APPLICATION NOTE



FT-NIR Spectroscopy

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Detection of Avocado Oil Adulteration Using FT-NIR Spectroscopy

Introduction

The production of avocados and related by-products has been continuously increasing over the recent years due to its rising popularity in both food and medicinal markets.¹ The high

content of healthy oleic acid in the fruit has been linked to improving heart health and reducing cholesterol.² Therefore, avocado, and its cold-pressed oil, has become a more prominent ingredient in cuisines worldwide.

Avocado oil, a high-value product, may be adulterated with lower-value oils in order to increase profit margins. These adulterant oils may include nut-containing oils which, if incorrectly advertised on the avocado oil packaging, may induce a dangerous allergic reaction for consumers with nut allergies. It is, therefore, important that an accurate and reliable adulteration detection method is available for manufacturers to routinely test their avocado oil.

Several analytical methods have been developed to detect adulteration in avocado oil. Many of these rely on chromatographic techniques which can require time-consuming sample preparation and may produce hazardous chemical waste.³ Near-Infrared spectroscopy, on the other hand, with Adulterant Screen[™] technology, can provide rapid detection of adulteration in avocado oil without the need for solvents.



Existing targeted approaches for adulterant screening, using Near-infrared spectroscopy, require a quantitative calibration to be developed for each potential adulterant. Alternatively, non-targeted screening approaches such as a SIMCA (Soft Independent Modelling of Class Analogy) algorithm can determine whether a sample has been adulterated but will neither identify nor quantify the adulterant. PerkinElmer's Adulterant Screen, on the other hand, provides a semi-targeted method which allows quick identification and estimation of adulteration levels.

Experimental

NIR spectra of pure avocado oil and four possible adulterant oils were collected using a PerkinElmer Spectrum Two N[™] FT-NIR spectrometer with the PerkinElmer Heatable Transmission Module (HTM), temperature controlled at 25 °C.



 $\it Figure~1.$ PerkinElmer Spectrum Two N spectrometer with PerkinElmer Heatable Transmission Module.

The samples were placed in 8 mm glass vials, allowed to reach thermal equilibrium within the heated transmission module and scanned using the parameters shown in Table 1.

Table 1. Scanning parameters for analysis of avocado oil and adulterant oils.

Scanning Parameters			
Spectral Range	10,000 – 4,000 cm ⁻¹		
Resolution	8 cm ⁻¹		
Number of Scans	32		

15 spectra of pure avocado oil (five replicates from three different commercially available brands) and one spectrum of each adulterant (groundnut oil, olive oil, rapeseed oil and sunflower oil) were collected for the Adulterant Screen method. The spectra were pre-processed adjusting the spectral range to 10,000-4,500 cm⁻¹, blanking regions with absorbance above 1.5 and using a first derivative baseline correction, as seen in Figure 2.

In addition, 16 pure avocado oil samples were spiked with each adulterant over a range of concentrations from 2-95 % (w/w). Spectra of each adulterated sample, 100 % avocado oil and 100 % adulterant were used to create quantitative Partial Least Squares (PLS1) models of each of the adulterant oils using PerkinElmer Spectrum Quant[™]. 15 samples were used for calibration and 3 (25 %, 55 % and 85 %) were used for independent validation of the model. Cross validation was also carried out for each of the models, using the Leave-1-Out method. In each model, the spectra were pre-processed using the parameters shown in Table 2.

Table 2. Pre-processing parameters for avocado oil adulteration models.

Adulterant Oil		Baseline Correction		
	Normalization	Derivative Order	Noise Reduction	
Groundnut	MSC	Second	Heavy	
Olive	MSC	Second	Medium	
Rapeseed	MSC	First	Light	
Sunflower	MSC	First	Light	



Figure 2. First derivative spectra of pure avocado oil (green) and adulterant oils (groundnut (black), olive (red), rapeseed (blue) and sunflower (pink)).

Adulterant Screen

An Adulterant Screen method was created for identifying and semi-quantitatively estimating the level of adulteration in avocado oil. Adulterant Screen is beneficial as it does not require the lengthy preparation and measurement of calibration standards. It simply requires a library of spectra of the pure material in order to represent the sources of natural variation that may occur in the samples. A pure adulterant library is also required, containing just one spectra of each possible adulterant.

All 15 spectra of pure avocado oil were inputted as 'material spectra' and the spectra of each of the 4 pure adulterants were entered as 'adulterant spectra'. The method was then tested using avocado oil samples spiked with known levels of each adulterant. The results are shown in Table 3.

Adulterant Oil	Level (%)	Detection Limit (%)	Adulterant Screen Pass/ Fail
Groundnut (20 %)	27.35	1.6.4	Fail
Groundnut (15 %)	22.82	1.04	Fail
Olive (20 %)	17.48	2 1 2	Fail
Olive (15 %)	12.50	2.12	Fail
Rapeseed (20%)	17.34	0.48	Fail
Rapeseed (15%)	12.18	0.40	Fail
Sunflower (20%)	18.89	0.27	Fail
Sunflower (15 %)	13.23	0.27	Fail
Avocado (100 %)	-	-	Pass

Table 3. Adulterant Screen results for a series of adulterated avocado oil samples.

The Adulterant Screen algorithm produced a "Fail" result in all cases, except pure avocado oil, signifying the presence of an adulterant. The method also correctly identified the adulterant oil in each case and gave an estimate of the level present in each sample. The detection limit provided by Adulterant Screen is relatively low for each of the adulterant oils. Although these detection limits are higher than those provided by more expensive analytical methods, economically motivated adulteration is typically performed at much higher levels in order to profit from it.

Adulterant Screen methods can also be incorporated into the Spectrum TouchTM software to provide a user-friendly and easyto-use interface for routine operators. Figure 3 illustrates the simple design of the workflow and the results provided by the software. These figures provide the estimated level and detection limits as a decimal.



Figure 3. Example of Spectrum Touch workflow and Adulterant Screen results for avocado oil adulteration.

PLS1 Calibration Models

A PLS1 calibration model was also created for each of the adulterant oils. Table 4 illustrates the regression data for all the calibration models. The R² values lie between 99.945-99.994 %, showing there is a very high level of agreement between the specified concentration of adulterant oil and the concentration predicted by the model. Table 5 shows the average independent validation results for the models. The standard error of prediction (SEP) is slightly higher for groundnut oil than the other adulterant oils. This may be due to the spectra of avocado oil being particularly similar to the spectra of groundnut oil.

Table 4. Regression summary for adulterant oil models (where SEC is standard error of calibration, SEP is standard error of prediction and CVSEP is cross validation standard error of prediction).

Adulterant Oil	Number of PCs	R² (%)	SEC (%)	SEP (%)	CVSEP (%)
Groundnut	3	99.938	1.014	1.381	2.646
Olive	3	99.945	0.955	1.196	1.695
Rapeseed	3	99.984	0.520	0.568	0.605
Sunflower	2	99.994	0.300	0.394	0.387

Table 5. Independent validation results from adulterant oil models.

Adulterant Oil	Average True Sample Property Value (%)	Average Predicted Sample Property Value (%)	SEP (%)
Groundnut	55.00	51.84	3.747
Olive	55.00	54.29	1.532
Rapeseed	55.00	54.85	0.187
Sunflower	55.00	54.69	0.673

Figure 4 shows an example correlation plot for the sunflower oil model, including the calibration and independent validation data points. The data points are distributed evenly about the unity line, further indicating that there is a very high level of correlation between the specified and predicted concentration of sunflower oil.



Figure 4. Correlation plot for sunflower oil model showing calibration (blue) and validation (red) data points with the solid black line indicating the unity line.

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Conclusion

The results show that Near-infrared spectroscopy with a HTM accessory can provide accurate detection and identification of adulterant oils in avocado oil. The PLS1 calibration models all gave accurate predictions of the level of adulteration present. However, this method requires time-consuming calibration standards to be prepared and measured. On the other hand, the Adulterant Screen algorithm rapidly identified the adulterant present and provided a relatively accurate estimate of the adulterant concentration. Should a sample fail the Adulterant Screen, it could then be subjected to further testing and if a new adulterant would need to be added to the pure adulterant library. Adulterant Screen is, therefore, a more suitable method to use for routine checks for avocado oil adulteration.

References

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