Ensuring the Integrity of the European food chain

NMR and MIR-ATR approaches to assess the integrity of saffron: a case study from the Food Integrity WP18 team

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Overview

Over the last years, food traceability has earned the attention of the international scientific community, as well as the interest of the consumers, which are more and more concerned about the origin, integrity and safety of the food. Instead of searching for a specific contaminant or anomaly, untargeted analytical methods aim to describe specific food "fingerprints" characterizing a particular food product. Within the activities of the European Project "Food Integrity", Work Package 18 aims at producing a consensus document (guidelines) on good practices and methodological procedures for the application and validation of untargeted analysis applied to food traceability. The Units involved in WP18 worked on different models using different untargeted analytical techniques, finally generating several data sets to be statistically processed. In November 2016 the USP "Guidance on developing and validating non-targeted methods for adulteration detection" has been released, with the aim of providing guidance on how to develop and implement one-class, non-targeted classification methods for the detection of economically-motivated adulteration (EMA)-related adulterants in food. Here we present the critical discussion of the results of a model study inspired by the indications reported in this guidance. For this, the processing through advanced mathematical tools of data generated using NMR and mid-infrared attenuated total reflectance (MIR-ATR) spectroscopy on pure and artificially adulterated saffron samples is implemented. Saffron is one of the most expensive spices throughout the world, and because of its limited production it is considered within the major candidates for economically motivated frauds.

EXPERIMENTAL DESIGN

Method development proposed by USP: collection of two separate sample sets

REFERENCE SET → **50 samples of pure Italian saffron powder**

TEST SET → **20 typical test samples of pure Italian saffron powder**

TEST SET → **18 atypical test samples of adulterated saffron powder**

Adulteration strategy

Spiking agent 1: **TURMERIC**

Spiking agent 2: **SAFFLOWER**

- **6 atypical test samples of saffron spiked at 5%**
- **6 atypical test samples of saffron spiked at 10%**
- **6 atypical test samples of saffron spiked at 20%**

Analytical flow

NMR

MIR-ATR

METHODOLOGY

- **NMR** analyses were performed using a Bruker Avance I 400 MHz Spectrometer (Bruker, Germany), operating at 400.13 MHz using BBI 5-mm probe and equipped with B-ACS 60 Autosampler (Bruker Automatic Sample Changer). Each spectrum is acquired using TOPSPIN 2.1 software (Bruker BioSpin GmbH, Rheinstetten, Germany) and processed using TOPSPIN 3.0 software (Bruker BioSpin GmbH, Rheinstetten, Germany).
- MIR-ATR analyses were performed….**complete**

- Statistical analyses were performed through 5 classifiers: Bayes Net **BN** with Cooper/Herskovits algorithm, max. 1 parent per node; Support Vector Machine **SMO** (Sequential Minimal Optimization), with Pearson Kernel and Platt's scaling for output prob.; Multi Layer Perceptron (**MLP**) with 1 hidden layer, Hidden units=(#features+#classes)/2; K Nearest Neighbor **KNN** with K=2, cross-validation and KDTree search strategy; Decision tree **J48** with confidence factor 0.25. Correlation-based feature selection has been applied before the analysis.

CLASSIFICATION RESULTS APPLYING A MULTICLASS APPROACH

Performance of classifiers on NMR data

Table 1: pure vs adulterated saffron

	Correctly Classified	Kappa	Precision	Recall	F-Measure	MCC
BN	98.86%	0.96	0.98	0.98	0.98	0.96
SMO	98.86%	0.96	0.98	0.98	0.98	0.96
MLP	100%	1.0	1.0	1.0	1.0	1.0
KNN	100%	1.0	1.0	1.0	1.0	1.0
J48	94.31%	0.83	0.94	0.94	0.94	0.84

Table 3: pure vs turmeric vs safflower (confusion matrix)

Pure	Safflower	Turmenic	
69	0	1	**P**
1	8	0	**S**
0	0	9	**T**

(a) BN

Pure	Safflower	Turmenic	
70	0	0	**P**
0	9	0	**S**
0	0	9	**T**

(b) MLP – KNN - SMO

Pure	Safflower	Turmenic	
65	2	3	**P**
2	7	0	**S**
0	0	9	**T**

(c) J48

Performance of classifiers on MIR-ATR data

Table 2: pure vs adulterated saffron

	Correctly Classified	Kappa	Precision	Recall	F-Measure	MCC
BN	77.27%	0.07	0.70	0.77	0.72	0.08
SMO	89.77%	0.63	0.89	0.89	0.88	0.66
MLP	88.63%	0.65	0.88	0.88	0.88	0.65
KNN	86.36%	0.54	0.85	0.86	0.85	0.55
J48	85.22%	0.49	0.84	0.85	0.84	0.50

Table 4: pure vs turmeric vs safflower (confusion matrix)

Pure	Safflower	Turmenic	
70	0	0	**P**
9	0	0	**S**
6	0	3	**T**

(a) BN

Pure	Safflower	Turmenic	
69	0	1	**P**
9	0	0	**S**
6	0	3	**T**

(b) MLP

Pure	Safflower	Turmenic	
69	0	1	**P**
9	0	0	**S**
5	0	4	**T**

(c) J48

CONCLUSIONS

- One-class classification using just reference samples as training and testing with test set provided 0 atypical test samples classified as not pure, both using NMR and using MIR-ATR data. The reason could be due either to the limited number of reference samples, or to the characteristics of the atypical tests, too similar to the reference samples to be identified as not belonging to that class.
- In order to provide indications about the analytical method, we set a multiclass approach by merging reference and test set, applying a 10-fold cross validation. Statistical analysis produced interesting results: very reasonable predictions with high recognition performance can be obtained by using the NMR dataset. Here, we are not only able to predict whether a sample is pure or adulterated (Table 1), but also to assess what type of contaminant has been used to spike the adulterated samples (Table 3). Using the MIR-ATR dataset, we could not obtain comparable results. In particular, the classifiers were all able to recognize pure samples, but they struggled to identify the adulterated ones. Therefore, the statistical analysis can take advantage of the NMR data, even if some risk of overfitting may arise, especially for MLP and KNN (Table 1).

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