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Chapter 4

Evaluation of the Rapid, High-Temperature Extraction of Feeds, Foods, and Oilseeds by the ANKOM^{XT20} Fat Analyzer to Determine Crude Fat Content

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Abstract

The process of extraction for the quantitative separation of fat/oil is the basis for the majority of official methods. The extraction process, which separates the sample into two fractions, permits two approaches to quantitative measurement. The analysis can be performed by either weighing the fat/oil fraction directly, or indirectly by measuring the loss of weight due to extraction. Acceleration of the extraction process has been achieved by elevating the temperature of the solvent. This chapter discusses a recently developed primary method called the Filter Bag Technique (FBT). This technique utilizes temperatures of up to twice the boiling point of petroleum ether to accelerate extraction. High sample throughputs are accomplished by batch processing of samples encapsulated in filter media formed in the shape of a bag. The extraction is performed automatically in an ANKOM^{XT20} Fat Analyzer, an instrument that can process 20 samples in 20–60 min. The fat/oil percentage is calculated indirectly from the loss of weight from the sample in the filter bag. Various studies related to the extraction and gravimetric measurements of these fractions are discussed in this chapter for both the conventional method and the FBT. The accuracy of the FBT depends on effective predrying and proper weighing of the sample. Studies of the conventional method suggest that samples containing polyunsaturated fatty acids are sensitive to oxidation particularly during the solvent evaporation step when the oil is heated in the presence of oxygen. Various studies of the ruggedness of the FBT indicate that the method is not sensitive to small changes in analytical conditions. The ruggedness of the method was confirmed in an experiment utilizing Youden's Ruggedness Test. When the accuracy of the FBT was compared to that of the conventional method with a wide variety of samples (n = 22) in a regression analysis, the two methods were highly correlated (R^2 = 0.9996). There was essentially no bias (–0.046 intercept) and no distortion over the range of the samples (slope 1.001). Two collaborative studies with laboratories from five countries provided similar evidence of the accuracy of the FBT. The second collaborative study, designed to evaluate the FBT as an AOCS official method, was conducted with 28 samples presented as 56 blind

duplicates. Twelve international collaborating laboratories used the FBT for the analysis, whereas three AOCS certified laboratories utilized the official methods. This study resulted in a similar highly significant R^2 of 0.9990 compared with the official methods, with an intercept of 0.046 and a slope of 1.005. The average repeatability within laboratories was S_r= 0.31 and reproducibility among laboratories was S_R = 0.46. These studies indicate that the FBT is an accurate and precise method capable of analyzing large quantities of samples in an efficient and automated fashion.

Introduction

Knowledge of the fat content of food and feed, or the oil content in oilseeds is of critical importance when evaluating the value of these materials. The oil content of oilseeds determines their commercial value, whereas the fat content is important in gaining an understanding of the nutritional value and energy metabolism of a diet. Both fat and oil represent the fraction of lipids generally associated with triacylglycerides and compounds of similar solubility in nonpolar solvents. In this chapter, the terms "fat" and "oil" will be used interchangeably.

The quantitative analysis of "Oil" as it is termed by American Oil Chemists' Society (AOCS) (1) or "Crude Fat," as designated by Association of Official Analytical Chemists (AOAC) (2), is based on separating the fat/oil from the sample matrix by extraction with nonpolar solvents. The amount of oil is determined either by directly weighing the extracted oil (Direct Method, AOAC Method 920.39a) or by measuring the loss of weight from the sample (Indirect Method, AOAC Method 920.39b, 948.22a). This process is described in the flow diagram in Figure 4.1. Each step in the process affects the accuracy and precision of the analysis. There are several critical drying, weighing, extraction, and evaporation steps. The process terminates with two fractions, i.e., the residue extracted by the solvent, for which the percentage can be calculated directly, and that portion of the sample not soluble in the solvent for which the percentage can be calculated indirectly. Because both values can be determined on the same sample, their agreement verifies the accuracy of the analysis.

Nonpolar solvents such as diethyl ether, petroleum ether, and hexane dissolve fats and oils and leave behind proteins, carbohydrates, and other compounds insoluble in these solvents. This fractionation is the basis for most of the "Official" analytical methods established by AOCS, AOAC, International Organization for Standardization (ISO) (3), German Fat Science Society (DGF) (4), and Federation of Oils, Seeds and Fats Associations (FOSFA) (5). These methods utilize either the Soxhlet extraction apparatus, developed by Franz Von Soxhlet (6) in 1939, the Butt-type apparatus (2), or the Goldfisch apparatus (7). All of these methods boil the solvent and utilize the condensed solvent to extract the sample. The Soxhlet apparatus allows the sample chamber to fill and periodically siphon off into the boiling flask; the others simply allow the condensed solvent to pass through the sample as the solvent is refluxed. The

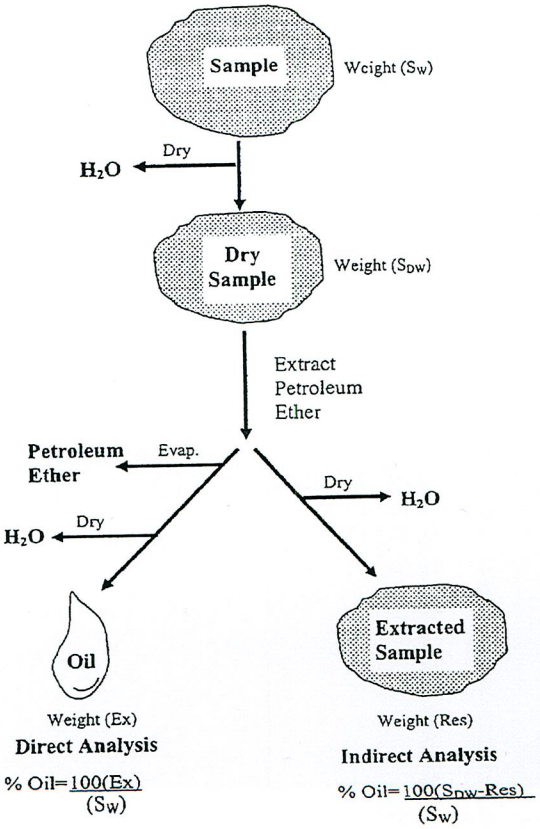

Fig. 4.1. A diagrammatic representation of the analysis of fat/oil by solvent extraction.

sample is therefore extracted with solvent at a temperature below the boiling point of the liquid, requiring extraction times from 4 to 16 h.

The rate of extraction has been increased by immersing the sample in the boiling solvent (8), thereby extracting the fat/oil at a higher temperature and reducing the extraction time. Further improvements in the kinetics of extraction have been achieved by performing the extraction in a sealed chamber at elevated pressures that permit extraction to be performed at temperatures well above the boiling point of the solvent [ANKOM (9) Dionex (10) and supercritical fluid extraction (11)]. This results in a further reduction in the extraction time.

A recently developed method that utilizes high solvent temperatures in an automated batch process is being evaluated as an Official AOCS Method. This technique responds to the need for a rapid, efficient, high-volume process for the analysis of fats/oils that is equivalent to a primary method using petroleum ether. The method is entitled, "Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction."

This method is performed by the ANKOM^{XT20} Fat Analyzer (XT20) and can also be performed by the ANKOM^{XT10} Extractor (XT10) (9). Batch processing is accomplished by encapsulating each sample in a special filter medium, preserving its quantitative identity while performing the high temperature extraction of multiple samples in a common extraction chamber. The filter media is made in the shape of a bag and is heat sealed after the introduction of the sample. This method of analysis will be referred to as the Filter Bag Technique (FBT) and has the capability of high sample throughput (>200 sample/d). This chapter will discuss the background of the extraction process and the evaluation of the precision (reproducibility among different laboratories in a collaborative study), accuracy (comparison with standard methods), and ruggedness of the FBT in laboratory and interlaboratory collaborative studies.

Materials and Methods

Conventional Method. The Goldfisch Method, conducted on a Labconco Goldfisch Fat Extraction Apparatus, was used in a number of studies as the conventional standard for comparison with the FBT (7). The apparatus functions essentially the same as the Butt-type apparatus, continually refluxing solvent over the sample during the extraction. The method can follow both paths, i.e., direct analysis and indirect analysis of fat/oil (Fig. 4.1). Extractions were performed over a 4- to 5-h period and the solvent was partially evaporated and recovered in a glass beaker. In earlier studies, the residual solvent (~10 mL) was evaporated above the hot plate on a holder in the apparatus. In subsequent studies, with sensitive samples, the residual solvent was evaporated on a steam bath under nitrogen. The analysis was conducted by weighing the sample in a tared thimble, drying the sample at 100°C for 3 h, and weighing it at ambient temperature from a desiccant pouch. The thimbles in these studies were made from the hydrophobic filter medium used for the filter bags. Typical cellulose thimbles are very hydroscopic and are difficult to weigh. The thimbles containing the samples were inserted into the apparatus and a tared glass beaker with 50 mL of petroleum ether was attached to each reflux unit. The cycle was started by turning on the hot plate. When the extraction was completed and the solvent evaporated, both the residual sample in the thimble and the fat/oil in the beaker were dried at 100°C for 30 min, cooled to room temperature in a desiccator, and weighed. Both direct and indirect analyses were performed on the same sample as a check for accuracy.

Filter Bag Technique. The FBT follows the path in Figure 4.1 of the indirect analysis and was performed in the XT20 (9). The sample was weighed in the filter bag, heat sealed, dried at 100°C for ~3 h, cooled in a desiccant pouch, and weighed. Samples (n = 20) were placed in a carousel in the extraction chamber. The temperature (90°C) and time of extraction, usually from 10 to 60 min, were selected and the instrument was started. The XT20 automatically processed the samples in the following fashion: sealed and purged the chamber, inserted and heated the solvent, rotated the bag carousel, and emptied when the extraction time was complete. Solvent was then added for the first rinse, emptied after 3 min and refilled with fresh solvent for a second rinse. After the solvent was emptied, the residual solvent was evaporated and the chamber was purged with nitrogen. When attached to an ANKOM^{XT} Recovery System, the instrument automatically distills and recycles the solvent. A similar process is performed by the ANKOM^{XT10} Extractor. The samples were then dried at 100°C for 30 min, cooled to room temperature in a desiccant pouch, and weighed.

The desiccant pouch was developed to more conveniently handle the filter bags during the weighing process. The pouches were made from resealable polyethylene bags containing desiccant and were used in all of the FBT studies. Filter bags were removed from the oven and placed directly in the desiccant pouch. The air was pressed out and the pouch was sealed. The samples rapidly equilibrated to room temperature and were effectively protected from ambient moisture by the limited head space in the pouch. The introduction of moist air during the removal of each bag was reduced by minimizing the size of the opening and pressing the pouch flat.

Solvents. Although other solvents can be used, petroleum ether is the preferred solvent for the FBT because of its safety, cost, and ease of recycling. Petroleum ether was used in all the studies reported in this chapter. The boiling point range of commercial petroleum ether is specified by the supplier as 35–65°C (12). The distribution of the solvent components over the temperature range was investigated in a fractional distillation study of both new and recycled petroleum ether (distilled to remove fat). Fractions were collected within 5°C increments from 36 to 80°C.

Sample Preparation. The objective of sample preparation is to provide a sample that accurately represents the "population" being studied and sufficiently disrupts the matrix to permit more efficient extraction. Meat samples were ground to a uniform consistency with a food processor and mixed thoroughly. For shipping convenience and sample uniformity, the meats in the international collaborative studies were dried for 3 h at 100°C and then ground in a cyclone mill to pass through a 2-mm screen. The feed samples were ground in a cyclone mill to pass through a 1-mm screen and mixed thoroughly. The food samples were processed with a food processor or cyclone mill to produce a representative sample of uniform consistency. Soybean samples were first dried at 130°C for 30 min and then ground in a cyclone mill to pass through a 1-mm screen. Other oilseeds were ground in a cyclone mill to pass through a 1- or 2-mm screen, depending on the level of screen occlusion.

The effects of grinding were demonstrated in a study with soybeans by processing them three ways. In the first treatment, soybeans were ground through a 2-mm screen and extracted. In the second treatment they were processed according to the AOCS procedure (13) by first heating the soybeans in a 130°C oven for 30 min and then grinding through a 1-mm screen followed by an extraction. The third

treatment involved regrinding the soybean samples from the second treatment through a 1-mm screen and then extracting a second time.

Conventional Method Weighing Procedures. The weighing procedure is critical to the gravimetric analysis of fats/oils. Accuracy of the analytical balance was verified and checked each day that weighing was performed. Accurate weighing of dried samples requires rapid processing directly from a desiccating environment, limiting exposure to moist ambient air. The glass beakers used in the conventional method were hydroscopic and can, under certain circumstances, carry a significant static charge. The effect of static charge was investigated in an experiment with samples of a pig diet. Samples were extracted for 4 h with petroleum ether, and the residual oil in beakers from six replicates was dried at 100°C for 30 min. After equilibration to room temperature in a desiccator, the beakers were weighed. The oil was then transferred with a small amount of petroleum ether to tared aluminum pans because they do not retain a static charge. After evaporation of the solvent, the samples in the aluminum pans were dried in the oven, equilibrated in a desiccator, and weighed.

Oxidation. A study designed to evaluate the relative accuracy of the direct and indirect measurements was conducted on duplicate samples of ground beef, hot dogs, potato chips, high-energy horse diet, pig diet, corn, oats, and soybeans. Both direct and indirect determinations were performed on the same sample using the conventional method. The oil was evaporated using the holder on the Labconco apparatus, which holds the beaker above the hot plate.

Due to the lack of agreement of the direct and indirect measurements with certain samples, studies were conducted to evaluate the role of oxidation in the elevated values of samples containing unsaturated lipids. An experiment was conducted with a corn sample that in previous studies had shown elevated direct values relative to the indirect values. A series of treatments were designed to first limit oxidation and then incrementally increase the opportunity for oxidation. It was observed that the bulk of the oil/fat was extracted at the beginning of the extraction period and that the oil/fat dissolved in the solvent was subjected to the boiling temperatures for hours during the refluxing of the solvent. Because the system was not anaerobic, there was a possibility that these conditions could present an opportunity for oxidation. In this experiment, extracted oil was removed from the apparatus during the extraction process in the first two treatments at 1.5 and 3.0 h, and continued with fresh solvent to complete the 5-h extraction. The remaining treatments were refluxed for 5 h without the removal of the first fraction. The last 10 mL of solvent was evaporated in several ways. For treatments 1, 2, and 3, solvent was evaporated on a steam bath with a nitrogen stream directed on the surface. In treatment 4, the solvent was evaporated on a steam bath without nitrogen. In treatment 5, solvent was evaporated above the hot plate in the Labconco holder. In treatment 6, the solvent was evaporated directly on the hot plate until all the solvent was observed to have been removed. After extraction, the samples for treatments 1 and 2 were dried in a desiccator and purged with nitrogen for 4 h.

The remaining treatments were dried in the oven at 100°C for 30 min. When samples were removed from the oven they were equilibrated to room temperature in a desiccator purged with nitrogen. The vacuum in the desiccator was returned to atmospheric pressure with nitrogen.

Oil recovered from treatments 1, 5, and 6 was analyzed by thin-layer chromatography (TLC). Samples were chromatographed on silica gel plates with methylene chloride and visualized with bromo thymol blue (14). This procedure separates the sterols, triacylglycerides, and the less polar fractions.

FBT Predrying. Before extraction, all samples were dried at 100°C for 3 h for both the conventional and the FBT methods. It is particularly important to remove the residual moisture from samples analyzed by the FBT because the moisture is removed during the extraction process, causing erroneously inflated values. A study was made of the effects of predrying on ground beef, a high-energy horse diet, corn, soybeans, and a pig diet for different periods of time and at different temperatures. Samples were weighed in a filter bag and dried at 100, 105, and 110°C. The samples were analyzed at intervals of 30 min up to 180 min and each treatment was replicated three times.

FBT Sample Size. The effect of sample size (1.00, 0.50, and 0.25 g) on the precision of the analysis of six corn and three soybean samples was investigated in a study with the FBT. The samples, analyzed in triplicate, were finely ground and had a uniform consistency. Because of the sensitivity of the analytical balance (capable of weighing to 0.1 mg) and the relatively small tare weight of the filter bags (0.5 g), it was expected that weighing errors would be minimized and the variance associated with this study would be related to sample handling and sample homogeneity.

FBT Extraction Temperature. Because elevated solvent temperatures enhance the extraction kinetics, the effects of extractions at three temperatures, 85, 90, and 95°C were studied. Samples were extracted in 15-min intervals over a 60-min period. The FBT analyses were conducted in triplicate on ground beef, soybeans, potato chips, and a high-energy horse diet.

FBT Postextraction Drying. After extraction and solvent evaporation in the XT20, samples can absorb weight from exposure to ambient moisture and can contain traces of solvent that must be removed. Postdrying periods of 10 and 20 min were studied. Samples were weighed directly upon removal from the XT20 and then placed in an oven at 100°C for two consecutive 10-min periods and weighed after cooling in a desiccant pouch. Samples (n = 10) were analyzed in duplicate (oat meal, brownie mix, crackers, dog food, pig diet, ham, turkey, corn, soybeans, and canola). A second study was conducted to determine the effect of drying at 100°C for intervals of 20, 40, 60, and 80 min. The FBT analyses were conducted in triplicate on soybeans, canola, potato chips, and horse feed.

FBT Youden's Ruggedness Test. Youden's Ruggedness Test (15) was performed to evaluate seven variables in the method and the effect of modest changes in these variables. The variables were sample size (0.8–0.9 g vs. 1.2–1.3 g), predry time (2.5 vs. 3.0 h), predry temperature (98 vs. 102°C), extraction time (25 vs. 35 min), extraction temperature (89 vs. 94°C), postdry time (25 vs. 35 min), and postdry temperature (98 vs. 102°C). Nine sample types were analyzed in triplicate, including ground beef, chicken thighs, hot dogs, corn, soybeans, potato chips, cattle feed, poultry feed, and dog food.

Comparison of the FBT with the Conventional Method. The relative accuracy of the FBT was evaluated by comparing the results of this method with those of the conventional method. Samples (n = 22) were analyzed by both methods; each was replicated five times to compare the relative precision. The samples included a range of samples encompassing meats, grains and oilseeds, feeds and foods. The data were analyzed by Regression Analysis.

Multilaboratory FBT Study. A study was designed to evaluate the precision and accuracy of the FBT by analyzing five samples in quadruplicate using the same protocol in 13 laboratories and completing the analysis within a 3-wk period. This study provided an opportunity for the laboratories to familiarize themselves with the FBT protocol to be used in the more extensive collaborative study. The laboratories were located in the United States, Canada, and Europe. The samples used were ground beef, cheese curls, soybeans, corn, and a horse diet. The conventional analysis was performed by ANKOM Technology.

FBT Collaborative Study. A collaborative study, performed in conjunction with AOCS, was designed to evaluate the precision and accuracy of the FBT with a wide variety of samples that represented foods, feeds, meats, and oilseeds. Samples (n = 28) were sent to 12 laboratories in the United States, Canada, and Europe in the form of 56 blind duplicates. Each laboratory was given a detailed protocol and had an opportunity to become familiar with the method in a preliminary study. These samples were also analyzed by three AOCS Certified Laboratories using the relevant official methods.

Results and Discussion

Reusing Solvent. The results of the solvent fractionation study of petroleum ether (Fig. 4.2) indicated that the majority of the solvent (~70%) distilled in the range of 36–40°C with no other fraction >8%. The distributions of all of the fractions were similar for both recycled and purchased solvent. This study indicates that petroleum ether can be recycled without significantly changing the distribution of the solvent components.

Sample Matrix Disruption. Fats and oils that are not hindered by the sample matrix or by various types of binding rapidly dissolve in fat solvents. Oils trapped

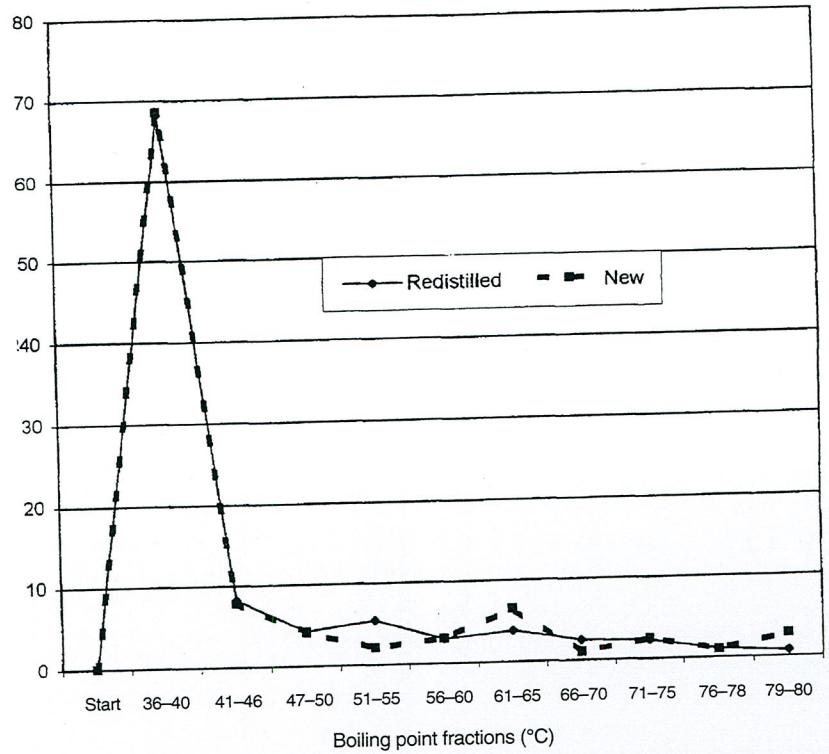

Fig. 4.2. The boiling point distribution of new reagent grade and recycled petroleum ether. The recycled petroleum ether was recovered by distilling waste solvent from fat extractions.

in plant cell matrices are particularly difficult to extract due to the cell wall. This microstructure can act as a semipermeable membrane where larger molecules have limited access to exit the structure even though the smaller solvent molecule can penetrate the structure. Plant matrices are difficult to disrupt on a cellular basis, and this has led to the development of extensive grinding procedures. The grinding and regrinding procedures required in the AOCS and FOSFA methods for certain oilseed samples attest to the difficulty of preparing these samples for analysis. The grinding study with soybeans illustrates the problem of sample preparation for complete extraction of the oil (Fig. 4.3). The drying of the whole soybean at 130°C for 60 min before grinding improved the yield by ~3%, whereas regrinding after extraction improved the recovery by another 2%. In both treatments, it would be expected that more extensive fracturing of the cell wall had occurred, enabling greater extraction of oil. Unfortunately, the oven treatment and extensive grinding