Authentication and detection of common adulterants in clove buds (Syzygium aromaticum L.) powders and oils using near IR combined to multivariate analysis

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Introduction

Clove buds are an important spice used in various cuisines and also have medicinal properties. Adulteration of clove buds can affect the quality, safety, and efficacy of the product. Therefore, it is important to detect the adulteration in clove buds to ensure their authenticity. The existence of commercialized products of way more inferior quality has a chance. Adulteration of essential oils is a major issue in today's markets, topping the list of problems facing the regulation of herbal products

Materials and Methods

Plant materials and sample preparation

Samples of Syzygium aromaticum in addition to samples of basil leaves, cinnamon bark, rosemary leaves, corn oil and olive oil samples were purchased. Clove stalks were separated, air-dried then ground followed by sieving. Some samples were exhausted by distillation. For preparation of adulteration mixtures, 7 clove powder samples were individually adulterated with clove stalks and exhausted clove powders with different adulterant percentage, samples were randomly divided into training and test sets.

Oil sample preparation

Clove, clove leaf, rosemary, basil and cinnamon essential oils samples were prepared by distillation using Clevenger apparatus. The obtained oils were clove mixed with different adulterant oils; in different concentrations. All samples were all subjected to NIR analysis. The obtained samples were randomly divided into training and test sets.

NIR spectroscopy measurements and data acquisition

Multipurpose analyzer FT-NIR spectrometer was used for NIR scanning within the wavenumber range of 12500–3600 cm − 1 . Spectral data were acquired through OPUS spectral acquisition software. Each sample was scanned three times and the spectra were mean centered. The average spectrum was then used. Weighted multiple scatter correction was performed.

Spectral data pre-treatment

Raw NIR spectral data were pre-processed using first order derivative transformations, as well as standard normal variate (SNV) transformations to using SIMCA-P + 14.1 software.

Multivariate statistical analysis

Class modeling of clove samples using soft independent modeling of class analogy (SIMCA) model

SIMCA was utilized for classification purposes, with the goal of authenticating clove samples. To accomplish this, a training set consisting of 16 samples from clove bud powder class to train the DD-SIMCA models was utilized. Additionally, a test set containing samples from clove bud powder in addition to adulterants and adulteration mixtures was used to evaluate the predictive performance of the constructed model for clove powder. Meanwhile, 16 samples from clove bud oil class to train the DD-SIMCA models was utilized in addition to, a test set containing samples from clove bud oil, other oil adulterants and adulteration mixtures was used to evaluate the predictive performance of the constructed model for clove oil.

Partial least squares (PLS) regression model of adulterated clove samples in terms of their adulterant content

The adulterant content was modelled in our investigation using PLS, with six PLS models built using PLS-1 for each of the six hypothesized adulterants

independently. The models' predictive performance was assessed using root mean squared error for calibration (RMSEC), root mean squared error of cross validation (RMSECV), and root mean squared error of prediction (RMSEP) of test set samples, RMSEC, RMSECV, and RMSEP for calibration, cross validation, and prediction, respectively. The absence of data over-fitting (noise modelling) was confirmed using permutations plots.

Results

NIR spectral features of clove and adulterants

The preprocessed spectrea showed distinct differences in the regions of approximately 6,100–6,020 cm-1 and 5,970–5,900 cm-1. We suggest that these differences are primarily related to eugenol.

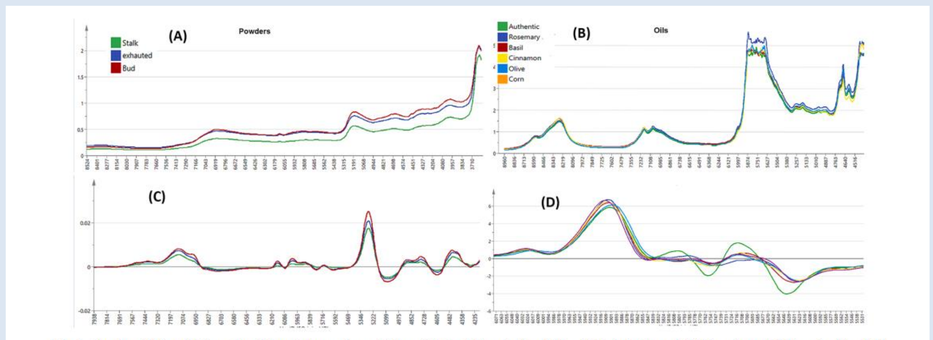

Fig. 1. Overlay of (A) raw NIR spectra of clove buds powders and oils and their adulterants (A and B) and first derivative and SNV transformed NIR spectra (C and D) of representative samples covering the range of 9000–4000 cm^{-1} acquired at resolution of 16 cm^{-1}.

Multivariate analysis

Unsupervised pattern recognition

The score scatter plot of PCA model for clove powder and its adulterants was valid . Both clove powder bud and exhausted clove powders were clustered together away from while clove stalk powder. The score scatter plot of PCA model for clove oil and its adulterants was also valid. It showed the clustering of authentic clove bud oil and clove leaf oil samples away from the rest of adulterants. The HCA dendrogram results were in accordance.

Class modeling using soft independent modeling of class analogy (SIMCA)

SIMCA model was built using data from 9000 to 4000 cm− 1 spectral region. For the clove bud powders model, the data matrix composed of 48 samples. A total of 3 principal components, capturing 83.5% of the total variance, were employed. As anticipated, all the training samples are situated within the acceptance area. The sensitivity and specificity parameters were 100%. Another DD-SIMCA analysis was conducted for the clove oil samples. A total of 2 principal components, capturing 78.2% of the total variance, were employed. All the samples were correctly identified, the sensitivity and specificity parameters were 100%.

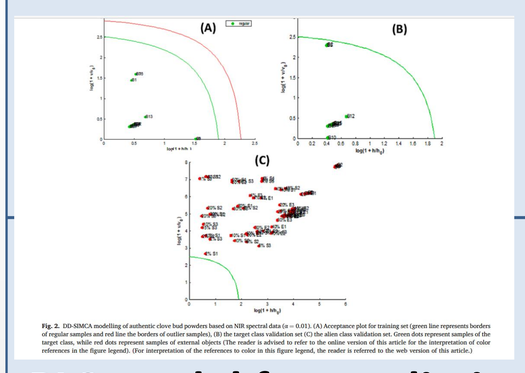

Fig. 2. DD-SIMCA modelling of authentic clove bud powders based on NIR spectral data (α = 0.01). (A) Acceptance plot for training set (green line represents borders of regular samples and red line the borders of outlier samples), (B) the target class validation set (C) the alien class validation set. Green dots represent samples of the target class, while red dots represent samples of external objects (The reader is advised to refer to the online version of this article for the interpretation of color references in the figure legend). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

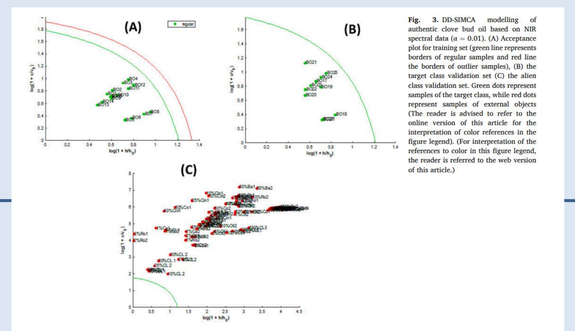

Fig. 3. DD-SIMCA modelling of authentic clove bud oil based on NIR spectral data (α = 0.01). (A) Acceptance plot for training set (green line represents borders of regular samples and red line the borders of outlier samples), (B) the target class validation set (C) the alien class validation set. Green dots represent samples of the target class, while red dots represent samples of external objects (The reader is advised to refer to the online version of this article for the interpretation of color references in the figure legend). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PLS model for predicting adulterant content in clove samples

The PLSR models constructed produced good results, with acceptable low values of RMSEC, RMSECV, LOQ and LOD for powder and oil models. Both calibration and cross validation data had R2 values indicating a good fit, and the optimal number of latent variables was chosen based on RMSEC, RMSECV, and R2 values. External validation was performed and the results were validated using permutation tests. The models can be used to detect sample adulteration and ensure authenticity.

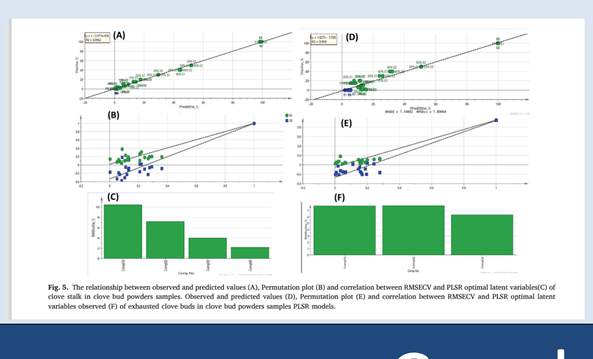

Fig. 5. The relationship between observed and predicted values (A), Permutation plot (B) and correlation between RMSECV and PLSR optimal latent variables(C) of clove stalk in clove bud powders samples. Observed and predicted values (D), Permutation plot (E) and correlation between RMSECV and PLSR optimal latent variables observed (F) of exhausted clove buds in clove bud powders samples PLSR models.

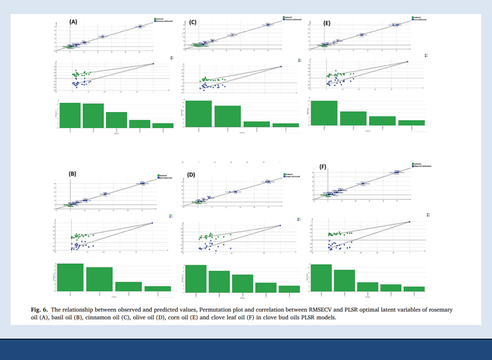

Fig. 6. The relationship between observed and predicted values, Permutation plot and correlation between RMSECV and PLSR optimal latent variables of rosemary oil (A), basil oil (B), cinnamon oil (C), olive oil (D), corn oil (E) and clove leaf oil (F) in clove bud oils PLSR models.

Conclusions

The NIR diffuse reflectance spectroscopy method linked to chemometry is simple, fast, and requires no sample preparation with good performance in classifying samples and detecting adulteration

References

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