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Authentication of Virgin Olive Oils Using Principal Component Analysis of Triglyceride and Fatty Acid Profiles: Part 2—Detection of Adulteration with Other Vegetable Oils

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ABSTRACT

Principal Component Analysis of triglyceride and fatty acid profiles has been reported as a method for the characterisation of virgin olive oils, and this approach is now extended to study adulteration of the same oils. The effect of incorporating 10 or 20% w/w of maize, cottonseed, sunflower, soyabean or rapeseed oil in olive oil on both the triglyceride and fatty acid compositions is studied. At the 20% level of incorporation PCA was able to show clearly the presence of a foreign oil, the distinction being less marked at the 10% level. The triglyceride profiles proved to be more discriminating than those of the fatty acids.

INTRODUCTION

The adulteration of edible oils with other cheaper oils may have severe health implications as well as the more obvious commercial importance. Spain has seen some 400 deaths and 20 000 casualties since May, 1981 from the disease ever since known as the 'Spanish Toxic Syndrome' caused by the

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consumption of adulterated cooking oil (Jimeno, 1982; Kochar & Rossell, 1984). As a result of this, and other incidents, the quality criteria of edible oils are being continuously updated in an attempt to counteract the increasing sophistication of falsification that is being perpetrated. Methods for the detection of adulteration based on simple colour reactions or physical properties may not be sufficient. Furthermore, methods using the qualitative analysis of fatty acids or triglycerides may also be inadequate as most vegetable oils contain the same fatty acids (mainly with carbon number 16 or 18) and the triglycerides are again often similar (carbon numbers 50, 52 or 54). The situation is improved when quantitative data are considered for both fatty acids and triglycerides with each profile providing a characteristic 'fingerprint' of the oil. However, if such profiles are to be used to study the effects of adulteration, powerful statistical methods are required to consider differences in the whole picture (chromatogram) rather than in individual components; i.e. multivariate statistics are mandatory, as illustrated in Part 1 of this study (Tsimidou *et al.*, 1987). The triglyceride composition of an oil contains more information as to its structural features than the corresponding fatty acid composition, and in many instances will prove to be more characteristic. HPLC offers considerable advantages as an analytical technique for the characterisation of oils with respect to their triglyceride profiles (El-Hamdy & Perkins, 1981; Herslof, 1981; Plattner, 1981; Aitzetmuller, 1982 and Dong & Dicesare, 1983), even though problems still exist due to incomplete resolution, lack of relevant standards and detector selectivity. A number of approaches have been developed to overcome these limitations (Wada *et al.*, 1978; Bezard & Ouedraogo, 1980; Karleskind & Blanc, 1980; Lie Ken Jie, 1980; Goiffon *et al.*, 1981; Plattner, 1981; Dong & Dicesare, 1983; Takahashi *et al.*, 1984 and Myher *et al.*, 1984) but, in general, these increase the complexity of the method and may, in fact, not be necessary for the detection of adulteration (Galanos & Kapoulas, 1965). In the present study a simple single-stage HPLC technique was used to produce triglyceride profiles of authentic and deliberately adulterated olive oil samples, and these data, together with the corresponding fatty acid compositions, were evaluated by Principal Component Analysis.

MATERIALS AND METHODS

Samples

Cottonseed oil (alkali washed), maize, sunflower, soyabean and rapeseed oils were kindly donated by Leatherhead Food Research Association (Leatherhead, Surrey, Great Britain) and Van der Berghs and Jurgens Ltd (Purfleet, Essex, Great Britain). Virgin olive oil (as a base for the adulteration

studies) was obtained from a Greek Co-operative which certified its authenticity. Adulterated samples were prepared by weight. Triplicate methyl esters were prepared from each sample for fatty acid analysis and each preparation was analysed once by GLC. Each oil was analysed three times for triglyceride profiles by HPLC.

The analytical methods and statistical techniques were as described in Part 1 (Tsimidou *et al.*, 1987).

RESULTS AND DISCUSSION

The five vegetable oils that were studied as adulterants; namely, maize, cottonseed, sunflower, soyabean and rapeseed oil, were analysed for both their fatty acid and triglyceride profiles. In each case the oil showed characteristic profiles which were distinctly different from those of olive oil. Representative chromatograms are shown in Fig. 1 for the triglyceride profiles obtained by HPLC. A similar degree of discrimination was obtained when the fatty acid profiles were considered. However, when mixtures of these oils with olive oil (at 10% and 20% levels of addition) were analysed for their fatty acid composition they could not be clearly distinguished from pure olive oil based on the acceptable ranges as given by the *Codex Alimentarius*, although minor anomalies in the GLC traces of the mixtures may have raised the suspicion of an expert. This result is a direct consequence of the small number of common fatty acids found in these oils and their natural variability from sample to sample of the same type of oil. The triglyceride compositional data obtained by reversed-phase HPLC proved to be far more informative, even at the lower level of adulteration studied (10% of adulterant in olive oil). The differences in the triglyceride profiles produced by the various oils are shown in Fig. 2; the 20% level of adulteration has been shown to illustrate the effects but they are all visible at the 10% level. Indeed, the adulteration of olive oil with groundnut oil, which is very similar in composition, could also be detected, although this adulterant was not studied in detail. Owing to the lack of all the necessary triglyceride standards, and to the possibility of co-elution of some components, the resulting triglyceride profiles were characterised only in terms of peak areas for the seven major peaks corrected for the sample weight injected. These data were then subjected to Principal Component Analysis (PCA) in the same manner as described in Part 1 (Tsimidou *et al.*, 1987). Figure 3 shows how the olive oil used for the base of the adulteration studies compares with all the other virgin olive oils previously studied (Tsimidou *et al.*, 1987). This particular oil was chosen as it was an average representative sample. Ideally, several olive oils should have been used to

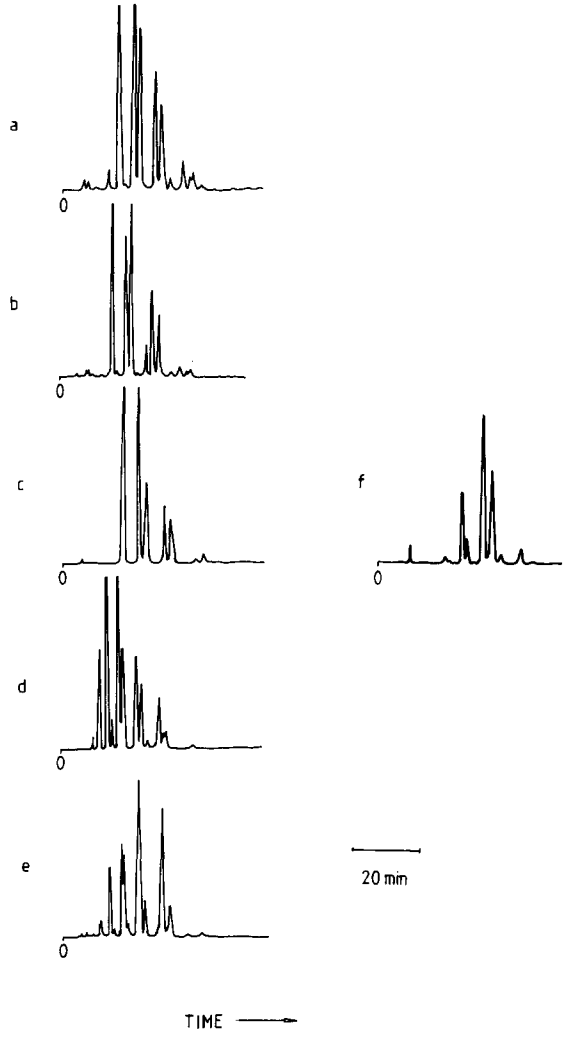

Fig. 1. HPLC triglyceride profiles of vegetable oils: a, maize; b, cottonseed; c, sunflower; d, soyabean; e, rapeseed; f, virgin olive oil.

cover the range available but this would have generated an impractical number of samples for analysis. The effects of the various adulterants can be seen in Fig. 4 which shows the plane of the first two Principal Components for the base olive oil and the mixtures at the 20% level. Here the discrimination is quite clear and the adulteration would have been detected irrespective of the position of the base olive oil on the sample plot. The further the base oil moves in the direction of the adulterant, the more difficult detection of the adulteration becomes. Also, as the level of the

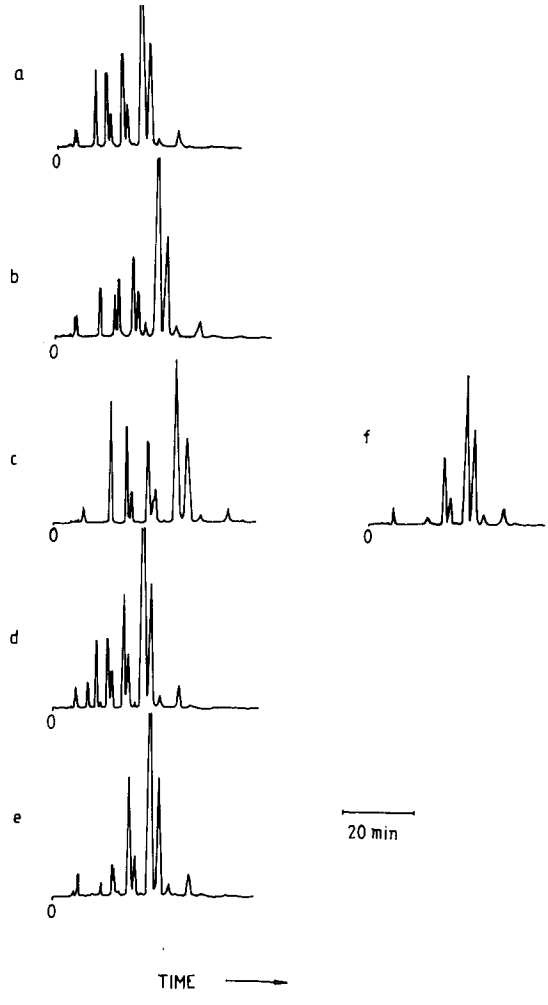

Fig. 2. HPLC triglyceride profiles of a virgin olive oil (f) adulterated with 20% of; a, maize; b, cottonseed; c, sunflower; d, soyabean; e, rapeseed oils.

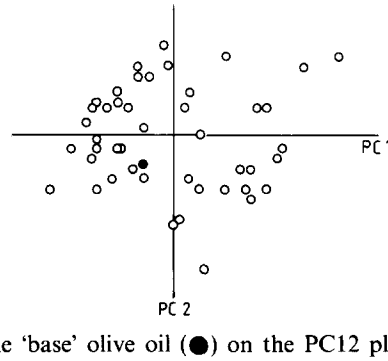

Fig. 3. Allocation of the 'base' olive oil (●) on the PC12 plane of the virgin olive oils previously studied (Tsimidou *et al.*, 1987).

adulterant is reduced, so the situation becomes less clear, as was observed in the present work when the 10% adulterated samples were examined.

PCA of triglyceride data, obtained by reversed-phase HPLC, would appear to provide the basis for a very powerful technique for the detection of gross adulteration.

The method relies on the establishment of a 'reference set' of olive oils as was carried out in Part 1 of this study (Tsimidou *et al.*, 1987). Once this has been completed it is not necessary to re-analyse the entire set when a further sample is to be incorporated as the addition or elimination of a single sample does not significantly affect the distribution of the samples in a given plane. This means that the loadings of the components may be considered as constants, which, in turn, means that they may be used to calculate the PC values of an unknown sample for location on the plot of the 'reference set'. Such calculations are relatively simple and do not require the use of computers, which would be needed for the analysis of the initial data set. Thus, once sufficient analyses have been carried out to establish the 'reference set' the data base may be continuously uprated from the results of further analyses obtained 'in the field'. The locations of the adulterated samples shown in Fig. 4 were calculated in this manner, and were found to be very close to those computed from the HPLC triglyceride data, including those of the unknown samples.

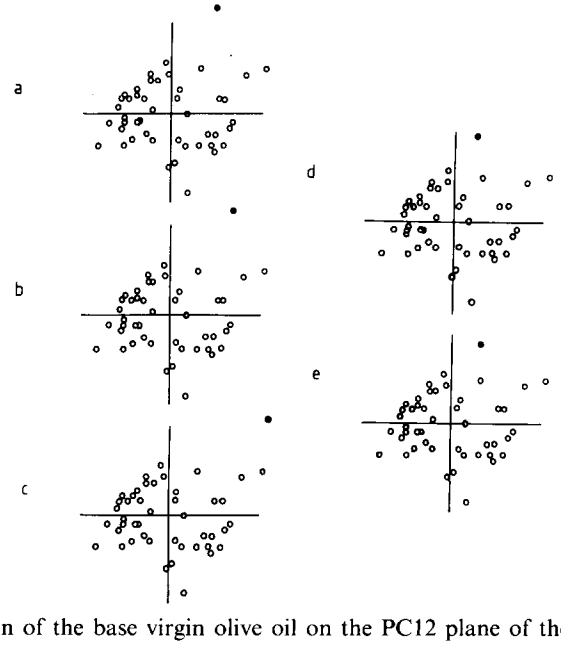

Fig. 4. Allocation of the base virgin olive oil on the PC12 plane of the virgin olive oils previously studied (Tsimidou *et al.*, 1987) after adulteration with: a, maize; b, cottonseed; c, sunflower; d, soyabean; e, rapeseed oils. (●) adulterated olive oil in each case.

CONCLUSION

The PCA of triglyceride data obtained by reversed-phase HPLC appears to offer a very simple and powerful technique for the detection of adulteration of vegetable oils. The analysis involved is very straightforward, requiring virtually no sample preparation, and detailed quantification of the chromatograms can also be avoided by using peak areas. The present study used the mass detector for detection but similar results could have been achieved, in this case, with refractive index detection as gradient elution was not required. The method relies on establishing a 'reference set' which is continuously uprated as it is used.

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