

# Update: Rapid Methods for the Determination of Fat, Moisture, and Protein

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The need for rapid methods for the determination of fat, moisture, and protein in meat continues to be of high interest and importance in industry operations and has led to the use of a wide variety of available methods and the introduction of new ones providing advantages such as speed, relative cost per analysis, convenience, simplicity, or low degree of hazard to the operator. Many of these methods have not gained official status, and their accuracy, precision, sensitivity, and applicability to a wide variety of products have not been established. A rapid method may be defined as one that provides a result in 5 minutes or less compared with a relatively rapid one requiring on the order of 15 minutes. Methods requiring hours to perform are far too slow for optimizing some processing operations where an ideal method would provide continuous analysis to facilitate maintaining quality control and for assuring regulatory compliance of a product.

Information which has become available since rapid analyses for fat and moisture were reviewed at the Reciprocal Meat Conference of 1973 is discussed in this presentation.

## Fat Determination

### Ether Extraction

Randall (1974) modified the ether extraction procedure to extract fat from meat samples in 30 minutes instead of 4 hours required with the Soxhlet method. He immersed a dried sample in a wire mesh cup directly into boiling ether in a reflux apparatus, raised the cup after 10 minutes, and permitted ether condensate to wash it for 20 minutes. A complete determination requires 2¼ hours, and 80 determinations a day may be made with a multiple extractor. Accuracy (0.2% fat between-methods mean difference) and standard deviation (0.6% fat between-methods) of his results were equivalent to those with Soxhlet reference method. The method was also evaluated by Ligugnana (1978). Equipment for the procedure is available from Tecator, Inc.\*, Boulder, Colo.

\*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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### Rendering

Fat determinations of 664 beef samples with the Banco (Anderson Laboratories, Inc., Ft. Worth, Tex.), modified Babcock, and Univex (Univex Corp., Salem, N.H.) methods were compared with those by the AOAC Soxhlet method (Marriott et al., 1975; Anon., 1977). With the Univex method, the most rapid (5 minutes) of the three, the height of rendered fat in a collection tube is measured after the sample is electrically heated. Results indicated the overall mean (20.6% fat) was high by 0.9% fat and correlated well ( $r = 0.97$ ) with reference determinations. Other statistics such as fat percent range of the samples and between-methods standard deviation of the paired data were omitted in the published report. However, on the basis of rapidity, simplicity, economy, and accuracy, the authors recommended the method for use in routine production control.

### Specific Gravity of Extract

The Foss-Let (Foss America, Inc., Fishkill, N.Y.) is designed to measure fat content as a function of specific gravity of a solvent extract of a meat sample. The 7-minute determination is accomplished with an orbital shaker that produces a fat extract in 2 minutes and a semi-automated, thermostatted hydrometer to measure specific gravity. Results of limited studies with the method on meat were published by Usher et al. (1973), Nilsson and Kolar (1973), Pfeiffer et al. (1973), Egberg et al. (1974), and Eslami-Matin et al. (1974). We critically compared the Foss-Let and AOAC Soxhlet methods and found excellent agreement (Pettinati and Swift, 1975a). Collaborative study of the method was carried out with 11 other laboratories. We found that accuracy was equivalent and precision was generally slightly better than with the AOAC method (Pettinati and Swift, 1977a,b). Standard deviations with the Foss-Let method were 0.2% fat within laboratory and 0.5% fat among laboratories. On the basis of these results, the Foss-Let procedure was adopted as a rapid, alternative standard method by the AOAC (AOAC, 1980) and the ASTM (ASTM, 1976).

### X-Ray Absorption

The Anyl-Ray Fat Analyzer (Anyl-Ray Corp., Davenport, Iowa) is specifically designed to determine the concentration of fat in fresh meat as a function of X-ray absorbance. Finny (1973) reviewed applications of X-ray methods for the determination of fat in various agricultural products and materials.

Measurement with the Anyl-Ray is made directly on a sample without extraction of fat so the method is truly rapid, requiring 5 minutes or less for a determination. In an evaluation of the unit at Kansas State College (Anon., 1970), repeatability was 0.5% fat and control of processed product could be maintained within 0.5%. Young et al. (1976) found from comparative analysis with AOAC Soxhlet method that Anyl-Ray determinations were low by 2% fat at the 15% level, 1.2% at the 30% level, and the between-methods standard deviation was 1.3% fat. Studies in our laboratory indicated that the accuracy of the method on samples containing 3 to 90% fat is good (0.2% fat mean difference), and the standard deviation relative to AOAC Soxhlet is 0.9% fat. Usefulness of the unit has been limited to large-scale meat packers and processors because of its cost (about \$20,000) and sample size requirement (13 pounds per test).

#### *Infrared Radiation Reflectance*

The application of the measurement of reflectance of infrared light is based on considerable research by Karl Norris at the Beltsville Instrumentation Laboratory, USDA, Beltsville, Md. Specialized instruments for the method introduced commercially in 1971 are produced by Neotec Instruments, Inc., Silver Spring, Md., and Technicon Instruments Corp., Tarrytown, N.Y. The simplest of these instruments, the Neotec Ground Meat Analyzer (Anon., 1973; Dempster, 1974) provides the most rapid fat analysis. Its production is aimed specifically for use in a supermarket. A determination of fat in ground meat is made in seconds by placing a 1-pound sample in transparent plastic wrap upon a window on the surface of the unit and reading the indicated fat content on a needle dial. Neotec claims that comparative analyses with the Ground Meat Analyzer and AOAC Soxhlet method agree with a standard deviation of 0.9% fat. A similar reflectance-measuring instrument yielded results that agreed with ether extraction reference determinations with a standard deviation of 2% fat (Massie, 1976) which would limit the method to screening use.

#### *Electrical Inductance*

A unique approach to determining fat in fresh meat developed by the EMME Co., Phoenix, Ariz., is based on the difference in electrolytic properties of lean and fatty tissues. Lean meat is a 20-fold better conductor of electrical current than fat. The amount of induced current combined with the weight of the sample being tested is used to calculate fat percent, which is displayed in seconds. Determinations can be made on samples weighing from 5 to 12 pounds, 60-pound boxes of meat, or small live animals. Information on evaluation of the method is limited to studies with an EMME small animal model and to the yield of carcass lean. Stiffler et al. (1974, 1976) reported a correlation coefficient of 0.28 in one study and 0.44 in a later study between EMME measurements of live hogs and the weight of fat-free lean determined from proximate analysis of carcass yield. The low correlations indicate considerable scatter in the compared data, which limits usefulness of this application. Koch and Varnadore (1976) compared EMME measurements on beef quarters from 66 carcasses with the total weight of trimmed primal cuts and

obtained a correlation of 0.82 between the two methods. However, they omitted results of proximate analysis in their report. Fredeen et al. (1979) reported that EMME measurements of live hogs and determinations of trimmed carcass fat by ether extraction method yielded a correlation of 0.42, apparently also with considerable scatter of data.

#### *Nuclear Magnetic Resonance*

Determination of fat in meat with this method has been principally of theoretical interest. The measuring procedure on a prepared sample is rapid, but preparatory steps tend to make the method impractical. Nilsson and Kolar (1971, 1974) measured meat samples (5-8g) which were oven dried 4 hours and tempered at 70°C for 1 hour. Their results averaged 0.3% fat high relative to reference method. Casey and Miles (1974) determined fat in the range from 1 to 12% in 24 samples of fresh beef, lamb, and pork. Sample preparation consisted of grinding, freeze drying, compression into a pellet, and tempering at 70°C for at least 30 minutes. Their results on 12-14g samples of dried product agreed well with determinations of total fat, with accuracy within 0.1% and standard deviation 0.2% fat.

#### *Total Fat by Elution*

Marmer et al. (1979) and Maxwell et al. (1980) developed a new method for determining total fat in meat and meat products by the extraction of polar and neutral lipids. Five grams of meat are mixed with Celite 545, placed in a glass column, the fatty constituents are eluted with a mixture of dichloromethane and methanol (9 + 1), and the solvent is evaporated from the eluate. Comparative analysis with AOAC method for crude fat yielded results which were high by 0.6% fat and had a standard deviation of 0.4% fat. Comparative analysis with an extraction method for total fat indicated excellent agreement, and the amount of phospholipid extracted was confirmed by chemical analysis. Important advantages of the method are simplicity, suitability for multiple determination, use of inexpensive equipment, and a nonflammable solvent. Although a determination requires 2.5 hours, it is more rapid than existing methods for total fat.

### **Moisture Determination**

#### *Gas Chromatography*

Khayat (1974) determined moisture in 10 minutes by means of gas chromatographic analysis of an anhydrous alcohol extract of a meat sample. Repeatability was reported to be improved with isopropanol extraction in place of either methanol or ethanol. In comparative, 5-replicate analyses of two samples of meat with isopropanol and the AOAC oven drying method, the means agreed within 0.2% moisture and the repeatability was 0.2%.

#### *Karl Fischer Titration and Capacitance*

Kirkbright et al. (1975) compared the determination of moisture by Karl Fischer titration (a 20-minute method with an automated instrument, and a 25- to 30-minute method when performed manually) and a British standard oven

method. Results of the comparative analysis on 10 meat samples indicated that moisture was determined low by 1.6% and the between-methods standard deviation was 0.9%. They also compared determination of moisture of the samples by measuring capacitance of dioxane extracts with a silica cell and standard method. Results of this comparison were low by 1.3% moisture and the between-methods standard deviation was 1.3%.

### Moisturefuge

Moisture determinations of 664 beef samples with toluene distillation, Ohaus moisture balance (Ohaus Scale Corp., Florham Park, N.J.), and Moisturefuge (Anderson Laboratories, Inc., Fort Worth, Tex.) methods were compared (Marriott et al., 1975; Anon., 1977) with results with AOAC oven drying method. With the Moisturefuge method, the most rapid (12 minutes) of the three, samples are dried in a heated centrifuge. Results of comparative analysis of this method indicated the overall mean (62.1%) was high by 0.1% moisture and correlated well ( $r = 0.96$ ) with reference determinations. Other statistics of the evaluation were omitted in the published report. The authors concluded that the method is useful for routine quality control analysis.

### Microwave Oven

Methodology involving use of a microwave oven for the rapid determination of moisture has undergone considerable development and acceptance in recent years. A procedure for meat and meat products developed at our laboratory requires only 2.5 minutes of heating in a 1,000-watt, domestic type microwave oven, followed by 1 minute of drying with forced air (Pettinati, 1975b). A sample is dispersed with sodium chloride and ferrous oxide in a weighing bottle. The salt prevents spattering during drying, and the oxide, a known strong absorber of microwave radiation, accelerates drying. Comparative analysis with this and the AOAC method indicated results were equivalent (between-methods mean difference 0.1% and standard deviation 0.6% moisture).

Lee and Latham (1976) also used a domestic type, 1,400-watt microwave oven for the rapid determination of moisture in canned pet food. A sample spread on tared filter paper was dried in 1.75 minutes. Comparative analysis of this method with the reference method yielded results that were equivalent (between-methods mean difference 0.1% and standard deviation 0.6% moisture). Steele (1976) developed a compact microwave oven for the rapid determination of moisture in foods. Results with samples of sausage and meat pastes, which required from 3.5 to 6 minutes drying time, indicated good agreement (mean difference 0.1% moisture) with reference method; however, standard deviation (0.9%) between the compared sets of data was not favorable. Photovolt Corp., 1115 Broadway, New York, N.Y., markets microwave ovens designed for analytical use. Kolar (1978) evaluated one of these units (Apollo model) for drying samples of meat and meat products in 4 to 6 minutes. Results of the study relative to the standard method indicated excellent accuracy (mean difference 0.01% moisture) and good correlation ( $r = 0.999$ ). Risman (1978) designed a compact microwave oven for drying food samples and determined that an input power of 260

watts was sufficient to dry 10g samples of meat products in 6 to 8 minutes. CEM Corp., Indian Trail, N.C., markets an analytical microwave oven with built-in balance, digital display, and optional microcomputer for calculating percent moisture. A 5- to 10-g sample is dried on a glass fiber filter pad in 3.5 minutes.

### Protein Determination

Recent advances in the rapid analysis of protein include automation of some part of, or in some cases the entire, older manual procedures. Methods for the determination of protein discussed here are based on measurement of total nitrogen.

#### Methodology Related to the Kjeldahl

The AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y.) provides an automated means of determining total nitrogen in an acid-digested food sample by the colorimetric reaction of ammonia at the rate of 80 per day. Gantenbein et al. (1974) evaluated the method collaboratively for protein determination of meat products. Based on their results, which were equivalent to those for the Kjeldahl, the method was adopted by the AOAC as an alternative procedure for total nitrogen. A number of manufacturers of laboratory equipment market high temperature block digestors which reduce time for conversion of amino nitrogen to ammonia from 2 hours to 45 minutes. Hambræus et al. (1976) reported that long-term accuracy and reproducibility of nitrogen determination of biological and food samples was good with a procedure involving a block digestor and AutoAnalyzer. Vincent and Shipe (1976) studied the effect of calibration procedures on the accuracy and precision of the AutoAnalyzer for a variety of formulated foods and concluded it is equivalent to the Kjeldahl method when care is exercised in the preparation of samples and calibration.

The Kjel-Foss analyzer (Foss America, Inc., Fishkill, N.Y.) was developed to automate the Kjeldahl procedure. After a sample is manually placed in the unit, the entire procedure is automated, permitting 120-160 determinations per day. All the steps of a macro-Kjeldahl procedure are duplicated in the unit except that 1-g instead of 2-g samples are used and hydrogen peroxide is added to sulfuric acid to accelerate sample digestion. The Kjel-Foss analyzer was described by Oberreith and Mermelstein (1974), evaluated for the determination of total nitrogen in meat by Montag (1974), and collaboratively studied by Noel (1976). Their results showed that the Kjel-Foss is equivalent to the macro-Kjeldahl method in accuracy and slightly better in between-laboratory precision, which led to its adoption by the AOAC as an alternative method.

#### Direct Distillation of Ammonia

Tecator, Inc., Boulder, Colo., developed the Kjeltec-DD system which permits determination of protein to be made in 10 minutes. The equipment steam distills and titrates ammonia liberated from free amino groups of a protein after it is dissolved and heated in an alkaline medium. The determination is based on an assumed constant relationship of free amino groups and total nitrogen content (Ronalds, 1974; Cipriani, 1979). Tecator data indicates that while results with the

method on meat and meat product samples correlated well ( $r = 0.991$ ) with Kjeldahl determinations, they were consistently low by 2.6% protein and the between-methods standard deviation was large (1.1%). These performance characteristics indicate that the method requires further development.

#### *Nitrogen Generation from Combustion*

Lee et al. (1972) determined protein of meat and meat products with a Coleman 29A nitrogen analyzer (Coleman Instruments Div., Perkin-Elmer Corp., Norwalk, Conn.), a 12- to 15-minute method by which total nitrogen in a sample after combustion is measured as gaseous volume. The procedure requires a 200- to 300-mg sample. Results of comparative analysis with the Kjeldahl procedures indicated that protein was determined consistently low by 0.6% and the between-methods standard deviation was 0.4%. Accuracy of the method could be improved, perhaps, by standardization with a meat powder in place of acetanilide.

Revesz and Aker (1977) determined protein of nonmeat foods with a Leco nitrogen analyzer (Leco Corp., St. Joseph, Mich.), a 7-minute method by which total nitrogen in a 1g sample after combustion is measured by thermal conductivity. Results of comparative analysis indicated that accuracy of the analyzer was equivalent to the Kjeldahl procedure and the between-methods standard deviation was good (0.15% protein).

#### *Dye Binding*

The dye binding procedure is an empirical photometric method which involves mixing a finely comminuted food product with an excess of acid dye in an acid medium. An insoluble dye-protein complex is formed by the dye and positively charged sites on proteins which are, namely, terminal amino acids and the basic amino acids histidine, lysine, and arginine. After reaction, the protein-dye complex is removed by filtration and the concentration of residual dye, inversely proportional to protein content, is measured photometrically. The speedy (15-minute) analysis that dye binding provides has led to its use as an unofficial method for protein in many foods and as an AOAC alternative method for milk. Recent studies of the method have involved Acid Orange 12 dye binding. Heller and Sherbon (1976) studied the method collaboratively with three other laboratories for protein determination in meat. Results indicated that while data from three of the laboratories correlated well ( $r = 0.976, 0.996, \text{ and } 0.995$ ) with Kjeldahl determinations and between-laboratory standard deviation was 0.6% protein, overall accuracy (low by 1% protein) was affected by lack of standardization of instrumentation. In our laboratory, use of the method with equipment marketed by the Baltimore Spice Co., Baltimore, Md., was evaluated (Pettinati and Swift, 1976) for the determination of protein in meat, and the procedure was studied collaboratively (Pettinati and Swift, 1977c). Overall results indicated that protein determination by dye binding compared favorably with Kjeldahl method analysis. The method is unaffected by factors such as type of meat (beef or pork), protein level (from fatty tissues to lean meat), and whether the product is fresh, cured, smoked, or processed. However, high levels of collagen, the principal protein of tendons and con-

nective tissue, lead to low determination. Collagen, being sufficiently different in amino acid composition from red muscle proteins, binds only 0.7 as much dye. For the majority of meat analyses, accuracy and repeatability of the dye binding and Kjeldahl methods are equivalent; the among-laboratory reproducibility, 0.4% protein, was twice that (0.2%) obtained with the Kjeldahl method. These results indicate that dye binding protein determination, while not fully equivalent to the Kjeldahl method, is very useful for relatively rapid screening analysis. Seperich and Price (1979) investigated factors fundamental to the determination of protein in fresh and processed meat. They showed that dye binding capacity differed for major protein fractions of muscle and was inversely proportional to protein concentration of an emulsion. However, it did not differ between beef and pork protein, between free or emulsified myofibrillar protein, between either raw emulsion or finished frankfurters, or for variations in fat content. They concluded that the method is useful for the determination of protein.

### **Multicomponent Determination**

#### *Fat and Moisture*

Farnell (1975) developed a 5 minute method for the determination of fat and moisture in meat by measuring the infrared attenuated total reflectance (fat, 5730 nm; moisture, 6080 nm) of an extract with a mixture of trichloroethylene and methanol. Results of comparative analysis with standard methods indicated that fat averaged 0.2% low and between-methods standard deviation was 0.4%; moisture averaged 0.02% low and between-methods standard deviation was 0.3%. These are highly desirable characteristics, useful in a rapid method.

#### *Fat and Protein*

The CEM Corporation recently developed an analyzer for fat and protein in meat for use in conjunction with their microwave oven for moisture determination cited earlier. After moisture is determined in a 5- to 10-g sample, the dried residue is transferred to the analyzer for the rapid determination of fat and protein in the same sample. The combination procedure permits analysis of the three components in 7 minutes. In operation, a dried residue and its supporting glass fiber pad are ground in a blender, extracted with a solvent such as dichloromethane, and the fat-free residue is collected onto a fresh filter pad by suction filtration. Protein percent is calculated from the weight of residue, and fat percent by difference. Repeatability of the method is claimed by the manufacturer to be 0.1% fat or protein.

#### *Fat, Moisture, and Protein*

Two instrumental procedures are available for the determination of fat, moisture, and protein in the same sample of meat or meat product. One involves measurement of reflectance of infrared radiation, and the other, heating with a microwave oven.

Neotec Instruments Inc., Silver Spring, Md., and Technicon Instruments Corp., Tarrytown, N.Y., market infrared reflectance instruments which utilize selected wavelengths specific

for fat, moisture, and protein. Until recently, the units have been limited in application to soy bean meals and cereal grains (Watson, 1977). Hauser and Weber (1978) studied the use of a Technicon InfraAnalyzer on 20-g samples of eight meat and meat products. From 181 determinations in their comparative analysis with reference methods, overall results were low by 0.4% moisture, fat, and protein, and between-methods standard deviations were 1.4% fat or moisture and 1.0% protein. These components of a sample are determined in several minutes, which makes the method a truly rapid one. However, the high standard deviations of the results indicate that further development of the method is required to improve precision.

An instrumental procedure involving use of a microwave oven for the rapid determination of fat, moisture, and protein in the same sample of meat or meat product was developed recently by the Hobart Corp., Troy, Ohio. Automated determination of the components is obtained in 2.5 to 5 minutes depending on the type of sample analyzed, and the results are digitally displayed in percent. During a heating cycle of the procedure, a sample of 70 to 80g, pressed between a special sample holder and cover, is dried, and a certain proportion of the fat content is rendered and collected in a weighing dish. Automated calculation and display of the three components is based on the relationship of weight loss due to moisture con-

tent, weight of moisture-free residue, and weight of rendered fat. Determinations can be made on fresh beef or pork containing up to 70% fat, blends of beef and pork in any combination and in the range of 15 to 50% fat, blends with up to 4% added salt and 18% water, and finished products such as frankfurter and bologna. Performance characteristics for the analyzer as exemplified by determinations of fat indicate agreement with AOAC method results with a standard deviation of 0.5 to 0.7% in the range of 5 to 50% fat and 1% at the 70% level; repeatability and reproducibility of the method are uniformly 0.5%. The rapid analysis and reasonable performance characteristics this method provide indicate its usefulness in process control operations which generate a heavy analytical demand.

### Summary

Eight methods cited for the determination of fat include ether extraction, rendering, specific gravity, X-ray absorption, infrared reflectance, electrical inductance, nuclear magnetic resonance, and column elution. Characteristics of the methods are summarized in Table 1. Of these the 7-minute specific gravity method (Foss-Let) is the most thoroughly evaluated and is standardized as an alternative method for crude fat in meat and meat products. The infrared reflectance

**Table 1. Characteristics of Methods for Determination of Fat**

Method	Time per determination	Sample size	% Fat	
			Mean difference from stand.	Standard deviation
Ether extraction	2.25 hr (80/day)	3.5 g	+0.2	0.6 <sup>a</sup>
Rendering	5 min	56.8 g	+0.9	(0.97) <sup>b</sup>
Specific gravity	7 min	45.0 g	+0.1	0.2 <sup>c</sup> , 0.5 <sup>d</sup>
X-ray absorption				
Kansas State Univ.	3 min	13 lb	—	0.5 <sup>c</sup>
Young et al.	same	same	-2.0 at 15% -1.2 at 30%	1.3 <sup>a</sup>
Pettinati et al. <sup>e</sup>	same	same	+0.2	0.9 <sup>a</sup>
Infrared reflectance	5 sec	1 lb	0	0.9 <sup>a</sup>
Electrical inductance				
Stiffler et al.	<5 min	hogs	—	(0.28, 0.44) <sup>b</sup>
Koch and Varnadore	same	beef quart.	—	(0.82) <sup>b</sup>
Fredeen et al.	same	hogs	—	(0.42) <sup>b</sup>
Nuclear Magnetic Resonance				
Nilsson and Kolar	5 hr	5-8 g	+0.3	—
Casey and Miles	24 hr	12-14g dry	+0.1	0.2 <sup>a</sup>
Column elution	2.5 hr	5 g	+0.1	0.4 <sup>a</sup>

<sup>a</sup>Between-methods standard deviation is calculated from comparative analysis with standard method.

<sup>b</sup>Correlation coefficient is calculated from comparative analysis with standard method.

<sup>c</sup>Within-laboratory precision, repeatability, of the method.

<sup>d</sup>Among-laboratory precision, reproducibility, of the method.

<sup>e</sup>Unpublished data.

(Neotec Ground Meat Analyzer) and X-ray absorption (Anyl-Ray) methods provide the most rapid analysis but less than optimal precision.

Five methods cited for the determination of moisture include gas chromatography, Karl Fischer titration, capacitance, heated centrifuge, and microwave oven. Characteristics of the methods are summarized in Table 2. A microwave oven procedure provides the most rapid (about 3.5 minutes) analysis. While it is not standardized, its usefulness is demonstrated by the good accuracy and precision reported by investigators.

Five methods cited for the determination of protein include the AutoAnalyzer and Kjel-Foss, direct distillation of ammonia, nitrogen from combustion measured by either gas volume or concentration, and dye binding. Characteristics of the methods are summarized in Table 3. The AutoAnalyzer and Kjel-Foss methods provide speedy, automated, and standardized analysis. The 7-minute nitrogen concentration method provides good accuracy and precision and is acceptable in laboratories where fewer analyses per day are required. The dye binding method (15 minutes) provides gener-

**Table 2. Characteristics of Methods for Determination of Moisture**

Method	Time per determination (min)	Sample size (grams)	% Moisture	
			Mean difference from stand.	Standard deviation
Gas chromatography	10	1-2	+0.2	0.2 <sup>a</sup>
Karl Fischer titration	20-30	2	-1.6	0.9 <sup>b</sup>
Capacitance	20-30	2	-1.3	1.3 <sup>b</sup>
Moisturefuge	12	4	+0.1	(0.96) <sup>c</sup>
Microwave oven				
Pettinati	3.5	5	+0.1	0.6 <sup>b</sup>
Lee and Latham	1.75	10	+0.1	0.6 <sup>b</sup>
Steele	3.5-6.0	3.5	+0.1	0.9 <sup>b</sup>
Kolar	4-6	5-20	+0.01	(0.999) <sup>c</sup>
Risman	6-8	10	-	-
CEM Corp.	3.5	5-10	-	-

<sup>a</sup>Repeatability of the method.

<sup>b</sup>Between-methods standard deviation is calculated from comparative analysis with standard method.

<sup>c</sup>Correlation coefficient is calculated from comparative analysis with standard method.

**Table 3. Characteristics of Methods for Determination of Protein**

Method	Time per determination (min)	Sample size (grams)	% Protein	
			Mean difference from stand.	Standard deviation
AutoAnalyzer	80/day	10	-0.1	0.2 <sup>a</sup> , 0.2 <sup>b</sup>
Kjel-Foss	120-160/day	1	+0.3	0.5 <sup>b</sup>
Alkaline digest	10	10	-2.6	1.1 <sup>c</sup>
Nitrogen gas volume	12-15	0.2-0.3	-0.6	0.4 <sup>c</sup>
Nitrogen gas conc.	7	1	0	0.2 <sup>c</sup>
Dye binding				
Heller and Sherbon	15	20	-1.0	0.6 <sup>d</sup>
Pettinati and Swift	15	40	+0.2	0.2 <sup>a</sup> , 0.4 <sup>b</sup>

<sup>a</sup>Within-laboratory precision, repeatability, of the method.

<sup>b</sup>Among-laboratory precision, reproducibility, of the method.

<sup>c</sup>Between-methods standard deviation is calculated from comparison analysis with standard method.

<sup>d</sup>Between-laboratory precision of the method.

Table 4. Characteristics of Methods for Determination of More Than One Constituent

Method	Time per determination (min)	Sample size (grams)	% Constituent	
			Mean difference from stand.	Standard deviation
Fat and moisture, infrared ATR	5	3	fat, -0.2 moist., -0.2	0.4 <sup>a</sup> 0.3 <sup>a</sup>
Fat and protein, pre-dried residue	3.5	5-10 wet	-	0.1 <sup>b</sup>
Fat, moisture, and protein Infrared reflectance	<5	20	-0.4 <sup>c</sup>	fat, 1.4 <sup>a</sup> moist., 1.4 <sup>a</sup> prot., 1.0 <sup>a</sup>
Microwave oven	2.5-5	70-80	0	0.5-1.0 <sup>a</sup> 0.5 <sup>d</sup>

<sup>a</sup>Between-methods standard deviation is calculated from comparison analysis with standard method.

<sup>b</sup>Within-laboratory precision, repeatability, of the method.

<sup>c</sup>Overall mean difference for fat, moisture, and protein content.

<sup>d</sup>Within- and among-laboratory precision of the method.

ally good results and is acceptable where an even lower analytical rate is required.

Determination of more than one constituent in the same sample of meat or meat product was outlined for four rapid, instrumental methods, and their characteristics are summarized in Table 4. One of these, the InfraAlyzer, applied extensively to the analysis of cereal grains, is new in application to meat foods. Continued investigation and development of this application is indicated to obtain improved precision in the determination of fat, moisture, and protein. The other three methods, an attenuated total reflectance procedure for fat and moisture, a CEM Corporation fat and protein analyzer, and a Hobart Corporation microwave oven for fat, moisture, and protein provide rapid analysis and either good or reasonable analytical results. These three methods are newly developed, and more information on their analytical performance in practice is desirable.

### Discussion

*A. W. Kotula, SEA/USDA:* Would you comment on the fact that in the official methods, a small size sample is used, whereas in the analray a better estimate of the sample may be obtained because 13 pounds are used.

*J. D. Pettinati:* The important point is that sampling is much more critical with smaller samples. If sample preparation is thorough and representative, then fat can be determined accurately by any of the methods.

*A. W. Kotula:* What I'm looking for is a method of sampling so 2-4 grams represents a 900 pound batch.

*J. D. Pettinati:* That's another 10 to 15 minute talk, but there are methods for determining if sampling procedures will yield adequate samples for a given purpose.

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