Spatially Offset Raman Spectroscopy (SORS) for sustainable Olive Oil authentication - tackling the challenges in on-site food control

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Key Words

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ABSTRACT

While olive oil production faces many challenges and prices are strongly increasing the demand for non-invasive analysing methods is high. The spatially offset Raman spectroscopy (SORS) can be a potential method for a sustainable food analysis since it can penetrate different kinds of containers while giving a good spectrum of the food of interest. In this study, we developed a SORS-based method for the authentication of olive oils. Based on a dataset of verified oils of four sample groups we developed an analysis strategy using plotting, principal component analysis as well as a classification and regression model. This analysis strategy was tested in food inspections of different companies. The results show, that our strategy was successful for on-site analysis and earned positive feedback from the involved parties. In an additional validation step, we analysed 30 online retail samples where we were able to differentiate between actual adulterated and authentic olive oil samples.

INTRODUCTION

The protection of consumers is the most important priority for all EU institutions involved in food safety, from producing companies to the regulatory authorities.[1] Like other processes, the steps involved are subject to constant optimization. The key concepts here are speed, costs, and, increasingly, the aspect of sustainability. For this reason, research into rapid methods for new analytical procedures and on-site analyses has been underway for a long time. Spectroscopic methods such as infrared (IR) or Raman spectroscopy are widely used.[2–5] These have the advantage that they deliver results quickly and can be used for a variety of issues, particularly in conjunction with algorithm-guided evaluation methods. Typical examples are the quantitation of nutritional parameters like the fat/protein content in meat products or the analysis of authenticity for example of edible oils.[6–9] The risk of possible fraud is particularly relevant for the high-quality and expensive product of olive oil, as lists for vulnerable food groups regularly show.[10–12] In 2015 adulterated samples of olive oil were discovered during routine analyses. These products were declared as olive oil but consisted of dyed oils from other plants, such as soy or sunflower. Over many years, a criminal organization had purchased several million liters of cheap vegetable oil, then dyed and flavoured it and sold it to restaurateurs as high-quality olive oil. Suspicious samples could still be found up until 2018.[13] This case impressively demonstrates the high potential for counterfeiting high-priced oils, which is already well-known in the case of olive oil. In 2023 the olive oil prices increased by nearly 50% due to lower production rates and emptiness of storages.[14,15] For these reasons, many spectroscopic methods have already been described in the literature to detect many conceivable adulterations of olive oil. Examples of this are the addition of other edible oils[16,17], the addition of inferior olive oils, or the claiming of an inferior olive oil as extra virgin olive oil.[18,19] However, these methods are invasive or require the packaging to be opened, meaning that a pre-packaged product is no longer available for sale. A non-invasive analysis method would have the advantage of further

improving risk-oriented sampling. Until now, the standard procedure has been randomly taking food samples and then analysing them in the laboratory. With on-site screening, conspicuous samples could be detected in place and, in the best-case scenario, no flawless samples would be taken. This would have the potential to reduce losses of safe food and would contribute to a more sustainable, faster, and also more comprehensive food analysis. Spatially offset Raman spectroscopy (SORS) is a fairly new development. With classic Raman spectroscopy, the application is limited to unpackaged goods, as the measurement must be carried out directly on the sample. SORS uses a spatially separated laser and detector so the scattering from the inside of the sample can also be detected and measurements can therefore be made through a wide variety of packaging.[20,21] However, the measurement principle results in reduced sensitivity.[22]

In this study, we present a methodology for determining the authenticity of olive oils by measuring through the original packaging. We also describe the challenges and solutions for a transfer to on-site controls. For this purpose, the described methodology was applied and verified in on-side measurements at eight different food businesses in Schleswig-Holstein. We were able to show that the SORS is very well suited for the identification of adulteration of olive oils and how a broad application in on-site control could be possible.

MATERIAL AND METHODS

2.1 Samples

For model building, we used in total 81 samples of linseed oil (12), rapeseed oil (25), sunflower oil (21), and olive oil (23). All samples were sold through the retail sector. No further validated metadata (e.g. organic, cold-pressed, etc.) was available. For mixture analysis, 120 mixtures from randomly selected samples were prepared. Every mixture sample contains an individual sample combination. Mixture ratios consist of four olive oil/sunflower oil ratios of 3/7 (30), 5/5 (30), 7/3 (30) and 9/1 (30). For validation, 30 additional samples of olive oil were procured

from online retailers. Of these samples, three were declared as olive pomace oils and the rest as extra virgin olive oils (EVOO).

2.2 Data Acquisition

All Raman spectra were measured with a VAYA Raman Handheld System (Agilent, Santa Clara, USA) equipped with a Near Infrared Laser operating at 830 nm. All spectra were measured with a laser intensity of 100% (475 mW), 1651 Data points and an integrated scaling to the highest signal intensity. Before sample measurement, the measuring method had to be prepared using the method development mode. The creation of a method is done in three steps. First, a typical sample is placed in a standardized glass vial and measured with the designated sample attachment without packaging. Then a typical package without a sample is measured. Finally, an originally packed sample is measured through the packaging. We used for this purpose an olive oil in a green glass bottle. In the following training mode, these three steps were repeated with a higher number of measurements. The following sample measurements were made in *batch mode* of the spectrometer. Before each batch measurement, a sample of polystyrene is measured to validate the spectrometer's functioning. Afterwards, the samples were measured. Each sample was measured in a triple measurement.

2.3 Data processing of Raman spectra

The spectrum of the packaging obtained is subtracted directly from the total spectrum by the device software and stored as a processed spectrum in addition to the raw data with an automatic baseline correction. Only the spectra corrected in this way were used for the multivariate data analysis. The comparison of the triple measurements showed very high conformity, so only single determinations were used for the following analyses. Matlab 2021b (Mathworks) was used for the following evaluation and further processing. The spectra were imported and the last data point was removed. The remaining data points were combined into bins of 10 data

points each. All signals are automatically normalised by the spectrometer to the highest signal with an intensity of 1.

2.4 Multivariate analysis of Raman spectra

First, the reference data set consisting of linseed oil (12), rapeseed oil (25), sunflower oil (21), and olive oil (23) samples as well as mixture samples (ratios 3/7, 5/5, and 7/3) were plotted using a principal component analysis.

In the next step, the data was divided into a trainings set (65 samples) and a test set (16 samples, four of each class). The classification model was built based on the trainings set using a linear support vector machine (Matlab classification learner app) and fivefold cross-validation. Afterwards, the model was validated using the test set. The confusion matrix of the trainings set is shown in Figure S1.

For regression analysis, the spectra of the pure sunflower and olive oil samples were used as well as the samples of the 120 mixture samples. The data was split again into a trainings set (134) and a test set (31) consisting of a constant amount of all sample groups. For regression analysis, a model was created using the regression learner app (Matlab) by applying a bagged tree ensemble learner (RMSE=1.948, fivefold cross-validation). The response plots are shown in S2. Afterward, the response of the test set was predicted.

RESULTS AND DISCUSSION

A total of 81 samples consisting of the four types linseed oil (12), rapeseed oil (25), sunflower oil (21), and olive oil (23) were measured to develop a multivariate evaluation model. The measurements on this reference data set were carried out in glass vials. A PCA was first carried out on these samples (Figure 1). It can be seen that the main component 1 is responsible for the

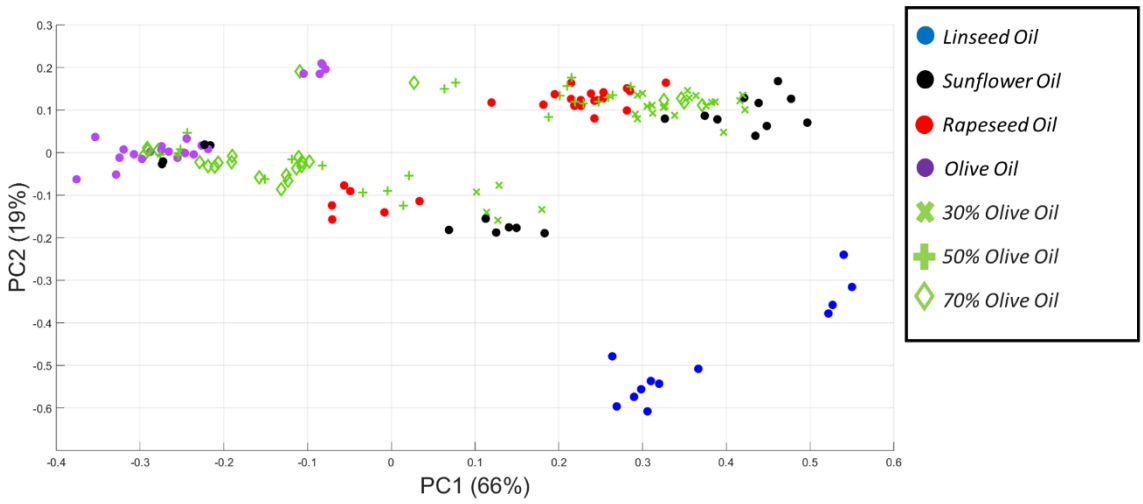

Figure 1: PCA plot of reference dataset and mixture samples. Four of the sunflower oil samples cluster close to the olive oil samples, probably due to a similar triglyceride composition. Mixtures are made of different ratios of sunflower and olive oil.

separation of the sample groups. However, principal component 2 also shows a dichotomy in the data. This bipartition of the data is based on intensity differences in the signal of 1260-1270 cm^{-1} and is a vibration of a double bond. In addition, it can be seen that four samples of sunflower oil cluster in the olive oil group. In the next step, a classification model was built. For this, 65 samples were randomly selected and a classification model was prepared using a linear support vector machine. The overall accuracy was determined using five-fold cross-validation and amounted to 91%. Four sunflower oil samples were incorrectly classified as olive oils. These are the oils that also cluster very closely to the olive oil samples in the PCA, probably due to a high amount of oleic acid triglycerides. The test set was subsequently assigned. A classification accuracy of 100% was achieved for all sample groups.

Then the 120 mixtures prepared were assigned using the classification model. The corresponding values are shown in Table S3. It was found that, as expected, the recognition rate of the prepared mixtures decreases as the degree of admixture decreases. Of the mixtures prepared in a ratio of 3/7, 100% were classified as other oil, of the 5/5 mixtures 80%, of the 7/3 mixtures 66%, and of the 9/1 mixtures only 23%. If we now take a closer look at the score values, we can see that the scores for many blends are close to each other. We define an uncertain classification as values less than -0.1 for olive oil and less than 0.05 away from the next lowest classification. Olive oils with these scores should therefore carefully be analysed, as an admixture of other oils is likely. Looking back at the mixtures, this definition does not change the detection rate for the 3/7 and 5/5 mixtures. For the other mixtures, however, the conspicuousness rate would increase to 80 (7/3) or 60% (9/1).

In the next step, a regression model was also developed for the analysis of olive oil adulteration. The focus here lies on sunflower oils, as rapeseed oils and linseed oils have very intense and characteristic aromas, so the addition of these oils to olive oil seems rather unlikely. Together

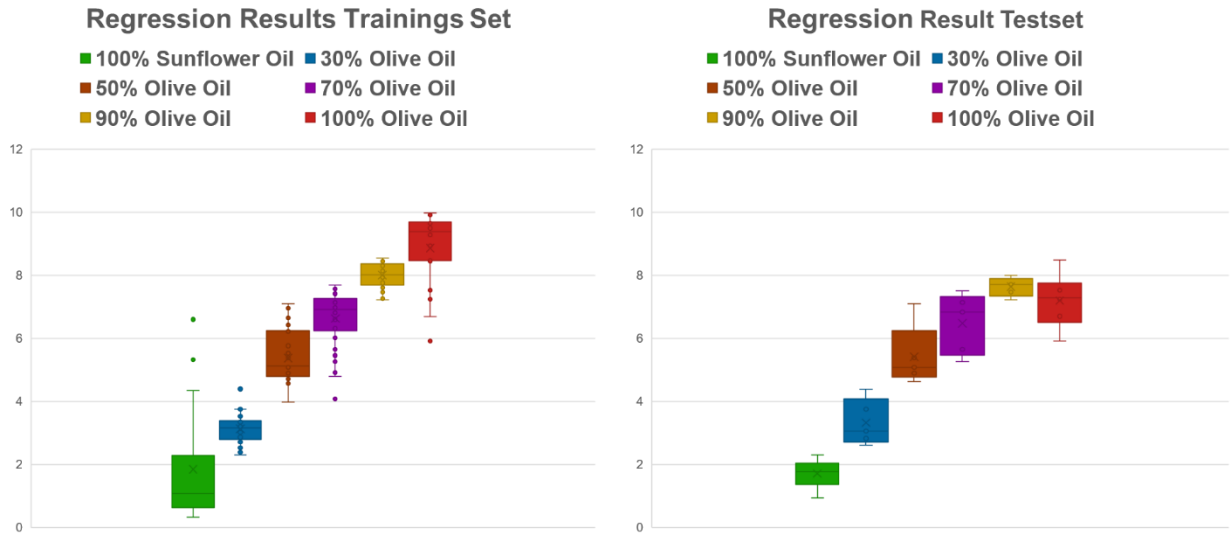

Figure 2: Regression results for trains set and test set of pure olive oils, sunflower oils, and their mixtures.

with the spectra of the pure olive and sunflower oils, the sample set was divided into a training set and a test set. A regression model was developed with the training set using a linear support vector machine (SVM).

As with the PCA results, some sunflower oil samples are classified very similarly to the olive oils, with regression values of up to 6.6 (Figure 2). This is a circumstance that must be taken into account in the final assessment of unknown samples. However, the test set values correspond very well to the trainings set results indicating a good suitability for mixture detection. The regression model already shows the substantial biological variance of the samples. Together with the classification results, however, a clearer overall picture emerges for assessing the measurement result.

For a final evaluation, the automated evaluation and the creation of a PDF report are carried out using a Matlab script (S7). The sample can then be evaluated based on the available information. The evaluation workflow is shown as an example in Figure 3. First, the spectrum is plotted

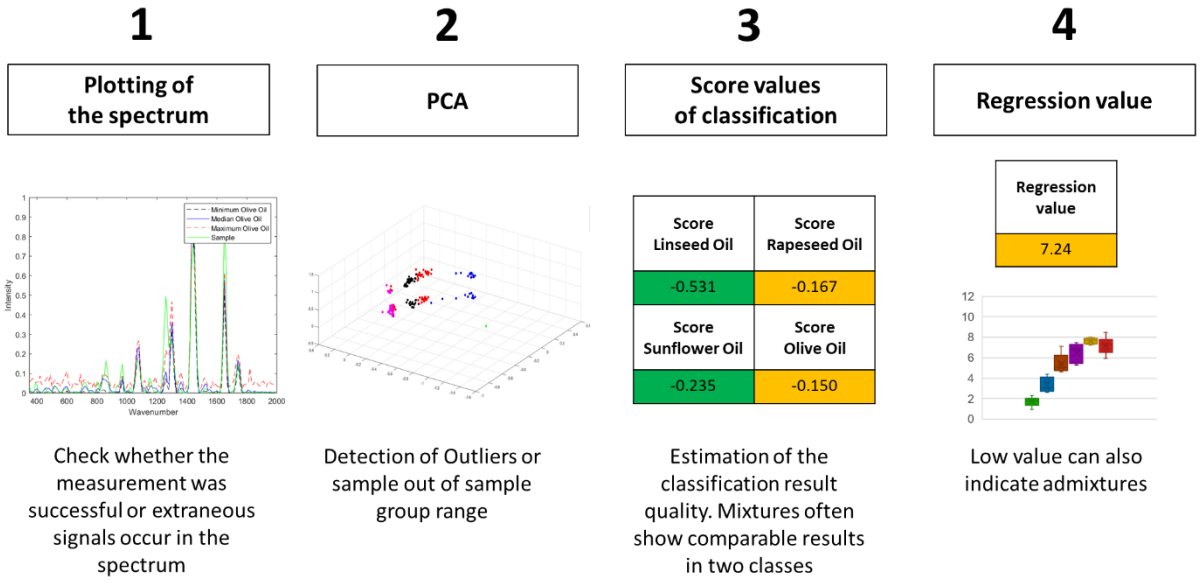

Figure 3: Workflow for analysis of olive oil samples. Firstly, each sample is plotted to check the quality of measurements or new signals appearing in the spectrum. Additionally, a PCA is done to detect outliers and a first check of the plausibility of the labeling of the sample. In the next steps, the classification scores and the regression result are analyzed to make a final decision for sample evaluation.

against the existing spectra. This is done separately for each of the four sample groups, whereby the measured sample, a minimum, the median, and a maximum spectrum are plotted. On the one hand, this serves to recognize when a measurement cannot be evaluated. Due to the large number of different types of packaging (composition, shape, etc.), it is possible that the laser does not penetrate the packaging sufficiently. This would result in a poor signal-to-noise ratio.

In addition, this display also shows when additional signals occur or signals are clearly outside the displayed range. This can be the case, for example, if oils other than the four sample groups tested are measured.

In the second step, a PCA is performed to detect outliers and to see whether the analyzed sample is outside of the stored sample groups. The sample is then classified and the score values for the respective sample groups are displayed. As a final point of reference, the value from the regression model is also given. In practice, it is often the case that all four points mentioned must be carefully considered to be able to recognize non-conforming samples. Due to the manual analysis caused by the large number of different types of outer packaging, an excessively high error rate is otherwise to be expected with this analysis.

Test of the method in on-site measurement and practical experience

For the on-site measurement test, a total of 8 different companies were visited and edible oil samples were measured. The primary aim here was to check the processes of the developed measurement routine and to identify possible disturbing factors that do not show up in laboratory operations. The companies involved were four restaurants, a wholesaler of olive oils, two food retailers, and a food production company that processes edible oils on a large scale. The greatest influence in the on-site analysis, as in the laboratory, was ambient light. Ambient light, such as sunlight or light from ceiling lights, often interferes with the analysis as soon as it falls on the detector. As a result, the device does not measure because the detector recognizes the light. One possibility is to shield the sample with an opaque outer packaging. A second possibility is to switch off the light source; in many of the companies investigated, an appropriate zone was found where this was possible and the measurements could be carried out. This sensitivity of the measurement is particularly evident with transparent packaging. The measurement conditions at one of the retailers were an exception to this. Here, LED lamps were used as lighting. These are characterized by a very narrow and specific radiation spectrum, which in

this case also enabled the measurement of olive oils in transparent glass packaging without further shielding contrary to our laboratory environment.

Many different types of packaging were measured on the site, which showed that almost all packaging can be penetrated to obtain a SORS spectrum that can be used for analysis. The packaging consisted of transparent and colored glass bottles, various transparent plastic packaging, as well as colored, opaque large containers, which consisted of approximately 1 mm thick, white-colored plastic packaging, which could also be penetrated. These investigations demonstrated the fundamental robustness of the methodology and evaluation for the outer packaging available in practice. However, there were exceptions here too. In some cases, we found very small and round containers with a nominal volume of less than 200 mL. These could not be measured with the spectrometer used, as the measuring unit has a flat surface and the curves of these containers meant that the detector and laser could not be placed on the container in such a way only the sample inside could be measured. Furthermore, some colored glass containers showed a very strong absorption of the laser light, so only very low-intensity spectra with a low signal-to-noise ratio were obtained. In such cases, multiple measurements and the addition of the spectra obtained could be considered in the future. No anomalies were found in the evaluations obtained during these investigations, as shown by the PCA of the samples from one business (Figure S4). The feedback from all the companies visited on this investigation was very positive.

Most of the measurements within the companies were carried out in separate rooms without public traffic. Examples are offices or storage rooms. But here too, as would have been expected in the sales rooms, there was always interference from operating personnel. This is not as relevant for the measurements as it is for the safety aspect. The laser used is a class 3B laser and therefore potentially dangerous. At this point, we therefore expressly recommend positioning the laser opposite doors or other openings during measurements and being prepared to cover

the laser at all times. From this practical experience, we were able to determine the time required for a measurement series of 20 samples, including starting the device, measuring, exporting the samples, and evaluating and documenting them, at around one hour. However, the practical measurements also showed that often only a significantly smaller number of samples were available.

Extended validation with samples from online trading

To test the effectiveness of the method, real samples with a high risk of adulteration should be examined in order to finally assess the suitability for the analysis of these samples. For this purpose, 30 samples of olive oil were purchased from online retailers. Of these samples, three were declared as olive pomace oils and the rest as extra virgin olive oils (EVOO). The samples were measured both in the packaging and in the vial and the results of the classifications were then compared. However, this was only possible for eight of the samples, as the rest were filled

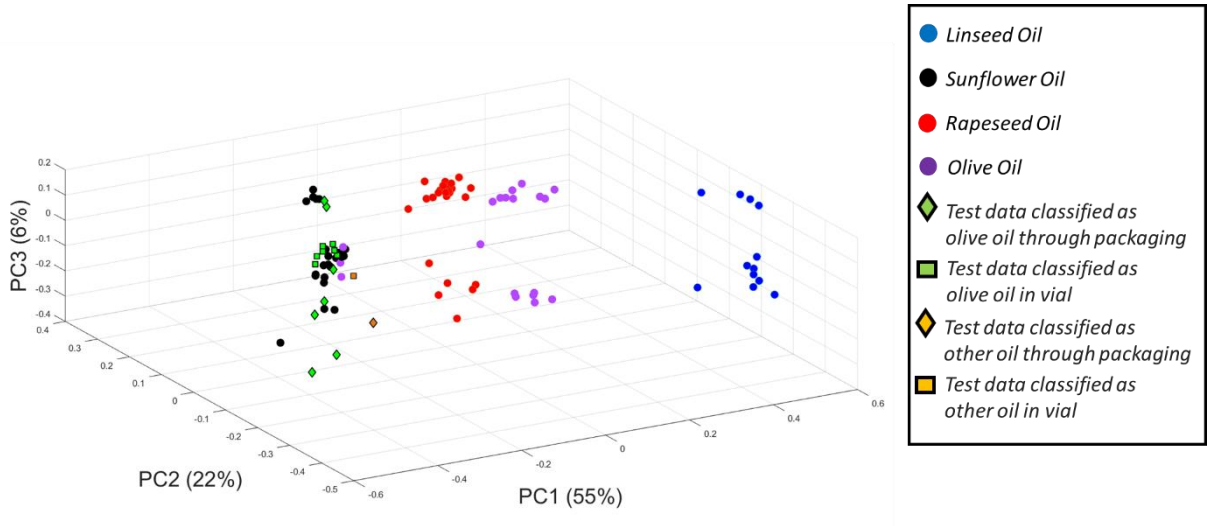

Figure 4: PCA of the reference dataset with eight samples of olive oil measured through the original packaging as well as the respective vial measurements. Visible is the higher variance of samples measured through the original container, than in vial measurements. One sample (two measurements) clusters in the direction of the rapeseed samples although near olive oils. This sample is likely adulterated.

in outer packaging that did not allow measurement through them (metal cans). The comparison of the measurements and their results, including the scores and regression results, is shown in S5 and S6. The PCA (Figure 4) shows a significantly higher scattering of the samples measured through the packaging compared to measurements of the same samples in the vial. Looking at

the classification and regression results of the samples, it is noticeable that one of these samples was classified as a different oil. Depending on the measurement, the sample was classified as sunflower oil (through packing) or rapeseed oil (in vial). This is in contrast to the declaration as olive oil. The comparison of the score values of all samples measured twice in this way shows that the samples received comparable values for both measurement conditions.

			Score Values			
		Classification result	Linseed Oil	Rapeseed Oil	Sunflower Oil	Olive Oil
Measure-ment through packaging	Sample 1	Sunflower Oil	-0.431	-0.278	-0.134	-0.157
	Sample 2	Olive Oil	-0.475	-0.195	-0.319	-0.035
	Sample 3	Olive Oil	-0.488	-0.265	-0.219	-0.086
	Sample 4	Olive Oil	-0.507	-0.150	-0.307	-0.084
	Sample 5	Olive Oil	-0.543	-0.125	-0.323	-0.073
	Sample 6	Olive Oil	-0.551	-0.164	-0.265	-0.076
	Sample 7	Olive Oil	-0.552	-0.167	-0.308	-0.028
	Sample 8	Olive Oil	-0.478	-0.212	-0.261	-0.070
Measure-ment in vial	Sample 1	Rapeseed Oil	-0.475	-0.126	-0.215	-0.192
	Sample 2	Olive Oil	-0.536	-0.143	-0.275	-0.096
	Sample 3	Olive Oil	-0.552	-0.146	-0.298	-0.061
	Sample 4	Olive Oil	-0.546	-0.186	-0.263	-0.055
	Sample 5	Olive Oil	-0.510	-0.167	-0.238	-0.113
	Sample 6	Olive Oil	-0.526	-0.196	-0.232	-0.083
	Sample 7	Olive Oil	-0.542	-0.182	-0.266	-0.056
	Sample 8	Olive Oil	-0.525	-0.122	-0.331	-0.079

A comparison of all samples (measured in the vial) shows that one of the three pomace samples is not classified as olive oil. In addition to the not-olive oil classified EVOO sample, another sample also shows a very small difference between the classification results. Although it was classified as olive oil, the score for classification as rapeseed oil is similar to the result for olive oil. However, for the three mentioned samples only the EVOO samples classified as other oil shows also a high variance in PCA as well as low regression values. For verification purposes, the fatty acid ratios of a total of 15 samples were determined. These were carried out using both GC-MS and NMR spectra analysis. Of these 15 samples, the sample classified as other oil, the

sample with the same small differences in the score values, two of the pomace oils (including the suspicious sample), and 11 inconspicuous samples were measured. The results show that all samples have an unremarkable fatty acid profile, with the exception of the sample classified as other (sunflower/rapeseed) oil. It shows a content of 1.16% (NMR) or 1.12% (GC) linolenic acid, indicating the addition of other oils, which confirms the results of the Raman spectroscopy.[23] Of the other 14 samples, none showed a conspicuous fatty acid profile. But the case of the two other conspicuous samples show clearly that our described four-parameter evaluation scheme should be used when analyzing a sample to omit false positive results.

CONCLUSION

The results of the on-site measurements and the analysis of samples obtained from online retailers clearly demonstrate the potential of the method presented for a more sustainable analysis of olive oils. In practice, it was shown that a large number of packages could be penetrated and that measurements outside the laboratory environment are also possible. It was also shown that all four evaluation parameters mentioned should be used for a sample evaluation, as this enables plausibility checks of the results and thus takes into account the variability of the packaging and the edible oils tested. The final validation of the measurements with real commercial samples showed the suitability of this method for detecting olive oil adulteration very well with the correct detection of one adulterated sample and the equally correct detection of 14 unremarkable samples. This described method thus enables the non-invasive analysis of olive oils for the addition of foreign oils together with a documentation and evaluation workflow. This could be an effective and sustainable building block for the prevention of food fraud, particularly in the case of high-priced olive oils or import controls.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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