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

Brett Murphy, Brian Dorich, and Bruce Richter
Thermo Fisher Scientific, Salt Lake City, UT, USA

Application Note 361

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. Accelerated solvent extraction 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.

Our newly updated flow-through solvent extraction system 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 accelerated solvent extraction technology uses a pH-hardened pathway with Thermo Scientific™ Dionium™ components to prevent this corrosion. The ability to extract these pretreated matrices significantly expands the capabilities of accelerated solvent extraction technology and widens the scope of accelerated solvent extraction 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

- Thermo Scientific™ Dionex™ ASE™ 150 or 350 Accelerated Solvent Extractor with pH-hardened pathway (PN 066401 or 066230)
- Dionium extraction cells (100 mL) (PN 068103)
- Glass fiber filters (PN 056781)
- Collection bottles (250 mL) (PN 056284)
- Collection vials (40 mL) (PN 048783)
- GC-MS
- Capillary GC column
- 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)
- Dionex ASE Prep DE (PN 062819)
- Dionex ASE Prep CR (PN 080024)
- 8.3 M HCL (Fisher Scientific)
- Toluene (Fisher Scientific)
- 12% BF_3 in MeOH (Fisher Scientific)
- Na_2SO_4 (Fisher Scientific)

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.

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Sample Prep with Dionex ASE Prep CR

After acid hydrolysis is complete, transfer the contents of the 40 mL vial to a mortar containing 30 g Dionex 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 Dionex ASE 350 Dionium extraction cell containing a cellulose filter and 6 g ASE Prep CR. Add 5 g Dionex ASE Prep CR to the top of the extraction cell and secure the top cell cap.

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 accelerated solvent extraction applications

Extraction

Pre-weigh the appropriate number of 250 mL collection bottles and place them in the Dionex ASE 150 or 350 Accelerated Solvent Extractor bottle carousel. Place the extraction cells in the Dionex ASE 150 or 350 Accelerated Solvent Extractor 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% 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 minutes. 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 minute.

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 minute. 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 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:	30 mm × 0.25 mm, d_f = 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 Mojonnier Techniques and accelerated solvent extraction (n = 3).

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

Results and Discussion

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

Conclusion

Combined with acid hydrolysis, accelerated solvent extraction 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, accelerated solvent extraction technology allows automation of the extraction procedure when compared to the Mojonnier method, which requires liquid-liquid separation funnels and uses significant amounts of solvent. The accelerated solvent extraction technique also provides substantial savings in labor and solvent costs. The newly developed Dionex ASE 350 Accelerated Solvent Extractor expands the capability of automated extraction technology and provides a degree of flexibility not found in other systems.

List of Manufacturers

- Dionex Corporation (now part of Thermo Scientific), Sunnyvale, CA USA
- Fisher Scientific International, Pittsburgh, PA USA
- ACE Glass Incorporated, Vineland, NJ USA

References

1. Dionex (now part of Thermo Scientific). *Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction*; Application Note 321; Sunnyvale, CA.
2. AOAC Method 9096.06.

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