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The identification of vegetable matter using Fourier Transform Infrared Spectroscopy

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A comparison of Fourier transform infrared methods for identifying vegetable matter is presented. Results from diffuse reflectance (DRIFTS) and FTIR microscopy on samples of cell wall material from 10 different species of fruits and vegetables are presented and compared with results from a KBr disc method. All three methods are able to discriminate between a test sample (apple) and non-apple samples. However, there are significant spectral variations from method to method which preclude the use of spectral libraries obtained by one method being used to identify spectra obtained by another method.

INTRODUCTION

The visual identification of fresh vegetable species is a straightforward matter and presents no special problems. However, when that matter is processed and mixed with food material from a variety of origins the problem is much more difficult. It is particularly exacerbated when high levels of maceration or cooking are used or the product is an extract such as a juice.

The need to identify the origins of vegetable matter arises from the need to control quality and to ensure authenticity of an ingredient or product. Ideally the identification technique should be rapid, easy to use and of low cost. The increasingly widespread use of Fourier Transform Infrared Spectroscopy (FTIR) in the food industry and its increasingly favourable costs indicate that it could be the technique of choice. The target indicator for speciation should be easily obtained from the food and should be a unique species marker. For vegetable matter cell wall material is a likely candidate. It is present in all vegetable matter preparations including juices, and is easily separated because of its insolubility. In addition it is to be expected that the variations in polysaccharide composition as well as associated minor components would be unique for each plant species.

Previous work (Defernez & Wilson, 1994; Defernez *et al.*, 1994; Wilson *et al.*, 1993) has shown that it is possible to discriminate between the different types of fruits used in jams and purees by FTIR. Transmission spectroscopy can discriminate the species origins of plant cell walls (Kemsley *et al.*, 1994). Thus the combination of plant cell wall material and FTIR has some potential for speciation. The previous work on cell walls used the KBr disc method of sample preparation. This method, although traditional, has the disadvantage that it is lengthy and is not suited to the problem of very small amounts of sample such as might be required for forensic purposes. In this paper the use of FTIR microspectroscopy and diffuse reflectance spectroscopy (DRIFTS) is examined as a the possible method for rapid sampling and forensic investigation.

As previously (Kemsley *et al.*, 1994), 10 different species were examined, to test the potential of the method. The approach taken was first to test the intra-species variation and then compare this with the inter-species variation. This was done by using one species (apple) as a reference material and then testing the power of the method to discriminate this from all the other species.

MATERIALS AND METHODS

Sample preparation

Ten different species of fruits and vegetables were used. These were: carrot, cucumber, kiwi fruit, capsicum,

orange, pear, tomato, grape, apple and courgette. All of these belong to the biological class of dicotyledons.

Cell wall material of parenchyma tissue was isolated from each sample. In each case, the outer skin and the inner core were removed, and the pulp (parenchyma tissue) retained. This material was homogenised in a Waring blender and washed over Miracloth to remove cytoplasmic contaminants. In order to break open remaining cells, the material was ground in liquid nitrogen in a pre-chilled mortar and pestle. After thawing, the material was thoroughly washed again to remove contaminants. Light microscopy was used to check that clean fragments were obtained. The examined material was stored at − 18°C prior to freeze-drying *in vacuo*.

FT–IR microspectroscopy

All spectra were collected using a Spectra-Tech Inc. 'IR-PLAN^{tm}' microscope combined with a Bio-Rad FTS40 FTIR spectrometer. The detector was a liquid nitrogen cooled-mercury cadmium telluride (MCT) detector. All transmission spectra were obtained at a resolution of 8 cm^{-1}, and 256 interferograms were co-added before Fourier transformation. For sampling, droplets of cell walls in aqueous suspension were placed on BaF_2 windows (13 mm diameter x 2 mm thickness) and left to dry at 30°C in an oven. The sample was then placed on the stage of the IR-plain microscope and spectra collected from areas of single cell walls of about 100 × 100 μm areas.

Diffuse reflectance

All spectra were collected using a Bruker IFS 66v FTIR spectrometer, equipped with a deuterated triglycine sulphate detector (DTGS). A Spectra-Tech 'Collector' DRIFT accessory with a macro sample cup was used. All spectra were obtained at a resolution of 4 cm^{-1} with 256 interferograms co-added before Fourier transformation. Freeze-dried cell wall material was ground with KBr in a variety of ratios before sampling. It has been shown previously that DRIFT spectra of insoluble matter from jams show high levels of discrimination between species when the undiluted materials are used (Wilson *et al.*, 1993). The spectra observed were, however, largely the result of specular reflectance effects and bore little resemblance to spectra obtained by other methods. Typically jams are prepared from whole fruits or fruit purees and will contain both cell wall material and seeds and seed fragments. This combination of materials will give rise to a particular specular reflectance pattern but this may not be reproduced in other fruit products where processing is different. In order to avoid factors arising from the physical state of the vegetable matter and to more accurately reflect the chemical composition, which is more likely to be processing independent (especially for fresh products such as juices) it was decided to dilute the cell wall material in KBr to reduce gross specular reflectance effects and emphasise chemical effects.

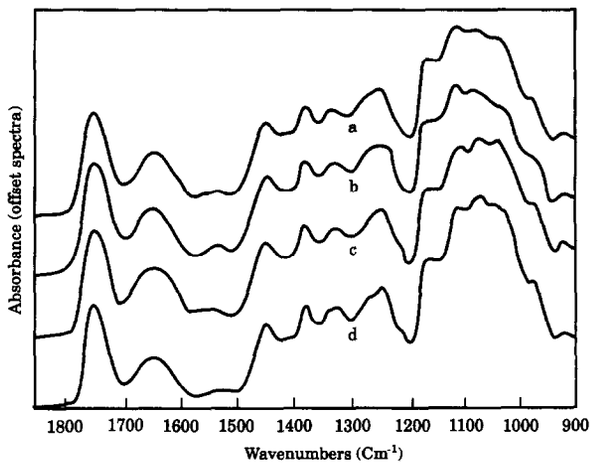

Fig. 1. The effects of sample preparation and dilution on diffuse reflectance spectra. (a) Dilution 1:5, sample not sieved. (b) Dilution 1:5, sieved sample. (c) Dilution 1:10, sieved sample. (d) Dilution 1:20, sieved sample. Spectra are offset for clarity.

RESULTS AND DISCUSSION

DRIFTS Spectra

The effects of sieving and dilution of the apple cell wall samples in a 100 μm sieve on the DRIFTS spectra diluted 1:5 in KBr are shown in Fig. 1. The effect is clearly quite small for this sample but it does ensure that no large flat surfaces are presented to the light beam which might distort the spectra by causing additional specular reflectance. It is a worthwhile precaution. Diluting the sample in potassium bromide results in some *spectral changes: intensity in the region 1000 to 1050 cm^{-1} is enhanced relative to the intensity in the 1050 to 1200 cm^{-1} region; however none of the band features are lost. These observations may be attributed to the effects of specular reflectance (Belton & Wilson, 1990) which can lead to apparent band suppression. Dilution results in deterioration of signal to noise ratio; it is thus desirable to dilute as little as possible if this results in an acceptable degree of discrimination between samples. Figure 2 (a) shows a set of DRIFTS spectra obtained from a number of apple cell samples using the 1:5 dilution. Clearly the spectra are all very similar. For the non-apple samples shown in Fig. 2(b) there is clearly considerable spectral variation between cell walls from different species present. We have shown previously (Kemsley *et al.*, 1994) that when cell walls are pressed into KBr discs it is possible to discriminate between the spectra obtained from 10 different apple samples and the set of non-apple samples. DRIFTS represents a more convenient sampling method than disc preparations and would be preferable in authentication experiments if suitable discrimination could be obtained. Therefore comparison was made to determine whether the variety of species shown in Fig. 2(b) could be discriminated from the apple sample shown in Fig. 2(a). Following Principal Component Analysis (PCA) and Discriminant Analysis (DA) the data were compared by measuring Mahalanobis D2 distances (Mardia, 1977)

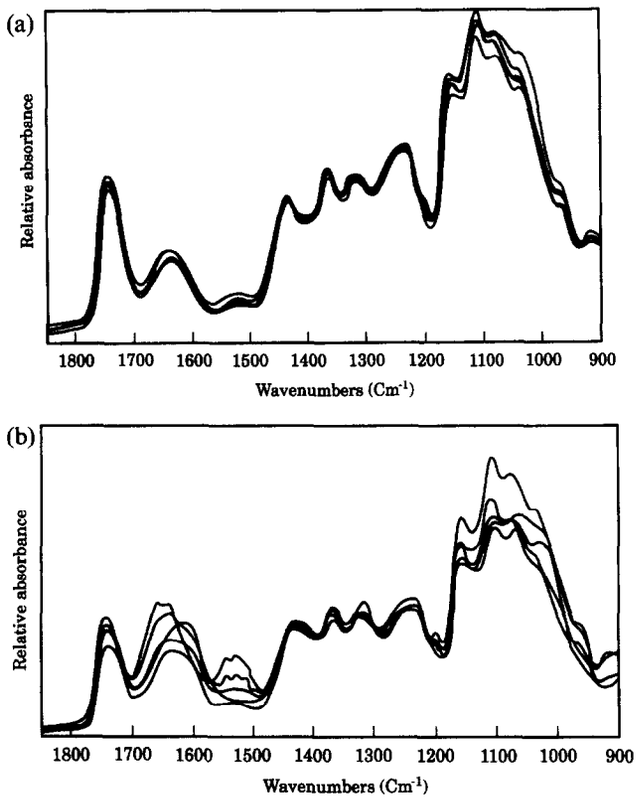

Fig. 2. (a) A set of spectra from five different apple samples. (b) A set of spectra from five different non-apple samples demonstrating the variation in non-apple spectra.

from group means. The results are shown in Fig. 3. The data represents calibration sets containing seven apple and seven non-apple spectra together with test sets of two non-apple and three apple samples. 99% of the significant information in the original data could be described by just 3 PC principal component scores. The group mean observations of each of the apple and non-apple groups were determined and the Mahalonobis D2 of each observation from the group means were calculated. After reassignment to the nearest group it was

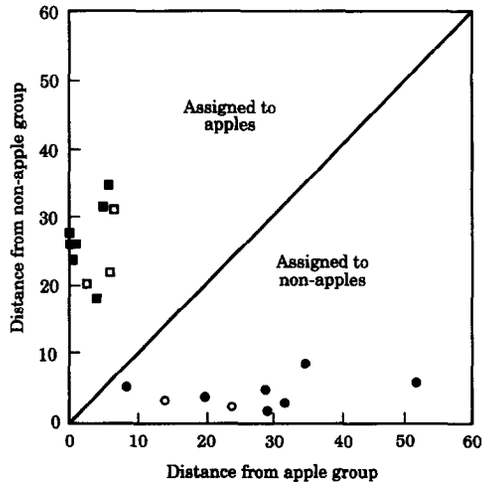

Fig. 3. A plot showing the differentiation of the set of apple DRIFTS spectra from non-apple spectra using Mahalanobis D2 distance. ■ Apple calibration set, ● non-apple calibration set, □ apple validation set, ○ non-apple validation set.

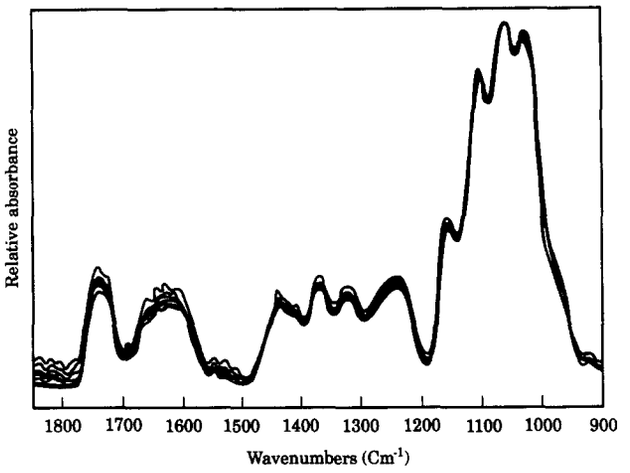

Fig. 4. Spectra obtained by FTIR microscopy from different parts of a pear cell wall sample.

found that none of the calibration sets were mis-classified. The observations on the test sets were transformed to PC space using the linear transformation determined by PCA on the calibration set, and the Mahalonobis D2 calculated from the group means of each calibration group. Again no mis-classification occurred.

These results clearly show that the spectral resolution obtained using the 1:5 dilution drifts spectra are adequate for discriminating between apple and non-apple cell walls.

FTIR microspectroscopy

Since the aperture used was quite small (100 × 100 μm) it is important to check that the area sampled is representative of the whole sample. Significant variations within a sample on a length scale greater than this would require the exclusive use of a bulk sampling method which would result in a spectrum from a much larger length scale. Such an observation might also invalidate the use of microscopy as a forensic method since a small fragment might not then be representative of the species. Observations on apple and pear cell walls indicate that the samples are homogeneous and that cell-to-cell variations are not important. This is illustrated in Fig. 4 which shows spectra obtained from different parts of a pear cell wall sample. Clearly there is relatively little variation within a sample. Similarly repeated experiments on samples obtained from different apples show very little variation. When apple spectra are compared with non-apple spectra using the procedures described above, clear discrimination is possible. This is illustrated in Fig. 5, which shows the same level of discrimination as in Fig. 3. It is concluded therefore that FTIR microspectroscopy is a viable method for testing small samples of cell wall material for authenticity.

Comparison of methods

Both DRIFTS and FTIR microspectroscopy are viable methods for authenticating cell wall samples. A

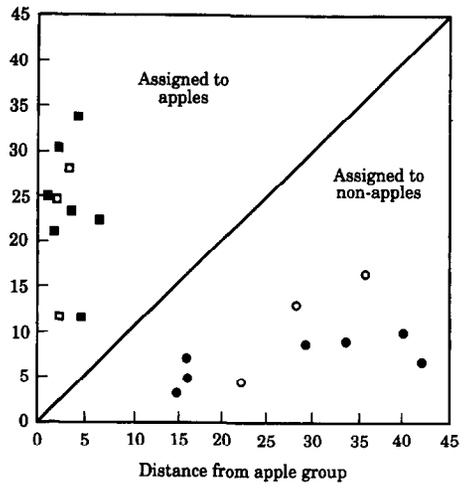

Fig. 5. Mahalanobis D2 plot obtained from microscopy. ■ apple calibration set, ● non-apple calibration set, ○ apple validation set, ● non-apple validation set.

previous publication (Kemsley *et al.*, 1994) showed that transmission methods using KBr discs also give useful results. However, as is shown in Fig. 6 the methods do not give identical spectra for the same sample. Microspectroscopy shows better resolution than transmission or diffuse reflectance in the 1000–1200 cm^{-1} region. In the 1550–1800 cm^{-1} region there is a shift of bands to lower wave number and a broadening of the band centred between 1600 and 1700 cm^{-1}. The broadening of the bands in the 1000–1200 cm^{-1} region may arise from scattering and specular reflectance effects in DRIFTS and KBr discs. Dilution of the sample in KBr causes the DRIFTS spectra to more nearly resemble the disc transmission spectra. This is consistent with the reduction of specular reflectance effects on dilution. The disc transmission spectra may themselves be affected by scattering (Belton & Wilson, 1990) which could distort the relative intensities in highly absorbing regions. The variation observed in the 1600–1800 cm^{-1} region is less easy to explain. The spectra illustrated are from apple but all samples showed similar behaviour. The observations suggest that some real chemical or physical difference exists between samples that are prepared by deposition onto a BaF_2 window and those which are

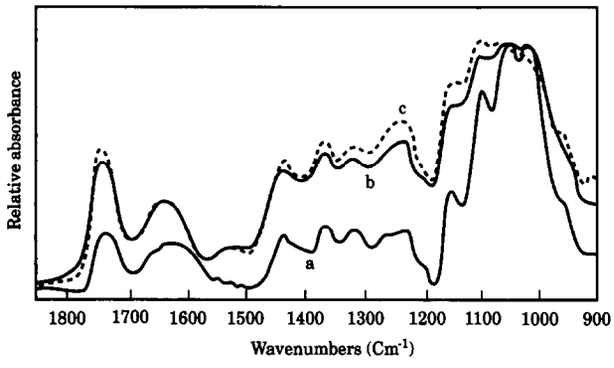

Fig. 6. Comparison of apple cell wall spectra obtained from (a) microscopy, (b) KBr disc and (c) DRIFTS using a 1:5 dilution.

prepared by intimate contact with potassium bromide. This may be due to the drying process used for preparing the sample. During the process, atmospheric CO_2 can dissolve and carboxylic acid residues may be transformed to carboxylate which absorbs at a lower frequency. A similar phenomenon has already been observed in systems containing citric acid (Delgadillo & Boto, 1994). These effects are significant for structural and chemical investigations of cell walls. If this is the case great care must be taken that the sample is reproducibly presented, since slight variations in presentation may lead to variable spectra in this region. Since the region 1550–1700 cm^{-1} appears to be sensitive to the species type this could lead to misclassification. This was not the case in the experiments reported here as intraspecies variation was always significantly less than interspecies variation. Since the nature and distribution of carboxylic residues is characteristic of the species it may be that the response of a particular species to the sample preparation, as described, is also characteristic and may enhance discrimination.

The variation of spectra between sampling methods implies that it is not practicable to compare spectra obtained with different sampling methods and that attempts to identify or discriminate a species using a spectrum obtained by one method and comparing it with a standard spectrum obtained by another method are likely to be misleading. Great care must therefore be taken in the compilation of spectral reference libraries.

The method of sampling which is most suitable depends upon the applications. Where the plant material is present in small quantities (as foreign matter), micromanipulation followed by microspectroscopy is clearly indicated. However, when larger quantities are present (which may contain more than one species) a bulk sampling method such as DRIFTS is preferable as a screening method. Further investigation of the sample may then require separation and sampling of individual cell fragments by microspectrometry. It may also be necessary to carry out some pre-separation process before the DRIFTS experiment to eliminate possible contaminating material such as seeds. The sieving procedure outlined above should serve this purpose. It is important to ensure that preparation and dilution of the sample does not result in the concentration or dilution of a particular species in a mixed sample. This is particularly important where the concentration of one species is low. Care should also be taken to ensure that, with a KBr-diluted sample, sufficient sample is interrogated by the IR beam to detect the presence other low levels of adulterants. Whereas microspectroscopy is attractive since individual cells can be selected, DRIFTS offers the advantage of measuring bulk samples, eliminating the need to identify suspect cell wall material. Dilution can lead to the loss of this advantage. In the future some attention needs to be given to the use of undiluted samples which, although not yielding data which is interpretable in chemical terms, may be better suited to the detection of adulteration.

CONCLUSIONS

Mid-infrared diffuse reflectance, transmission and microspectrometry may be used to discriminate between cell walls derived from apples and a variety of other species. The use of FTIR spectroscopy thus has considerable potential as a method for the verification of the origins of foods which contain plant cell wall debris. However, before such methods can be considered as robust methods of authentication the effects of such factors as plant maturity, agronomic history and climatic effects must be considered.

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