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Rapid scan correlation NMR spectroscopy for food analysis

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The technique of rapid scan correlation (RSC) NMR combines the speed advantage of Fourier transform NMR with the dynamic range advantage of the continuous wave method. In this paper, results of quantitative studies are reported along with qualitative results from a variety of foods and food-related systems. It is concluded that RSC NMR is a rapid, non-invasive method, which requires no sample preparation, for the qualitative and quantitative analysis of a number of food components.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy today plays a vital role in every research laboratory world-wide. A high-resolution NMR spectrum can give detailed information on, amongst other things, chemical structure, composition (i.e. what is present and in what quantity) and dynamics of the system under study. It is possible to obtain complete structural determination of unknown compounds and now even three-dimensional solution-state structures of complex proteins (Clore & Gronenborn, 1991).

In most spectrometers in current use, an NMR spectrum is obtained by placing the sample under study in a highly uniform strong magnetic field (1·4–12 T). The sample is then perturbed by giving it a short (μs) intense (approximately 100 W) pulse of radio frequency energy (60–500 MHz). The return of the excited system to equilibrium is recorded. This decaying response is known as free induction decay (FID). To obtain the NMR spectrum in its most useful form a mathematical operation, a Fourier transform (FT) is next carried out on the FID. This converts the decay (intensity vs time) into a spectrum (intensity vs frequency) from which useful chemical information can be extracted. The short intense pulse used in the FT NMR experiment excites all the nuclei in the sample at once and the resulting FID thus contains information from all nuclei in the sample. Because the decay is rapid a number of these FIDs can be co-added to increase signal-to-noise ratio (S/N). This, along with the fact that all the absorbing nuclei contribute to each point in the FID, gives the FT NMR technique the well-known multiplex advantage (Sheppard, 1990). In contrast to this, the continuous wave (CW) method of obtaining NMR spectra sweeps the frequency while keeping the field constant. Here each point in the spectrum has contributions only from nuclei which absorb at that frequency, thus there is no multiplex advantage. In practice, it is often easier to sweep the magnetic field and maintain a constant detection frequency. This is, however, an exactly equivalent operation to the frequency sweep.

Compared with pure research, the use of FT NMR for the routine analysis of foods has had limited applications. One of the reasons for this is the cost. FT NMR requires sophisticated electronic hardware which must produce pulses of radiation with precisely controlled phase and duration, and often involves the use of a superconducting magnet. The high capital outlay is prohibitive when routine simple tasks are required for applications such as quality control. Another major problem with FT NMR is the dynamic range problem, which results from the multiplex nature of the experiment. If there is a large signal in the presence of a small one the large signal has to be scaled to fit the analogue to digital convertor; this may prevent adequate digitisation of the small signal and in the worst case the small signal may not be digitised at all. This situation, i.e. large signals in the presence of small, occurs in many food systems, water often being the major signal.

The problems of cost and dynamic range can be overcome by using CW NMR. CW NMR spectrometers are cheap and, by partially scanning a region of a spectrum, can avoid large signals. However, the major problems with CW NMR are its poor S/N and slow speed of data acquisition. In CW NMR spectral acquisition times can be very long because the field sweep rate must be much less than $1/T_1$ or $1/T_2$ (T_1 is the spin–lattice and T_2 the spin–spin relaxation time) for

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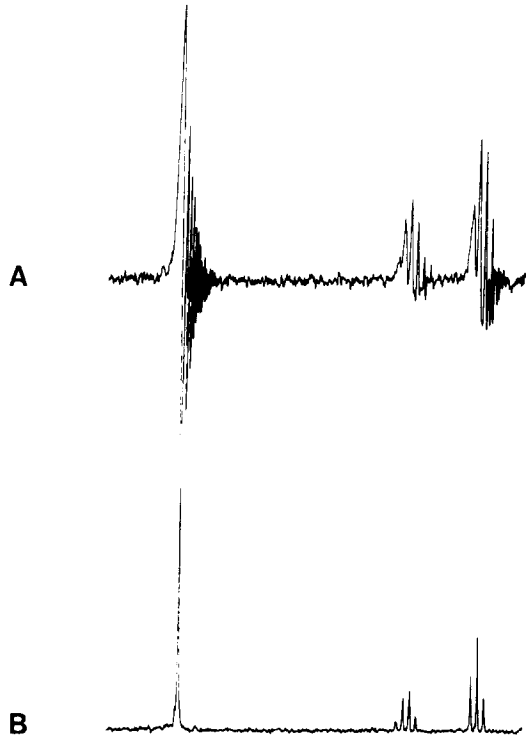

Fig. 1. Rapid scan spectrum of ethyl benzene before (A) and after (B) correlations.

the sample, otherwise distortion of the peaks occurs. This distortion appears as 'ringing' around the base of the peaks, which makes spectral interpretation and quantification difficult (Fig. 1(A)). Rapid scan correlation (RSC) NMR (Dadok & Sprecher, 1974; Gupta *et al.*, 1974) is a technique which overcomes both problems associated with CW NMR. It is, in essence, based upon a CW spectrometer but with the added advantage of the ability to co-add rapidly scanned spectra and thus achieve a much larger S/N ratio in the same time it would take to acquire one high-resolution CW spectrum. Rapidly scanned spectra give rise to distortion which must be removed. This is achieved by a mathematical process which cross-correlates the rapidly scanned spectrum with a function

$$E(t) = \exp(-ibt^2) \quad (1)$$

where b is the sweep rate in radians per second and t is the time after peak maximum. The actual correlation process is carried out by simply multiplying the inverse FT of the rapidly scanned spectrum by (1). The resulting decay, in the time domain, is forward Fourier transformed to give the undistorted spectrum. Figure 1 shows a spectrum before and after correlation. Thus there is now a way of rapidly scanning spectra which can then be co-added before correlation to remove distortion. This results in an increase in S/N when compared with CW spectroscopy per unit time. The speed of data acquisition for RSC NMR rivals that for FT NMR. It must be noted, however, that RSC NMR does not have the true multiplex advantage of FT NMR. However, under favourable conditions the S/N ratio available from RS NMR can be similar to that from FT methods per unit time (Gupta *et al.*, 1974).

In this paper, RSC NMR is applied to the analysis of various foodstuffs. Examples of aqueous systems where the peaks of interest are far from the interfering water peak (ethanol) and where they are nearer (glucose) were chosen to investigate quantitative aspects of this form of spectroscopy. Oils and fats are also analysed. RSC NMR is shown to be a rapid, sensitive, non-invasive method for food analysis.

MATERIALS AND METHODS

A Hitachi (Nissai Sangyo) R-1200 rapid scan spectrometer was used in all experiments. The spectrometer operated at 60 MHz, and the permanent magnet was thermostated at 35°C. The samples were equilibrated at 35°C before spectral acquisition.

The mayonnaise, margarine, wine and beer samples were all obtained from a local retail source. The ethanol used was 95% v/v (Hayman). The glucose and fructose were obtained from BDH. The ethanol determinations, other than by NMR, were carried out using standard methods in an independent analytical laboratory.

An NMR spectrum is obtained on an RSC NMR spectrometer in exactly the same way as a normal CW NMR spectrum. The resolution was adjusted on a tetramethylsilane (TMS) standard before the samples were run, then the r.f. power, amplitude, and start and stop positions for the field sweep were set for each sample and the spectrum was acquired. A detailed discussion on choice of acquisition parameters and how they affect the spectra has been given elsewhere (Barjat *et al.*, 1993). The spectral parameters varied for each sample and are given where appropriate in the results section. The only difference between this RSC spectrometer and a conventional CW spectrometer, apart from the extra computing processing needed, is the field drift compensation adjustment. If a large number of scans are being co-added any drift in the magnetic field must be compensated for. In CW NMR, because of the slow data acquisition field drift, compensation is achieved by using a deuterium lock. For RSC NMR, it is achieved by selecting a peak in the spectrum for the spectrom-eter to use as a reference and shifting subsequent scans so that the peak maxima overlap.

RESULTS AND DISCUSSION

The analysis of the ethanol–water system typifies that of many systems by the food industry in which a small amount of dissolved mater must be analysed in the presence of a large quantity of water. The spectrum of a 0·475% v/v solution of ethanol is shown in Fig. 2. The ethanol cannot be seen under normal acquisition conditions, that is, wide spectral scan and sweep rate of 10 s per scan with the receiver gain adjusted so that no overloading was caused by the large water signal. However, by avoiding the water region and concentration on the region of interest, the receiver gain could be

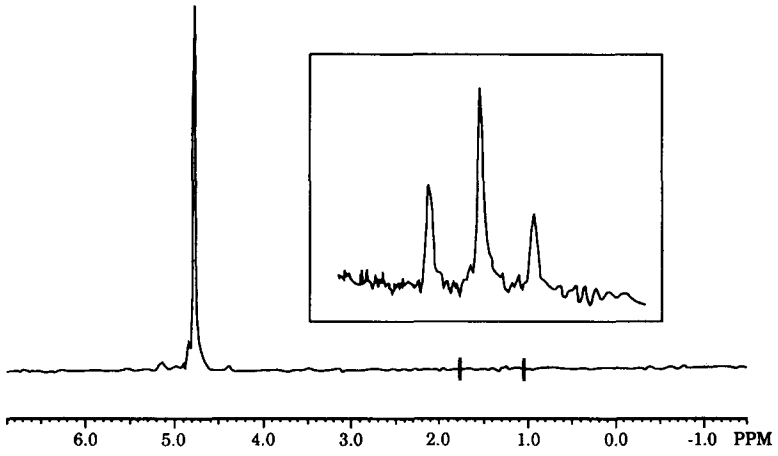

Fig. 2. Spectrum of 0·475% v/v ethanol in water. The expanded region shows the result of increased amplification and partial scanning.

increased along with the r.f. power so that the methyl peak of ethanol could be observed (expanded region of Fig. 2). The methyl peak of ethanol was chosen for the quantitative study as it is furthest from the intense water peak (approximately 4·7 ppm). A calibration set was prepared from 15 to 3% v/v ethanol–water and peak areas were measured. The acquisition conditions were a 300-Hz expansion at 15 s per scan, low r.f. power and 16 scans being co-added. Each measurement took a total of 4 min. Detailed discussion on the determination of the optimum acquisition conditions has been given elsewhere (Barjat *et al.*, 1993). A calibration curve is shown in Fig. 3. A number of wine and beer samples were then analysed and the results compared with those obtained from other standard methods and the values given by the manufacturers on the label. These results are displayed in Table 1. The results obtained by RSC NMR are in good agreement with those given by the manufacturer. The results from gas chromatography (GC) and distillation/specific gravity (SG) are, within experimental error, the same as from RSC NMR, illustrating that at this stage in the development of the RSC NMR method the results are at least as good as those from standard methods.

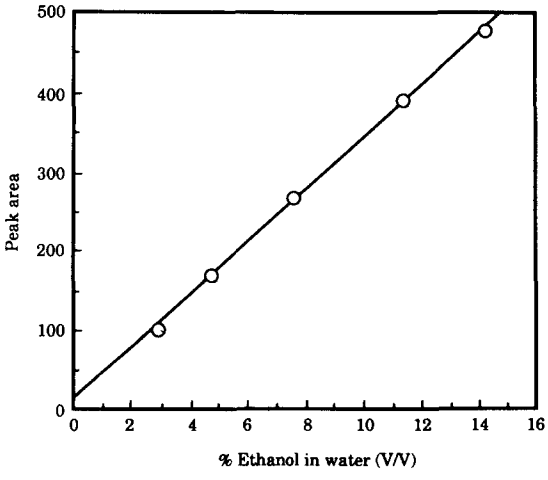

Fig. 3. Calibration curve for ethanol in water from 2·85 to 14·25% v/v. The line has gradient 32·95, intercept 13·02 and a correlation coefficient of 0·999.

The distance of the methyl peak of ethanol (approximately 1·5 ppm) from the intense water peak (approximately 4·7 ppm) makes analysis less complicated than the case where peaks of interest are near the water peak. An example of this kind of problem is glucose in water. The major peaks of glucose (or any sugar) appear around 3–5 ppm, close to the water peak. The spectra of five glucose solutions are shown in Fig. 4. The acquisition conditions were a 300-Hz expansion at 60 s per scan with amplitude 12, r.f. power normal and 16 scans co-added. The sloping baseline results from the tail of the water peak appearing in this region. For the glucose system the peaks in the 3–4 ppm region were too weak to be used for field drift compensation so 5 μl of acetone was added to each sample to act as a reference. As a result of overlapping peaks in this region, peak intensities were measured and plotted against concentration rather than peak areas. The results are shown in Fig. 5. Good correlation is observed. In the case of glucose, good shimming is important, as the tail of the water peak, if the shimming is bad, will produce serious baseline distortions which can affect the measurement of peak areas or heights, thus resulting in errors in calibration.

Table 1. Comparison of ethanol percentages (v/v) obtained by RSC NMR with results from other techniques

Sample	RSC NMR[a] (% ethanol)	GC[b] (% ethanol)	Distillation/SG[c] (% ethanol)	Ethanol content claimed on label (% ethanol)
Muscadet	12·5	12·1	12·1	12·5
Niersteiner	9·3	9·2	9·2	9·5
Côtes du Rhône	12·4	12·1	12·1	12·0
Lager	5·2	5·1	5·1	5·0

[a] Estimated error ±0·22%.
[b] Error not reported.
[c] Estimated error ±0·35% for wine samples and ±0·14% for the beer.

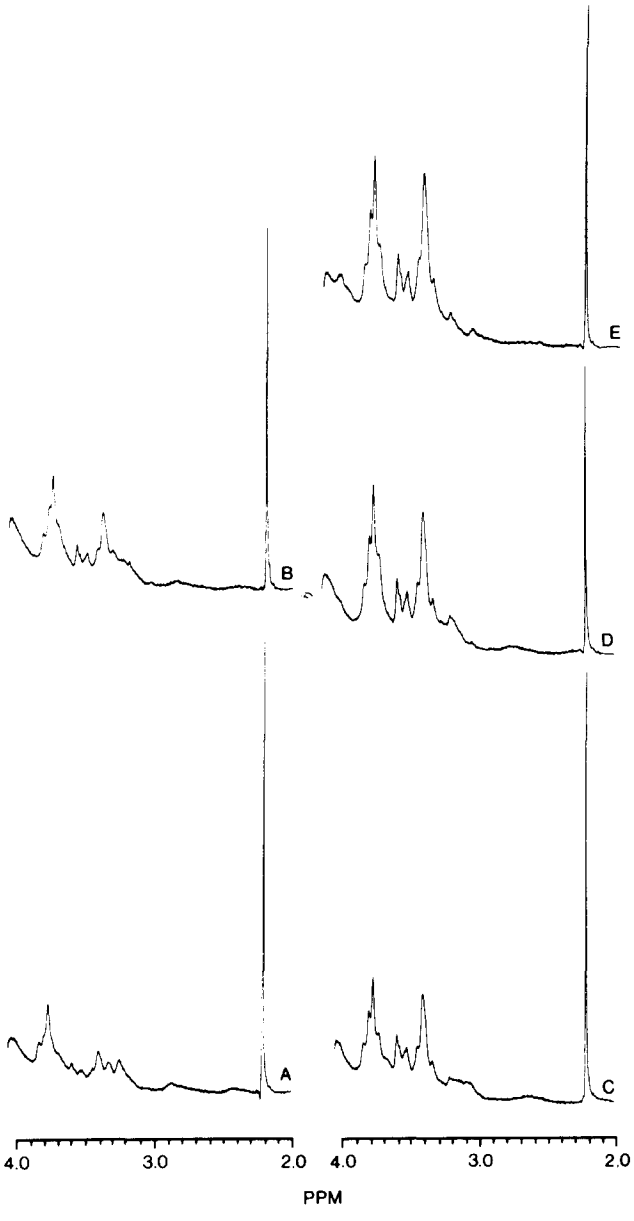

Fig. 4. Spectra of glucose in water at concentrations (A) 0·17, (B) 0·33, (C) 0·5, (D) 0·67 and (E) 0·83 mol dm^{-3}.

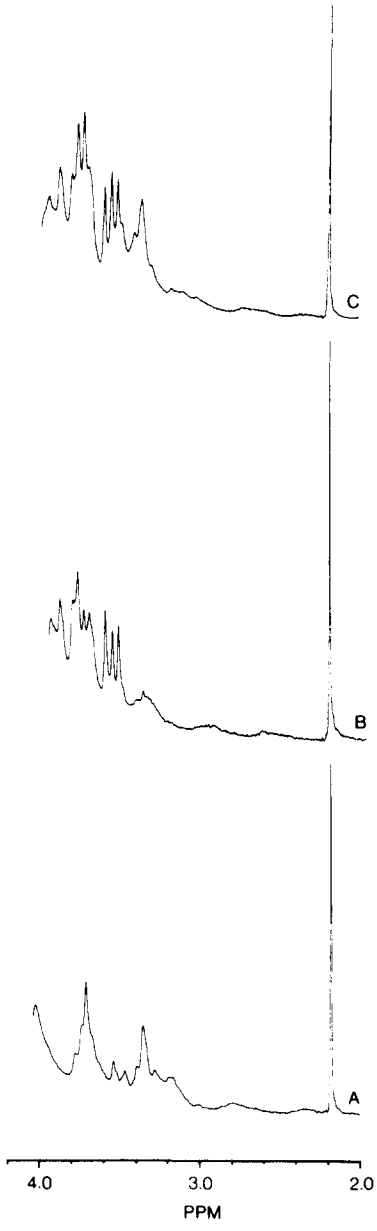

Fig. 6. (A) Glucose in water at 0·33 mol dm^{-3}. (B) Fructose in water at 0·33 mol dm^{-3}. (C) Glucose + fructose in water (1 : 1) at 0·33 mol dm^{-3}.

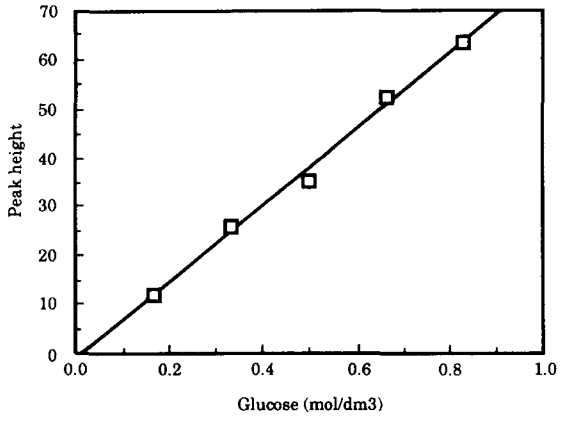

Fig. 5. Calibration curve for glucose in water from 0·17 to 0·83 mol dm^{-3}. The line has gradient 77·10, intercept −0·95 and correlation coefficient 0·993.

In real food systems sugars are usually present as a mixture. Figure 6 shows the spectra of glucose, fructose and a mixture of the two. It may be possible to measure levels of glucose in the presence of fructose using peaks at 3·4 and 3·78 ppm and the peaks at 3·55, 3·60 and 3·95 ppm for fructose in the presence of glucose; this suggests that quantitative analysis of mixtures of glucose and fructose in, for example, soft drinks may be possible.

Oils and fats are an important class of materials for analysis in the food industry. To investigate the possibilities for using RSC NMR for analysis of such materials, samples were taken of three fatty acid esters (18 : 1, 18 : 2 and 18 : 3) dissolved in CCl_4. The experimental conditions chosen were the same as for glucose, with the exception that the scan rate was 30 s per scan and the expansion was 400 Hz. The results are shown in Fig. 7. Inspection shows that the intensity of the

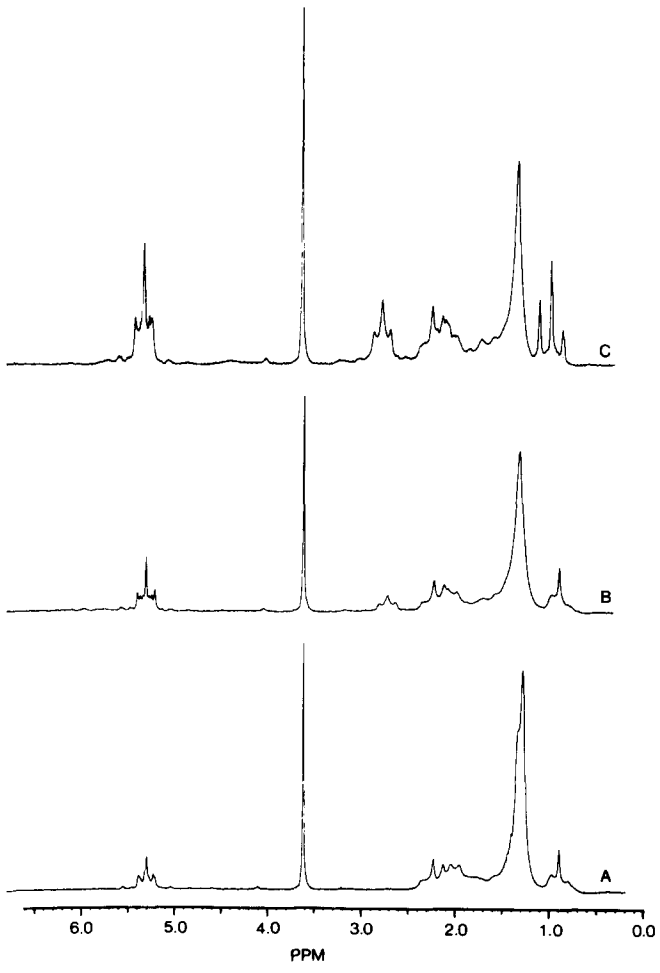

Fig. 7. (A) Oleic acid (18:1) ester in CCl_4. (B) Linoleic acid (18:2) ester in CCl_4. (C) Linolenic acid (18:3) ester in CCl_4.

peaks was at 5·3 ppm associated with olefinic linkages decreases with the level of unsaturation. There is a corresponding relative increase in the intensity of the bulk methylene protons at 1·2 ppm. The observed intensity ratios (height and area) were 6·56, 2·92 and 1·66 for 18:1, 18:2 and 18:3, respectively.

In many cases the samples of interest will be neat oils. Samples of neat linseed, soya and olive oil were run, and the results are shown in Fig. 8. The spectra show six main groups of peaks: approximately 0·9 ppm (terminal CH_3 groups); 1·3 ppm (protons from CH_2 groups bound to two saturated carbons CH_2—CH_2—CH_2); 2·2 ppm (protons from CH_2 groups in alpha position to the carboxyl groups, —O—C=O—CH_2— and protons of CH_2 groups attached to the double bond carbons, —CH_2—CH=); 2·9 ppm (protons on carbons situated between two double bond carbons, =CH—CH_2—CH=); 4·2 ppm (CH_2 protons at the ester end of the triglyceride molecule); 5·3 ppm (protons on double bond carbons and the proton on the middle carbon of the triglyceride unit). These spectra show variations in peak intensity for the different samples. It can be seen that the intensity of the peak at 5·3 ppm decreases on going from A to E (Fig. 8); this result suggests decreasing unsaturation. Table 2 shows the ratio of the intensities at the peaks at 5·3 and 4·2 ppm

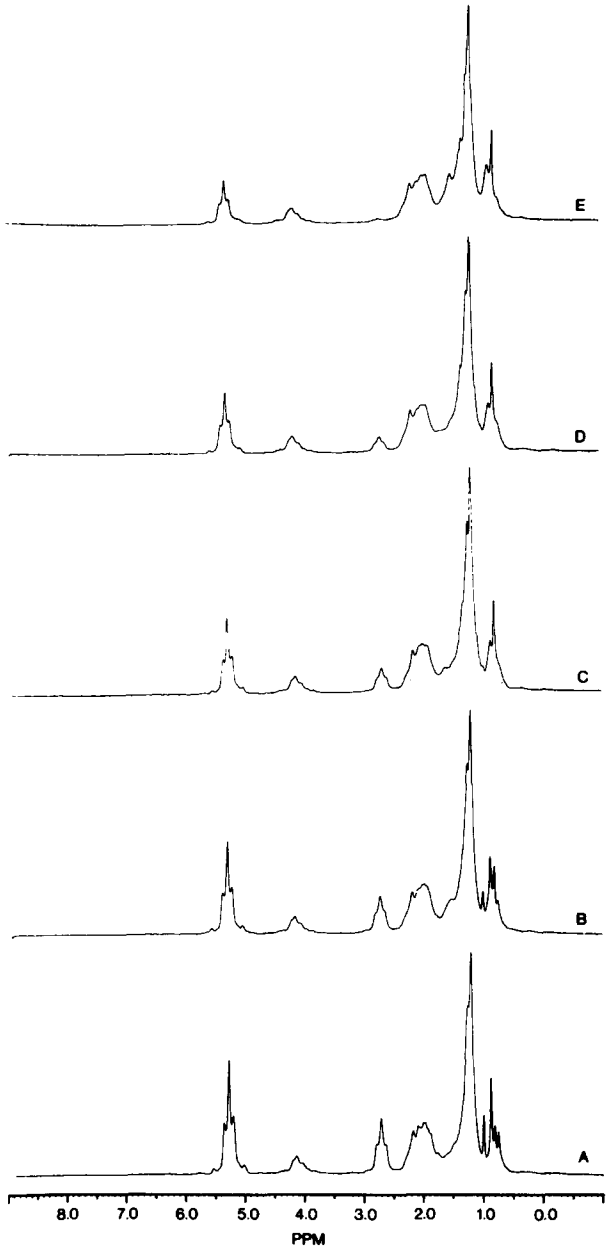

Fig. 8. (A) Linseed oil; (B) linseed–soya (50/50) oil; soya oil; (D) soya–olive (50/50) oil; (E) olive oil.

for three of the oil samples. Previous studies (Gunstone *et al.*, 1986) have shown that unsaturation increases in the order olive > soya > linseed; our results are in agreement with this.

Finally, real food systems which contain fat and water were studied. All samples needed no prior treatment before analysis. Figure 9 shows spectra of normal and low-fat margarine at a scan rate of 30 s per scan with 16 scans co-added. The water peak was scaled to

Table 2. Peak height ratio measurements for three pure oil samples

Sample	$I_{5.3}/I_{4.2}$	Spectra (Fig. 8)
Linseed	3·25	A
Soya	2·31	C
Olive	1·31	E

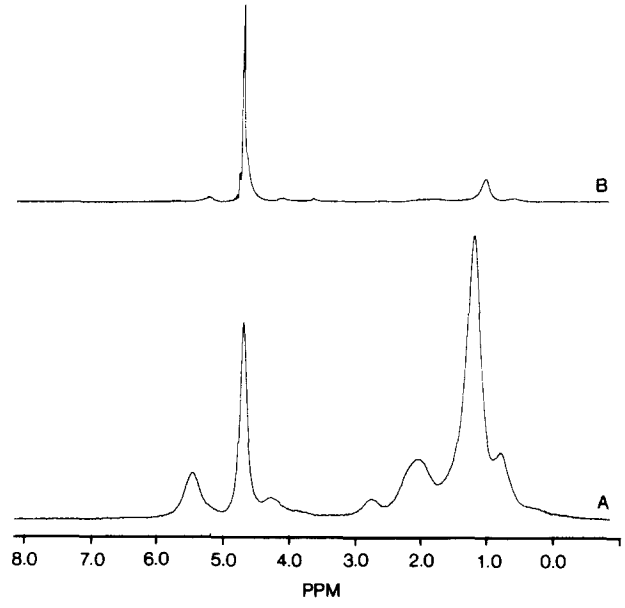

Fig. 9. (A) Margarine; (B) low-fat margarine.

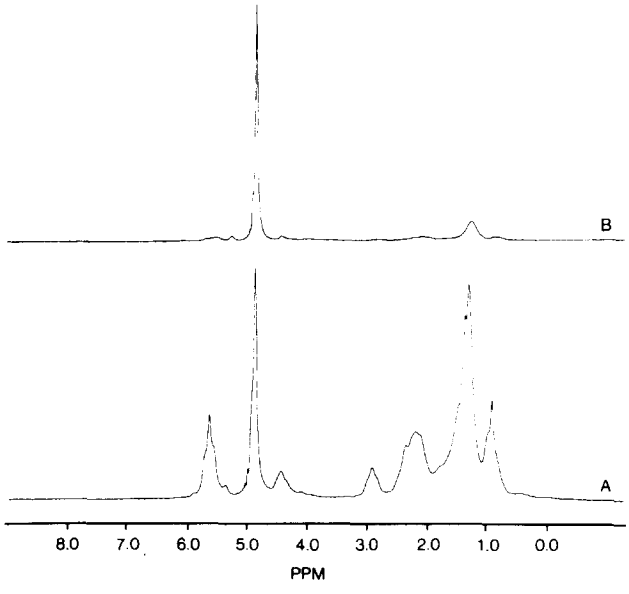

Fig. 10. (A) Mayonnaise; (B) low-fat mayonnaise.

be the same in both spectra. Figure 10 shows normal and low-fat mayonnaise. Both sets of spectra clearly show the decrease in fat levels for the low-fat samples.

CONCLUSIONS

The results obtained show that RSC NMR is a rapid, non-invasive method for the qualitative and quantitative analysis of a range of foods. The ethanol and glucose studies indicate the quantitative nature of the technique for aqueous systems, and the analysis of fats in a range of foods and food systems suggests that quantitative determination may also be possible in these cases. The nature of RSC NMR means that the signals are obtained, at some point, in the time domain. Thus techniques used in FT NMR, such as filtering and resolution enhancement, can be applied to increase spectral quality. No prior sample preparation is needed and the hardware requirements for RCS NMR result in the cost being much reduced when compared with FT NMR.

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