Excellence in Science

PO-CON1811E

Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

ASMS 2018 MP 521

Simona Salivo[1]; Tom K. Abban[1]; Ismael Duque[2]; Luis Mancera[2]; Matthew E. Openshaw[1]
[1]Shimadzu, Manchester, UK;
[2]Clover Bioanalytical Software, Granada, Spain

Introduction

Extra virgin olive oil (EVOO) is known for its nutraceutical properties, which associate it with several health benefits and a high economic value. For these reasons, EVOO is often a target of adulteration with cheaper, lower-grade vegetable oils, typically, sunflower, corn and soybean. Within the quality control process of EVOO products, it is fundamental to develop rapid, simple and robust analytical methods to detect any fraud. Here, we present a simple approach based on the profiling of triacylglycerols (TAGs) using MALDI-TOF mass spectrometry and an evolved neural network based on a logistic regression machine learning algorithm to reveal the adulteration of extra virgin olive oils by seed oils.

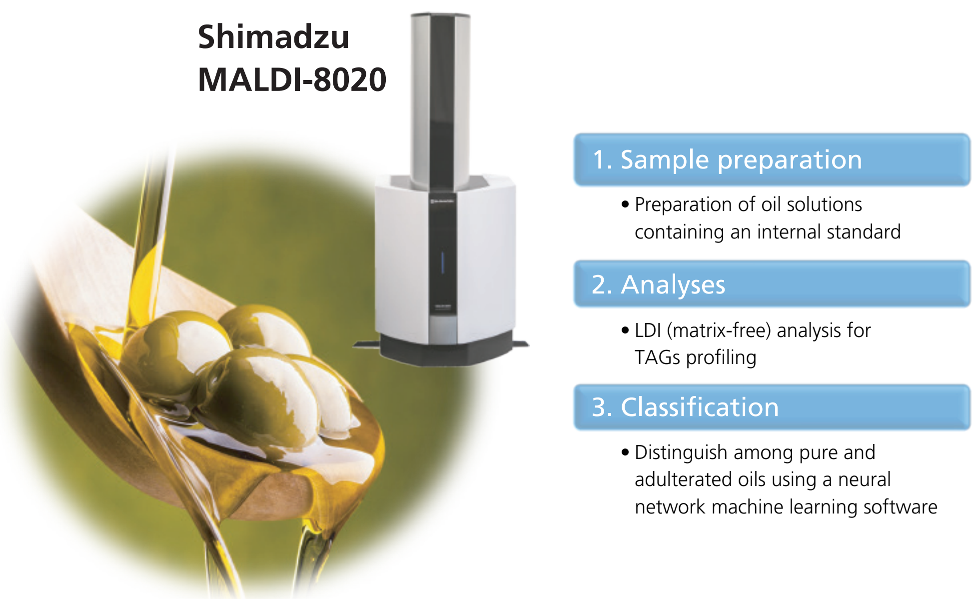

Figure 1. Left: MALDI-8020 Benchtop Linear MALDI-TOF mass spectrometer. Right: sample analysis workflow. (Picture taken from: https://www.iobenessereblog.it/olio-extravergine-di-oliva-benefici/26429).

Methods and Materials

EVOO and sunflower oils were purchased from local stores. Sample preparation involved dissolution of oil aliquots in chloroform. To simulate the adulteration, mixtures of EVOOs containing 5%, 10% and 20% of sunflower oil were prepared. Tricaprin was used as internal standard for mass alignment and the semi-quantitative analyses. LDI (matrix-free) analyses were conducted on a MALDI-8020 benchtop linear MALDI-TOF mass spectrometer (Shimadzu, Manchester, UK; Figure 1), by spotting the oil sample solutions directly onto the MALDI target which was previously pre-coated with NaTFA. Data were acquired in quadruplicates for each scenario and processed using Clover MS software (Clover Bioanalytical Software, Granada, Spain) for peak area calculation and classification with neural networks.

Results and Discussion

MALDI analyses

Figure 2a shows a comparison between the TAG profiles of an EVOO and a sunflower oil. It can be seen how, in EVOO (red trace), naturally rich in palmitic (P) and oleic (O) acids, the TAGs at *m/z* 881 and 907, i.e. most likely OPO and OOO, are predominant. In sunflower oil (blue trace), highly rich in linoleic acid (L), the most representative TAGs are those at m/z 901, 903 and 905, i.e. most likely LLL, OLL and OLO, respectively. In the oil mixture scenario (Figure 2b), the alteration of the natural TAG ratios in EVOO, e.g. *m/z* 877/907, 881/907, 903/907 and 905/907, can be observed. Interestingly, the TAG at *m/z* 901 (LLL), characteristic of sunflower oil but not normally present in EVOO, is revealed in the EVOO/sunflower mixtures even at the smallest adulteration level.

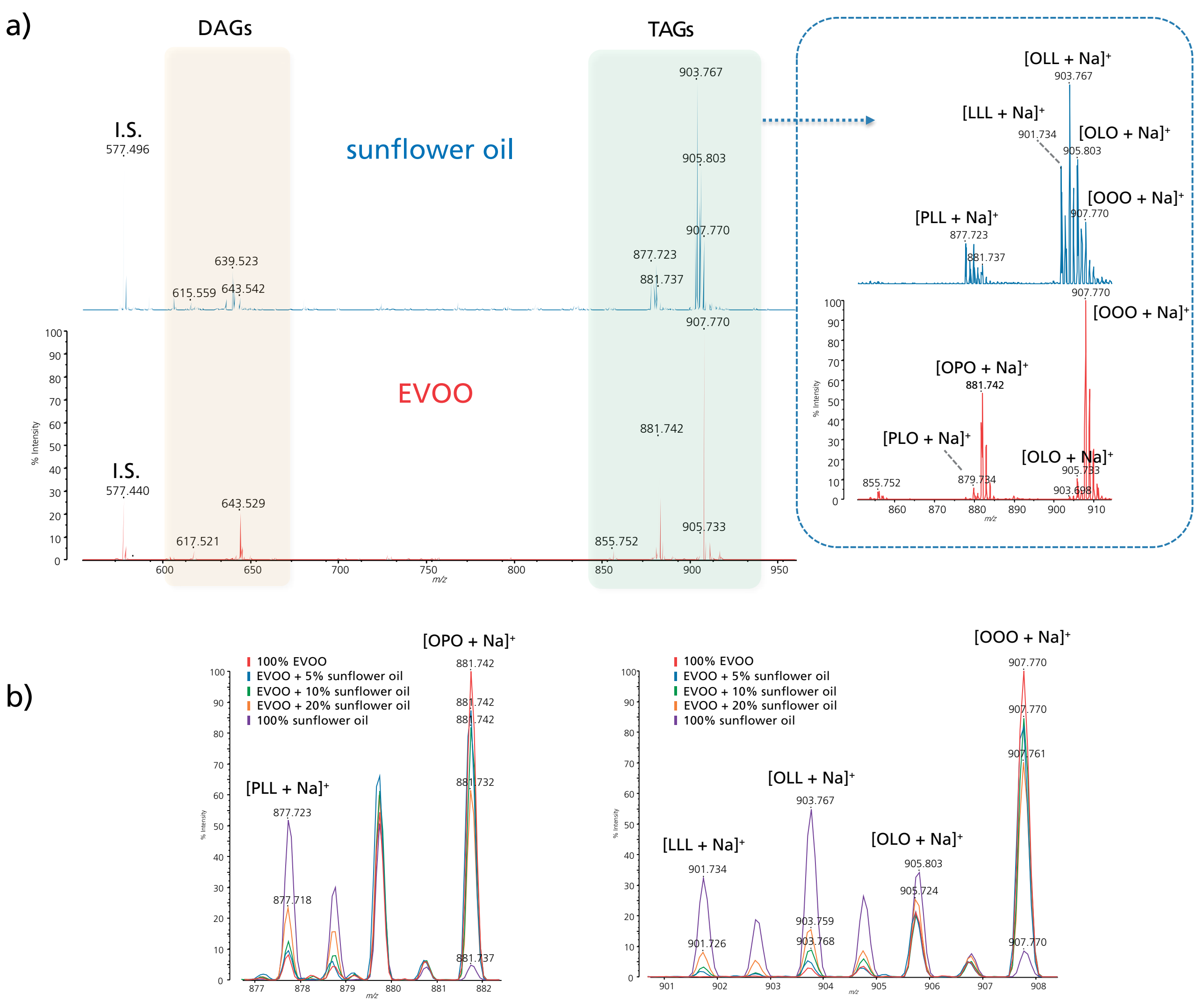

Figure 2. a) LDI MS spectra of a pure EVOO (red trace) and sunflower oil (blue trace). Right panel: expansion of the mass spectra showing the region of representative TAGs of EVOO (red trace) and sunflower oil (blue trace). b) Expansions of the overlaid mass spectra of EVOO (red trace), EVOO + 5% sunflower oil (blue trace), EVOO + 10% sunflower oil (green trace), EVOO + 20% sunflower oil (orange trace), sunflower oil (purple trace), showing the variation of TAGs and their ratios.
P = palmitic acid; O = oleic acid; L = linoleic acid.
I.S. = internal standard.

Semi-quantitative analyses

Figure 3 shows the plots of the ratios of EVOO's TAG markers and the TAG at *m/z* 907 (the most abundant and representative in EVOO), versus the different levels of adulterant oil (from 0%, i.e. pure EVOO, to 20% sunflower oil). All TAGs were normalised against the internal standard using the area of the peaks from quadruplicate analyses. A good linearity has been achieved along with good coefficients of determination (R^2).

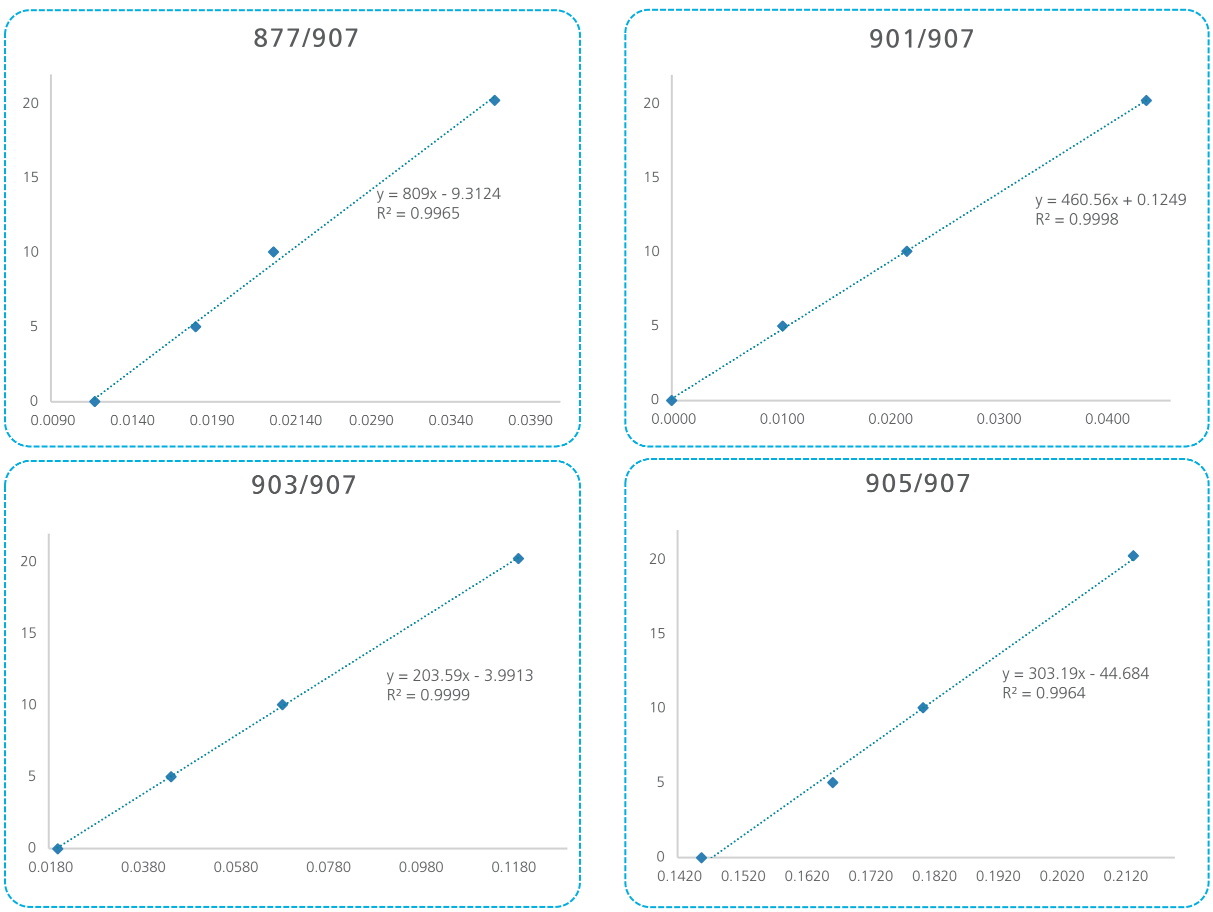

Figure 3. Plots of *m/z* 877/907, 901/907, 903/907 and 905/907 ratios (*x*-axis) versus percentage of EVOO adulteration (from 0%, i.e. pure EVOO, to 20% sunflower oil; *y*-axis).

Neural Network Training and Classification

Artificial Neural Networks (ANNs) are one of the well-known cutting edge technologies used for classification problems given the huge amount of data available nowadays. They are able to learn specific features from a given dataset. On the other hand, logistic regression models have been typically used for binary classification on linearly separable datasets. We show that the use of ANNs with a logistic regression model seems to be a fast and efficient combination to detect different types of oil samples including the adulterated ones. We have created a three layers neural network able to classify between the EVOO, adulterated EVOO and sunflower oil categories (Figure 4). Prior to the classification, all spectra were aligned and normalised by the 903 Da mass. A total of 267 spectra were used to train and validate the neural network. Thirty single-blinded spectra were used to test the model accuracy (Figure 5).

Use of MALDI-TOF mass spectrometry and machine learning to detect the adulteration of extra virgin olive oils

NN Parameters Definition:

- Input Data: (1188, 222)
- Validation Data: (1188, 45)
- Categories: 3
- Epochs: 75
- Batch Size: 10
- Nodes Hidden I: 50
- Nodes Hidden II: 25
- Learning Rate: 0.00025

NN Accuracy and Testing:

- Validation Accuracy: 97.78%
- 30 single-blinded spectra: 100%

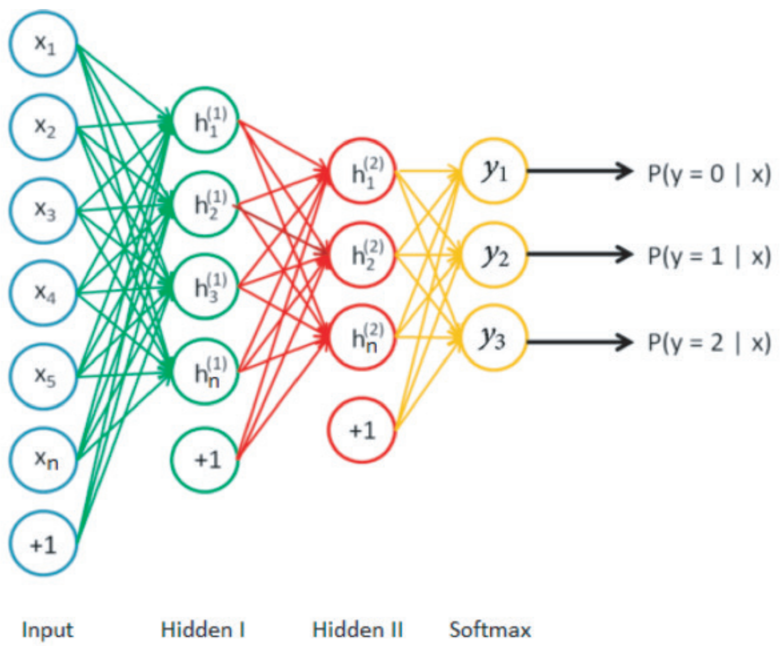

Figure 4. Representation of the neural network defined.

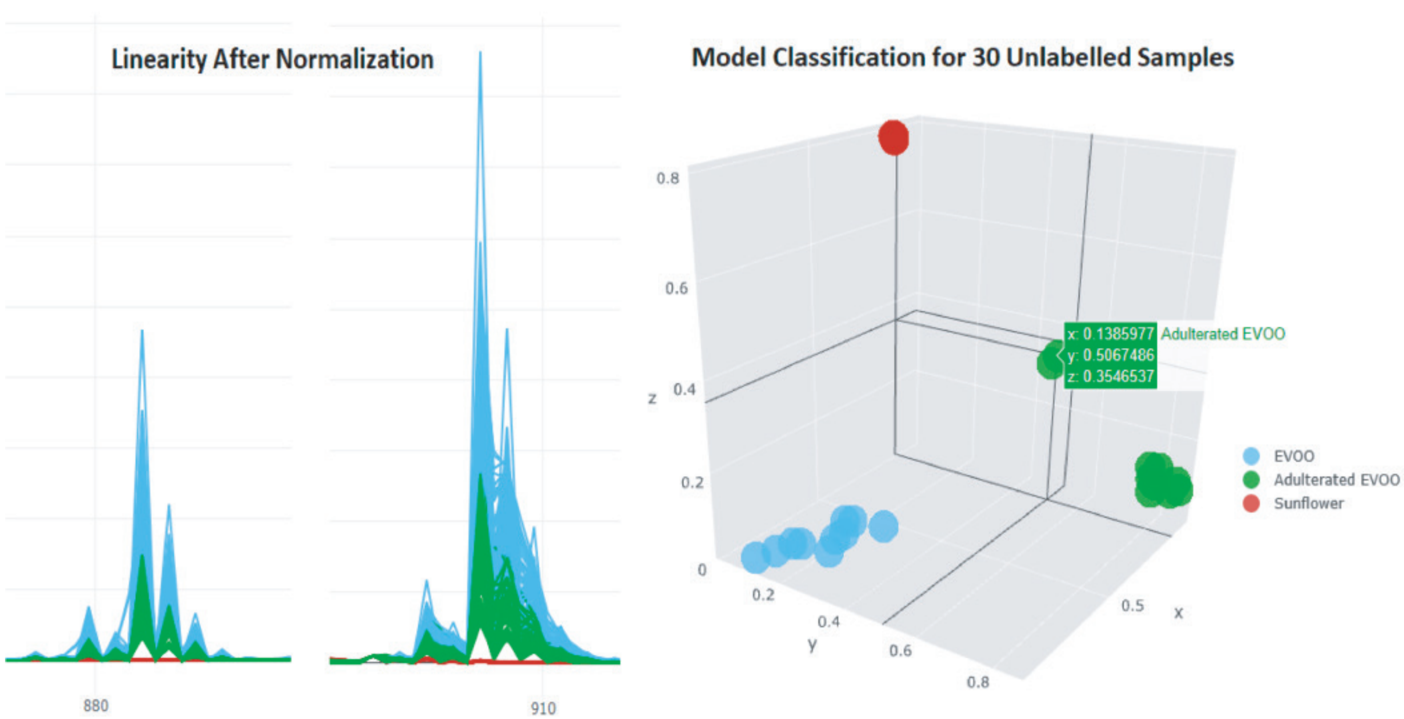

Figure 5. The linearity shown in the spectrum (left) after alignment and normalization of the input data using the Clover MS Software.
The trained model classification results (left) over the 30 single-blinded samples.

Conclusions

The combination of MALDI-TOF MS and the use of a cutting edge machine learning technique has been proven to be suitable for the detection of adulterated EVOO. The efficiency and simplicity of the methodology proposed is the key point of this research. The promising results achieved, and the expansion of the dataset and categories to be detected will determine the future viability of the system and its introduction into the oil industry.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/