Rapid Determination of Total Fat from Dairy Products

INTRODUCTION

Sample preparation—specifically, solvent extraction—is an important step in analytical processes. For many years, analysts have used an array of solvent extraction techniques, such as Soxhlet, automated Soxhlet, or sonication. Accelerated Solvent Extraction (ASE®) systems from Dionex provide flow-through solvent extraction that increases productivity, decreases costs, and provides an excellent platform for automation.

The newly updated flow-through solvent extraction systems (ASE 350 and ASE 150 systems) allow extraction of samples that require acidic or alkaline pretreatments. It is well known that mineral acids and caustic solutions corrode stainless steel cells and tubing. Dionex ASE systems are equipped with pH-hardened tubing and corrosion-resistant extraction cells to allow the use of mineral acids and caustics in automated sample preparation. The ability to extract pretreated samples significantly expands the capabilities of ASE systems and as a result, lab productivity is increased by streamlining the entire sample preparation process, while providing high quality, reproducible results.

Previous studies show that ASE systems can successfully extract fat from a number of different food matrices after an acidic hydrolysis treatment step.[1] The work shown here demonstrates that ASE systems can also be used to extract dairy products following base hydrolysis. The current methods for determining fat in dairy products are adequate but have several drawbacks: they are labor intensive, consume large amounts of expensive solvents, and are time consuming.

Dairy products present a set of complex matrices. Pretreatment steps are needed to denature the casein (a phosphoprotein found in dairy products), allowing greater exposure of the fat to the extraction solvent. For example, a cheese sample must be treated in several steps before solvent extraction. The entire manual extraction process usually requires 2 to 3 h and more than 100 mL of solvent per sample (AOAC Official Method 933.05).[2] Thus, the standard fat extraction methods, such as Roese-Gottlieb, Gerber, Babcock, and Mojonnier, do provide satisfactory results but are very time consuming and labor intensive.

Solvents that are currently used with standard fat extraction methods can also be used with the ASE systems; however, ASE system technology uses significantly less solvent and extracts samples in approximately a quarter of the time. This is possible due to the increased temperature and pressure of the ASE system, which in turn, enhances the efficiency of the extraction. Because the ASE system is automated, it allows unattended extraction of up to 24 samples for small cells and up to 19 samples when using the larger extraction cells (66 or 100 mL).

In this work, fat was extracted from sour cream, cream cheese, coffee creamer, heavy whipping cream, and low-fat milk using an ASE 350 system technique developed by Dionex. The same sample matrices were also extracted by the traditional Mojonnier technique. The ASE system results reported here are statistically equivalent to those of the Mojonnier method. The resulting extracts were derivatized and analyzed by gas chromatography-mass spectrometry (GC/MS) according to AOAC Official Method 996.06.[3]

EQUIPMENT

ASE 350 Accelerated Solvent Extraction System (Dionex P/N 066050)

Dionium extraction cells (100 mL) (Dionex P/N 068103)

Glass fiber filters (Dionex P/N 056781)

Collection bottles (250 mL) (Dionex P/N 056284)

Collection vials (40 mL) (Dionex P/N 048783)

Analytical balance (to read to the nearest 0.0001 g or better)

Mortar and pestle (Thermo Fisher Scientific or equivalent)

GC-MS (Agilent P/N 6890/5973)

Stabilwax® capillary GC column (Restek Corporation P/N 10623)

Pressure tubes (ACE Glass Inc. P/N 8648-03)

REAGENTS AND STANDARDS

Chloroform

Pyrogallol (Sigma-Aldrich)

Ethyl alcohol, reagent-grade

Water (Milli-Q® Water System), 18 MΩ

Hexane

Ethyl ether

ASE Prep DE (diatomaceous earth) (Dionex P/N 062819)

ASE Prep CR H^+ form (Dionex P/N 071397)

Ammonium hydroxide (58% w/w)

Toluene

Boron trifluoride, 12% in methanol

Sodium sulfate (baked prior to use)

All solvents are pesticide-grade or equivalent and were purchased from Thermo Fisher Scientific.

SAMPLES

Whipping cream

Cream cheese

Non-fat milk

Sour cream

Coffee creamer

All samples were purchased from a local grocery store.

SAMPLE PREPARATION

Base Hydrolysis Procedure

According to AOAC Official Method 996.06, approximately 100 to 200 mg of fat are required for the fatty acid methyl ester (FAME) analysis. The actual weight of the sample can be adjusted accordingly.

Weigh the sample into a 40 mL vial. Add 0.1 g of pyrogallol (to prevent oxidative losses during hydrolysis). Add 2 mL reagent alcohol to the vial and mix contents thoroughly. Next, add 4 mL of water and again mix thoroughly. Two mL of ammonium hydroxide are then added. Heat the vials for 30 to 45 min at 75 to 80 ºC using a hot plate or water bath, shaking the samples continuously. After the samples have cooled sufficiently, add 6 ml of reagent alcohol to the 40 mL vial and shake the mixture thoroughly.

Sample Preparation with ASE Prep CR H^+

Gently mix 22 g of ASE Prep CR H^+ and 12 g of ground ASE Prep DE in a mortar with a spatula until a uniform mixture is obtained. After base hydrolysis is complete, transfer the contents of the 40 mL vial to the mortar. Rinse the vial with two 2 mL portions of reagent alcohol and add each portion to the mortar. Again, gently mix the contents of the mortar with a spatula until there are no clumps. Add the contents of the mortar to a 100 mL ASE 350 Dionium™ extraction cell containing a glass fiber filter and 5 g ASE Prep CR H^+ form. Any additional room at the top of the extraction cell should be filled (leaving a 3 to 4 mm space) with additional resin to capture any excess base. Then, secure the top cell cap.

ASE CONDITIONS

Cell Size:	100 mL
Cell Type:	Dionium
Pressure:	1500 psi
Temperature:	100 °C
Solvent:	Hexane
Static Time:	5 min
Static Cycles:	3
Flush:	60 %
Purge:	150 s

EXTRACTION

Load the extraction cells onto the ASE 350 system and extract using the method specified above. If performing a gravimetric determination, preweigh the 250 mL collection bottles to determine the tared weight. After the extractions are complete, blow to dryness using nitrogen with the hot plate set at 60 °C. Then reweigh the sample bottles. Use the difference in weights to determine the amount of fat in each sample.

ESTERIFICATION PROCEDURE

Dissolve the fat contained in the collection bottles by adding 3 mL chloroform followed by 3 mL diethyl ether and transfer this solution to an ACE pressure tube. Wash the bottle a second time with chloroform and ether to ensure complete transfer of the hydrolyzed fat to the pressure tube. Evaporate the chloroform/ether mixture to dryness using a water bath. Once dry, add 2 mL 12% BF_3 in methanol and 1 mL toluene to the pressure tube. Seal the tube and place in an oven set to 100 °C for 55 min, shaking gently every 10 min. Allow the tube to cool to room temperature.

Add 5 mL H_2O, 2 mL hexane, and 1 g Na_2SO_4 to each tube. Shake or vortex for 1 min. Allow the two layers to separate, decant the top (hexane) layer, and transfer it to a 40 mL vial containing 1 g Na_2SO_4. Add a second 2 mL portion of hexane to the pressure tube. Shake or vortex for 1 min. Again, allow the layers to separate, decant the top layer, and transfer to the vial containing 1 g Na_2SO_4 and the first hexane portion. Accurately measure a final volume of the hexane/toluene mixture before analysis by GC/MS. This value will be used to calculate the amount of fat found in the samples.

Note: A 10× dilution was performed on all samples prepared for FAME analysis. All calculations used to determine the percent recovery of fat were according to AOAC Official Method 996.06 section G.[3]

GC/MS Analysis Parameters

GC:	Agilent 6890
MSD:	Agilent 5973
Source Pressure:	10-5 Torr
Column:	Stabilwax, 30 m × 0.25 mm, df = 0.25 µm
Injection Port	Temperature: 240 °C
Injection Mode:	Split, 25:1
Gas Flow Rate:	1.6 mL/min, constant flow
Temperature Program:	125 °C (0.5) – 7 – 210 °C 15 min)
MS Transfer	
Line Temperature:	240 °C
MS Conditions:	Full scan, 40 to 550 amu
Electron Multiplier:	1365 v

RESULTS AND DISCUSSION

Table 1 shows extraction recovery results obtained using the ASE system and Mojonnier techniques. The average results are expressed as a percentage of the values from the food packaging label and were determined by FAME analysis based on AOAC Official Method 996.06 section G. The Mojonnier extractions were done in duplicate, whereas the ASE system results are all in triplicate.

Table 1. Comparison of Extraction Results for Dairy Products using ASE and Mojonnier Methods

	Average	SD	%RSD
Sour Cream			
Mojonnier-FAME	102.4	1.29	1.26
ASE-FAME	98.2	2.82	2.93
ASE-Gravimetric	100.1	0.19	0.88
Cream Cheese			
Mojonnier-FAME	103.9	2.21	2.13
ASE-FAME	98.1	1.70	1.74
ASE-Gravimetric	98.0	0.32	1.30
Whipping Cream			
Mojonnier-FAME	103.2	1.04	1.01
ASE-FAME	102.8	1.48	1.44
ASE-Gravimetric	104.6	0.32	3.02
Coffee Creamer			
Mojonnier-FAME	98.5	0.39	0.39
ASE-FAME	99.2	1.77	1.79
ASE-Gravimetric	99.3	0.50	1.39
Milk			
Mojonnier-FAME	101.8	1.08	1.06
ASE-FAME	98.4	0.64	0.65
ASE-Gravimetric	104.1	0.03	0.76

CONCLUSION

Combined with base hydrolysis, the ASE system yields equivalent results for determination of lipids from dairy products, when compared to more time-consuming extraction techniques. In addition, the ASE system technique is automated and extracts were completed in 25 min compared to 2 to 3 h for the other techniques. Compared to the Mojonnier method, which requires liquid-liquid separation funnels, the ASE system technique provides substantial savings in labor and solvent costs. The newly developed ASE 350 system from Dionex expands the capability of automated extraction technology and provides a degree of flexibility unmatched in other extraction systems.

SUPPLIERS

Thermo Fisher Scientific International, 81 Wyman Street, Waltham, MA 02454, U.S.A.

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, U.S.A.

Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, 95051, CA U.S.A.

ACE Glass Incorporated, P.O. Box 688, 1340 North West Blvd., Vineland, NJ 08362-0688, U.S.A.

REFERENCES

1. Dionex Corporation, *Extraction of Total Fat from Food Samples After Acid Hydrolysis Using Accelerated Solvent Extraction (ASE) with GC-MS Analysis.* Application Note 361, LPN 2008, Sunnyvale, CA, 2008.
2. AOAC Official Method 933.05, *Fat and Cheese,* 2005.
3. AOAC Official Method 996.06, *Fat (Total, Saturated, and Unstaurated) in Foods,* 2001.

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