

## Moisture and Fat Analysis of Meat and Meat Products: A Review and Comparison of Methods

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Published analytical methods for moisture and fat analysis of meat and meat products and reviews of the methodology are surveyed. The methods are briefly described and characteristics such as time required for an analysis, accuracy, and precision are given. The discussion of instrumental methods includes methods which have not been fully developed for meat but which may become useful. Several of the considerations and limitations that are involved in moisture and fat analysis of meat are discussed. From the large number of moisture, fat, and combined methods available, the most promising were selected for a close inspection and compiled in two tables of data. The most useful rapid methods for meat industry quality control are the following: For moisture determination, high temperature mechanical convection oven drying, hot plate drying, moisture balance, and azeotropic distillation methods have the most advantages. For fat content determination, the modified Babcock procedures, X-ray transmission, specific gravity of heptane extracts, and determination of fat in the extract from azeotropic moisture analysis (a combined method) offer the most advantages.

The meat packing industry has an urgent need for rapid, relatively accurate, and simple methods for moisture and fat determination. Their availability underlies compliance with statutory requirements of regulatory agencies, quality control in manufacturing meat products, and good business management (1-4). Many methods currently available for analysis of moisture and fat content of meat and meat products were originally developed for other agricultural products and are not necessarily optimal for meats. In general, moisture and fat methods are chosen for either rapidity or accuracy, whereas obtaining both is the ultimate goal.

Desirable parameters for both ideal moisture and fat methods were outlined by Everson *et al.* (5), who indicated that the methods should perform as follows:

- (1) Determine moisture in 15 min and fat in 30 min,
- (2) Be applicable to the broadest range of unprocessed meats and formulated product,
- (3) Be performable by any technical, and preferably nontechnical, employee with brief training,
- (4) Use readily available apparatus of low initial investment and low cost per test,
- (5) Have reasonable accuracy and good reproducibility, and
- (6) Present few hazards easily controlled.

A profusion of analytical methods embodying varying numbers of these parameters can be found in the scientific literature. It was an objective of this review to delineate the salient features of reported methods of moisture and fat analysis, briefly using this framework of desirable parameters as the basis for comparison. It would be impossible to discuss all of these parameters for each method cited here; however, for those with some knowledge and experience in the analytical field, many of the features will be immediately obvious. It was another objective of this review to reduce available data on accuracy and precision to a uniform basis to permit comparison. The majority of methods cited reported results of comparison with official methods. In order to provide the desired uniform basis, results that were reported in many of the original publications were normalized. More specifically, differences in means between the experimental and standard method were determined to ascertain whether a tendency existed towards a constant positive or negative difference in relation to the standard method. One value of this is that a method capable of achieving acceptable precision, but which consistently yields low or high values, may be

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shown to be useful if a constant factor can be applied to correct the results to agree with reference values. As a result of this treatment, three numerical values are shown in many of the method reviews: (1) average accuracy (amount lower or higher than reference method), (2) difference range, and (3) standard deviation calculated from the normalized differences by the standard formula,  $s.d. = \pm \sqrt{\Sigma d^2 / (n - 1)}$ .

#### Moisture Analysis

Meat tissue holds moisture in various states which were characterized by Joslyn (6) as follows: (1) a solvent for dispersion of crystalloids such as sugars, salts, and acids of low molecular weight or a dispersing medium for colloidal solutions of hydrophilic macromolecules such as proteins and gums; (2) adsorbed thinly as a mono- or polymolecular layer on tissue structural surfaces; and (3) in chemical combination as water of hydration. The energy required to free chemically and physically bound water and/or to force it through physical barriers such as cell walls and capillaries limits the diffusion of water from tissue. At the surfaces, solutes tend to concentrate at the evaporating surfaces and "case-harden" or seal the sample. After some portion of the moisture content has been driven off, the forces that hold the remaining water actually become stronger. At this point, the energy requirements are greater to drive off the last of the moisture. To merely apply higher temperatures can lead to inaccurate results through fat spattering, sample decomposition, and oxidation. Hence, the removal of water from tissue by heating is complicated. However, oven methods, though time consuming, are simple to perform, relatively reproducible, and useful as primary reference methods for evaluating new methods. For practical purposes, then, moisture content of meat and meat products can be arbitrarily defined as the quantities determined by the official oven methods. The variety of chemical and physical approaches that has been investigated as alternative methods will be related in the following sections.

#### Non-Instrumental Moisture Methods

Although moisture determination is one of the most commonly applied food assays, there are few reviews of the methodology and even fewer devoted to meat and meat products. Churchward (7) reviewed moisture methods for foods including meat. Methods involving electrical resistance measurement, vacuum and atmospheric ovens, and azeotropic distillation were discussed. The last of these methods was reviewed in depth and the historical development of the method was summarized. Churchward stated that the distillation method has

many advantages and proposed the preferred conditions for the procedure.

Joslyn (6) presented a thorough review of moisture methodology and suggested the conditions that are important to the analyst concerned with food products.

Everson *et al.* (5), reviewing moisture methods applied to meats, selected azeotropic distillation for evaluation and comparison. Ethylene dichloride, toluene, and 2-octanol were compared as solvents for the distillations. 2-Octanol was recommended for a 10-15 min moisture determination. Results for 4 samples of meat product averaged 1.0% moisture lower than those by the official method. Normalized difference ranged from -1.4 to +2.2;  $s.d. = \pm 1.6\%$  moisture.

Klima *et al.* (8) reviewed 9 current methods of moisture analysis applied to meat and meat products for time of analysis, accuracy, and simplicity of procedure. Methods reviewed were the Czechoslovakian standard procedure (105°C oven method), azeotropic distillation, electrical resistance, dielectric constant, nuclear magnetic resonance, fast neutron absorption, 170°C oven, dielectric heating, and moisture balance. Results of their own investigations to improve the oven method were also presented. They preferred drying samples at 150°C, although the meat industry in their country uses a 170°C oven temperature routinely. Heat transfer to the sample was improved by use of a thick metal plate for the floor of the oven and by using metal drying dishes. Heating coils in the ceiling of the oven reduced drying time further, and they reported a 20 min drying time for samples. This oven was used to analyze 150 meat samples in a comparison with their standard method. Mean moisture values were essentially identical; difference ranged from -0.66 to +0.72 and  $s.d. = \pm 0.33\%$  moisture. A rapid moisture balance which would hold 8 samples on a circular platform was also developed; it allowed a moisture determination to be made in 20 min, or, when samples were done serially, results were obtained at 3½ min intervals.

The Swiss standard methods used for meat and meat products by the regulatory agencies of that country were reviewed by Wyler (9). Moisture methods he cited were 101°C oven, infrared radiation moisture balance, Karl Fischer titration, and azeotropic distillation. Karl Fischer titration was stated to be of no interest because both free and bound moisture were measured. Azeotropic distillation with tetrachloroethylene as the solvent was stated to be the Swiss standard method. Accuracy by this method was reported to be "1%", using 15 g samples distilled for 1 hr.

Pearson (10) reviewed methods for moisture determination in fresh sausage and evaluated 3 oven

procedures: heating of samples at  $103 \pm 2^\circ\text{C}$  to constant weight (4–6 hr),  $135^\circ\text{C}$  for 30 min, and  $104^\circ\text{C}$  (with vacuum) to constant weight (30 min). Reported mean moisture content values and difference ranges were 44.7% (–2.2 to +1.5), 44.5% (–2.3 to +1.8), and 46.9% (no difference range reported), respectively. The  $135^\circ\text{C}$  procedure was recommended for adoption as a rapid method.

#### Oven-Drying Methods

Four drying procedures for meat product samples were evaluated by Windham (11) in a study with 11 collaborators. In two of the procedures, samples were dried with air ovens and in the other two with vacuum ovens, as follows: (1) 16–17 hr drying in a  $100\text{--}102^\circ\text{C}$  mechanical convection oven, (2) 2.5–3.5 hr drying in a  $125 \pm 5^\circ\text{C}$  mechanical convection oven, (3) 16–17 hr drying in a  $69\text{--}71^\circ\text{C}$  vacuum oven, and (4) about 6 hr drying in a  $98\text{--}100^\circ\text{C}$  vacuum oven. The procedures were found to be satisfactory for a variety of meat products except for the last of these, which was not suitable for high fat products such as pork sausage. This study led to the adoption of several of the present official methods. Results reported by procedures 2, 3, and 4 were compared with those obtained by procedure 1. By procedure 2, 108 values averaged 0.16% moisture higher; normalized difference ranged from –0.97 to +1.76 and s.d. =  $\pm 0.33\%$  moisture. By procedure 3, 101 values averaged 0.14% moisture lower; normalized difference ranged from –1.40 to +3.42 and s.d. =  $\pm 0.55\%$  moisture. By procedure 4, 72 values of products other than those with high fat content, such as pork sausage, averaged 0.16% moisture higher; normalized difference ranged from –1.45 to +1.09 and s.d. =  $\pm 0.36\%$  moisture.

Four methods of moisture determination on fresh meat samples were evaluated by Benne *et al.* (12). Results of vacuum oven-drying at temperatures of 50, 70, or  $100^\circ\text{C}$  for 6, 18, and 24 hr showed values which were generally low after 6 and 18 hr at  $50^\circ\text{C}$ . After 24 hr, values agreed well with results obtained by drying samples 6 hr at  $100^\circ\text{C}$ . Hot air oven at  $100\text{--}105^\circ\text{C}$  required 5 hr to dry high fat meat samples and 24 hr to dry lean samples. Azeotropic distillation using toluene and a Bidwell-Sterling receiver took 2 hr for a determination. Two of the 3 analyses they made were low compared with the  $100^\circ\text{C}$  vacuum oven, 6 hr method. Vacuum desiccator drying of samples over sulfuric acid, at room temperature, required 8–15 days. Even after 15 days results for 2 of the 3 samples were low.

Drying of meat samples by mechanical convection

oven at  $200^\circ\text{C}$  was proposed by Perrin and Ferguson (13). They stated that after some experience with the method, an analyst could determine moisture with good results in 15 min. A 2600W mechanical convection oven with an inside volume of 1 cu. ft was used. Results for 25 samples, compared with results by the official method, averaged 0.1% moisture higher; difference (without normalizing) ranged from –0.4 to +0.5 and s.d. =  $\pm 0.02\%$  moisture.

The International Organization for Standardization (14) adopted the following oven procedure for moisture determination of meat: Samples are dried at  $103 \pm 2^\circ\text{C}$  for 2 hr, cooled in a desiccator, and re-dried for 1 hr periods until the dried sample weight differs no more than 0.1% from the previous weight. Duplicate determinations were stated to differ no more than 0.5% moisture.

Moisture determination by the British standard method (15) for meat samples cited use of a  $103^\circ\text{C}$  oven. The procedure reported is as follows: 5–10 g of sample, minced twice through a grinder, is mixed with 3–4 times its weight of sand and dried 30 min in a  $103 \pm 2^\circ\text{C}$  oven; 5–10 ml of ethanol is added to the dried residue; ethanol is evaporated on a  $60\text{--}80^\circ\text{C}$  water bath and the residue is heated 2 hr in a  $103 \pm 2^\circ\text{C}$  oven. Heating, cooling, and weighing is repeated until 1 hr of heating does not reduce weight by more than 0.1% the weight of the test portion. Difference between duplicate determinations should not be more than 0.5 g moisture/100 g sample.

Six rapid methods of moisture analysis were compared to the official method by Cohen (16). Three methods were of the non-instrumental type: gravity convection oven at various temperatures, specific gravity, and hot plate. Ground beef and frankfurter samples, 5 g each, were dried and weighed in the oven experiment for up to 4 hr and at temperatures up to  $200^\circ\text{C}$ . Results obtained with 2 hr drying at  $125^\circ\text{C}$  averaged 0.1% lower moisture than by the official method; normalized difference ranged from –0.8 to +0.7 and s.d. =  $\pm 0.5\%$  moisture. The specific gravity method was evaluated with 25–100 g samples homogenized 30 sec with cold anhydrous ethanol. Portions of the extracts were weighed in 10 ml volumetric flasks at  $20^\circ\text{C}$ . Results averaged 3.6% higher moisture than by the official method; normalized difference ranged from –4.0 to +4.8 and s.d. =  $\pm 3.9\%$  moisture. The same products were also analyzed by the hot plate method. Samples of 10 g each were placed in aluminum dishes, on aluminum foil to prevent loss by spattering; these were heated on a hot plate which maintained  $200^\circ\text{C}$  at the heating surface for 45 min, cooled 10 min, and weighed. Results averaged 0.7% moisture higher than by the official method; normalized difference ranged from –0.9 to +0.7 and s.d. =  $\pm 0.7\%$  moisture.

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.

### Acetyl Chloride Reaction

A preliminary report of the acetyl chloride method adapted to a 1½ hr moisture analysis of meat products was made by Lórant and Pollak (17). Essentially, acetyl chloride was reacted with the moisture of the sample to form acetic acid and HCl. Ethanol was then reacted with residual acetyl chloride to form ethyl acetate and HCl, which was titrated with sodium hydroxide solution. The difference between this result and a blank indicated the amount of acetic acid generated and hence the amount of moisture in the sample. Results for 8 samples averaged 0.3% moisture lower than those by the Hungarian standard oven procedure and azeotropic distillation with toluene; normalized difference ranged from -0.7 to +0.6 and s.d. = ±0.4% moisture.

### Saponification

A 30 min moisture determination for meat, using titration, was reported by Glass and Addis (18). Samples were mixed with anhydrous methanol, homogenized, and centrifuged. To a portion of the extract, methanolic sodium methoxide and ethyl acetate were added, and after tempering in a 50°C water bath, the solution was titrated with ethanolic hydrochloric acid. Of 8 samples analyzed, 6 were compared with results by an 18 hr, 108°C oven-drying method and 2 were compared with azeotropic distillation. Results averaged 0.6% moisture higher than reference method values; normalized difference ranged from -0.6 to +1.4 and s.d. = ±0.6% moisture.

### Azeotropic Distillation

This technique has been applied for moisture analysis of many food products. The first review of its analytical application was published by Hoffman in 1908 (19). He referred to a German patent issued to him in 1901 on the distillation technique. Applications which he and other investigators made in analyzing cereal grains, hops, malt, butter, and cellulose were discussed. Applications reported by Marcusson (20) who analyzed oils, fats, and soaps with xylene as the solvent were also reviewed. Another early investigator of azeotropic distillation was Young (21) who published a series of papers on the characteristics of azeotropic mixtures. More recent reviews of the development and applications of the technique were presented by Othmer (22), Churchward (7), Fetzer (23), and Joslyn (6). The theory of azeotropic distillation is treated in detail by Carlson and Stewart (24).

Many of the early studies of azeotropic distillation for moisture distillation were conducted with low boiling solvents to prevent hydrolysis of carbohydrates, for example, levulose in molasses and fruit products (25, 26). As a result, the use of the higher

boiling xylene was limited, and hence toluene was favored. Xylene is the preferred solvent for azeotropic distillation of meat samples because the higher boiling temperature and higher water-to-solvent ratio of the azeotrope allow moisture to be distilled more rapidly. Other solvents have been used in various procedures, such as cyclohexane and benzene for rapeseed oil (27) and isopropyl ether for fish (28). The use of liquids heavier than water was proposed by some investigators to prevent localized overheating in the boiling flask. With such solvents some samples will float on the surface within the boiling flask. Tetrachloroethylene was used for this reason by Phillips and Enas (29) and Wyler (9), and for its low flammability. The distillation procedure using this and other chlorinated hydrocarbons, however, requires special calibrated glassware, since water in the receiver tube will float upon the solvent; furthermore, the use of denser solvents prolongs distillation time.

A 4% mixture of *n*-amyl alcohol in toluene was used by Miller (30, 31) for moisture determination. The alcohol content was sufficient to prevent water films from adhering to the condenser inner surface. Similarly, Calderwood and Piechowski (32) used 2 drops of 95% ethanol at the end of xylene distillations for the same purpose.

Azeotropic distillation with toluene was compared to mechanical convection oven-drying at 100°C for 16-18 hr by Hill (33), using 25 g meat samples which had been ground 3 times. Averaged results were the same as by the reference method. For fresh meat, the range of difference was ±0.7 and s.d. = ±0.3% moisture. For frozen meat, the range of difference was ±1.3 and s.d. = ±0.6% moisture. Others who used toluene as the solvent were Kerr (34, 35), Benne *et al.* (12), Thompson and Corsi (36), Lunder (37), and Cohen (16). Cohen analyzed 10 g samples of ground beef, frankfurter, and pork sausage for an azeotropic distillation of 15 min to 1 hr. The analyses were determined to be complete when no additional water collected in the receiver upon continued distillation for intervals of 5 min. Results averaged 2.1% moisture lower than values obtained by the official method. Normalized difference ranged from -0.9 to +1.0 and s.d. = ±0.8% moisture.

An evaluation of 27 selected solvents for suitability in azeotropic distillation was reported by Cohen and Kimmelman (38). Ground beef, frankfurter, and pork sausage were analyzed for moisture content. Six of the solvents—ethylbenzene, cumene, 2-ethyl-1-hexanol, 1-heptanol, 1-octanol, and 2-octanol—yielded excellent results for 10 g samples using 15 min distillations. Some of the other solvents evaluated were found to be satisfactory for distillations of 30 min or more, such as nonane, *m*-xylene, and butyl ether. Results for the 3 solvents—cumene, 1-octanol,

and 2-octanol—for all 3 meat products, using 15 min distillations, were essentially the same; pooled results averaged 0.2% moisture lower than results by the official method. Normalized difference ranged from -0.7 to +1.3 and s.d. =  $\pm 0.5\%$  moisture. When these same distillations were continued for 30 min, results averaged 0.2% moisture higher than those by the official method. Normalized difference range and standard deviation were the same as for the 15 min distillations reported above.

### **Instrumental Moisture Methods**

#### ***Instruments of Potential Use for Meats***

A large variety of instruments can be used to measure moisture content of products by the application of well known physical principles including measurement of the response of the water molecule to some form of applied energy. Some of the instrumental methods have been readily applied to food products of simpler composition than meat, such as products that are free of fat. Other instrumental methods are not applicable to meat because the moisture content range of meat is higher than the instrument can measure.

The operating range of various instruments for moisture determination is among the characteristics reviewed by Roth (39). Operating range is important because analysis of meat samples may require determinations ranging between 0 and 80% moisture. At the time of his review, the ranges for some of the instruments were as follows: electrolytic hygrometer 0-10%; resistance electrode and cobalt chloride colorimetry 0-20%; capacitance meter 0-25%; and conductivity meter 0-50%. All of these are too limited for meat analysis. Radio frequency absorption instruments were reported as being usable for up to 60% moisture and nuclear reflection instruments for up to 80%. However, neither of these latter 2 instruments measured water specifically. Microwave absorption instruments have a 90% moisture upper limit and therefore might possibly be developed for moisture analysis of meats. Two other methods, reviewed by Roth, which will analyze up to 100% moisture use moisture balances and Karl Fischer titrators. These 2 types of apparatus will be discussed later in this review.

Webb (40) reviewed instrumental methods that were applied to specific moisture analyses and could possibly be adapted for use with meat. In addition to a number of the methods discussed above, others using refractometry, coulometry, and nuclear magnetic resonance were discussed.

#### ***Moisture Balance***

The Ohaus Model 770 moisture balance was compared to the official oven method by Solberg and Riha (41) and Cohen (16). The apparatus embodies

a triple-beam balance, a shielded infrared lamp, and a moisture calculator which can be read to 0.1% moisture. In the former report, results for frankfurters and ground meat were compared to values obtained by the oven method. Results averaged 0.01% moisture lower than the reference method; difference ranged from -0.9 to +0.7% and s.d. =  $\pm 0.4\%$  moisture. In the latter report, samples of 2 lots of beef, 1 frankfurter, and 1 pork sausage were analyzed and compared with the results by the official method. Results averaged 0.4% moisture higher than by the reference method. Normalized difference ranged from -0.3 to +0.4 and s.d. =  $\pm 0.3\%$  moisture. Results with the Dynatronic infrared radiation moisture balance (oven-type) and the official oven method were also compared by Cohen, using 12 g samples of 3 types of meat product. The samples were dried at  $100 \pm 10^\circ\text{C}$  for 45-60 min periods. Results averaged 0.1% moisture higher than by the reference method. The range of normalized difference was  $\pm 0.6$  and s.d. =  $\pm 0.5\%$  moisture. Bloemer (42) evaluated the Brabender moisture balance. Bartels and Gerigk (43) evaluated a similar moisture balance, marketed both in Germany and in this country, the Ultra-X unit, which is discussed later in this review (see combined methods).

#### ***Karl Fischer Titrator***

This technique was described by Mitchell (44) and applied to moisture content analysis of meat samples by Cook *et al.* (45). In a discussion of the determination of moisture in grain, the National Bureau of Standards (46) stated that the titrator was unsatisfactory because of the frequent restandardization that was necessary.

#### ***Refractive Index***

This optical method of determining moisture content has been used in dried fruit analysis by Bolin and Nury (47) and in turkey meat analysis by Ning and Marion (48). The latter report revealed that a method depending on the refractive index of tissue slurries requires uniformity of type and preparation of samples. The results showed a difference between red and white muscle. Addis *et al.* (49) reported the refractive index of anhydrous isopropanol extracts of meat samples.

#### ***Infrared Absorption***

Spectrophotometry of solvent extracts of food products other than meat has been reported by Gold (50) using methanol, by Rader (51) using dimethylformamide, and by Vornheder and Brabbs (52) using dimethylsulfoxide.

#### ***Gas Chromatography***

Moisture determination in grain was discussed in a communication by the National Bureau of Stan-

dards (46). The gas chromatographic method used on methanol extracts was reported to overcome many of the shortcomings of earlier laboratory methods and provided a good reference method. The method was stated to be highly specific for water. The nature of the apparatus and equipment may limit the usefulness of the method to standardizing equipment used for field testing. Gas chromatographic procedures using methanol extracts were also reported for food products including dog food by Schwecke and Nelson (53), for fruits by Brekke and Conrad (54), and for meat samples by Addis *et al.* (49).

#### **Nuclear Magnetic Resonance (NMR)**

Steffa *et al.* (55) evaluated a wide variety of meats and meat products in a study of the possible use of NMR in moisture and/or fat content determination. The method did not appear promising because measurements were affected by differences in free or bound water content, whether the sample was beef or pork, and whether the sample was pre-chilled, or warmed after chilling. They also reported an indication that ionic constituents would affect measurements.

#### **Radioactivity of Natural Potassium-40**

Studies of the relationship of naturally occurring  $^{40}\text{K}$  isotope to the composition of meat were reported by Kulwich (56) and Kirton *et al.* (57). The basis for the method is the measurement of radioactive counts primarily emitted from the lean portion of meat rather than the fat portion. Kulwich reviewed the known potassium content of various species and suggested that studies of  $^{40}\text{K}$  content of lean, fat and bone, and different muscles could lead to estimates of lean content. The estimates would then serve to calculate moisture and fat content. Kirton *et al.* evaluated 20 pork and 15 lamb samples to estimate moisture, fat, and protein content by measuring  $^{40}\text{K}$  counts. The results on pork, but not lamb samples, were reported to be promising.

#### **Electrolytic Hygrometry**

Preliminary results with a micro-procedure of electrolytic hygrometry, based on coulometry, were described in detail by Fraade (58) for food products. The instrument measured the current required to electrolyze water vapor introduced as a gaseous stream.

#### **Fat Analysis**

In the same respect that moisture content of meat is not neatly and exactly defined, fat extraction is not simply an extraction of fat per se. The factors that make this so will be apparent from the following considerations.

Solvent extractions of fat involve dissolving constituents of various solubilities. In addition, meat constituents such as phospholipids can be extracted. Also, factors that affect the analysis are particle size of the meat product, the product's previous processing treatment, the barrier effect of water, and the effect of colloidal and electrolytic constituents in the product.

While free lipids are readily solvent-soluble, that portion of the fat which is encapsulated in its native cell is more difficult to extract. If the product is of the emulsion-based type, fat is emulsified in the form of discrete globules as discussed in detail by Swift and Sulzbacher (59) and Swift (60).

The constituents of meat and meat products are structurally arranged in a highly organized fashion. Nonpolar fat solvents cannot rapidly penetrate polar or electrostatically bonded arrangements because such sites are similar to physical barriers. On the other hand, polar solvents, which can penetrate electrostatic barriers, may dissolve constituents other than fat. Thus care must be exercised in the choice of a solvent. Diethyl ether, for example, tends to extract water-soluble constituents unless it is absolutely free of water. Petroleum ether is preferred by many analysts because it extracts fewer nonfat components (61).

In an extensive study of chloroform extracts of the residues obtained from alcohol-ether extraction of nerve tissue, Brante (62) identified the presence of many constituents other than triglycerides including phospholipids, lecithin, choline, ethanolamine, inositol, and cholesterol. Free fatty acids were not tested.

Bloor (63) was the first to suggest that a polar solvent such as ethanol be added to a conventional lipid solvent such as diethyl ether to obtain more effective solvent penetration and more efficient extraction. The use of chloroform-methanol mixed solvent was reported by Folch *et al.* (64, 65) for extraction of brain lipids. Arnold and Hsia (66) reported higher estimates of fat from beef by using chloroform and tetrachloroethylene in a Soxhlet apparatus. However, samples were freeze-dried rather than oven-dried. Bligh and Dyer (67) homogenized fish tissue with chloroform-methanol solvent to form a miscible system. Water was then added to this system which resulted in a chloroform-lipid layer and an aqueous methanol layer. A number of other investigators used chloroform-methanol solvent for a variety of food products (68-78). Ostrander and Dugan (79) investigated fats from different parts of meat animals by precipitating protein with zinc acetate and extracting in a blender with chloroform-methanol solvent. Giam and Dugan (80) developed a procedure using either diethyl ether or hexane to extract free lipids, and 95% ethanol or hexane-

ethanol to extract bound lipids. Hagen *et al.* (81) studied the extraction of lipids from beef samples. They evaluated 6 extraction solvent combinations (including diethyl ether, petroleum ether, mixed ethers, and chloroform), 3 drying procedures, and 2 methods of sample preparation. Chloroform-methanol extraction yielded the highest amounts of extracted fat. However, this extract also yielded the highest amount of phospholipid; samples which were acid-hydrolyzed yielded the lowest amount. Among the 6 different extraction methods, the phospholipid content ranged from 2.8 to 10.1% of the extracted solids.

Southgate (82) reviewed the use of chloroform-methanol for fat extraction of foods. In that report, the mixed solvent was distilled from the extract; the residue was then re-extracted with petroleum ether and shaken with sodium sulfate. The re-extraction with petroleum ether was not quantitative with some fats unless the residues were warmed into solution for several min. Bixby *et al.* (83) extracted liver samples by using the solvent mixture of the Mojonier method in a Goldfish extractor. The solvent mixture consisted of diethyl ether, petroleum ether, and ethanol, 5+5+2, respectively. Fat content by this method averaged 5.7% fat compared with 5.5% by Mojonier method and 3.7% by extraction with diethyl ether in a Goldfish extractor. The residue obtained by the 3-component solvent mixture was redissolved in diethyl ether. An average of 98% of the residue redissolved.

A number of these investigators used freeze-drying instead of oven-drying prior to extracting (66, 79, 81). This was done to avoid exposing the samples to heat when the fat extracts were to be further investigated for identification of constituents. It is possible that removal of the moisture barrier by this means would also leave tissue structure more porous than by oven-drying. Watts *et al.* (84) investigated this point by extracting fat by the Mojonier method from fresh or freeze-dried ground beef and several other food products. The results were not significantly different: average yield from fresh product was 11.54% fat, and from freeze-dried product, 11.50% fat.

As the preceding discussion indicates, an evaluation of methods for fat analysis requires definition of the meaning of fat. For the present purpose of evaluating methods for industrial and regulatory use, fat is arbitrarily considered to be measurable as the ether-extractable material that is yielded by the official method.

## Fat Determination Methods

### Reviews of Fat Methods

Recent reviews of fat determination in meat products include one by Everson *et al.* (5) who discussed

the Mojonier method, a modified Babcock method, and the capacitance measurement procedure. Klima *et al.* (8) reviewed modified butyrometric methods extensively as well as a number of other methods. They discussed special Babcock bottles to contain up to 28 g of meat sample, the use of acetic-perchloric acids in place of sulfuric acid, alkaline digestion reagents, and pepsin enzyme digestion. They also reviewed refractometry, specific gravity, and capacitance measurements on solvent extracts of the fat, rapid rendering devices, potassium-40 content, and nuclear magnetic resonance. Of these methods, 2 were selected for further evaluation and results were compared with Soxhlet ether extraction. The methods were: (1) rapid extraction with carbon tetrachloride after mincing the samples in a top-driven homogenizer, followed by determination of specific gravity of the extract; and (2) butyrometric method using a 5 g cream bottle and 18*N* sulfuric acid which digested the meat sample in 25-30 min. Results for 31 samples analyzed by the specific gravity method averaged 0.3% fat lower than reference values. Normalized difference ranged from -1.9 to +2.9 and s.d. =  $\pm 1.0\%$  fat. Results for 8 samples analyzed butyrometrically averaged 0.15% fat higher than reference values. Normalized difference ranged from -1.25 to +0.65 and s.d. =  $\pm 0.8\%$  fat.

Whitehead (85) discussed 8 methods used for meat fat determination and described the specific gravity method developed at the Honeywell Corporation. Methods he reviewed included the Babcock, modified Babcock, capacitance, gamma-ray penetration, ultrasonic, empirical determination from moisture content, rapid rendering devices, and specific gravity.

Wyler (9) reviewed Swiss standard methodology for determining fat content in meat products. He stated that direct Soxhlet extraction is unsuitable for meat because bound lipids are not extracted from tissue. He also stated that butyrometric methods with either sulfuric or perchloric acid yielded inexact results and that the Mojonier method required a special centrifuge. Standard methods, in his opinion, were those by which meat samples were digested with hydrochloric and sulfuric acids and subsequently were extracted with chlorinated hydrocarbons or with ether.

Smith (86) presented a broad survey of rapid methods for total fat determination in foods including meat and concluded that the most promising areas for further investigation are the volumetric, refractometric, and rapid extraction methods. Reviewed in detail were the butyrometric method and modifications of it, solvent extraction followed by refractive index measurement, specific gravity, infrared radiation and absorption, nuclear magnetic reso-

nance, capacitance, X-ray absorption, microwave absorption, rapid extraction, photometric, and ultrasonic methods. The review cited 106 references. Joslyn (87) presented a discussion of extraction methods for fat determination of food products including meat. The factors that affect solvent extraction of fat were reviewed. The chapter is well documented with 137 references. Pearson (10) reviewed methods of fat analysis for fresh sausage and evaluated 2 of these, namely, Soxhlet extraction with petroleum ether after drying at 100–105°C for 3–8 hr and the Gerber procedure on 2 g samples using a milk testing bottle (45 min method). The mean values and difference range obtained by the 2 methods, 34.1% ( $\pm 5.9$ ) and 34.8% ( $-5.4$  to  $+4.9$ ) fat, respectively, were not significantly different.

### **Extraction**

Use of a series of 5 extractions with diethyl ether followed by partition in a separatory funnel having a fritted glass filter plate fused into it was suggested by Ernst (88) for meat samples. The procedure required 1.5–2 hr for a determination. Results for 23 samples averaged 0.2% fat higher than by the official method. Normalized difference ranged from  $-1.1$  to  $+1.5$  and s.d. =  $\pm 0.7\%$  fat.

Windham (89) compared extraction of meat samples dried 6 hr with samples dried 16–18 hr at 100–102°C before Soxhlet extraction. Samples dried 6 hr were extracted with either ethyl ether or petroleum ether and samples dried 16–18 hr, with petroleum ether only. Mean values and difference range for 60 samples were:  $24.2 \pm 0.5\%$  fat for samples dried 6 hr and extracted with ethyl ether,  $24.3 \pm 0.4\%$  fat for samples dried 6 hr and extracted with petroleum ether, and  $24.1 \pm 0.5\%$  fat for samples dried 16–18 hr and extracted with petroleum ether.

Fat extraction by means of *n*-hexane or petroleum ether (boiling range 40–60°C) was selected for meat samples by the International Organization for Standardization (90). The procedure states that the solvent should be distilled from the extract and fat content should be determined gravimetrically.

Chromic acid oxidation of extracted fat was originally developed by Bloor (91) for determination of fatty acids. Paul (92) adapted the procedure for fat determination in small samples of tissue and O'Shea and Maguire (93) used it for 1.5 g samples of meat. The oxidation reaction was conducted on the fat extracted from the samples to keep chromic acid from reacting with other organic matter. The procedure required 2 hr for a determination. Pork, beef, and lamb samples, 34 determinations, were analyzed and compared with results by the official method. Results averaged 0.1% fat higher than the reference method. Normalized difference ranged from  $-1.0$  to  $+1.6$  and s.d. =  $\pm 0.6\%$  fat.

Two British standard methods (94) for determination of total fat content of meat products both specify digestion of a minced sample with hydrochloric acid and gravimetric determination of the extracted fat. The analyst is directed to either digest a 3–5 g sample for 1 hr and, continuously or semicontinuously, extract the filtered residue with hexane or petroleum ether, or to digest a 2–3 g sample for 30 min and manually extract, 3 times, the digest with diethyl ether. For determination of the free fat of meat samples, the British standard method (95) cited Soxhlet extraction with hexane or petroleum ether for at least 6 hr of a 5–10 g minced sample dried as in the moisture determination and gravimetric determination of the extracted fat.

Modification in both the drying and extraction time of the official method for meat was evaluated by Cohen and Swift (96) to reduce time of analysis without loss of accuracy. Samples of ground beef, frankfurter, and pork sausage were dried for periods of 15–90 min and solvent-extracted for periods of 15 min to 16 hr. Replicate analyses were compared with results by the official method. Results showed that ground beef required 30 min drying time and 45 min extraction; frankfurter required either 30 min drying and 30 min extraction, or 45 min drying and 15 min extraction; and pork sausage required either 15 min drying and 30 min extraction, or 30 min drying and 15 min extraction. For these analyses recoveries were 99.2–100.5% of results by the official method. In each case, 15 min less drying time or 15 min less extraction time yielded fat recoveries of 95.8–98.6%.

### **Babcock Method Modifications**

Emulsification of ground meat with Oakite brand household cleanser was proposed by Oesting and Kaufman (97). The emulsion that was formed was weighed into a Babcock bottle and the procedure was continued as in the usual Babcock method. Results of 20 analyses, compared with values obtained by the official method, averaged 0.04% higher fat content, difference ranged from  $-1.6$  to  $+1.1$  and s.d. =  $\pm 0.7\%$  fat.

A modified Babcock procedure was evaluated as a rapid method for fat determination in meat by Windham (89, 98). The earlier report was a collaborative study of the method compared with ether extraction methods by 7 collaborators. Quadruplicate analyses on 60 samples averaged 0.01% higher fat with a difference range of  $\pm 0.64$  and s.d. =  $\pm 0.4\%$  fat. In the later report, Windham evaluated the same method along with 2 other rapid methods, perchloric-acetic acid modification of the Babcock and capacitance measurement. Results for 18 meat samples (4 different meat products) averaged 0.15% higher fat. No explanation was offered for this higher average difference compared to the previous report.

Normalized difference ranged from  $-0.6$  to  $+1.2$  and s.d. =  $\pm 0.4\%$  fat.

Use of perchloric and acetic acids in place of sulfuric acid was proposed by Salwin *et al.* (99). Meat fat contents from 19 analyses were compared to values obtained by the official method. Results averaged  $0.01\%$  lower fat; difference ranged from  $-0.4$  to  $+0.5$  and s.d. =  $\pm 0.2\%$  fat. However, Windham (98) and Krol and Meester (100) evaluated the method and found consistently higher values than those obtained by Soxhlet extraction. Windham reported finding  $5\%$  acid, calculated as acetic, in the fat layer so that fat content values had to be corrected by this amount; results for 18 meat samples averaged  $1.1\%$  higher fat. Normalized difference ranged  $\pm 1.0$  and s.d. =  $\pm 0.5\%$  fat.

The "Banco test", a modified Babcock procedure, was devised by Anderson *et al.* (101) to prevent charring of samples by sulfuric acid. Samples were treated with papain powder, sodium hydroxide, a solution containing urea, sodium carbonate, disodium phosphate and ethylenediaminetetraacetic acid in aqueous methanol, and a detergent. The procedure reportedly required 30 min for an analysis. It was evaluated by 4 laboratories, using 8 meat products, with the official method as reference. Results averaged  $0.2\%$  higher fat. Normalized difference ranged from  $-0.8$  to  $+1.2$  and s.d. =  $\pm 0.4\%$  fat.

Another modification of the Babcock procedure was proposed by Whalen (102), who digested meat samples with hot hydrochloric acid and diluted the digest with dimethylsulfoxide in a procedure requiring 10 min. Results from 98 samples were compared with results obtained by the Mojonnier extraction method. Results averaged  $0.17\%$  lower fat. Normalized difference ranged from  $-1.3$  to  $+1.6$  and s.d. =  $\pm 0.6\%$  fat.

#### Rapid Screening Devices

A number of devices have been developed for rapid screening of meat samples and have a limited acceptance in field inspection. One of these units is the "Fat-Alyzer" designed as an abbreviated Babcock procedure kit (103) to analyze a meat sample in 15 min.

In a rendering device made by the Univex Corporation (104, 105) the meat sample was heated between 2 electrodes, fat content and juices dripped into a flask, and fat column height was read directly in units of per cent fat. This unit was tested at our laboratories with unfavorable results.

The Hobart Mfg. Co. (106, 107) markets a similar rendering device, which was tested and reported by Bellis *et al.* (108). The unit utilized an inverted hot plate to render the meat sample in 15 min. Samples at 16 fat levels (14–29% fat content) were analyzed.

Results reported showed that difference ranged from  $-0.49$  to  $-4.10$  with an average difference of  $2.3\%$  lower fat than values obtained by the official method.

#### Capacitance Measurement

Furgal (109) extracted fat from meat samples with *o*-dichlorobenzene and related the capacitance of the extract, corrected for temperature of extract, to fat concentration. Results for 20 samples of meat products averaged  $0.2\%$  higher fat than values obtained by the official method. Normalized difference ranged from  $-1.9$  to  $+1.2$  and s.d. =  $\pm 0.8\%$  fat.

The above method was also evaluated by Everson *et al.* (5). In the 30 min analysis, 50 g samples of 20 meat and meat products were blended with 100 ml *o*-dichlorobenzene and also 5 g filter aid for 4 min, the blend was filtered, and the filtrate was measured for capacitance. Results averaged  $0.6\%$  fat higher than values obtained by the official method. Normalized difference ranged from  $-4.5$  to  $+4.0$  and s.d. =  $\pm 2.0\%$  fat.

Another evaluation of this method was reported by Windham (98), who analyzed 18 meat samples. Results averaged  $0.3\%$  fat higher than values obtained by the official method. Normalized difference ranged from  $-0.7$  to  $+1.5$  and s.d. =  $\pm 0.7\%$  fat.

#### Specific Gravity Measurement

Gipr and Lukashova (110) proposed determining fat content by measuring the specific gravity of solvent extracts of meat products. Trichloroethylene was used as the solvent and accuracy was reported to be within  $1\%$  of the Soxhlet fat extraction method. This was confirmed when Mahmood-ul-Hassan and Pearson (111) evaluated the procedure. Results for 8 samples of beef and 6 samples of pork averaged  $0.1\%$  lower fat for beef and  $0.3\%$  higher fat for pork than values by the official method. The range of normalized difference was  $\pm 0.6$  for beef and  $-0.8$  to  $+0.6$  for pork; s.d. =  $\pm 0.5\%$  fat for both meats.

Determination of fat content in meat by measurement of the specific gravity of the meat itself was developed by Whitehead (112). The apparatus measured weight, compacted volume, and temperature of 750 g samples. From these data, fat content was computed automatically after corrections were applied for animal species and the section of the animal sampled. The procedure was described as useful on the processing floor because measurements could be made in 30 sec on samples which contained no frozen mixture or foreign ingredients. Results on 69 samples of beef chuck averaged  $0.06\%$  higher fat than values obtained by the official method; range of difference was  $\pm 2.4$  and s.d. =  $\pm 1.2\%$  fat.

The above specific gravity apparatus was also evaluated by Malanoski and Greenfield (113) on meat samples of varied origins. Results on 56 sam-

ples averaged 1% fat lower than by the official method. Normalized difference ranged from -3.4 to +3.6 and s.d. =  $\pm 1.5\%$  fat.

A method based on specific gravity was reported by Bittenbender (114) for measuring fat content of meat. Heptane extracts of samples were made and custom-made hydrometers were used to determine specific gravity at 34°C in a 15 min procedure. Temperature of the water bath was closely controlled because a variation of  $\pm 0.01^\circ\text{C}$  in the extract influenced the accuracy by  $\pm 0.05\%$  fat. Results on 17 meat samples averaged 0.3% fat higher than values obtained by the official method. Normalized difference ranged from -0.9 to +0.8 and s.d. =  $\pm 0.5\%$  fat.

#### Refractometry

Fat determination of foods including meat was reported by various investigators (115-117). Samples were ground with monobromonaphthalene and sodium sulfate. The refractive index of the filtered extract was then read with a reported accuracy of  $\pm 0.5\%$  fat.

The above procedure was modified and evaluated by Mahmood-ul-Hassan and Pearson (111) who used a 1+1 mixture of 1-bromonaphthalene and mineral oil as the extracting solvent. Results on 6 beef and 6 pork samples averaged 0.5% lower fat for beef samples and 0.7% lower fat for pork samples than values obtained by the official method. Normalized difference ranged from -1.0 to +0.5 for beef and -0.8 to +0.65 for pork; s.d. =  $\pm 0.6\%$  fat for beef and  $\pm 0.5\%$  fat for pork.

A refractometric procedure, a German standard method, was described by Rudischer (118) for fat analysis of meat samples. A mixture of perchloric and phosphoric acids and a low flame were used to digest a sample in 1-3 min. Without cooling the digest, 1-bromonaphthalene (or mixtures of mono- and dibromonaphthalene) was used to extract fat. Calcium carbonate and sodium sulfate were added to the extract which was then filtered, and the filtrate was read on a refractometer at a temperature of 50°C.

#### X-Ray Absorption

The Anyl-Ray Analyzer was reported to utilize a dental-type X-ray source to measure fat content of meat samples (119, 120). The instrument measured a 13 lb sample and was reported to yield values for fat content with a difference range of  $\pm 1.5$  and s.d. =  $\pm 0.5\%$  fat.

#### Reflectance Photometry

A photoelectric method of determining fat content was devised by Knudsen (121) for meat trimmings arranged on a conveyor band. As the meat was car-

ried past a fluorescent tube, reflected light was measured by photoelectric cells and the signal was converted to indicate fat content. Results with the method are not available.

#### Combined Method for Moisture and Fat Determination

A combined method for moisture and fat content determination of oil seeds was proposed by Kaufman and Keller (27). Moisture was determined by azeotropic distillation with heptane, and fat content was determined gravimetrically after removal of solvent from a portion of the extract.

Azeotropic distillation using 2-octanol for moisture determination reported by Everson *et al.* (5) and their procedure for determining fat content by capacitance measurement of an *o*-dichlorobenzene extract of the sample were discussed earlier in this review. In a combined method for determining both moisture and fat content of the same sample, they reported that azeotropic distillation with a 1+7 mixture of 1-octanol and 2-octanol to determine moisture content, followed by capacitance measurement on the solvent extract obtained during the distillation, would yield the fat content in a procedure requiring 30 min. Using 15 g portions of 19 meat samples and 100 ml solvent for each assay, they compared results with values obtained by official methods. Results averaged 0.7% moisture lower and 0.3% fat lower than reference values. The range of normalized difference was  $\pm 1.9$  for moisture and -2.1 to +2.9 for fat; s.d. =  $\pm 1.1\%$  moisture and  $\pm 1.3\%$  fat.

Wistreich *et al.* (122) also recommended azeotropic distillation as part of a combined method for moisture and fat content determination of meat samples. A special 2-compartment flask and 10 g samples were used. Water volume was read from a Bidwell-Sterling type receiver, and fat content was determined either gravimetrically after evaporating solvent from the extract, or by difference after drying and weighing the residue of the extracted sample. A distillation time of 2 hr was recommended. This procedure was later modified (36) so that a Florence flask with a center well was used to contain the sample and 250 ml solvent in place of the 2-compartment flask. Dry toluene was used for a distillation time of 30-90 min. Moisture and fat content values were obtained as with the earlier procedure.

Bartels and Gerigk (43) determined moisture and fat content in meat in a 2-part method by first determining moisture in a moisture balance (the "Ultra-X" unit) and then extracting fat from the residue with carbon tetrachloride. Samples were dried 15-25 min depending on type of meat product. The dried material was weighed and then extracted for 10-20 min. After extraction, the solvent was

evaporated from the residue by infrared radiation and fat was determined by difference. Results for 23 samples averaged 0.5% moisture lower than their reference method (4–6 hr drying in a 105°C oven) and 0.1% fat lower than Soxhlet petroleum ether extraction. Normalized difference ranged from -1.7 to +2.2 for moisture and from -1.7 to +1.8 for fat; s.d. =  $\pm 1.0\%$  moisture and  $\pm 1.0\%$  fat.

Moisture and fat content of meat samples were determined in a combined method by Davis *et al.* (123). The procedure made use of azeotropic distillation with *n*-butyl ether and a vacuum oven to dry the sample residue. Distillation time varied (2–2.5 hr) according to type of sample analyzed. After distillation, during which moisture and fat are removed, the sample residue was placed in a 140°C vacuum oven for 10–15 min and then weighed. Results for 46 samples averaged 1% moisture higher and 1% fat higher than values obtained by official methods. Normalized difference ranged from -1.7 to +1.3 for moisture and from -1.6 to +1.7 for fat; s.d. =  $\pm 0.8\%$  moisture and  $\pm 0.8\%$  fat.

An instrumental approach to develop a combined method for moisture and fat determination was proposed by Ben-Gera and Norris (124). Infrared absorbance of 2 g samples of comminuted meat was read in a spectrophotometer with a sample layer 2 mm deep. Moisture was determined by the difference in absorption at 1.725 and 1.800  $\mu\text{m}$  and fat was determined by the difference at 1.650 and 1.725  $\mu\text{m}$ . Accuracy was reported to be  $\pm 2.1\%$  moisture and  $\pm 1.4\%$  fat compared with values obtained by official methods. Technical problems of sample preparation and low sample transmittance were said to require further investigation.

Determination of moisture and fat content of meat by a combined method was also evaluated by Cohen and Kimmelman (125). Moisture content was determined by azeotropic distillation. Fat content was determined gravimetrically on an aliquot of the solvent extract. The suitability of 13 water-immiscible solvents was evaluated for the combined procedure. Ground beef, frankfurter, and pork sausage were analyzed by taking 10 g samples and 100 ml solvent in each determination. Moisture content of all three products was determined after 15–30 min of distillation. Recoveries of 95–100% of the moisture contents were obtained with a number of the solvents. Fat content was determined by taking 20 ml of the solvent extract in each case, evaporating the solvent by boiling under a stream of nitrogen (6–15 min), and weighing the residue. Of the 13 solvents evaluated, 95–100% recoveries of the fat contents were obtained with 4 of the solvents on ground beef samples and with 5 of the solvents on frankfurter samples after 15–30 min distillation; 95–100% recoveries were also obtained for pork sausage

when distillation was continued an additional 15–30 min with 3 of the solvents. Optimum results for both moisture and fat content, for all 3 products, were obtained with the solvents *m*-xylene and cumene in a comparison with values obtained by official methods: (a) With *m*-xylene solvent, results averaged 0.6% moisture lower and 0.7% fat lower; range of normalized difference was  $\pm 0.9$  for both analyses; s.d. =  $\pm 0.6\%$  moisture and  $\pm 0.4\%$  fat. (b) With cumene solvent, moisture content was estimated to be the same as by the official method and fat content was estimated to be 0.3% fat lower; the range of difference was  $\pm 0.7$  for moisture and the range of normalized difference was  $\pm 0.5$  for fat; s.d. =  $\pm 0.4\%$  moisture and  $\pm 0.5\%$  fat.

#### Evaluation of Methods

This survey reviewed the large number of methods available in order to identify the most promising, and to make an in-depth comparison of those which most closely meet the needs of the meat industry for quality control work. Data relevant to the selected methods were compiled and are shown in Tables 1 and 2. The moisture and fat methods were selected as useful for the following reasons: (1) simple to perform, (2) inexpensive, (3) rapid, (4) reasonably accurate and precise, and (5) applicable to a broad range of meat products. Also listed are the official methods to which they were compared.

#### Moisture Analysis

*Method 1*, AOAC official method 24.003(a), requires drying a meat or meat product sample 16–18 hr at 100–102°C in an air oven (mechanical convection preferred), cooling in a desiccator, and weighing to determine loss of weight as moisture. The method is indirect and is empirical. It is one of the reference methods that serves as the accepted standard for the meat industry and as the reference with which new methods are compared. It is by no means a rapid method. However, reproducibility of results is excellent.

*Method 2*, AOAC official method 24.003(b), requires drying a meat or meat product sample 2–4 hr, depending on product, in a mechanical convection oven at about 125°C. Similar to Method 1, it is an indirect and empirical method. However, it is more rapid than Method 1 and provides accuracy and reproducibility equivalent to Method 1.

*Method 3*, a modification of Method 2, uses a 125–150°C oven and is advantageous because the

Table 1. Comparison of methods of analysis for moisture content of meat

No.	Method <sup>a</sup> Type	Reference	Essential characteristic	Time required for a single analysis	Sample size, <sup>b</sup> g	No. of analyses <sup>c</sup>	Mean difference, <sup>d</sup> % moisture	Range, <sup>e</sup> % moisture	Std dev., <sup>f</sup> % moisture	Cost of equipment <sup>g</sup> and training <sup>h</sup>
1	AOAC official	126, 11	100-102° air oven	16-18 hr	4-6	108	0	not available	0.25	>\$250, 1
2	AOAC official	127, 11	125° mechanical convection oven	2-4 hr	4-6	108	0.2	-1.0 to +1.8	0.33	>\$500, 1
3	Modified Method No. 2	16	125-150° gravity oven	2-4 hr	4-6	12	0	-0.3 to +1.0	0.7	>\$250, 1
4	High temperature oven	13	200° mechanical convection oven	>15 min	25	25	0	-0.4 to +0.5	0.2	>\$500, 1
5	AOAC official	128, 11	95-100° vacuum oven	5 hr	4-6	72	0.2	-1.5 to +1.1	0.4	>\$150, 1
6	Low temperature vacuum oven	11	70° vacuum oven	16-18 hr	4-6	101	-0.1	-1.4 to +3.4	0.6	>\$150, 1
7	Hot plate	16	200° at surface	30-45 min	10	24	0.7	-0.9 to +0.7	0.7	<\$100, 1
8	Infrared radiation oven (Dynatron)	16	IR heater, built-in balance	40-60 min	12	24	0.1	±0.6	0.5	>\$600, 1
9	Infrared radiation lamp (Ultra-X)	43	IR lamp, built-in balance	15-25 min	2.5	23	-0.5	-1.7 to +2.2	1.0	>\$400, 1
10	Infrared balance (Ohaus)	16	IR lamp, separate balance	30-45 min	10-20	24	0.4	-0.3 to +0.4	0.3	\$100, 1
11	Azeotropic distillation (Si-Mo-Fat)	123	distillation with butyl ether	2 hr	10	46	1.0	-0.7 to +2.3	0.8	>\$200, 2
12	Azeotropic distillation (AMIF)	5	distillation with 2-octanol	15 min	15	19	-0.7	±1.9	1.1	>\$100, 2
13	Azeotropic distillation (Cohen)	38	distillation with various solvents	15 min 30 min	10 10	24 24	0	-0.7 to +1.3	0.5	<\$100, 2

<sup>a</sup> Method identified by common name, type, author, or manufacturer.

<sup>b</sup> Sample size as reported in the reference.

<sup>c</sup> Number of analyses of samples reported in the reference cited.

<sup>d</sup> Mean difference is the average algebraic sum of differences between results by the experimental and reference methods, indicating average higher (+) or lower (-) results with the experimental method.

<sup>e</sup> Range of differences between results by the compared methods, after results by the experimental method were adjusted (made normal) to reference method results by adding or subtracting the method's mean difference to or from the individual differences.

<sup>f</sup> Standard deviation was calculated on normalized differences.

<sup>g</sup> Cost quoted is on a unit basis, unless noted differently.

<sup>h</sup> Training required to perform the method indicated as 1, 2, and 3 for brief, moderate, and thorough, respectively.

Table 2. Comparison of methods of analysis for fat content of meat

No.	Method <sup>a</sup> Type	Refer- ence	Essential characteristic	Time required for a single analysis	Sample size, <sup>b</sup> g	No. of analyses <sup>c</sup>	Mean difference, <sup>d</sup> % fat	Range, <sup>e</sup> % fat	Std dev., % fat	Cost of equipment <sup>f</sup> and training <sup>g</sup>
1	AOAC official	129, 89	Soxhlet or Goldfisch extraction	7 hr	3-4	240	0	±0.7	0.25	\$75 per Soxhlet unit; \$650 per Goldfisch unit, 2 same as above
2	Modified Method No. 1	96	rapid 125° drying and rapid extraction	¾-1¼ hr	3-4	27	0	-0.8 to +0.2	0.6	same as above
3	Modified Babcock	89, 98	modified Paley bot- tle, with or without centrifugation	20-30 min	9	{ 240 18 }	-0.1 0.2	±1.0 -0.6 to +1.2	0.3 0.4	<\$50 (for 6 sam- ples), 1
4	Modified Babcock, HClO <sub>4</sub> -HOAc	99	same as above	30 min	9	19	0	-0.4 to +0.5	0.2	same as above
5	Modified Babcock, HCl-DMSO	102	same as above	15 min	9	98	-0.2	-1.3 to +1.6	0.6	same as above
6	Modified Babcock (Banco)	100	same as above, Banco reagents	35 min	9	32	0.2	-0.8 to +1.2	0.4	\$300, with centri- fuge, 2
7	Rendering (Hobart)	108	inverted hot plate and collector	15 min	56.6 (2 oz)	64	-2.3	-4.1 to -0.5	not available	\$195, 1
8	Rendering (Goss)	105	resistance heater and collector	15 min	15-25	not available	not available	±1.5	not available	<\$100, 1
9	Moisture balance (Ultra-X)	43	CCl <sub>4</sub> extraction after drying	35-55 min	2.5	23	-0.1	-2.4 to +1.8	1.0	\$1250, 1
10	Separatory funnel	88	extraction 5X with ether	1.5-2 hr	5	23	0.2	-1.1 to +1.5	0.7	<\$50, 1
11	Infrared absorption	124	spectrophotometry	<5 min	1	34	not available	not available	1.4	>\$3,000, 3
12	X-ray absorption (Anyi-Ray)	119	electronic unit	5 min	5902 (13 lb)	12	not available	±1.5	0.5	\$5,000/year rent- al, 1
13	Specific gravity (Honeywell)	112, 113	semiautomatic weight and volume	3 min	750	{ 69 56 }	0.1 -1.0	±2.4 ±3.4	1.2 1.5	\$10,000 or rental, 1
14	Specific gravity of extracts	114	heptane extracts, hydrometers	15 min	20-40	17	0.3	-0.9 to +0.8	0.5	>\$300, 2
15	Capacitance (Steinlite)	109	solvent extraction	20 min	50	20	0.2	-1.9 to +1.2	0.8	>\$500, 2
16	Capacitance (AMIF)	5	distillation extract is measured	30 min	15	19	0.3	-2.1 to +2.9	1.3	>\$500, 2
17	Extracted residue (Si-Mo-Fat)	123	micro vacuum oven	1-2 hr	10	46	-1.0	-2.6 to +0.7	0.8	>\$200, 2
18	Distillation extract residue (Cohen)	125	azeotropic distilla- tion-extraction, evaporation	¾-1 hr (for both moisture and fat)	10	{ 16 16 }	-0.7 -0.3 (cumene)	±0.9 ±0.5	0.4 0.5	\$75, 2

<sup>a-f</sup> Footnotes same as those of Table 1.

commonly available gravity convection oven is utilized. This oven is less expensive than a mechanical convection oven, required for Method 2 (AOAC Method 24.003(b)). Time for an analysis is the same as for Method 2 without loss of accuracy.

*Method 4*, the high temperature oven method, 15 min per determination, is rapid but it also requires the more expensive oven used in Method 2. Drying samples at 200°C may cause fat spattering and produce errors unless care is exercised.

*Method 5*, the vacuum oven procedure at 95–100°C, is accurate but requires 5 hr per analysis and the method is stated to be limited to lean meat samples because high fat samples will tend to spatter.

*Method 6*, the vacuum oven procedure at 70°C which provides results of fair accuracy with overnight drying, is not a rapid technique.

*Method 7*, drying of samples on a hot plate, yields results approaching, though not equalling, those by the official method. It may be preferred to the infrared radiation oven Methods 8 and 9 because a hot plate is commonly found in laboratories and more than one sample at a time can be put on a unit. A higher correction factor is required for the hot plate method than is necessary for Methods 8 and 9 and the reproducibility is intermediate.

*Method 10*, drying of samples by means of a moisture balance, gave good accuracy and better reproducibility than Methods 7–9. Multiple infrared lamps can be used for multiple sample analysis in conjunction with a single balance.

*Methods 11, 12, and 13*, azeotropic distillation, may be performed economically and have the added advantage that analysis requires only 15–30 min. Moisture content is measured directly as volume of water distilled from the sample and collected as condensate. This directness and relative simplicity has led to the widespread adoption of azeotropic distillation as a moisture method by various segments of the food industry. This, and the frequency with which reviews of moisture methods have concluded that azeotropic distillation has the most promise, led to the extensive evaluation of the technique in our laboratories (16, 38, 125). Another inherent advantage of azeotropic distillation is that the moisture receiver can be used indefinitely after an initial calibration.

### Fat Analysis

*Method 1*, AOAC official method 24.005(a), requires: (1) drying a meat sample 6 hr at 100–102°C or 1.5 hr at 125°C; (2) ether extraction for 4–16 hr, depending on condensation rate; (3) drying the extracted fat for 30 min at 100–102°C; (4) cooling the fat and weighing. The method is a direct, gravimetric determination of fat content. It is not a rapid method but reproducibility of results with the method is  $\pm 0.25\%$  fat.

*Method 2* is a modification of Method 1 in which a sample is dried in a 125°C gravity oven in 30 min as efficiently as in the preceding procedure. Fat can then be ether-extracted in 45 min or less, depending on type of meat product, without loss of accuracy. This procedure reduces analysis time by  $4\frac{1}{4}$  hr as compared with the official method.

*Methods 3, 4, 5, and 6*, the Babcock-type methods, have the advantage that an analysis can be made in 15–35 min. These are widely used, direct methods of analysis with a fair degree of accuracy. Concentrated sulfuric acid yields the most rapid digestion of meat samples but this occasionally leads to sample charring, which clouds the meniscus and affects accuracy. Use of dilute sulfuric acid minimizes charring but prolongs digestion time. Meat product samples containing spices cannot be determined accurately by these methods because the spice particles float at the interface between the 2 liquid phases. When sulfuric acid is replaced by mixed acetic and perchloric acids (Method 4), a correction is required for acetic acid dissolved in the fat column. In Method 5, sulfuric acid charring is eliminated by digesting the meat sample with hydrochloric acid. The constituents of the digested sample are then diluted with dimethylsulfoxide, which readily dissolves all digest constituents except fat. The problem of spice particles at the fat-aqueous interface remains and, in addition, there is a hazard to the analyst in working with dimethylsulfoxide. Method 6 utilizes a number of protein solubilizing agents in place of the customary acid and introduces less hazard to the analyst. Digestion of the sample is relatively rapid but the method is unduly complicated by the need for 3 separate water baths (55–60°C, 95–100°C, and boiling water bath).

*Methods 7 and 8*, which utilize fat-rendering devices, are relatively inaccurate. The devices do not provide a means for pressing the residue of rendered samples. Therefore, more fat content

remains unmeasured than if a press-cake were utilized. Use is limited to making crude approximations preliminary to laboratory analysis. Normal variation of meat type and processed meat product composition directly affects amount of fat drip.

*Method 9* utilizes an infrared radiation moisture balance to remove moisture from a sample. Fat is extracted from the dried residue with carbon tetrachloride. The extracted residue is then dried with infrared radiation so that fat is determined by difference. The principle used in the Ultra-X method was evaluated at our laboratory with a moisture balance. Extraction was found to be incomplete. The method is fairly rapid but reproducibility is marginal.

*Method 10*, extraction by means of a modified separatory funnel, shows that since samples are not dried prior to extraction, the moisture barrier to ether extraction can be overcome by the physical action of vigorously shaking the sample and ether in the funnel. However, the procedure requires up to 2 hr for an analysis.

*Method 11*, spectrophotometric measurement of infrared absorption, may require further development for use as a rapid method. The technique is capable of very sensitive measurement but it is also very sensitive to interfering substances. The cost of present instruments would tend to limit its acceptance and use.

*Method 12*, X-ray transmission analysis, in the preliminary reports in the literature appears to have considerable potential as a rapid, simple, and nondestructive method for fat determination. A thorough evaluation of the instrument on a number of meats and meat products would benefit the interests of meat analysts. The instrument cost is high, and this factor would limit its use to large processors.

*Method 13*, semiautomatic specific gravity measurement, was reported to be rapid, simple, and nondestructive of the sample. Cost of the instrument tends to limit its use to the very large processor. It requires programming for use with each type of fresh meat being measured. Foreign ingredients, such as spices and frozen moisture, introduce error. From the two reports in the literature concerning the method, standard deviation values of  $\pm 1.2$  and  $\pm 1.5\%$  fat indicate that accuracy of the method is marginal.

*Method 14*, specific gravity of the fat extracts by means of hydrometers in a 15 min procedure,

has the elements of a good, rapid method. A fire hazard exists when heptane is used as the extractant. The cost of a tempering bath which will maintain a temperature of  $\pm 0.01^\circ\text{C}$  and a series of hydrometers is not excessive.

*Methods 15 and 16*, capacitance measurement of fat extracts by means of *o*-dichlorobenzene or 1+7 mixture of 1-octanol and 2-octanol, is fairly rapid but the solvents have a very unpleasant odor and the sensitivity of the method to temperature variation can lead to error. Only fair accuracy was reported.

*Method 17*, vacuum oven drying and weighing of the residue that remains after distilling off moisture and extracting fat from the meat sample with *n*-butyl ether, was reported to require 2-2.5 hr.

*Method 18*, gravimetric determination of fat in the extract obtained by azeotropic distillation, involves evaporation of solvent from an aliquot of the extract. Distillation for 15-45 min is required to determine moisture and to obtain a representative fat extract, depending on type of product. Gravimetric determination of fat in the extract is obtained by solvent removal in 6-15 min, depending on type of solvent. Accuracy of the results was good. Total time for determining both moisture and fat is between 30 min and  $1\frac{1}{4}$  hr, depending on type of product. Evidence that azeotropic distillation prevails as the preferred method for combined moisture and fat determination is noted in the combined methods review section where 5 of the 7 procedures reviewed applied this technique.

### Conclusions

Until careful comparative studies have been made of many of the methods evaluated above, a precise order of value cannot be given to some methods which now appear equally satisfactory. Methods presently available that meet the needs of the meat industry for rapid analytical methods and newly developed methods which may merit this status upon further investigation are as follows:

#### Moisture Analysis

*Method 4*, high temperature mechanical convection oven-drying, is one of the most rapid for moisture determination. Its accuracy and precision are quite satisfactory. The method should be more thoroughly evaluated, since data on only 25 samples were reported.

*Method 7*, hot plate drying with a 200°C heating surface, is not as rapid as Method 4 but it has a good degree of accuracy and it can be a useful and economical method for moisture analysis.

*Methods 8, 9, and 10*, drying by infrared radiation, require 15–60 min for moisture analysis. Method 10 provides results in 30–45 min with better reproducibility than Methods 8 and 9.

*Methods 11, 12, and 13* yield moisture results by azeotropic distillation. Method 11 requires 2 hr for an analysis whereas Methods 12 and 13 require 15–30 min. The accuracy and precision of the results shown for Methods 11 and 12 are not as good as those of Method 13. This may be attributed to factors incidental to the method, such as cleanliness of glassware, or imperfections, such as scratches on the condenser inner surfaces.

#### **Fat Analysis**

*Method 2*, modified official method, is not one of the most rapid fat analysis methods, but good accuracy and a fair degree of precision can be obtained by drying samples 30 min in a 125°C gravity oven and extracting 45 min with ether.

*Methods 3, 4, 5, and 6* are all modified Babcock-type procedures. These methods have been very commonly used for rapid analysis and will continue to be used in the future. The procedures are fairly rapid and simple, and provide a good degree of accuracy. Within this group of 4, Method 5 with a 15 min analysis time apparently is the most rapid, although its reproducibility is the poorest.

*Method 11*, infrared absorption spectrophotometry, will remain an expensive instrumental method but it is potentially a very rapid method as a combined procedure for determining both moisture and fat if it is further developed to improve accuracy.

*Method 12*, X-ray transmission analysis, is also an expensive instrumental method and even more rapid than infrared absorption, since no special preparation of the sample is required for the measurement. The method is limited to measuring fat content but analyses are very rapid and simple.

*Method 13*, semiautomatic specific gravity measurement of meat, appears to be the most rapid method for assaying fat content, but from the published results, it is one of the least accurate of the available methods.

*Method 14*, specific gravity of heptane extracts

measured by hydrometers, is very advantageous for measuring fat only. It is fairly economical, rapid, and simple, and provides acceptable accuracy.

*Methods 15 and 16*, capacitance measurement of solvent extracts, are fairly rapid and simple methods for fat content determination but accuracy of results is poor.

*Methods 17 and 18* permit fat analysis in combined methods after determining moisture content by azeotropic distillation. The procedures are most useful when both quantities are to be determined; otherwise many of the above procedures are more rapid for determining fat alone. Of these two, Method 18 is more rapid, accurate, and precise.

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