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A rapid FTIR quality-control method for determining fat and moisture in high-fat products

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A general-purpose, automated Fourier-transform infra-red (FTIR) quality-control method was developed and evaluated for the rapid analysis of fat and/or moisture in high-fat products, typified by mayonnaise and peanut butter. 1-Propanol was used to solvate fat in peanut butter and fat and moisture in mayonnaise, and the sample was then pumped through a 100-μm CaF_2 flow cell and the C=O stretching (1748 cm^{-1}) and HOH bending (*c.* 1650 cm^{-1}) absorptions were measured after the solvent spectrum had been ratioed out. Calibrations were based on relating the peak heights of the absorption bands to the known fat and moisture values of calibration standards prepared by dissolving pure components (peanut oil, canola oil/water) in propanol or by adding to propanol varying amounts of a representative sample pre-analyzed by the Mojonnier method. The time of sample preparation and analysis was 5–7 min per sample, and the predictions obtained by FTIR were within ±0·30% of values determined by the Mojonnier method. The FTIR portion of the method was automated by programming the instrument by using macro-command language, leaving the operator only to follow menu-driven instructions to carry out the calibration and analysis. As such, the method is rapid and routine and requires no knowledge of FTIR spectroscopy, which makes the procedure suitable for process-quality-control monitoring of high-fat products. Extension of this general approach to other high-fat food systems, such as butter, margarine, and chocolate, should be feasible.

INTRODUCTION

The rapid analysis of food products is crucial in food processing in order to adjust manufacturing processes while production is underway. Our laboratory has been pursuing the development of rapid quality-control methodologies for the food industry through the application of Fourier-transform infra-red (FTIR) spectroscopy (van de Voort *et al.*, 1992*a*,*b*). The basic principles of FTIR spectroscopy have been well described by Griffiths and de Haseth (1986), and their book is recommended as a general reference for FTIR theory and techniques. Developed in the early 1970s but still relatively unexploited by the food industry, FTIR spectroscopy has come of age in terms of price, performance, and ease of use (van de Voort & Ismail, 1991). Coupled with an attenuated-total-reflectance (ATR) accessory or a transmission flow cell, and through the use of instrument macro-programming, an FTIR spectrometer can be as simple to operate as a conventional visible spectrophotometer and can provide compositional information in terms of fat, protein, carbohydrate and moisture content in foods (van de Voort, 1992). As such, FTIR spectroscopy can now be considered a potentially cost-effective tool for quality-control applications in the food industry.

In response to an industry query for a simple and rapid method for butter analysis, a method was developed that was based on the addition of 1-propanol to butter in a 10 : 1 ratio, followed by measurement of fat and moisture in the solvent by FTIR spectroscopy (van de Voort *et al.*, 1992*b*). This method was based on the use of a 45° ZnSe ATR crystal, and the instrument was programmed to automate the analysis, which made it suitable for plant operators to carry out routine analyses. On the basis of the experience gained from the development of the ATR-based butter-analysis protocol, it was considered that a flow-based transmission system might be more advantageous from a sample-handling viewpoint and of more general applicability to high-fat food systems such as mayonnaise, salad dressings, peanut butter, butter, and margarine. Such products require ongoing analysis during the manufacturing process to ensure that product quality and regulatory specifications are adhered to. Traditional methods such as the Mojonnier method (Anon., 1922), which can be used to analyze for fat and moisture, the Soxhlet method (AOAC, 1975), for analysis of fat, or the vacuum-oven method (AOAC, 1975), for moisture determination, all present constraints in terms of time, manpower, and/or the need for substantial amounts of solvents. If timely analytical results are not available, batches in production may need to be reworked if out of specification, involving additional time and expense. For these very practical reasons, there is a need in the food industry for simple, rapid methodologies capable of monitoring an ongoing manufacturing process.

For the assessment of an FTIR flow-based transmission method capable of measuring fat and/or moisture, two high-fat food systems were considered: peanut butter, a product that contains no appreciable levels of moisture but is high in fat and solids, and mayonnaise, an oil-in-water emulsion that is relatively low in solids but contains substantial amounts of water. Peanut butters commonly have a fat content of 48–54%, dependent upon the peanut type and degree of roast, and are largely composed of finely ground peanuts, 4–7% sugar (usually dextrose), *c.* 1% salt and some emulsifier to stabilize the product and prevent oil separation. Mayonnaise is usually prepared from three base materials: vegetable oil (75–80%), vinegar (9–12%) and a pre-mix composed of water, egg yolk, starch, spices, and stabilizers (10–11%). In 'light' versions, the oil content is reduced to 35–40%, usually by substituting more water and starch. These products thus represent two high-fat products, which present an analytical challenge in terms of the rapid determination of fat and/or moisture. This paper describes a simple, automated transmission-based FTIR method capable of determining fat in peanut butter and moisture and fat in mayonnaise and suitable for quality-control applications.

MATERIALS AND METHODS

Instrumentation

A Nicolet 8210 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI, USA) was used for this study, equipped with a CaF_2 demountable transmission-flow cell (Wilmad Glass Co., Buena, NJ, USA) with a 100-μm Teflon spacer. The cell was connected to a Spectra-Tech sipper accessory (Spectra-Tech Ltd, Stamford, CT, USA) by using 1·6-mm-i.d. tubing to aspirate the sample through the cell and automate sample-handling. The suction end of the sipper was equipped with a small sintered glass filter to prevent any suspended insoluble particulates in the sample from entering the line and clogging the cell. The instrument was purged of CO_2 and water vapor by using a Balston dryer (Lexington, MA), and the cell was operated at ambient temperature. Spectral acquisition was carried out at a resolution of 16 cm^{-1} by using 32 scans for the samples. Sample-emittance spectra were ratioed against the solvent background-emittance spectrum, recorded by using 64 scans to minimize the noise level in the ratioed spectra. The spectrometer was operated under Nicolet DXFTIR software (Anon., 1988) and programmed by using the Nicolet macro-command language to automate spectral-acquisition and data-handling operations for both calibration and analysis.

Calibration standards

Anhydrous 1-propanol, stored over molecular sieves (type 4A, J. T. Baker Chemical Co., Phillipsburg, NJ, USA), was used as a solvent for the preparation of calibration standards. Two sets of peanut butter calibration standards were prepared, one by simply adding varying weights of commercial peanut oil to 1-propanol (6–9 g per 75 g), and the second by adding varying weights of a pre-analyzed peanut butter to propanol. Similarly, two sets of mayonnaise calibration standards were prepared, one by adding canola oil and water to propanol in random ratios to avoid any direct correlation between fat and moisture, and a second set by adding the mayonnaise-base ingredients (vegetable oil (canola), vinegar, and a manufacturer's pre-mix) in random proportions to propanol. In the case of the standards prepared from peanut butter and the mayonnaise-base ingredients, sonication was carried out to extract the fat and moisture. This was done by first shaking the samples and placing the sealed vials in a sonic bath (Branson Ultrasonics Ltd, Danbury, CT, USA) for exactly 5 min. After sonication, the insoluble solids were allowed to settle out of solution.

Samples

Commercial peanut butters and mayonnaises were obtained from a local manufacturer. As the composition of the commercial products was not sufficiently variable for a rigorous validation of the FTIR method, the base ingredients used in their formulation were also obtained in order to prepare products with a wider range of composition in the laboratory. Ground-roasted peanut base, dextrose, and stabilizer were used to make peanut butter, and vegetable oil (canola), vinegar, and a manufacturer's pre-mix were used to make mayonnaise. Both products were prepared by using a polytron (Brinkmann Instruments, Inc., Westbury, NY, USA) in accordance with the manufacturer's instructions. All peanut-butter and mayonnaise samples and base ingredients were subsequently analyzed for fat and/or moisture by the Mojonnier method (Anon., 1922) to confirm the expected composition.

For the analysis of peanut-butter or mayonnaise samples by FTIR, *c.* 2·5 g of the sample were placed in a tared 40-ml vial followed by the addition of *c.* 25 g of propanol delivered by a re-pipette, with the exact weights of the sample and sample + propanol recorded.

These weights are required to convert the percentage fat and moisture determined to be present in propanol by FTIR analysis to their respective values in the products. After preparation, the vials were capped, shaken, and sonicated for exactly 5 min to extract fat and/or moisture and were ready for analysis after 1–2 min, when the majority of the solids had settled.

Calibration and analysis

Prior to analysis, the CaF_2 flow cell was cleaned by aspirating *c.* 20 ml of a 1% Triton X-100 detergent solution (Sigma Chemical Co., St. Louis, MO, USA) through the cell, this being followed by a similar volume of dry propanol. The cell was then filled with dry propanol and its single-beam spectrum recorded as a reference background and stored to disk. Owing to the hygroscopic nature of propanol, it is important that the propanol used for recording the reference background be kept over molecular sieves in order to ensure that it is maintained in an adequately anhydrous state. A spectroscopic check for the presence of moisture in the propanol was incorporated into the analysis routine. This involves ratioing the single-beam spectrum of the propanol being used against a stored reference single-beam spectrum of anhydrous propanol (dried by using recently microwaved molecular sieves) and recording the absorbance at 1650 cm^{-1}. An absorbance value greater than 0·005 (corresponding to *c.* 0·05% moisture) indicates that the propanol is unsuitable for analytical use and must be dried further. Propanol stored over molecular sieves routinely passes this check on a 100-μm cell.

Each calibration standard was aspirated into the cell (*c.* 5 ml) and the emittance spectrum recorded and ratioed against the propanol reference background spectrum to produce the absorbance spectrum of the standard. The absorbance spectrum of the standard was subsequently baseline corrected by using 1600 and 1850 cm^{-1} as anchor points and the peak heights of the carbonyl ester-linkage signal of fat (*c.* 1748 cm^{-1}) and the HOH-bending absorption of water (*c.* 1650 cm^{-1}) were measured by using a 'peak-pick' routine (Anon., 1988). The peak-pick routine ensures that the maximum absorbance is measured and accounts for any minor shifts in the positions of the maxima of the peaks. When all the standards had been run, the peak-height values were regressed against the chemical values of the standards. Linear regression was used to calibrate for fat in peanut butter, and multiple linear regression was used for mayonnaise because two components (fat and moisture) are being analyzed for. Both regressions were forced through the origin to allow a direct comparison of the slopes of the various calibration methods (Barbano & Clark, 1989). Regressions and subsequent comparative statistical analyses of the data were carried out by using Statgraphics, a general-purpose statistical and graphics package (Statgraphics, STSC Inc., Rockville, MD, USA).

Similar sample- and spectral-handling procedures were used for the products. The product spectral-absorbance data were first transformed into percentage fat or moisture in propanol by using the appropriate calibration equations, and the values obtained were then converted to percentage fat and moisture in the product by using the weight of product added to propanol and the total weight of product plus propanol.

All spectral operations and calculations described above were programmed by using Nicolet macro-command language to automate the analysis. As programmed, the operator is led through the analysis by a menu-driven program, which includes the propanol dryness-check routine, the calibration procedure, and then sample analysis and the input of the weights for calculation of the results. After each analysis, the fat and moisture results are printed. As structured, instrument operation is rote and requires no prior knowledge of FTIR spectroscopy. Only the regression analysis of the calibration data is separate from the pre-programmed routine; however, the regression coefficients obtained can be entered interactively once they have been determined. A simplified listing of the basic elements of the program is presented in Fig. 1.

RESULTS

Spectra

Representative absorbance spectra of mayonnaise and peanut butter dissolved in propanol after the dry-propanol background spectrum has been ratioed out are shown in Fig. 2(a) and (b). Propanol, which is a relatively innocuous solvent and is capable of extracting both fat and moisture, does not have any substantial absorptions in the 1850–1550-cm^{-1} region, where both fat and water are measured. The fat and moisture absorption peaks are quite apparent in the mayonnaise spectrum in Fig. 2(a), whereas there is no discernible water peak in the spectrum of the peanut butter (Fig. 2(b)), since it does not contain any appreciable levels of moisture. Absent from these spectra are features due to any of the other components (i.e. sugar, starch, or protein) since they are insoluble in propanol and settle out of solution.

Peanut butter

Chemical analysis

Seventeen commercial peanut-butter samples were analyzed in duplicate for fat by the Mojonnier method (Anon., 1922), which is based on an ethyl ether/petroleum ether extraction. The resulting data were analyzed in terms of mean difference (MD_r) and standard deviation of the difference (SDD_r) as suggested by the AOAC (Youden & Steiner, 1975). Ideally, over a series of analyses, the mean difference between duplicates should average out to zero, the SDD_r being a measure of the variability of the method. The results obtained for the seventeen samples were an MD_r of −0·44% and

```
                FTIR FAT/MOISTURE ANALYSIS PROGRAM

     ENTER DATE
     ENTER OPERATOR NAME
     ENTER PRODUCT NAME
     ENTER BATCH #
[1]  PROPANOL ASSESSMENT
[2]  FILL CELL WITH DRY PROPANOL - SCAN PROPANOL
[3]  RATIO AGAINST STORED REFERENCE SPECTRUM OF DRY
     PROPANOL
[4]  ABSORBANCE @ 1650 CM-1 > 0.0050?
[5]  YES - PROPANOL IS NOT DRY ENOUGH, STOP ANALYSIS
[6]  NO - CONTINUE
[7]  CALIBRATION OR ANALYSIS?
     FOR CALIBRATION GO TO [8]
     FOR ANALYSIS GO TO [19]
[8]  CALIBRATION ROUTINE - CLEAN FLOW CELL
[9]  PUMP IN PROPANOL (~5 ML)
[10] SCAN PROPANOL
[11] HOW MANY STANDARDS ARE THERE? X
[12] x = 1
[13] PUMP IN STANDARD # x
[14] SCAN SAMPLE
[15] PRINT PEAK HEIGHTS
[16] x = x + 1
[17] IF x < X GO TO [13]
[18] END - CARRY OUT REGRESSION -> CALIBRATION COEFFICIENTS

[19] ANALYSIS ROUTINE
[20] INPUT/UPDATE CALIBRATION COEFFICIENTS IF NECESSARY
[21] PUMP IN PROPANOL
[22] SCAN PROPANOL
[23] HOW MANY SAMPLES ARE THERE? Y
[24] y = 1
[25] ENTER SAMPLE WEIGHT; WEIGHT SAMPLE+PROPANOL
[26] PUMP IN SAMPLE # y
[27] SCAN SAMPLE
[28] PRINT %FAT, %WATER
[29] y = y + 1
[30] IF y < Y GO TO [25]
[31] END
```

Fig. 1. A simplified listing of macroprogram used for the analysis of peanut butter and mayonnaise.

an SDD_r of ±0·48%. These results are relatively poor for the Mojonnier method, which is considered a very accurate analytical procedure, and the differences in the degree of grind of the peanut butters, and hence the degree of extraction efficiency, were suspected as the cause of the Mojonnier variations. Three pairs of replicate Mojonnier analyses of a single product of fine grind reduced the MD_r to *c.* 0·03% and the SDD_r to *c.* ±0·25%, which indicates that a substantial part of the variability in the over-all analysis was due to variations in extraction efficiency. Subsequent extraction experiments were carried out by adding 10:1 propanol to three products having fine, medium and coarse grinds. These products were treated by shaking for 1 min, sonicating for 5 min, and sonicating for an additional 15 min, with the fat extracted determined gravimetrically after evaporation of the solvent. Table 1 presents the means and standard deviations for percentage fat in each of the three products as determined from the amount of fat extracted by each treatment based on five replicate analyses. Sonication for 5 min increased the amount of fat extracted by 0·5, 2·3, and 3·1% for the fine, medium, and coarse samples, respectively, relative to a shaking treatment, whereas an additional 15 min of sonication led to no significant change in the fat content. These results confirmed the relationship between extraction efficiency and particle size, and this accounts for the high variability observed in the Mojonnier results of samples having varying grinds. These data also indicate that a 5-min sonication treatment is an effective means of rapidly achieving the maximum extraction of fat into propanol for a specific grind, although it does not guarantee that the fat has been extracted to the same degree in samples of different grinds.

FTIR analysis

The FTIR/propanol method assumes that fat can be extracted to a consistent degree under standard conditions. In terms of calibrating the spectrometer, the ideal

Table 1. Effect of grind and extraction treatment on the amount of fat extracted from peanut butters by adding propanol in a 10:1 ratio.

	Percentage fat in peanut butter					
Grind	Fine		Medium		Coarse	
Treatment	Mean	SD	Mean	SD	Mean	SD
Shake	51·66	0·16	49·05	0·43	48·48	0·63
Sonicate (5 min)	51·93	0·14	50·66	0·18	49·67	0·21
Sonicate (15 min)	52·01	0·08	50·74	0·19	49·81	0·22

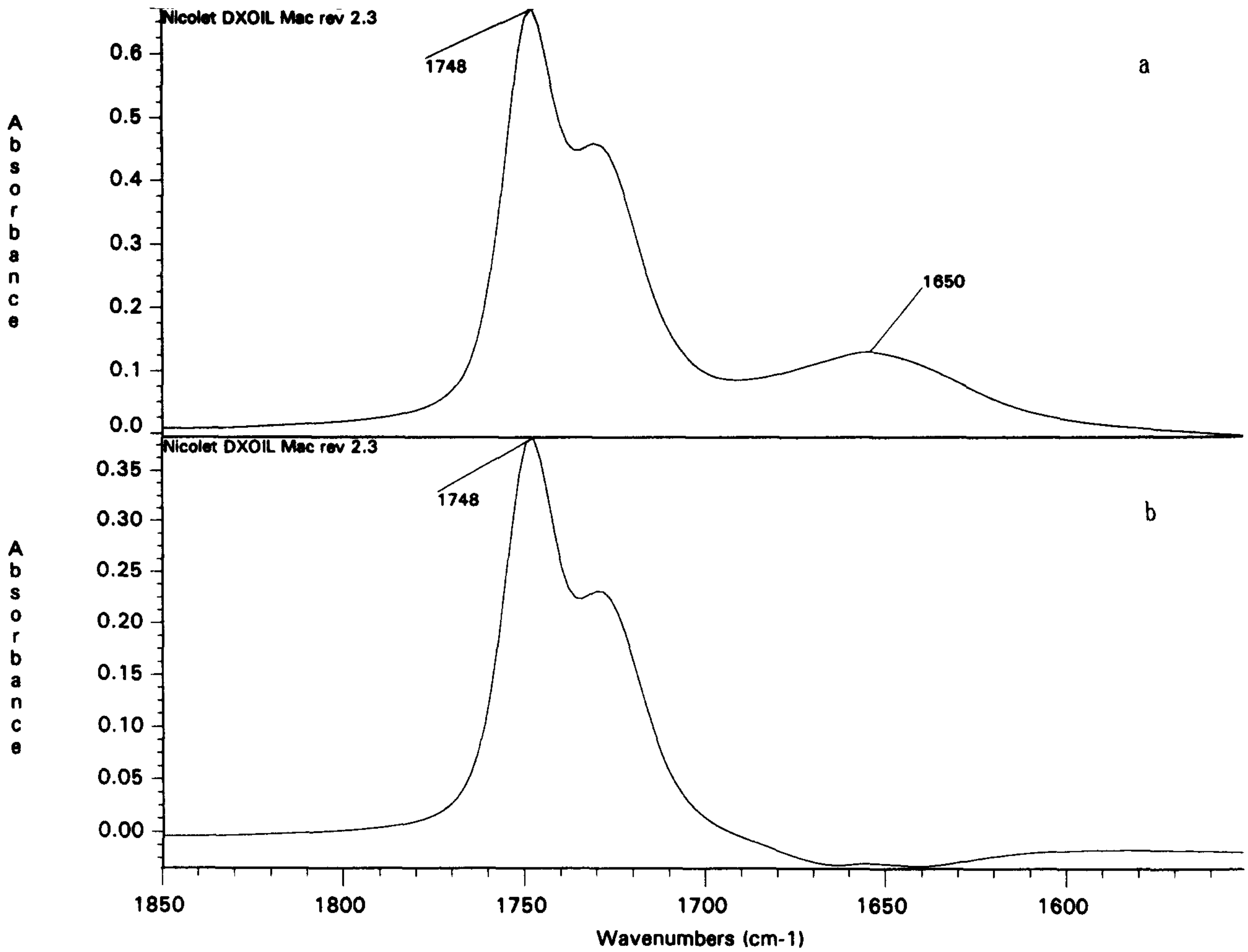

Fig. 2. Typical FTIR spectra of mayonnaise (a) and peanut butter (b) in the 1850–1550-cm^{-1} region, showing the peaks used for analysis of fat (1748 cm^{-1}) and moisture (ca. 1650 cm^{-1}).

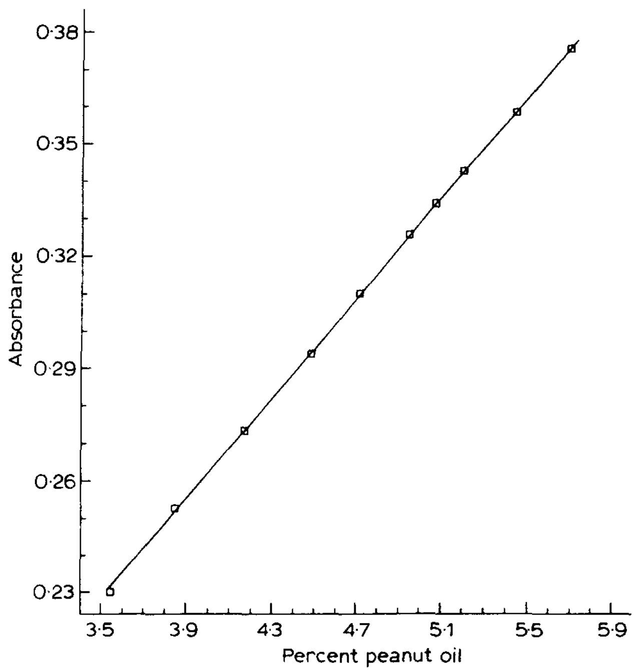

Fig. 3. A representative standard curve of the fat-absorbance signal (1748 cm^{-1}) plotted against the weight of peanut oil added to propanol.

approach is to calibrate directly against the component of interest, in this case peanut oil. Figure 3 illustrates a representative calibration curve of absorbance against percentage peanut oil in propanol, the regression equation for which is:

$$F = 15{\cdot}1978F_i \qquad \mathrm{SE} = 0{\cdot}019 \tag{1}$$

where F = % fat in propanol; F_i = absorbance at 1748 cm^{-1}; and SE = standard error.

The plot is linear and the SE associated with the calibration is <0·02%. This is thus an excellent standard curve for predicting the amount of fat in propanol. However, conversion of this value into percentage fat in the actual product requires taking into account any displacement effects caused by the insoluble solids and the relative extraction efficiency of the solvent system. For a completely soluble or miscible system, concentration is normally determined by using eqn (2):

$$F_s = \{F_p(S + P)/S\}100 \tag{2}$$

where F_s = % fat in the product; F_p = % fat in propanol determined from the standard curve; P= weight of propanol added to sample, and S =weight of sample.

However, when there are insoluble solids in the sample that are not dispersed homogenously throughout the propanol solution, but rather have settled out of the solution and thus displaced propanol, the weight of the solution in which the fat is dissolved is less than that assumed in eqn (2), so that the fat concentration will be overestimated by this equation. For a system containing an ideal insoluble solid, the equation has to be modified to:

$$F_s = [(F_p P/(100 - F_p))/S]100 \qquad (3)$$

to account for the displacement of propanol by the solids. Since peanut butter contains substantial amounts of solids (40–50%), the error caused by the displacement effect will be significant. In addition, the question of extraction efficiency arises, since a portion of the fat is present in the peanut cotyledon matrix and only a limited volume of solvent is available for its extraction (10 : 1 solvent-to-sample ratio). One means of combining the effects of both solids displacement and extraction efficiency is to calibrate by adding varying weights of a selected peanut-butter sample to propanol, which thereby make both effects an inherent part of the calibration. The calibration equation obtained for this system was:

$$F = 14{\cdot}9960 F_i \qquad \mathrm{SE} = 0{\cdot}018 \qquad (4)$$

Table 2 presents the fat contents calculated by using eqns (1) and (2), (1) and (3), and (4) and (2) for ten peanut-butter samples prepared in the laboratory. Both the peanut-butter samples and the peanut butter used to prepare the peanut-butter calibration standards had been prepared from a finely ground peanut base. Table 2 indicates that the uncorrected predictions based on the peanut-oil calibration (eqns (1) and (2)) overpredicted the chemical results by *c.* 0·8 % whereas correcting for displacement (eqns (1) and (3)) underpredicted the chemical values by *c.* 1·8% and increased the SDD_a from 0·13 to 0·40%. This underprediction can only be explained by a limitation in the extraction efficiency. By using the fat contents determined by FTIR for the peanut-butter standards by means of eqns (1) and (3) and comparing them to the formulated values, the average relative extraction efficiency was determined to be 96·1% ± 0·10%. Adjusting the displacement-corrected predictions for the peanut-butter samples for the extraction efficiency (eqns (1) and (3c)) produced results very similar to the chemical values, with a slight negative bias of *c.* 0·16% and a reduction of the standard deviation to *c.* 0·28%. Hence consideration of both displacement and extraction efficiency brought the predicted and chemical-fat compositions into line with each other. On the other hand, the peanut-butter calibration predictions (eqns (4) and (2)) are a good match to the chemical values without further manipulation, indicating that a standard curve prepared in this manner builds in both displacement and extraction-efficiency effects directly.

Table 2. Predicted fat contents (g per 100 g) of prepared peanut-butter samples obtained by using peanut-oil calibration equation with and without displacement correction and peanut-butter calibration equation

	FCHEM	Eqns (1) and (2)	Eqns (1) and (3)	Eqns (1) and (3c)*	Eqns (4) and (2)
1	46·16	47·10	44·96	46·78	46·48
2	47·32	48·17	45·93	47·79	47·53
3	48·23	49·07	46·74	48·64	48·41
4	49·48	50·41	47·87	49·81	49·74
5	51·53	52·40	49·64	51·65	51·70
6	52·56	53·27	50·52	52·57	52·56
7	53·51	54·09	51·20	53·28	53·37
8	54·44	55·36	52·31	54·44	54·62
9	55·64	56·35	53·59	55·66	55·60
10	56·79	57·81	54·47	56·68	57·04
MD_a		−0·836	1·850	−0·161	−0·140
SDD_a		0·133	0·402	0·279	0·148

* Corrected for extraction efficiency.

These results indicate that calibrating with a representative peanut-butter sample of predetermined fat content produces good predictions directly to within ± 0·2% fat, whereas calibrating with peanut oil introduces a bias, since it does not take into account the extraction efficiency, which would have to be determined experimentally. Even with this limitation, it may still be preferable to calibrate with peanut oil, since one calibration can be used for all products and the results corrected by determining the bias for a particular type of product through the analysis of a representative product of predetermined fat content.

Figure 4 presents a plot of the FTIR-predicted fat values against the Mojonnier results for seventeen commercial peanut-butter samples obtained by using the peanut-oil calibration. The slope of the plot is close to 1·0; however, the SE is in the order of 0·70%. Better results could not be obtained for these samples because the Mojonnier results themselves have a variability of

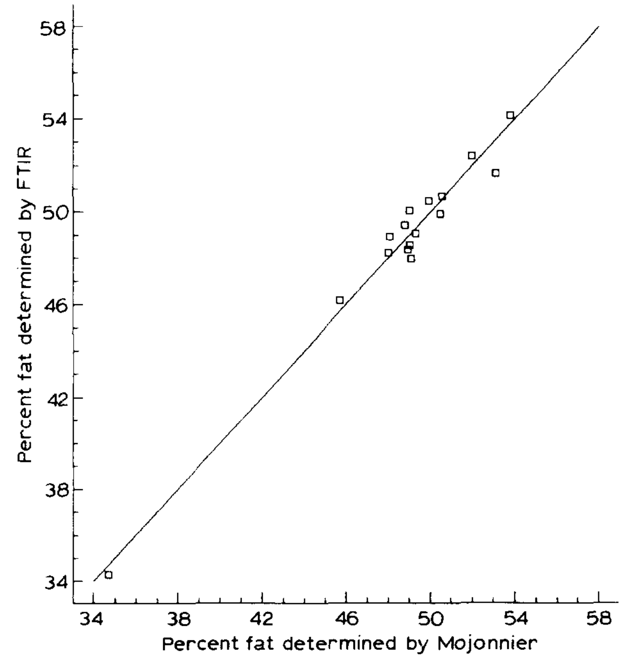

Fig 4. A plot of predicted-FTIR fat results for commercial peanut butter samples plotted against their respective Mojonnier chemical values.

± 0·50% owing to variations in extraction efficiency caused by grind variations. Even with these inherent errors, Fig. 4 clearly illustrates that the FTIR predictions follow the trend obtained by the reference method. When these confounding variations are absent, as in our formulated products (Table 2), excellent predictions for the fat content in peanut butter can be obtained (±0·20%).

Mayonnaise

The Mojonnier fat-chemical results for mayonnaise were substantially better than those for peanut butter and produced an MD_r value of 0·03% and an SDD_r value of ±0·12%, since there is no particulate matrix to extract and the solids content is low. As a consequence, one would expect the displacement and extraction-efficiency effects to be minimal compared with those in peanut butter.

The FTIR calibration equations obtained for canola-oil-and-water mixtures in propanol were:

$$F = 14{\cdot}9991F_i - 0{\cdot}1457W_i \qquad SE = 0{\cdot}010 \qquad (5)$$

$$W = 0{\cdot}2469F_i + 10{\cdot}2054W_i \qquad SE = 0{\cdot}016 \qquad (6)$$

where F_i = instrumental fat-absorbance signal, and W_i = instrumental water-absorbance signal.

Both equations have small secondary coefficients accounting for the interaction of fat and water or the overlap of their absorbances and give rise to linear calibration plots. Since mayonnaise contains two soluble components, the addition of varying weights of a representative sample to propanol cannot be used as an alternative means of calibration, as the calibration will build in a fixed relationship between fat and water, whereas this relationship would be variable in real samples. To avoid this problem, calibration standards were prepared by using randomized amounts of the mayonnaise base ingredients so that any correlation between fat, moisture, and solids was eliminated. The respective equations obtained for the standard curves were:

$$F = 14{\cdot}7966F_i - 0{\cdot}2119W_i \qquad SE = 0{\cdot}020 \qquad (7)$$

$$W = -0{\cdot}0147F_i + 10{\cdot}3549W_i \qquad SE = 0{\cdot}016 \qquad (8)$$

These equations are slightly different from those for the oil–water standards, eqns (5) and (6). Since the mayonnaise standards contain varying proportions of the base components of mayonnaise, these calibration equations should take into consideration all the compositional variations that may be encountered. The analytical results obtained by FTIR for mayonnaises prepared in our laboratory by using eqns (5)–(8) to determine the fat and water concentrations in propanol and eqn (2) to calculate the corresponding values in the product are presented in Table 3. These results follow the same trend as observed for peanut butter, with a bias obtained from the oil–water calibration, which is largely compensated for when the calibration is based on mayonnaise ingredients. In this case, the biases for both fat and water are substantially higher than can be accounted for by displacement effects only, even if one assumes a high extraction efficiency. Although the origin of such a high bias is not directly understood, the acetic acid in vinegar, which has a strong unionized carboxyl band at 1710 cm^{-1}, in the midst of the measurement region, could be a cause of the larger bias. This bias is largely accounted for when the calibration is based on mayonnaise ingredients. Both approaches indicate that the relative accuracy of the FTIR predictions is ca. ±0·30% for both components, with any bias ignored.

Table 3. FTIR mean difference and standard deviation of the difference obtained for fat and moisture in ten mayonnaise samples by using eqns (5) and (6) and (7) and (8) relative to the formulated chemical values

	Eqn (5F)	Eqn (6W)	Eqn (7F)	Eqn (8W)
MD_a	−0·984	−0·906	0·191	0·221
SDD_a	0·277	0·302	0·281	0·285

A set of twenty commercial mayonnaises pre-analyzed by the Mojonnier method were analyzed by the FTIR method by using eqns (7) and (8). The MD_a and SDD_a values were 0·208% and 0·310%, respectively, for fat and 0.200% and 0.264%, respectively, for moisture. There was very little compositional variability in these samples since they all met the specifications legislated for mayonnaise. In order to extend the compositional range, a separate validation set consisting of mayonnaise samples prepared in the laboratory was employed, and Fig. 5 presents plots of the predicted

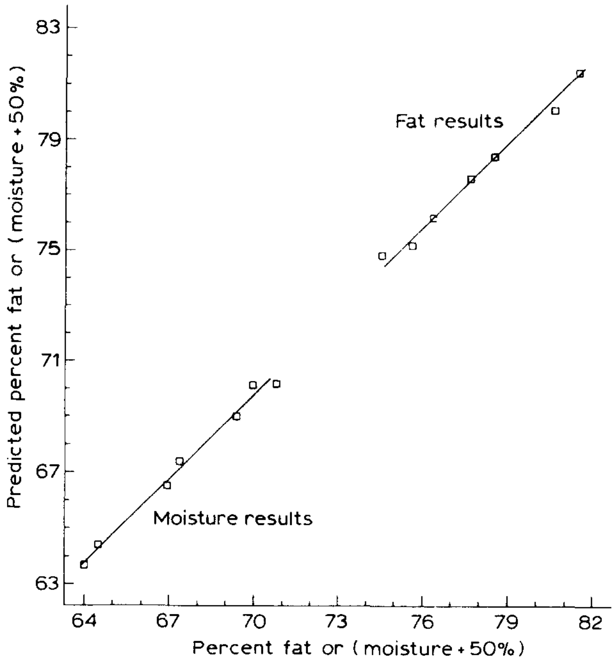

Fig 5. A combined plot of the FTIR results for fat and moisture in mayonnaise samples plotted against their respective Mojonnier chemical values. Moisture content has been increased by a constant (50%) to allow the two plots to be shown on one graph.

against the chemical values for both fat and moisture in these samples. Both plots are linear, with a slope close to 1·0, and the predictions obtained have an SE of <0·3% relative to the chemical values determined by the Mojonnier method. These results are in line with those obtained for the commercial samples and clearly indicate that the FTIR method responds to changes in both fat and moisture.

DISCUSSION

The results obtained in this study parallel those obtained for butter analysis by using a ZnSe ATR crystal, with mixtures of anhydrous butter fat and water in propanol employed as calibration standards (van de Voort *et al.*, 1992*b*). On comparing the ATR approach with the flow-cell-transmission technique, the latter is simpler, more reproducible, and more efficient in terms of sample-handling. In addition, the increased path length provides a stronger IR signal, and the cell is much easier to clean and does not suffer from fat build-up over time, which was a particular problem with the hydrophobic and slightly porous ZnSe ATR crystal. Residual fat was a concern with the ATR cell, since substances on the crystal surface make a major contribution to the IR signal in ATR measurements, whereas only a small portion of the solution, i.e. ca. 1–3 μm in depth, is actually measured. In the transmission mode, any substances on the window surface make only a minor contribution, with the solution (ca. 100 μm) contributing most of the signal. As a result, the 'clean-cell-check routine' required to monitor and account for any residual fat on the ATR crystal after each analysis was not required in this system, which saved time and the need for a residual-fat-correction routine.

For FTIR/propanol-based analyses, maintaining propanol in an anhydrous state is crucial, especially if moisture is analyzed for, since any water absorption in the propanol background spectrum depresses the moisture predictions accordingly. The propanol-dryness-check routine built into the macroprogram ensures that, if the propanol being used contains more than 0·05% moisture, the operator is warned not to proceed. Another factor that has an influence on the FTIR predictions is the ratio of sample to solvent, ideally set at 1:10, owing to the extraction-efficiency and solids-displacement effects, which are affected by this ratio. In practice, especially with a product such as peanut butter, attempting to obtain a specified target weight is difficult and adjusting the weight of propanol delivered accordingly is inconvenient. Our results indicate that delivery of the product weight to within 15% of its target weight, while keeping the weight of propanol constant, did not induce any significant error in the calculated results, which gave some leeway in the weighing procedure.

On the basis of the experience gained in this study, the use of component-based calibrations (i.e. oil; oil–water) is recommended. This suggestion runs counter to the traditional calibration approach based on relating chemically determined values of a series of 'real samples' to the instrumental signals. Using real samples makes the variability in the chemical analyses an inherent part of the error in the instrumental calibration. In FTIR analysis, the instrument is generally more precise than the chemical method; for instance, excellent standard curves for fat and moisture in propanol can be obtained by using component-based calibrations, allowing fat and moisture in propanol to be measured with a high degree of accuracy (0·015%) and reproducibility (0·005%). The subsequent step of converting from component concentrations in propanol to those in the product by using the equation for an ideal system (eqn (2)) will, in most real cases, result in a bias due to such effects as extraction efficiency and solids displacement. A bias-correction factor must then be determined to bring the FTIR data into line with the values obtained from the primary chemical method. This can be achieved, without bringing in the error associated with the chemical analysis, by using a single well-defined chemical value for a representative sample of a particular product (i.e. the mean value obtained by carrying out a comprehensive series of replicate chemical analyses). As a consequence, a direct conversion of instrumental predictions into product compositional data is achieved while maintaining the precision provided by the instrumental method. This advantage can only be properly exploited by the appropriate choice of a representative sample based on a sound understanding of the variables affecting the product being analyzed (e.g. particle size in peanut butter). Thus, formulation differences (e.g. salad dressings with more starch and vinegar against mayonnaise) have to be taken into consideration, and it must be recognized that the same bias correction may not be applicable to different formulations. Calibrating in this manner allows a single calibration to be used for a number of products within a given category (e.g. fine, medium, and coarse peanut butters), requiring only that appropriate bias corrections be determined for each type of product. In circumstances where the oil used in a product (e.g. mayonnaise) is changed (e.g. palm oil substituted for canola oil), the calibration equation obtained by using the original oil will no longer be valid if the substitute oil has an appreciably different saponification number (weight-average molecular weight) from that of the original oil. This is due to the fact that the carbonyl ester-linkage signal, on which the FTIR calibration for fat is based, is a function of the number of ester linkages per unit weight. Accordingly, in such a case, it would be necessary to prepare a new set of calibration standards by using the substitute oil.

An additional advantage of using component calibrations is that the standards are simple to prepare in bulk and are stable as long as they are not exposed to moisture, and tedious chemical analyses of a set of calibration standards are not required. From a day-to-day analytical perspective, one of the standards can be used as a routine-check sample to ensure that both the instrument and calibration are performing consistently.

The discussion above demonstrates significant advantages of the FTIR approach using component calibrations. The inevitable problem faced with any secondary method is the degree of concurrence that one can obtain with respect to a chosen primary method, in this case the Mojonnier method. Primary methods are not immune to factors that may affect their results, as was demonstrated in the case of peanut butter. On the other hand, out of necessity, primary-method results are traditionally considered directly comparable from product to product. In the case of peanut butter, comparing the more variable Mojonnier results with the more consistent FTIR data limits the degree of concurrence one can ultimately obtain. Through the use of a bias correction, one can overcome the major portion of the inherent differences in the factors affecting the two methods (different extraction solvents and procedures and FTIR compared with gravimetric measurements) and obtain predictions from the FTIR method that resemble and parallel the results obtained with a primary method; however, one cannot expect a direct match. Despite these limitations, the FTIR/propanol-extraction method provides fat and moisture results close to the Mojonnier chemical values (*c.* ± 0·30%), and thus can be considered adequate for quality-control purposes.

CONCLUSION

The results from this study and previous work on butter indicate that mid-FTIR spectroscopy of high-fat products added to propanol in a 10:1 ratio provides a sound, simple, and rapid means for the analysis of fat and/or moisture under standardized conditions. All operations considered (weighing, sonication, pumping, and scanning), preparation and analysis of a sample take about 5–7 min and require no particular operator skills, other than using a balance and following a menu-driven program. By using an FTIR spectrometer programmed for this type of analysis, the method described here is particularly suitable for quality-control applications and is rapid enough to monitor an ongoing manufacturing process. It is likely that other high-fat products, such as salad dressings, butter, margarine, chocolate and chocolate crumb, could be analyzed by this technique.

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