

# Near infrared spectroscopy — a potentially useful method for rapid determination of fat and protein content in homogenized diets

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**Objective:** To investigate the potential of near-infrared reflectance (NIR) spectroscopy as a rapid and non-destructive method to determine total fat and protein in mixed, homogenized and freeze-dried human diets.

**Design:** 29 students collected duplicate portions of their diet for four consecutive days. In addition, a detailed food diary was kept. The duplicate portions were analysed for total protein and fat content both by traditional chemical analysis (Kjeldahl and Folch methods) and through the recently developed NIR spectroscopy method. In addition, traditional computerized estimation of nutrient composition was performed.

**Results:** Plotting of the NIR-predicted fat content against the chemically analysed fat content gave a correlation coefficient of 0.99. Plotting of the NIR-predicted protein content against the Kjeldahl-analysed protein gave a correlation coefficient of 0.81.

**Conclusion:** NIR-spectroscopy seems to be able to determine fat content in mixed, homogenized diets to a high degree of accuracy. In surveys involving duplicate portion sampling this will save time and money. The prediction accuracy for protein was less convincing, but acceptable depending on the need for accurate individual data.

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## Introduction

The determination of the quantitative and qualitative composition of the diet is essential to dietary research. The necessary analytical procedures are, however, often complex and time consuming. Recently, near-infrared reflectance (NIR) spectroscopy has been introduced as an alternative to or even a replacement for the traditional chemical methods for nutrient measurements in stools (Benini *et al*, 1989, 1992) and foods (Lanza, 1983). NIR spectroscopy is a rapid, non-destructive method, which has found widespread use in compositional analyses such as fat, protein, water and carbohydrates (Osborne & Fearn, 1986; Williams & Norris, 1987). The method uses the wavelength region from 700 to 2500 nm. The spectrum is a result of specific light absorption from C–H, O–H, N–H and C=O overtones and combinations of bands at different wavelengths (Murray & Williams, 1990). These chemical analytes have strong, overlapping peaks in the NIR spectra, in

addition to other physical phenomena in the samples, e.g. light scatter can cause interference problems. The NIR method thus has to be calibrated towards reference methods for quantitative measurements. By using multivariate calibration techniques the problem of interferences is solved by combining the information from several wavelengths when predicting e.g. the fat content of the sample (Martens & Naes, 1989). After calibration, a routine analysis is easily performed by loading the finely ground sample into the sample holder, inserting it into the instrument, waiting approximately half a minute and recording the results.

To date, NIR spectroscopy has turned out to be an excellent method for nutrient analysis of relatively homogenous food items, e.g. ground meat or fish products (Osborne & Fearn, 1986). The method is therefore widely used in the food industry for routine quality analysis. However, to our knowledge, no reports have been published which evaluate to what extent NIR spectroscopy can replace traditional chemical analysis in dietary research where analysis of less homogenous samples is required. In several types of dietary studies, mixed diets are analysed for nutrient content.

We have previously conducted dietary studies in which mixed diets were carefully sampled and analysed (Meltzer *et al*, 1990, 1993, 1997) using the so-called duplicate portion method (Borgström *et al*, 1975, Cameron & van Staveren, 1988). This implies that the subjects gather a duplicate of all foods they eat and drink for a given number of days. The duplicate portions are then homogenized and

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can be analysed for whatever nutrient is requested. The method is extremely time consuming and demands a high degree of motivation and accuracy from the participants. In addition, traditional chemical analysis of nutrients are not trivial, neither timewise nor economically. The duplicate portion method would probably be used more often in dietary research if the time spent on nutrient analysis could be shortened and costs reduced. Although the method has the potential of giving a very precise estimate of what people are actually eating, it is mostly used today when there is a need to know the dietary content of food components not normally found in food composition tables, e.g. trace elements, *trans* fatty acids, heavy metals or other non-nutrients. The method can also be used as a quality control in controlled dietary intervention studies in order to check the estimated vs the actual nutrient intake (Almendingen *et al*, 1995).

In one of the studies mentioned earlier, duplicate portions of human 4-day diets were analysed for several nutrients with traditional chemical analyses and the results compared to the calculated nutrient content (Meltzer, 1995). In this study, we got the opportunity to compare our previous results with NIR analysis on the same samples. The aim of the present study was to investigate the potential of NIR spectroscopy as a rapid and non-destructive method of determining total fat and protein in mixed, homogenized and freeze-dried human diets.

## Materials and methods

As part of a selenium bioavailability study (Meltzer *et al*, 1997), 29 female nutrition undergraduate students collected duplicate portions of their regular diet for four consecutive days. The food was gathered in 2L cartons and frozen consecutively by the end of each day. After being stored at  $-20^{\circ}\text{C}$ , the duplicate portions were carefully thawed, homogenized and freeze dried. Depending on the analysis, these were performed on wet weight or freeze-dried samples.

In addition, all food and drink was weighed and weights, brands and preparation methods accurately written down in a 'food diary'. The software package 'Fiber', developed by T Ydersbond and The Norwegian Association of Clinical Nutritionists, was used for all nutrient calculations. All calculations were based on the Food Composition Tables of the National Nutrition Council (1991).

The participants were all healthy and living at home. They were encouraged to maintain their usual dietary habits. All participants gave informed consent and the study protocol was approved by the Regional Ethics Committee of Medical Research.

### Chemical analysis

The total fat content of the duplicate portions were determined by a modified Folch procedure on freeze-dried material (Folch *et al*, 1957). Freeze-dried samples (7.5 g) were extracted three times with 100ml hot ( $65^{\circ}\text{C}$ ) chloroform:methanol, 2:1, 1:1 and 1:2, respectively. After evaporation of the organic layer the residue was weighed. The nitrogen content was determined (per wet weight) by the Kjeldahl technique (Borgström *et al*, 1975). The factor used for conversion of nitrogen content to protein was 6.25. Each individual value used for statistical analysis represents the mean of minimum two parallels for fat and three parallels for protein. The protein concentrations in the

figures are corrected for water content and described as g protein/dry weight.

### NIR spectroscopy

The NIR spectra of freeze dried samples were measured on a Technicon 500 instrument (Technicon Industrial Systems, New York, USA), from 1100 to 2500nm at 4nm intervals in closed sample cups. Each sample was measured in three repacked replicates and the mean of the spectra obtained was used in the regressions.

### Data analysis

Multiplicative scatter correction (MSC); (Martens *et al*, 1983) was performed on the spectral data. Previous work (Geladi *et al*, 1985; Isaksson & Naes, 1988) has demonstrated that MSC of spectral data results in better linear fit between NIR data and chemical concentrations, better spectral interpretability and improved prediction results compared with uncorrected data. The multivariate calibration method PLS (partial least squares) regression was used. PLS is a linear modelling method, which handles the collinearity problem by data compression (Martens & Naes, 1989). The method projects the spectra onto factors which express the main sources of variation in the spectra (*X*-data) which are relevant for the *Y*-data, and then uses a number of these estimated components as *X*-variables.

Cross-validation (Martens & Naes, 1989) was used to validate the calibration models. The prediction accuracy of the calibration model is described by the root mean square error of prediction (RMSEP; Martens & Naes, 1989), which is defined as:

$$RMSEP = \left( I^{-1} \sum_{i=1}^I (\hat{y}_i - y_i)^2 \right)^{0.5}$$

where  $\hat{y}_i$  is the predicted value of the chemical constituent (fat or protein) by NIR analysis and  $y_i$  is the actual amount of the chemical constituent (fat or protein) determined by traditional chemical analysis for prediction sample *i* and *I* is the number of cross-validations (which in this case, equals the number of samples).

The MSC and PLS regressions were performed by the Unscrambler Software Version 5.5 (Camo A/S, Trondheim).

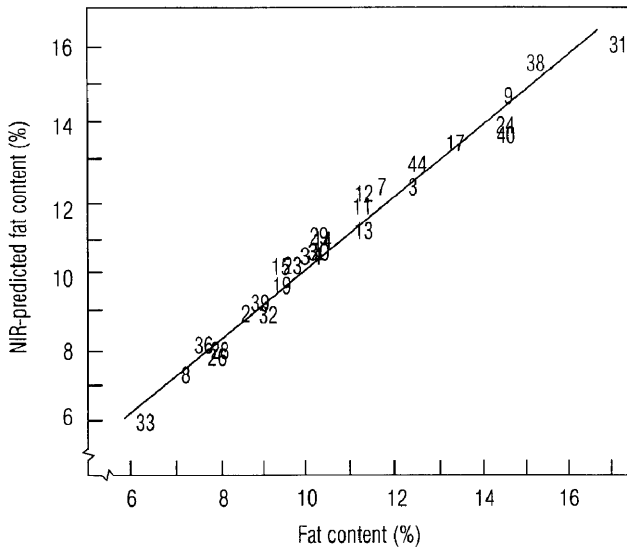
## Results

### Fat

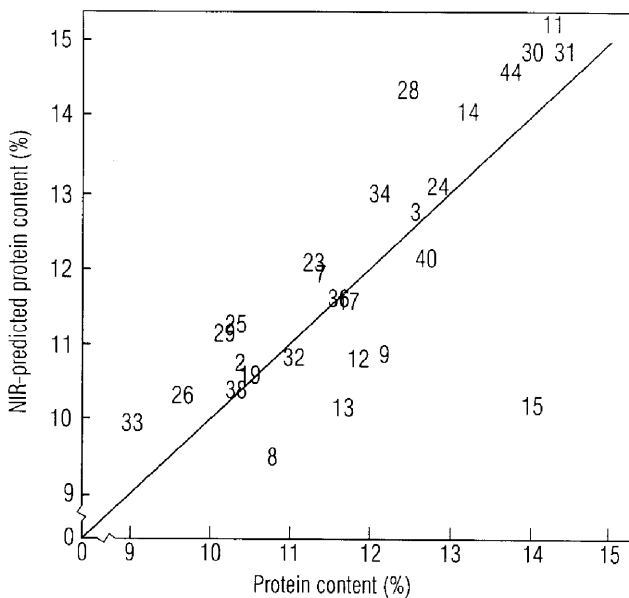
The NIR method was able to determine the fat content of the 29 samples with a prediction accuracy (RMSEP) of 0.45 when the fat content of the samples varied between 6.50 and 17.11%. This was achieved by using a PLS model of six factors. By plotting the NIR-predicted fat content towards the fat content determined by chloroform-methanol extraction (Folch *et al*, 1957), a correlation coefficient of 0.99 was achieved (Figure 1).

### Protein

The protein content of the 29 samples varied between 9.4 and 15%. One of the samples (20) was an obvious outlier and was not included in further analysis. The protein content was determined with an accuracy (RMSEP) of 0.97 using a PLS model of four factors. By plotting the NIR-predicted protein content towards the Kjeldahl-



**Figure 1** NIR-determined fat content plotted against the Folch-analysed fat content of 29 duplicate portions. The line of equality is drawn.



**Figure 2** NIR-determined protein content plotted against the Kjeldahl-analysed protein content of 28 duplicate portions. The line of equality is drawn.

analysed protein content (Figure 2), a correlation coefficient of 0.81 was achieved. The plot shows that there is a discrepancy between the predicted and the measured protein content, especially for sample 15.

#### The calculated dietary intake of fat and protein

In Table 1 we have shown that the calculated intake of fat and protein (based on the food composition tables of the National Nutrition Council, 1991) among these subjects was highly significantly correlated with the analysed values obtained by the traditional chemical methods (both  $P < 0.001$ , Table 1). Urinary nitrogen content may be used as an indicator and biomarker of nitrogen or 'true' protein intake (FAO/WHO/UNU, 1985). A highly significant correlation was found between the analysed dietary and the analysed urinary content of nitrogen ( $P < 0.001$ ).

**Table 1** Comparison between fat and protein data obtained with food composition table data (National Nutrition Council, 1991) and the data obtained with traditional chemical methods.<sup>a</sup> Values are given as means  $\pm$  s.d.

<b>Dietary fat</b>	
Calculated (g/d)	52.7 $\pm$ 14.7
Analysed (g/d)	50.7 $\pm$ 13.0
Correlation, calculated vs analysed fat	0.93 ( $P < 0.001$ )
<b>Dietary protein</b>	
Calculated (g/d)	73.7 $\pm$ 14.6
Analysed (g/d)	73.4 $\pm$ 14.2
Correlation, calculated vs analysed protein	0.94 ( $P < 0.001$ )
<b>Urinary vs dietary content of nitrogen</b>	
Analysed urinary N (g/d)	10.683 $\pm$ 1.524
Analysed dietary N (g/d)	11.744 $\pm$ 2.272
Correlation, urine N vs dietary N	0.71 ( $P < 0.001$ )
Percentage urinary N of dietary N (%)	91

<sup>a</sup>Fat analyses were performed according to a modified Folch procedure and the protein analysis according to the Kjeldahl technique (Borgström *et al*, 1975). Data are taken from Meltzer (1995).

## Discussion

The present results indicate that NIR spectroscopy is able to determine the fat, and to some extent also the protein content of mixed, homogenized diets. Today most of the information concerning the composition of the diet is collected from tables specifying the nutrient composition. Usually such tables are based on chemical analysis of raw food. There is no guarantee that the figures apply to mixed food served on the plate—the original material may differ, preparation by industrial procedure or in the kitchen may have destroyed or changed certain components. Although indispensable for mass production and mass surveillance of food, such tables may not give a true picture of what the individual is actually eating (Borgström *et al*, 1975). Because of this, and the disadvantages of the traditional chemical analysis, NIR spectroscopy offers a cost-effective alternative in dietary research where sampling of duplicate portions of any kind is involved.

The correlation between Folch-analysed and NIR-measured fat content was remarkable. On the other hand, we have few explanations for the protein comparisons not being equally good. The Kjeldahl method we used for chemical analysis is well established and by all standard evaluation methods considered good enough (Borgström *et al*, 1975). However, the method analyses the nitrogen content, not protein *per se*. Most proteins contain about 16% nitrogen. Thus the total dietary N multiplied by 6.25 gives a reasonable estimate of the protein content. Most food tables and analyses base their estimates on this conversion factor of 6.25 (FAO/WHO/UNU, 1985), as do the ones used in this work. However, some proteins contain a higher or lower percentage of nitrogen and some foods also contain non-protein nitrogen. We may have got a slightly different protein content by analysis if we had differentiated the diets more carefully, and taken into account the individual protein-containing foods. Some participants had a predominantly vegetarian diet, while others had a more typical meat-rich diet. Accordingly, by applying more individualized conversion factors, we might have obtained slightly different protein values. We neglected this approach because of the high correlation between the chemical analysis and calculations according to Norwegian Food Composition Tables (Table 1).

The outlier, no. 15 (Figure 2), had a very low fat content, in absolute grams, the lowest content of all. It also had an almost vegetarian profile with little or no meat and fish and a very high fibre content. On the other hand, the excluded outlier, no. 20, was characterized by its extremely high protein concentration, with a very high percentage of it coming from animal foods. Earlier studies have shown that NIR is sensitive to amino acid profiles (Alfaro *et al*, 1990, Martens & Paulus, 1988) and to changes in the meat proteins during denaturation (Ellekjær & Isaksson, 1992). It might thus be that the various diets which contained proteins from different origins might have added noise to the protein content determination.

In addition to the heterogeneity of the diets, the present study consists of few samples. This makes it very sensitive to outliers. Including more samples in the calibration model would probably have increased the prediction accuracy. The accuracy of the predicted property is not only affected by sample size, but by a sum of different error terms, e.g. sampling errors, instrumental errors, reference method errors and regression model fit errors (Williams & Norris, 1987). It can be excluded that the observed differences between the methods used in the present study were real differences because we used aliquots collected from the same freeze-dried homogenates. Because it is simple and quick to perform, the NIR method is already in wide use within other areas, in the food, agriculture, pharmaceutical, chemical and polymer industries (Hall & Pollard, 1992). Although the NIR analyses of protein showed some disagreement with the Kjeldahl method for some of the samples, the NIR method undoubtedly can replace the Kjeldahl method for certain purposes, for example in relatively homogenous samples. Further studies are, however, needed in order to determine to what extent this method can be fully applied to mixed heterogeneous diets in controlled dietary research.

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