AOAC Official Method 2011.04
Protein in Raw and Processed Meats
Automated Dye-Binding Method
First Action 2011

Caution: The Sprint Protein Analyzer is designed to operate safely under normal laboratory conditions. The instrument utilizes high voltages. Only technicians trained in repair and maintenance of high-voltage systems should perform instrument service. If the instrument is used in a manner not specified in the operation manual, the protection provided by the equipment may be impaired.

See Table **2011.04** for the results of the interlaboratory study supporting acceptance of the method.

A. Scope

Applicable for the determination of protein in raw and processed meat products of beef, pork, and poultry in the protein range of 9 to 40%.

B. Principle

The method uses the CEM Sprint Protein Analyzer (CEM Corp., Matthews, NC, USA; www.cem.com). The method is based on protein-tagging technology where the sample is mechanically mixed with an anionic dye that binds to cationic groups of the basic amino acid residues (histidine, arginine, and lysine) and to free amino terminal groups. The amount of unbound dye remaining in solution after the insoluble protein has been removed by filtration is determined by measuring its absorbance. The amount of protein present in the original solution is proportional to the amount of dye removed from the solution. The method is calibrated to **981.10** (*see* 39.1.19).

C. Apparatus

(**a**) *Sprint Protein Analyzer, with optional meat homogenizer*.—0.2 mg protein sensitivity; range of 0.01 to 99.99% in liquids, solids, and slurries; 0.01% resolution; microprocessor computer control.

(**b**) *Electronic balance*.—0.1 mg readability, serial cable for communication with Sprint Protein Analyzer.

(**c**) *Sprint Pak*.—Includes 50 sample cups with lids, 50 filters, optics cleaning solution, homogenizer cleaning solution, and dye-binding agent.

(**d**) *Certified reference standard.*

Items (**a**), (**c**), and (**d**) are available from CEM Corp.

D. Preparation of Analytical Sample

Prepare laboratory sample using **983.18** (*see* 39.1.01).

E. Procedure

Different types of sample matrixes and proteins exhibit different responses on the Sprint Protein Analyzer and require differing analytical conditions. This is accommodated through the use of methods that are loaded into the system. These sample-type-specific methods contain established, linear relationships for protein determination and a series of analytical conditions that ensure accurate results. It is necessary to establish calibration methods for validation of user-specific product blends. To create user-specific calibration methods, at least two or more reference materials with known protein values (as determined by the Kjeldahl method) are required. Each reference material must be tested a minimum of three times in the Sprint Protein Analyzer to establish a calibration line.

F. Determination

(*1*) Fill all reservoirs with the appropriate solutions provided in each Sprint Pak.

(*2*) On the Sprint MAIN MENU screen, select LOAD METHOD, then select the desired, preprogrammed method. Follow the prompt on the Sprint's screen.

(*3*) Place an empty cup on the balance pan of the electronic balance and press TARE on the Sprint keypad. Serial communication is established between the Sprint and the balance as a convenience to the operator and to minimize the possibility of transcription

Table 2011.04. Interlaboratory study results for the determination of protein in raw and processed meat products

Sample	No. labs	Mean, %	s_r[a]	r[b]	RSD_r, %[c]	s_R[d]	R[e]	RSD_R, %[f]	HorRat	Recovery, %
Beef hot dog	10	9.80	0.17	0.47	1.71	0.25	0.70	2.55	0.90	104.14
Pork sausage	10	16.05	0.49	1.36	3.04	0.55	1.53	3.41	1.30	101.71
Ham	10	17.21	0.25	0.71	1.48	0.49	1.38	2.86	1.10	96.26
Pork, raw	10	17.26	0.24	0.67	1.38	0.35	0.99	2.04	0.78	102.19
Turkey, raw	10	18.03	0.16	0.46	0.91	0.29	0.81	1.60	0.62	99.23
Beef, raw	10	18.06	0.23	0.64	1.26	0.30	0.84	1.65	0.64	98.90
Semi-dry summer sausage	10	18.29	0.23	0.63	1.23	0.27	0.77	1.50	0.58	99.56
Chicken, raw	10	22.25	0.34	0.94	1.51	0.34	0.94	1.51	0.60	102.39
Dry hard salami	10	21.48	0.38	1.07	1.77	0.38	1.07	1.77	0.70	101.42
Beef jerky	10	39.11	0.41	1.14	1.04	0.61	1.71	1.56	0.68	101.09

[a] s_r = Repeatability standard deviation.

[b] r = Repeatability; $r = 2.8 \times s_r$.

[c] RSD_r = Repeatability relative standard deviation.

[d] s_R = Reproducibility standard deviation.

[e] R = Reproducibility; $R = 2.8 \times s_R$.

[f] RSD_R = Reproducibility relative standard deviation.

errors.

(*4*) With a stainless steel spatula, transfer 0.5–1.0 g sample to the center of the sample cup. The precision of the results benefits from properly placing the sample at the bottom of the cup or on the side of the cup near the bottom. This position ensures successful homogenization of the sample with the iTAG solution.

(*5*) Place the cup with the weighed sample on to the balance pan of the electronic balance and press BALANCE on the Sprint keypad. This action records the weight of the sample in the Sprint's electronic memory.

(*6*) Remove the cup and sample from the balance and place them in the cup holder in the Sprint.

(*7*) Place a new filter in the filter holder in the Sprint.

(*8*) Begin analysis by pressing RUN on the keypad.

(*9*) When analysis is complete, cover the sample cup with a cup lid and discard.

G. Calculations

The Sprint software calculates the results for protein as percentages (g/100 g) to two decimal places.

H. Calibration

(*1*) Determine protein content of a certified reference standard using the Sprint Protein Analyzer. Use the "Check Standard" method loaded within the system. Results should be within ±0.1% of the protein value provided on the certificate of analysis.

(*2*) If the result of the certified reference standard is not within specifications, restandardize the dye-binding agent by following the on-screen instructions of the Sprint Protein Analyzer.

(*3*) An optics cleaning operation should be performed and a dye-binding agent weight confirmed before proceeding.

(*4*) Analyze the certified reference standard again using the Sprint Protein Analyzer.

(*5*) If the result of the protein content of the certified reference standard is not within specifications after restandardizing the dye-binding agent and maintenance of the system, a technician should be consulted.

(*6*) Test a known certified reference standard weekly and perform routine maintenance and cleaning of the system as check on validity of calibration.

Alternative calibration.—Verify an in-house control material by sending out to three laboratories for Kjeldahl analysis using **981.10** (*see* 39.1.19).

Follow steps (*1*)–(*5*) above.

Reference: *J. AOAC Int*. (future issue)