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Fat Extraction from Acid- and Base-Hydrolyzed Food Samples Using Accelerated Solvent Extraction

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ABSTRACT: This paper describes a new in-cell method for pursuing accelerated solvent extraction (ASE) prior to lipid analysis from food samples. It is difficult to pursue direct ASE with acid- or base-hydrolyzed samples due to the corrosive nature of the reagents and material limitations. In this study ion exchange based materials were used to remove acid or base reagents in-cell without compromising the recovery of lipids. The performance data are presented here for the new methods for lipid extraction for a variety of food samples and compared to the Mojonnier method. NIST Standard Reference Materials (SRM-1546 and SRM-1849) were used to validate the ASE methods. Excellent fat recoveries were obtained for the ASE methods. The new methods presented here enhance the utility of ASE and eliminate labor intensive protocols.

KEYWORDS: ASE, fat extraction, acid hydrolysis, base hydrolysis, gravimetry, fat analysis, sample preparation

INTRODUCTION

The Nutrition Labeling and Education Act (NLEA) of 1990, under the jurisdiction of the U.S. Food and Drug Administration (FDA), describes total fat as one of the mandatory requirements for nutrition labeling for most food products.[1] Total fats and total lipids are defined as the sum of all fatty acids expressed as triglyceride equivalents and include the sum of all fatty acids from mono-, di-, and triglycerides, free fatty acids, phospholipids, and sterols. The determination of lipid content in food products is a commonly used analytical technique; however, the methods are usually multistep protocols and are labor intensive. There are many extraction protocols described in the literature for fat extraction such as the Gerber, Babcock, Roese-Gottlieb, Mojonnier, Folch, and Bligh and Dyer methods.[2−6] Some of these methods involve reading volumetrically the fat content. Gravimetry is a popular and more commonly pursued method for determining total fats since it is more quantitative and less reliant on user interpretation relative to volumetric methods. Furthermore, gravimetry methods could be implemented with minimal equipment and require less skilled workers, making the method simpler to implement when compared to gas chromatography with mass spectrometric detection (GC-MS) based techniques. GC-MS is the preferred method, though, when the classification of the fat types is pursued.

Automated extraction techniques, such as supercritical fluid extraction, and pressurized liquid extraction (PLE), such as accelerated solvent extraction (ASE), have also been used to extract food samples.[7−11] With ASE, by pursuing extractions at higher temperatures and pressures the technique allows for faster extraction of analytes relative to conventional liquid−liquid-based extraction techniques, which are implemented at low pressures and slightly elevated temperatures.[12] The gravimetric determination of total fat involves solvent extraction of fat with a suitable solvent from food matrices, followed by evaporation of the solvent and measurement of the residual extracted fat by weight. From a sample extraction perspective some samples are pretreated with concentrated acids, bases, or other chemicals such as enzymes prior to extraction so that the analytes of interest are freely available for extraction by organic or aqueous solvent systems.[2,4] Pursuing extraction of lipids in some food samples is difficult because the lipids are bound with carbohydrate or proteins. Disintegration of the sample with an acid or base and heat hydrolyzes the proteins and starch and liberates the fat to allow its easy extraction.[2,4] The purpose of the hydrolysis is to release bound lipids. Association of Official Analytical Chemists (AOAC) methods 922.06, 989.05, and 996.06 describe acid or base hydrolysis steps for food samples followed by a liquid−liquid extraction method.[2,4] In the liquid−liquid extraction method the lipids are extracted away from the acidic or basic matrices by intimately mixing the sample with an organic solvent selective to the analyte such as an ether-based solvent mixture. The lipids preferentially dissolve in the ether-based solvent, whereas the acid or base reagents remain in the aqueous matrix. The ether-based solvent containing the lipid analytes are separated visually (manually) from the aqueous fraction containing the acid or base matrix. The extracted solvent mixture is evaporated, leaving the lipids available for further analysis by gravimetry or by GC or by GC-MS after the lipids have been converted to the fatty acid methyl ester form.[2,4]

Solvent extraction involves equilibrating the solvent with the sample matrix containing the analytes of interest. The oldest methodology of pursuing solvent extraction is Soxhlet extraction and is widely used in both AOAC and American Oil Chemists' Society (AOCS) official methods.[2,3] The Soxhlet extractions are typically pursued at or near atmospheric pressure and with only slightly elevated temperatures. As a result, the extractions performed under these conditions take longer than those typically

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pursued with a pressurized solvent extraction technique such as ASE. ASE uses an organic solvent at elevated temperature and pressure. ASE utilizes the elevated temperature to increase the solubility and mass transfer while decreasing the viscosity of solvent to accelerate the extraction. The elevated pressure keeps the solvent at a liquid state, thereby preventing boiling, making faster extractions feasible at high temperatures. Solvent consumption and extraction time is significantly reduced compared to Soxhlet extraction based methods.[12]

Whereas ASE has shown utility in the direct extraction of fats for some sample matrices, under acid or base hydrolysis conditions, the ASE instrument would be exposed to the concentrated acid or base reagents, which can cause corrosion issues and in some cases interfere with reliable operation of the system. Further presence of acid or base that can become concentrated during the solvent evaporation process also is detrimental to the sample processing step and can be hazardous to the practioner. It would therefore be useful to eliminate these harsh conditions in the pursuit of lipid analysis so that the benefits of ASE can be realized.

In this paper we show a new in-cell extraction approach. This approach eliminated the exposure of the system to harsh conditions, and a robust platform was available to pursue fast extraction of fat from various food samples. In this study, meat homogenate standard reference material (SRM-1546) and infant/adult nutritional formula standard reference material (SRM-1849) were used to validate the ASE methods.

MATERIALS AND METHODS

Materials. All reagents used in this work were of ACS grade. All dilutions were accomplished using 18.2 MΩ · cm water. Eight molar HCl reagent was prepared from concentrated HCl (J. T. Baker, Philipsburg, NJ), and 58% NH_4OH (~28−30% NH_3 content, CAS Registry No. 1336-21-6) was purchased from Sigma-Aldrich (St. Louis, MO). Hexane, ACS grade (Sigma-Aldrich, St. Louis, MO) was the extraction solvent for ASE. A FAME standard (AOCS#1) was purchased from Restek (Bellefonte, PA). Individual standards and an internal standard, C19 trinonadecanoin (T-165), were purchased from Nu-Check Prep, Inc. (Elysian, MN). Chloroform, petroleum ether, toluene, and ethanol were purchased from Fisher Scientific (Pittsburgh, PA). Diethyl ether, pyrogallol, and hexane were purchased from Sigma-Aldrich. All of the food samples for gravimetry and GC-FAME analyses were purchased from local supermarkets in two different cities, Sunnyvale, CA, and Salt Lake City, UT. Food samples such as corn chips, mayonnaise, parmesan cheese, bologna, and infant formula samples were used for acid hydrolysis experiments, and dairy-based foods, whipping cream, half-and-half, milk, cream cheese, cottage cheese, and sour cream, were used for base hydrolysis experiments. The accelerated solvent extractions were carried out with ASE instrument 150, 350, and 300 (Dionex Corp., Sunnyvale, CA). Nitrogen gas for the ASE instrument was supplied by Airgas NCN (Sacramento, CA). The ASE Prep CR in H^+ or Na^+ forms are proprietary in-house ion exchange products from Dionex (Sunnyvale, CA). Diatomaceous earth (DE) was a commercial product (P/N 062819) from Dionex (Sunnyvale, CA). The ASE cells (100 mL) and extract collection bottles (250 mL) are products available from Dionex (Sunnyvale, CA). A VWR (West Chester, PA) 370 hot plate was used for a water bath setup for evaporating the solvent extract mixture. A Mettler Toledo (Columbus, OH) AG 204 analytical balance was used for weighing the samples, collection bottles, and fat residue. An oven from Tenney Engineering (Union, NJ) was used for drying the fat residue.

Standard Reference Materials. Standard Reference Material 1546 (SRM-1546), meat homogenate, was obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, MD. SRM-1546 was used for acid hydrolysis (AH) followed by ASE. Standard Reference Material 1849 (SRM-1849), infant/adult nutritional formula, was also obtained from NIST and was used for base hydrolysis (BH) followed by ASE.

Acid Hydrolysis of Food Samples for Gravimetric Determination. The food samples (usually 1−2 g) were weighed to the nearest 0.0001 g in a 40 mL clear glass bottle (Dionex, Sunnyvale, CA), 2 mL of ethanol was added, and the contents were lightly mixed by swirling. To the above mixture was added 10 mL of 8 M HCl, and the contents were mixed thoroughly. Hydrolysis proceeded for 40 min at 80 °C. The vial was vortexed every 10 min to ensure that the food sample was hydrolyzed effectively. After the hydrolysis was complete, the acid-hydrolyzed samples were cooled to room temperature. All samples were hydrolyzed using the above procedure regardless of the extraction procedure used (Mojonnier or ASE).

Acid Hydrolysis Procedure for GC-MS. A ground and homogenized sample containing 100−200 mg of fat was weighed into a labeled 40 mL clear glass bottle (Dionex, Sunnyvale, CA). To the above sample was added 0.1 g of pyrogallol to prevent oxidative losses during hydrolysis followed by the addition of 2 mL of internal standard solution (C19 fatty acid in chloroform). To the above mixture was added 2.0 mL of ethanol, and the contents were mixed, followed by the addition of 10 mL of 8 M HCl. The contents of the vial were mixed thoroughly. Hydrolysis conditions were similar to the gravimetry procedure. All samples were hydrolyzed using these conditions regardless of the extraction procedure used (Mojonnier or ASE).

Base Hydrolysis of Food Samples for Gravimetric Determination. The food samples (usually 1−2 g) were weighed to the nearest 0.0001 g in a 40 mL clear glass bottle, 2 mL of ethanol was added, and the contents were lightly mixed. For liquid milk samples 4−6 g was the sample size. To the above sample was added 4 mL of water, and the contents were mixed, followed by the addition of 2 mL of 58% NH_4OH. The contents of the vial were thoroughly mixed. Hydrolysis proceeded for 10−20 min at 80 °C. The vial was vortexed every 10 min to ensure that the food sample was hydrolyzed effectively.[2,4] After the hydrolysis was complete, the base-hydrolyzed samples were cooled to room temperature. All samples were hydrolyzed using these conditions regardless of the extraction procedure used (Mojonnier or ASE). For ASE based on this work it is recommended to add 6 mL of ethanol to the base-hydrolyzed sample vial. The SRM-1849 sample was base-hydrolyzed similarly using the above procedure. The sample portion used was about 0.5 g as recommended by NIST for SRM-1849.

Base Hydrolysis Procedure for GC-MS. A ground and homogenized sample containing 100−200 mg of fat was weighed into a labeled 40 mL clear glass bottle. To this sample was added 0.1 g of pyrogallol to prevent oxidative losses during hydrolysis followed by the addition of 2 mL of internal standard solution (C19 fatty acid in chloroform). A further 2.0 mL of ethanol was added followed by 4 mL of water, and the contents were mixed. To this mixture was added 2 mL of 58% NH_4OH, and the contents were mixed thoroughly. The vial cap was fixed firmly to avoid any leaks. Hydrolysis proceeded for 10−20 min at 80 °C. The vial was vortexed every 10 min to ensure that the food sample was hydrolyzed effectively.[2,4] All samples were hydrolyzed using these conditions regardless of the extraction procedure used (Mojonnier or ASE). After the hydrolysis was complete, the base-hydrolyzed samples were cooled to room temperature. For ASE based on this work it is recommended to add 6 mL of ethanol to the base-hydrolyzed sample vial and proceed to the sample preparation procedure prior to ASE.

Acid-Hydrolyzed Sample Preparation Procedure for ASE. The extraction of acid-hydrolyzed food samples were pursued in a 100 mL zirconium extraction cell (Dionex, Sunnyvale, CA). The bottom cell end-cap was fixed and a filter inserted. A plug (5 g) of ASE Prep CR Na^+ form resin (Dionex, Sunnyvale, CA) was added at the bottom of the cell.

The cell was ready for the sample matrix. To prepare the sample, 15 g of ASE Prep Diatomaceous Earth, DE (Dionex, Sunnyvale, CA) was taken into a mortar and ground thoroughly. To this was added 30 g of ASE Prep CR Na^+ form resin (Dionex, Sunnyvale, CA) and mixed by pestle without aggressive grinding. The acid-hydrolyzed sample was added evenly over this resin/DE mixture in the mortar. Any residual sample in the hydrolysis container was rinsed with a 1−2 mL portion of 1:1 diethyl ether and petroleum ether. The contents of the mortar were mixed by pestle. This sample matrix was loaded into the cell from above. The cell was tapped on the laboratory counter during loading of the sample matrix to ensure adequate compaction for loading the whole contents of the mortar. After the contents of the mortar had been transferred, a small amount of DE was added to the mortar, swirled, and ground by pestle to remove any residual sample from the mortar and loaded into the ASE cell. Another plug (about 5 g) of resin was added to top off the cell, and the top end-cap was affixed.

Base-Hydrolyzed Sample Preparation Procedure for ASE. The extraction of base-hydrolyzed food samples was pursued in a 100 mL zirconium extraction cell. The bottom cell end-cap was fixed, and a filter was inserted. A plug (5 g) of ASE Prep CR H^+ form resin (Dionex, Sunnyvale, CA) was added at the bottom of the cell. The cell was ready for the sample matrix. To prepare the sample, a 15 g of ASE Prep DE was taken into a mortar and ground thoroughly. To this was added 15 g of ASE Prep CR H^+ form resin and mixed by pestle without aggressive grinding. The base-hydrolyzed sample was added evenly over this resin/DE mixture in the mortar. The hydrolysis sample bottle was rinsed with a 1−2 mL portion of ethanol to rinse out any residual sample. The ethanol was then added into the mortar. The whole contents of the mortar were mixed by pestle. This mixture was loaded into the same 100 mL zirconium cell from above. The cell was tapped on the laboratory counter during loading of the sample mixture to ensure adequate compaction for loading the whole contents of the mortar. After the contents of the mortar had been transferred, a small amount of DE was added to the mortar, swirled, and ground by pestle to take out the residual sample from the mortar and loaded into the ASE cell. Another plug (about 5 g) of resin was added to top off the cell, and the top end-cap was affixed.

Extraction. The prepared cell was loaded into the ASE instrument (Dionex ASE 350, 150, or 300) upper carousel, and a preweighed 250 mL collection bottle (Dionex, Sunnyvale, CA) was loaded in the lower carousel for collection of the extract. The weight of the collection bottle was recorded. Automated methods were created for running the samples in ASE.

Extraction Condition for Acid-Hydrolyzed Samples. Hexane was used as the extraction solvent. The extraction was pursued at 100 °C (heating time, 5 min) using a pressure of 1500 psi for a 5 min static time and using a 30% flush volume for three cycles. A 120 s nitrogen purge was used to displace residual solvent after the extraction was complete. The extracts were collected in a 250 mL collection bottle. The total extraction time was about 26 min per sample. Extraction conditions such as static time, cycles, and flush volume can be tailored for a particular sample and extraction.

Extraction Condition for Base-Hydrolyzed Samples. Hexane was used as the extraction solvent. The extraction was pursued at 110 °C (heating time, 6 min) using a pressure of 1500 psi for a 15 min static time and using a 30% flush volume for 1 cycle. A 120 s nitrogen purge was used to displace residual solvent after the extraction was complete. The extracts were collected in a 250 mL collection bottle. The total extraction time was about 27 min per sample. Extraction conditions such as static time, cycles, and flush volume can be tailored for a particular sample and extraction.

Mojonnier Extraction. The contents of the 40 mL vial containing the acid- or base-hydrolyzed samples were transferred to a Mojonnier extraction flask. The vial was rinsed with two portions of ethanol (12 mL), and the ethanol was transferred to the Mojonnier flask. Additional ethanol was added to fill the bottom reservoir of the flask, and the solvent was mixed gently, followed by the addition of 25 mL of ethyl ether. The inlet of the flask was sealed with a stopper, and the Mojonnier flask was shaken manually for 5 min, after which 25 mL of petroleum ether was added to the flask. The flask was again shaken for 5 min. The Mojonnier flask was allowed to sit for at least 60 min until the organic ethers and acid/base aqueous layers separated and the top organic layer was free of particulates. The top layer was decanted into a preweighed glass bottle. The fat-containing ethers were evaporated to dryness under a stream of nitrogen at 40−50 °C in a water bath. The evaporation was started on the low end of the temperature range to prevent boiling of the ether. After all of the ether was evaporated, the glass bottle was placed in an oven set at 80 °C to dry for 20−30 min.

Evaporation and Drying of ASE Extract. The collected extract from ASE was evaporated in a water bath at about 80 °C under a nitrogen gas stream until all of the solvent was evaporated. The collection bottle was further dried in an oven at 80 °C for 20−30 min. The collection bottle was allowed to cool in the laboratory to room temperature. The dried bottle was weighed, and the difference between the measured weight of the bottle and the initial bottle weight was recorded as the amount of fat extracted. The weight percent of fat extracted expressed as fat content (%) was calculated from the sample weight. The recovery (%) was calculated by using the measured fat content (%) and the label fat content (%) information.

Esterification Procedure. After the total fat had been extracted from the respective food matrix and the solvent completely evaporated, the extracted fat was dissolved in 3 mL of chloroform and 3 mL of diethyl ether. This solution was transferred to a pressure tube. The 40 mL vial was washed a second time with chloroform and ether to ensure complete transfer of any residual fat, and the rinsed solvents were transferred to the pressure tubes (ACE Glass Inc., Vineland, NJ). The solvent in the pressure tube was evaporated to dryness under a stream of nitrogen at 40 °C. Once the contents of the pressure tube were dry, 2.0 mL of 12% boron trifluoride (BF_3) in methanol and 1.0 mL of toluene were added. The pressure tube was sealed and placed in an oven set to 100 °C for 45 min with gentle shaking every 10 min. The pressure tube was allowed to cool to room temperature, and 5.0 mL of H_2O, 2.0 mL of hexane, and 1.0 g of Na_2SO_4 were added. The tube was shaken or vortexed for 1 min. The two layers were allowed to separate, and the top hexane layer was removed and placed into a 40 mL vial containing 1.0 g of Na_2SO_4. A second 2.0 mL portion of hexane was added to the pressure tube. The pressure tube was shaken or vortexed for 1 min. The two layers were allowed to separate, and the top hexane layer was removed and placed into the 40 mL vial containing 1.0 g of Na_2SO_4 and the first hexane portion. A final volume of the hexane/toluene mixture was accurately measured before analysis by GC-MS. This value was used to calculate the amount of fat found in the samples. A 10× dilution was performed on all samples prepared for FAME analysis.

GC-MS Analysis Parameters. FAMEs were analyzed by an Agilent 6890 series GC interfaced to an Agilent 5973 mass selective detector (Santa Clara, CA). An RTX-Wax column from Restek (Bellefonte, PA) was used in all analyses. The column was 30 m long with an internal diameter of 0.25 mm and a film thickness of 0.25 μm. The flow rate was set to 1.4 mL/min. A 25:1 split was used in GC. The initial oven temperature was held at 125 °C for 30 s. The oven was then heated at a rate of 7 °C/min to 210 °C and then held for 15 min. The total analysis time was 27 min and 40 s. The injection port was at 220 °C, whereas the GC-MS interface was at 230 °C. The MS was set to scan from 40 to 550 atomic mass units (amu). The electron multiplier (EM) voltage was set to 1365 V. Total fat was calculated as the sum of all fatty acids expressed as triglycerides.[4] The recovery (%) was calculated by using the measured fat content (%) against the label fat content (%) information.

Table 1. Gravimetric[a] Results for Percent Fat Recovery (n = 3) in Acid-Hydrolyzed Food Samples for the ASE Method and Mojonnier Method

		ASE method		Mojonnier method	
food sample	label claim, % fat	fat recovery (%)	RSD	fat recovery (%)	RSD
corn chips	35.3	99.95	0.66	98.95	0.65
mayonnaise	76.9	100.1	0.55	99.99	0.88
parmesan cheese	30.0	96.97	0.80	97.98	0.98
bologna	28.6	99.05	1.35	100.7	1.01
infant formula	28.5	101.7	1.58	102.6	1.73

[a] The ASE and Mojonnier gravimetry data were generated in the Sunnyvale laboratory of Dionex Corp.

RESULTS AND DISCUSSION

In-Cell Neutralization. The acid- or base-hydrolyzed food samples have different needs in terms of neutralization. For example, the acid hydrolysis method requires neutralization of 80 mequiv of the acid. Additionally, there is a need to remove about 10 mL of aqueous product. The aqueous content may be higher depending on the water content of the sample analyzed. In-cell neutralization and removal were achieved by mixing the acid- or base-hydrolyzed samples with cation exchange resins of the appropriate form and DE. For example, for acid hydrolysis the cation exchange resin is in the sodium form (40 g of resin with a capacity of about 4 mequiv/g) and reacts with the acid to convert it to a salt form. Furthermore, the addition of DE allows the absorption of the neutralized reaction products, namely, salt and water. Thus, the acid stream no longer interferes with the extraction and there is no acid escaping out of the cell. In contrast, if the acid stream is not neutralized, the stainless steel components of the ASE systems are attacked by the acids, particularly at the higher temperatures (>40 to <200 °C) employed during the extraction. The blackening of the stainless steel components is observed with the cell, tubing, filters, and frits. In some extreme cases there is also clogging of the tubing and associated valve issues. Furthermore, there are associated issues with evaporating or boiling the solvent (hexane) with an aliquot of acid as the water in the acid would tend to boil and the release of the water vapor from the lower water layer would tend to create a splash on the container during the evaporation process, causing a charring on the container walls. Presumably, the fats are dispersed on the container and are superheated during the water evaporation process, causing a charring effect and affecting the total recovery of fats. Other means of removing the acid prior to GC analysis may also be needed because the presence of acid can cause issues with GC analysis. The present approach addresses these limitations by completely removing the acid and associated water in situ in the extraction cell. Similarly for base-hydrolyzed samples the need is to neutralize approximately 30 mequiv of base and remove about 6 mL of water. The water content may be higher depending on the sample water content. The use of cation exchange resin in hydronium form (25 g of resin with a capacity of about 3.5 mequiv/g) allowed the conversion of the base to water. Again, the presence of the DE allowed removal of the water (reaction product). Neutralizing the sample averts the above-cited issues, and robust operation of the method and instrument became feasible. It should be noted that anion exchange resins were unsuitable for this work because they retain the fatty acids and result in poor recoveries. It should be noted that the final resin and DE quantities were inferred experimentally after a series of experiments that showed complete removal of the hydrolysis reagents (data not shown). The layering of the cell with neutralizing medium on the top of the cell and the bottom ensured that there was no residual acid or base stream escaping out of the cell and that the cell components were not exposed to the acid or base stream.

Table 2. FAME Results by GC-MS[a] for Percent Fat Recovery (n = 3) in Food Samples for the ASE Method and Mojonnier Method

		ASE method		Mojonnier method	
food sample	label claim, % fat	fat recovery (%)	RSD	fat recovery (%)	RSD
mayonnaise	76.9	96.53	0.58	97.65	1.18
bologna	28.6	100.1	1.31	100.0	0.97

[a] The GC-FAME analysis was completed in the Salt Lake City laboratory of Dionex Corp.

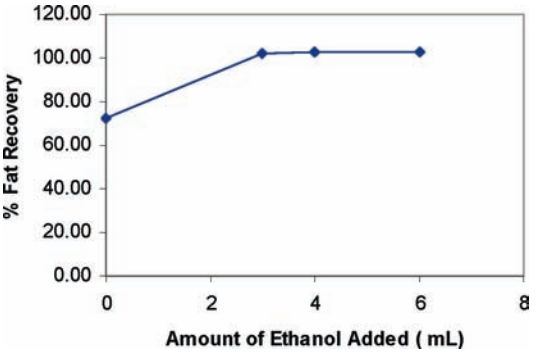

Figure 1. Effect of ethanol addition in milk sample vial after base hydrolysis.

pH Measurement. The collected ASE solvent fractions from the acid- or base-hydrolyzed samples were equilibrated with small aliquots of DI water and shaken thoroughly. The pH of the aqueous fraction was measured. The pH values of the aqueous fractions were similar to that of the DI water (near neutral), thus verifying that there was no intrusion of acids or bases in the collection vessel after pursuit of the ASE.

Acid Hydrolysis Gravimetry. Table 1 compares the performance of the new ASE in-line method with the Mojonnier method. Several food samples varying in fat content from 28.5% (infant formula) to 76.9% (mayonnaise) were analyzed. The recovery is

reported here as a percentage of the label claim. Excellent recoveries were observed for all five samples with good reproducibility. The ASE method results also compared well with the Mojonnier method. Overall, the ASE method was easier to implement and enjoyed the advantage of automation and speed versus the Mojonnier method. In contrast, the Mojonnier method was labor intensive and was a multistep process.

Acid Hydrolysis GC-MS. Two samples were analyzed by the GC-MS technique after derivatizing the fat residues, and the results are shown in Table 2. Excellent recovery and reproducibility were observed for the bologna sample. The results for the mayonnaise samples were slightly lower in terms of overall recovery by both extraction techniques. There was, however, good correlation between the ASE and Mojonnier results. Overall, the above results demonstrate good performance of the method. In general terms, in the pursuit of food analysis variances are observed from the sampling process. For example, viscous samples such as mayonnaise require intimate mixing during the sampling process. This will ensure consistent recoveries. Another variance in recovery originated from how the sample was stored and how long the container was used. The variance may originate from sample fat content differing from batch to batch. Moreover, the samples were purchased from local supermarket in two different cities, Sunnyvale and Salt Lake City, which may incorporate some variance between gravimetry and GC-FAME results. Nevertheless, the overall utility of the method could be inferred from the above results.

Base Hydrolysis Gravimetry. The AOAC 996.06 method recommends adding 2 mL of ethanol during base hydrolysis.[4] The extraction of base-hydrolyzed milk samples showed poor lipid recoveries when hexane was the solvent and with the addition of 2 mL of ethanol. To address the poor recovery, we pursued experiments to study the effect of ethanol on extraction. It should be noted that addition of ethanol to hexane increases the polarity of the solvent.[8,9,13,14] Repeating the extraction method with increasing amounts of ethanol added in addition to the recommended 2 mL improved the recovery as evident from Figure 1. The added ethanol imparts some polarity and aids an improved extraction of the fat by possibly disrupting the stabilizing proteins around the fat globules.[14] It is evident from Figure 1 that the good recovery is achieved somewhere in the 3−4 mL range. To ensure that we have good recovery under a variety of sample conditions, we increased the amount of ethanol to 6 mL. Studies pursued with and without ethanol added for a variety of samples indicated that this addition was needed only with milk samples. For other samples such as half-and-half the recovery improvement was insignificant upon the addition of ethanol. Similarly, for whipping cream and cream cheese samples no change in recovery was observed upon the addition of ethanol. Table 3 compares the fat recovery (%) obtained from the in-cell ASE resin method and the Mojonnier method for various base-hydrolyzed dairy food samples. Comparable recoveries were obtained for all samples. Some samples recovered >100%, possibly from extracting other compounds in the sample.

Base Hydrolysis GC-MS. Four samples were analyzed by the GC-MS technique after derivatizing the samples. The GC-FAME method showed comparable recoveries for both the ASE and Mojonnier methods (Table 4). The recoveries were within a couple of percent to each other for all of the samples used. The critical factors that may account for the variances remained the same for base-hydrolyzed sample as discussed above.

Standard Reference Material. The new ASE method was validated using standard reference materials after acid and base hydrolysis. Table 5 shows the gravimetric results of the total fat extraction in ASE for SRM-1546 and SRM-1849. The acid hydrolysis−ASE of SRM-1546, meat homogenate sample,

Table 3. Gravimetric[a] Results for Percent Fat Recovery (n = 3) in Base-Hydrolyzed Food Samples for the ASE Method and Mojonnier Method

		ASE method		Mojonnier method	
food sample	label claim, % fat	fat recovery (%)	RSD	fat recovery (%)	RSD
heavy whipping cream	36.0	101.2	0.16	100.2	0.11
half-and-half	10.5	100.8	0.52	100.8	0.92
milk	3.24	103.0	0.16	104.7	0.63
cream cheese	29.0	98.25	1.47	98.33	0.65
cottage cheese	4.13	97.86	0.57	96.65	2.15
sour cream	20.0	95.94	0.64	94.22	1.34

[a] The ASE and Mojonnier gravimetry data were generated in the Sunnyvale laboratory of Dionex Corp.

Table 4. FAME Results by GC-MS[a] for Percent Fat Recovery in Base-Hydrolyzed Food Samples for the ASE Method and Mojonnier Method

		ASE method, resin (n = 3)		Mojonnier method (n = 2)	
food sample	label claim, % fat	fat recovery (%)	RSD	fat recovery (%)	RSD
heavy whipping cream	36.0	99.23	1.79	98.54	0.39
half-and-half	10.5	97.44	1.44	97.82	1.01
milk	3.24	98.40	0.65	101.8	1.06
cream cheese	29.0	98.05	1.70	103.9	2.13

[a] The GC-FAME analysis was completed in the Salt Lake City laboratory of Dionex Corp.

Table 5. Weight Percent (n = 3) of Fat Extracted for NIST SRM-1546 and SRM-1849 by ASE Gravimetric Methods

sample	sample treatment−extraction−analytical method	% fat	NIST value % fat
SRM-1546	acid hydrolysis−ASE−gravimetry	20.9 ± 0.2	21.0 ± 1.4
SRM-1849	base hydrolysis−ASE−gravimetry	31.2 ± 0.1	31.0 ± 0.5

showed a fat content of 20.9 ± 0.2%. The measured fat content is very close to the NIST certified value of 21.0 ± 1.4%. The base hydrolysis−ASE of SRM-1849, infant/adult nutritional formula, showed a fat content of 31.2 ± 0.1%. The measured fat content is very close to the NIST value of 31.0 ± 0.5%. Overall, the above results validate the present method as a good tool for measuring fat content for food products.

In conclusion, the traditional Mojonnier method uses ambient temperature and atmospheric pressure for liquid−liquid extraction of fat from food samples. The Mojonnier method is a labor intensive, low-throughput extraction method. Also, the Mojonnier method exposes the practitioner to concentrated reagents and solvent by requiring (a) shaking of the concentrated reagents solvent mixture and (b) handling of the reagents solvent mixture after the extraction process. Furthermore, the fractionation step in the Mojonnier method is highly technician/technique dependent. A new in-cell method allows acid- or base-hydrolyzed samples to be easily extracted by ASE. By pursuing in-cell neutralization and removal, the instrument is no longer exposed to the corrosive acid or base conditions. Furthermore, the ASE method is fully automated and does not require shaking or fractionation steps, thus eliminating human errors. The ASE method is simple, reliable, and fully automated. We have shown here that ASE is a suitable alternative to Mojonnier extraction and provides similar fat recoveries for various food products and standard reference materials.

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