FOOD COMPOSITION AND ADDITIVES

Determination of Fat, Moisture, and Protein in Meat and Meat Products by Using the FOSS FoodScan™ Near-Infrared Spectrophotometer with FOSS Artificial Neural Network Calibration Model and Associated Database: Collaborative Study

SHIRLEY ANDERSON[1]
FOSS North America, 8091 Wallace Rd, Eden Prairie, MN 55344

Collaborators: S. Aldana; M. Beggs; J. Birkey; A. Conquest; R. Conway; T. Hemminger; J. Herrick; C. Hurley; C. Ionita; J. Longbind; S. McMaignal; A. Milu; T. Mitchell; K. Nanke; A. Perez; M. Phelps; J. Reitz; A. Salazar; T. Shinkle; M. Strampe; K. Van Horn; J. Williams; C. Wipperfurth; S. Zelten; S. Zerr

A collaborative study was conducted to evaluate the repeatability and reproducibility of the FOSS FoodScan™ near-infrared spectrophotometer with artificial neural network calibration model and database for the determination of fat, moisture, and protein in meat and meat products. Representative samples were homogenized by grinding according to AOAC Official Method 983.18. Approximately 180 g ground sample was placed in a 140 mm round sample dish, and the dish was placed in the FoodScan. The operator ID was entered, the meat product profile within the software was selected, and the scanning process was initiated by pressing the "start" button. Results were displayed for percent (g/100 g) fat, moisture, and protein. Ten blind duplicate samples were sent to 15 collaborators in the United States. The within-laboratory (repeatability) relative standard deviation (RSD_r) ranged from 0.22 to 2.67% for fat, 0.23 to 0.92% for moisture, and 0.35 to 2.13% for protein. The between-laboratories (reproducibility) relative standard deviation (RSD_R) ranged from 0.52 to 6.89% for fat, 0.39 to 1.55% for moisture, and 0.54 to 5.23% for protein. The method is recommended for Official First Action.

Traditional chemical analysis methods involve lengthy processes and may take several hours to generate results, which in many food production industries hinder real-time production and product-quality decisions. Near-infrared (NIR) technology offers rapid results, in 50 s, for multiple constituents and is well suited to high-capacity production environments. The meat industry has been routinely using NIR technology for many years for processing, packaging, and labeling. Currently, there are no Official AOAC NIR methods for meat and/or meat products. Therefore, it seemed appropriate that this method should be evaluated by collaborative study, and if the results were acceptable, that the method should be made an AOAC Official Method for meats and meat products.

This collaborative study was conducted to evaluate the performance of the FOSS FoodScan™ NIR spectrophotometer (Eden Prairie, MN) with artificial neural network (ANN) calibration to support the official use of the instrument within the meat industries. The FoodScan analysis is a secondary method based on NIR transmittance technology for the simultaneous determination of moisture, protein, and fat content in meat and meat products.

FoodScan Hardware

From a tungsten-halogen lamp housed at the back of the instrument, light is guided through an optical fiber into the internal moving-grating monochromator, which provides monochromatic light in the spectral region between 850 and 1050 nm. Through a second optical fiber, light is then guided through a collimator lens positioned over the sample cup in the sample chamber. The light is transmitted through the sample, and the unabsorbed light strikes a detector. The detector measures the amount of light and sends the result to the digital signal processor, which communicates with the personal computer (PC) where the final results are calculated.

The sample is placed in a cup and positioned inside the FoodScan sample chamber. The sample cup is rotated during the analysis process to subscan various zones of the test sample, which are then merged together for the final result. This procedure provides a more representative result from potentially nonhomogeneous samples.

Submitted for publication May 2007.
The recommendation was approved by the Methods Committee on Commodity Foods and Commodity Products as First Action. *See* "Official Methods Program Actions," (2007) *Inside Laboratory Management*, July/August issue.
Corresponding author's e-mail: mbusch@fossnorthamerica.com
[1] Deceased.

Table 1. Results of the ANN calibration validation

Parameter	N[a]	SEP[b]	Mean[c]	Min.[d]	Max.[e]	r^{2f}
Fat	1403	0.78	21.10	0.30	70.60	0.9949
Moisture	1197	0.72	59.30	21.10	82.00	0.9968
Protein	1226	0.62	16.00	6.40	31.40	0.9744

[a] N = Number of samples in the independent validation data set.
[b] SEP = Accuracy expressed as standard error of prediction (SEP) in g/100 g.
[c] Mean = Average value of the validation set in g/100 g.
[d] Min. = Minimum value in the validation set in g/100 g.
[e] Max. = Maximum value in the validation set in g/100 g.
[f] r^2 = Coefficient of determination between FoodScan results and chemical analysis results.

Artificial Neural Network Calibration

ANN calibration is a technique designed to emulate the basic function of the human brain to solve complex problems. The ANN model has the ability to describe both linear and nonlinear relationships between spectral characteristics and compositional analysis. As demonstrated by Borggaard and Thodberg (1, 2), ANN models have been found to offer superior performance, compared with other calibration techniques such as partial least squares (PLS) regression models. Further, PLS calibration models often cannot adequately describe a very broad range of sample types, physical characteristics, and composition within a single calibration.

The ANN technique was chosen for the FoodScan meat calibrations because of its ability to generate a single, global, multiproduct, full-range calibration for each constituent. Thus, ANN technology eliminates the need for development and maintenance of separate calibrations for specific sample types and/or analyte levels.

ANN calibrations require a database where all calibration samples are represented by spectra and chemical analysis results for each analyte. Calibration development uses chemometric techniques to correlate spectral characteristics of the sample with compositional analysis. It is important that calibration samples describe as much variability as reasonable and practical. This variability includes sample type, sample composition, and results of chemical analysis and will give the calibration robustness with respect to these variables. The FoodScan database contains over 21 000 meat and meat product samples collected throughout the world. Constituent ranges of calibration samples in the database are for fat, 0.1–86%; moisture, 10–81%; and protein, 3–49%.

The laboratory procedures used for the ANN calibrations were officially recognized methods for chemical analysis of meat and were predominately International Standards Organization (ISO) and AOAC methods; however, because samples came from many countries, many locally approved methods were also used. Methods included combustion and Kjeldahl for protein, Soxhlet with and without acid hydrolysis for fat, and drying- and vacuum-oven methods for moisture. The specific chemical analysis methods used in this study are identified in the *Collaborative Study* section.

The ANN calibration is developed and optimized by an iterative training process on this database until calibration errors have been minimized. To ensure the calibration has not been "overfitted," it is validated against an independent data set representing samples and reference laboratories not included in the calibration set. In routine analysis, the test sample is expressed as a result through a mathematical conversion of the spectral data by using the ANN calibration model.

Precollaborative Calibration Validation

Before the collaborative study was conducted, the accuracy of the FOSS FoodScan and ANN calibration was evaluated. By using independent validation data sets (i.e., samples that were not part of the calibration data set), the results predicted by FoodScan were compared with results from laboratory methods of chemical analysis. The validation sets comprised between 1200 and 1400 samples from several countries. They included various sample types (beef, poultry, pork, fresh, in-process, and finished products) and multiple instruments, plant locations, and laboratories providing chemical analysis values. The results of the validation study are summarized in Table 1.

The accuracy of the ANN calibration is quantified in terms of standard error of prediction (or performance; SEP) and is calculated as defined by Mark and Workman (3):

$$SEP = \left(\sum (Y_i - \hat{Y}_i)^2 / N \right)^{1/2}$$

where Y_i = chemical analysis value of the ith sample; $\hat{Y}_i$ = predicted value of the ith sample obtained from the calibration [each sample in the validation set will have a Y (chemical analysis) and a corresponding $\hat{Y}$ (NIR) value]; and N = number of samples in the validation set.

In routine usage, an SEP is calculated and compared with the chemical analysis laboratory error for each constituent. As described by Mark and Workman (4), the SEP of a good calibration is generally 1.0–1.5 times the laboratory error as determined by a blind duplicate study from a minimum of 10 samples.

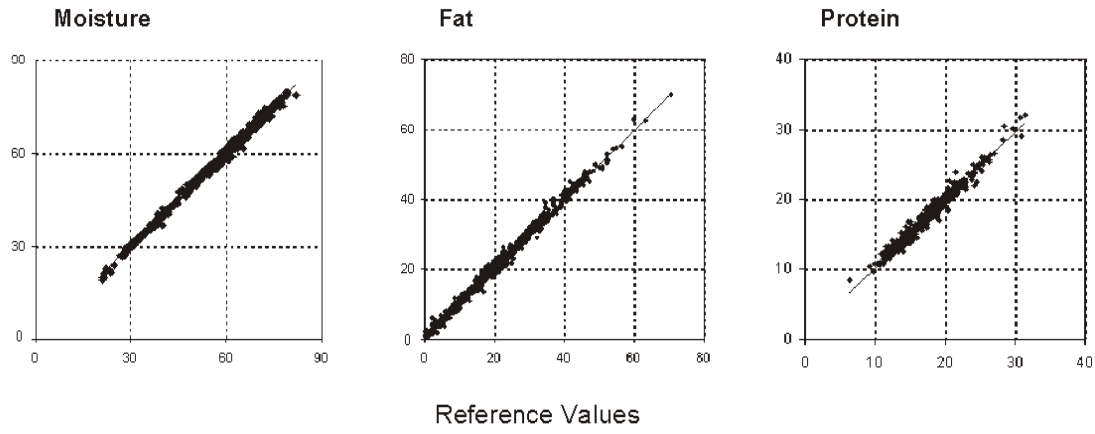

Figure 1. Correlation of chemical analysis values with FoodScan predicted values.

The SEPs as shown in Table 1 are in terms of constituent percent (g/100 g) and were calculated from the validation set as 0.62 for protein, 0.78 for fat, and 0.72 for moisture.

The coefficient of determination (r^2) values from Table 1 quantify the proportion of the sample variance explained by the calibration and were found to be 0.9949 for fat, 0.9968 for moisture, and 0.9744 for protein. These values were found to be acceptable on the basis of the criteria suggested by Williams (5) for NIR applications where r^2 values of >0.96 are deemed "usable in most applications, including quality assurance." The graphs in Figure 1 plot chemical analysis values (x) versus the FoodScan predicted values (y) for moisture, fat, and protein for the validation sets.

Collaborative Study

The Study Director and 15 meat-plant laboratories participated in the collaborative study. Laboratories were selected to be representative of those that would routinely use the proposed method and on the basis of availability of the necessary instrumentation.

Practice samples for testing were sent to each collaborator before the actual study samples, and results were reported to the Study Director. This was done to rehearse all aspects of the study including sample preparation, transportation, handling, and actual analysis protocol as well as to determine the collaborator's capability to perform the study. In addition, it ensured that all collaborators' instruments contained the same instrument settings, version of operating software, and ANN calibration, and that all slope and intercept (bias) adjustment options were set to 1 and 0, respectively. The configuration of instrument parameters is identified in the FoodScan software as a "Profile," which was identified as "AOAC" and used for all practice and study samples.

The study samples were chosen to represent the majority of products from the commercial meat industry (beef, pork, and poultry) and included raw meats, emulsions, and finished products. All samples were natural and real-world, and none were adulterated. The collaborative study samples consisted of 10 meat study samples prepared as blind duplicate pairs, resulting in 20 test samples.

The study samples were ground according to AOAC Official Method **983.18** (6). Of these samples, 6 were fresh meat, 2 were emulsions, and 2 were finished (cured) products. The use of fresh meat samples presented several logistical and practical challenges. Because of shipping and import/customs restrictions, participants in the study were required to be U.S. based. All study samples had to be held at refrigerator temperature (2–4 C) to avoid deterioration and/or water purge. Samples were not to be frozen. Samples were cold packed and shipped via overnight carriers to the collaborators, who were notified to expect delivery and instructed to place them under refrigeration and conduct the analyses within 24 h. There was a 10-day turnaround for the reference laboratory conducting the chemical analyses of the study samples. Because the samples could not be withheld from the collaborators until the results of the analyses by the reference laboratory were obtained, they were shipped to the reference laboratory and the collaborators simultaneously. Another challenge was to avoid the plethora of issues associated with interspecies contamination and hygiene from the presence of "nonproduction" meats in the meat plants. These problems were overcome by sending the samples to the collaborators preground and sealed in the sample cups. After analyzing the samples, the collaborators returned them to the Study Director in biohazard bags. For several collaborators, permission from the onsite U.S. Department of Agriculture (USDA) inspector was required for them to participate in the collaborative study, and only if this sample management protocol was observed.

The samples were evaluated for homogeneity and to determine chemical analysis reference values by using an independent analytical laboratory (Covance Laboratories, Inc., Madison, WI). Covance is accredited by the USDA Food Safety and Inspection Service in food chemistry for meat and poultry products.

To determine homogeneity, 10 sample aliquots for each constituent were taken in a random manner from approximately 5 lb ground sample material. Ten replicate analyses for each sample and constituent were performed.

Table 2. Results obtained by the reference laboratory for the determination of homogeneity, fat, moisture, and protein

		Protein			Fat			Moisture		
Sample	Type	Mean, g/100 g[a]	s_r	CV, %	Mean, g/100 g[a]	s_r	CV, %	Mean, g/100 g[a]	s_r	CV, %
Beef, low fat	Fresh, ground	17.80	0.23	1.29	16.79	0.19	1.13	65.23	0.65	1.00
Beef, high fat		15.64	0.31	1.98	29.30	0.57	1.95	54.42	0.39	0.72
Pork, low fat		17.17	0.37	2.16	22.25	0.36	1.62	61.17	0.22	0.36
Pork, high fat		14.68	0.44	3.00	31.92	0.37	1.16	53.98	0.44	0.30
Chicken breast	Fresh, whole muscle	22.36	0.15	0.67	3.27	0.19	5.81	73.75	0.40	0.54
Turkey breast		24.47	0.12	0.49	1.48	0.14	9.46	73.86	0.33	0.45
Pepperoni	Finished product	20.50	0.60	2.93	41.86	1.17	2.80	28.91	0.83	2.87
Hard salami		20.41	0.37	1.81	33.82	0.56	1.66	39.63	0.42	1.06
Hot dog	Emulsion	16.42	0.15	0.91	15.39	0.13	0.84	63.29	0.32	0.51
Summer sausage		17.31	0.21	1.21	8.07	0.10	1.24	68.51	0.26	0.38

[a] Ten replicate determinations.

AOAC Official Methods for meat analysis were used for these determinations: protein, combustion method, **992.15** (7); fat, Soxhlet method, **960.39** (8); and moisture, 125 C air dry, **950.46B**(**b**) (9). For each sample and constituent, the mean chemical analysis value, repeatability standard deviation (s_r), and coefficient of variation (CV) were obtained (Table 2).

Sample constituent values ranged from 1.48 to 41.86% for fat, from 28.91 to 73.86% for moisture, and from 14.68 to 24.47% for protein. As suggested by Thiex et al. (10), an acceptability criterion of 2.0% for the CV was applied to evaluate homogeneity. Several observations were noted during this study. The comparatively high CVs for fat in chicken (5.81%) and turkey (9.46%) appeared to be due to low mean results for fat. The s_r values of 0.19 and 0.14 for fat in chicken and turkey, respectively, compare satisfactorily with the Soxhlet results from the AOAC collaborative study as reported by Foster and Gonzales (11). Because the CVs for the other constituents in these samples were under the 2% limit, these samples were considered homogeneous. In general, the CVs for protein were somewhat high, specifically the CVs for the high- and low-fat pork and the pepperoni, which were above the 2% criterion. This was likely due to the 0.3 g sample size that the reference laboratory used for protein determination, which was not discovered until after the chemical analyses were performed. For meat samples, including most products in this study, 0.3 g should not be considered a representative sample size for the chemical determination of protein. When the CVs for moisture in the pork samples were considered, these samples were also considered homogeneous. Pepperoni was the only sample for which the CVs for all 3 constituents were >2%.

The collaborators were asked to report all results on an "as is" basis, to perform only 1 analysis per sample, and to report results for each constituent to 2 decimal places. Two of the 15 collaborators had 2 instruments at their facilities and analyzed the study samples on each instrument; these additional analyses resulted in a total of 17 data sets.

Study sample materials were prepared and shipped to collaborators on July 17, 2006. Collaborators were asked to analyze the samples within 24 h after receipt of the samples and to hold them, if necessary, in the refrigerator. Study sample results were received from the collaborators within 72 h.

AOAC Official Method 2007.04
Fat, Moisture, and Protein in Meat and Meat Products

FOSS FoodScan™ Near-Infrared (NIR) Spectrophotometer with FOSS Artificial Neural Network (ANN) Calibration Model and Associated Database
First Action 2007

[Applicable to the simultaneous determination of fat, moisture, and protein in meat and meat products (fresh meat, beef, pork, and poultry, emulsions, and finished products) in the constituent ranges of 1–43% fat, 27–74% moisture, and 14–25% protein.]

Caution: (*1*) The FoodScan is designed to operate safely under the following conditions: indoor use only; altitude, 2000 m; temperature, 5–40 C; maximum relative humidity, 80% for temperatures of 31 C, decreasing linearly to 50% relative humidity at 40 C; (*2*) because of electric shock hazard, covers or panels must be removed by qualified personnel only; (*3*) the instrument is equipped with an AC power connector containing a protective ground against electric shock hazard; (*4*) if the equipment is used in a manner not specified in the documentation, the protection provided by the equipment may be

Table 2007.04. Interlaboratory study results for the determination of fat, moisture, and protein in meat and meat products

Sample	Constituent determined	No. of labs[a]	Mean, %	s_r[b]	r[c]	RSD_r, %[d]	s_R[e]	R[f]	RSD_R, %[g]	HorRat	Rec., %
Beef, low fat	Fat	17	17.46	0.28	0.78	1.60	0.33	0.92	1.87	0.72	104.17
	Moisture	17	62.30	0.41	1.15	0.66	0.64	1.79	1.02	0.48	95.51
	Protein	17	18.92	0.33	0.92	1.76	0.64	1.79	3.36	1.32	106.68
Beef, high fat	Fat	17	29.99	0.26	0.73	0.87	0.29	0.81	0.95	0.40	102.37
	Moisture	17	51.96	0.48	1.34	0.92	0.66	1.85	1.26	0.57	95.48
	Protein	17	16.13	0.22	0.62	1.37	0.40	1.12	2.51	0.95	103.10
Pork, low fat	Fat	17	21.99	0.16	0.45	0.73	0.17	0.48	0.75	0.30	98.81
	Moisture	17	60.51	0.20	0.56	0.33	0.33	0.92	0.55	0.25	98.92
	Protein	17	16.71	0.20	0.56	1.18	0.44	1.23	2.61	1.00	97.40
Pork, high fat	Fat	17	31.32	0.07	0.20	0.22	0.17	0.48	0.55	0.23	98.11
	Moisture	17	53.29	0.22	0.62	0.40	0.38	1.06	0.71	0.32	98.73
	Protein	17	14.53	0.13	0.36	0.88	0.35	0.98	2.44	0.91	99.00
Chicken breast	Fat	17	3.25	0.07	0.20	2.10	0.14	0.39	4.08	1.22	102.44
	Moisture	17	73.48	0.19	0.53	0.26	0.32	0.90	0.43	0.21	99.63
	Protein	17	22.74	0.09	0.25	0.40	0.18	0.50	0.78	0.31	101.68
Turkey breast	Fat	17	1.89	0.05	0.14	2.67	0.13	0.36	6.89	1.90	127.84
	Moisture	17	73.69	0.17	0.48	0.23	0.29	0.81	0.39	0.19	99.78
	Protein	16(1)	24.86	0.18	0.50	0.71	0.22	0.62	0.90	0.36	101.60
Pepperoni	Fat	17	43.42	0.15	0.42	0.35	0.22	0.62	0.52	0.23	103.72
	Moisture	17	27.29	0.20	0.56	0.74	0.42	1.18	1.55	0.64	94.40
	Protein	15(2)	20.87	0.14	0.39	0.69	0.33	0.92	1.56	0.62	101.80
Hard salami	Fat	17	32.22	0.16	0.45	0.50	0.29	0.81	0.89	0.37	95.26
	Moisture	16(1)	38.81	0.13	0.36	0.35	0.16	0.45	0.41	0.18	97.93
	Protein	16(1)	19.66	0.07	0.20	0.35	0.11	0.31	0.54	0.21	96.31
Hot dog	Fat	17	15.05	0.14	0.39	0.92	0.25	0.70	1.64	0.62	97.82
	Moisture	17	62.17	0.20	0.56	0.32	0.30	0.84	0.48	0.22	98.23
	Protein	17	15.25	0.26	0.73	1.69	0.52	1.46	3.42	1.29	92.86
Summer sausage	Fat	17	7.79	0.11	0.31	1.43	0.44	1.23	5.60	1.91	96.53
	Moisture	17	68.48	0.56	1.57	0.82	0.72	2.02	1.06	0.50	99.95
	Protein	17	15.03	0.32	0.90	2.13	0.79	2.21	5.23	1.97	86.82

[a] Number of laboratories retained after removal of the number of laboratories in parentheses.

[b] s_r = Repeatability standard deviation.

[c] r = Repeatability; r = 2.8 s_r.

[d] RSD_r = Repeatability relative standard deviation.

[e] s_R = Reproducibility standard deviation.

[f] R = Reproducibility; R = 2.8 s_R.

[g] RSD_R = Reproducibility relative standard deviation.

impaired; (*5*) do not touch identified hot surfaces; (*6*) when lifting or moving the instrument, use proper lifting equipment.

Results of interlaboratory study.—Values for within-laboratory [repeatability relative standard deviation (RSD_r)]: 0.22–2.67% for fat, 0.23–0.92% for moisture, and 0.35–2.13% for protein; values for between-laboratories [reproducibility relative standard deviation (RSD_R)]: 0.52–6.89% for fat, 0.39–1.55% for moisture, and 0.54–5.23% for protein; HorRat: 0.23–1.91 for fat, 0.18–0.64 for moisture, and 0.21–1.97 for protein.

See Table **2007.04** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

The method uses the FOSS FoodScan™ (FOSS North America, 8091 Wallace Rd, Eden Prairie, MN 55344, USA) with artificial neural network (ANN) calibration and associated database. The method is based on near-infrared (NIR) transmission spectroscopy, a secondary, correlative technique to predict the concentration of various constituents in biological or organic samples. The ground sample is placed in a cup and positioned inside the FoodScan sample chamber. The sample cup is rotated during the analysis process to subscan 16 zones of the test sample, which are then merged together for the final result. The ANN calibration model is derived from a database of sample spectra and associated chemical analysis values. The ANN calibration quantifies the relationship between the spectral characteristics and the constituent values to interpret the test spectra and return the results for protein, fat, and moisture.

B. Apparatus

(**a**) *FOSS FoodScan system.*—NIR transmission, with a moving grating monochromator scanning the region from 850 to 1050 nm.

(**b**) *FoodScan ANN calibration for meat and meat products, version 3.00, with the associated database.*—The FoodScan for meat comes complete with the operating software, ANN calibration, and required accessories.

(**c**) *Polysulfone or glass-bottom sample cups*.—140 mm (diameter) 17.5 mm (height). Because of optical variations, the use of polystyrene dishes, such as Petri dishes, is not recommended.

(**d**) *Personal computer (PC).*—With the following minimum specifications: XP (SP2) operating system, Intel Celeron or Pentium 4 processor, 2.8 GHz, 512 Mb RAM, 40 MB hard-drive space, CD and floppy drives, and USB ports.

Items (**a**)–(**d**) are from FOSS Analytical (Slangerupgade 69, DK-3400 Hillerød, Denmark) from FOSS North America (Tel: 1-952-974-9892, Fax: 1-952-974-9823, www.foss.dk).

C. Preparation of Analytical Sample

Grind or homogenize representative sample, using standardized protocol as described in **983.18**. Pack approximately 180 g sample into the FoodScan sample cup. Avoid air pockets in the sample, and pack the sample level with the top of the sample cup and in a consistent manner. The optimal sample temperature is 10–20 C; however, if measurements outside this range are needed, ensure that the temperatures of the samples do not vary by more than ±5 C and/or condensation on the collimator lens.

D. Determination

(*1*) Turn power on for the unit, allow unit to warm up, and perform self-test diagnostics.

(*2*) Select the appropriate operator ID and product profiles. The product profile must specify the use of the FoodScan ANN calibration for meat and meat products, version 3.00.

(*3*) Place prepared sample into the sample cup.

(*4*) Place the sample cup in the holder in the instrument. Ensure that the sample cup engages the index pin in the holder. Close and lock the door.

(*5*) Start the analysis by pressing the "Start" button.

(*6*) Enter sample ID and/or sample description.

(*7*) When analysis is complete, remove sample from the instrument.

(*8*) Process and/or record results.

E. Calculations

The FoodScan software calculates the results for fat, moisture, and protein, which are displayed as percentages (g/100 g) to 2 decimal places.

F. Calibration Validation

NIR analysis is a secondary, correlative technique, and the results must be validated against those obtained by chemical analysis methods. It is important to use chemical analysis methods that are officially approved and standardized such as AOAC Official Methods for Protein (**992.15**), Fat (**960.39**), and Moisture [**950.46B**(**b**)]. The purpose of validation is to determine the degree of agreement of the FoodScan results with those from chemical analyses, based on analysis of r^2 values, standard error of prediction (SEP) values, and bias statistics.

Calculate the coefficient of determination (r^2) between chemical analysis methods and FoodScan predictions from a minimum of 10 samples. Compare this value to general calibration guidelines (>0.96) and/or historical performance.

The SEP is compared with the laboratory error for each constituent and is calculated as follows:

$$(\ (Y_i - \hat{Y}_i)^2/N)^{1/2}$$

where Y_i = chemical analysis value of the *i*th sample; $\hat{Y}_i$ = predicted value of the *i*th sample obtained from the calibration [each sample in the validation set will have a Y (chemical analysis) value and a corresponding $\hat{Y}$ (NIR) value]; and N = number of samples in the validation set.

A satisfactory SEP is 1.0–1.5 times the laboratory error as determined by a blind duplicate study.

Determine the bias from the difference between the mean of chemical analyses and the FoodScan results from a minimum of 10 samples (mean laboratory – mean FoodScan). Evaluate the bias for significance by determining the ±bias limit, using the following formula:

$$(3 \quad S)/\sqrt{N}$$

where S = standard deviation of the differences between the FoodScan prediction and the chemical analysis result in the validation set; N = number of samples in the validation set (minimum of 10).

When this limit is exceeded, a bias (or offset) is implemented in the FoodScan software, and the validation process is then repeated.

Reference: *J. AOAC Int*. **90**, 1073(2007).

Results and Discussion

The results obtained from the collaborative study are shown in Table 3. In the first analysis of the collaborative data, a laboratory ranking was obtained by using the procedure described by Youden and Steiner (12) to detect any bias among collaborators' instruments. Only protein for Laboratory 3 was found to have a small but significant bias. However, because none of the other constituents from this laboratory demonstrated a bias (in which case an instrument issue may have been indicated), this laboratory was included in the study. Further analysis of the collaborative study data found 4 pairs of results that were identified as outliers by either the Cochran or the Grubbs test. All statistics and outlier detection, except laboratory bias, were determined by using worksheets obtained through AOAC INTERNATIONAL for blind duplicate collaborative study design (13).

The statistical analysis of the collaborative study results is summarized in Table **2007.04**. The within-laboratory RSD_r ranged from 0.22 to 2.67% for fat, from 0.23 to 0.92% for moisture, and from 0.35 to 2.13% for protein. The between-laboratories RSD_R ranged from 0.52 to 6.89% for fat, from 0.39 to 1.55% for moisture, and from 0.54 to 5.23% for protein.

HorRat values, as described by Horwitz (14), were calculated and found to be acceptable, ranging from 0.23 to 1.91 for fat, from 0.18 to 0.64 for moisture, and from 0.21 to 1.97 for protein. Three HorRat values were found to approach the acceptability limit of 2: fat (1.91) and protein (1.97) in summer sausage and fat (1.90) in turkey breast. For summer sausage, these results have been due to possible homogeneity, ingredient, or other optical characteristics. The high HorRat value for fat in turkey was caused by the very low mean value.

The pepperoni sample, which demonstrated high CVs of 2.93% for protein, 2.80% for fat, and 2.87% for moisture in the homogeneity study, yielded very good HorRat values of 0.62, 0.23, and 0.64, respectively. These results could be due to the FoodScan's use of a larger sample size than was used in the chemical analyses and/or to the higher mean values, compared with (for example) those for protein and fat in the summer sausage.

Percent recovery was determined by comparing the overall mean of the collaborator values for each study sample constituent with its chemical analysis value. In most cases, the recovery was very good, between 94 and 107%. Three results demonstrated either high or low recovery: fat in turkey (128%), protein in hot dog (93%), and protein in summer sausage (87%).

Bias or inherent systematic error, as described by Youden and Steiner (15), is exhibited when the predicted results for a specific sample group or product show a mean offset value when compared with their reference values.

The bias, i.e., difference between the mean of chemical analyses and the FoodScan results from a minimum of 10 samples (mean laboratory – mean FoodScan), may or may not be statistically significant. Based on the procedure described in AOAC Official Method **972.16** (16), the ±bias limit can be calculated by using the following formula:

$$(3 \quad S)/\sqrt{N}$$

where S = standard deviation of the differences between the FoodScan prediction and the chemical analysis in the validation set; N = number of samples in the validation set (minimum of 10).

When this limit is exceeded, a bias (or offset) is implemented in the FoodScan software, and the validation process is repeated. Refer to the Foss FoodScan User Manual (17) for this procedure.

Comparison of FoodScan and Chemical Analysis Precision

The precision values of the FoodScan study results were compared with those of official chemical analysis methods for both the study samples and prior AOAC and ISO collaborative studies. Because AOAC Official Method **950.46B**(**b**) for moisture in meat includes no precision data, ISO 1442 (18) was used. This method, based on drying of the sample at 102–105 C for 16–18 h, had been collaboratively studied in the early 1990s, and precision statistics were determined. Table 4 compares the precision parameters found for the study sample chemical analysis and collaborative studies prior to the FoodScan collaborative study.

For protein and moisture, the FoodScan collaborative study data demonstrated similar repeatability and reproducibility compared with the corresponding data for chemical analysis methods. For fat determinations, the FoodScan demonstrated better repeatability and reproducibility.

Collaborators' Comments

There were relatively few collaborator comments. Collaborator 4 reported that when the samples arrived, their temperature was found to be 59 F (15 C), and the following was noted: Sample 4: "Top of dish has red-colored meat";

Table 3. Interlaboratory study results for the determination of fat, moisture, and protein in 20 test samples prepared as 10 blind duplicate pairs

	Beef, high fat		Beef, low fat		Chicken		Hard salami		Hot dog, emulsion		Pepperoni		Pork, high fat		Pork, low fat		Summer sausage, emulsion		Turkey	
Lab	20	4	5	17	8	16	2	6	1	10	11	7	13	19	12	3	9	15	14	18
									Fat, g/100 g											
A	30.30	30.11	17.60	17.53	3.18	3.13	32.20	32.09	14.96	14.95	43.22	43.23	31.49	31.33	21.88	21.94	7.99	7.72	1.71	1.71
B	30.22	30.19	17.69	17.21	3.27	3.29	32.07	32.05	14.93	14.92	43.17	43.41	31.50	31.43	22.19	21.96	7.78	7.73	1.89	1.94
C	30.37	30.13	17.92	17.88	3.50	3.29	32.66	32.89	15.09	15.39	43.86	43.68	31.37	31.48	22.07	21.88	8.03	8.29	2.03	2.04
D	30.10	29.89	17.77	17.26	3.56	3.50	32.30	32.69	15.17	15.20	43.73	43.57	31.16	31.27	21.84	21.89	8.65	8.48	2.04	2.06
E	29.93	29.95	17.41	17.00	3.41	3.44	31.88	32.01	14.78	14.57	42.97	43.02	31.10	31.03	22.31	21.96	7.78	7.56	1.86	1.86
F	29.77	30.06	17.97	17.91	3.42	3.30	32.20	32.34	15.67	15.56	43.64	43.62	31.19	31.30	21.96	22.05	7.29	7.11	1.91	1.98
G	29.50	29.87	17.64	17.43	3.26	3.32	31.77	32.02	14.81	14.99	43.28	43.37	31.29	31.08	21.99	21.78	7.94	7.95	1.73	1.73
H	30.13	30.09	17.69	17.21	3.21	3.34	32.27	32.22	14.65	14.76	43.69	43.38	31.14	31.15	22.07	21.63	7.91	7.88	1.89	1.84
I	30.27	29.43	17.65	17.01	3.45	3.27	32.03	32.43	15.19	15.25	43.42	43.43	31.01	31.07	21.75	21.97	7.02	7.07	1.99	1.94
J	30.22	30.45	17.97	18.12	3.17	3.28	31.99	31.99	15.34	15.06	43.61	43.81	31.53	31.60	22.46	22.00	7.17	7.04	1.67	1.77
K	29.61	29.63	16.97	17.05	3.57	3.70	32.11	32.06	15.30	15.15	43.27	43.31	31.58	31.60	22.25	22.06	7.68	7.76	2.25	2.12
L	30.04	30.56	17.01	17.79	3.09	3.16	32.11	32.41	15.30	14.96	43.21	43.47	31.50	31.47	21.87	22.09	7.23	7.36	1.69	1.87
M[a]	29.65	30.17	17.57	17.21	3.49	3.39	31.90	32.02	15.14	14.92	43.46	43.26	31.39	31.47	22.06	22.20	7.77	7.86	1.93	1.87
M[b]	29.98	30.14	17.51	17.17	3.28	3.34	32.83	32.54	15.19	15.02	43.57	43.44	31.28	31.27	21.87	21.86	8.00	8.10	1.78	1.87
N[a]	30.05	29.82	17.48	17.49	3.33	3.31	32.32	31.95	14.97	14.93	43.09	43.54	31.36	31.27	21.89	21.94	8.03	7.81	1.85	1.84
N[b]	30.03	29.34	17.27	17.62	3.41	3.37	32.14	31.85	15.01	14.69	43.07	43.50	31.25	31.14	22.05	21.97	8.10	7.92	1.91	1.89
O	29.75	30.05	17.07	17.61	3.42	3.44	32.47	32.56	14.89	15.12	43.44	43.46	31.28	31.34	22.01	21.80	8.50	8.34	1.91	1.96
									Moisture, g/100 g											
A	52.10	52.05	62.08	61.85	73.41	73.36	38.82	38.68	62.60	61.97	27.55	27.46	53.24	53.21	60.40	60.20	69.48	68.58	73.49	73.47
B	51.20	51.36	62.08	62.46	73.54	73.39	39.02	38.90	62.44	62.31	27.44	27.31	53.57	53.10	60.33	60.76	69.53	69.09	73.67	73.80
C	51.75	52.33	62.48	62.56	73.35	73.76	38.90	38.66	61.98	61.96	27.69	27.75	53.85	53.80	60.84	61.04	67.30	67.65	73.96	73.68
D	52.03	51.99	62.47	62.91	73.84	73.70	39.04	38.82	61.97	61.94	27.77	27.78	53.45	53.56	60.55	60.94	67.99	69.23	74.11	74.04
E	51.30	52.31	61.78	62.35	73.69	73.50	38.76	38.69	62.32	62.82	27.15	27.12	52.97	53.11	60.01	59.98	67.24	69.23	73.61	73.81
F	52.27	51.58	61.63	62.06	73.77	73.50	38.80	38.64	62.25	62.29	26.83	26.93	53.77	53.34	60.90	60.97	67.67	67.55	73.72	73.84
G	53.04	52.40	62.23	62.53	73.37	73.27	39.11	38.97	62.74	62.37	27.72	27.70	53.50	53.79	60.60	60.58	68.80	69.20	73.51	73.66
H	52.33	52.05	61.92	62.89	73.46	73.49	38.81	38.73	62.38	62.26	27.14	27.40	53.37	53.79	60.05	60.73	68.47	67.66	73.34	73.56
I	51.16	52.07	62.19	63.30	73.45	73.88	37.22[a]	38.63[a]	62.44	62.41	26.41	26.82	53.31	52.93	60.60	60.31	68.10	69.11	73.80	73.94
J	50.89	50.45	60.60	60.42	72.97	72.73	38.83	38.66	61.89	62.09	26.97	27.20	52.65	52.68	60.12	60.16	69.28	69.37	73.07	73.34
K	52.79	53.41	62.69	62.98	73.77	73.94	39.00	38.95	62.15	62.20	26.43	26.46	53.55	53.22	59.82	60.24	68.14	67.71	74.40	74.12

Table 3. (*continued*)

	Beef, high fat		Beef, low fat		Chicken		Hard salami		Hot dog, emulsion		Pepperoni		Pork, high fat		Pork, low fat		Summer sausage, emulsion		Turkey	
Lab	20	4	5	17	8	16	2	6	1	10	11	7	13	19	12	3	9	15	14	18
L	52.25	51.86	62.73	61.33	73.07	72.84	38.86	38.36	61.55	62.06	27.69	27.79	53.21	53.44	60.56	60.33	67.72	68.42	73.53	73.09
M[a]	52.01	51.35	62.44	62.54	73.56	72.86	38.89	38.75	62.26	62.14	27.03	26.95	52.94	53.07	60.62	60.53	67.84	68.49	73.41	73.66
M[b]	52.49	51.99	62.94	62.98	73.35	73.43	38.81	38.57	61.96	61.78	27.51	26.95	53.83	53.78	60.88	60.96	67.65	68.49	73.76	73.73
N[a]	51.10	52.19	62.41	62.32	73.41	73.40	38.66	38.66	62.08	62.01	27.64	26.99	53.02	53.38	60.75	60.78	68.79	69.40	73.65	73.46
N[b]	50.91	52.32	62.29	62.07	73.75	74.00	38.94	39.02	62.23	62.61	27.76	27.27	52.94	52.29	60.77	60.46	69.40	69.20	74.04	73.58
O	52.63	52.67	63.18	62.49	73.74	73.74	38.89	38.75	61.61	61.77	27.77	27.52	53.11	53.16	60.20	60.36	68.07	68.41	73.77	73.99
											Protein, g/100 g									
A	16.22	16.32	19.64	19.81	22.75	22.81	19.69	19.74	14.24	15.25	20.74	20.73	14.79	14.81	16.85	17.14	14.09	14.56	25.10	25.19
B	16.83	16.68	19.85	19.68	22.86	22.87	19.67	19.64	15.21	15.15	20.72	20.70	14.72	14.98	16.95	16.93	14.10	14.39	25.28	25.08
C	15.76	15.83	17.94	18.23	22.85	22.76	19.72	19.74	15.58	15.61	20.74	20.62	13.91	13.77	15.95	15.96	15.84	15.53	24.81	24.84
D	15.94	15.96	17.92	17.91	22.85	22.91	19.83	19.75	15.63	15.43	20.78	21.16	14.14	14.16	16.25	16.27	15.19	15.44	25.14	24.88
E	16.65	16.31	19.64	19.53	22.68	22.74	19.46	19.56	14.73	14.43	20.36	20.85	14.65	14.97	17.06	17.21	14.44	14.68	25.03	24.84
F	15.81	16.17	18.70	18.53	23.07	23.00	19.61	19.64	15.83	16.00	21.51	21.54	14.45	14.71	16.80	16.69	16.13	16.34	25.04	24.96
G	15.94	16.06	19.47	18.93	22.65	22.66	19.65	19.75	14.22	14.41	21.03	20.85	14.73	14.49	16.56	16.64	14.25	14.09	25.04	24.83
H	15.58	15.79	18.92	18.31	22.66	22.95	19.61	19.66	15.14	14.92	20.98	20.75	14.48	14.20	16.29	16.86	14.63	15.20	25.01	24.69
I	16.64	16.47	19.07	18.67	22.68	22.82	19.43	19.67	15.89	15.67	20.26[a]	21.23[a]	14.52	14.38	16.83	16.99	16.26	15.38	24.51	24.70
J	15.53	15.58	18.58	19.04	22.78	22.74	17.64[b]	17.79[b]	15.88	15.46	19.16[b]	19.22[b]	14.97	14.71	16.66	17.38	16.45	16.94	25.03	24.52
K	16.31	16.03	18.09	18.98	22.36	22.23	19.53	19.37	16.03	15.99	20.94	21.01	14.85	14.95	17.17	17.56	15.16	15.49	23.86[b]	24.30[b]
L	16.41	16.45	19.56	20.07	22.49	22.60	19.73	19.71	15.09	14.76	20.90	21.05	14.39	14.48	16.38	16.44	15.08	14.16	24.82	24.56
M[a]	16.01	16.58	19.65	19.02	22.86	22.95	19.61	19.68	15.71	15.30	21.14	21.41	14.94	14.76	17.08	17.01	15.09	14.80	25.09	24.96
M[b]	15.68	16.05	18.70	18.68	22.59	22.70	19.72	19.77	15.61	15.16	20.39	20.29	13.91	13.81	16.05	15.74	15.54	15.06	24.81	24.74
N[a]	16.72	16.09	18.48	19.51	22.83	22.75	19.80	19.75	14.92	15.43	20.51	20.42	14.68	14.62	16.71	16.59	14.30	14.17	24.96	24.52
N[b]	16.75	16.16	19.03	19.09	22.74	22.44	19.54	19.67	14.62	14.76	20.77	20.79	14.84	14.81	17.13	17.08	14.11	14.38	24.68	24.38
O	15.52	15.42	18.20	17.96	22.75	22.65	19.69	19.63	15.14	15.24	21.27	21.12	14.32	14.23	16.41	16.64	15.08	14.64	24.82	24.67

[a] Excluded as an outlier by the Cochran test.

[b] Excluded as an outlier by single Grubbs test.

Table 4. Comparison of chemical analysis and FoodScan methods

Method	Ranges of statistical parameters			
	s_r	RSD_r, %	s_R	RSD_R, %
Protein				
Chemical analysis				
Study samples using **992.15**	0.12–0.60	0.49–3.00	—	—
AOAC **992.15** collaborative study	0.12–0.41	0.60–2.23	0.18–0.46	1.32–3.35
FoodScan				
Collaborative study data	0.07–0.33	0.35–2.13	0.11–0.79	0.54–5.23
SEP (from precollaborative validation)	0.62			
Fat				
Chemical analysis				
Study samples using **960.39**	0.10–1.17	0.84–9.46	—	—
AOAC **960.39** collaborative study	0.12–2.48	1.71–8.75	0.15–3.13	2.26–11.05
FoodScan				
Collaborative study data	0.05–0.28	0.22–2.67	0.13–0.44	0.52–6.89
SEP (from precollaborative validation)	0.78			
Moisture				
Chemical analysis				
Study samples using **950.46B**(**b**)	0.22–0.83	0.30–2.87	—	—
ISO 1442 collaborative study	0.24–0.26	0.34–0.54	0.36–0.41	0.55–0.81
FoodScan				
Collaborative study data	0.13–0.56	0.23–0.92	0.16–0.72	0.39–1.55
SEP (from precollaborative validation)	0.72			

Sample 5: "Blood leaked from dish to plastic bag"; Sample 17: "Moisture leaked from dish to plastic bag." These samples were scanned, and the results were reported with those for the remaining study samples. Collaborator 11 initially ran samples, using an incorrect FoodScan profile; samples were immediately rescanned under the correct profile, and the results were reported to the Study Director. Collaborator 15 ran Sample 9 twice and reported the results as those for Samples 9 and 10. Sample 10 was run, and the results were submitted to the Study Director. Both of these sample-handling errors were immediately discovered upon initial review of the data.

Recommendations

The Study Director recommends that this method for the Determination of Fat, Moisture, and Protein in Meat and Meat Products by Using the FOSS FoodScan Near-Infrared Spectrophotometer with the FOSS Artificial Neural Network (ANN) Calibration Model and Associated Database be adopted as Official First Action.

Acknowledgments

The Study Director thanks Michael Busch, Mark Host, Jeff Boedigheimer, Andree Behrens, and Christian Harpsøe (FOSS North America, Eden Prairie, MN) for their assistance with preparation of the practice and study samples and Jakob Jons (FOSS Analytical, Hillerod, Denmark) for contributing sample cups for the study.

The Study Director also thanks the following collaborators for their participation in this study:

Susan Aldana and Ashley Perez, Beef Products Inc., Holcomb, KS

John Birkey, Cargill Meat Solutions, Friona, TX

Ryan Conway, Cargill Meat Solutions, Ft. Morgan, CO

Tony Hemminger and Todd Shinkle, Pierre Foods, Cincinnati, OH

Jeremy Herrick, Hormel Foods, Algona, IA

Crystal Hurley, SSI Foods, Inc., Wilder, ID

Jennifer Longbind and Marshall Beggs, Beef Products Inc., Amarillo, TX

Scott McMaignal, Beef Products Inc., South Sioux City, NE

Kevin Nanke and Tonya Mitchell, Lopez Foods, Oklahoma City, OK

Mara Phelps, Cristian Ionita, Andi Milu, James Williams, and Andreas Conquest, Tyson Fresh Meats Inc., Goodlettsville, TN

Josh Reitz, Beef Products Inc., Waterloo, IA

Andy Salazar, Cargill Meat Solutions, Plainview, TX

Matthew Strampe and Krista Van Horn, Hormel Foods, Austin, MN

Steve Zelten and Christy Wipperfurth, Fair Oaks Farms, Pleasant Prairie, WI

Steve Zerr, Cloverdale, Mandan, ND

References

(1) Borggaard, C., & Thodberg, H.H. (1992) *Anal. Chem.* **64**, 545–551

(2) Borggaard, C. (2001) in *Near Infrared Technology in the Agriculture and Food Industries*, P. Williams & K. Norris (Eds), American Association of Cereal Chemists, Inc., St. Paul, MN, pp 101–107

(3) Mark, H., & Workman, J. (1991) *Statistics in Spectroscopy*, Academic Press, San Diego, CA, p. 299

(4) Mark, H., & Workman, J. (1991) *Statistics in Spectroscopy*, Academic Press, San Diego, CA, p. 302

(5) Williams, P.C. (2001) in *Near Infrared Technology in the Agriculture and Food Industries*, P. Williams & K. Norris (Eds), American Association of Cereal Chemists, Inc., St. Paul, MN, pp 162–163

(6) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **983.18**

(7) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **992.15**

(8) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **960.39**

(9) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **950.46**

(10) Thiex, N.J., Manson, H., Anderson, S., & Persson, J.A. (2002) *J. AOAC Int.* **85**, 309–317

(11) Foster, M., & Gonzales, S. (1992) *J. AOAC Int.* **75**, 288–292

(12) Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, AOAC INTERNATIONAL, Arlington, VA, p. 74

(13) Lynch, J.M. (2001) *AOAC Statistical Program for Blind Replicates*, Version 1.14, Ithaca, NY

(14) Horwitz, W. (1982) *Anal. Chem.* **54**, 67A–76A

(15) Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, AOAC INTERNATIONAL, Arlington, VA, pp 26–27

(16) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **972.16**

(17) FOSS FoodScan User Manual (2004) FOSS Analytical A/S, Hillerod, Denmark

(18) ISO 1442:1997(E), Meat and Meat Products—Determination of Moisture Content (Reference Method), 2nd Ed., International Organization for Standardization, Geneva, Switzerland