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A Rapid Method for the Determination of Fat in Foodstuffs by Infrared Spectrometry

D. A. Cronin & K. McKenzie

Department of Agricultural Chemistry and Soil Science,
University College, Belfield, Dublin 4, Republic of Ireland

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ABSTRACT

A rapid method for the determination of the fat content of foodstuffs by transmittance infrared spectrometry is described. Fat is extracted with a chloroform–methanol (2:1) solvent containing an apolar methylsilicone oil as an internal standard. After partitioning of both components into the chloroform layer, the areas of a strong absorption band at 5·72 μm representing carbonyl ester stretching of the fat and that of a strong band in the internal standard at 7·88 μm due to CH_3Si stretching are recorded. Fat content is estimated from the band area ratio (fat/internal standard) using suitable calibration plots. The calibration strategy used is discussed in some detail. The method was tested on a diverse range of foodstuffs including dairy products, processed meats, biscuit and cakes, chocolate, salad dressings, etc., and the results obtained were generally in good agreement with those given by standard reference methods.

INTRODUCTION

Recent years have seen a remarkable growth in the applications of infrared (IR) spectrometry as an analytical technique in industrial, forensic, food and agricultural laboratories. The use of diffuse reflectance in the near infrared (NIR) region of the electromagnetic spectrum (0·72–2·5 μm) utilising dedicated analysers operating at preselected wavelengths and carefully calibrated for each specific product, is a well established approach of

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increasing importance for the rapid quantitative analysis of macroconstituents such as moisture, fat, protein, fibre, etc., in food and agricultural products (Osborne & Fearn, 1986; Williams & Norris, 1987. Advances in IR instrumentation—in particular the exploitation of the Fourier Transform Technique (Hirschfield, 1983, 1984)—and in accessories for handling difficult samples (Coates *et al.*, 1987) have also led to a resurgence of interest in the analytical capabilities of absorptions occurring in the fundamental IR region (2·5–16 μm). The usefulness of this region in the analysis of natural lipids is well documented (Freeman, 1968; MacLeod, 1971; Christie, 1982).

In 1968 Freeman discussed the potential for the development of quantitative IR methods for estimating different lipid classes; one particular determination considered was that of total esterified fatty acids based on the strong ester carbonyl absorption band. While individual esterified lipid classes such as triacylglycerols, phospholipids and cholesteryl esters do exhibit different absorption maxima (Fig. 1), they appear in the IR spectrum as an essentially unresolved composite band. Free fatty acids, which show a

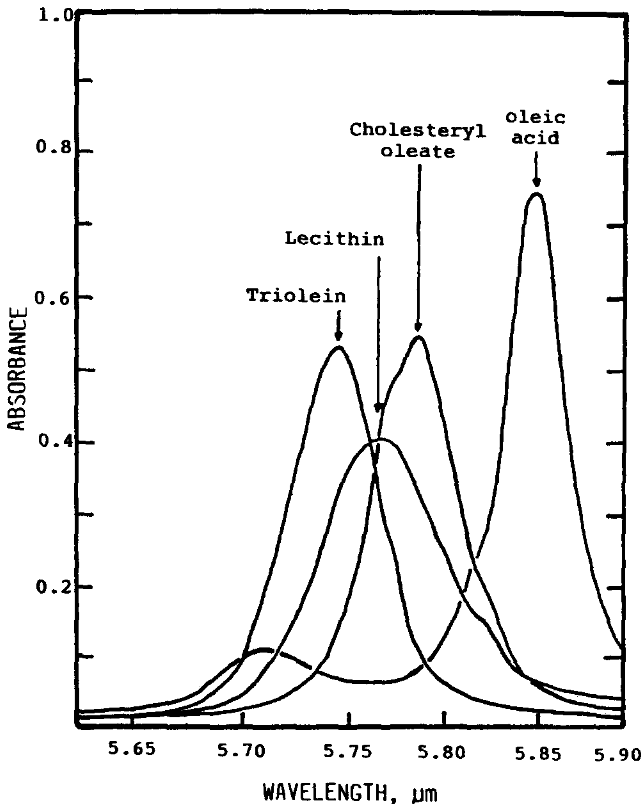

Fig. 1. Carbonyl absorption bands of representative lipid classes. From Freeman (1968).

carbonyl frequency having a maximum at 5·85 μm, are reasonably well resolved from the esterified lipids. Using computerised techniques and integrated carbonyl absorption band areas it was shown by Freeman that total esterified lipids could be determined to within an error of ±5% if the free fatty acids did not exceed about 10% of the mixture.

The primary objective of the present study was to investigate the possibility of developing a quick procedure, applicable to foodstuffs in general, for the determination of fat content based on measurement of the total esterified fatty acids by IR spectrometry. The method proposed here involves the extraction of the fat from the foodstuff with an organic solvent to which a suitable internal standard has been added. The choice of internal standard was critical since it had to satisfy the following requirements:

(a) Possess similar solubility properties to the fat in order to allow non-discriminative coextraction of both components into the extracting solvent.
(b) Exhibit a strong analytically useful absorption band in a region free from interference by major absorption bands of the fat when present at concentrations similar to that of the latter.

Among several compounds examined, the substance which best satisfied both criteria was a low viscosity apolar methylsilicone oil (MS 550), a material which has been extensively used in gas–liquid chromatography as an apolar stationary phase. The oil exhibits a strong sharp absorption band representing CH_3—Si stretching with a maximum at 7·88 μm. This region is largely free of significant interfering absorption bands due to the analyte. The fat content of the extract is obtained from the ratio of the area of the triacylglycerol carbonyl ester stretching band (maximum at 5·72 μm) to that of the selected band of the silicone oil with the aid of suitable calibration plots. This study describes the potential of this new approach for the rapid determination of the fat content of a fairly broad range of common food products.

MATERIALS AND METHODS

Materials

Food materials were obtained from local retail outlets and pure fats from commercial suppliers to the food industry. Homogeneous samples for analysis were prepared using a mill or grinder for solid low moisture material, a pestle and mortar for semi-solid products and a whisking method for liquid foodstuffs. An electric blender was required for certain heterogeneous foods, such as processed meats and layered cakes and biscuits.

Reference methods of analysis

Replicate analyses using a standard method appropriate for the particular product were performed on all samples. Reference procedures were taken from Pearson (1976) and included the Gerber method for milk and gravimetric methods based on acid or alkali digestion and solvent extraction such as the Werner–Schmid and Rose–Gottlieb procedures.

Infrared method for fat analysis

Internal standard solutions
Solutions of methylsilicone oil, MS550 (Phase Separations, Deeside Industrial Estate, Clwyd, UK) were prepared in chloroform (AR grade). Four concentrations representing 5, 10, 15 and 20 mg/ml of the fluid were adequate to cover the range of fat levels present in the products investigated.

Procedure
The sample (2–3 g) was transferred accurately to a 250 ml separating funnel fitted with a Teflon (PTFE) stopcock, followed by 30 ml of a solution of the internal standard, containing the latter at a concentration in the region of the expected fat content of the foodstuffs. Methanol (15 ml) was added, the funnel stoppered and the contents shaken vigorously for 1–2 min in order to extract the fat. Distilled water (150 ml) was then added, and the contents were allowed to settle so as to produce a biphasic system comprising a lower chloroform lipid layer and an upper methanol–water layer of non-lipid material. A little of the (usually turbid) chloroform layer was allowed to percolate through a small bed of anhydrous sodium sulphate supported in a high-porosity micro-sintered glass funnel and 1–2 ml of clear chloroform extract was collected.

Two KBr windows (diameter 25 mm) were placed on tissue paper on a flat surface in a fume hood directly under a laboratory air gun or hair drier. Approximately 0·2 ml of the chloroform extract was applied from a hypodermic syringe in a stepwise manner to the faces of both windows, allowing solvent to evaporate under a stream of cool air from the air gun between each application. A short sleeve of narrow-bore Teflon tubing was fitted over the tip of the hypodermic needle to protect the surfaces of the KBr windows from being scratched. The two windows were pressed together, gently moving the top one over the bottom face to ensure an even film of coating on each surface. A small drop (approx. 10 mg) of liquid paraffin (IR grade), applied to one of the KBr windows directly after coating the sample, was found to facilitate uniform dispersion of the sample over the window

surfaces, especially for solid fats which tended to be somewhat 'sticky' in nature.

Infrared determination

Infrared analysis was carried out using a double-beam Pye Unicam SP 1100 Infrared Spectrometer which operates in the range 2·5–30 μm. The sample was scanned over the wavelength range of interest in this study (5·5–9 μm) using the maximum scanning speed of the instrument (3 min per full range scan). A typical scan of a biscuit fat extract recorded on plain chart paper is shown in Fig. 2. The areas under the internal standard band at 7·88 μm and carbonyl ester band of the fat at 5·72 μm were determined by triangulation (height × width at half height). Band widths were measured with the aid of a magnifying eye glass incorporating 0·1 mm scale graduations. From the band area ratio (esterified fatty acids/internal standard) the fat content of the sample was estimated with the aid of an appropriate calibration plot.

Preparation of calibration graphs

At least five samples of pure reference fat covering the range 40–180 mg were accurately weighed into 10 ml screw-cap vials. To each was added 5 ml internal standard solution (20 mg/ml) and the samples mixed thoroughly. Infrared analysis was carried out as described above and a graph of fat concentration (mg) against band area ratio was plotted.

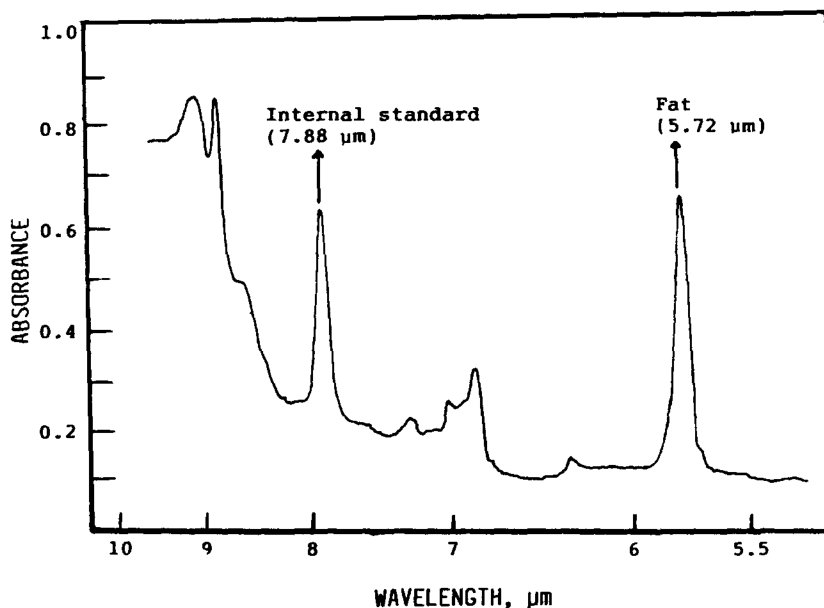

Fig. 2. Infrared scan of biscuit fat extract.

RESULTS AND DISCUSSION

Calibration of the method

At the outset of this study some doubt existed as to whether it would be possible to devise a calibration strategy of sufficient accuracy to accommodate the analysis of certain processed foodstuffs where there was considerable uncertainty as to the actual composition of the fats used therein. While calibration plots (weight of fat (mg) vs band area ratio) for individual fats were found to exhibit excellent linearity over the range examined and only began to deviate at ratios of greater than about 3·5 at the upper end, it was also observed that the plots had somewhat different slopes which were related to the fatty acid composition of the fats and specifically, to their Saponification Numbers (SN). The largest variations occurred between fats differing widely in SN values as exemplified in Fig. 3 for lard (200), butter oil (227) and coconut oil (268).

In order to assess the extent of slope variations which might arise in the analysis of fairly diverse food types, calibration plots were prepared for a range of 17 common pure and commodity specific fats and their slopes and intercepts calculated. On the basis of their SN values it was possible to group the fats as shown in Table 1. The major group (Group A) had 11 fats with SN values in the region of lard or beef-tallow while Group B consisted of five

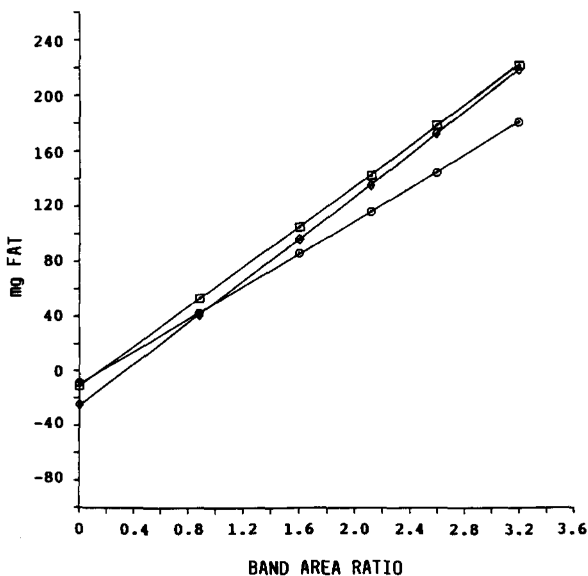

Fig. 3. Calibration plots for fats which differ considerably in saponification numbers. □ = lard; ◇ = butter oil; ○ = coconut oil.

TABLE 1
Calibration Data on Reference Fats

Fat	*Saponification number*	*IR calibration plots*	
		Slope (*m*)	*Intercept* (*c*)
Group A			
Dough fat I	207·1	66·5	1·06
Dough fat II	198·0	72·25	−6·74
Dough fat III	204·6	74·13	−7·99
Toffee fat I	200·5	76·83	−8·67
Toffee fat II	203·2	74·28	−10·75
Margarine fat I	196·9	86·19	−19·92
Margarine fat II	190·7	74·60	−5·81
Frying oil	197·7	80·43	−16·22
Chocolate fat	198·8	85·31	−20·00
Lard	200·0	72·50	−10·99
Beef tallow	199·9	70·52	−2·07
Composite A		74·36	−8·57
Group B			
Palm kernel oil	247·2	72·79	−28·14
Ice-cream fat	241·2	66·15	−18·0
Whipped topping fat	235·1	67·31	−16·0
Confectionery fat	246·1	60·9	−9·5
Composite B		67·01	−17·85
Group C			
Butter oil	227	75·94	−25·46
Group D			
Coconut oil	268	59·83	−10·64

fats with SN values close to that of palm kernel oil. Butter oil with an intermediate and coconut oil with a very high SN value were placed in separate groups. The superimposed plots for the eight most divergent fats of Group A are shown in Fig. 4. It is of interest that at band area ratios in the approximate range 0·9–1·4, representing roughly comparable amounts of fat and internal standard, the slopes for all the fats converge into a fairly narrow band. A similar situation arises for Group B fats at a band area ratio 1·3–1·8. These data suggested that, provided the amount of internal standard incorporated into the product was in the region of the expected fat content, the latter might be determined with an acceptable degree of accuracy in the majority of foodstuffs with the aid of a limited number of calibration plots.

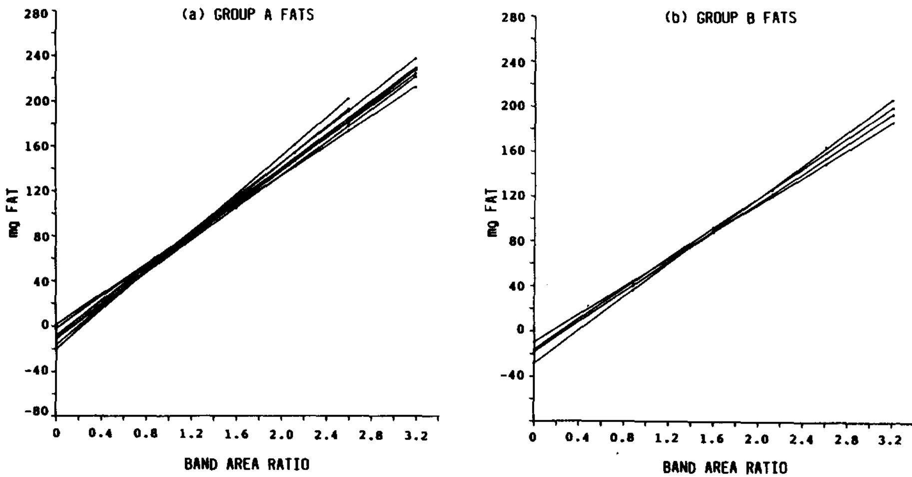

Fig. 4. Calibration plots for reference fats showing convergence at band area ratios in the region 0·9–1·4 (Group A fats) and 1·3–1·8 (Group B fats).

These would consist of: (a) a butter oil calibration for dairy products (b) a composite plot from Group A fats which should be suitable for a large proportion of non-dairy products and (c) a composite plot for Group B fats which might be more useful for foodstuffs containing relatively large amounts of fats with high saponification numbers.

Composites were prepared by linear regression analysis and the calculated slopes (m) and intercepts (c) are given in Table 1. The latter, together with the band area ratio (x) were used to calculate the weight of fat (y) in the extracts from the expression:

$$y = mx + c$$

Analysis of foodstuffs

Results for the analysis of fat levels in a representative range of foodstuffs are presented in Table 2. Standard deviations are given in brackets for each product.

In general, there was good agreement between reference and IR methods of analysis with 15 out of 20 products examined giving results which were not significantly different at the 5% level of significance ($p = 0{\cdot}05$). Five products (indicated by asterisks) fell outside this range with liquid whole egg showing the largest discrepancy. Precision was somewhat variable, being excellent for some products and less satisfactory for others. Taking all foodstuffs, the average standard deviation was 0·22 for the reference methods and 0·28 for the IR procedure. Apart from dairy products, where

TABLE 2
Results for Analysis of Fat Content in Various Foodstuffs by Infrared Spectrometry

Foodstuff	*Fat content (g/100 g) (reference method)*	*Fat content (g/100 g) (IR method)*	*Calibration used*
Dairy Products			
Milk	3·30 (0·01)[c]	3·30 (0·01)	Butter oil
Cottage cheese	4·35 (0·1)[a]	4·17 (0·1)	Butter oil
Cheddar cheese	28·94 (0·04)[a]	29·36 (0·37)	Butter oil
Meat Products			
Frankfurters	19·71 (0·1)[a]	19·42 (0·79)	Composite A
Beefburger	32·54 (0·13)[a]	32·75 (0·40)	Composite A
Sausages	21·75 (0·27)[a]	21·01 (0·65)	Composite A
Biscuits and Cakes			
Shortcake	20·02 (0·68)[a]	19·68 (0·07)	Composite A
*Marietta	12·94 (0·01)[a]	11·84 (0·41)	Composite A
Raspberry creams	23·45 (0·66)[a]	23·03 (0·16)	Composite A
*Nice	16·36 (0·27)[a]	17·40 (0·54)	Composite A
Cream crackers	13·20 (0·11)[a]	13·40 (0·08)	Composite A
Madeira cake	15·81 (0·10)[a]	15·58 (0·04)	Composite A
*Ripple Swiss roll	8·80 (0·11)[a]	8·20 (0·14)	Composite A
Miscellaneous Products			
Chocolate	30·37 (0·36)[a]	29·13 (0·65)	Composite A
Chocolate ice-cream	19·17 (0·38)[b]	20·39 (0·15)	Butter oil
		19·00 (0·15)	Composite B
Salad cream (I)	27·35 (0·49)[a]	26·86 (0·42)	Composite A
Salad cream (II)	24·77 (0·16)[a]	24·30 (0·01)	Composite A
*Salad dressing (low calorie)	9·48 (0·02)[a]	8·75 (0·04)	Composite A
'Instant' custard powder	8·42 (0·26)[a]	8·14 (0·12)	Composite B
*Whole liquid egg	10·84 (0·19)[b]	9·03 (0·25)	Composite A
		10·58 (corrected)	See text

[a] Werner–Schmid Method; [b] Rose–Gottlieb method; [c] Gerber method.
* Significantly different at $p = 0{\cdot}05$.

the butter oil calibration was most appropriate, the composite calibration for Group A fats was satisfactory for most of the other foodstuffs examined. Moreover, provided the band area ratios were not significantly outside the ranges mentioned above, there was generally little difference between results calculated from the composite calibration and those obtained from a product specific fat, e.g. lard or tallow for meat products, dough fat composites for confectionery, etc. The composite calibration for Group B fats gave better agreement with the reference method for only two products,

chocolate ice-cream and 'instant' custard powder—the latter was labelled as containing palm kernel oil as the major fat component.

The data in Table 2 also show that fat contents estimated by the IR method are lower than those given by the reference procedure in about 75% of the foods analysed. This is not surprising since extraction/gravimetric methods will estimate all lipid material in a product, whereas the IR technique, which is based on the determination of total esterified fatty acids, is perhaps a better measure of 'true fat'. The substantially lower result given by the IR method for liquid egg is explained by the complex nature of egg lipids which comprise 66% triacylglycerol esters, 28% phospholipids, 5% cholesterol and 0·5% free fatty acids. A phospholipid such as lecithin will, on a per g basis, yield only 0·75 g of fatty acids compared to 0·96 g from a triacylglycerol of similar fatty acid composition. Since the IR method was much more rapid than the Rose–Gottlieb reference procedure and gave quite good precision, it is suggested that the IR approach is, in fact, a feasible one for estimating total fat in egg if a correction of approximately +17% is added to the figure given by the composite calibration. A mixture of equal weights of extracted egg lipid and internal standard gave a band area ratio which was 17% lower than that predicted by the composite calibration for the same relative amounts of fat and internal standard.

In the early stages of evaluating the IR procedure an acid digestion treatment, followed by extraction of the fat into a solution of hexane containing the internal standard, was used. Besides being much slower than the chloroform–methanol technique, there was also a tendency to liberate small amounts of free fatty acids by hydrolysis; the latter appeared as a small band at 5·85 μm on the leading edge of the ester band. The presence of a little free fatty acid was only observed in one product (sausages) using chloroform/methanol extraction.

In this study the time required—from weighing of the sample to acquisition of the result—was approximately 20 min for replicate analysis of most of the products examined. There is the potential to shorten this time considerably, perhaps by as much as 50%. For example, some saving in time could result from direct analysis of the chloroform extracts using matched liquid cells with pure solvent in the reference beam. More significant benefits would accrue from the use of an IR spectrometer with a facility for integration of band areas; the rather slow and tedious triangulation method adopted here was only used because the 12 year old instrument did not have such a facility. It is very likely that the use of a modern instrument with integration mode and computerised data handling system incorporating facilities for storage and selection of calibration data and for quantitative analysis would yield significant improvements in respect of speed, accuracy and precision.

CONCLUSIONS

This study has shown that, with the aid of a suitable internal standard, a food extract could be rapidly prepared in a form suitable for quantitative estimation of fat content by infrared spectrometry. Provided the internal standard and the fat were present in the extract at roughly similar concentrations, a relatively simple calibration strategy was required to produce results which were in good agreement with those given by standard reference methods for the majority of products examined. The proposed method, which is capable of further improvement and refinement, extends the utility of IR spectrometry as a versatile tool in the analysis of food lipids.

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