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Quantitative analysis of potential adulterants of extra virgin olive oil using infrared spectroscopy

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The determination of food authenticity and the detection of adulteration are problems of increasing importance in the food industry. This is especially so for 'value-added' products, where the potential financial rewards for substitution with a cheaper ingredient are high. In this paper, the potential of infrared spectroscopy as a rapid analytical technique for the quantitative determination of adulterants in extra virgin olive oil is demonstrated. The method uses Fourier transform infrared spectroscopy, combined with attenuated total reflectance and partial least squares regression. Model systems comprising two types of 'contaminant' oil — refined olive and walnut — are investigated.

INTRODUCTION

The determination of food authenticity and the detection of adulteration are problems of increasing importance in the food industry. This is especially so for 'value-added' products, where the potential financial rewards for substitution with a cheaper ingredient are high. Olive oil, extracted from the fruit of the olive tree, *Olea europea* L., is an economically important product. The International Olive Oil Council (IOOC) has established criteria for the categorisation of olive oil into various grades (IOOC, 1984). The best quality oil is called 'extra virgin', and is derived from the first, cold pressing of the olive. This oil must be of perfect flavour and odour, with a maximum acidity, in terms of oleic acid of 1 g/100 g. Other grades include 'virgin' and 'refined' olive oils. Blends of virgin and refined oil are also found, the characteristics of which are determined by mutual agreement between buyers and sellers.

Relationships between the various grades of olive oil and certain sensory and chemical criteria have been established (Kiritsakis & Markakis, 1987). Extra virgin commands a relatively high price compared with other grades of olive oil, such that adulteration of the high value product with other, cheaper olive grades or other seed oils, can be an attractive proposition. Adulteration has been known to exist for a long time (Poutet, 1819), and various physical and chemical tests have been devised to address the problem (Gracian, 1968). Ultraviolet spectroscopy is widely used to detect the adulteration of extra virgin with refined olive oil (Passaloglou-Emmanouilidou, 1990). Other analytical procedures including gas chromatography (Kapoulas & Passaloglou-Emmanouilidou, 1981; Morchio *et al.*, 1989; Mariani *et al.*, 1991), and high-performance liquid chromatography (HPLC) (Casadei, 1987; Serani & Staiano, 1989; Sanchis Rodriguez & Rodriguez Serrano, 1991; Salivaras & McCurdy, 1992) have more recently been developed. However, all these methods are time-consuming and require skilled operators. More recently, Fourier transform infrared (FTIR) spectroscopy has been shown to have potential for discriminating between extra virgin and other olive oils (Lai, 1993; Lai *et al.*, 1994). Although previous attempts had been made to use infrared spectroscopy for the determination of oil authenticity (Gracian, 1968), the chemical variation between extra virgin and other olive oils is such that very high spectral quality is required, imposing rigorous experimental demands, in order to successfully apply discriminant analysis. However, Lai *et al.*, were able to develop a successful discriminant model and provide a method suitable for use by an untrained user, with an analysis time of approximately 2–3 min. The object of this paper is to demonstrate the potential of infrared spectroscopy for the quantitative determination of the levels of adulterant in extra virgin olive oil. The method uses FTIR spectroscopy, combined with attenuated total reflectance (ATR) (Wilson, 1990) and partial least squares (PLS) multivariate analysis (Martens & Naes, 1989). Two 'adulterant' oils were used at various levels of addition. The oils chosen were refined olive and walnut, both known adulterants of extra virgin olive oils.

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MATERIALS AND METHODS

All spectra were collected on a Spectra-Tech (Applied Systems Inc) Monitir FTIR spectrometer, equipped with room temperature source and deuterated triglycine sulphate detector. The system featured a sealed and desiccated interferometer chamber and transfer optics to the ATR accessory. Potassium bromide windows built into the top of the instrument allowed the ATR crystal to be removed for cleaning without disturbing the desiccated optical path. This enabled spectra to be collected with a minimum of absorptions due to water vapour. The ATR crystal was a 10-reflection, 45° zinc selenide parallelogram with mirrored angled faces. All spectra were acquired at 4 cm^{-1} resolution, between 4800 and 800 cm^{-1}. A total of 256 interferograms were co-added before Fourier transformation and triangular apodisation was employed. All sample single-beam spectra were ratioed to a single-beam collected with a clean ATR crystal. Following the ratioing, the spectra were converted into absorbance units. Approximately 2 ml of each sample was applied directly to the crystal. Between samples, the ATR crystal was cleaned by wiping with hexane, 2% Triton X-100 solution, distilled water and acetone.

Table 1. Concentrations of olive oil in extra virgin olive oil used for PLS analysis

Sample	Concentration of added olive oil (g/100 g)
Calibration	
C1	0·00
C2	0·93
C3	1·84
C4	3·32
C5	5·05
C6	7·41
C7	8·66
C8	11·48
C9	12·75
C10	14·18
C11	16·79
C12	18·11
C13	22·02
C14	3·96
C15	5·72
C16	8·72
C17	12·66
C18	1·25
C19	1·56
C20	3·26
C21	4·46
C22	6·09
C23	20·16
C24	10·07
C25	5·80
C26	13·41
C27	15·82
C28	20·11
C29	5·59
C30	5·56
C31	5·02
C32	10·30
C33	10·81
C34	11·74
V1	1·88
V2	3·91
V3	8·26
V4	10·36
V5	12·45
V6	15·11
V7	17·22
V8	5·63
V9	5·30
V10	11·62

The samples used in this experiment (extra virgin olive, refined olive and walnut oils) were purchased from a local retailer. Initially, a series of mixtures of extra virgin and refined olive oil were analytically prepared, with the concentration of refined olive oil in the range of 0–22 g/100 g extra virgin olive oil. A total of 44 samples were produced, with 34 assigned for use as a calibration set for method development, and 10 as a validation set. The concentrations are given in Table 1. The validation samples were chosen such that their concentrations fell within the concentration range of the calibration set. The concentration range of the calibration set was chosen to model the likely range of added adulterant. A similar set was prepared by mixing walnut oil with extra virgin olive oil; a total of 37 samples were prepared, with 27 assigned to a calibration and 10 to a validation set.

PLS analysis was accomplished after transferring the data into the UNSCRAMBLER II package (Computer-Aided Modelling (CAMO) A/S, Trondheim, Norway). Orthogonalised PLSI (Martens & Naes, 1989) was applied to raw and pre-treated data sets. Pretreatment consisted of standardisation (mean centring and scaling to unit variance all spectral data points). In both cases, models were constructed using up to a maximum of 16 PLS factors. The optimum number of factors was selected using the process of internal cross-validation (a detailed description of this and other terms can be found in Martens and Naes (1989). The variance of the concentration residuals versus the number of factors used in the model was plotted, and from this the optimum number of factors was selected. The best model was used to determine the concentration of the samples in the independent validation set. The same procedure was applied to the system comprising extra virgin adulterated with walnut oil.

RESULTS AND DISCUSSION

The variance of the concentration residuals versus the number of factors used in the PLS model for refined olive in extra virgin olive oil was plotted for the raw and pre-treated data analyses. A typical plot is shown in Fig. 1. In this example it can be seen that the variance reaches a stable minimum after eight factors. A plot of predicted versus actual concentrations for the calibration set using this number of factors is shown in Fig. 2. A summary of the results obtained from the different calibration models is given in Table 2. The best model is

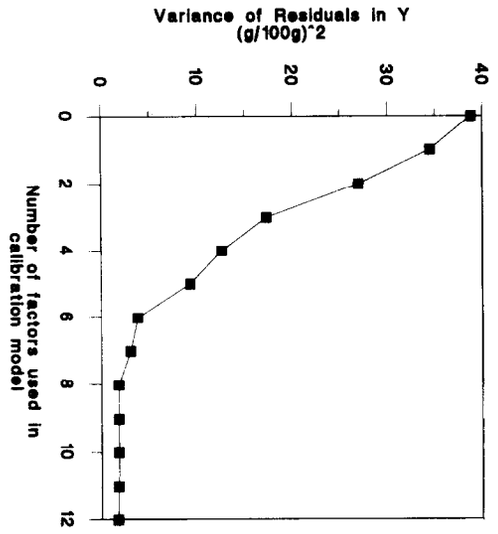

Fig. 1. Plot of concentration residuals variance versus number of PLS factors used in calibration model (pre-treated calibration data).

developed from pre-treated data, yielding the lowest standard error of prediction (SEP) with eight factors. Therefore, the model using pre-treated data and eight factors was used to predict the concentration of refined olive oil in the independent validation set. The results are shown in Fig. 3. The SEP obtained was 0·92 g/100 g.

Table 2. Summary of the effect of data pre-treatment on PLS calibration models for olive in extra virgin oil

Model details	Data	
	Pre-treated	Raw data
Model centre	Mean	Origin
Variance scaling	1/standard deviation	1
Optimum number of factors	8	6
Standard error of prediction (g/100 g)	1.29	2.80
Correlation coefficient (predicted versus actual)	0.98	0.89

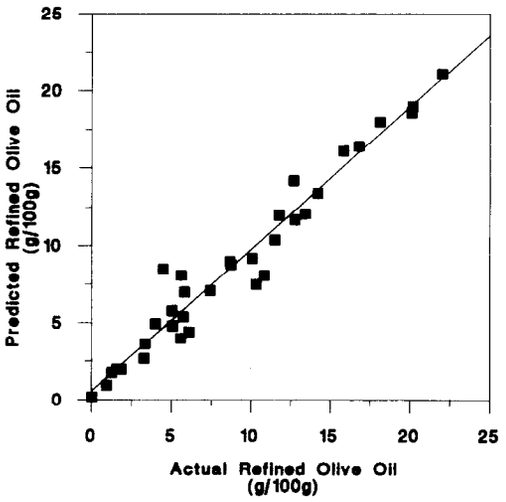

Fig. 2. Plot showing predicted versus actual refined olive oil contents, by internal cross-validation on the pre-treated calibration data (eight PLS factors).

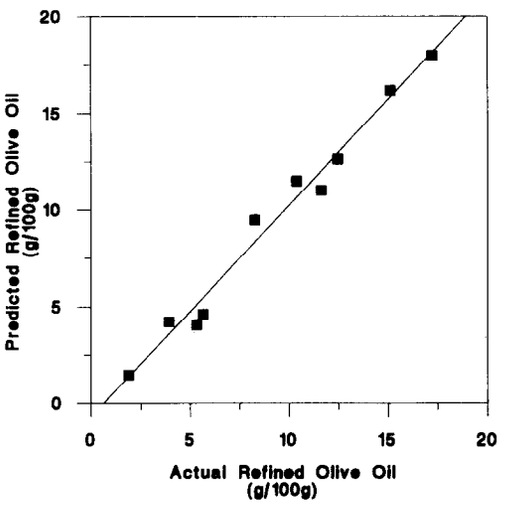

Fig. 3. Predicted versus actual refined olive oil for the independent validation set (pre-treated data, eight PLS factors).

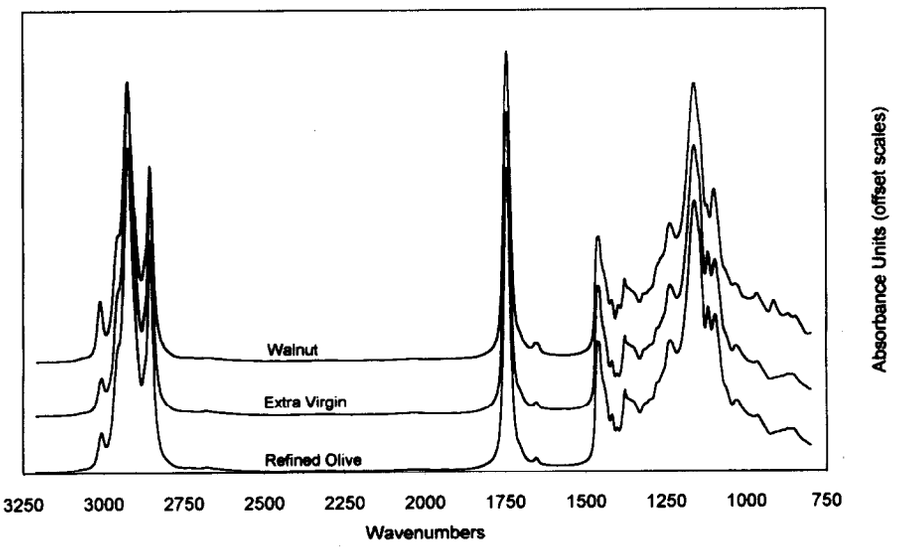

Fig 4. Example spectra of extra virgin olive, refined olive and walnut oils.

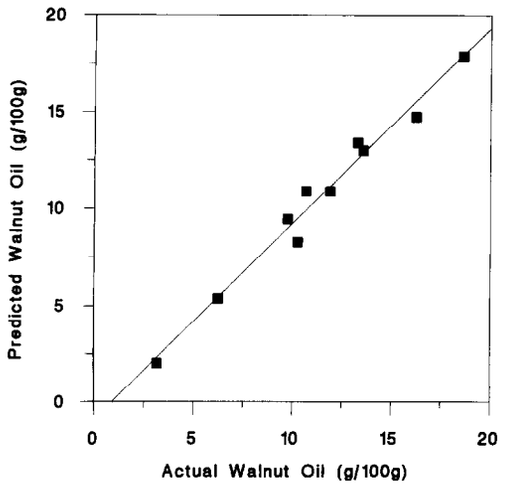

Fig. 5. Predicted versus actual walnut oil for the independent validation set (pre-treated data, three PLS factors).

The extra virgin and walnut oil mixtures were subjected to the same calibration procedure. Pre-treated data was again found to give the best results, but the optimum number of factors was only three. This is not surprising, since the spectra of refined and extra virgin olive oils were almost superimposable to the eye, whereas the walnut spectrum exhibited clearly observable differences (Fig. 4). Any spectral differences between the olive oils will largely arise from the presence of low levels of minor components (e.g. free acids, protein, etc.), but the fatty acid composition will be much the same. However, walnut oil has, in addition, a different overall fatty acid composition which is reflected in the spectra. The greater spectral differences between walnut and extra virgin olive oil are reflected by the fewer number of factors required for calibration in this system. The results for the analysis of the validation set are shown in Fig. 5, where the SEP was 0·68 g/100 g.

CONCLUSIONS

In previous work, infrared spectroscopy has been shown to be able to discriminate between different vegetable oils, and types of olive oil in particular. It has now been shown that it is also possible to determine, quantitatively, the level of a typical adulterant in extra virgin olive oil. Although the lowest SEP (0·68 g/100 g) was observed when walnut oil was added to extra virgin olive oil, the technique clearly also has the potential to determine the addition of low levels of refined olive oil. This does not imply that the calibration produced here is applicable to the determination of added olive oil to any extra virgin oil sample, but the fact that it can be accomplished at all and with such sensitivity enhances the potential of FTIR for the detection of adulteration of vegetable oils.

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REFERENCES

Casadei, E. (1987). Analysis of triglycerides to detect adulteration of olive oil by hazelnut oil or esterified oils. Preliminary research. *Riv. Ital. Sostanze Grasse*, **64**(9), 373–6.

Gracian, J. (1968). The chemistry and analysis of olive oil. In *Analysis and Characterization of Oils, Fats and Fat Products* (Vol. 2), ed. H. A. Boekenoogen. Wiley Interscience Publishers, New York, USA, pp. 315–606.

IOOC (1984). *International Trade Standards Applying to Olive Oils and Olive-Residue Oils* (COI/T.15/NC NO 1). International Olive Oil Council. Madrid, Spain.

Kapoulas, V. M. & Passaloglou-Emmanouilidou, S. (1981). Detection of adulteration of olive oil with seed oils by a combination of column and gas chromatography. *J. Am. Oil Chem. Soc.*, **58**, 694.

Kiritsakis, A. & Markakis, P. (1987). Olive oil: A review. *Adv. Food Res.*, **31**, 453–82.

Lai, Y. W. (1993). Application of FTIR spectroscopy and multivariate analysis in the authentication of extra virgin olive oil. MSc. thesis, School of Chemical Sciences, University of East Anglia, Norwich, UK.

Lai, Y. W., Kemsley, E. K. & Wilson, R. H. (1994). Potential of FTIR spectroscopy for the authentication of vegetable oils. *J. Agric. Food Chem.*, **42**, 1154–9.

Mariani, C., Venturini, S. & Fedeli, E. (1991). Detection of low sterol seed oils in olive oil, Note 2. *Riv. Ital. Sostanze Grasse*, **68**(6), 283–6.

Martens, H. & Naes, T. (1989). *Multivariate Calibration*. John Wiley and Sons Ltd., New York, USA.

Morchio, G., DiBello, A., Mariani, C. & Fedeli, E. (1989). Detection of refined oils in virgin olive oil. *Riv. Ital. Sostanze Grasse*, **66**(5), 251–7.

Passaloglou-Emmanouilidou, S. (1990). A comparative study of UV spectrophotometric methods for the detection of olive oil adulteration by refined oils. *Z. Lebensm. Unters. Forsch.*, **191**(2), 132–4.

Poutet, M. (1819). *Ann. Chim. Phys.*, **12**(2), 58.

Salivaras, E. & McCurdy, A. R. (1992). Detection of olive oil adulteration with canola oil from triacylglycerol analysis by reversed phase HPLC. *J. Am. Oil Chem. Soc.*, **69**(9), 935–8.

Sanchis Rodriguez, J. & Rodriguez Serrano, J. (1991). Rapid HPLC procedure for the detection of adulteration of olive oils by seed oils. *Alimentaria* (Madrid), **28**(224), 27–9.

Serani, A. & Staiano, G. (1989). Correlation between the triglyceride composition (determined by HPLC) and the fatty acid composition as an indicator of the genuineness of olive oil. *Riv. Ital. Sostanze Grasse*, **66**(6), 327–33.

Wilson, R. H. (1990). Fourier transform mid-infrared spectroscopy for food analysis. *Trends Anal. Chem.*, **9**(4), 127–35.