Fast determination of the fat contents of milk and food products by near infrared spectrometry

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Introduction

The quantification of dioxins in milk and food products is based on the analysis of the fat fraction after a Soxhlett or supercritical fluid extraction. The fat percentage of the samples is usually determined by wet-chemical methods, but these techniques are rather elaborate and sometimes inaccurate.

In the food industry, near infrared spectrometry (NIR) is rapidly gaining acceptance as a valuable tool for the quantitative analysis of fat and fatty acids in bulk samples [1]. A major advantage of NIR is that it hardly requires any sample preparation while spectra can be acquired and processed in the order of seconds. It was, therefore, a logical step to study the potentials of NIR for the quantitative determination of the fat constituents in milk and food in relation to the analysis of dioxins.

A pilot study has been carried out on a series of freeze-dried samples of cow's milk, mother's milk and food diets, vegetable oils and fish oils. NIR appears to be a viable and fast alternative for current more elaborate wet-chemical methods. The relative error of prediction of unknown samples, obtained from a quantitative NIR-model based on cow's milk samples, varied between 1 and 10% (0.6-2.6% absolute) while the time of analysis including spectrum acquisition, processing and calculation is less than a minute per sample.

Methods and materials

Experiments were carried out on samples of cow's milk, mother's milk, vegetable oils (soy bean oil, palm oil), fish oil (herring, red eel) and duplicate diets. The fat percentage varied between 0.06 and 35%. All samples were freeze dried prior to NIR analysis.

Measurements were carried with a NIR-spectrometer model Identicheck (Perkin-Elmer) in the wavenumber region 12.000-3000 cm^{-1}. Spectra were acquired with a data

point resolution of 8 cm^{-1} at 64 scans. Solids were measured in reflection in upper mode in closed glas vials (Alltech) and in lower mode in open metal cups (Perkin-Elmer). Liquids were analysed in transmission in a CaF_2 liquid cell, pathlength 500 µm.

Samples were quantified by the integrated area of the NIR absorption band around 4258 cm^{-1} after a 2-point baseline correction at 4293 and 4220 cm^{-1}. A quantitative model, based on cow's milk samples, was used for calculation of the unknown samples. NIR-results were compared with wet-chemically determined values.

Results and discussion

All compounds give rise to NIR absorption bands around 4330 and 4258 cm^{-1}. According to literature these bands can be attributed to overtones correlated to fat absorptions [2]. The bandmaxima appear to be constant, i.e., independent of the sample type (Figure 1). Besides, the transmission spectra of the pure oils point to identical extinction coefficients for all samples. It implies that a cow-milk model is, in principle, valid for quantification of other, non cow-milk products.

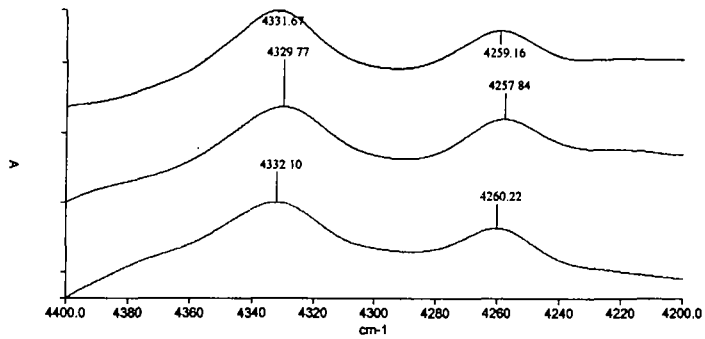

Figure 1. Absorptionmaxima at 4330 en 4258 cm^{-1} of freez dried fish oil (upper), mother's milk (mid) and 24 hour duplicate diet (bottom).

The effect of the instrumental and sampling conditions on the reproducibility of the integrated peak area of a freeze-dried cow-milk sample with a fat content of about 25% is presented in Table 1. The effect of the instrument on the reproducibilty is negligiable compared to sampling effects. The effect of (cryogenic) grinding and "packing" , however, is evident, indicating that homogeneity and a uniform particle size are crucial factors to improve the reproducibility.

Table 1. The effect of instrument and sampling on the reproducability of vial measurements. A_{4258}: integrated area of the NIR-absorption band at 4258 cm^{-1} after baseline correction. *n*: number of measurements, s_d : standard deviation.

Experiment	A_{4258}	*n*	s_d
In instrument	0.61288	5	0.0015
In/out instrument	0.63306	5	0.0179
Different batches	0.63320	5	0.0200
Packing	0.63284	10	0.0138
Cryogenic grinding	0.64314	10	0.0129

The calibration curve of the cow's milk samples is shown in Figure 2. The results of the unknown samples, calculated from the cow-milk model, are summarised in Table 2. The relative error of prediction of the cow's milk samples, compared to the wet-chemically determined values, is between 2 and 10%. Similar values are obtained for the mother's milk and duplicate diet samples (1-9%). All values are slightly smaller than the wet-chemical percentages, which might indicate a systematical error.

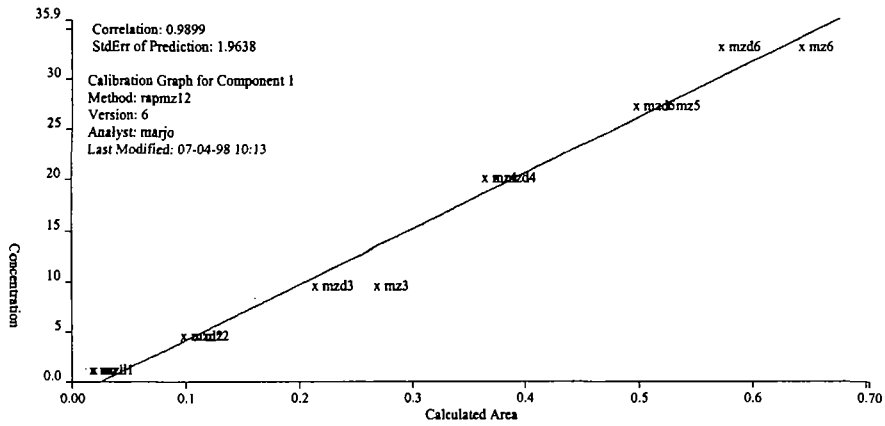

Figure 2. Calibration curve of freeze-dried cow's milk samples based on the NIR-absorption band at 4258 cm^{-1} after baseline correction. Fat percentage 0.06-35%; number of calibration samples: 12.

Tabel 2. Fat percentage of freeze dried milk and food duplicate as determined by NIR and wet chemically.

Sample	% (NIR)	% (wet chemical)	% error (relative)	% error (absolute)
cow milk	15.2	13.6	10	1.6
cow milk	26.7	24.9	7	1.8
cow milk	28.7	28.1	2	0.6
24 hour food duplicate	19.6	18.0	8	1.6
24 hour food duplicate	20.0	18.8	6	1.2
mother milk	28.9	26.2	9	2.7

Conclusions

NIR is a viable technique for the quantification of fat in freeze-dried milk and food samples. The fat contents of a variety of samples can be determined with a relative error of 2-10% (0.6-2.6% absolute) using a model based on cow's milk samples. The NIR technique is extremely easy to operate while the time of analysis including spectrum acquisition, processing and calculation is less than a minute per sample. As such NIR can be a valuable time-saving alternative for the wet-chemical methods, currently used in the analysis of dioxins. The variety in particle size and homogeneity are the main limiting factors for a higher accuracy but improvement can be achieved by (cryogenic) grinding prior to NIR measurement.

References

1. B.G. Osborne, T. Fearn, Near-Infrared Analysis in Food Industry, 1986, ISBN 0-582-49489-3.
2. Robert P., Bertrand D. and Devaux M.F.; *Anal.Chem.* **1987**, 59, 2187-2191.

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