Dendrogram of the cluster analysis performed based on the normalized FT-NIR spectral signals of all Chamomile, Ginseng, and Quebra-pedras samples.

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