Application of near infrared reflectance spectrometry in the analysis of meat products

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INTRODUCTION

While the use of Near Infrared (NIR) analysis of water, protein etc. has become widespread in the cereal industries, the potential applications on meats have up to recent date been largely unexplored[1-5]. In this paper we will update the present situation on NIR-applications on meat products in Norway. That will include recent results from the Norwegian Food Research Institute, concerning inter-product and inter-laboratory variations, but also the practical implementation of the technique in the process control of a meat processing company (Stabburet A/S).

NIR spectrometry may give fast and easy determination of all major chemical constituents after a minimum of sample preparation work. In commercial NIR instruments, the light reflectance is measured very precisely from the surface of a sample at several different wavelengths in the Near Infrared (1400-2600 nm) wavelength region, and combined, in a microprocessor, to yield the concentration of the food constituents e.g. fat, water and protein. The microprocessor has to be "taught", in a calibration procedure, how to recognize the fat, water and protein percentages from the NIR spectral data. This "calibration" procedure involves multivariate statistical comparisons between the NIR spectra and the known chemical compositions of a set of calibration samples. The "obtained knowledge" (the calibration constants) is later used for predicting the composition of unknown samples from NIR measurements alone. The calibration step of NIR analysis is expensive and laboursome, because many samples must be analyzed by traditional "wet-chemistry"-methods for e.g. fat, water and protein. But once calibrated, the NIR instrument is fast and easy to use.
The quality of the calibrations in decisive for the performance of the NIR analysis. The present paper discusses calibration from two angles: A food control laboratory, analyzing a very wide range of meat products, cannot possibly calibrate for each and every product type separately, and may want a general, multi-product calibration for rapid screening purposes. A process control laboratory, on the other hand, may choose to perform individual calibrations for a few product types, thereby expecting increased analytical precision. Preliminary multi-product calibrations for two laboratories, and single-product calibrations from one laboratory will here be described.

MATERIALS AND METHODS

Multi-product calibrations

A total of 181 meat products of many different kinds, including raw, cooked, fried, smoked and fermented products, - made from both bovine and porcine meat cuts were analyzed. Water, salt, spices, starch and meat extenders like caseinates and soy isolates were used as other ingredients. The samples originated from many different meat processing plants, and were analyzed chemically for fat, water and protein in 2 different laboratories - "Lab. A" (the official health authority of Oslo) and "Lab B" (the central laboratory of the farmers' cooperative meat processing company). Fat content was determined by Foslet, water content by drying and protein content by the standard Kjeldahl analysis. The homogenized samples were stored at -20°C until NIR analysis, thawed to 20°C, homogenized for about 30 sec. in a Janke & Kunkel Ultra-Turrax blendor and analyzed on a Technicon Infralyzer 400 (equipped with 19 different optical filters) at the Norwegian Food Research Institute. Calibrations were performed in a HP 9825T computer using the authors' software.

Single-product calibrations

About 100 meat samples of 4 different product types from Stabburet A/S, a private meat processing company, were homogenized in a laboratory meat chopper with horizontally mounted knives for 3-4 minutes and analyzed directly on the company's NIR-instrument (Technicon Infralyzer 400, 19 filters). The chemical composition of the samples were analyzed for fat (Foslet), water (drying) and protein (Kjeldahl) in the company's laboratory ("Lab. C"). Calibrations were performed in a HP 9815 calculator using Technicon software.

Statistical methods

These abbreviations will be used in the following:
SEE is the standard error of estimation; the average (rms) difference between the calibration samples' NIR-based concentration predictions and their chemically obtained concentrations. SEE is given in percent of wet weight. SEP is the standard error of prediction, i.e. the corresponding difference obtained when testing the calibration for new known samples. SEP is given in the same units as SEE. SEP gives a more realistic description of the actual precision of the whole analysis than does SEE.
The calibration consisted in a downward stepwise multiple linear regression starting with all 19 filters and an offset term. In each step the filter showing the least significant regression coefficient was eliminated, until all remaining filters showed statistically significant coefficients (by student-t test, at the 95% confidence level). Samples yielding calibration residuals > 2 SEE were taken as "outliers" and deleted before recalibration. Samples yielding prediction residuals > 2 SEP were likewise deleted before renewed calculation of SEP, when multi-product calibrations were tested. This estimation of outliers was repeated successively twice for each data set.

RESULTS AND DISCUSSION

Multi-product calibrations

The 90 samples from Lab. A were split at random in two approximately equal subsets, and the 91 samples from Lab. B were likewise split at random in two approximately equal subsets, yielding a total of 4 subsets, each of which were used for calibration. Each subset contained different types of comminuted, cooked meat products (smoked and unsmoked), in addition to various types of sandwich saussages, leverpatê etc. Tables 1-3 give the results for fat, water and protein respectively.

In each table, the calibration standard errors between chemically and NIR-determined percentages (SEE) in the calibration procedures are given along the diagonals for the 4 data sets. Below the SEE the number of statistically significant filters is given for each calibration. In paranthesis the number of deleted samples is given.

Each of these 4 calibrations were tested for data from the 3 other subsets and the standard errors between chemically and NIR-determined percentages (SEP) are given off the diagonals.

When the concentrations in one set of samples were predicted by the calibrations obtained from the other set of samples from the same laboratory, the average prediction error (SEP) was 1.30% for fat, 1.49% for water and 0.77% for protein, when on the average 6.3 samples were deleted as "outliers" for fat, 6.3 for water and 5.0 for protein, out of an average total of 45 samples. The prediction error (SEP) was, on the average, larger than the calibration error (SEE) by a factor of 1.45 for fat, 1.63 for water and 1.82 for protein. This indicates as expected, that some of the measurement noice in the calibration data was incorporated into the calibration constants instead of being counted as residual error. This is characteristic for the statistical calibration method used.

For prediction of samples from one laboratory with calibrations from the other laboratory the prediction error (SEP) was, on the average, 1.37% for fat, 1.94% for water and 0.87% for protein, with, on the average, 6.3, 5.8 and 4.7 "outliers", respectively. This means that SEP was, on the average, only slightly higher for predictions between laboratories than for predictions within laboratories. In general these preliminary multi-product calibrations were not as good as desired, especially so for the fat and water analyses, which yielded many "outliers" in addition to high SEP values. This may indicate large systematic variations between product types with respect to NIR reflectance. The 4 calibrations for a given constituent also varied in choice of filters, possibly because the calibration procedure is somewhat instabile with respect to outliers etc., when the samples are as few and as heterogenous as in the present study. Work is in progress to test alternative calibration methods for the same data[6].

Table 1. Multi-product NIR calibrations for fat

Standard error of NIR-predicted fat percentage compared to Foslet analysis, in meat products of different origins and types, analyzed chemically in two different laboratories.

Calibration set		Prediction set: Lab. A Set 1	Lab. A Set 2	Lab. B Set 1	Lab. B Set 2
Lab. A	Set 1	1.13(5) 6F	1.31(7)	1.75(5)	2.24(7)
	Set 2	1.67(5)	0.66(5) 8F	0.96(9)	0.85(7)
Lab. B	Set 1	1.29(6)	0.82(7)	1.05(5) 5F	1.08(8)
	Set 2	1.76(5)	1.27(4)	1.17(5)	0.76(6) 5F

Calibration SEE is placed along the diagonal, with number of deleted samples in paranthesis and number of significant filters (F) directly below; test SEP is placed off the diagonal.

Table 2. Multi-product NIR calibration for water

Standard error of NIR-predicted water percentage, compared to percentages obtained by drying, in meat products of different origins and types, analyzed chemically in two different laboratories.

Calibration set		Prediction set: Lab. A Set 1	Lab. A Set 2	Lab. B Set 1	Lab. B Set 2
Lab. A	Set 1	0.87(1) 5F	1.17(10)	1.78(10)	1.79(6)
	Set 2	1.34(4)	0.75(1) 8F	1.71(9)	2.26(6)
Lab. B	Set 1	2.33(6)	2.46(2)	1.06(7) 9F	1.60(4)
	Set 2	1.69(6)	1.47(2)	1.84(7)	0.93(4) 5F

Calibration SEE is placed along the diagonal, with number of deleted samples in paranthesis and number of significant filters (F) directly below; test SEP is placed off the diagonal.

Single-product calibrations

To increase the overall precision of the NIR-analysis, the meat products at Lab. C were divided into product subgroups. Separate calibrations were performed on each subgroup.

Table 4 shows the results of the calibration for the four main subgroups, each consisting of 50-60 samples from the processing lines of up to three different factories (of the same company). The SEE's were of the same agnitude as in the previous experiment, ighest for water and lowest for protein. he multiple correlation coefficient (MCC) or the calibrations were between 0.9 and .0 for the components, except for the pro- ein calibrations for cooked and fried pro- ucts, which were lower.

n the basis of the promising calibration esults, the company decided to use the NIR- nstrument in the processing control of ominuted meats. Control samples, which ere also analyzed by the standard techni- ues, were daily taken from the processing ines over a period of several months, and he precision of the predictions were cal- ulated (Table 5). The maximum deviations om the standard analysis were considered easonably low, and were distributed fairly ymmetrically around the zero point.

Table 3. Multi-product NIR calibrations for protein

Standard error of NIR-predicted protein percentage, compared to Kjeldahl-analysis, in meat products of different origins and types, analyzed chemically in two different laboratories.

Calibration set	Prediction set	Lab. A Set 1	Lab. A Set 2	Lab. B Set 1	Lab. B Set 2
Lab. A	Set 1	0.30(1) 11F	0.70(4)	0.78(3)	0.88(10)
	Set 2	0.61(4)	0.64(1) 5F	0.88(4)	1.26(10)
Lab. B	Set 1	0.79(4)	1.05(3)	0.51(2) 5F	1.10(9)
	Set 2	0.54(3)	0.79(2)	0.65(3)	0.27(8) 12F

Calibration SEE is placed along the diagonal, with number of deleted samples in paranthesis and number of significant filters (F) directly below; test SEP is placed off the diagonal.

NCLUSION

he preliminary multi-product calibrations ere not reliable enough. As expected, the recision of the NIR analysis was improved hen the samples were divided into suitable roduct subgroups and analyzed in a given aboratory. However, it appears that the ocessing origin needs not to be critical o the precision of the analysis, - as long s representative samples from the different igins are included in the calibration.

e NIR-instrument has rationalized a large part of the Stabburet company's routine analyses, and the company s quite pleased with the instrument today. The instrument is easy to operate and the speed of the analysis is ppreciated in the processing control. However, several aspects of the use of the NIR-technique in meats re- uires further attention. The homogenization of raw samples easily results in fat separation, and the presence f pieces of sinew, bone and cartilage interferes with the analysis. Also the effects f e.g. types and physical states of protein, fat and water need to be further examined, and the statistical thod used is not quite satisfactory for sample types as complex as meat products.

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able 4. Single-product NIR calibrations
IR-calibration for different meat product groups

RODUCT GROUP	COMPONENT	NO. OF SAMPLES	NO. OF DELETES	NO.OF FILTERS USED	SEE†	MCC*
AW COMMINUTED MEAT with other ingredients included (1 factory)	Protein	53	8	9	0.493	0.946
	Water	53	10	7	0.896	0.973
	Fat	53	12	9	0.605	0.988
OOKED COMMINUTED MEAT PRODUCTS (3 factories)	Protein	55	4	10	0.448	0.835
	Water	55	8	9	0.748	0.981
	Fat	55	7	8	0.601	0.990
RIED COMMINUTED MEAT PRODUCTS (3 factories)	Protein	56	7	11	0.582	0.872
	Water	56	10	9	0.924	0.970
	Fat	56	6	10	0.521	0.984
RY FERMENTED SAUSAGES (2 factories)	Protein	57	10	7	0.677	0.979
	Water	57	8	10	1.083	0.985
	Fat	57	13	7	0.963	0.988

† SEE = standard error of estimate
* MCC = multiple correlation coefficient.

Table 5. Test of single-product NIR calibrations

Predictions of the protein, fat and water contents in meat products by the NIR-technique, using the calibrations from Table 4

PRODUCT GROUP	NO. OF SAMPLES	COMPONENT	Deviation from standard analysis		SEP+
			Max. pos. dev.	Max. neg. dev.	
RAW COMMINUTED MEAT	20	Protein	0.7	1.2	0.574
	55	Water	2.3	2.8	1.139
	55	Fat	1.9	2.0	0.806
COOKED COMMINUTED MEAT PRODUCTS	20	Protein	0.6	1.0	0.602
	38	Water	2.1	1.9	0.908
	38	Fat	0.9	1.3	0.853
FRIED COMMINUTED MEAT PRODUCTS	21	Protein	0.2	1.8	0.853
	29	Water	3.3	2.6	1.341
	29	Fat	1.3	1.7	0.682

+ SEP = standard error of prediction

LITERATURE

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