8:13 Detection of errors in the NIR-analysis of meat components

HILDRUM, K.I., MARTENS, H. and LEA, P.

Norwegian Food Research Institute, 1432 Ås-NLH, Norway

Introduction

Near infrared (NIR) reflectance spectroscopy was developed to replace laborious conventional methods for food analysis e.g. methods for the analysis of protein, fat and water. The use of the NIR technique has been growing rapidly in the recent years and has also found applications in the analysis of meat products (Lanza, 1983; Kruggel et al. 1981).

NIR reflectance analysis has many advantages to its use, such as speed, reproducibility and no use of chemicals. However, since NIR is an indirect method the instrument has to be carefully calibrated in order to achieve reliable results.

Since 1980 NIR reflectance spectroscopy has been used in the process control in a meat processing company in Norway. The use has included composition analysis of raw materials, of preblends for comminuted meat products, and of the finished products. The accuracy of the analysis has been considered satisfactory for the intended use (Martens et al., 1981).

Recent controls of the performance of the overall NIR analysis of raw, comminuted meat samples showed increased standard errors of predictions, which indicated lower accuracy of the analysis. This paper discusses the reasons for this and suggests steps to improve overall calibration. The importance of introducing effective error detecting systems is stressed.

Materials and methods

The instrument used is InfraAlyzer 400 (Technicon Instrument Co.) with 19 narrow bandpass interference filters. The light source in the instrument is a tungsten lamp. The diffusely reflected light from the sample at each wavelength is collected by means of an integrating sphere and focussed into a lead sulphide detector. The sample is packed into a sample holder without a glass cover.

The control samples were obtained under the same conditions as the calibration and prediction samples. Fifty samples each of 2-3 kilograms were sampled from 1500 kilograms preblended batches of ground (8 mm) beef and pork - added 2% sodium chloride and 17% water. The batches were preblended for 15 min in horizontal mixers with double wings moving in opposite directions. The samples were homogenized for 2-3 minutes in a laboratory meat chopper (Robot-Coupe, No. 4) and analyzed for protein, water and fat contents by standard chemical methods and by the NIR-technique in the company's quality control laboratories. The latter analysis was based on the NIR calibration parameters originally determined in 1980, using stepwise multiple linear regression.

The standard chemical methods were Foslet for fat, Kjeldahl for protein and drying for 14 hours at 102-105°C for water (Nordisk Metodik-Komite for Levnedsmidler, 1955). The ranges for each component in the samples were as follows, as analyzed by the standard methods:

Water	54.5-72.4%
Fat	8.0-30.9%
Protein	10.4-16.8%

SEP is the root mean square standard error of the original prediction; the standard deviation of the differences between the values of the standard methods and the NIR-values in the prediction set. SEPC is the root mean square standard error of the 50 recent control samples. Both terms are given in per cent of wet weight.

The presence of outliers in the control data set was tested by a sample kurtosis test (Barnett and Lewis, 1978):

$$T_{N15} = \frac{\Sigma(x_i - \bar{x})^4}{n \cdot s^4}$$

where

n = number of control samples
s = standard deviation between samples
x_i = observation of a certain property in sample i
$\bar{x}$ = mean of observations over all samples.

If T_{N15} exceeds the critical value tabulated in (Barnett and Lewis, 1978), the observation with the largest value of $|x_i - \bar{x}|$ is considered an outlier and can be deleted from the data set. This test is suitable for consecutive use in the presence of more than one outlier. T_{N15} is then computed repeatedly with n, $\bar{x}$ and s based on the reduced data set.

Data from 10 additional meat samples within the same ranges were used to test the predictive ability of the fat calibration obtained, using partial least square regression (PLS). These 10 samples were considered 'normal' samples. Data from 10 other samples of a different quality were also used in the same way. These samples did not contain any added salt and water, and were analyzed in a different laboratory with respect to NIR and fat content. The latter 10 samples were considered 'abnormal'.

The NIR data of each of the 10 'normal' and 10 'abnormal' samples were fitted to the PLS calibration model whereby two different outlier criteria were obtained. One was the NIR lack-of-fit residuals averaged over the 19 wavelengths, which is termed d. The second is leverage (h). In addition, the difference between chemically and NIR determined fat percentages (e) was determined for each sample.

Results and discussion

As earlier studies have not indicated significant differences in absorption properties for beef and pork in the NIR region (Lanza, 1983 and Martens et al., 1981), one calibration was used for preblends containing both beef and pork.

Table 1 shows the standard errors of prediction in 1980 and 1983 (SEP and SEPC, respectively) in the analysis of meat preblends, both in original form and normalizing the sum of water, fat and protein to 100%. In the calibration control data set of 1983, the SEPC's have increased considerably for water, fat and protein. The fat and water error terms have more than doubled over the period. This was a reason for serious concern to the company as NIR analysis is regularly used to adjust the composition of the meat preblends in the processing lines. Normalizing the sum of protein, fat and water to 100% decreased the SEPC of the water analysis, while the corresponding term for fat and water essentially remained constant.

In table 2 the deviations between the individual measurements from the NIR and standard chemical analysis are desribed in more detail. For the fat and particularly the water analyses the mean biases were high and indicated systematic errors either in the NIR or the standard analysis. In addition, certain individual samples yielded abnormally large differences between chemically and NIR-determined results. Whether these abnormalities were due to errors in the standard chemical analysis or in the NIR-determinations, was studied by inspection of the sums of fat, water and protein.

The average sums of fat, water and protein for chemically and NIR-determined data over all samples were 95.7% and 97.1%, respectively. Hence a bias of 1.4% was observed. The sums of water, fat and protein in different cuts of beef and pork are reported to be in the range 99.5-100.5% (Livsmedelstabeller, 1978). Taking into account that 2% salt was added to the meat preblends, this means that one or several of the components systematically were underestimated by the standard chemical methods, while the NIR determinations only had a minor sum bias.

The frequency distribution of the individual deviations between the sums is given in figure 1. One sample with a deviation of 7.7, which is excluded from this figure, was found to be an outlier at the 95% significance level according to the sample kurtosis test (T_{N15}) (5, figure 2). Inspection of the individual analysis of that sample revealed a large overestimation of water by the standard chemical method. Seven of the remaining 49 samples showed up as a heavy tail in the frequency distribution. These samples were not judged to be outliers in the formal sense of the T_{N15}-test, but we found it worthwhile to study them separately as the reason for their non-outlying status might just as well be a consequence of the distribution at hand not being normal.

Plotting the deviations of the individual constituents in each sample as functions of the chemically determined constituents gave additional information. The protein deviation was small over the whole concentration range of protein. The fat deviation was small at low contents of fat, - but a larger, negative deviation between the NIR-and chemical technique was observed at high fat contents (>22% fat). A high water deviation was found at low moisture levels in the meat (<60% water). This is in agreement with earlier studies where a less reliable NIR-prediction was observed at higher fat levels (>17% fat) (Hildrum et al., 1982).

The relationship between leanness and water content of meat has long been known (Karmas et al, 1961). Figure 2 shows this relationship as straight lines estimated by linear regression over the 50 samples, one for the standard chemical analysis and another for the NIR analysis. The mean deviation for the water in the NIR and standard analyses was 1.3%, which is very close to the mean bias between the sums of all three chemical constituents (1.4%), indicating that water was a primary error source. The best fit was obtained between the NIR-determined fat and water contents with a correlation coefficient $r_{NIR} = -0.99$. For the chemical analysis the corresponding coefficient was $r_{chem} = -0.96$, which increased to -0.98 when the above mentioned outlier was excluded.

In figure 2 the individual data for seven samples with a sum deviation exceeding 3.0 are plotted. For the NIR-analysis, a good fit between fat and water contents was obtained even for these samples. However, for the standard analysis negative deviations in moisture content were observed in 6 out of the 7 samples. This indicated underestimation of water by the standard chemical method. In the seventh sample, the high sum deviation was probably caused by an error in the NIR-determination of protein (6, figure 2).

The direction of lines connecting the chemical results and the NIR-results in figure 2, indicated that the water deviations were larger than the fat deviations for 5 of the 7 apparent outliers, while two displayed fat deviations in their chemical data.

The reasons for the underestimation of water by the standard chemical method might be several, where incomplete drying of the samples was the most probable factor. The drying at 102-105°C was done for all samples without the addition of sand. For high-fat samples it is recommended to mix in sand to ensure a complete drying (Nordisk Metodik-komité for Levnedsmidler, 1955). Another reason might be variations in salt contents in the samples, as salt is known to give shifts in the water-peaks in the NIR-region (Lanza, personal communication). These possibilities are presently being explored.

This study shows that regular control and adjustments of the calibrations are necessary to keep them reliable and accurate. Also care must be taken to incorporate new, representative sample types to the calibration and to update calibration results which are outdated. There is a human tendency to have blind faith in the results generated by computers. As the instruments get more complex, this danger will be even more serious. The need for reliable error detection or alarm systems in the future is evident.

Table 3 shows the control results obtained when comparing the 'normal' and the 'abnormal' meat samples to the PLS calibration. The 'normal' meat samples yielded fat determinations of the same accuracy as that obtained during calibration, while the 'abnormal' samples gave higher prediction errors. However, these abnormalities could be determined automatically. While every 'normal' control sample gave NIR residuals (in absorbance units), and NIR leverages (in relative units) close to the levels obtained during calibration, every 'abnormal' control sample gave much higher residuals and leverages. This confirms results obtained with cereals (Martens and Jensen,

1982), indicating that outlier detection is possible by multivariate pattern recognition techniques.

Acknowledgements

The authors want to thank Ms Ingebjørg Pedersen and Ms Mari Buer for technical assistance and Ms Iris Sigdestad and Ms Ulla Bråthe for typing and technical drawing, respectively.

Literature

Barnett, V. and Lewis, T.
Outliers in statistical data
John Wiley & Sons, 1978, p. 101.

Hildrum, K.I., Valland, M. and Martens, H.
Analysis of meat components in near-infrared and infrared regions by multivariate spectrometri.
In: 'Food Research and Data Analysis'
Eds. Martens, H. and Russwurm, H., jr. Proc. IUFoST Symposium, Oslo September 1982.
Appl. Sci. Publ., London, 1983, p. 416.

Karmas, E., Thompson, J.E., and Wistreich, H.E.
Relationship between pork leanness and moisture content.
Food Technol 15 (1961) 8.

Kruggel, W.G., Field, R.A., Riley, M.L. Radloff, H.D., and Horton, K.M.
Near-infrared reflectance determination of fat, protein, and moisture in fresh meat.
J. Assoc. of Anal. Chem. 64 (1981) 694.

Lanza, E.
Determination of moisture, protein, fat and calories in raw pork and beef by near infrared spectroscopy
J. Food Sci. 48 (1983) 471

Lanza, E.
Personal Communication.

Livsmedelstabeller
Statens Livsmedelsverk
Liber Tryck, 1978, Stockholm

Martens, H., Bakker, E.A., and Hildrum, K.I.
Application of near infrared reflectance spectrometry in the analysis of meat products. 27th. Eur. Meet. Meat Res. Workers, Vienna 1981, vol. 2, 561.

Martens, H. and Jensen, S.Aa.
Partial least squares regression: A new two-stage NIR calibration method.
Proc. 7th World Cereal and Bread Congr. Prague, June 1982. Eds. J. Hotas and J. Kratochvil. Elsevier, 1983, Amsterdam, p. 607.

Nordisk Metodik-Komité for Levnedsmidler
Kemiska analysmetoder för kött og charkuterivaror. Forskrift 23.
Teknisk Forlag, 1955, Copenhagen.

	PROTEIN	FAT	WATER
SEP (1980)	0.57	0.81	1.14
SEPC (1983)	0.95	1.48	2.87
SEPC (1983) (normalizing sum of protein, fat and water to 100%)	0.97	1.66	1.98

TABLE 1. Standard errors of prediction of original prediction (SEP, 1980) and of a calibration control prediction (SEPC, 1983), given in per cent wet weight.

	Maximum deviation	Minimum deviation	Range of deviation	Bias
PROTEIN	4.3	-1.8	6.1	-0.11
FAT	2.1	-5.0	7.1	-0.61
WATER	5.7	-7.3	13.0	2.14
SUM OF THE 3 COMPONENTS	4.6	-7.7	12.3	1.42

TABLE 2 Deviations between the individual measurements from the NIR and standard chemical analysis

	NIR		FAT
	$d \cdot 10^2$	h	e
1. Calibration samples (n=50)			
average	1.2[a]	0.1	0.5[a]
2. 'Normal' control samples (n=10)			
MINIMUM	0.5	0.1	0.0
MAXIMUM	2.0	0.5	0.8
average	1.2[a]	0.2	0.3[a]
3. 'Abnormal' control samples (N=10)			
MINIMUM	9.6	3.4	0.3
MAXIMUM	17.5	13.1	2.6
average	12.3[a]	7.8	1.9[a]

TABLE 3 Ability of PLS-calibration to predict fat and detect outliers in 'normal' samples and 'abnormal' samples (not added salt and water). [a] Root-mean-square averages.

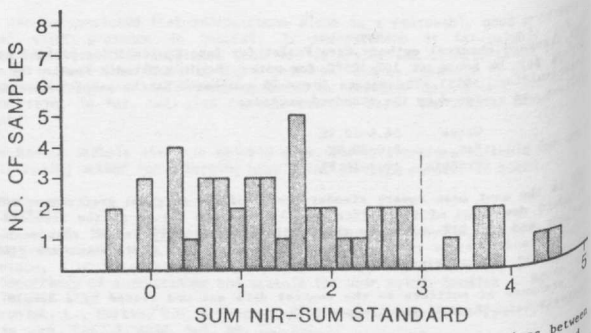

Figure 1 The frequency distribution of the individual deviations between the sums of water, fat and protein contents for the NIR and chemically determined data for 50 meat samples (abscissa interval = 0.17%).

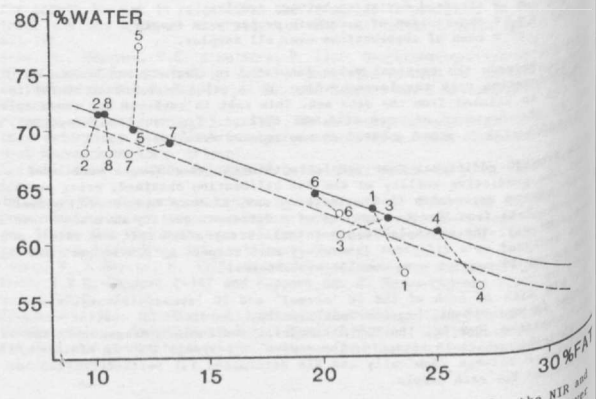

Figure 2 Relationships between the fat and water contents for the NIR and chemically determined data, estimated by linear regressions over 50 meat samples.
——— NIR determined data, - - - - Chemically determined data
Samples with a difference between sum NIR determined data and sum chemically determined data exceeding 3.0 are plotted individually.
• NIR determinations, o Chemical determinations.