Spectroscopy Letters

An International Journal for Rapid Communication

ISSN: 0038-7010 (Print) 1532-2289 (Online) Journal homepage: www.tandfonline.com/journals/lstl20

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To cite this article: Stephanie Jaen , Sulaf Assi , Dhiya Al-Jumeily , Luis Vargas Canelos & Rachel Leeson (03 Jun 2026): On the feasibility of near-infrared spectroscopy for the identification of *Nicotiana* species, Spectroscopy Letters, DOI: 10.1080/00387010.2026.2676027

To link to this article: https://doi.org/10.1080/00387010.2026.2676027

SPECTROSCOPY LETTERS
https://doi.org/10.1080/00387010.2026.2676027

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RESEARCH ARTICLE

On the feasibility of near-infrared spectroscopy for the identification of *Nicotiana* species

Stephanie Jaen[a], Sulaf Assi[b], Dhiya Al-Jumeily[b], Luis Vargas Canelos[c] and Rachel Leeson[a]

[a]Oral and Maxillofacial Surgery Department, Eastman Dental Institute, University College London, London, UK; [b]Pharmacy and Biomolecular Science, Liverpool John Moores University Liverpool, Liverpool, UK; [c]Achuar Nationality of Ecuador, Puyo-Pastaza, Ecuador

ABSTRACT

This study evaluates the feasibility of near-infrared (NIR) spectroscopy for identifying biochemical differences in *Nicotiana* species (*N.* spp.) in ceremonial, handcrafted Indigenous smokeless tobacco products from Ecuador and Peru. NIR spectra of three *N.* spp. *Nicotiana tabacum* (*N. tabacum*), *Nicotiana glauca (N. glauca*), *Nicotiana rustica,* (*N. rustica*) and three smokeless tobacco samples (SLT), (Ecuadorian, Peruvian white and Peruvian brown) were collected. For each sample, more than 20 subsamples were taken. Spectra were exported to MATLAB 2024b, where multiplicative scatter correlation and first-derivative was applied prior to spectral analysis. Spectral analysis involved evaluating spectral quality, spectral interpretation, and applying correlation in wavenumber space (CWS) and principal component analysis (PCA). Spectral interpretation showed similar absorption bands in both wet (*N.* species) and dry (SLT) samples; however, dry samples showed stronger absorbance due to reduced interference from water. Bands corresponding to water showed absorbance around 4716 cm^{-1} in the wet samples. CWS applied to the NIR spectra showed close correlation between Ecuadorian sample and *N. rustica* and between Peruvian white and *N. glauca*. However, Peruvian brown sample did not correlate with any species. PCA showed distinct clusters for each of the *N.* species and the tobacco samples corresponding to biochemical differences in the samples. This is the first study to investigate the NIR spectral signatures of *N.* glauca and *N.* rustica, as previous studies have been limited to *N.* tabacum. In addition, this work provides the first chemical characterization of handcrafted shamanic tobacco produced by the Achuar Indigenous community in Ecuador and Ashaninkas in Peru. NIR spectral signatures showed key differences in alkaloids, proteins, and moisture bands. However, due to sampling limitations, future work will focus on evaluating the findings of the study with larger sample set.

ARTICLE HISTORY
Received 24 January 2026
Accepted 19 April 2026

KEYWORDS
Nicotiana species; Smokeless tobacco; Near-infrared spectroscopy; Correlation in wavenumber space; Principal component analysis

Introduction

Chewing is one of the oldest forms of tobacco consumption, with evidence of its use among South American Indigenous populations prior to 1500.[1] Historical records indicate that it was primarily used by shamans as an entheogen in religious rituals or for medicinal rather than recreational purposes. Ceremonial tobacco practices remain today in many Indigenous communities across the globe.[2]

To acknowledge the cultural significance of tobacco while addressing the health risks of its industrial forms, indigenous communities, researchers, and advocates now distinguish between "ceremonial tobacco" and "commercial tobacco".[3] Before industrialization, tobacco was primarily consumed through pipe smoking, chewing, and snuff, and in Europe was largely restricted to the aristocracy due to its high cost.[4] Colonization subsequently industrialized tobacco production, converting it into a plantation-based crop.

The SLT products analyzed included one sample originated from the Achuar nationality in Ecuador and two samples from the Ashaninka nationality in Peru. The handcraft smokeless tobacco, (locally known as maso), is produced through a complex process of approximately two to three weeks (Supplementary Appendix A). The process involves

CONTACT Rachel Leeson r.leeson@ucl.ac.uk Oral and Maxillofacial Department, Eastman Dental Institute, University College London, London, UK.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/00387010.2026.2676027.

drying tobacco leaves for around one week, maceration for three to four days, then crushing by foot to initiate fermentation. These three steps produce a liquid extract, which is poured over additional tobacco leaves. The resulting mixture is then subjected to a low-fire curing process lasting two to three days. During curing, *maso* acquires a brown coloration and is carefully wrapped using the *Huaco or bejuco* plant that possesses healing properties (according to local traditions).[5] Maso's modality of use includes chewing or as an oral snus that is held under the mucosa of the tongue for sublingual absorption.[5] Oral absorption of nicotine is rapid with 40% oral bioavailability, and this can be attributed to its adverse events[6]

According to the World Health Organization (WHO, 2023), tobacco is linked to eight million deaths annually.[7] SLT is also strongly associated with cancers of the oral cavity and oral potential malignant lesions. However, tobacco comprises multiple species which nicotine content varies according to both species and cultivation conditions. Key factors involve the use of pesticides and fire-curing techniques, which can increase levels of polycyclic aromatic hydrocarbons (PAH'S). In addition, the inclusion of specific plants, ashes or mescaline-containing substances can increase the pH of tobacco products, enhancing nicotine and facilitating absorption across oral membranes.[8,9]

The *Nicotiana* genus includes approximately 95 known species worldwide, with around 75 originating in South America. Commonly referenced species include *N.* rustica (Aztec tobacco), and *N. tabacum*, the latter being predominantly cultivated for commercial purposes.[10] In contrast, *N. rustica* is known to be used among Indigenous populations due to their high nicotine content. *N.* glauca has also reported to be used in Navajo, Tarahumara and Cahuilla ritual practices.[2] Previous analyses have demonstrated that *N.* rustica contains significantly higher nicotine levels (47.7 mg/g) compared to *N. tabacum* (25.6 mg/g).[11] Notably, *N.* glauca is atypical among *N.* spp. because anabasine, rather than nicotine, is its predominant alkaloid, with reported concentrations ranging from 1–3 mg/g, whereas nicotine is present only at trace levels, with an average concentration of 0.001 mg/g.[12–14]. Given the variation in *N.* spp. it is vital to apply validated analytical methods for accurate identification and chemical characterization before assessing health impacts.[15]

Subsequently, to predict the harm related to the different species chemical analysis is essential to understand the physical and chemical properties of SLT. Traditional chemical analysis of *N.* spp. was applied using colourimetry,[16] GC-MS,[17] and ATR-FTIR.[10] More recently, NIRS has been used for characterization of fresh *N. tabacum* leaves,[18] and quantification of nicotine in *N. tabacum*.[19,20] However, no study has used NIRS to characterize different *N.* spp.

Consequently, this study builds on the finding of the NIR-based studies in characterizing new species of *Nicotiana* and relating tobacco samples to these species. Thus, this study represented the first NIR spectroscopic application for profiling *N. rustica* and *N. glauca* that looks at biochemical differences between these species alongside *N. tabacum*. NIR was chosen because of its ability to characterize the sample measured based on their physicochemical properties. Three types of tobacco samples were evaluated and obtained from Peru and Ecuador. These samples were compared to the three most common *N.* spp. (*N. glauca*, *N. rustica* and *N. tabacum*).

Materials and methods

Sample collection

Samples were collected during field works to Indigenous communities, sample collection posed logistical challenges, as the communities involved—the Achuar (Ecuador) and the Ashaninka (Peru)—reside in remote areas of the Amazon. Cross-border coordination and the need for translation into Indigenous languages further complicated fieldwork.

It is estimated that approximately five of the fourteen Indigenous nationalities in Ecuador and at least ten of the fifty-five in Peru have been reported to engage in the production of handcrafted SLT. One Ecuadorian SLT sample was collected from an Achuar Indigenous community in the Amazon region of Ecuador. Two Peruvian SLT samples were collected from the Ecuadorian Peruvian border region and

originate from an Ashaninka Indigenous community (Supplementary Appendix B).

Tobacco leaves used in the preparation of SLT products were collected from Indigenous community crops in Ecuador and Peru for comparative analysis. Standard reference *N.* spp. used for identification and comparison were certified tobacco specimens obtained from Royal Horticultural Society (RHS) nursery suppliers in England. The plant material collected included: genus *Nicotiana,* family *Solanaceae*, *N. tabacum* (Virginian/common tobacco), *N. rustica* (wild/Aztec tobacco), and *N. glauca* (tree tobacco).

To control variability, *N.* spp. were cultivated for six to twelve months under consistent, automated, and controlled environmental conditions. Reference *N.* spp. growing conditions and analytical instruments (Supplementary Appendix C), included maintaining temperatures between 38 °C and 40 °C, regulating moisture via an automatic irrigation system, and providing exposure to full-spectrum red-blue light for twelve to twenty-four hours daily. Given the perishable nature of the leaves, samples were dried to remove moisture and extend shelf life while preserving quality. The process involved soaking the leaves in a water-glycerin solution (2:1) for three to five days, followed by a thorough two-step rinsing procedure to remove any residues, and then exposed to natural air-drying for three days.[21] The whole dried leaves were then ground and homogenized using a handheld grinder before storage in VWR 2 mL glass vials. A Minimum of 20 vials per *N.* spp. were prepared and analyzed (n = 180 vials). These 180 vials assessed within-sample spectral repeatability and heterogeneity related to sample presentation, packing, pathlength, scatter and instrument variation, Samples were handled and stored according to the CORESTA technical guide N°11: "Technical guide for sample handling of smokeless tobacco and smokeless tobacco products".[22] Cultivation conditions are known to influence the chemical composition of *N.* spp. through variations in nutrient availability, environmental factors, and metabolic activity. These differences may directly affect the resulting NIR spectra. For this reason, the sample sources and origins are presented in Supplementary Table S1, and growth conditions are described above to minimize variability unrelated to the objectives of the study.

Instrumentation

Measurements were conducted using a PerkinElmer Spectrum Two Fourier Transform-Near-Infrared (FT-NIR) spectrometer equipped with the Near-Infrared Reflectance Module (NIRM) (Supplementary Appendix C). Each spectrum was generated by the sum of 32 scans over the range of 10,000–4,000 cm^{-1}. The advantage of NIR lies in its ability to determine the physiochemical properties of the tobacco samples non-destructively. The collected NIR spectral signature required less than a minute of measurement. Hence, within the context of tobacco sampled, the NIR spectrometer used could measure both raw and undried leaves multiple times without destroying the samples.

Procedure

A total of nine distinct sample groups were evaluated, comprising fresh leaf material (n = 6) and dried smokeless tobacco (SLT) products (n = 3). For each fresh-leaf species, a minimum of 100 individual leaves were collected and pooled per sample group prior to homogenization, resulting in approximately 600–800 leaves across all fresh-leaf groups. Each SLT group consisted of 10 individual products, yielding a total of 30 SLT. Homogenization reduced intra-plant variability that was essential to obtain a representative biochemical profile. All tobacco material, including fresh leaves and dried products, were homogenized prior to spectral acquisition, for each subject group, 20 subsamples (vials) were prepared and measured to increase the effective sample size and capture within-group variability appropriate for a feasibility study. This resulted in a total of 180 vials analyzed in the study. This design was intended to capture within-group heterogeneity while maintaining feasibility constraints.

NIR spectra collected through glass vials and exported into MATLAB 2024b for spectral pretreatment and treatment. Multiplicative scatter correction-first derivative (MSC-D1) minimized the spectral variability arising from physical effects

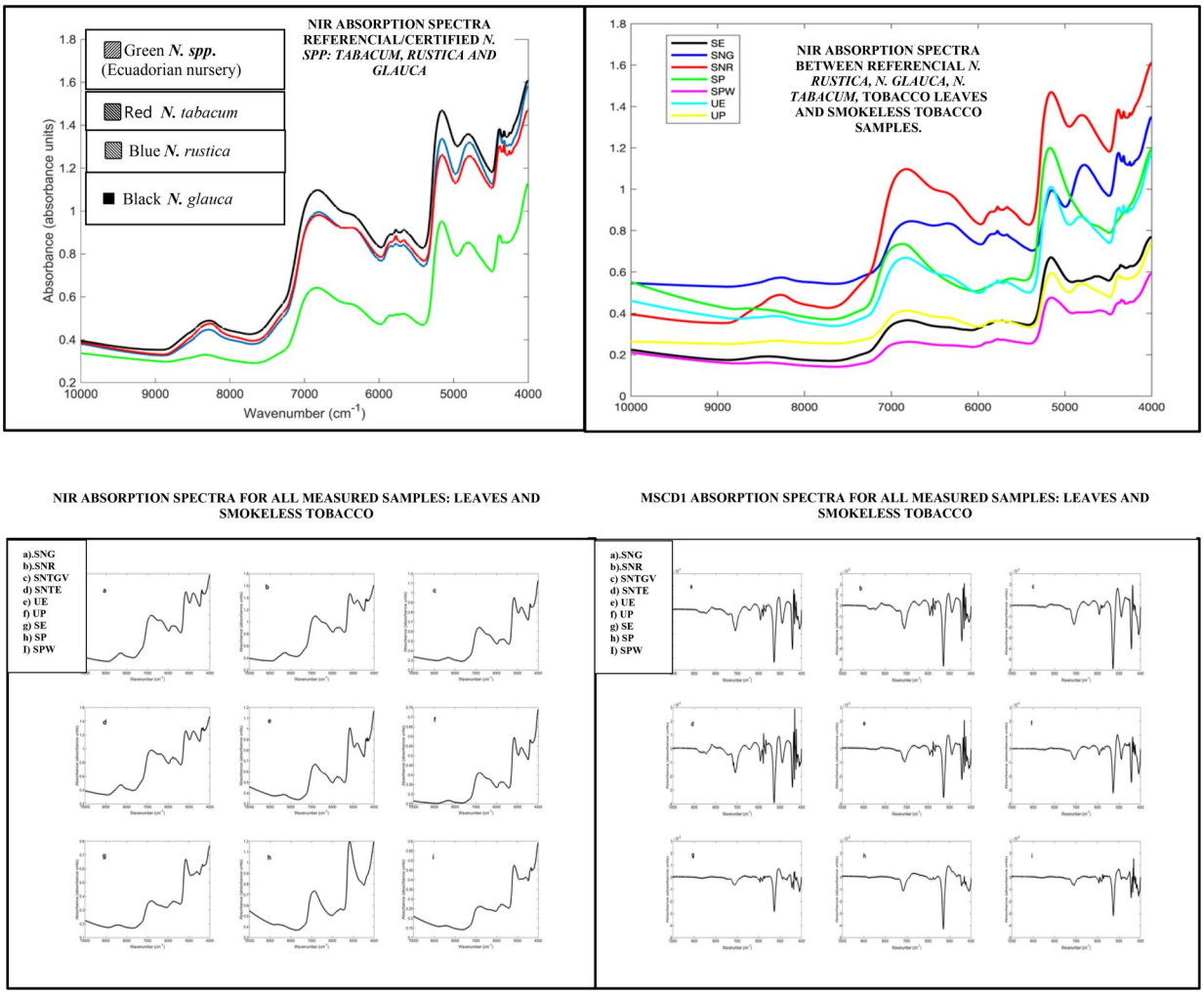

Figure 1. Absorption spectra of various *N. spp.* and smokeless tobacco products. **SNG:** *N. glauca* **SNR:** *N. rustica* **SNTGV:** *N. tabacum* **SNTE:** *N. spp.* Ecuadorian nursery. **UE:** *N. spp.* Achuar crops. **UP:** *N. spp.* Ashaninca crops. **SE:** Smokeless tobacco from Ecuador. **SP:** Brown Smokeless tobacco from Peru. **SPW:** White smokeless tobacco from Peru.

relating to scattering and pathlength and thus enhancing comparability between spectra. MSC corrected the offset by constructing a new spectrum that is a linear combination of the original spectrum. This was followed by first derivative (D1) that corrected both the offset and baseline of the NIR spectra using Savitzky–Golay method where a second-order polynomial was fitted to the data by least square using 13 data points (Jee, 2004).

The spectral pretreatment algorithms applied were the multiplicative scatter correlation first-derivative (MSC-D1). Post-spectral pretreatment, data analysis was applied using correlation in wavenumber space (CWS) and principal component analysis (PCA).[23,24] A table detailing the number of vials per sample prior analysis, along with the source and country of sample collection, is presented as Supplementary Table S1.

Results

Spectral interpretation

The absorption spectra of Nicotiana samples and smokeless tobacco (SLT) products are presented in Fig. 1. The NIR spectra demonstrated good analytical quality, with absorbance values ranging from approximately 0.2 to 1.6 absorbance units and well-defined overtone and combination bands. Clear differences in absorbance intensity were observed between wet and dried tobacco samples.

For wet tobacco leaves, prominent absorption features were observed near 6846 cm^{-1}, 5173 cm^{-1}, and 4815 cm^{-1}. These bands are primarily

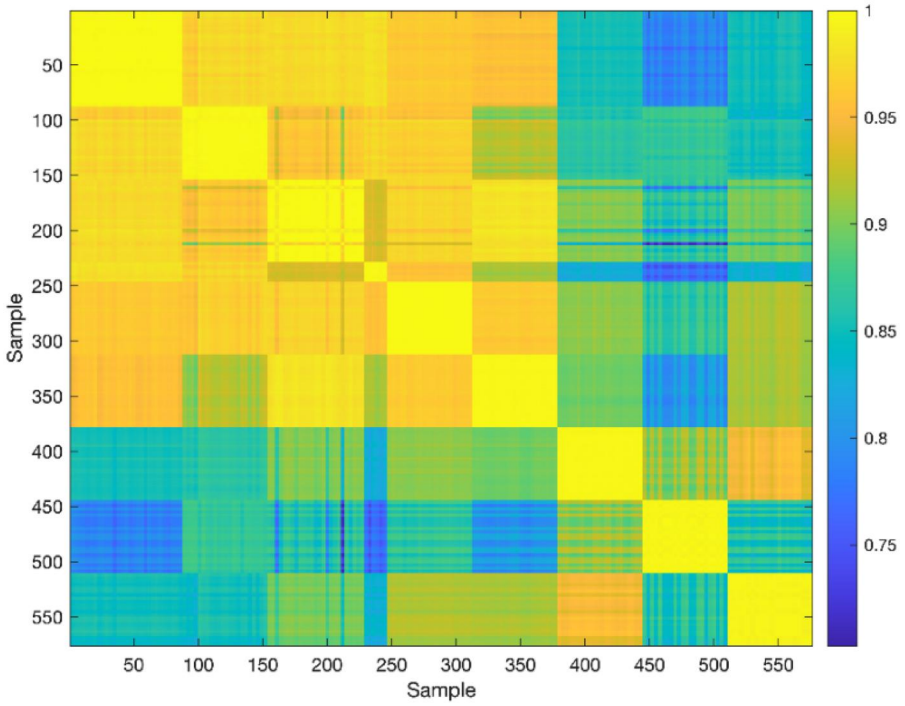

Figure 2. Correlation map of the MSC-D1 near-infrared spectra for various *Nicotiana spp.* and smokeless tobacco samples measured using the PerkinElmer Spectrum II FT-NIR spectrometer. Spectral ranges correspond to: *N. glauca* (1–87), *N. rustica* (88–153), *N. spp.* Ecuadorian nursery (145–228), *N. tabacum* (229–246), *N. spp.* Achuar crops (247–312), *N. spp.* Ashaninka crops (313–378), Ecuadorian smokeless tobacco (379–444), Peruvian brown smokeless tobacco (445–510), and Peruvian white smokeless tobacco (511–576).

associated with O–H and C–H overtone and combination vibrations arising from water and organic matrix constituents. Although the overall spectral patterns of wet and dried samples were similar, a notable difference was observed near 4716 cm^{-1} in the wet samples. This feature is predominantly attributed to O–H combination modes involving stretching and bending vibrations of water and hydroxyl-containing components within the plant matrix, consistent with contemporary NIR band assignments reported in the literature.[23,24] The increased intensity of this band in wet samples reflects the higher moisture content and associated hydrogen-bonding interactions, which significantly influence NIR spectral response.

Correlation in wavenumber space (CWS) analysis

CWS method was applied to investigate sample-sample similarity between NIR spectra of *N.* spp. This approach involved evaluating the closeness in spectra of different *N.* spp. Correlation coefficient values (r) were calculated by comparing each tobacco sample spectrum against all others, producing values ranging from −1 to +1. An r value of −1 indicates complete dissimilarity, while +1 denotes similar spectral characteristics.

The resulting correlation map (Fig. 2) uses a color gradient to represent different levels of spectral similarity: dark blue (r = 0.64–0.73), light blue (0.74–0.80), dark green (0.80–0.85), light green (0.85–0.89), orange (0.89–0.95), and yellow (0.95–1.00). Darker colors correspond to higher similarity, indicating that these products share similar composition and matrix characteristics. A correlation coefficient threshold of 0.95 was established to determine significant similarity. Values at or above this threshold suggest that the samples are highly comparable, while a correlation coefficients threshold of 0.95 is considered a match, this correlation is an angle and not a probability. By applying the CWS method, it was possible to assess the degree of similarity between tobacco samples based on their geographical origin and *N.* spp.

The Ecuadorian and Peruvian white SLT samples exhibited the highest correlation coefficients ($r > 0.95$; $r = 0.951$). Similarly, the Achuar

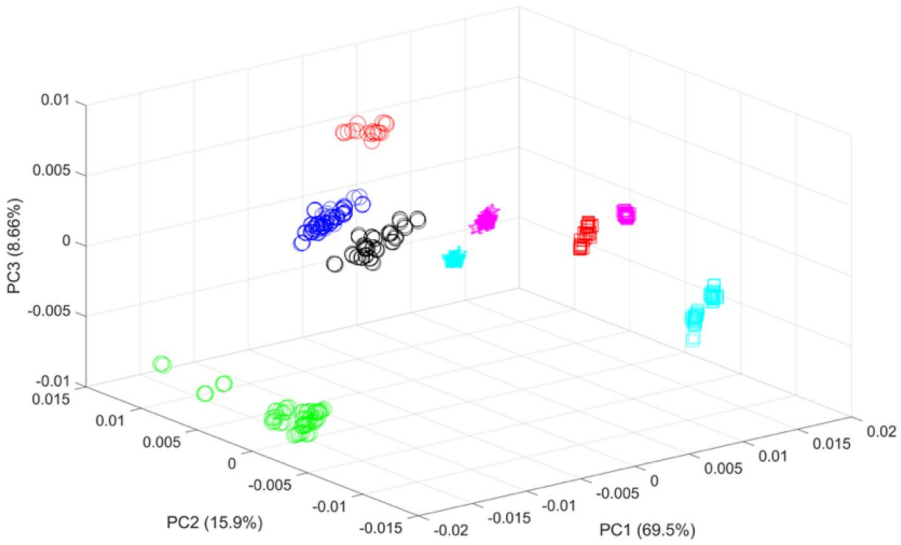

Figure 3. PC scores plot of the MSC-D1 NIR spectra of *Nicotiana glauca* (blue circle), *Nicotiana rustica* (black circle), *N. spp. Ecuadorian nursery* (green circle), *Nicotiana tabacum* (red circle), *N. spp. Achuar* crops (magenta star), *N. spp. Ashaninka* (cyan star), Ecuadorian smokeless tobacco (magenta square), Peruvian brown smokeless tobacco (cyan square) and Peruvian White smokeless tobacco (red square) measured using the Perkin Elmer Spectrum II FT-NIR spectrometer.

(Ecuadorian) and Ashaninka (Peruvian) leaf samples also showed a strong correlation (r = 0.974). However, when the Ecuadorian and Peruvian leaves were compared with the three reference *N.* spp, the Peruvian leaves showed a lower correlation with *N.* rustica (r = 0.80), indicating weaker similarity, but a stronger correlation with *N.* glauca (r = 0.958). In contrast, the Achuar Ecuadorian leaf samples showed the highest correlation with *N.* rustica (r = 0.951), suggesting a closer chemical profile to this species. This contrasts with the Peruvian sample's greater similarity to *N. glauca,* despite the known spectral similarities between these two *N.* spp.

Principal component analysis (PCA)

PCA comprised a data reduction technique that served as an unsupervised exploratory tool to visualize differences between *N.* spp. PCA was adapted in this study for understanding patterns between the different spectra of *N.* spp. Previous studies have used PCA for categorization of tobacco varieties, species and products. In all of the aforementioned four studies, clear clusters corresponded to the different tobacco categories evaluated. Likewise, PCA in this study showed clusters between different *N.* spp. that corresponded to differences between these species. PCA split the original dataset into a scores plot and a loading plot. The scores plot showed patterns in spectral data; whereas, the loading plot indicated what absorbances played a key role in the model (Fig. 3).[23,24] Previous Gas chromatography–mass spectrometry (GC-MS) analysis were also performed. The PCA results based on GC-MS data are presented in Figs. 4 and 5, and a summary of the findings is provided in Supplementary Tables S3 and S4. Additional analytical results by GC-MS are included in Supplementary Appendix D.

For the NIR data, the first PCs contributed to 94% of the variance collectively. PC1 (69.5%) accounted for the majority of variance in the dataset, PC2 (15.9%) adds further differentiation, and. PC3 (8.6%) provided additional nuance to the clustering. Clear species differentiation was observed**:** *N. tabacum, N. glauca* and *N. rustica* formed distinct clusters, well separated along PC1 and PC2, reflecting spectral differences likely due to intrinsic or structural variation among species. Minor overlap between *N. rustica* and *N. glauca* suggests some shared spectral features, although still clearly distinguishable.

The tight clustering observed within each group indicates high analytical reproducibility. As each group represents a different *N.* spp. or smokeless tobacco sample the PCA results confirms they are distinct. The presence of non-overlapping clusters provides supporting evidence

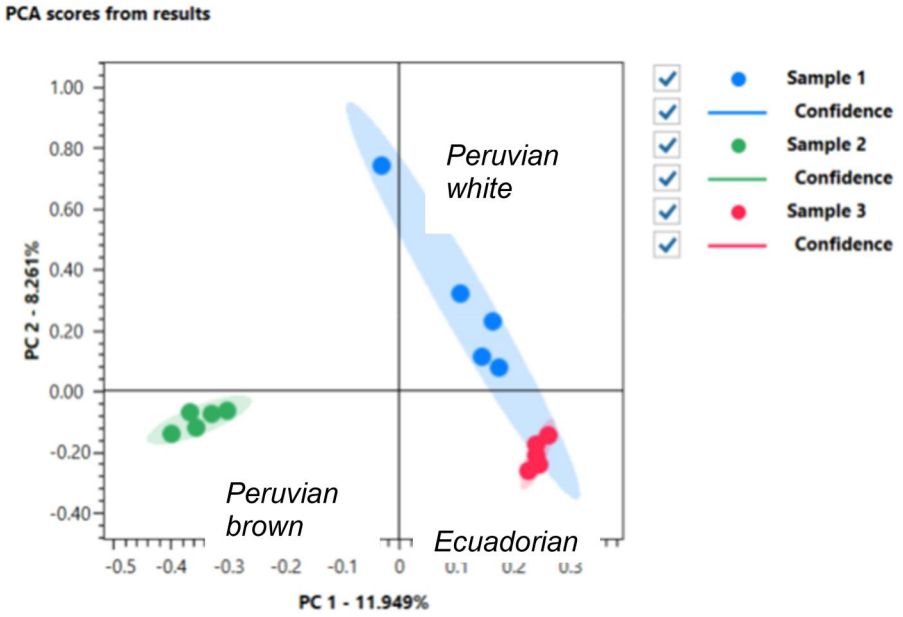

Figure 4. PCA scores results obtained by GC-MS, Analyzer Pro XD software, Version 1.134.

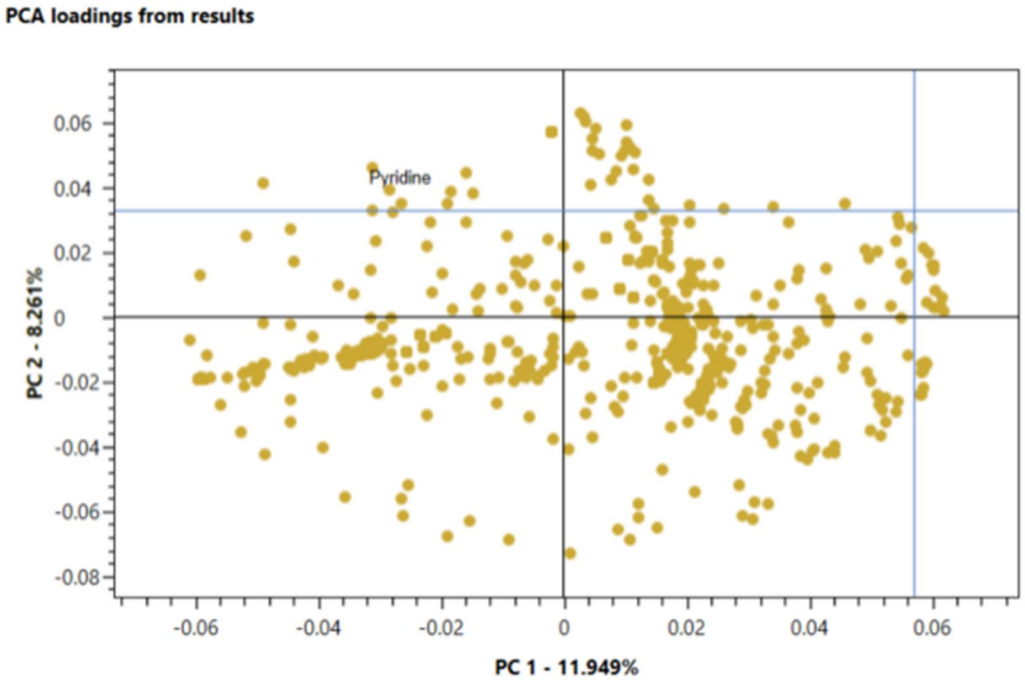

Figure 5. PCA loading scores results obtained by GC-MS, Analyzer Pro XD software, Version 1.134.

that PCA can effectively categorize and differentiate the sample types based on NIR data or similar high-dimensional input.

Discussion

The present study extends previous literature by evaluating the applicability of NIR spectroscopy for the biochemical characterization of tobacco species beyond *N.* tabacum. Specifically, this study is the first to explore the NIR biochemical signatures of *N.* glauca and *N.* rustica alongside *N.* tabacum. Overall, the findings demonstrate the feasibility of NIR spectroscopy for discriminating among *N.* spp. and capturing species-specific biochemical fingerprints. Spectroscopic spectra and cluster analysis suggested correlation between the Ecuadorian *N.* spp. Achuar leaves (source of SLT Ecuadorian sample) and *N.* rustica, a species known to contain two to three times higher

concentrations of nicotine and TSNAs than other *N.* spp. These correlations were further supported by previous GC-MS analysis and quantifications, which showed ionized nicotine concentrations of 65 mg/g, around three times higher than those detected in the two Peruvian samples. Conversely, the Peruvian *N.* spp. Ashaninka leaves (source of Peruvian white SLT sample), showed the strongest correlations with *N.* glauca. This species is characterized by low nicotine content and unusually high concentrations of anabasine. GC-MS quantifications revealed average ionized nicotine levels of 19 mg/g for this sample. The Peruvian brown SLT sample, however, did not correlate with any of the *N. spp.* examined in this study. According to Asháninka community members, it is primarily used as a medicinal ointment rather than as a SLT product. GC-MS analysis confirmed the lowest ionized nicotine levels among all samples, averaging 14.5 mg/g (Supplementary Appendix D).

These trends were consistently reflected in the PCA models derived from both NIR and GC–MS data (Figs. 1–5). Clear clustering patterns were observed in the respective score plots, indicating discrimination among *N.* spp. exhibiting greater variability in nicotine content tended to form adjacent but distinguishable clusters. This agreement between NIR and GC–MS PCA results suggests that differences in nicotine composition contribute substantially to the observed spectral differentiation and play a key role in species categorization.

Several limitations were encountered in the study related to confounding factors and sample size. Confounding factors encountered in the study were variability associated with the biological nature of the plants obtained and the differences cultivation conditions of these plants. To minimize their impact on analysis, relevant physicochemical details of these samples were recorded and considered during data analysis. Moreover, the sample size was technically low and included nine independent samples. From each sample, 20 replicates were obtained per sample to assess instrumental repeatability. Nonetheless, the independent experimental unit of the original sample remained nine. Therefore, replicate spectra were treated as technical replicates rather than independent observations.

Conclusion

In summary, the study showed the potential of NIR spectroscopy as a rapid exploratory screening tool for *N.* spp. When combined with chemometrics, NIR was able to identify and categorize different species according to nicotine content. Future work will expand on the number of samples and species to assess the potential of NIR as an analytical method.

We also acknowledge, the collaboration of Dr Jimmy Jaen Noblecilla (†), Mrs. Maritza Jaen Noblecilla (†), and Mr. Antonio Montalvo Ibarra for their support during the planning and coordination of the fieldwork. Our heartfelt thanks to Mr. Luis Antonio Vargas Canelos founder, and first president of the Achuar Association, for his warm welcome into the community and for providing the smokeless tobacco samples and tobacco leaves used in this research.

This work would not have been possible without the trust and cooperation of the Achuar people. Finally, we also acknowledge the collaboration of the Research Committee of the Ecuadorian Institute of Sciences and Indigenous Cultures, *Amawta Runakunapak Yachay (ARY)* during the revision of this manuscript. We remain committed to conducting research that honors and respects Indigenous knowledge, traditions, and sovereignty.

Acknowledgements

First and foremost, we wish to express our sincere gratitude to Dr. Karu Kersti (Department of Mass Spectrometry, University College London) for facilitating the interdisciplinary collaboration that was essential to the successful execution of this study. The authors extend their sincere gratitude to the Achuar community *El Chumpi* for their invaluable collaboration in sharing knowledge about smokeless tobacco products and traditional cultural practices. We are especially thankful to Illanes Waakiach and Kaitian Ana for their participation in the preparation of smokeless tobacco and their contributions to ritual demonstrations.

Ethical considerations

This research involved a field visit to Indigenous communities, during which cultural data, images, videos, and smokeless tobacco samples were collected. Free, prior, and informed consent (FPIC) was obtained from all participants in accordance with ethical research standards and Indigenous rights protocols. The findings were disseminated and published with the prior written approval of an

Indigenous governance body—the Achuar Nationalities of Ecuador (NAE)—under the leadership of the association's president, Waakiach Kuja Jawirit.

NAE represents the collective interests of 91 Achuar communities and includes the following member associations: AAC, Punkir, Kasak, Jurijri, Tawasap, ACAP, MAANA, Makusar, Pump, Tsunk, Sapap, MAWIKNA, MAINA, MAWAK, Kaiptach, MAPTIN, Tunkian, MAIK, MASNIK, and MASHUMARENTSA. In addition, the research received approval for international data dissemination and publication from the Research Committee of the Ecuadorian Institute of Sciences and Indigenous Cultures, *Amawta Runakunapak Yachay (ARY)*.

Authors' contributions

CRediT: **Stephanie Jaen**: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing; **Sulaf Assi**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Dhiya Al-Jumeily**: Data curation, Formal analysis, Software, Validation; **Luis Vargas Canelos**: Project administration; **Rachel Leeson**: Project administration, Supervision.

Disclosure statement

The authors of this manuscript confirm that there are no known conflicts of interest associated with this publication.

Funding

This research did not receive any specific funding from public, commercial, or not-for-profit funding agencies.

Data availability statement

The aim of this study was to document the use of tobacco products among Indigenous communities, to identify the specific *Nicotiana* species used in their traditional smokeless tobacco preparations, and to correlate the associated alkaloid content by previous GC-MS analysis. The study sought to detect environmental pollutants and potentially carcinogenic compounds to better understand the health risks faced by these often-under-researched populations. This study was performed without inappropriate use of cultural information supporting and encouraging respect to Indigenous ancestral traditions and knowledge.

The data used in this research—including biological, spectral, and cultural findings—were presented to the Ecuadorian Institute of Sciences and Indigenous Cultures *Amawta Runakunapak Yachay (ARY)* and the offices of the Achuar Nationalities of Ecuador (NAE) for documentation, storage, and dissemination to the communities involved.

In accordance with the Collective Benefit, Authority to Control, Responsibility, and Ethics (CARE) Principles for Indigenous Data Governance and Article 31 of the United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP), all data published in this study, including biological specimens, plant specimens, smokeless tobacco samples, spectral data, and cultural knowledge, are owned by the Indigenous communities who contributed to this research. The data supporting the findings of this study include culturally sensitive information shared by members of the Achuar and Asháninka communities. Due to ethical obligations and agreements with community representatives, these data are not publicly available. Access may be granted upon reasonable request and with explicit approval from the relevant Indigenous authorities.

References

[1] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines. Lyon (FR): International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 89; 2007.

[2] Joseph, C. W. Tobacco Used by Native North Americans, Sacred Smoked and Silent Killer, University of Oklahoma Press Norman, Part One, Traditional Uses of Tobacco by Native Americans; 2000, vol. 236, p. 81–87, University of Oklahoma Press, US.

[3] Nez Henderson, P.; Lee, J. P.; Soto, C.; O Leary, R.; Rutan, E.; D Silva, J.; Waa, A.; Henderson, Z. P.; Nez, S. S.; Maddox, R. Decolonization of Tobacco in Indigenous Communities of Turtle Island (North America). *Nicot. Tobacco Res. Off. J. Soc. Res. Nicot. Tobacco.* **2022**, *24*(2), 289–291. PMID: 34516637; PMCID: PMC8807169. DOI: 10.1093/ntr/ntab180.

[4] Martyn, C. N. Smoking in British popular culture 1800–2000. *BMJ (Clin. Res. ed.).* **2000**, *321*(7257), 389.

[5] Luis Vargas Canelos. *La Posición Achuar, la Corriente de mi Vida, Autobiografia, Primera Edición*. Tabaco Kasak: Ediciones Abya-Yala, 2019, p. 48–54.

[6] Olsson Gisleskog, P. O.; Perez Ruixo, J. J.; Westin, Å.; Hansson, A. C.; Soons, P. A. Nicotine Population Pharmacokinetics in Healthy Smokers After Intravenous, Oral, Buccal and Transdermal Administration. *Clin. Pharmacokinet.* **2021**, *60*(4), 541–561. DOI: 10.1007/s40262-020-00960-5.

[7] World Health Organization. Tobacco; 2023. https://www.who.int/news-room/fact-sheets/detail/tobacco.

[8] Geiss, O.; Kotzias, D. European Commission Joint Research Centre, Tobacco, Cigarettes and Cigarettes Smoke, an Overview; 2007.

[9] Chuah, A.; Arora, M.; Neha, J.; et al. The Global Impact of Tobacco Control Policies on Smokeless Tobacco Use: A Systematic Review. *Lancet Global*

Health J. **2023**, *11*, e953-68. DOI: 10.1016/S2214-109X(23)00205-X.

[10] Stanfill, S. B.; Connolly, G. N.; Zhang, L.; Jia, L. T.; Henningfield, J. E.; Richter, P.; Lawler, T. S.; Ayo-Yusuf, O. A.; Ashley, D. L.; Watson, C. H.; et al. Global Surveillance of Oral Tobacco Products: Total Nicotine, Unionised and Tobacco Specific N-Nitrosamines. *Tobacco Control.* **2011**, *20*(3), e2. DOI: 10.1136/tc.2010.037465.

[11] Sierro, N.; Battey, J. N. D.; Bovet, L.; Liedschulte, V.; Ouadi, S.; Thomas, J.; Broye, H.; Laparra, H.; Vuarnoz, A.; Lang, G.; et al. The Impact of Genome Evolution on the Allotetraploid Nicotiana Rustica, An Intriguing Story of Enhanced Alkaloid Production. *BMC Genom.* **2018**, *19*(1), 855. DOI: 10.1186/s12864-018-5241-5.

[12] Stanfill, S. B.; Oliveira da Silva, A. L.; Lisko, J. G.; Lawler, T. S.; Kuklenyik, P.; Tyx, R. E.; Peuchen, E. H.; Richter, P.; Watson, C. H. Comprehensive Chemical Characterization of Rape Tobacco Products: Nicotine, Un-ionized Nicotine, Tobacco-specific N'-Nitrosamines, Polycyclic Aromatic Hydrocarbons and Flavour Constituents. *Food Chem. Toxicol. Int. J. Publish. British Indus. Biol. Res. Associat.* **2015**, *82*, 50–58. DOI: 10.1016/j.fct.2015.04.016.

[13] Furer, V.; Hersch, M.; Silvetzki, N.; Breuer, G. S.; Zevin, S. Nicotiana Glauca (Tree Tobacco), Intoxication, Two Cases in One Family. *J. Med. Toxicol. Offi. J. Am. College Med. Toxicol.* **2011**, *7*(1), 47–51. DOI: 10.1007/s13181-010-0102-x.

[14] Massadeh, R. K.; El-Elimat, T.; Al-Gharaibeh, M.; Tawaha, K.; Alali, F. Q. UPLC-HRESI-MS and GC-MS Analysis of the Leaves of *Nicotiana glauca*. *Acta Pharmaceutica (Zagreb, Croatia)* **2022**, *72*(1), 97–108. DOI: 10.2478/acph-2022-0001.

[15] Asthana, S.; Labani, S.; Kailash, U.; Sinha, D. N.; Mehrotra, R. Association of Smokeless Tobacco Use and Oral Cancer: A Systematic Global Review and Meta-Analysis. *Nicot. Tobacco Res. Offi. J. Soc. Res. Nicot. Tobacco.* **2019**, *21*(9), 1162–1171. DOI: 10.1093/ntr/nty074.

[16] Ariq, M.; Ahmad, Z.; Shah, S. A.; Gul, Z.; Khan, S. A. Phytochemical Analysis and Antibacterial Activity of *Nicotiana tabacum* and *Nicotiana rustica*. *RADS J. Biol. Res. Appl. Sci.* **2021**, *12*(1), 60–65.

[17] Camlica, M.; Yaldiz, G. Analyses and Evaluation of the Main Chemical Components in Different Tobacco (*Nicotiana tabacum* L.) Genotypes. *Grasas y Aceites.* **2021**, *72*(1), e389. DOI: 10.3989/gya.0801192.

[18] Chen, Y.; Bin, J.; Zou, C.; Ding, M. Discrimination of Fresh Tobacco Leaves with Different Maturity Levels by Near-Infrared (NIR) Spectroscopy and Deep Learning. *J. Anal. Meth. Chem.* **2021**, *2021*, 9912589–9912511. DOI: 10.1155/2021/9912589.

[19] Falcioni, R.; Moriwaki, T.; Antunes, W. C.; Nanni, M. R. Rapid quantification method for yield, calorimetric energy and chlorophyll *a* fluorescence parameters in *Nicotiana tabacum* L. using Vis-NIR-SWIR hyperspectroscopy. *Plants (Basel, Switzerland).* **2022**, *11*(18), 2406. Article DOI: 10.3390/plants11182406.

[20] Rodrigues, M.; de Oliveira, R. B.; dos Santos, G. L. A. A.; de Oliveira, K. M.; Reis, A. S.; Furlanetto, R. H.; Júnior, L. A. Y. B.; Coelho, F. S.; Nanni, M. R. Rapid Quantification of Alkaloids, Sugar and Yield of Tobacco (*Nicotiana tabacum* L.) Varieties by Using Vis–NIR–SWIR Spectroradiometry. *Spectrochim. Acta Part A. Mol. Biomol. Spectroscop.* **2022**, *274*, 121082. DOI: 10.1016/j.saa.2022.121082.

[21] Babu, A. K.; Kumaresan, G.; Antony Aroul Raj, V.; Velraj, R. Review of Leaf Drying: Mechanism and Influencing Parameters, Drying Methods, Nutrient Preservation, and Mathematical Models. *Renew. Sustain. Energy Rev.* **2018**, *90*, 536–556. DOI: 10.1016/j.rser.2018.04.002.

[22] Cooperation Centre for Scientific Research Relative to Tobacco, CORESTA, Technical Guidelines Number 11, Title: Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products. Version 2; 2020.

[23] Lu, M.; Zhou, Q.; Chen, T.; Li, J.; Jiang, S.; Gao, Q.; Wang, C.; Chen, D. Qualitative Discrimination of Intact Tobacco Leaves Based on Near-Infrared Technology. *J. Spectros.* **2021**, *2021*, 1–9. DOI: 10.1155/2021/8807199.

[24] Luan, L.; Wang, Y.; Li, X.; Hu, W.; Li, K.; Li, J.; Yang, K.; Shu, R.; Zhao, L.; Lao, C.; et al. Application of Multiple Classifier Fusion in the Discriminant Analysis of Near Infrared Spectroscopy for Agricultural Products. *J. Near Infrar. Spectros.* **2016**, *24*(4), 363–372. DOI: 10.1255/jnirs.1236.

[25] NREDH. Apuntes sobre la explotación petrolera en el Ecuador Quito: Fundación Regional de Asesoría en Derechos Humanos (INREDH); 2016. https://inredh.org/apuntes-sobre-la-explotacion-petrolera-en-el-ecuador.

[26] IWGIA. The Indigenous World 2025: Ecuador. Copenhagen: International Work Group for Indigenous Affairs; 2025. https://iwgia.org/en/ecuador/5732-iw-2025-ecuador.html.

[27] Maddox, R.; Waa, A.; Henderson, P. N.; Calma, T.; Kennedy, M. Commercial Tobacco and Cancer. In: *Indigenous and Tribal Peoples and Cancer*; Garvey, G., Ed.; Springer, Switzerland, 2024, pp 171–175. DOI: 10.1007/978-3-031-56806-0_36.

[28] Woodworth, J. R.; Bloch, R. W. Effects of Light Intensity, Temperature, and Root Gaseous Environment on Growth of *Nicotiana tabacum*. *Agronomy J.* **1969**, *61*(2), 184–187. DOI: 10.2134/agronj1969.00021962006100020032.

[29] Drapal, M.; Enfissi, E. M. A.; Fraser, P. D. The Chemotype Core Collection of Genus Nicotiana. *Plant J. Cell Mol. Biol.* **2022**, *110*(5), 1516–1528. Epub 2022 Apr 7. PMID: 35322494; PMCID: PMC9321557. DOI: 10.1111/tpj.15745.