

Extraction of Total Fat from Food Samples After Acid Hydrolysis Using Accelerated Solvent Extraction (ASE) with GC-MS Analysis

INTRODUCTION

Sample preparation—specifically, solvent extraction—is an important step in the analytical process. For many years, analysts have used an array of solvent extraction techniques including Soxhlet, shaking, sonication, and blending. ASE[®] technology provides a flow-thru solvent extraction system that increases productivity while decreasing cost and providing a platform for automation.

Complex matrices such as food typically require acid hydrolysis or pretreatment prior to solvent extraction. Pretreatment or hydrolysis of these matrices is often necessary to facilitate complete extraction of lipids from the sample. Time-consuming and labor-intensive liquid extraction techniques such as Soxhlet, automated Soxhlet, and Mojonnier extraction are typically used to extract fatty acids after acid hydrolysis.

The newly updated flow-through solvent extraction system from Dionex allows extraction of matrices which require acidic or alkaline pretreatment. While these pretreatment techniques can corrode the stainless steel cells and pathways found in other extraction systems, a recent innovation for ASE technology uses a pH-hardened pathway with Dionium[™] components to prevent this corrosion. The ability to extract these pretreated matrices significantly expands the capabilities of ASE technology and widens the scope of ASE applications.

This Application Note describes methods used for extraction of total fat from food samples and determination of fat content by FAME (Fatty Acid Methyl Ester) analysis based on AOAC Official Method 996.06 section G.

EQUIPMENT

ASE 150 or 350 with pH-hardened pathway
(Dionex PN 066401 or 066230)
Dionium extraction cells (100 mL) (Dionex PN 068103)
Glass fiber filters (Dionex PN 056781)
Collection bottles (250 mL) (Dionex PN 056284)
Collection vials (40 mL) (Dionex PN 048783)
GC-MS
Rtx[®]-Wax capillary GC column (Restek Corp. PN 12423)
Pressure tubes (ACE Glass Inc.)

SOLVENTS AND REAGENTS

Chloroform (Fisher Scientific)
Pyrogallol (Sigma Aldrich)
Alcohol; reagent-grade (Fisher Scientific)
Hexane (Fisher Scientific)
Ethyl ether (Fisher Scientific)
ASE Prep DE (Dionex PN 062819)
ASE Prep CR (Dionex PN 080024)
8.3 M HCL (Fisher Scientific)
Toluene (Fisher Scientific)
12% BF₃ in MeOH (Fisher Scientific)
Na₂SO₄ (Fisher Scientific)

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SAMPLES

(All samples were purchased from a local grocery store.)

- Mayonnaise
- Fried Corn Chips
- Parmesan Cheese
- Baked Shortcake
- Bologna

SAMPLE PREP

Hydrolysis Procedure

Weigh between 0.1 and 0.5 g of each food sample into 40-mL vials. Add 0.1 g pyrogallol (to prevent oxidative losses during hydrolysis). Add 2 mL alcohol to the vial and mix contents thoroughly. Next, add 10 mL 8 M HCl to the vial and mix thoroughly. Heat the vials for 60 min at 75–80 °C using a hot plate or water bath, shaking the samples continuously.

Sample Prep with ASE Prep CR

After acid hydrolysis is complete, transfer the contents of the 40-mL vial to a mortar containing 30 g ASE Prep CR and 15 g ground ASE Prep DE. Gently mix the contents of the mortar with a pestle until a uniform mixture is obtained. Rinse the vial with two 2-mL portions of diethyl ether and add each portion to the mortar. Again, gently mix the contents of the mortar with the pestle. Add the contents of the mortar to a 100-mL ASE 350 Dionium extraction cell containing a cellulose filter and 6 g ASE Prep CR. Add 5 g ASE Prep CR to the top of the extraction cell and secure the top cell cap.

ASE CONDITIONS

Pressure:	1500 psi*
Temperature:	100 °C
Solvent:	Hexane
Static Time:	5 minutes
Static Cycles:	3
Flush:	70%
Purge:	120 sec

**Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE extractions*

EXTRACTION

Pre-weigh the appropriate number of 250-mL collection bottles and place them in the ASE 150 or 350 bottle carousel. Place the extraction cells in the ASE 150 or 350 cell tray and extract using the conditions listed above.

After extraction, evaporate the contents of the pre-weighed extraction vials to dryness. If gravimetric analysis is required, the weight of the residue in the collection bottle can be used to calculate the amount of fat in the original samples. If direct analysis of the lipids is desired, the fatty acids can be esterified and determined using GC or GC-MS

ESTERIFICATION PROCEDURE

Dissolve the fat contained in the vials by adding 3 mL chloroform followed by 3 mL diethyl ether and transfer this solution to a pressure tube. Wash the vial 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. Once dry, add 2 mL 12% BF₃ 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 minutes. Allow the tube to cool to room temperature. Add 5 mL H₂O, 2 mL hexane, and 1 g Na₂SO₄ to each tube. Shake or vortex for 1 minute.

Allow the two layers to separate, decant the top (hexane) layer and transfer it to a 40-mL vial containing 1 g Na₂SO₄. Add a second 2 mL portion of hexane to the pressure tube. Shake or vortex for 1 minute. Again, allow the layers to separate, decant the top layer and transfer to the vial containing 1 g Na₂SO₄ 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 10x dilution was performed on all samples prepared for FAME analysis. All calculations used to determine the percent recovery of fat were taken from AOAC Official Method 996.06 section G.)

GC-MS ANALYSIS PARAMETERS

Source Pressure:	10 ⁻⁵ Torr
Column:	Rtx-Wax, 30 m x 0.25 mm, d _r = 0.25 μm
Injection Port Temperature:	220 °C
Injection Mode:	Split, 25:1
Column Flow Rate:	1.4 mL/min; constant flow
Temperature program:	125 °C (0.5) – 7 – 210 °C (15 min)
MS Transfer	
Line Temperature:	230 °C
MS Conditions:	Full scan, 40 to 550 amu
Electron Multiplier:	1365 v

Table 1. Extraction Results Using Mojonier Techniques and ASE (n = 3)

Mayonnaise	Average	RSD	%RSD
Mojonnier	75.1	0.89	1.18
ASE	74.2	0.43	0.575
Corn Chips			
Mojonnier	30.41	0.37	1.21
ASE	29.85	0.33	1.10
Parmesean Cheese			
Mojonnier	26.41	0.284	1.08
ASE	26.27	0.220	0.839
Baked Shortbread			
Mojonnier	13.95	0.033	0.238
ASE	14.07	0.451	3.20
Bologna			
Mojonnier	25.58	0.275	0.968
ASE	28.60	0.375	1.31

RESULTS AND DISCUSSION

The table at left shows extraction recovery results obtained using Mojonier techniques and ASE. The average values are expressed as weight percent, and were determined by FAME analysis based on AOAC Official Method 996.06 section G.

CONCLUSIONS

Combined with acid hydrolysis, ASE with the new pH-hardened pathway yields equivalent results for determination of lipids from food, often with better precision than other, more time-consuming extraction techniques. Additionally, ASE technology allows automation of the extraction procedure when compared to the Mojonier method, which requires liquid-liquid separation funnels and uses significant amounts of solvent. The ASE technique also provides substantial savings in labor and solvent costs. The newly developed ASE 350 from Dionex expands the capability of automated extraction technology and provides a degree of flexibility not found in other systems.

LIST OF MANUFACTURERS

Dionex Corporation, Sunnyvale, CA USA
Fisher Scientific International, Pittsburgh, PA USA
Restek Corporation, Bellefonte, PA USA
ACE Glass Incorporated, Vineland, NJ USA

REFERENCES

Dionex Application Note 321: Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction (ASE).

AOAC Method 9096.06

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