RAPID DETERMINATION OF MOISTURE AND FAT IN MEATS BY MICROWAVE AND NUCLEAR MAGNETIC RESONANCE ANALYSIS

A Thesis

By

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ABSTRACT

Determination of moisture, fat, protein, and other components of meat is important for the evaluation of the quality of raw materials and finished products, the assessment of process control, and for ensuring regulatory compliance of meat products. Standard methods of analysis may be time- or labor-intensive, expensive, potentially harmful to the user or environment, or may require advanced training for operation of analytical equipment, but technology has allowed the introduction of more rapid methods that require less time, labor, skill, and cost. Microwave drying and nuclear magnetic resonance technologies for the determination of moisture and fat in meat products, respectively, have been incorporated into the CEM Smart Trac 5 System®, an instrument designed for the rapid analysis of moisture and fat in various food products.

The CEM Smart Trac 5 System®, approved as an AOAC Peer Verified Method, was used in a collaborative study for the rapid determination of moisture and fat in a variety of raw and processed meat products of beef, pork, chicken, and turkey origin. The objective of the study was to determine if the CEM Smart Trac 5 System® could analyze moisture and fat in meat products with the same accuracy and precision as standard methods of analysis as specified by the Association of Official Analytical Chemists (AOAC). Meat products were obtained from various commercial sources, homogenized, and distributed to 10 collaborative laboratories. Each collaborative laboratory evaluated the fat and moisture content of each meat product samples provided using the CEM Smart Trac 5 System®. Two standard methods of analysis, Forced Air

Drying Method (AOAC Official Method 950.46) and Soxhlet Extraction of Crude Fat (AOAC Official Method 960.39), were performed on each sample for comparison to the Smart Trac 5 System®. Ten replicates were analyzed by the reference methods to achieve an analytical variance of no more than $\pm$ 2%. Data collected from the reference methods for moisture (AOAC 950.46) and fat (AOAC 960.39) were used for the calibration of each of the CEM Smart Trac 5 Systems® and for comparison to the results produced by the Smart Trac 5 System® in each of the collaborative laboratories.

The results indicated that the CEM Smart Trac 5 System® compares favorably with the AOAC methods for moisture and fat determination. The CEM Smart Trac 5 System® would be suitable for the rapid determination of moisture and fat in a variety of commercially produced raw and processed meat and poultry products. Statistical analysis confirmed the within-laboratory repeatability qualities of AOAC methods and provided a baseline for comparing the between-laboratory reproducibility potential of the CEM Smart Trac 5 System®.

For all samples evaluated, the within-laboratory (repeatability) results and between-laboratory (reproducibility) results for moisture were acceptable. With the exception of low-fat ham, diluted low-fat ham, low-fat pork, diluted low-fat pork, diluted low-fat chicken, low-fat turkey, and diluted low-fat turkey, the within-laboratory (repeatability) results and between-laboratory (reproducibility) results for fat were acceptable. This study revealed that meat samples that have a very low concentration of fat (i.e. <3% fat) yielded relative standard deviation values (> 2%) that were not acceptable by AOAC standards.

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CHAPTER I

INTRODUCTION

Analyses for the determination of moisture, fat, protein, salt, and other analytes in meat and poultry products are important for evaluating raw material quality, ensuring process control and finished product composition, and meeting regulatory compliance in meat plant operations. Such analyses are also valuable for scientists in governmental agencies and academia conducting food related research.

Regulatory requirements are in place to protect the health and safety of consumers by ensuring that the food produced is safe for human consumption, complies with government regulations, and conforms to information provided on the label. Ensuring compliance with regulatory requirements and meeting customer specifications are two of the most important purposes for analyzing meat and poultry products.

In addition, analysis of raw materials and final products can assist the producer in more effectively manufacturing a product that fulfills product specifications and that is consistent in taste, quality, and appearance batch after batch. Analyses also assist the producer in controlling costs through use of appropriate ingredients, meeting the expectations of customers, minimizing waste, and avoiding unnecessary expense.

Standard methods of analysis are universally accepted and provide a known degree of accuracy and precision. However, some standard methods of analysis of meat and poultry products can be time- or energy-intensive, require highly trained personnel, involve the use of harmful or toxic chemicals, or generate wastes that have expensive

disposal fees. Other methods of analysis may not provide the same degree of accuracy, but may have greater ease of use or require less time or less training of employees. Laboratories in plant operations and other entities may select a method of analysis for use in their facility based upon the following criteria: the types of products manufactured, specific component(s) in the product, product specifications, budget, training of personnel, laboratory capacity, number of samples to be analyzed, time requirements, degree of accuracy, reliability of the equipment or method, or other important factors.

Rapid analytical methods with the potential to produce results with the same precision and accuracy as standard methods would be of great benefit to researchers, plant operations, governmental agencies, academia, and other entities. Of particular interest for this study was comparison of rapid analytical methods for the determination of moisture and fat in raw and processed meat and poultry products to standard methods of analysis. The intent was to verify the accuracy and precision of the CEM Smart Trac 5 System® by comparing same sample analytical results to those of accepted standard methods, such as the Association of Official Analytical Chemists (AOAC) Official Methods. To accomplish this, an Association of Official Analytical Chemists (AOAC) collaborative study involving 10 laboratories was conducted to analyze various raw and processed meat products commonly produced and distributed by the meat industry.

We hypothesized that the combined use of microwave drying technology and nuclear magnetic resonance (NMR) technology incorporated into the CEM Smart Trac 5 System® would be effective in rapidly determining the moisture and fat content of meat

products, respectively, with comparable accuracy and precision to the standard methods of analysis (AOAC Official Methods 950.46 for moisture and 960.39 for fat). Previously, CEM Corporation (Matthews, NC) obtained AOAC Peer-Verified Method (PVM) approval for the CEM Smart Trac 5 System® that was used in this study, indicating the instrument's potential for use as an acceptable method for rapid analysis of moisture and fat in meat and poultry products.

The objective of this study was to compare the moisture and fat content of a variety of raw and processed meat products using standard and rapid methods of analysis as part of a 10 laboratory collaborative study for the fulfillment of AOAC requirements to become an official method of analysis. This study evaluated the accuracy and precision of the CEM Smart Trac 5 System® for rapid determination of moisture and fat in meat products in comparison to the standard methods of analysis (AOAC official methods 950.46 and 960.39, respectively). Knowledge from this study may provide food processors, governmental and regulatory laboratories, and researchers in academia a tool that will improve testing efficiency, save time, and reduce the cost of labor without compromising the accuracy or precision of the results.

CHAPTER II

LITERATURE REVIEW

Analytical methods for proximate (fat, water, protein, ash) composition are essential in the meat industry for good product control. Consistency in the formulation and blending stages of meat for further processed products is of great importance for controlling costs, maintaining process control, and ensuring compliance with governmental regulations (Sebranek 1998). Meat and further processed meat products can go from raw material to finished product within a few hours depending on plant operation capabilities, thus it is vital to have rapid analytical methods that provide quick and efficient results throughout the production process.

In the evaluation of meat and meat products, standard methods of analysis have been universally accepted and used in research, analytical laboratories, and the food industry, but the standard methods for moisture and fat analysis can be time-consuming, costly, inconvenient, and require trained personnel, specialized laboratory equipment, and harmful chemicals. Such methods are not efficient or practical in fast-paced plant operations, so rapid methods of analysis with similar precision and repeatability are essential.

The use of rapid methods for proximate composition would have obvious advantages, including time and labor savings, collection of a greater number of sample measurements, better control over multi-step processes, more precise formulations, and more frequent checks on processes (Sebranek 1998). Two important factors that should be considered in obtaining usable analytical information, regardless of the analytical

method to be used, are sample preparation and method performance. Samples should be collected and prepared in a manner to provide a homogenous, uniform, and representative portion of the larger lot. This becomes increasingly important as sample size becomes limited to 1 g or 2 g for some methods of analysis. The second factor, method performance, should be evaluated objectively before selecting a method of analysis and then continuously monitored for performance (Sebranek 1998).

Determination of Moisture Content

Lean muscle tissue of beef, pork, lamb, and chicken is comprised of approximately 68-74% moisture, 19-23% protein, 4-11% lipid, and 1.0-1.6% ash (Foegeding and others 1996). Water is the most abundant chemical component of meat, and the predominant component of lean muscle, but not fatty tissue. The total moisture content of a meat cut varies inversely with the amount of fat in the tissue, so the leaner the meat cut, the higher the moisture content (Romans and others 2001).

Water is a common ingredient added to further processed meat products to help facilitate chopping and mixing, to control meat batter temperature (when added as ice), to hydrate binders and extenders within a product formulation, to achieve a specific texture, to improve product juiciness, and to assist salt in solubilizing meat proteins for batter stability (Romans and others 2001).

Multiple factors can affect the moisture content of a meat product. These factors include fat percentage (inverse relationship), addition of water and non-meat ingredients, and degree and type of processing and cooking. The moisture content of a meat or food

product is important because of the properties of water, its interaction with other components in the product, and its contribution to the chemical, biological, and physical properties of foods (Cornejo and Chinachoti 2003).

Several analytical methods, including drying methods and spectroscopic methods, have been developed and used in the food industry to determine the moisture content of meat and meat products. Drying methods offer accurate moisture values, are relatively easy and inexpensive to perform, permit the simultaneous analyses of large numbers of samples, and do not require calibration of equipment. Although drying methods offer many advantages, the greatest disadvantage to drying is the length of time required to perform the analysis in comparison to spectroscopic methods. The drying process is often lengthy (generally several hours), since the degree of heating for moisture determination is generally performed at 95-105°C to prevent decomposition of lipids, proteins, or other components within the sample that could release compounds and falsify moisture results (Honikel 2009).

In comparison to drying, spectroscopic methods from moisture determination are more rapid and often enable the simultaneous measurement of other analytes (such as fat or protein) within the same sample. However, spectroscopic methods often involve more expensive and specialized equipment and are dependant on careful and proper calibration of each instrument with samples similar to the unknowns to be measured. Additionally, if the sample material being analyzed falls outside of the range of the samples used to calibrate the equipment, then re-calibration of the equipment based on new parameters may be necessary (Honikel 2009).

The following paragraphs will discuss specific methods for determining moisture content in meat and meat products, including drying (Forced Air Drying, Rapid Microwave Drying, and Drying Under Vacuum) and spectroscopic methods (Near Infrared Transmittance and Guided Microwave Spectrometry).

Forced Air Drying (AOAC Method 950.46(B))

The AOAC standard reference method 950.46(B), Forced Air Drying Method, is a gravimetric method that uses an air oven (mechanical convection is preferred) to dry a meat sample for the determination of moisture content. In this method, a small amount (ca 5-6 g) of homogenized meat sample is evenly distributed in a small aluminum dish ($\geq$ 50 mm diameter and $\leq$ 40 mm deep), then dried in an air oven (16-18 hr at 100-102°C with lids removed) to produce ca 2 g of dried material. When drying is complete, the dishes are covered (with their corresponding lid), cooled to a constant weight in a desiccator, and weighed (AOAC 2006a). The weight loss is reported as the moisture content (as a percentage of total sample weight) and is calculated by subtracting the weight of the wet meat sample (includes weight of dish and lid) and the weight of the dried meat sample (includes weight of dish and lid), dividing the difference by the weight of the wet meat sample (includes weight of dish and lid), and then multiplying the quotient by 100.

$$\text{Moisture} = \frac{\text{Wet Meat Wt.} - \text{Dried Meat Wt.}}{\text{Wet Meat Wt.}} \times 100$$

The two greatest advantages to using this method include its low cost and the accurate and precise results that it provides for a wide variety of products. In addition,

multiple 5-6 g homogenized meat samples can be dried simultaneously in an oven. The greatest disadvantage to using this method is the amount of time (16-18 hr) required to dry the samples, which is inconvenient for fast-paced operations and may hinder real-time production and product quality decisions. In addition, the dried sample obtained from this method cannot be used in subsequent fat determination. The temperature and time at which a sample is dried are very important when considering drying methods for the determination of moisture in a sample. High temperatures and long drying times could result in oxidation of fat in the sample, which could lead to erroneous moisture results.

If a mechanical convection oven or gravity oven with single shelf, set at 125°C, is available for use, then the drying time may be reduced from 16-18 hr to 2-4 hr (depending on the product), as indicated in AOAC 950.46(B)(b). Procedures for sample preparation, desiccation, and moisture calculation are the same as described above and the resultant dried sample cannot be used for subsequent fat determination (AOAC 2006a).

Rapid Microwave Drying Method (AOAC 985.14)

The AOAC standard reference method 985.14, Rapid Microwave Drying Method, is a gravimetric method that uses microwave energy to rapidly dry a homogenized meat sample. The CEM Smart Trac 5 System®, which was the instrument used in this collaborative study for the fulfillment of AOAC requirements to become an official method, uses focused microwave drying technology for moisture determination.

The instrument is comprised of a computerized microwave moisture analyzer, an automatic tare electronic balance, a microwave drying system, and a microprocessor digital computer control (AOAC 2006c).

To perform the analysis, two glass fiber pads (9.8 x 10.2 cm, CEM Corp. or equivalent) are tared on the instrument's internal electronic balance. A homogenized meat sample (ca 4 g) is spread evenly across one glass fiber pad using a Teflon coated spatula. The second glass fiber pad is then placed over the sample (to produce a 'sandwiched' appearance). The prepared sample 'sandwich' is transferred to the instrument's drying chamber and dried per manufacturer's instructions. The microwave moisture analyzer automatically calculates moisture content based on weight loss during drying (determined by the electronic balance readings before and after drying) and displays the result on the equipment's digital readout panel (AOAC 2006c).

The four greatest advantages to using this method include the rapid determination of moisture content (3-5 min), the ease of use of the instrument (does not require highly trained or skilled personnel), comparable degree of precision and accuracy to traditional drying methods, and the resultant dried sample can be used for subsequent fat analysis in the instrument's NMR chamber. The greatest potential disadvantage to using this method is that it is limited to analyzing one sample at a time, so the equipment may not have the capacity to perform moisture and fat analysis in settings that require numerous product sample analysis (i.e. > 100 samples per day). A second potential disadvantage to using the CEM Smart Trac 5 System® is the initial cost required to purchase the

instrumentation. In addition, consumable materials, such as the glass fiber pads, would also need to be replaced.

Drying Under Vacuum at 95-100°C (AOAC 950.46(A))

The AOAC standard reference method 950.46(A), Drying Under Vacuum Method, is a gravimetric method that uses a vacuum oven to dry a homogenized meat sample for the determination of moisture content. In this method, ca 5-6 g homogenized meat sample is evenly distributed in a small aluminum dish ($\geq$ 50 mm diameter and $\leq$ 40 mm deep), then dried under pressure (<100 mm Hg at 95-100°C with lids removed, ca 5 hr) to produce ca 2 g of dried material. When drying is complete, the dishes are covered (with their corresponding lid), cooled in a desiccator to a constant weight, and weighed. The loss of weight is reported as moisture content (as a percentage of total sample weight) and is calculated by subtracting the weight of the wet meat sample (includes weight of dish and lid) and the weight of the dried meat sample (includes weight of dish and lid), dividing the difference by the weight of the wet meat sample (includes weight of dish and lid), and then multiplying the quotient by 100.

$$\text{Moisture} = \frac{\text{Wet Meat Wt.} - \text{Dried Meat Wt.}}{\text{Wet Meat Wt.}} \times 100$$

The greatest advantage to using this method is the shorter drying time required in comparison to the forced air drying method. By drying under reduced pressure, more complete removal of water and volatiles can be obtained without decomposition (Bradley 2003). The three greatest disadvantages to using this method are that it is not suitable for high fat products (i.e. pork sausage), the dried sample obtained cannot be

used for subsequent fat determination (AOAC 2006a), and the number of samples that can be analyzed at a time or throughout the day may be limited due to the size and capacity of the vacuum chamber.

Near Infrared Transmittance (FOSS FoodScan™, AOAC 2007.04)

Near Infrared (NIR) Transmittance technology offers a newer and more sophisticated spectroscopic method for the analysis of moisture content in meat samples. NIR measurements are based on the principle that almost all organic functional groups (i.e. ketones, alcohols, etc.) have a specific absorption band in the near infrared region and that the infrared absorption spectra is unique for many different components in meat (Sabrenek 1998).

One example of instrumentation utilizing NIR Transmittance technology is the FOSS FoodScan™ Meat Analyzer (Eden Prairie, MN). This instrument is an NIR Spectrophotometer with artificial neural network (ANN). The FOSS FoodScan™ Meat Analyzer recently received AOAC approval as an official method for the determination of moisture, fat, and protein content in meat and meat products (AOAC Method 2007.04). Parameters for meat products evaluated included fresh meat, beef, pork, poultry, emulsions, and finished products in the constituent ranges of 1-43% fat, 27-74% moisture, and 14-25% protein (Anderson 2007).

To perform this method using the FOSS FoodScan™ Meat Analyzer instrument, ca 180 g of homogenized meat sample is evenly distributed in the instrument's round sample dish and loaded into the instrument's sample chamber. The operator selects the

appropriate meat product profile from the instrument's menu screen or personal computer (depending on instrument version), presses the 'START' button to initiate the scan, and waits for analysis to be completed and the results calculated and reported for moisture, fat, and protein percent (Anderson 2007).

The FOSS FoodScan™ instrument contains a tungsten-halogen lamp and optical fibers that guide emitted light into an internal moving-grating monochromater (which provides monochromatic light in the spectral region between 850 and 1050 nm) and a collimator lens positioned over the sample cup in the sample chamber. Light that is not absorbed by the sample in the sample chamber strikes a detector that measures the amount of unabsorbed light. The results are sent to the instrument's digital signal processor, which calculates the final results and communicates them with the personal computer. The results are displayed as % moisture, % fat, and % protein on the digital screen or personal computer (Anderson 2007).

The ANN calibration technique used for this system eliminates the need for development and maintenance of separate calibrations for specific sample types. The ANN calibration generates a single, global, multi-product, full-range calibration for each constituent based on a database containing calibration sample data represented by spectra and chemical analysis results (Anderson 2007).

The FOSS FoodScan™ instrument offers multiple advantages, including rapid results (ca 50 sec) for multiple constituents, is well-suited to high-capacity production environments, is easy to use and not dependent on highly skilled personnel to operate the instrument, is cost effective (no consumable materials and low operation costs), and

demonstrates similar repeatability and reproducibility compared with chemical analysis reference methods. In addition, an on-line version of this instrument is available, which may be used for continuous, flow-by measurements of material in a pipe or tube system, such as in meat grinders and blenders.

One disadvantage to using this method is that it is limited to analyzing one sample at a time, but fortunately each sample can be analyzed in a very short amount of time. A second potential disadvantage to using the FOSS FoodScan™ instrument is the initial cost required to purchase the instrumentation. Additional disadvantages may exist, such as affected instrumentation performance due to addition of non-meat ingredients (such as spices, colorants, or other ingredients that affect meat batter color) or the presence of ice crystals in the sample. Although not discussed in the literature, ice crystals, non-meat ingredients, or other factors may potentially interfere with the instrument's infrared absorption performance. In such cases, it may be necessary to calibrate the equipment specific to the type of product being analyzed in order to obtain the most reliable results.

NIR transmission instruments are believed to perform better than NIR reflectance instruments because a greater amount of sample may be scanned and a highly sensitive detector can be used (Sebranek 1998). In many cases, sample scans with infrared instruments must be calibrated against known values for sample composition with reference methods. In addition, a large number of samples similar to those to be measured need to be included for calibration (Sebranek 1998).

Guided Microwave Spectrometry (GMS)

Guided Microwave Spectrometry (GMS) is a method that is based on microwave energy absorption. It utilizes a broad range of frequencies (up to 750 frequencies) in the microwave spectrum to measure molecular electromagnetic properties (such as the dielectric constant of water, conductivity, and molecular relaxation time) and relate those properties to composition (such as moisture, fat, and protein) through the use of a transmitter and receiver within the GMS instrument (Sebranek 1998).

The E-Scan In-line Food Analyzer™ (Guided Microwave Spectrometer) by Thermo Fisher Scientific, Inc. is an example of an instrument that uses GMS technology for the determination of moisture, fat, and protein in ground meat. The instrument includes a GMS chamber, electronics control module, and PC-based software for developing calibrations and configuring the analyzer (ThermoElectron Corp. 2003).

The instrument is installed within processing equipment and can provide rapid, real-time data feedback from analysis of samples flowing through pipe-type processes. Commercial applications for the meat industry have been for meat grinders where product composition can be measured as the meat exits the grinder (Sebranek 1998). Calibration of the instrument prior to sample measurement is needed, and the results are validated with daily checks against the results from an off-line measurement tool. GMS has been used to measure moisture in milled corn, dog food, candy-coated peanuts, dough, peppercorns, and other foods, but measurement of moisture, fat, and protein in ground meat is a newer application (Food Eng Mag 2004).

Microwave energy is sensitive to the concentration of polar, semi-polar, and non-polar molecules such as water, protein, fat, oil, and ion/salt concentration in a process or sample (ThermoElectron Corp. 2003). When electromagnetic energy of various frequencies in the microwave spectrum is transmitted through sample material in a GMS instrument, the polar molecules (such as water) within the sample rotate and align with the electromagnetic field. The movement of the molecules cause the microwave signal to be attenuated or weakened, and the velocity of the wave decreases as it passes through the sample (Food Eng Mag 2004).

The attenuation of the microwave signal through the sample and the reduction in velocity of the energy (which changes the wavelength) results in what is known as the cut-off region, which is one of two characteristic features of the GMS spectrum. The cut-off region is the characteristic high slope 'rise' in the spectrum and is determined by the dielectric constant of the sample. The cut-off region is generally sensitive to moisture (ThermoElectron Corp. 2003).

The second characteristic feature of the GMS spectrum is the pass-band region, which is generally horizontal and shifts in the vertical direction with small changes in the slope. The amplitude (intensity) of the pass-band region is determined by the conductivity of the sample and the amount of energy that is lost in the transmission of electromagnetic energy from the GMS instrument transmitter to the receiver antennae of the GMS chamber (ThermoElectron Corp., 2003). The pass-band region is generally sensitive to constituents other than water.

The GMS system is 'trained' to interpret readings it receives from the product using multiple regression analysis to compare the plotted cut-off and pass-band regions with results from off-line analysis of the same sample (Food Eng Mag 2004). Using a well-defined calibration, the changes in the pass-band and cut-off regions are correlated to the amount of change in the concentration of the component of interest (i.e. moisture, fat) in the sample material being analyzed (ThermoElectron Corp. 2003). The slope and the intercept of the cut-off and pass-band regions are plotted to derive a rapid response analysis. The cut-off band shifts to the right on the X-axis (a measure of frequency) as moisture content decreases. The strength of the signal in the pass-band region indicates other constituents in the sample, each with its own electromagnetic signature (Food Eng Mag 2004).

The greatest advantage offered by the E-Scan In-Line Food Analyzer equipment is that it can be incorporated into on-line processing equipment so that rapid, real-time results can be achieved. This method can also analyze multiple constituents (moisture, fat, and protein) simultaneously.

The greatest disadvantage to this instrument is that it is limited to raw ground meat, so it would not be an acceptable method to use to evaluate constituents in raw meat blends containing non-meat ingredients or in cooked meat products. In addition, this method is not an AOAC-approved method for the analysis of moisture, fat, protein, or other constituents, which may be a disadvantage for food manufacturers seeking AOAC-approved methods for their operation's processes and products.

Determination of Fat Content

One important component of meat and meat products is fat. Fat influences palatability (i.e. flavor, aroma, tenderness) and keeping quality (i.e. development of off-flavors due to oxidation) of meat and meat products and serves many important functions in human nutrition (i.e. carrier of fat soluble vitamins, source of energy, insulation) (Romans and others 2001). Fat is also the basis for many formulation decisions in further processed meat and meat products.

The amount of fat in meat is variable and inversely related to the amount of moisture. Fat content of various grades and cuts of meat and poultry can vary widely. Raw poultry without skin and lean cuts of red meat (beef, pork, lamb) are generally low in fat (<10% fat), whereas poultry with skin and red meat cuts that have greater amounts of marbling and trim can be much higher in fat (<20%). Further processed meat and poultry products can also vary greatly in fat percentage, depending on government regulations, labeling and nutritional goals, product formulation, and degree and type of processing and cooking.

The physical and chemical properties of fat are very different from the other components of meat, so different measurement techniques are needed to analyze or quantify fat content. Many procedures for the determination of fat are available, but only a limited number of methods are approved as an 'official method'. Some techniques involve the use of organic solvents to extract and gravimetrically measure fat, whereas other methods involve sophisticated instrumentation to determine fat content. Considerations for choosing a method include: cost, equipment, waste and disposal,

speed of testing, accuracy and reproducibility, and level of skill required to perform the test.

The following paragraphs will discuss specific methods for determining fat content in meat and meat products, including extraction methods (Soxhlet Extraction of Crude Fat, Extraction of Fat with Chloroform and Methanol (Folch), Rapid Specific Gravity Method, Rapid Microwave-Solvent Extraction Method, Supercritical Fluid Extraction) and non-extraction methods (Low-Resolution Nuclear Magnetic Resonance and Near Infrared Transmittance).

Soxhlet Extraction of Crude Fat (AOAC Official Method 960.39)

The AOAC standard reference method 960.39, Soxhlet Extraction of Crude Fat Method, is a solvent-based extraction method for the determination of fat in meat and meat products that is often considered the standard method by which other methods are evaluated (Min and Boff 2003). This method involves the use of petroleum ether (a flammable, non-polar solvent with a low boiling point (35°C/95°F) to extract fat from a dried, homogenized meat sample. Although petroleum ether is not as good of a solvent as diethyl ether, petroleum ether is more often used because it is selective for more hydrophobic lipids, and less expensive, less hygroscopic, and less flammable than diethyl ether (Min and Boff 2003).

Prior to Soxhlet extraction, a homogenized meat sample (ca 3-4 g) is weighed into a small, disposable aluminum dish, mixed with a small amount of laboratory-grade sand (the sand increases surface area, allowing for moisture escape and prevention of fat

entrapment), and dried in an air oven (100-102°C for 16-18 hr). The dried sample is cooled in a desiccator and prepared for subsequent Soxhlet extraction.

The Soxhlet extraction system involves five important components, including a heating mantel, a flat-bottom flask, a Soxhlet, a condenser, and rubber tubing connected to a functioning water faucet. The extraction system operates by heating the petroleum ether to create a vapor, then cooling the vapor back to liquid form via the condensers. The condensed liquid ether drips into the Soxhlet and completely surrounds the dried meat sample, providing a soaking effect, then 'fluxes' back into the flask (ca 40-50 mL of ether at a time), taking with it fat from the meat sample. This 'fill and flux' process continues for 4-6 hr to completely extract the fat from the meat sample. Once the extraction process is complete, the flasks are disconnected from the condensers, the petroleum ether evaporated, the flasks weighed, and the fat content of the meat sample calculated.

This method has several advantages, including precision, repeatability, recognition as a standard reference method, and the ability to analyze multiple samples simultaneously. However, there are multiple disadvantages to using this method, including the use of a flammable solvent, the length of time required to prepare and extract the sample (drying time + extraction time), the need for a trained and skilled analyst, and the need for special accommodations (i.e. fume hood, running water). Despite extensive efforts to develop analytical methods for fat using new technology, the extraction of fat followed by measurement is still the most successful general approach (Sebranek 1998).

Folch or Modified Folch (Extraction With Chloroform/Methanol)

The Folch Method was originally developed as a method for the preparation and purification of brain lipids by the biochemist, Jordi Folch, but has been applied to the extraction of lipids in other biological tissues. Folch described his extraction method in his article, "A Simple Method For the Isolation and Purification of Total Lipides From Animal Tissues" (Folch and others 1957).

The extraction method described by Folch is a solvent-based extraction method that uses chloroform:methanol (2:1, v/v) and water (or adequate salt solution) to extract lipid components from a meat sample. Unlike extraction methods that use diethyl ether or chloromethane, the Folch method also extracts phospholipids from a meat sample. When a meat sample is homogenized with the solvent mixture, the mixture separates into two phases that can be divided and further analyzed. The lower phase (chloroform) contains the lipid material while the upper phase (methanol and aqueous) contains the non-lipid material and water (Castera 1995). For lipid quantification, the upper phase (methanol and aqueous) is siphoned off, allowing the lower phase to be evaporated and lipid content gravimetrically determined.

This procedure is performed by homogenizing a meat sample with a chloroform-methanol (2:1, v/v) mixture. The chloroform-methanol mixture is added in an amount that is 20 times the volume of the tissue sample (i.e. 20 mL of solvent mixture is needed for a 1 g meat sample). In his publication, Folch indicated performing homogenization in a Potter-Elvehjem type homogenizer for samples that are $\leq$ 1 g or in an adequate blender for larger samples ($\geq$ 1 g) (Folch and others 1957).

After homogenization, the homogenate is filtered (through a funnel with folded filter paper) or centrifuged to recover the liquid phase. The crude extract is washed with 0.2 volume (4 mL for 20 mL) of water or NaCl solution (0.9%) and the mixture is allowed to separate into two phases (by centrifugation or prolonged standing). Upon separation, the upper phase is removed by siphoning and the inside wall of the tube is rinsed three times with methanol/water (1/1). Care should be taken to avoid mixing of the upper and lower phases during rinsing. The upper phase that forms after rinsing is also siphoned off. In this washing procedure, the proportions of chloroform, methanol, and water (including water from the meat sample) are 8:4:3, which is critical and must be kept constant (Folch and others 1957).

The lower phase (which contains the lipid constituents) is then combined with the rest of the rinsing fluid and made into one phase by the addition of methanol. The lower phase is then evaporated (in a rotary evaporator or under nitrogen stream) until all detectable traces of solvent are gone. Final traces of solvent and water are then removed by flushing with nitrogen then vacuum suctioning to complete dryness (Iverson and others 2001). Lipid content is then determined gravimetrically**.**

Several researchers have used various extraction procedures and have found that the chloroform/methanol procedure worked best for extracting all classes of lipids (King and Min 1998). The combination of polar and non-polar solvents (2:1 chloroform:methanol) to extract fat has made this method very efficient. The Folch method, when compared to other solvent extraction methods, may yield higher results for fat due to more complete extraction (Mann and others 1991). Polar solvents

(chloroform) disrupt hydrogen bonding between lipids and proteins and lipids and carbohydrates, so when a non-polar solvent (methanol) is introduced after a polar solvent has been used, the non-polar solvent can access the lipid components and fully extract them (Mann and others 1991).

The two greatest advantages to this method are the complete extraction of all classes of lipids from a tissue sample and the opportunity to further analyze the extracted lipids. This method of fat extraction is very efficient and would be especially useful if further analysis of the lipids is needed, as very few other methods offer the complete extraction of all classes of lipids.

Despite the greatest advantages to this method, multiple disadvantages exist for this method, including the use of two organic solvents (potentially harmful to health, costs and dangers associated to storing, handling, and disposing of them), the greater degree of difficulty in performing the fat extraction, the need for more highly trained personnel, the sensitive nature of the method (must maintain specific solvent proportions, avoid mixing the two phases when rinsing), and the amount of time required to perform the procedure. If further analysis of the lipids is not needed, other methods may be more ideal and user-friendly for the general quantification of fat in a meat-production environment. In addition, this method has not received AOAC approval for the analysis of fat in meat and meat products, which may be an additional disadvantage for analytical laboratories or food manufacturers seeking to use AOAC-approved methods for their product analysis.

Rapid Specific Gravity Method (Foss-let, AOAC 976.21)

The AOAC standard reference method 976.21, Rapid Specific Gravity Method, is a solvent-based extraction method for the determination of fat content in meat and poultry products. This method is known as the Foss-let procedure and is very effective in determining fat content (Sebranek 1998). This procedure involves the Foss-let Fat Analyzer system, a solvent (tetrachloroethylene) for the extraction of fat, an anhydrous salt (anhydrous calcium sulfate) for the absorption of moisture droplets from the sample, and a specific gravity read-out unit for determining fat content of the sample.

To perform the Foss-let procedure, a test sample is weighed into a tared stainless steel Foss-let cup. For meat products containing $\leq$ 60% fat, a 45.0 g test sample is used. For meat products containing $\geq$ 60% fat, a 22.5 g test sample is used. Upon weighing the meat sample into the cup, 80 g of anhydrous calcium sulfate ($CaS0_4$) and 120 mL of tetrachloroethylene are added to the cup. The cup is covered and placed in the mechanical orbital shaker, which facilitates the rapid extraction of fat through strong mechanical action. After 2 min. in the orbital shaker, the cup containing the sample is removed and immersed in an ice-water bath to cool the contents to ca 40°C (from 47-52°C out of the orbital shaker).

The contents of the cup are poured into an assembled filter and filtered under pressure until 10 mL of extract is retained in the measuring chamber. The measuring chamber, which is thermostatically maintained at 37°C, contains a miniature hydrometer that measures the specific gravity of the extract (Pettinati and Swift 1976). Three to five specific gravity readings are obtained and averaged, then converted to percent fat by

means of the conversion chart provided with the equipment. For high-fat products in which a 22.5 g portion was used, the chart percent fat should be multiplied by 2 (AOAC 2006b). A reference standard oil (specific gravity at 23°C = 0.915) is provided for use as a periodic check of the potentiometer calibration.

The three greatest advantages to using this method include its recognition as an AOAC standard method, results can be obtained rather quickly (7-10 min), and it is efficient in extracting fat. Two of the greatest disadvantages include the use, handling, and disposal of the chemical solvent and the need for more skilled personnel to perform the analysis.

Rapid Microwave-Solvent Extraction Method (CEM Automated System, AOAC 985.15)

The AOAC standard reference method 985.15, Rapid Microwave-Solvent Extraction Method, is a solvent-based extraction method that uses methylene chloride and the CEM Automated Solvent Extraction System (CEM Corp., Mathews, NC). The CEM apparatus is an enclosed, self-contained, thermostatically controlled fat extraction and solvent recovery system with a 0.5 mg fat sensitivity and 0-100% fat measurement range (AOAC 2006d). The CEM apparatus also includes a microwave moisture analyzer, which is used to dry the test sample in preparation for solvent extraction. This method is less time-intensive compared to other traditional extraction methods.

To perform the analysis, three glass fiber pads [two rectangular (9.8 x 10.2 cm) and one round (11 cm), CEM Corp] are tared on the internal electronic balance of the apparatus. A homogenized meat sample (ca 4 g) is spread evenly across one rectangular

glass fiber pad and covered with the second rectangular glass fiber pad (to produce a 'sandwiched' appearance). The 'sandwiched' sample preparation is weighed on the internal balance, dried for ca 3-5 min in the apparatus's microwave drying chamber, then transferred to the automated solvent extraction chamber. In the extraction chamber, the dried meat sample and rectangular glass pads are blended with a sufficient amount of methylene chloride to extract fat. During the extraction cycle, which takes ca 1-2 min, the extracted fat is collected on the round glass fiber pad. Upon completion of the extraction cycle, the round glass fiber pad containing the fat extract is transferred to the balance pan in the microwave moisture analyzer chamber and dried (ca 30 s) to remove residual solvent or moisture. The apparatus' microprocessor converts the weight loss due to solvent extraction to % fat and displays the result on the digital read-out panel.

An adjustment factor is needed for certain product classes in order to produce more accurate results with this method. An adjustment factor of 0.40 is needed for fresh meats, pre-blends, emulsions, and cured cooked meats, whereas an adjustment factor of 0.80 is needed for cooked sausages (AOAC 2006b).

This method efficiently extracts triglycerides, but fails to fully extract phospholipids. This was realized when fat extraction with the CEM automated system was compared to the modified Folch method. During a study that compared the CEM automated system to the modified Folch method, it was observed that the CEM automated system consistently yielded lower values for fat compared to the modified Folch method. The researchers explained that the Folch method uses a combination of polar and non-polar solvents (2:1 chloroform: methanol) to extract fat. Polar solvents

disrupt hydrogen bonding between lipids and proteins and lipids and carbohydrates, so when a non-polar solvent is introduced after a polar solvent has been used, the non-polar solvent can access the lipid components and fully extract them, which is demonstrated in the Folch method (Mann and others 1991). The Folch method also exposes the test sample to solvent for 24 hr, so a longer exposure to solvents may improve extraction results.

In comparison, the CEM automated system uses a slightly polar solvent (methylene chloride), which is efficient in extracting triglycerides, but does not completely extract phospholipids. In addition, the CEM automated system exposes the test sample to the solvent for a short amount of time (1-2 min), which may not allow sufficient time for the solvent to disrupt the membrane material and extract the phospholipids completely (Mann and others 1991).

Although the CEM automated system has limitations, it is able to efficiently extract triglycerides, which are the primary lipids of interest for evaluating raw material quality, process control, finished product composition, and regulatory compliance in meat plant operations. The CEM automated system offers four main advantages: (1) it is an AOAC approved method of analysis, (2) it provides reliable results in a short amount of time, (3) it uses less solvent than other extraction methods, so less waste is generated, and (4) it does not require a highly skilled technician to perform the procedure. Three primary disadvantages to using this method include: (1) it requires the use of a potentially harmful chemical, (2) it is limited to analyzing one constituent at a time, and (3) the sample cannot be retained for other analytical procedures.

Super Critical Fluid Extraction (AOAC Peer Verified Method – PVM 3:2000)

More recent developments have led to extraction methods, such as supercritical fluid extraction (SFE), that do not require the use of organic solvents. SFE has been published by AOAC International as a Peer Verified Method (PVM 3:2000) for the determination of crude fat in meat and meat products. This method involves the use of the TFE2000 fat determinator SFE system (LECO Corp., St. Joseph, MI), carbon dioxide (CO_2), and granular diatomaceous earth (LECO-dry or equivalent). In addition, a household microwave oven (1000 W) is also needed. The peer verified study involved the analysis of raw, cooked, and processed meat products containing 5-28% crude fat (Chandrasekar 2001).

SFE uses a supercritical fluid for the selective extraction of a constituent from a test sample. A supercritical fluid is a substance that has exceeded its critical point for temperature and pressure, giving rise to liquid-like densities and gas-like properties. Carbon dioxide (an inert, non-toxic, inexpensive fluid) is commonly used in SFE, including the extraction of fat from meat samples, because of the high solubility of lipids in this medium (King 2001).

Through a specific pressure-temperature combination, CO_2 is transformed into a substance with supercritical fluid properties. The liquid-like density of the supercritical CO_2 enhances fat extraction from the sample matrix and the gas-like properties promote separation of the solubilized fat from the solvent fluid after extraction (Min and Boff 2003). When a fluid is close to its critical point, these properties can be altered or 'fine-

tuned', which can promote selectivity of specific components because small changes in pressure or temperature can result in large changes in density (Min and Boff 2003).

To perform fat extraction using the TFE2000 instrument, ca 1.0-1.5 g of ground, homogenized meat sample is weighed and thoroughly mixed with 2.2 g diatomaceous earth (to absorb moisture and increase surface area of the sample). However, a smaller sample (≤1.0 g) should be used if the meat sample contains >70% moisture. Higher sample moisture content was noted by Chandrasekar and others to impede lipid extraction. In their study, when total moisture was >1.0 g in a test sample, incomplete extraction and reduced extraction efficiency occurred (Chandrasekar 2001).

Once the meat sample has been prepared, it is transferred to a high-pressure extraction thimble and placed in the SFE instrument according to the manufacturer's instructions. Once the 'START' key on the instrument's panel is pressed, the extraction process begins automatically. The system draws liquid CO_2 from a 'dip-tube' tank into a refrigerated pump head, preheats and compresses the CO_2 to 9,000 psi, then passes it through the heated high-pressure extraction thimbles (95-105°C) containing the sample. As the compressed CO_2 passes through the sample (a process that lasts for 45 min), it removes fat and carries it to the collection system, where the CO_2 is depressurized. The sudden change in pressure separates the dissolved fat from the supercritical solvent (Min and Boff 2003), allowing for the collection of the fat into a vial containing glass wool. Once the extraction process is complete, the collection vial is removed from the SFE instrument, placed into a microwave oven for 2 min (at 1000 W) to remove residual

moisture and solvent, cooled for 15 min, then weighed. Fat percentage is determined based on the weight gain of the collection vial (Chandrasekar 2001).

The SFE method described above involves the use of a 'wet' homogenized meat sample. However, some SFE methods may require that the sample be dried prior to extraction. For example, Min and Boff (2003) describe a procedure in which ca 3-5 g of dried homogenized meat sample is placed into the extraction cell of the instrument, extracted for 20 min at the proper temperature-pressure settings, then rotary dried and weighed to determine fat content. For methods using dried meat samples, use of diatomaceous earth or other desiccants is not necessary since moisture was removed from the sample during the drying process.

The greatest advantage to using SFE is that no harsh solvents are required, which (1) eliminates exposure of lab personnel to harmful chemicals, and (2) eliminates storage, tracking, and disposal costs and dangers associated with organic solvents and waste. In addition, SFE methods can match the overall precision and accuracy of traditional extraction methods (Min and Boff 2003). Depending on the instrument used, multiple samples may be extracted simultaneously (Sebranek 1998). One additional advantage for analytical laboratories or food manufacturers seeking AOAC-approved methods for use in their operations is that the TFE2000 fat determinator SFE system has been accepted as an AOAC Peer Verified Method, indicating the potential of this method to become and AOAC-approved method in the future.

SFE offers a more rapid method for determining fat content in meat and meat products when compared to traditional extraction methods that use organic solvents.

SFE and similar methods may gain more acceptance as regulations by the U.S. Environmental Protection Agency (EPA) and other federal entities encourage the reduction of organic solvents in laboratories (Min and Boff 2003).

Low Resolution Nuclear Magnetic Resonance (CEM Corporation Instrumentation)

Low Resolution-Nuclear Magnetic Resonance (LR-NMR) offers rapid fat analysis for meat and meat products. The CEM Smart Trac 5 System® has been accepted as an AOAC Peer-Verified Method (PVM 1:2003) for the rapid determination of moisture and fat in meats by microwave and Nuclear Magnetic Resonance analysis. The CEM Smart Trac 5 System®, which was the instrument used in this collaborative study for the fulfillment of AOAC requirements to become an official method, uses LR-NMR technology for fat determination. It is the goal of this collaborative study to recommend that this method be adopted as an AOAC-approved reference method for the rapid determination of fat.

Nuclear Magnetic Resonance (NMR) is based on the observation that certain nuclei will re-absorb and re-emit radio frequency (RF) energy over a narrow band of frequencies when placed in a static magnetic field. NMR does not involve the emission of ionizing radiation as the name may suggest, but rather is caused by the interaction between the nuclear magnetic dipole of a nucleus and the magnetic field it experiences. The strength of a magnetic field produces a specific frequency at which the NMR effect occurs for a given nuclear isotope (Leffler and others 2008).

In NMR spectroscopy, a phenomenon known as a chemical shift effect occurs, and can be used to distinguish different hydrogen-containing constituents within a

sample. Differences in the electronic structure of molecules cause small variations in the magnetic field that ^{1}H nuclei experience in different molecules and in different parts of the same molecule. This leads to small differences in the NMR frequencies of ^{1}H nuclei in different molecules, which can be used to distinguish between the different constituents within a meat sample (Leffler and others 2008).

Unlike NMR spectroscopy, LR-NMR cannot detect chemical shift effects in samples containing ^{1}H nuclei due to the low field strength and homogeneity of the magnet used to generate the static magnetic field. In LR-NMR, the NMR signals from different constituents (moisture, fat, protein, carbohydrates) within the sample are distinguished by differences in the rate of decay of the signal from the different constituents (commonly known as transverse relaxation or T_2 decay). There are significant differences between the proton transverse relaxation times (T_2) of these constituents. More specifically, protein and carbohydrates have a very short transverse relaxation time and therefore decay very quickly, whereas fat has a much longer transverse relaxation time and decays much more slowly.

Transverse relaxation can generally be approximated as an exponential decay with time constant T_2. The transverse relaxation times for fat are considerably longer (typically of the order of 10 mS or greater) than the transverse relaxation times of protein and carbohydrates (typically of the order of 10 mS or less). When the system is excited, the signals associated with protein and carbohydrates decay first, which make it possible to acquire the remaining signal from fat. The NMR signal acquired from a dried food sample using the NMR methodology will be directly proportional to the number of

protons within the fat contained in the sample and thus directly proportional to the fat content of the sample (Leffler and others 2008).

The CEM Smart Trac 5 System® removes moisture from a food sample using the microwave drying technique prior to fat analysis using NMR. Moisture and its corresponding protons are removed from a meat sample, leaving fat, protein, and carbohydrate as the remaining constituents that contain significant protons in the sample to be analyzed. The protons associated with the fat, protein, and carbohydrates in the dried meat sample will produce a signal when a magnetic field is applied to the dried meat sample in the NMR chamber.

To perform fat analysis using the CEM Smart Trac 5 System®, a homogenized meat sample is first dried in the microwave drying chamber per manufacturer's instructions (and as described previously in this report). After drying, the sample (including the glass fiber pads) is placed on a single sheet of Teflon film and tightly rolled into a cylindrical shape, placed and pounded into a special Teflon tube fitted for the instrument, placed into the NMR chamber, and the instrument initialized to begin the analysis of fat content. The instrument uses LR-NMR technology that sends pulses of magnetic energy through the sample, creating a free induction decay (FID) of the hydrogen protons associated with the lipid, ash, and protein components of the meat sample.

The FID relates to the scattering and realignment of the protons in response to the pulsing on and off of the magnetic energy through the sample. The magnetic energy causes the protons to align while the removal of the magnetic energy causes the protons

to "scatter" or resume their natural position. Due to the longer FID time of the hydrogen protons of the lipid components in comparison to the shorter FID time of the hydrogen protons of the protein and ash components, the equipment is able to identify and measure the lipid components separate from the other protons and use the information to calculate the fat percentage of the sample using the programmed equations.

The advantages to using the CEM Smart Trac 5 System® NMR instrument include: the speed in which it can determine the fat content of a wide variety of meat product samples; organic solvents are not required for extraction, which (1) eliminates exposure of lab personnel to harmful chemicals, and (2) eliminates storage, tracking, and disposal costs and dangers associated with organic solvents and waste; the ease of use of the instrument (no complicated procedures are involved and highly skilled personnel are not needed); the low cost of operating the instrument; and it is recognized as an AOAC peer verified method.

Disadvantages associated with this method include the relatively high initial cost of purchasing the instrumentation, the expense associated with replenishing consumable materials (i.e. glass fiber pads, Teflon film), and the limitation of analyzing one sample at a time.

Near Infrared Transmittance (FOSS FoodScan™, AOAC 2007.04)

Near Infrared Transmittance was discussed previously in this report in the section regarding moisture analysis using NIR. NIR will be discussed again in this section regarding fat analysis, as NIR Transmittance technology offers a newer and more sophisticated spectroscopic method for the analysis of fat content in meat samples.

NIR measurements are based on the principle that almost all organic functional groups (i.e. ketone, alcohols, etc.) have a specific absorption band in the near infrared region and that the infrared absorption spectra is unique for many different components in meat (Sebranek 1998).

One example of instrumentation utilizing NIR Transmittance technology is the FOSS FoodScan™ Meat Analyzer (Eden Prairie, MN). This instrument is an NIR Spectrophotometer with artificial neural network (ANN). The FOSS FoodScan™ Meat Analyzer recently received AOAC approval as an official method for the determination of moisture, fat, and protein content in meat and meat products (AOAC Method 2007.04). Parameters for meat products evaluated include fresh meat, beef, pork, poultry, emulsions, and finished products in the constituent ranges of 1-43% fat, 27-74% moisture, and 14-25% protein (Anderson 2007).

To perform this method using the FOSS FoodScan™ Meat Analyzer instrument, ca 180 g of homogenized meat sample is evenly distributed in the instrument's round sample dish and loaded into the instrument's sample chamber. The operator selects the appropriate meat product profile from the instrument's menu screen or personal computer (depending on instrument version), presses the 'START' button to initiate the scan, and waits for analysis to be completed and the results calculated and reported for moisture, fat, and protein percent (Anderson 2007).

The FoodScan™ instrument contains a tungsten-halogen lamp and optical fibers that guide emitted light into an internal moving-grating monochromater (which provides monochromatic light in the spectral region between 850 and 1050 nm) and a collimator

lens positioned over the sample cup in the sample chamber. Light that is not absorbed by the sample in the sample chamber strikes a detector that measures the amount of unabsorbed light. The results are sent to the instrument's digital signal processor, which calculates the final results and communicates them with the personal computer. The results are displayed as % moisture, % fat, and % protein on the digital screen or personal computer (Anderson 2007).

The ANN calibration technique used for this system eliminates the need for development and maintenance of separate calibrations for specific sample types. The ANN calibration generates a single, global, multi-product, full-range calibration for each constituent based on a database containing calibration sample data represented by spectra and chemical analysis results (Anderson 2007).

The FOSS FoodScan™ instrument offers multiple advantages, including rapid results (ca 50 sec) for multiple constituents, is well-suited to high-capacity production environments, is easy to use and is not dependent on highly skilled personnel to operate the instrument, is cost effective (no consumables and low operation costs), and demonstrates similar repeatability and reproducibility compared with chemical analysis reference methods. In addition, an on-line version of this instrument is available, which may be used for continuous, flow-by measurements of material in a pipe or tube system, such as in meat grinders and blenders.

One disadvantage to using this method is that it is limited to analyzing one sample at a time, but fortunately each sample can be analyzed in a very short amount of time. A second potential disadvantage to using the FOSS FoodScan™ instrument is the

initial cost required to purchase the instrumentation. Additional disadvantages may exist, such as affected instrumentation performance due to addition of non-meat ingredients (such as spices, colorants, or other ingredients that affect meat batter color) or the presence of ice crystals in the sample. Although not discussed in the literature, ice crystals, non-meat ingredients, or other factors may potentially interfere with the instrument's infrared absorption performance. In such cases, it may be necessary to calibrate the equipment specific to the type of product being analyzed in order to obtain the most reliable results.

NIR transmission instruments are believed to perform better than NIR reflectance instruments because a greater amount of sample may be scanned and a highly sensitive detector can be used (Sebranek 1998). In many cases, sample scans with infrared instruments must be calibrated against known values for sample composition with reference methods. In addition, a large number of samples similar to those to be measured need to be included for calibration (Sebranek 1998).

CHAPTER III

MATERIALS AND METHODS

An AOAC collaborative study involving 10 laboratories representing private industry, government agencies, and academia was conducted to determine if the CEM Smart Trac 5 System®, which performs rapid analyses of moisture and fat, is comparable to universally accepted standard methods of analysis. The primary goal of the collaborative study was to determine if a method for the rapid determination of moisture and fat in raw and processed meat products could produce results of comparable accuracy and precision to standard methods. A variety of raw and processed meat products representing the primary meat categories of beef, pork, chicken, and turkey were selected for analysis in this study. The meat products selected for use are commonly produced and distributed by meat plant operations.

Meat samples were obtained fresh from commercial sources and stored frozen until homogenized, packaged, frozen, and distributed to participating collaborative laboratories for analysis on the Smart Trac 5 System®. Ten replicates were analyzed in the Department of Animal Science at Texas A&M University (TAMU) using the following standard methods of analysis for the determination of moisture and fat, respectively: Forced Air Drying, AOAC Official Method 950.46 and Soxhlet Extraction of Crude Fat, AOAC Official Method 960.39. Data collected from replicate analyses using the AOAC official methods provided by TAMU were used for the programming and calibration of all CEM Smart Trac 5 Systems® used in this study and for

comparative analysis of the data collected from ten different collaborators using their calibrated CEM Smart Trac 5 Systems®. Each of the 10 collaborative research laboratories owned a CEM Smart Trac 5 System® and was familiar with sample preparation and instrument operation. Each laboratory independently completed an analysis of the moisture and fat content of each meat product specified using the calibrated CEM Smart Trac 5 System®. All meat samples evaluated were taken from a larger composite sample and handled in a similar manner by each laboratory per detailed instructions. Thus, the only differences observed should have been due to individual sample handling and the inherent limitations of the instrument.

The following paragraphs will discuss the collection, homogenization, packaging, storage, distribution, and analysis of the meat samples. The statistical design used to analyze the data will also be discussed.

Meat Product Specification

Raw and further processed meat products representing four primary categories of red meat and poultry (beef, pork, chicken, and turkey) were obtained commercially for analysis for this study. The raw meat products obtained included: ground beef (high fat and low fat), fresh pork (high fat and low fat), fresh pork sausage (high fat and low fat), fresh chicken (high fat and low fat), and fresh turkey breasts (low fat). Other further processed meat products obtained included: mechanically deboned turkey (high fat), bone-in ham (high fat), formed ham with natural juices (low fat), beef frankfurters (high fat), beef frankfurters (low fat), and potted meat (medium fat). For each of the meat

products listed, a diluted counterpart was prepared by adding four percent (w:w) of distilled, deionized water during homogenization for use as blind samples and required by AOAC for the study.

Collection of Meat Products

Approximately 6.8 kg (15 lbs) of each meat product specified above were obtained from meat plant operations and local grocery stores. The meat products purchased from local grocery stores were homogenized within one day of purchase. The meat products obtained from meat plant operations were shipped frozen to Texas A&M University, stored in a freezer (-10^{o}C) upon receipt, and thawed in a refrigerator (4^{o}C) one to two days prior to homogenization and sample preparation. The fresh pork sausage (low fat) used in this study was prepared fresh at the Rosenthal Meat Science and Technology processing facility using lean pork, a pre-blended sausage spice blend, and by following standard processing practices for ground products. Low fat fresh pork sausages are not commonly produced by meat operations, so for the purpose of this study, a fresh pork sausage (low fat) was specially made.

Preparation of Meat Aliquots (Homogenization of Meat Products)

Each meat product was kept refrigerated (4° C) immediately prior to size reduction and homogenization. As written previously, some products were frozen (to prevent spoilage) and then thawed in the refrigerator two days prior to sample preparation. The fresh pork, fresh chicken, fresh turkey, ham, and frankfurter products

required size reduction prior to homogenization. Each of these products were individually removed from their respective package, manually cut with a knife into smaller pieces (ca. 5 cm x 5 cm), and ground through a table-top meat grinder (Hobart® 4612 Chopper, Hobart Corporation, Troy, OH), using a 1.27 cm (1/2 inch) grinding plate. The ground beef, fresh pork sausage, mechanically deboned turkey, and potted meat products did not require size reduction (cutting and grinding) prior to homogenization.

Approximately 3.18 kg (7 lbs) of material was placed in a 10 quart capacity stainless steel commercial food processor (Robot Coupe® R10 Vertical Cutter-Mixer, Robot Coupe USA, Inc., Jackson, MS) and chopped for 30 seconds using a two blade, high speed (4.5 HP) setting. A rubber spatula was used to recover and return the meat that had collected on the inner sides and bottom of the bowl. The sample was then chopped for an additional 30 seconds on high speed. Products with "skin" (processed and bone-in hams, frankfurters) were more difficult to homogenize and required an additional 60 seconds (30 seconds of chop, wipe sides, 30 seconds of chop) of chopping.

For each meat product, a 0.4% diluted counterpart was prepared for use as blind samples for calibration and testing of the CEM Smart Trac 5 System® to ensure detection of modest variations in the moisture content of each meat product. The diluted counterpart for all of the meat products was prepared by placing 3.18 kg (7 lbs) of the remaining ground material into the commercial food processor and adding 0.127 kg (0.28 lbs) of distilled, deionized water. Each diluted sample was then chopped in the

same manner as its undiluted counterpart (as indicated in the previous paragraph) for the same amount of time to maintain consistency between products of the same kind.

The temperature of each meat product was taken immediately prior to and after homogenization. The temperature of each meat sample prior to homogenization was between 4°C and 10°C. Each meat sample experienced an average of 9°C temperature increase while chopping in the commercial food processor, likely due to the heat generated by the friction of the blades against the meat.

Packaging of the Meat Aliquots

The homogenized material was tightly packed into forty 2-oz and five 4-oz sterile, screw capped polyurethane specimen containers. During packaging, the homogenized material was covered to minimize moisture escape, evaporation, or absorption. Each specimen container was filled to minimize airspace and to limit oxidation and risk of sample deterioration during storage. Each container was labeled with a self-adhesive label indicating the title of the study, the meat product and fat level, the packaging date, and the name of the laboratory to which it would be sent. The containers of homogenized material were then transferred to a -40^{o}C freezer for storage.

Storage and Distribution of the Meat Aliquots

All containers of homogenized material were transferred to a -40^{o}C freezer for storage within 25 minutes of packaging and were undisturbed until the date of distribution to collaborating laboratories. Samples were distributed to collaborating

laboratories on two separate days. On the first day of distribution, two 2-oz containers of each of the following homogenized samples were packed into Thermosafe™ insulated boxes: ground beef (high fat and low fat), pork (high fat and low fat), chicken (high fat and low fat), and ham (high fat and low fat). On the second day of distribution, two 2-oz containers of each of the following homogenized samples were packed into Thermosafe™ insulated boxes: turkey (high fat and low fat), frankfurters (high and low fat), pork sausage (high fat and low fat), and potted meat.

On each of the two days of distribution, the homogenized meat samples and ice packs were packed into insulated boxes. The empty top space inside the boxes was packed with crumpled newspaper to aid in insulation and to prevent shifting of the sample containers during transport. Taped onto the outer top portion of the Styrofoam lid were the following instructions for the storage and handling of the meat samples shipped to the collaborating laboratories:

1. Receive samples from Texas A&M University.
2. Place the samples in the freezer immediately upon receipt. Store the samples in the freezer (0°F or below is preferred) until one day prior to sample analysis on the CEM Smart Trac 5 System®. [*Please note: Your lab will receive more than one product sample per shipment. Remove the sample to be analyzed the following day (for example, High Fat Beef sample) from the freezer and place in the refrigerator the day before analysis. Leave the remaining samples (for example: Low Fat Beef, High Fat Pork, and Low Fat*

Pork samples) in the freezer until the day before analysis of the specific product sample]

3. One day prior to sample analysis, place the sample to be analyzed in the refrigerator ($\leq$ 40°F) to thaw overnight. Do not try to speed up the thawing process by using a microwave, running water, or other thawing method.
4. On the day of analysis, remove the sample container from the refrigerator and place it in ice at least two-thirds of depth of the container to keep the sample cold. Keep the container in ice until the analysis of all replications (10) of the sample is complete. (Ice will help keep the product a constant temperature throughout the analysis of all replications).
5. After placing the sample in ice, open the container lid and stir the sample thoroughly with a small spatula since ice crystals may have condensed on the side. (*Note: Avoid transferring moisture from the ice to the sample).* Replace the lid between each replication to prevent moisture loss. Stir the sample before beginning the next replication.
6. After stirring the sample, follow the instructions provided by CEM to begin the analysis (i.e. tare the pads, spread 3-4 g of sample on the pads, place on scale, press 'START', etc).

The outer box flaps were taped closed, shipping labels attached, and the packages shipped for next-day delivery via Federal Express. The project leader at each of the collaborative laboratories was notified via electronic mail that the package was en route to their facility and the date in which the samples would be delivered. Per the

instructions listed above, the collaborators, upon receipt of the samples, stored them in a freezer at their facility until one day prior to analysis on the CEM Smart Trac 5 System®.

Analytical Methods

Moisture Analysis – Standard Method (AOAC Official Method 950.46)

Ten replicates of each meat product and their diluted counterparts were analyzed at Texas A&M University in the Department of Animal Science using the Forced Air Drying Method (AOAC Official Method 950.46) for the determination of moisture in meat products. The AOAC official method for moisture determination was performed to obtain data for programming and calibrating the CEM Smart Trac 5 System® and for comparative analysis of results collected from collaborators using their individual CEM Smart Trac 5 System®. For statistical purposes, 10 replicates of each meat product (undiluted and diluted) were analyzed simultaneously using the standard method for moisture analysis.

One day prior to analysis, a 4-oz polyurethane specimen container of homogenized meat sample was transferred from the -40^{o}C freezer to a refrigerator (4^{o}C) for thawing. The following day, the sample was stirred thoroughly with a stainless steel spatula to evenly redistribute any moisture that may have migrated during storage and subsequent thawing. It is also important to note that most, if not all, samples had moisture droplets collect on the inward-facing specimen cup lid. The moisture that had collected on the lid was carefully transferred from the lid and reincorporated into the

sample by stirring. Maintaining sample temperature was important in preventing data inconsistencies that could have been caused by the warming of the sample to room temperature. Potential moisture transfer from the chilling ice to the sample was of concern, so a sheet of waxed paper was placed between the sample container and the ice to serve as a barrier against moisture transfer.

Ten aluminum pans (5.08 cm diameter) and corresponding lids were numbered, washed, dried in a forced-air oven (103^{o}C), and desiccated until cooled to room temperature. Latex gloves and tongs were used in the handling of all pans, lids, spatulas, and other utensils to prevent transfer of sweat and oils from hands and fingers onto equipment and utensils. The importance of using gloves cannot be understated in quantitative methods of analyses, as fingerprints have weight (i.e. moisture, oils, residues) and can affect results. The amount of sample to be weighed into each pan was determined using the following equation:

$$\text{Amount of sample} = \frac{200}{100\% - \text{Predicted moisture \% of sample}}.$$

Note: The AOAC official method indicates that approximately two grams of dried material remain after drying. In most cases, five grams of meat material was used for high fat meat products and six grams of meat material was used for low fat meat products.

The combined weight of the first pan and corresponding lid was obtained and recorded to the nearest 0.0001 g using an analytical balance. Approximately 5-6 grams of homogenized material were weighed into the pan and spread in an even layer using a small metal spatula. The combined weight of the pan, lid, and sample was then recorded

to the nearest 0.0001 g. This process was repeated until 10 replicates were prepared. The meat sample in the polyurethane specimen container was stirred between each replicate sample and the lid kept in place to minimize moisture escape, evaporation, or absorption during sample preparation.

The pans containing the prepared samples were transferred to a forced air drying oven and the samples dried for 16 hours. The lids were removed and placed alongside the corresponding pans during drying. Upon completion of the drying process, the lids were fitted onto the corresponding pans, the pans removed from the oven, transferred to a desiccator, and allowed to cool to room temperature (for at least 6 hours to ensure a stable balance reading). The pans (with the corresponding lids tightly fitted) containing the dried meat material were weighed on the balance and the weight recorded. The following calculation was used to determine the percent moisture in each of the samples:

$$\frac{A - B}{C} \times 100 = D$$

A= Wet weight (pan + lid + wet sample)

B = Dry weight (pan + lid + dry weight)

C = Initial sample weight [Wet weight – (pan + lid weight)]

D = % Moisture

After the data was collected and the results determined, the dry sample material was removed from the pan and discarded. The aluminum pans and corresponding lids were washed with soap and water, rinsed with distilled de-ionized water, dried in the forced air drying oven, and placed in a desiccator until needed for further use.

Moisture Analysis – Rapid Method (CEM Smart Trac 5 System®)

Each of the 10 collaborative laboratories involved in the study analyzed the moisture for each of the aforementioned meat products using their CEM Smart Trac 5 System®. To begin analysis of the meat samples, the appropriate program method was selected using the digital menu and touchpad on the CEM Smart Trac 5 System®. Two square-shaped glass fiber pads (stored at room temperature, undesiccated) designed specifically for use with the CEM Smart Trac 5 System® were placed on the internal balance and tared by pressing the "TARE" button on the touchpad. Approximately 3-4 g of sample was spread in a thin, even layer with a Teflon-coated spatula on one of the glass fiber pads. The second glass fiber pad was placed on top of the first so that the meat sample was sandwiched between the two glass fiber pads. The lid to the equipment was then closed and locked in place and the "START" button on the touchpad pressed to initiate drying of the sample. After drying (approximately 3 ½ - 4 min), the internal computer in the CEM Smart Trac 5 System® automatically calculated the percentage of moisture in the sample and displayed the results on the digital screen. The "READY" button was then pressed and the dried sample material prepared for fat analysis, as described in the section titled "Fat Analysis – Rapid Method (CEM Smart Trac 5 System®)."

Fat Analysis – Standard Method (AOAC Official Method 960.39)

The standard method for fat analysis is a lengthy process that requires the use of a potentially hazardous chemical. Great care should be exercised when performing the

analysis to avoid risk of damage or injury. Among the ten collaborative laboratories involved in the study, the research laboratory at Texas A&M University was designated to perform the AOAC Official Method 960.39 (Soxhlet Extraction of Crude Fat) for the determination of fat in each of the meat products. Ten replicate samples for each meat product (undiluted and diluted) were analyzed for statistical purposes.

The two fume hoods used for Soxhlet extraction were inspected and serviced prior to use for this study to ensure adequate ventilation and operation. The apparatus for the simultaneous Soxhlet extraction of five to six samples per hood was assembled and several analyses performed on generic meat samples to ensure that the system was operating correctly.

For the actual replications, a homogenized meat sample packaged in a 4-oz polyurethane specimen container was thawed overnight in a refrigerator. The following day, the sample was stirred thoroughly and kept on ice for the duration of sample preparation. A sheet of waxed paper was placed between the container and the ice to prevent moisture transfer from the ice to the sample. Ten disposable aluminum pans (5.05 cm diameter) were labeled in numerical order with a permanent marker. A small glass rod (ca. 4 cm in length) was placed in each pan. The first pan with a glass rod was placed on the analytical balance, tared, and approximately 3.34 g of homogenized material weighed into the pan. (AOAC Official Method 960.39 indicated between 3.0-3.5 g of sample be added). The weight of the homogenized material was recorded to the nearest 0.0001 g. Approximately 3.34 to 3.35 g of laboratory grade sand was added to the pan and mixed into the homogenized material using the glass rod. The glass rod was

then used to spread the mixture into an even layer on the bottom of the pan. The glass rod was kept in the pan with the mixture when the spreading of the sample was complete. This set of steps was repeated until 10 replicate samples had been prepared. The ten prepared samples were then transferred to a forced air drying oven (103^{o}C) for 16 hours (overnight) to dry.

Flat-bottom 250 mL glass flasks for the extraction system were numbered, washed with soap and water, rinsed thoroughly with distilled water, and rinsed a second time with distilled, deionized water. Approximately 5 to 7 g of porous boiling chips (VWR Scientific, Inc.) were added to each flask, which were then placed into a forced air drying oven (103^{o}C) until dry. The flasks were then transferred to a desiccator and allowed to cool overnight.

The following day, the ten samples were removed from the oven and transferred to a desiccator to cool to room temperature. Meanwhile, each flask containing porous boiling chips was weighed on an analytical balance and each weight recorded to the nearest 0.0001 g. Each of the ten flasks was then transferred to the fume hood and filled with 150 ml of petroleum ether. Each of the pans containing the dried sample material was removed from the desiccator, folded and fitted into a cellulose thimble to prevent escape of pan contents, then placed into a Soxhlet apparatus corresponding to the appropriate flask. For example, sample #1 would be placed in a Soxhlet to correspond with flask #1. The setup of the Soxhlet extraction system was then completed by connecting the flasks to the Soxhlet condensers and tightly wrapping the junctions between the three pieces of glassware with Parafilm® to prevent escape of the petroleum

ether. The heating mantels were adjusted to an initial setting of "6", the water for the condensers were turned on, and the extraction process allowed to proceed for a minimum of four hours without interruption. After the petroleum ether started to condense and drip into the Soxhlet, the mantels were adjusted as necessary to maintain a consistent rapid drip of approximately 4 to 6 drops/sec. At this rate, the ether dripped as rapid individual drops, but not so fast that a continuous, unbroken stream was formed.

At the end of the extraction period, the water and heating mantels were turned off and the flasks containing the petroleum ether allowed to cool. The Parafilm® was removed and the condenser disconnected from its corresponding Soxhlet. The cellulose thimble was then removed from the Soxhlet with large stainless steel tweezers and the remaining petroleum ether in the Soxhlet carefully fluxed back into the flask. The Soxhlet was then set aside and the flask placed back onto the heating mantel. This series of steps was repeated for each of the samples. The flasks were then slowly heated on the mantels to aid in the evaporation of the remaining petroleum ether to near dryness. When near dryness was reached, the heating mantels were shut off and the flasks were gently removed. The flasks were kept in the vent hood overnight to allow the residual petroleum ether to evaporate unassisted and undisturbed overnight.

After all of the ether had evaporated from the flasks, the flasks were placed into the forced air drying oven (103^{o}C) for an hour to evaporate any moisture that may have collected in the flask. The flasks were removed from the oven, transferred to a desiccator, and allowed to cool to room temperature so a stable balance reading could be obtained. When cooled, each flask containing the extracted fat was weighed and the

weight recorded. The difference between the pre-extract flask weight and the post-extract flask weight was the direct weight of the fat extracted from the sample. The percentage of fat in the sample was calculated using the following equation:

$$\% \text{ Fat} = \frac{\text{Post-extract Flask Wt. (g)} - \text{Pre-Extract Flask Wt. (g)}}{\text{Wet Sample Wt. (g)}} \times 100$$

Fat Analysis – Rapid Method (CEM Smart Trac 5 System®)

Each of the 10 collaborative laboratories involved in the study used their CEM Smart Trac 5 System® to analyze a specimen of each of the aforementioned meat products for fat percentage. Each sample was prepared for fat determination using the steps described in the previous section titled "Moisture Analysis – Rapid Method (CEM Smart Trac 5 System®)" and was analyzed for fat immediately after the sample's moisture content had been determined by the microwave drying process.

After the sample had been dried, the glass fiber pad "sandwich" was removed from the internal balance, placed onto a sheet of Smart Trac® Teflon film (CEM Corporation, Matthews, NC), tightly rolled into a cylindrical shape per manufacturer's instruction manual, and placed into the polyurethane tubing designed specifically for the NMR portion of the system equipment. The sample was then tightly compacted into the tubing, and the tubing inserted into the appropriate opening in the NMR portion of the system equipment. The "START" button was pressed to initiate the measurement of fat in the product. Upon measurement completion, the internal computer of the CEM Smart Trac 5 System® computed the percentage of fat in the meat sample using results from

the NMR procedure. The equipment automatically displayed the results on the digital display screen and printed the results for the moisture and fat analyses of the sample.

The results from the AOAC Official Methods for Moisture (950.46) and Fat (960.39) analyses performed in the Dept. of Animal Science at TAMU were used as reference values for each sample type in the Smart Trac 5 System®. The reference values for each sample type (i.e. "ham", includes reference values for low fat ham and high fat ham) were loaded into the Smart Trac 5 System® and encompassed the entire range of fat into which the aliquot samples could fall. Additional samples were used in conjunction with the reference values in order to determine the appropriate NMR signal for that specific sample type (i.e. "ham") and to establish a standard curve (y=mx+b) for fat determination of that specific sample type. This was to ensure that each program was equal and that variations would be due to preparation of the sample, the instrument, or both.

Each collaborating laboratory was then instructed to perform one analysis from each sample aliquot (non-diluted and diluted) and report the results to the Study Director for comparative analysis against data collected using the AOAC Official Methods. Statistical analysis of the study results for moisture and fat were performed to determine repeatability and reproducibility of the Smart Trac 5 System® for the determination of moisture and fat.

Statistical Analysis

The following statistical analyses were performed to fulfill requirements set forth by AOAC International for calculating and reporting data results for collaborative

studies: mean, repeatability (within-laboratory) standard deviation (SD_r), reproducibility (between-laboratory) standard deviation (SD_R), repeatability (within-laboratory) relative standard deviation (RSD_r), reproducibility (between-laboratory) relative standard deviation (RSD_R), and percent recovery (%Recovery) (AOAC Int. 2002). Acceptability criteria for repeatability (within-laboratory) relative standard deviation (RSD_r) and reproducibility (between-laboratory) relative standard deviation (RSD_R) are values <2%.

The mean is the average of the results, such as the average among 10 replicate analyses for moisture for a specific meat sample type (i.e. low-fat ham). It is calculated by adding the individual result values and dividing the sum of the values by the number (n) of individual values, as follows:

$$\text{Mean} = \frac{x_1 + x_2 + x_3 + x_4 \ldots..}{n}$$

Repeatability (within-laboratory) is a measure of how well an analyst in a given laboratory can check himself using the same analytical method to analyze the same test sample at the same time (AOAC Int 2002). The repeatability (within-laboratory) standard deviation (SD_r) was calculated as follows, where x_1, x_2, x_3, etc. represent individual results obtained for an analyte (i.e. moisture, fat), and where $\bar{x}$ is the mean of the values obtained within the data set:

$$SD_r = \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \ldots..}{n-1}}$$

Reproducibility (between-laboratory) is a measure of how well an analyst in one laboratory can check the results of another analyst in another laboratory using the same analytical method to analyze the same test sample at the same or different time (AOAC

Int 2002). The reproducibility (between-laboratory) standard deviation (SD_R) was calculated as follows, where x_1, x_2, x_3, etc. represent individual results obtained for an analyte (i.e. moisture, fat), and where $\bar{x}$ is the mean of the values obtained within the data set:

$$SD_R = \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \ldots}{n-1}}$$

AOAC describes relative standard deviations as the most useful measures of precision in chemical analytical work because the RSD values are usually independent of concentration and therefore can facilitate comparison of variabilities at different concentrations (AOAC Int 2002). The RSD is often times more convenient than standard deviations since it is expressed in percent. The repeatability (within-laboratory) relative standard deviation (RSD_r) was calculated by multiplying the standard deviation by 100 and dividing this product by the mean, as follows:

$$RSD_r = \frac{100 \times SD_r}{\bar{x}}$$

AOAC describes relative standard deviations as the most useful measures of precision in chemical analytical work because the RSD values are usually independent of concentration and therefore can facilitate comparison of variabilities at different concentrations (AOAC Int 2002). The RSD is often times more convenient than standard deviations since it is expressed in percent. The reproducibility (between-laboratory) relative standard deviation (RSD_R) was calculated by multiplying the standard deviation by 100 and dividing this product by the mean, as follows:

$$RSD_R = \frac{100 \times SD_R}{\bar{x}}$$

The percent recovery indicates how much of the analyte was recovered using the study methods (i.e. CEM SMART Trac 5 System) in comparison to the amount of analyte recovered using the reference method (i.e. AOAC methods 950.46 and 960.39). To obtain the percent recovery for moisture and fat using for the CEM SMART Trac 5 System in comparison to AOAC reference methods, the following calculation was used:

$$\% \text{ Recovery} = \frac{\text{Study method mean}}{\text{Reference method mean}} \times 100$$

An additional AOAC International requirement for calculation and reporting of data for collaborative studies is the HORRAT (Horwitz Ratio). For purposes of this study, the HORRAT value was not determined for moisture and fat analysis because AOAC indicates that the HORRAT value is not applicable to empirical methods (i.e. fiber, enzymes, moisture, methods with indefinite analytes (i.e. polymers), quality measurements (i.e. drained weight), or physical properties (i.e. viscosity, density, pH, absorbance, etc.) (AOAC Int 2002).

CHAPTER IV

RESULTS AND DISCUSSION

Four primary categories of red meat (beef and pork) and poultry (chicken and turkey) were analyzed for moisture and fat for this study, including: ground beef (high fat and low fat), fresh pork (high fat and low fat), fresh pork sausage (high fat and low fat), fresh chicken (high fat and low fat), fresh turkey breasts (low fat), mechanically deboned turkey (high fat), bone-in ham (high fat), formed ham with natural juices (low fat), beef frankfurters (high fat), pork/chicken/turkey frankfurters (low fat), and potted meat (medium fat). For each of the meat products listed, a diluted counterpart was also analyzed as blind samples.

AOAC methods for moisture (AOAC 950.46) and fat (AOAC 960.39) were performed by the TAMU laboratory. The results from these methods of analysis were used as reference values for each sample type. Results from AOAC methods for each sample type are provided in Tables 1-26.

AOAC methods for moisture and fat for four sample types (high-fat chicken, low-fat frankfurters, high-fat turkey, and low-fat sausage) were performed by the TAMU laboratory, but were not analyzed by the 10 collaborative laboratories. These samples were used in conjunction with the reference values in order to determine the appropriate NMR signal for that specific sample type (i.e. “chicken”) and to establish a standard curve for fat determination of that specific sample type to ensure that each program was equal and that variations would be due to preparation of the sample, the instrument, or

both. The moisture and fat results for these four sample types are provided (see tables on pages 83, 87, 94, and 97).

Study methods for moisture (rapid microwave method) and fat (NMR method) were performed by 10 collaborative laboratories using the CEM Smart Trac 5 System®. Results from the study methods are provided in Tables 1-26.

Data were analyzed and the statistical summaries for within-laboratory repeatability (see table on page 105) and for between-laboratory reproducibility (see table on page 106) are provided. The values shown for each product type include means, standard deviations (SD), and relative standard deviations (RSD).

The percent recovery of moisture and fat was calculated based on data obtained from the AOAC methods and CEM SMART Trac 5 System® methods. The percent recovery for moisture and fat is provided (see table on page 108).

Discussion of Ham Results

AOAC methods yielded 74.93% and 75.91% mean total moisture values and 2.54% and 2.37% mean total fat values for the low-fat ham and diluted low-fat ham samples, respectively (Tables 1 and 2). The CEM Smart Trac 5 System® yielded comparable results, with 74.28% and 75.59% mean total moisture values and 2.60% and 2.36% mean total fat values, respectively.

For the high-fat ham and diluted high-fat ham samples, AOAC methods yielded mean total moisture values of 58.41% and 60.18% and mean total fat values of 16.34%

Table 1. Comparative Moisture and Fat Analysis Values of Low Fat Ham Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)

	Method 950.46		Method 960.39	
Sample ID	Wt., g	Moisture %	Wt., g	Fat %
1	6.0003	74.94	3.3479	2.43
2	5.9992	74.86	3.3458	2.66
3	6.0007	74.90	3.3440	2.63
4	6.0020	74.92	3.3482	2.53
5	6.0012	75.00	3.3449	2.63
6	5.9971	74.85	3.3422	2.61
7	5.9985	74.92	3.3430	2.44
8	5.9979	74.90	3.3468	2.57
9	5.9972	74.98	3.3480	2.49
10	6.0026	75.04	3.3438	2.42
Mean		74.93		2.54
SD_r		0.0598		0.0925
RSD_r		0.0798		3.6391

Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)

		Microwave	NMR
Lab ID	Wt., g	Moisture %	Fat %
A	4.1945	74.35	2.63
B	4.2167	74.57	2.57
C	4.0277	74.36	2.61
D	4.1785	74.29	2.59
E	4.3281	74.03	2.68
F	3.8787	74.14	2.71
G	3.6790	74.10	2.66
H	3.7222	74.07	2.64
I	3.8335	74.36	2.38
J	4.0436	74.59	2.54
Mean		74.29	2.60
SD_R		0.1990	0.0929
RSD_R		0.2678	3.5721

Table 2. Comparative Moisture and Fat Analysis Values of Low Fat Ham – Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	6.0002	75.95	3.3479	2.35	A	3.6494	75.31	2.49
2	6.0058	75.91	3.3458	2.50	B	4.0622	75.39	2.53
3	5.9992	76.05	3.3440	2.48	C	4.3697	75.64	2.43
4	5.9960	75.93	3.3482	2.32	D	4.5874	75.34	2.35
5	6.0016	75.98	3.3449	2.50	E	4.3857	76.05	2.24
6	5.9994	75.84	3.3422	2.46	F	3.7969	76.04	2.37
7	5.9945	75.88	3.3430	2.33	G	4.3782	75.86	2.22
8	6.0031	75.72	3.3468	2.20	H	4.4130	74.95	2.50
9	5.9972	75.83	3.3480	2.20	I	3.6778	75.92	2.10
10	6.0073	75.96	3.3438	2.36	J	4.1226	75.38	2.32
Mean		75.91		2.37	Mean		75.59	2.36
SD_r		0.0937		0.1144	SD_R		0.3701	0.1388
RSD_r		0.1234		4.8267	RSD_R		0.4897	5.8949

Table 3. Comparative Moisture and Fat Analysis Values of High Fat Ham Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9961	58.48	3.3466	16.40	A	4.4982	58.44	16.03
2	5.0050	58.44	3.3443	16.30	B	4.0837	58.38	16.41
3	4.9941	58.35	3.3421	16.42	C	3.9018	58.40	16.21
4	4.9958	58.37	3.3436	16.33	D	3.8314	58.05	16.42
5	5.0003	58.45	3.3438	16.44	E	4.0873	58.31	16.56
6	5.0007	58.37	3.3444	16.37	F	3.4584	58.28	16.29
7	4.9976	58.40	3.3476	16.25	G	4.2823	58.47	16.21
8	5.0042	58.42	3.3438	16.29	H	3.7693	58.15	16.44
9	5.0078	58.43	3.3447	16.27	I	3.7742	58.45	16.15
10	4.9977	58.41	3.3468	16.33	J	3.8725	57.38	15.97
Mean		58.41		16.34	Mean		58.23	16.26
SD_r		0.0411		0.0644	SD_R		0.3286	0.1899
RSD_r		0.0703		0.3944	RSD_R		0.5643	1.1675

Table 4. Comparative Moisture and Fat Analysis Values of High Fat Ham – Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9976	60.07	3.3472	16.09	A	4.4955	59.71	15.89
2	5.0061	60.14	3.3484	16.17	B	3.7876	60.06	16.16
3	4.9975	60.18	3.3427	16.21	C	4.2110	59.31	15.66
4	4.9992	60.09	3.3420	16.06	D	4.2291	59.45	15.98
5	4.9924	60.13	3.3438	15.94	E	3.5634	59.80	15.86
6	4.9946	60.29	3.3473	16.14	F	3.5143	59.95	15.87
7	4.9950	60.16	3.3457	16.03	G	3.7094	59.58	15.83
8	4.9949	60.21	3.3486	15.84	H	3.3293	59.67	16.17
9	5.0046	60.34	3.3488	15.95	I	4.0497	59.85	15.86
10	4.9944	60.18	3.3440	15.91	J	4.7705	59.09	16.01
Mean		60.18		16.03	Mean		59.65	15.93
SD_r		0.0836		0.1203	SD_R		0.2979	0.1554
RSD_r		0.1389		0.7501	RSD_R		0.4994	0.9755

and 16.03%, respectively (Tables 3 and 4). The CEM Smart Trac 5 System® yielded comparable results, with 58.23% and 59.65% mean total moisture values and 16.27% and 15.93% mean total fat values, respectively.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 1 and 2) for moisture content of low-fat ham (0.0598, 0.0798%) and diluted low-fat ham (0.0937, 0.1234%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for moisture content of low-fat ham (0.1990, 0.2678%) and diluted low-fat ham (0.3701, 0.4897%) samples. The between-laboratory RSD_R values were slightly higher than the within-laboratory RSD_r values, but collectively, the very low RSD_r and RSD_R values for moisture observed for the low-fat ham and diluted low-fat ham samples were likely due to the high level of moisture among these two sample types. Based on the RSD_r and RSD_R values for moisture, the AOAC reference method and CEM SMART Trac 5 System® method met AOAC's acceptability criteria of < 2.00% for low-fat ham and diluted low-fat ham samples.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 1 and 2) for fat content of low-fat ham (0.0925, 3.6391%) and diluted low-fat ham (0.1144, 4.8267%) samples trended similarly to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of low-fat ham (0.0929, 3.5721%) and diluted low-fat ham (0.1388, 5.8949%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values exceeded AOAC's acceptability criteria of 2.00% for low-fat ham and diluted low-fat ham samples and were therefore not acceptable for fat analysis. The high RSD_r and RSD_R values were likely due to the very small levels of fat in the

low-fat ham and diluted low-fat ham samples and the inherent variability of weighing minute amounts of fat to the fourth decimal place. The small amount of fat in the low-fat ham and diluted low-fat ham samples could have also reduced fat extraction efficiency in the AOAC method, thus making fat weights more variable (less precise) among samples.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 3 and 4) for moisture content of high-fat ham (0.0411, 0.0703%) and diluted high-fat ham (0.0836, 0.1389%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for moisture content of high-fat ham (0.3286, 0.5643%) and diluted high-fat ham (0.2979, 0.4994%) samples. Between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, likely due to the variation among different laboratory conditions and multiple individuals performing the analyses among the collaborative laboratories, but the RSD_r and RSD_R values were < 2.00% and therefore met AOAC's acceptability criteria for moisture for the AOAC reference method and the CEM SMART Trac 5 System® method for moisture analysis.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 3 and 4) for fat content of high-fat ham (0.0644, 0.3944%) and diluted high-fat ham (0.1203, 0.7501%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for fat content of high-fat ham (0.1899, 1.1675%) and diluted high-fat ham (0.1554, 0.9755%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values met AOAC's acceptability criteria of < 2.00% for the high-fat ham and diluted high-fat ham samples. The smaller RSD_r and RSD_R values for these samples, in comparison to the

low-fat ham samples, indicate that the AOAC reference method and CEM SMART Trac 5 System® method were more precise in determining fat content in the high-fat ham samples than in the low-fat ham samples. This can be attributed to the higher fat content in the high-fat ham samples, which produced less variability when handling samples with higher fat content.

Among all ham samples (low-fat, diluted low-fat, high-fat, diluted high-fat), the between-laboratory (CEM®) SD_R and RSD_R values were higher in comparison to within-laboratory (AOAC) SD_r and RSD_r values for moisture and fat. The higher SD_R and RSD_R (between-laboratory) values were likely a result of variation among different laboratory conditions and multiple individuals performing the analyses in comparison to the carefully controlled laboratory conditions and a single individual performing within-laboratory analyses.

The data indicates that the CEM SMART Trac 5 System® performed very well in recovering moisture and fat from the ham samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the ham samples, the percent recovery for moisture by the CEM SMART Trac 5 System® ranged from 99.12% to 99.69% while the percent recovery for fat by the CEM SMART Trac 5 System® ranged from 99.38% to 102.36% among all ham samples.

The CEM SMART Trac 5 System® was found to be acceptable based on AOAC's acceptability criteria ($RSD_R < 2.00\%$) for moisture analysis in all ham samples. It was also found to be acceptable for fat analysis for the high-fat ham and diluted high-

fat ham samples, but failed to meet the acceptability criteria for fat analysis for the low-fat ham and diluted low-fat ham samples.

Discussion of Fresh Pork Results

AOAC methods yielded 74.49% and 75.40% mean total moisture values and 2.26% and 2.18% mean total fat values for the low-fat pork and diluted low-fat pork samples, respectively (Tables 5 and 6). The CEM Smart Trac 5 System® yielded comparable results, with 74.19% and 75.24% mean total moisture values and 2.28% and 2.11% mean total fat values, respectively.

For the high-fat pork and diluted high-fat pork samples, AOAC methods yielded mean total moisture values of 60.07% and 61.54% and mean total fat values of 22.30% and 21.88%, respectively (Tables 7 and 8). The CEM Smart Trac 5 System® yielded comparable results, with 60.15% and 61.42% mean total moisture values and 22.44% and 21.73% mean total fat values, respectively.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 5 and 6) for moisture content of low-fat pork (0.0445, 0.0598%) and diluted low-fat pork (0.0370, 0.0491%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for moisture content of low-fat pork (0.2211, 0.2980%) and diluted low-fat pork (0.1106, 0.1470%) samples. The between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, but collectively, the very low RSD_r and RSD_R values for moisture observed for the low-fat pork and diluted low-fat pork samples were likely due

Table 5. Comparative Moisture and Fat Analysis Values of Low Fat Pork Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9844	74.41	3.3500	2.30	A	3.6650	73.91	2.29
2	5.0043	74.51	3.3472	2.24	B	3.9470	74.39	2.40
3	5.0003	74.50	3.3414	2.27	C	4.0674	74.37	2.31
4	4.9925	74.53	3.3450	2.29	D	4.3584	74.15	2.21
5	4.9944	74.49	3.3419	2.23	E	3.8669	74.33	2.22
6	5.0166	74.50	3.3432	2.29	F	3.6057	74.14	2.30
7	4.9872	74.55	3.4224	2.28	G	4.3342	74.33	2.26
8	5.0342	74.53	3.4239	2.22	H	3.8028	74.43	2.19
9	5.0142	74.49	3.3328	2.24	I	4.3173	74.13	2.33
10	5.0215	74.42	3.3402	2.20	J	3.9272	73.76	2.28
Mean		74.49		2.26	Mean		74.19	2.28
SD_r		0.0445		0.0345	SD_R		0.2211	0.0626
RSD_r		0.0598		1.5303	RSD_R		0.2980	2.7476

Table 6. Comparative Moisture and Fat Analysis Values of Low Fat Pork - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0388	75.45	3.3402	2.20	A	4.0884	75.16	2.13
2	5.0216	75.39	3.3595	2.22	B	4.4983	75.27	2.13
3	5.0495	75.40	3.3349	2.23	C	4.1782	75.37	2.13
4	5.0213	75.36	3.3524	2.22	D	4.5243	75.08	2.17
5	5.0057	75.33	3.3485	2.20	E	3.9424	75.15	2.10
6	5.0286	75.42	3.3495	2.16	F	3.5038	75.40	2.11
7	5.0179	75.43	3.3426	2.14	G	3.9015	75.19	2.17
8	5.0251	75.38	3.3432	2.16	H	3.9845	75.16	2.03
9	5.0119	75.38	3.3446	2.13	I	4.1816	75.37	2.08
10	5.0329	75.45	3.3412	2.18	J	3.9654	75.21	2.04
Mean		75.40		2.18	Mean		75.24	2.11
SD_r		0.0370		0.0361	SD_R		0.1106	0.0479
RSD_r		0.0491		1.6541	RSD_R		0.1470	2.2734

Table 7. Comparative Moisture and Fat Analysis Values of High Fat Pork Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0660	59.96	3.3707	22.27	A	4.2022	60.27	22.16
2	5.0899	60.09	3.3200	22.17	B	3.8433	60.43	22.30
3	5.1278	60.23	3.3555	22.30	C	3.8020	60.22	22.53
4	4.8629	60.16	3.3170	22.45	D	4.1862	59.72	22.50
5	5.3052	59.91	3.3467	22.37	E	3.9279	60.47	22.53
6	4.9273	59.83	3.3265	21.84	F	3.7719	59.94	22.88
7	5.0832	60.10	3.3490	22.39	G	3.9483	60.17	22.40
8	5.0719	60.08	3.3652	22.29	H	4.0830	60.34	22.08
9	4.9299	60.28	3.3553	22.64	I	4.1278	60.26	22.37
10	5.0880	60.07	3.3059	22.27	J	4.1576	59.72	22.64
Mean		60.07		22.30	Mean		60.15	22.44
SD_r		0.1410		0.2065	SD_R		0.2713	0.2327
RSD_r		0.2348		0.9258	RSD_R		0.4510	1.0372

Table 8. Comparative Moisture and Fat Analysis Values of High Fat Pork - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.5890	61.66	3.3876	21.81	A	4.0962	61.80	21.46
2	5.1292	61.37	3.3634	21.89	B	4.0751	61.21	22.02
3	4.1887	61.52	3.3566	21.79	C	4.5518	61.41	22.04
4	4.8817	61.71	3.3329	22.06	D	4.0726	61.09	21.90
5	5.2047	61.48	3.3153	21.55	E	4.1692	61.52	21.53
6	5.1786	61.38	3.3229	22.49	F	3.6203	61.58	21.65
7	5.1946	61.72	3.3180	21.73	G	4.1204	61.43	21.61
8	5.0325	61.53	3.3491	21.69	H	4.2085	61.14	21.69
9	5.1671	61.52	3.3364	22.05	I	3.8355	61.57	21.67
10	5.0143	61.54	3.3883	21.70	J	4.0487	61.40	21.76
Mean		61.54		21.88	Mean		61.42	21.73
SD_r		0.1219		0.2679	SD_R		0.2197	0.1969
RSD_r		0.1981		1.2247	RSD_R		0.3577	0.9058

to the high level of moisture among these two sample types. Based on the RSD_r and RSD_R values for moisture, the AOAC reference method and CEM SMART Trac 5 System® method met AOAC's acceptability criteria of < 2.00% for low-fat pork and diluted low-fat pork samples.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 5 and 6) for fat content of low-fat pork (0.0345, 1.5303%) and diluted low-fat pork (0.0361, 1.6541%) samples were lower than between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of low-fat pork (0.0626, 2.7476%) and diluted low-fat pork (0.0479, 2.2734%) samples.

Within-laboratory RSD_r values met AOAC's acceptability criteria (RSD_r < 2.00%) for fat in the low-fat pork and diluted low-fat pork samples, but the between-laboratory RSD_R values for fat exceeded AOAC's acceptability criteria (RSD_R < 2.00%) and was therefore not acceptable for fat for the low-fat pork and diluted low-fat pork samples. The higher RSD_R values indicate that the CEM SMART Trac 5 System® was less precise than the AOAC reference method in determining fat content, likely due to the very small levels of fat in the low-fat pork and diluted low-fat pork samples and the greater variability that existed between laboratories and analysts. Sample preparation and handling would have also been critical, as small variations in the sample could produce large data inconsistencies when fat content is at such a low level.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 7 and 8) for moisture content of high-fat pork (0.1410, 0.2348%) and diluted high-fat pork (0.1219, 0.1981%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R

(reproducibility) values for moisture content of high-fat pork (0.2713, 0.4510%) and diluted high-fat pork (0.2197, 0.3577%) samples. Between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, likely due to the variation among different laboratory conditions and multiple individuals performing the analyses among the collaborative laboratories, but the RSD_r and RSD_R values were < 2.00% and therefore met AOAC's acceptability criteria for moisture for the AOAC reference method and the CEM SMART Trac 5 System® method for moisture analysis.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 7 and 8) for fat content of high-fat pork (0.2065, 0.9258%) and diluted high-fat pork (0.2679, 1.2247%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for fat content of high-fat pork (0.2327, 1.0372%) and diluted high-fat pork (0.1969, 0.9058%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values met AOAC's acceptability criteria of < 2.00% for the high-fat ham and diluted high-fat ham samples. The smaller RSD_r and RSD_R values for these samples, in comparison to the low-fat pork samples, indicate that the AOAC reference method and CEM SMART Trac 5 System® method were more precise in determining fat content in the high-fat pork samples than in the low-fat pork samples. This can be attributed to the higher fat content in the high-fat pork samples, which produced less variability when handling samples with higher fat content.

Among all pork samples, with the exception of diluted high-fat pork samples, the between-laboratory SD_R and RSD_R values were higher in comparison to within-laboratory SD_r and RSD_r values for moisture and fat. The higher SD_R and RSD_R

(reproducibility) values were likely a result of variation among different laboratory conditions and multiple individuals performing the analyses in comparison to the carefully controlled laboratory conditions and a single individual performing within-laboratory analyses.

The data indicates that the CEM SMART Trac 5 System® performed very well in recovering moisture and fat from the pork samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the pork samples, the percent recovery for moisture by the CEM SMART Trac 5 System® ranged from 99.60% to 100.13% while the percent recovery for fat by the CEM SMART Trac 5 System® ranged from 99.31% to 100.88% among all pork samples.

The CEM SMART Trac 5 System was found to be acceptable based on AOAC's acceptability criteria ($RSD_R < 2.00\%$) for moisture analysis in all pork samples. It was also found to be acceptable for fat analysis for the high-fat pork and diluted high-fat pork samples, but failed to meet the acceptability criteria for fat analysis for the low-fat pork and diluted low-fat pork samples.

Discussion of Fresh Beef Results

AOAC methods yielded 67.31% and 68.86% mean total moisture values and 11.23% and 10.63% mean total fat values for the low-fat beef and diluted low-fat beef samples, respectively (Tables 9 and 10). The CEM Smart Trac 5 System® yielded very comparable results, with 67.11% and 68.58% mean total moisture values and 11.30% and 10.73% mean total fat values, respectively.

Table 9. Comparative Moisture and Fat Analysis Values of Low Fat Beef Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9901	67.30	3.3971	11.19	A	3.9731	66.57	11.51
2	5.0514	67.24	3.3245	11.35	B	4.3489	67.09	11.22
3	4.9766	67.23	3.3283	10.96	C	4.0628	67.13	11.40
4	5.0150	67.15	3.3273	11.14	D	4.3097	66.95	11.36
5	5.0094	67.40	3.3291	11.31	E	4.2080	67.41	11.16
6	4.9682	67.25	3.3217	11.30	F	3.8955	67.16	11.12
7	5.0408	67.15	3.3243	11.24	G	4.6510	67.54	11.20
8	5.0347	67.28	3.3220	11.07	H	4.2399	66.97	11.41
9	5.0050	67.48	3.3265	11.21	I	3.7519	67.18	11.28
10	4.9835	67.60	3.3216	11.49	J	4.0059	67.10	11.33
Mean		67.31		11.23	Mean		67.11	11.30
SD_r		0.1444		0.1494	SD_R		0.2625	0.1245
RSD_r		0.2145		1.3309	RSD_R		0.3911	1.1018

Table 10. Comparative Moisture and Fat Analysis Values of Low Fat Beef - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9946	68.87	3.3206	10.65	A	4.0521	68.37	10.74
2	5.0011	68.30	3.3222	10.72	B	4.9856	68.94	10.37
3	4.9430	69.00	3.3212	10.77	C	4.3503	68.64	10.60
4	4.9965	68.92	3.3191	10.68	D	4.3530	68.26	10.80
5	4.9838	69.01	3.3301	10.53	E	3.7710	68.96	10.87
6	4.9680	68.89	3.3229	10.62	F	3.7252	68.63	10.64
7	5.0042	68.93	3.3255	10.68	G	3.7224	68.91	10.73
8	4.9871	68.90	3.3226	10.42	H	3.7017	68.70	10.74
9	5.0051	68.87	3.3243	10.81	I	4.2452	68.36	10.85
10	5.0107	68.86	3.3315	10.44	J	4.3877	68.02	10.98
Mean		68.86		10.63	Mean		68.58	10.73
SD_r		0.2016		0.1331	SD_R		0.3183	0.1687
RSD_r		0.2928		1.2516	RSD_R		0.4642	1.5720

For the high-fat beef and diluted high-fat beef samples, AOAC methods yielded mean total moisture values of 57.84% and 60.16% and mean total fat values of 26.56% and 25.44%, respectively (Tables 11 and 12). The CEM Smart Trac 5 System® yielded very comparable results, with 57.96% and 59.59% mean total moisture values and 26.55% and 25.43% mean total fat values, respectively.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 9 and 10) for moisture content of low-fat beef (0.1444, 0.2145%) and diluted low-fat beef (0.2016, 0.2928%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of low-fat beef (0.2625, 0.3911%) and diluted low-fat beef (0.3183, 0.4642%) samples. The within-laboratory RSD_r values and between-laboratory RSD_R values for moisture were very low, with between-laboratory RSD_R values being slightly higher, likely due to the variation among different laboratory conditions and multiple analysts among the collaborative laboratories. Based on the RSD_r and RSD_R values for moisture, the AOAC reference method and CEM SMART Trac 5 System® method met AOAC's acceptability criteria of < 2.00% for moisture analysis in low-fat beef and diluted low-fat beef samples.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 9 and 10) for fat content of low-fat beef (0.1494, 1.3309%) and diluted low-fat beef (0.1331, 1.2516%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of low-fat beef (0.1245, 1.1018%) and diluted low-fat beef (0.1687, 1.5720%) samples. The within-laboratory RSD_r and between-laboratory RSD_R values for fat analysis were within AOAC's acceptability criteria

Table 11. Comparative Moisture and Fat Analysis Values of High Fat Beef Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9973	57.82	3.3419	26.85	A	4.3275	57.78	26.52
2	5.0031	57.55	3.3345	26.69	B	4.6886	58.30	26.92
3	4.9979	57.96	3.3882	26.78	C	3.7874	57.56	26.45
4	5.0029	57.71	3.3839	26.76	D	4.2676	58.31	25.95
5	5.0003	57.66	3.3891	26.70	E	3.9380	57.92	26.87
6	4.9997	57.89	3.3783	26.34	F	3.6041	58.01	26.76
7	4.9998	58.15	3.3244	26.23	G	3.7313	58.34	26.56
8	4.9989	57.80	3.3570	26.06	H	4.5678	57.63	26.55
9	5.0032	57.82	3.3291	27.02	I	3.9628	57.52	26.90
10	5.0058	58.02	3.3462	26.21	J	4.2296	58.25	25.99
Mean		57.84		26.56	Mean		57.96	26.55
SD_r		0.1783		0.3256	SD_R		0.3275	0.3481
RSD_r		0.3083		1.2257	RSD_R		0.5651	1.3112

Table 12. Comparative Moisture and Fat Analysis Values of High Fat Beef - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.3424	60.28	3.3391	26.02	A	4.7892	59.16	25.22
2	5.0626	59.35	3.3379	25.75	B	4.4993	59.81	25.44
3	4.8691	59.79	3.3187	25.40	C	4.4521	58.97	25.64
4	5.1590	60.31	3.3976	25.28	D	4.5670	59.58	24.92
5	5.0889	60.14	3.3666	25.55	E	3.7458	60.05	25.51
6	5.0110	60.88	3.3788	25.63	F	3.6157	59.69	25.88
7	4.8938	60.31	3.3335	25.32	G	3.9466	59.98	24.81
8	4.9029	60.33	3.3504	25.57	H	3.9156	59.91	25.28
9	4.9475	60.08	3.3674	25.02	I	4.0508	59.31	25.63
10	5.2606	60.16	3.3810	24.84	J	4.2901	59.39	26.00
Mean		60.16		25.44	Mean		59.59	25.43
SD_r		0.3939		0.3447	SD_R		0.3670	0.3843
RSD_r		0.6547		1.3552	RSD_R		0.6159	1.5109

(<2.00%) for the low-fat beef and diluted low-fat beef samples.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 11 and 12) for moisture content of high-fat beef (0.1783, 0.3083%) and diluted high-fat beef (0.3939, 0.6547%) samples were similar to between-laboratory SD_r and RDS_R (reproducibility) values for moisture content of high-fat beef (0.3275, 0.5651%) and diluted high-fat beef (0.3670, 0.6159%) samples. Between-laboratory RSD_R values and within-laboratory RSD_r values were < 2.00% and therefore met AOAC's acceptability criteria for moisture for the AOAC reference method and the CEM SMART Trac 5 System® method for moisture analysis.

Within-laboratory SD_r and RSD_r (repeatability) values (Tables 11 and 12) for fat content of high-fat beef (0.3256, 1.2257%) and diluted high-fat beef (0.3447, 1.3552%) samples were similar to between-laboratory SD_R and RSD_R (reproducibility) values for fat content of high-fat beef (0.3481, 1.3112%) and diluted high-fat beef (0.3843, 1.5109%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values for fat analysis met AOAC's acceptability criteria of < 2.00% for the high-fat beef and diluted high-fat beef samples.

In comparing the RSD_r and RSD_R values for fat analysis between the four beef samples (low-fat beef, diluted low-fat beef, high-fat beef, and diluted high-fat beef), there did not appear to be as great of a difference between the low-fat RSD (RSD_r and RSD_R) values as compared to the low-fat RSD (RSD_r and RSD_R) values observed for other meat sample types (i.e. ham samples, pork samples). For example, the RSD_r value for fat analysis of the low-fat beef sample (1.3309%) was similar to the RSD_r value of

the high-fat beef sample (1.2257%). In comparison to the ham samples and fresh pork samples, a much greater difference between the low-fat RSD_r fat values (i.e. low-fat pork RSD_r = 1.5303%) was much greater than the high-fat RSD_r fat values (i.e. high-fat pork RSD_r = 0.9258%). This difference in trend may indicate that the beef samples were more variable in nature than other meat samples (such as ham or fresh pork), with one possible explanation being the presence of connective tissue in the beef samples.

Among all beef samples (low-fat, diluted low-fat, high-fat, diluted high-fat), the between-laboratory SD_R and RSD_R values were higher in comparison to within-laboratory SD_r and RSD_r values for moisture and fat, with the exception of RSD_R values for fat analysis in the low-fat beef samples. The RSD_R value for fat analysis of the low-fat beef samples was slightly lower than the RSD_r value for fat analysis, indicating that the CEM SMART Trac 5 Systems® among collaborative laboratories performed with slightly greater precision than AOAC within-laboratory methods.

The data indicates that the CEM SMART Trac 5 System® performed very well in recovering moisture and fat from the beef samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the beef samples, the percent recovery for moisture by the CEM SMART Trac 5 System® ranged from 99.05% to 100.21% while the percent recovery for fat by the CEM SMART Trac 5 System® ranged from 99.96% to 100.94% among all beef samples.

The CEM SMART Trac 5 System® was found to be acceptable based on the AOAC's acceptability criteria ($RSD_R < 2.00\%$) for moisture and fat analysis in all beef samples.

Discussion of Fresh Chicken Results

AOAC methods yielded 74.99% and 75.90% mean total moisture values and 2.91% and 2.79% mean total fat values for the low-fat chicken and diluted low-fat chicken samples, respectively (Tables 13 and 14). The CEM Smart Trac 5 System® yielded very comparable results, with 74.66% and 75.63% mean total moisture values and 2.92% and 2.73% mean total fat values, respectively.

For the high-fat chicken, AOAC methods yielded a mean total moisture value of 61.62% and a mean total fat value of 23.30% (Table 15). Moisture and fat data were not obtained from the collaborative laboratories' CEM SMART Trac 5 Systems®. The high-fat chicken sample results from AOAC methods were used only for establishing a reference value in order to determine the appropriate NMR signal for that specific sample type (i.e. "chicken") and to establish a standard curve for fat determination of that specific sample type on the CEM SMART Trac 5 Systems®. Diluted high-fat chicken samples were not analyzed by AOAC methods or by the CEM SMART Trac 5 Systems® among collaborative laboratories.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 13 and 14) for moisture content of low-fat chicken (0.0388, 1.3327%) and diluted low-fat chicken (0.0382, 1.3695%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of low-fat chicken (0.1552, 0.2079%) and diluted low-fat chicken (0.2225, 0.2942%) samples. The between-laboratory RSD_R values were slightly higher than the within-laboratory RSD_r values, but both values were within AOAC's acceptability criteria of < 2.00% and were therefore

Table 13. Comparative Moisture and Fat Analysis Values of Low Fat Chicken Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0029	75.02	3.3436	2.92	A	4.6249	74.75	2.98
2	5.0016	74.85	3.3460	2.99	B	3.9349	74.76	2.97
3	4.9983	74.96	3.3423	2.88	C	3.8794	74.76	2.98
4	5.1334	74.95	3.3436	2.93	D	2.9766	74.68	2.84
5	5.0057	74.94	3.3467	2.93	E	4.2604	74.66	2.91
6	5.0315	75.02	3.3452	2.85	F	3.8469	74.80	2.87
7	4.9689	75.11	3.3458	2.90	G	3.5562	74.81	2.90
8	4.9765	75.01	3.3447	2.88	H	3.9174	74.45	2.91
9	4.9890	75.03	3.3479	2.93	I	4.2915	74.55	2.93
10	5.0354	74.99	3.3492	2.89	J	4.1324	74.36	2.93
Mean		74.99		2.91	Mean		74.66	2.92
SD_r		0.0668		0.0388	SD_R		0.1552	0.0464
RSD_r		0.0891		1.3327	RSD_R		0.2079	1.5873

Table 14. Comparative Moisture and Fat Analysis Values of Low Fat Chicken - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.9951	76.04	3.3461	2.80	A	3.9567	75.50	2.84
2	6.0088	76.01	3.3491	2.82	B	4.2728	75.68	2.75
3	6.0264	76.01	3.3458	2.80	C	3.8645	75.99	2.70
4	6.0252	76.03	3.3431	2.85	D	3.9658	75.44	2.63
5	6.0007	76.02	3.3460	2.80	E	4.3841	75.63	2.65
6	6.0043	75.97	3.3455	2.77	F	3.9554	75.79	2.71
7	6.0025	75.99	3.3473	2.82	G	3.7329	75.93	2.73
8	5.9965	75.20	3.3446	2.73	H	3.7147	75.41	2.82
9	6.0017	75.84	3.3435	2.80	I	4.4424	75.61	2.71
10	5.9950	75.93	3.3481	2.73	J	3.7582	75.32	2.72
Mean		75.90		2.79	Mean		75.63	2.73
SD_r		0.2541		0.0382	SD_R		0.2225	0.0655
RSD_r		0.3348		1.3695	RSD_R		0.2942	2.4037

Table 15. Reference Moisture and Fat Analysis Values of High Fat Chicken Samples Analyzed By AOAC Method 950.46 and AOAC Method 960.39.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0024	61.70	3.3446	23.65	A	n/a	n/a	n/a
2	5.0027	61.56	3.3469	23.52	B	n/a	n/a	n/a
3	4.9975	61.70	3.3455	23.32	C	n/a	n/a	n/a
4	4.9938	61.74	3.3475	23.66	D	n/a	n/a	n/a
5	5.0044	61.49	3.3464	22.73	E	n/a	n/a	n/a
6	5.0001	61.65	3.3433	23.09	F	n/a	n/a	n/a
7	5.0017	61.63	3.3448	23.16	G	n/a	n/a	n/a
8	4.9936	61.56	3.3490	23.78	H	n/a	n/a	n/a
9	5.0078	61.50	3.3429	22.97	I	n/a	n/a	n/a
10	5.0007	61.62	3.3476	23.13	J	n/a	n/a	n/a
Mean		61.62		23.30				
SD_r		0.0868		0.3428				
RSD_r		0.1409		1.4713				

acceptable for moisture analysis. Collectively, the very low RSD_r and RSD_R values for moisture observed for the low-fat chicken and diluted low-fat chicken samples were likely due to the high level of moisture among these two sample types.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 13 and 14) for fat content of low-fat chicken (0.0388. 1.3327%) and diluted low-fat chicken (0.0382, 1.3695%) samples trended similarly to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of low-fat chicken (0.0464, 1.5873%) and diluted low-fat chicken (0.0655, 2.4037%) samples. Within-laboratory RSD_r values for low-fat chicken and diluted low-fat chicken and between-laboratory RSD_R value for low-fat chicken were within AOAC's acceptability criteria of < 2.00% for fat analysis. However, the between-laboratory RSD_R value for diluted low-fat chicken exceeded AOAC's acceptability criteria of 2.00% and was therefore not acceptable for fat analysis. The high RSD_R value for the between-laboratory fat analysis of the diluted low-fat chicken samples was due to the very small amount of fat in the sample and the variation among different laboratory conditions and multiple analysts. It was not unexpected to see a high RSD_R value in the diluted low-fat chicken sample, as this trend was observed in other meat samples in which fat content was very low (i.e. low-fat ham, low-fat pork).

Within-laboratory SD_r and RSD_r (repeatability) values (Table 15) for moisture content (0.0868, 0.1409%) and fat content (0.3428, 1.4713%) of high-fat chicken samples were acceptable based on AOAC's acceptability criteria of RSD_r < 2.00%. Moisture and fat data were not obtained from the collaborative laboratories' CEM

SMART Trac 5 Systems® for high-fat chicken, so between-laboratory reproducibility cannot be determined. Moisture and fat data were also not obtained from AOAC methods or from the collaborative laboratories' CEM SMART Trac 5 Systems® for diluted high-fat chicken.

The data indicates that the CEM SMART Trac 5 System® performed well in recovering moisture and fat from the low-fat chicken and diluted low-fat chicken samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the chicken samples, the percent recovery for moisture by the CEM SMART Trac 5 System® ranged from 99.56% to 99.64% while the percent recovery for fat by the CEM SMART Trac 5 System® ranged from 97.85% to 100.34%. Based on this data, the percent fat recovery by the CEM SMART Trac 5 System® was more variable for the low-fat chicken and diluted low-fat chicken samples compared to other meat samples analyzed. This is most likely due to the very small amount of fat in the samples.

The CEM SMART Trac 5 System® was found to be acceptable for moisture analysis for low-fat chicken and diluted low-fat chicken samples based on AOAC's acceptability criteria ($RSD_R < 2.00\%$). The CEM SMART Trac 5 System® was also acceptable for fat analysis for low-fat chicken samples, but was not acceptable for fat analysis for diluted low-fat chicken samples due to exceeding AOAC's acceptability criteria.

Discussion of Frankfurter Results

AOAC methods yielded a 74.97% mean total moisture value and a 2.68% mean total fat value for the low-fat frankfurter samples, respectively, based on three replicate determinations (Table 16). Moisture and fat data were not obtained from the collaborative laboratories' CEM SMART Trac 5 Systems®. The low-fat frankfurter sample results from AOAC methods were used only for establishing a reference value in order to determine the appropriate NMR signal for that specific sample type (i.e. "frankfurter") and to establish a standard curve for fat determination of that specific sample type on the CEM SMART Trac 5 Systems®. Diluted low-fat frankfurter samples were not analyzed by AOAC methods or by the CEM SMART Trac 5 Systems® among collaborative laboratories.

For the high-fat and diluted high-fat frankfurter samples, AOAC methods yielded mean total moisture values of 54.03% and 55.54% and mean total fat values of 29.79% and 28.80%, respectively (Tables 17 and 18). The CEM Smart Trac 5 System® yielded comparable results, with 53.64% and 55.52% mean total moisture values and 29.93% and 28.78% mean total fat values, respectively.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 17 and 18) for moisture content of high-fat frankfurter (0.0722, 0.1337%) and diluted high-fat frankfurter (0.0787, 0.1416%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of high-fat frankfurter (0.2704, 0.5041%) and diluted high-fat frankfurter (0.3709, 0.6680%) samples. The between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, but overall,

Table 16. Reference Moisture and Fat Analysis Values of Low Fat Frankfurter Samples Analyzed By AOAC Method 950.46 and AOAC Method 960.39.

TAMU Lab Results For AOAC Methods (For Calibration of SMART Trac System instruments)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.9989	75.02	3.3467	2.6832	A	n/a	n/a	n/a
2	6.0008	75.31	3.3460	2.6509	B	n/a	n/a	n/a
3	6.0037	74.59	3.3492	2.6962	C	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	D	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	E	n/a	n/a	n/a
6	n/a	n/a	n/a	n/a	F	n/a	n/a	n/a
7	n/a	n/a	n/a	n/a	G	n/a	n/a	n/a
8	n/a	n/a	n/a	n/a	H	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	I	n/a	n/a	n/a
10	n/a	n/a	n/a	n/a	J	n/a	n/a	n/a
Mean		74.97		2.6768				
SD_r		0.3638		0.0233				
RSD_r		0.4852		0.8705				

Table 17. Comparative Moisture and Fat Analysis Values of High Fat Frankfurter Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0070	53.94	3.3437	29.85	A	3.8665	53.63	30.00
2	5.0003	54.07	3.3438	29.65	B	3.9047	53.70	29.83
3	5.0010	54.19	3.3442	29.79	C	4.1362	53.27	29.84
4	5.0070	53.98	3.3424	29.73	D	4.3709	53.27	30.31
5	4.9994	54.00	3.3442	29.95	E	3.9357	53.85	29.85
6	5.0027	54.07	3.3426	29.93	F	4.3307	53.35	29.91
7	4.9968	53.96	3.3410	30.03	G	3.9830	53.92	29.76
8	4.9992	54.07	3.3454	29.73	H	3.9218	53.53	29.86
9	4.9951	53.98	3.3434	29.63	I	3.4103	53.97	29.83
10	4.9995	54.03	3.3439	29.66	J	4.1807	53.87	30.06
Mean		54.03		29.79	Mean		53.64	29.93
SD_r		0.0722		0.1397	SD_R		0.2704	0.1615
RSD_r		0.1337		0.4688	RSD_R		0.5041	0.5396

Table 18. Comparative Moisture and Fat Analysis Values of High Fat Frankfurter - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	4.9975	55.60	3.3459	28.53	A	4.4579	55.40	28.92
2	5.0059	55.47	3.3443	29.16	B	4.3911	54.87	28.97
3	5.0043	55.45	3.3438	28.52	C	4.4177	54.94	28.93
4	5.0036	55.53	3.3474	28.72	D	4.3976	55.70	28.67
5	5.0007	55.48	3.3470	28.76	E	4.1753	55.84	28.49
6	5.0032	55.54	3.3444	28.91	F	4.2986	55.67	28.88
7	4.9936	55.60	3.3434	28.89	G	3.9425	56.00	28.70
8	4.9973	55.71	3.3457	28.76	H	4.4674	55.75	28.98
9	5.0036	55.51	3.3417	29.02	I	3.5102	55.42	28.82
10	5.0006	55.54	3.3427	28.72	J	3.9421	55.64	28.46
Mean		55.54		28.80	Mean		55.52	28.78
SD_r		0.0787		0.2011	SD_R		0.3709	0.1929
RSD_r		0.1416		0.6984	RSD_R		0.6680	0.6701

the very low RSD_r and RSD_R values met AOAC's acceptability criteria (< 2.00%) for moisture analysis and reflect a great level of precision for moisture analysis among the AOAC method and CEM Smart Trac 5 System® method.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 17 and 18) for fat content of high-fat frankfurter (0.1397, 0.4688%) and diluted high-fat frankfurter (0.2011, 0.6984%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of high-fat frankfurter (0.1615, 0.5396%) and diluted high-fat frankfurter (0.1929, 0.6701%) samples. The low RSD_r and RSD_R values met AOAC's acceptability criteria (< 2.00%) for fat analysis and reflect a great level of precision among the AOAC method and CEM Smart Trac 5 System® method for fat analysis in frankfurter samples.

The data indicates that the CEM Smart Trac 5 System® performed well in recovering moisture and fat from the frankfurter samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the frankfurter samples, the percent recovery for moisture by the CEM Smart Trac 5 System® ranged from 99.28% to 99.96% while the percent recovery for fat by the CEM Smart Trac 5 System® ranged from 99.93% to 100.47% among the frankfurter samples.

The CEM SMART Trac 5 System® was found to be acceptable for moisture analysis and fat analysis for high-fat frankfurter samples based on AOAC's acceptability criteria (RSD_R < 2.00%).

Discussion of Fresh Turkey Results

AOAC methods yielded 74.67% and 75.43% mean total moisture values and 1.00% and 0.74% mean total fat values for the low-fat turkey samples and diluted low-fat turkey samples, respectively (Tables 19 and 20). The CEM Smart Trac 5 System® yielded very comparable results, with 74.37% and 75.16% mean total moisture values and 0.95% and 0.87% mean total fat values, respectively.

For the high-fat turkey samples, AOAC methods yielded a mean total moisture value of 65.79% and a mean total fat value of 18.52% for the high-fat turkey samples based on three replicate determinations (Table 21). Moisture and fat data were not obtained from the collaborative laboratories' CEM SMART Trac 5 Systems® for high-fat turkey samples. The high-fat turkey sample results from AOAC methods were used only for establishing a reference value in order to determine the appropriate NMR signal for that specific sample type (i.e. "turkey") and to establish a standard curve for fat determination of that specific sample type on the CEM SMART Trac 5 Systems®. Diluted high-fat turkey samples were not analyzed by AOAC methods or by the CEM SMART Trac 5 Systems® among collaborative laboratories.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 19 and 20) for moisture content of low-fat turkey (0.0564, 0.0755%) and diluted low-fat turkey (0.0687, 0.0911) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of low-fat turkey (0.2708, 0.3641%) and diluted low-fat turkey (0.2399, 0.3191%) samples. The between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, which is a trend that has

Table 19. Comparative Moisture and Fat Analysis Values of Low Fat Turkey Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.9992	74.70	3.3457	0.99	A	3.6998	74.98	0.89
2	6.0014	74.61	3.3489	0.99	B	3.5556	74.18	1.05
3	6.0003	74.61	3.3446	1.05	C	4.5618	74.31	0.95
4	5.9989	74.70	3.3477	1.07	D	5.3125	74.38	0.91
5	5.9942	74.64	3.3458	1.03	E	3.9651	74.21	1.03
6	5.9943	74.80	3.3454	1.04	F	3.9223	74.43	0.91
7	6.0020	74.65	3.3447	0.94	G	3.7962	74.68	0.92
8	6.0009	74.68	3.3451	0.95	H	3.8354	74.18	1.11
9	6.0035	74.63	3.3465	0.96	I	3.6082	74.28	0.99
10	6.0034	74.67	3.3442	0.98	J	4.2368	74.09	0.73
Mean		74.67		1.00	Mean		74.37	0.95
SD_r		0.0564		0.0445	SD_R		0.2708	0.1052
RSD_r		0.0755		4.4407	RSD_R		0.3641	11.0902

Table 20. Comparative Moisture and Fat Analysis Values of Low Fat Turkey - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.9944	75.39	3.3450	0.81	A	4.5629	75.40	0.92
2	6.0044	75.49	3.3469	0.74	B	4.1247	75.07	0.80
3	6.0016	75.45	3.3464	0.76	C	5.6173	74.99	0.86
4	6.0002	75.41	3.3464	0.73	D	5.2797	75.29	0.88
5	6.0005	75.27	3.3443	0.77	E	4.2696	75.23	0.89
6	6.0035	75.43	3.3472	0.76	F	4.1348	75.47	0.86
7	5.9951	75.53	3.3472	0.71	G	4.5045	75.40	0.87
8	5.9960	75.43	3.3426	0.71	H	4.1187	75.06	1.00
9	5.9989	75.43	3.3456	0.70	I	3.8006	75.03	0.79
10	5.9976	75.43	3.3482	0.73	J	4.1246	74.69	0.85
Mean		75.43		0.74	Mean		75.16	0.87
SD_r		0.0687		0.0342	SD_R		0.2399	0.0594
RSD_r		0.0911		4.6280	RSD_R		0.3191	6.8124

Table 21. Reference Moisture and Fat Analysis Values of High Fat Turkey Samples Analyzed By AOAC Method 950.46 and AOAC Method 960.39.

TAMU Lab Results For AOAC Methods (For Calibration of SMART Trac System instruments)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0051	65.81	3.3482	18.61	A	n/a	n/a	n/a
2	4.9993	65.64	3.3431	18.45	B	n/a	n/a	n/a
3	4.9958	65.92	3.3428	18.51	C	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	D	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	E	n/a	n/a	n/a
6	n/a	n/a	n/a	n/a	F	n/a	n/a	n/a
7	n/a	n/a	n/a	n/a	G	n/a	n/a	n/a
8	n/a	n/a	n/a	n/a	H	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	I	n/a	n/a	n/a
10	n/a	n/a	n/a	n/a	J	n/a	n/a	n/a
Mean		65.79		18.52				
SD_r		0.1423		0.0821				
RSD_r		0.2164		0.4433				

been observed in almost all other meat samples. Overall, the RSD_r and RSD_R values were very low due to the very high moisture content in the low-fat turkey and diluted low-fat turkey samples and met AOAC's acceptability criteria of <2.00%.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 19 and 20) for fat content of low-fat turkey (0.0445, 4.4407%) and diluted low-fat turkey (0.0342, 4.6280%) samples trended similarly to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of low-fat turkey (0.1052, 11.0902%) and diluted low-fat turkey (0.0594, 6.8124%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values exceeded AOAC's acceptability criteria of 2.00% for low-fat turkey and diluted low-fat turkey samples and were therefore not acceptable for fat analysis. The very high RSD_r and RSD_R values were likely due to the very small levels of fat in the low-fat turkey and diluted low-fat turkey samples, as these samples contained the least amount of fat of all meat samples analyzed. High RSD_r and RSD_R values were also seen in other very low-fat meat samples, such as low-fat ham, low-fat pork, and low-fat beef, so the high RSD_r and RSD_R values observed for fat analysis in the low-fat turkey samples followed the same trend as other low-fat meat samples and were therefore expected.

The data indicates that the CEM Smart Trac 5 System® performed well in recovering moisture from the turkey samples (see table on page 108), but that its performance was much more variable in recovering fat from the turkey samples. Using AOAC method mean values as the reference values for moisture and fat in the turkey samples, the percent recovery for moisture by the CEM Smart Trac 5 System® ranged

from 99.60% to 99.64% while the percent recovery for fat by the CEM Smart Trac 5 System® ranged from 95.00% to 117.57% among the low-fat turkey and diluted low-fat turkey samples.

The CEM Smart Trac 5 System® was found to be acceptable based on AOAC's acceptability criteria ($RSD_R < 2.00\%$) for moisture analysis in the low-fat turkey and diluted low-fat turkey samples. However, CEM Smart Trac 5 System® was found to be not acceptable for fat analysis in low-fat turkey and diluted low-fat turkey samples based on the very high RSD_R values for these samples.

Discussion of Sausage Results

AOAC methods yielded a 72.87% mean total moisture value and a 2.30% mean total fat value for the low-fat sausage samples, based on three replicate determinations (Table 22). Moisture and fat data were not obtained from the collaborative laboratories' CEM SMART Trac 5 Systems®. The low-fat sausage sample results from AOAC methods were used only for establishing a reference value in order to determine the appropriate NMR signal for that specific sample type (i.e. "sausage") and to establish a standard curve for fat determination of that specific sample type on the CEM SMART Trac 5 Systems®. Diluted low-fat sausage samples were not analyzed by AOAC methods or by the CEM SMART Trac 5 Systems® among collaborative laboratories.

For the high-fat sausage and diluted high-fat sausage, AOAC methods yielded mean total moisture values of 55.08% and 57.12% and mean total fat values of 27.92% and 26.99%, respectively (Tables 23 and 24). The CEM Smart Trac 5 System® yielded

Table 22. Reference Moisture and Fat Analysis Values of Low Fat Sausage Samples Analyzed By AOAC Method 950.46 and AOAC Method 960.39.

TAMU Lab Results For AOAC Methods (For Calibration of SMART Trac System instruments)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.9958	72.88	3.3418	2.29	A	n/a	n/a	n/a
2	6.0032	72.88	3.3472	2.29	B	n/a	n/a	n/a
3	6.0069	72.84	3.3466	2.31	C	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	D	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	E	n/a	n/a	n/a
6	n/a	n/a	n/a	n/a	F	n/a	n/a	n/a
7	n/a	n/a	n/a	n/a	G	n/a	n/a	n/a
8	n/a	n/a	n/a	n/a	H	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	I	n/a	n/a	n/a
10	n/a	n/a	n/a	n/a	J	n/a	n/a	n/a
Mean		72.87		2.30				
SD_r		0.0236		0.0096				
RSD_r		0.0324		0.4171				

Table 23. Comparative Moisture and Fat Analysis Values of High Fat Sausage Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0133	55.25	3.3429	27.84	A	4.2446	55.14	28.08
2	4.9879	54.99	3.3446	27.95	B	4.5464	54.99	27.96
3	5.0526	54.95	3.3441	28.25	C	4.6631	54.83	28.02
4	4.9907	55.08	3.3457	28.04	D	4.7448	54.78	27.84
5	5.0060	54.97	3.3412	27.87	E	3.6834	55.04	27.88
6	4.9831	55.16	3.3465	27.86	F	4.0932	55.08	27.10
7	5.0426	55.22	3.3424	27.68	G	3.9364	55.13	28.08
8	4.9466	54.97	3.3421	28.05	H	3.9166	55.24	27.74
9	4.9677	55.13	3.3425	27.70	I	3.7842	54.95	28.02
10	4.9674	55.08	3.3422	27.95	J	3.8815	55.12	27.88
Mean		55.08		27.92	Mean		55.03	27.86
SD_r		0.1071		0.1691	SD_R		0.1443	0.2889
RSD_r		0.1945		0.6056	RSD_R		0.2622	1.0370

Table 24. Comparative Moisture and Fat Analysis Values of High Fat Sausage – Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0054	56.92	3.3434	26.95	A	4.2379	56.83	26.83
2	4.9967	58.27	3.3469	27.15	B	4.0120	56.55	27.11
3	5.0028	56.94	3.3415	27.12	C	4.2778	56.28	27.35
4	5.0030	57.10	3.3487	26.74	D	4.1757	56.14	27.07
5	5.0051	57.06	3.3459	27.04	E	4.0293	57.05	26.36
6	4.9970	56.86	3.3435	27.20	F	4.1172	57.15	26.86
7	4.9998	56.96	3.3421	26.68	G	4.0107	56.71	27.15
8	4.9982	57.12	3.3455	26.92	H	3.9572	56.66	27.01
9	4.9973	56.84	3.3484	27.00	I	3.9956	56.56	26.74
10	5.0043	57.12	3.3481	27.12	J	3.8916	56.76	27.22
Mean		57.12		26.99	Mean		56.67	26.97
SD_r		0.4182		0.1738	SD_R		0.3109	0.2843
RSD_r		0.7322		0.6439	RSD_R		0.5485	1.0540

comparable results, with 55.03% and 56.67% mean total moisture values and 27.86% and 26.97% mean total fat values, respectively.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 23 and 24) for moisture content of high-fat sausage (0.1071, 0.1945%) and diluted high-fat sausage (0.4182, 0.7322%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of high-fat sausage (0.1443, 0.2622%) and diluted high-fat sausage (0.3109, 0.5485%) samples. The between-laboratory RSD_R values were higher than the within-laboratory RSD_r values, which is a trend observed in nearly all other meat sample results. The RSD_r and RSD_R values for moisture analysis were all < 2.00% and therefore were acceptable based on AOAC's acceptability criteria (RSD_r and RSD_R < 2.00%).

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 23 and 24) for fat content of high-fat sausage (0.1691, 0.6056%) and diluted high-fat sausage (0.1738, 0.6439%) were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for fat content of high-fat sausage (0.2889, 1.0370%) and diluted high-fat sausage (0.2843, 1.0540%) samples. Within-laboratory RSD_r and between-laboratory RSD_R values were within AOAC's acceptability criteria of < 2.00% and were therefore acceptable for fat analysis.

The data indicates that the CEM Smart Trac 5 System® performed well in recovering moisture and fat from the sausage samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the sausage samples, the percent recovery for moisture by the CEM Smart Trac 5 System® ranged

from 99.21% to 99.91% while the percent recovery for fat by the CEM Smart Trac 5 System® ranged from 99.79% to 99.93% among the sausage samples.

The CEM SMART Trac 5 System® was found to be acceptable for moisture analysis and fat analysis for high-fat sausage samples based on AOAC's acceptability criteria ($RSD_R < 2.00\%$).

Discussion of Potted Meat Results

AOAC methods yielded 59.99% and 62.20% mean total moisture values and 18.01% and 17.21% mean total fat values for the potted meat and diluted potted meat samples, respectively (Tables 25 and 26). The CEM Smart Trac 5 System® yielded comparable results, with 60.73% and 62.29% mean total moisture values and 18.05% and 17.17% mean total fat values, respectively.

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 25 and 26) for moisture content of potted meat (0.1833, 0.3055%) and diluted potted meat (0.1907, 0.3066%) samples were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for moisture content of potted meat (0.1561, 0.2570%) and diluted potted meat (0.1550, 0.2489%) samples. The between-laboratory RSD_R values followed the same trend of being higher in comparison to within-laboratory RSD_r values observed in nearly all other meat samples analyzed. Based on the RSD_r and RSD_R values for moisture, the AOAC reference method and CEM SMART Trac 5 System® method met AOAC's acceptability criteria of < 2.00% for moisture analysis of potted meat and diluted potted meat samples.

Table 25. Comparative Moisture and Fat Analysis Values of Potted Meat Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0046	59.96	3.3484	18.08	A	3.6324	60.74	18.12
2	5.0051	60.19	3.3426	18.08	B	4.2511	60.64	17.94
3	4.9961	59.87	3.3433	18.06	C	4.5724	60.48	18.34
4	4.9971	59.85	3.3453	17.97	D	4.3954	60.73	18.04
5	5.0017	59.77	3.3463	18.00	E	3.8326	60.61	17.98
6	4.9967	60.17	3.3460	18.16	F	4.3378	61.01	18.21
7	4.9975	59.79	3.3430	17.97	G	3.6830	60.91	18.02
8	5.0007	60.02	3.3453	17.97	H	3.4259	60.84	17.92
9	5.0028	60.31	3.3467	17.87	I	3.6243	60.65	17.87
10	4.9978	59.99	3.3475	17.91	J	3.7385	60.66	18.09
Mean		59.99		18.01	Mean		60.73	18.05
SD_r		0.1833		0.0855	SD_R		0.1561	0.1428
RSD_r		0.3055		0.4747	RSD_R		0.2570	0.7908

Table 26. Comparative Moisture and Fat Analysis Values of Potted Meat - Diluted Samples Analyzed By AOAC Method 950.46, AOAC Method 960.39, and SMART Trac 5 System® Among 10 Collaborative Laboratories.

TAMU Lab Results For AOAC Methods (Within-Laboratory Repeatability Determination)					Collaborative Lab Results For SMART Trac Methods (Between-Laboratory Reproducibility Determination)			
	Method 950.46		Method 960.39				Microwave	NMR
Sample ID	Wt., g	Moisture %	Wt., g	Fat %	Lab ID	Wt., g	Moisture %	Fat %
1	5.0035	62.45	3.3466	17.49	A	4.0983	62.08	17.15
2	5.0009	62.28	3.3480	17.14	B	3.8038	62.35	17.13
3	5.0022	62.31	3.3427	17.04	C	4.1789	62.25	17.33
4	5.0019	61.78	3.3462	17.19	D	4.6142	62.29	16.75
5	5.0027	62.29	3.3448	17.25	E	3.7752	62.12	17.29
6	4.9957	62.24	3.3439	17.46	F	4.1531	62.63	17.28
7	4.9998	62.09	3.3445	17.20	G	3.6902	62.30	17.39
8	5.0002	62.01	3.3454	17.11	H	4.3668	62.21	17.14
9	5.0046	62.30	3.3440	17.07	I	3.8761	62.25	17.03
10	5.0026	62.20	3.3446	17.16	J	3.5230	62.41	17.18
Mean		62.20		17.21	Mean		62.29	17.17
SD_r		0.1907		0.1521	SD_R		0.1550	0.1824
RSD_r		0.3066		0.8837	RSD_R		0.2489	1.0625

Within-laboratory (AOAC) SD_r and RSD_r (repeatability) values (Tables 25 and 26) for fat content of potted meat (0.0855, 0.4747%) and diluted potted meat (0.1521, 0.8837%) were similar to between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values for potted meat (0.1428, 0.7908%) and diluted potted meat (0.1824, 1.0625%) samples. The between-laboratory RSD_R values followed the same trend of being higher in comparison to within-laboratory RSD_r values observed in nearly all other meat samples analyzed. Based on the RSD_r and RSD_R values for fat, the AOAC reference method and CEM SMART Trac 5 System® method met AOAC's acceptability criteria of < 2.00% for fat analysis of potted meat and diluted potted meat samples.

The data indicates that the CEM SMART Trac 5 System® performed well in recovering moisture and fat from the potted meat samples (see table on page 108). Using AOAC method mean values as the reference values for moisture and fat in the potted meat samples, the percent recovery for moisture by the CEM SMART Trac 5 System® ranged from 100.14% to 101.23% while the percent recovery for fat by the CEM SMART Trac 5 System® ranged from 99.77% to 100.22% for the potted meat and diluted potted meat samples.

The CEM SMART Trac 5 System® was found to be acceptable for moisture analysis and fat analysis for potted meat samples based on AOAC's acceptability criteria (RSD_R <2.00%).

Discussion of Overall Results

A summary of the statistical analysis results is provided in Tables 27 and 28.

Table 27. Summary of TAMU Laboratory Results for Moisture (AOAC 950.46) and Fat (AOAC 960.39) Analysis Using AOAC Reference Methods.

Sample Name	% Moisture			% Fat		
	Mean	SD	RSD	Mean	SD	RSD
Ham, Low Fat[a]	74.93	0.0598	0.0798	2.54	0.0925	3.6391
Ham, Low Fat, Diluted[a]	75.91	0.0937	0.1234	2.37	0.1144	4.8267
Ham, High Fat[a]	58.41	0.0411	0.0703	16.34	0.0644	0.3944
Ham, High Fat, Diluted[a]	60.18	0.0836	0.1389	16.03	0.1203	0.7501
Pork, Low Fat[a]	74.49	0.0445	0.0598	2.26	0.0345	1.5303
Pork, Low Fat, Diluted[a]	75.40	0.0370	0.0491	2.18	0.0361	1.6541
Pork, High Fat[a]	60.07	0.1410	0.2348	22.30	0.2065	0.9258
Pork, High Fat, Diluted[a]	61.54	0.1219	0.1981	21.88	0.2679	1.2247
Beef, Low Fat[a]	67.31	0.1444	0.2145	11.23	0.1494	1.3309
Beef, Low Fat, Diluted[a]	68.86	0.2016	0.2928	10.63	0.1331	1.2516
Beef, High Fat[a]	57.84	0.1783	0.3083	26.56	0.3256	1.2257
Beef, High Fat, Diluted[a]	60.16	0.3939	0.6547	25.44	0.3447	1.3552
Chicken, Low Fat[a]	74.99	0.0668	0.0891	2.91	0.0388	1.3327
Chicken, Low Fat, Diluted[a]	75.90	0.2541	0.3348	2.79	0.0382	1.3695
Chicken, High Fat[a]	61.62	0.0868	0.1409	23.30	0.3428	1.4713
Hot Dog, Low Fat[b]	74.97	0.3638	0.4852	2.68	0.0233	0.8705
Hot Dog, High Fat[a]	54.03	0.0722	0.1337	29.79	0.1397	0.4688
Hot Dog, High Fat, Diluted[a]	55.54	0.0787	0.1416	28.80	0.2011	0.6984
Turkey, Low Fat[a]	74.67	0.0564	0.0755	1.00	0.0445	4.4407
Turkey, Low Fat, Diluted[a]	75.43	0.0687	0.0911	0.74	0.0342	4.6280
Turkey, High Fat[b]	65.79	0.1423	0.2164	18.52	0.0821	0.4433
Sausage, Low Fat[b]	72.87	0.0236	0.0324	2.30	0.0096	0.4171
Sausage, High Fat[a]	55.08	0.1071	0.1945	27.92	0.1691	0.6056
Sausage, High Fat, Diluted[a]	57.12	0.4182	0.7322	26.99	0.1738	0.6439
Potted Meat[a]	59.99	0.1833	0.3055	18.01	0.0855	0.4747
Potted Meat, Diluted[a]	62.20	0.1907	0.3066	17.21	0.1521	0.8837

[a] Ten replicate determinations
[b] Three replicate determinations

Table 28. Summary of Collaborative Laboratory Results for Moisture (Microwave) and Fat (NMR) Analysis Using the SMART Trac System®.

Sample Name	% Moisture			% Fat		
	Mean	SD_R	RSD_R	Mean	SD_R	RSD_R
Ham, Low Fat[a]	74.29	0.1990	0.2678	2.60	0.0929	3.5721
Ham, Low Fat, Diluted[a]	75.59	0.3701	0.4897	2.36	0.1388	5.8949
Ham, High Fat[a]	58.23	0.3286	0.5643	16.27	0.1899	1.1675
Ham, High Fat, Diluted[a]	59.65	0.2979	0.4994	15.93	0.1554	0.9755
Pork, Low Fat[a]	74.19	0.2211	0.2980	2.28	0.0626	2.7476
Pork, Low Fat, Diluted[a]	75.24	0.1106	0.1470	2.11	0.0479	2.2734
Pork, High Fat[a]	60.15	0.2713	0.4510	22.44	0.2327	1.0372
Pork, High Fat, Diluted[a]	61.42	0.2197	0.3577	21.73	0.1969	0.9058
Beef, Low Fat[a]	67.11	0.2625	0.3911	11.30	0.1245	1.1018
Beef, Low Fat, Diluted[a]	68.58	0.3183	0.4642	10.73	0.1687	1.5720
Beef, High Fat[a]	57.96	0.3275	0.5651	26.55	0.3481	1.3112
Beef, High Fat, Diluted[a]	59.59	0.3670	0.6159	25.43	0.3843	1.5109
Chicken, Low Fat[a]	74.66	0.1552	0.2079	2.92	0.0464	1.5873
Chicken, Low Fat, Diluted[a]	75.63	0.2225	0.2942	2.73	0.0655	2.4037
Hot Dog, High Fat[a]	53.64	0.2704	0.5041	29.93	0.1615	0.5396
Hot Dog, High Fat, Diluted[a]	55.52	0.3709	0.6680	28.78	0.1929	0.6701
Turkey, Low Fat[a]	74.37	0.2708	0.3641	0.95	0.1052	11.0902
Turkey, Low Fat, Diluted[a]	75.16	0.2399	0.3191	0.87	0.0594	6.8124
Sausage, High Fat[a]	55.03	0.1443	0.2622	27.86	0.2889	1.0370
Sausage, High Fat, Diluted[a]	56.67	0.3109	0.5485	26.97	0.2843	1.0540
Potted Meat[a]	60.73	0.1561	0.2570	18.05	0.1428	0.7908
Potted Meat, Diluted[a]	62.29	0.1550	0.2489	17.17	0.1824	1.0625

[a] Ten replicate determinations

For nearly all samples analyzed, the between-laboratory (CEM®) SD_R and RSD_R (reproducibility) values were larger in comparison to the within-laboratory (AOAC) SD_r and RSD_r (repeatability) values, suggesting that the CEM method is less precise than the AOAC methods for moisture and fat analysis. It should be noted that AOAC methods for moisture and fat analysis were performed in a single laboratory by a single individual under carefully controlled laboratory conditions whereas the CEM methods for moisture

and fat analysis were performed in multiple laboratories with different CEM SMART Trac 5 Systems® and by multiple analysts among different laboratory conditions. Lower precision would likely result from such variations. As the data indicates, the CEM SMART Trac 5 System® was found to be acceptable for moisture analysis for all samples analyzed and acceptable for fat analysis for meat samples containing more than 3% fat based on AOAC's acceptability criteria ($RSD_R < 2.00\%$). The CEM SMART Trac 5 System® was found not to be acceptable for fat analysis of meat samples containing less than 3% fat (i.e. low-fat ham, low-fat pork, low-fat turkey).

The percent recovery (% Recovery) for moisture and fat reported in Table 29 was determined by comparing the overall mean of the collaborative laboratory values (obtained from CEM Smart Trac 5 System® analysis) to the reference values (obtained from AOAC Methods 950.46 and 960.39). The recovery for moisture was excellent for all meat sample types analyzed and ranged from 99.05% to 101.23%. Recovery for fat was more variable, ranging from 95.00% to 117.57%, with the greater variation due to some sample types containing very low levels of fat (i.e. low-fat ham, low-fat turkey).

Overall review of the results indicates that the CEM Smart Trac 5 System® compares favorably to the AOAC methods for moisture and fat determination. The CEM Smart Trac 5 System® would be suitable for the rapid determination of moisture and fat in a variety of commercially produced raw and processed meat and poultry products. Statistical analysis confirmed the within-laboratory repeatability and precision qualities of AOAC methods and provided a baseline for comparing the between-laboratory reproducibility and precision potential of the CEM Smart Trac 5

Table 29. Comparison of Mean Moisture Values and Mean Fat Values From Study Methods (SMART Trac 5 System®) to Reference Methods (AOAC Methods 950.46 and 960.39)

Sample Name	% Moisture			% Fat		
	Study Mean	Reference Mean	% Recovery	Study Mean	Reference Mean	% Recovery
Ham, Low Fat[a]	74.29	74.93	99.15	2.60	2.54	102.36
Ham, Low Fat, Diluted[a]	75.59	75.91	99.58	2.36	2.37	99.58
Ham, High Fat[a]	58.23	58.41	99.69	16.27	16.34	99.57
Ham, High Fat, Diluted[a]	59.65	60.18	99.12	15.93	16.03	99.38
Pork, Low Fat[a]	74.19	74.49	99.60	2.28	2.26	100.88
Pork, Low Fat, Diluted[a]	75.24	75.40	99.79	2.11	2.18	96.79
Pork, High Fat[a]	60.15	60.07	100.13	22.44	22.30	100.63
Pork, High Fat, Diluted[a]	61.42	61.54	99.81	21.73	21.88	99.31
Beef, Low Fat[a]	67.11	67.31	99.70	11.30	11.23	100.62
Beef, Low Fat, Diluted[a]	68.58	68.86	99.59	10.73	10.63	100.94
Beef, High Fat[a]	57.96	57.84	100.21	26.55	26.56	99.96
Beef, High Fat, Diluted[a]	59.59	60.16	99.05	25.43	25.44	99.96
Chicken, Low Fat[a]	74.66	74.99	99.56	2.92	2.91	100.34
Chicken, Low Fat, Diluted[a]	75.63	75.90	99.64	2.73	2.79	97.85
Hot Dog, High Fat[a]	53.64	54.03	99.28	29.93	29.79	100.47
Hot Dog, High Fat, Diluted[a]	55.52	55.54	99.96	28.78	28.80	99.93
Turkey, Low Fat[a]	74.37	74.67	99.60	0.95	1.00	95.00
Turkey, Low Fat, Diluted[a]	75.16	75.43	99.64	0.87	0.74	117.57
Sausage, High Fat[a]	55.03	55.08	99.91	27.86	27.92	99.79
Sausage, High Fat, Diluted[a]	56.67	57.12	99.21	26.97	26.99	99.93
Potted Meat[a]	60.73	59.99	101.23	18.05	18.01	100.22
Potted Meat, Diluted[a]	62.29	62.20	100.14	17.17	17.21	99.77

[a] Ten replicate determinations

System®. For moisture analysis, the within-laboratory (AOAC) precision results (RSD_r) and between-laboratory (CEM®) precision results (RSD_R) were acceptable for all samples evaluated based on AOAC's acceptability criteria (RSD < 2.00%). For fat analysis, the within-laboratory (AOAC) precision results (RSD_r) and between-laboratory (CEM®) precision results (RSD_R) were acceptable for high-fat ham, diluted high-fat ham, high-fat pork, diluted high-fat pork, low-fat beef, diluted low-fat beef, high-fat beef, diluted high-fat beef, low-fat chicken, high-fat frankfurter, diluted high-fat frankfurter, high-fat sausage, diluted high-fat sausage, potted meat, and diluted potted meat samples based on AOAC's acceptability criteria (RSD <2.00%).

Samples containing very small amounts of fat (< 3% fat) were found to yield higher relative standard deviation (RSD) results, and therefore lower precision, for within-laboratory (RSD_r) and between-laboratory (RSD_R) fat analysis. The samples that yielded RSD_r and/or RSD_R values that exceeded AOAC's acceptability criteria of <2.00% included: low-fat ham, diluted low-fat ham, low-fat pork, diluted low-fat pork, diluted low-fat chicken, low-fat turkey, and diluted low-fat turkey samples.

Although RSD_r and RSD_R values were calculated to determine if the method met the acceptability criteria for each meat sample type, it is interesting to note that the difference between the highest value and lowest value for fat results in a high fat sample set (i.e. AOAC fat results for high fat ham) yields a greater value than the difference between the highest value and lowest value for fat results in the corresponding low fat sample set (i.e. AOAC fat results for low fat ham). For example, the difference between the high and low values for fat analysis using the AOAC method for high fat ham was

0.50 whereas the difference between the high and low values for fat analysis using the AOAC method for low fat ham was 0.24. The same trend was observed for fat values obtained using the CEM SMART Trac System® for fat analysis. This trend would suggest that although RSD_r and RSD_R values for fat analysis of low fat samples exceed the 2.00% limit for acceptability, the range in which the individual fat results are being measured is much tighter for low fat samples compared to high fat samples.

When analyzing a meat sample using AOAC methods, the CEM Smart Trac 5 System® methods, or any other method available, it is important to collect and prepare samples in a manner that provides a homogenous, uniform, and representative portion of the larger lot and to handle samples and equipment with care to achieve the most accurate and reliable results for the selected method. One of the most notable observations made upon reviewing the statistical results of this study data was that samples that have a very small amount of fat (<3% fat) have the largest relative standard deviation (RSD) in comparison to meat samples that have a larger amount of fat (>10% fat). For fat analysis methods used in this study (AOAC and CEM®), the larger RSD_r and RSD_R values observed in the low-fat samples were primarily due to the very small amount of fat in the samples, in which small variations in the sample or procedure could significantly impact data results. In addition, the inherent variability of weighing minute amounts of fat to the fourth decimal place or possible reduced fat extraction efficiency associated with the AOAC extraction method may have also contributed to higher RSD_r values for fat analysis in low-fat samples. For the CEM® method, higher RSD_R values for fat analysis likely resulted, in part, from the variation among different laboratory

conditions and multiple individuals performing analyses among the collaborative laboratories.

When analyzing the fat content of very low-fat products, it is critical that the sample be homogenized and great care be taken in the handling of the sample through each step of the analytical procedure, as small deviations can results in large data gaps. Such care should be taken no matter which method is selected for analysis.

CHAPTER V

SUMMARY

Analyses for the determination of moisture, fat, protein, salt, and other analytes in meat and poultry products are important for evaluating raw material quality, ensuring process control and finished product composition, and meeting regulatory compliance in meat plant operations. Such analyses are also valuable for scientists in governmental agencies and academia conducting food related research.

Standard methods of analysis are universally accepted and provide a known degree of accuracy and precision. However, some standard methods of analysis of meat and poultry products can be time- or energy-intensive, require highly trained personnel, involve the use of harmful or toxic chemicals, or generate wastes that have expensive disposal fees. Other methods of analysis may not provide the same degree of accuracy, but may have greater ease of use or require less time or less training of employees.

Rapid analytical methods with the potential to produce results with the same precision and accuracy as standard methods would be of great benefit to researchers, plant operations, governmental agencies, academia, and other entities. This study evaluated the accuracy and precision of the CEM Smart Trac 5 System® for rapid determination of moisture and fat in meat products in comparison to the standard methods of analysis (AOAC official methods 950.46 and 960.39, respectively). An AOAC collaborative study involving 10 laboratories representing private industry, government agencies, and academia was conducted to determine if the CEM Smart Trac

5 System®, which performs rapid analyses of moisture and fat, is comparable to universally accepted standard methods of analysis. A variety of raw and processed meat products representing the primary meat categories of beef, pork, chicken, and turkey were selected for analysis in this study.

Overall review of the results and data analysis indicated that the CEM Smart Trac 5 System® compares favorably with the AOAC methods for moisture and fat determination and would be suitable for the rapid determination of moisture and fat in a variety of commercially produced raw and processed meat and poultry products. Statistical analysis confirmed the within-laboratory repeatability qualities of AOAC methods and provided a baseline for comparing the between-laboratory reproducibility potential of the CEM Smart Trac 5 System®. For all samples evaluated, the within-laboratory (AOAC) RSD_r (repeatability) results and between-laboratory (CEM®) RSD_R (reproducibility) results for moisture met AOAC acceptability criteria (RSD < 2.00%). With the exception of meat products containing very small amounts of fat (i.e. low-fat ham, low-fat pork, low-fat turkey), the within-laboratory (AOAC) and between-laboratory (CEM®) results for fat met AOAC's acceptability criteria (RSD < 2.00%).

CHAPTER VI

CONCLUSIONS

The data from this study indicate that the moisture and fat results obtained from the CEM Smart Trac 5 System® compare favorably to moisture and fat results obtained by AOAC methods. We conclude that the CEM Smart Trac 5 System® would be suitable for determining total moisture and fat in a variety of commercially produced raw and processed meat and poultry products. This method offers similar accuracy and precision of moisture and fat results in comparison to AOAC methods, with the additional benefit of producing results more rapidly, with greater ease of use and less personnel training, and without the use of harmful or toxic chemicals or wastes. Therefore it is our recommendation that the CEM Smart Trac 5 System® be accepted as an AOAC-approved official method of analysis for moisture and fat in meat and meat products.

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APPENDIX

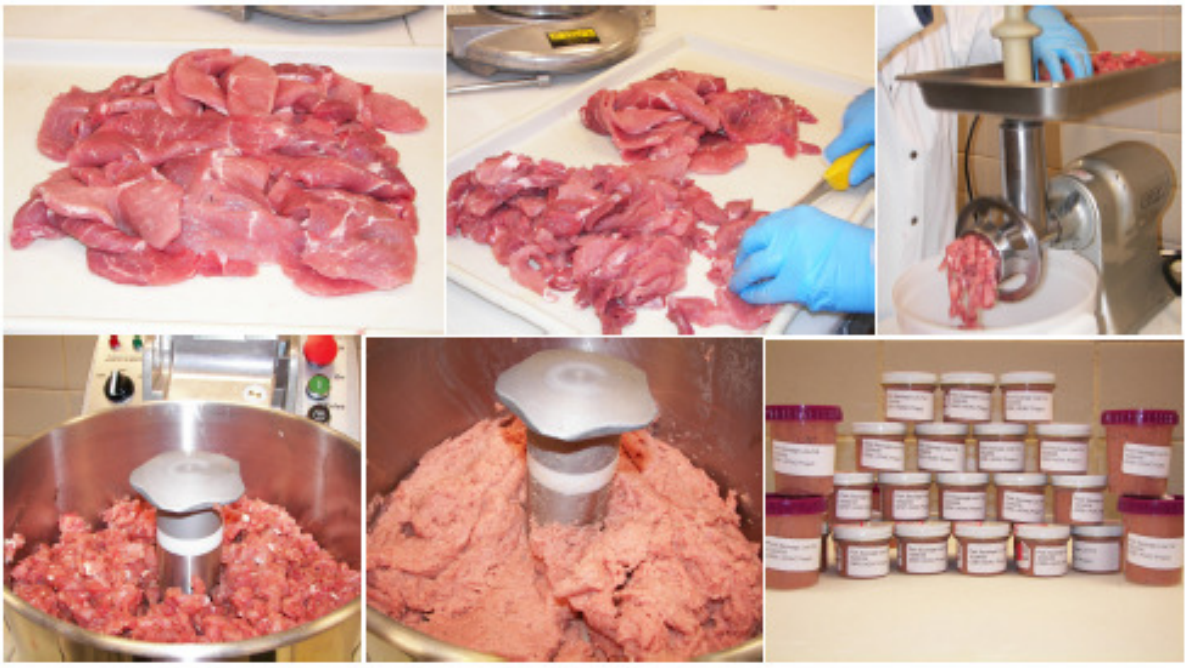

Figure A-1. Preparation of Meat Aliquots (Homogenization and Packaging of Meat Products)
Meat products were removed from their package, manually cut with a knife into smaller pieces (if necessary), and ground through a table-top meat grinder using a ½" grinding plate. Approximately 3.18 kg (7 lbs) of material was placed in a 10 quart capacity stainless steel commercial food processor and chopped on high speed until the material was well homogenized (paste-like appearance). The homogenized material was then packed into 2-oz and 4-oz sterile, screw capped polyurethane specimen containers, labeled, and transferred to a freezer for storage.

Figure A-2. Sample Preparation for Moisture Analysis Using the Oven Drying Method (AOAC 950.46).
An aluminum pan and corresponding lid were placed onto an analytical balance. Approximately 5-6 grams of homogenized meat material was placed into the pan and the combined weight of the pan, lid, and sample was recorded. The homogenized meat material was spread evenly across the bottom of the pan with a metal spatula, the lid was replaced, and the aluminum pan, lid, and sample were returned to the analytical balance to obtain the final weight. The aluminum pans containing the homogenized meat material were placed in a drying oven with lids removed and dried overnight. The lids were fitted onto the corresponding aluminum pans immediately prior to removing the samples from the oven. The aluminum pans containing the dried sample material were then transferred to a desiccators and allowed sufficient time to cool to room temperature.

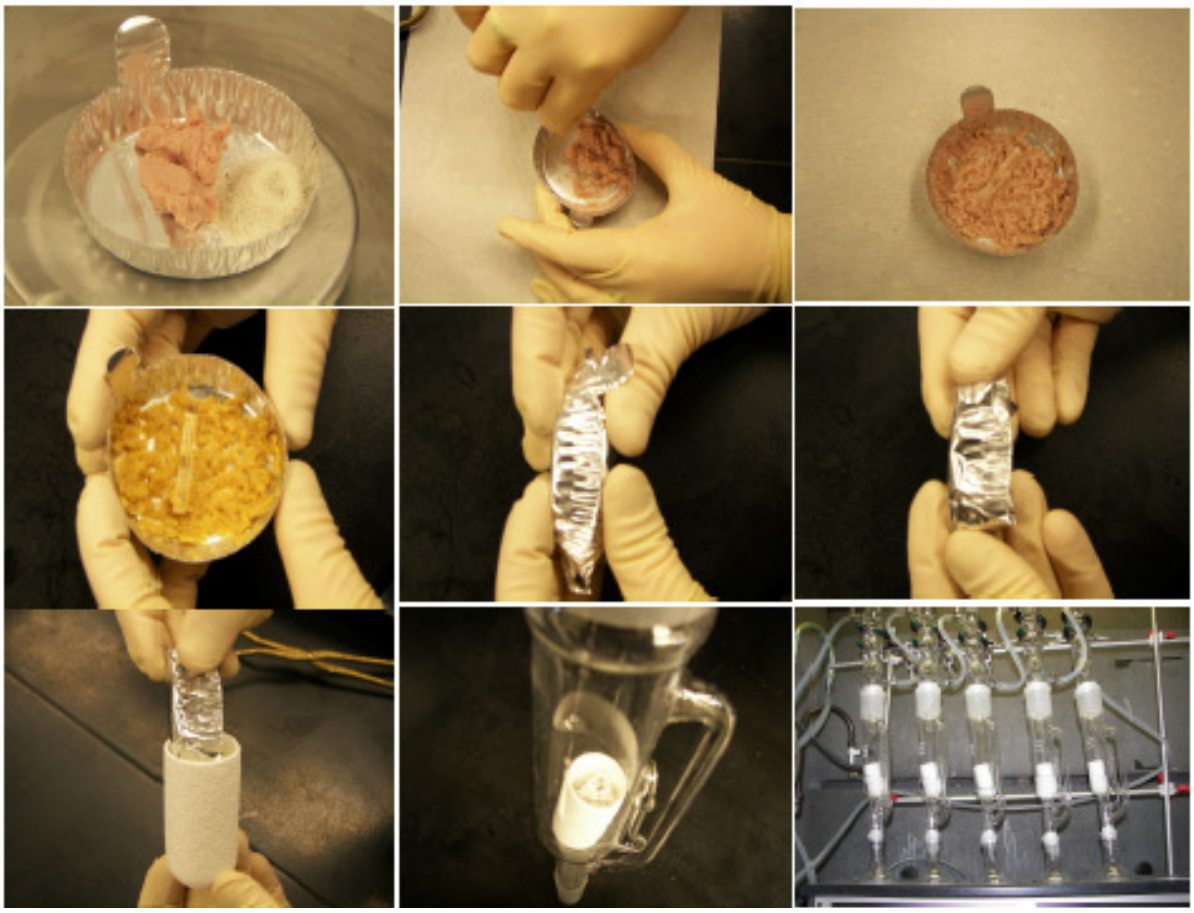

Figure A-3. Sample Preparation for Fat Analysis Using the Soxhlet Extraction Method (AOAC 960.39).

Approximately 3-5 g of homogenized meat material was placed into a disposable pan and the weight recorded. Approximately 3-5 g of laboratory grade sand was added to the pan, mixed into the homogenized meat material using a glass rod, and the mixture spread into an even layer on the bottom of the pan. The meat sample was then dried in a drying oven and desiccated to room temperature (picture not shown). The disposable pan containing the dried meat material, sand, and glass rod was folded into thirds with the ends folded upward to prevent escape of the pan contents. The folded pan was then placed in a cellulose thimble and transferred to the Soxhlet portion of the fat extraction system. The fully assembled Soxhlet extraction system (bottom right) is comprised of: a heating mantle, a 250 mL glass flask containing 150 mL of petroleum ether and porous boiling chips, a Soxhlet, and a condenser connected to tubing in which cool running water flows through continuously.

Figure A-4. CEM Smart Trac 5 System®.
The CEM Smart Trac 5 Microwave Moisture Analyzer instrumentation (right) and the CEM Smart Trac 5 Nuclear Magnetic Resonance Fat Analyzer instrumentation (left) at Texas A&M University.

Illustration I

If the sample is in a paste, semi-solid, or crumb form or a raw or skeletal meat product such as fresh pork, ground beef, or chicken, place the sample on the end of a spatula and spread it across one end of the pad. Then spread the sample to a uniform thickness covering approximately 90% of the pad surface area.

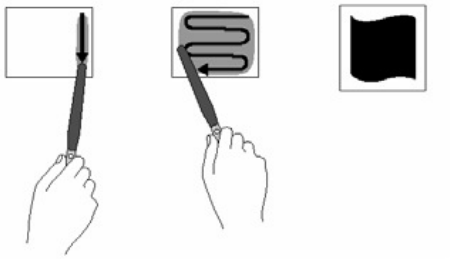

Illustration II

For samples containing bound water such as an all-meat emulsion, cooked all-meat sausage, sausage with extenders, semi-dry sausage, or ham, place the sample on the end of the spatula and apply the sample to the middle of the pad. Then spread the sample around the pad in a circle.

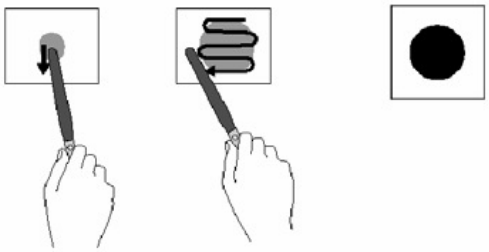

Figure A-5. Sample Preparation for Moisture Analysis Using CEM Smart Trac 5 System® (Leffler and others 2008)

Illustration III

Place the 2 square pads and dried sample in the center of the Trac film. Fold the left corner of the film and pads as illustrated. Fold the right corner. Pull the lower edge of the film and sample pads toward the top and begin to roll them into a tube.

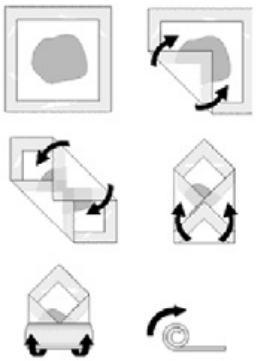

Illustration IV

For samples that are rigid after being dried and more difficult to roll into a cylinder, prepare the pads as illustrated.

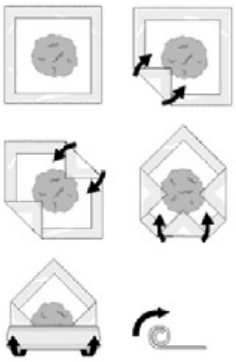

Figure A-6. Sample Preparation for Fat Analysis Using CEM Smart Trac 5 System® (Leffler and others 2008)

Table A-1. Meat Sample Aliquots Prepared for Analysis Among Collaborative Laboratories and For Use in Programming CEM SMART Trac 5 System®.

"Regular" meat products	**"Diluted" meat products (diluted with 0.4% deionized water)**	**Additional products for program setup (not analyzed by the collaborative laboratories)**
Beef (high fat)	Beef (high fat)	Chicken (high fat)
Beef (low fat)	Beef (low fat)	Turkey (high fat)
Pork (high fat)	Pork (high fat)	Beef Frankfurter (low fat)
Pork (low fat)	Pork (low fat)	Pork Sausage (Low fat)
Chicken (low fat)	Chicken (low fat)	Ham (low fat)
Turkey (low fat)	Turkey (low fat)	
Beef Frankfurter (high fat)	Beef Frankfurter (high fat)	
Pork Sausage (high fat)	Pork Sausage (high fat)	
Ham (high fat)	Ham (low fat)	
Potted Meat	Potted Meat	

Table A-2. Participating Collaborative Laboratories for Moisture and Fat Analysis Using SMART Trac 5 Systems for Comparative Analysis to AOAC Method 950.46 and AOAC Method 960.39.

Lab A	Jones Dairy Farm, Fort Atkinson, WI
Lab B	USDA, Blakely, GA
Lab C	Wayne Farms, Douglas, GA
Lab D	Five Star Custom Foods, Fort Worth, TX
Lab E	Wayne Farms, Dobson, NC
Lab F	Quality Sausage, Dallas, TX
Lab G	Texas A&M University, College Station, TX
Lab H	Diebel Laboratories, Madison, WI
Lab I	CEM Corporation, Matthews, NC
Lab J	Tyson Prepared Foods, North Richland Hills, TX