ION MOBILITY SPECTROMETRY AS A TOOL FOR RAPID AND ACCURATE BLACK PEPPER AUTHENTICATION

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Summary

This contribution describes a non-targeted method for authentication of black pepper by Gas Chromatography Ion Mobility Spectrometry (GC-IMS) coupled to multivariate statistical analysis. A total of 88 samples were provided by a well-knows spices supplier and analyzed by GC-IMS. The sample set included 39 authentic black pepper samples (from 8 different countries and 4 harvesting seasons) and 17 samples spiked with non-functional endogenous material (pinhead and spent) and 32 exogenous materials (green lentil, olive kernel, black mustard, sesame, garlic, corn flour, rice flour, chili, papaya). The percentage of adulteration ranged between 5% and 30%.

Materials and Methods

A total of 88 samples were analyzed by using the GC-IMS (FlavourSpec® G.A.S. Gesellschaft für Analytische Sensorsysteme mbH, Dortmund Germany) which allows the analysis of volatiles in the headspace without any sample pre-treatment. Each sample was analyzed in duplicate. An amount of 0.5 g of sample was incubated for 3 minutes at 60°C. Each run lasts 20 minutes, with a nitrogen carrier gas gradient as follows: ramping from 2 mL/min to 5 mL/min within the first 5 min and from 5 mL/min to 17 mL/min in the following 17 minutes. We applied a drift gas constant flux of 150mL/min. The data were acquired over one month (in 4 different days of analysis). The acquired data were analyzed by Python 3.9.12 using the GC-IMS-Tools package. The spectra were randomly split in training and test set (70:30). Specifically, the whole dataset was split in training (n=64) and test set (n=24). The spectra were aligned along the drift time coordinate and repeated spectra of each sample were averaged. The retention time, considered for statistical analysis, ranged between 1.3 min to 8.3 min and a relative drift time between 1.025 and 1.7. A Pareto normalization was applied. While the test set was withheld for further testing of the model, the training set was submitted to partial least squared discriminant analysis (PLS-DA) with the aim to discriminate the samples as authentic, exogenously-adulterated and endogenously-adulterated was carried out.

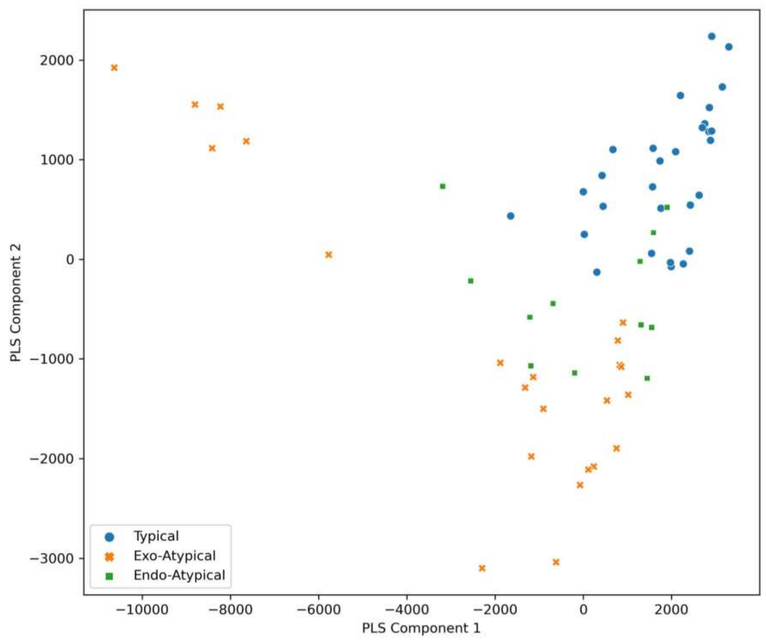

Figure 1. *PLS-DA score plot showing the clustering of the three groups of samples (typical, endogenously-adulterated and exogenously-adulterated) analysed by GC-IMS and included in the training set*

Introduction

Economically motivated adulteration of spices and herbs encompasses the fraudulent deliberate substitution of valuable commodity with cheaper materials or plant subproducts (1). The European Commission published in 2021 the results of the first coordinated control plan on the authenticity of herbs and spices on the European market carried out by 21 EU Member States, Switzerland and Norway (2). As resulted from this large investigation 17% of black pepper is suspicious of adulteration. Many techniques can be used to verify spices authenticity as NGS, GC-MS FTIR, LC-MS/MS but generally they are consuming, slow and costly.
Simple, reliable, robust and rapid methods are needed to reveal the presence of suspicious samples in the market or check the raw materials prior their application in food industry. In this contribution we test GC-IMS on authentic samples (typical), endo-atypical and exo-atypical samples.

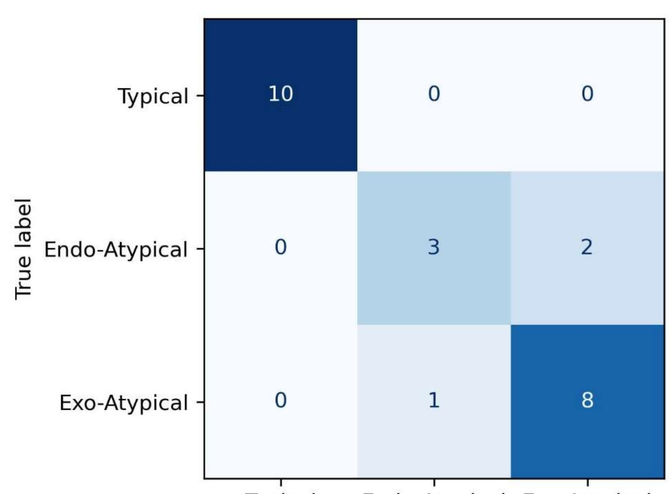

Table 1. *Predictions of the PLS-DA classifier on test samples.*

Results and Discussion

A good clustering of authentic, exogenously-adulterated and endogenously-adulterated groups was observed in the PLS-DA score plot (Figure 1). The model, tested on the samples withheld previously, achieved a good overall accuracy (87.5%) with 21 samples out of 24 correctly classified. The classifier also showed high sensitivity and specificity on authentic and exogenously-adulterated samples (Table 1 and 2).
The low discrimination rate achieved between endo-adulterated and exogenously-adulterated samples is a minor issue, as we reached 100% of accuracy when taking into account a binary classifier of authentic and adulterated samples.

PERFORMANCES ON TEST SET			
	Typical	**Endo-atypical**	**Exo-atypical**
Accuracy	87.5%		
Sensitivity	100%	60.0%	88.9%
Specificity	100%	94.7%	86.7%

Table 2. *Performances of the PLS-DA classifier established on test set samples*

Conclusions

GC-IMS coupled to chemometrics allowed a good discrimination of authentic, exogenously-adulterated and endogenously-adulterated black pepper. The classifier achieved a discrete overall accuracy on the withheld test set. Further validation with an independent set of samples will be carried out.

References

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2. A. Maquet, A. Lievens, V. Paracchini, G. Kaklamanos, M.B. De La Calle Guntinas, L. Garlant, et al. 2021. Contract No.: EUR 30877 EN