

# Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction (ASE®)

## **INTRODUCTION**

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped into an extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for cleanup or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption. Up to 24 samples can be loaded and extracted sequentially without requiring operator intervention.

Recently, the requirements for accurate labeling of fats in foods were revised by the U.S. Food and Drug Administration (U.S. FDA) and the U.S. Department of Agriculture (US DA). This occurred as a result of the Nutrition Labeling and Education Act, which requires the labeling of total saturated and unsaturated fats contained in foods.<sup>1</sup> Though these laws do not directly affect food sold outside of the U.S., there seems to be increased awareness worldwide of fat content in foods. In addition, food manufacturers require a method for the consistent determination of fat content for quality-control purposes.

In currently used methods such as Soxhlet and automated Soxhlet, the fat content is determined gravimetrically after extraction with organic solvents such as chloroform or petroleum ether. The current methods require large volumes of solvents and time periods from 2 to 16 h. Many analysts want faster techniques that also require less solvent.

ASE is a relatively new extraction technique that utilizes elevated temperature and pressure to expedite the removal of analytes from various matrices. It has been accepted by the U.S. EPA as Method 3545 for environmental samples, and it has been applied to other sample extraction areas.

The procedures described in this application note apply to the determination of fat content in various solid or semisolid foods such as snack foods and dog biscuits. The fat content is determined by collecting the extracts in preweighed vials, evaporating the solvent with a nitrogen stream, and reweighing the vials.

## **EQUIPMENT**

ASE 200 Accelerated Solvent Extractor equipped with either 11- or 22-mL cells

Analytical balance

Dionex vials for collection of extracts (40 mL, P/N 49465; 60 mL, P/N 49466)

Cellulose filter disks (P/N 49458)

## **SOLVENTS**

Petroleum ether, chloroform, hexane, isopropanol, ethanol (all pesticide-quality or equivalent)

## EXTRACTION CONDITIONS

Oven Temperature:	125 °C
Pressure:	6.7 MPa (1000 psi)
Oven Heatup Time:	6 min
Static Time:	5–25 min
Flush Volume:	60%
Purge Time:	60 s
Solvent:	Petroleum ether, chloroform, hexane, or hexane/isopropanol (3:2), chloroform/ethanol (1:1), depending on application
Static Cycles:	1 to 3

*Note: If the samples contain unsaturated fats that may be oxidized during extraction, and these fats are to be analyzed, the solvent should be degassed to avoid potential oxidative degradation. Methanol should not be used with ASE for the extraction of fats from foods because adverse reactions may occur.*

## SAMPLE PREPARATION

It is important to reduce the particle size of all samples by grinding or another appropriate procedure. The particle size should be less than 2–3 mm. Samples should also be mixed with an inert filler such as clean sand to prevent sample compaction during extraction. Wet samples should be dried in an oven at 105 °C for at least 2 h, or mixed with a desiccant such as ASE Prep DE (diatomaceous earth), P/N 062819 (1:1 w/w) prior to extraction. Depending on the density of the samples, it may be necessary to use the 22-mL cells.

## EXTRACTION PROCEDURE

Make sure that the sample is ground to an appropriate particle size (less than 2–3 mm). Place a cellulose disk at the outlet end of the extraction cell. Weigh out 3 to 10 g of sample in a beaker. Mix with ASE Prep DE if the sample

**Table 1. Extraction of Fat from Snack Foods by ASE**

Sample (n=5)	Avg. %Fat (wt. %)	Std. Dev.	RSD (%)
Potato Chips	34.0	0.11	0.33
Corn Chips	32.8	0.08	0.25
Cheese Snacks	33.3	0.17	0.51
Tortilla Chips	21.5	0.07	0.34
Snack Chips	19.2	0.10	0.53

\*Conditions: 3-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 10-min static, 100% flush, 60-s purge, chloroform, 3 static cycles.

**Table 2. Extraction of Fat from Cookies:  
Comparison of Results by Soxhlet and ASE**

Method	Solvent	Avg. %Fat (wt. %)	Std Dev.	RSD (%)
Soxhlet	Methanol/ chloroform (2:1)	20.0–22.0	N/A	N/A
ASE, n = 3*	Hexane/ isopropanol (3:2)	20.8	0.18	0.85

\*Conditions: 3-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 25-min static, 60% flush, 60-s purge, 1 static cycle.

**Table 3. Extraction of Fat from Dog Biscuits:  
Comparison of Results by Soxhlet and ASE**

Sample	Method	Solvent	Avg. %Fat (wt. %)	Std. Dev.	RSD (%)
Standard Reference Material	Soxhlet	Petroleum ether	8.80	0.50	5.7
Standard Reference Material	ASE*	Petroleum ether	9.12	0.15	1.6
Brand X	Soxhlet	Petroleum ether	10.3	N/A	N/A
Brand X	ASE*	Hexane/ IPA (3:2)	10.4	N/A	N/A

\*Conditions: 7-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 25-min static, 60% flush, 60-s purge, 1 static cycle.

is wet. If not, mix with sand (approximately 1:1) and load into the extraction cell. Fill any void volume with clean sand, which will further reduce the amount of solvent used.

Place closed cells into the upper carousel and the appropriate number of clean, preweighed collection vials in the lower carousel. For low fat samples, the best precision will be obtained if each vial is weighed without its lid and septum before extraction and then again after solvent evaporation. Set the method conditions on the ASE 200 system and initiate the run. The solvent is evaporated completely, and the vials are reweighed once they reach a constant weight to determine the amount of extracted material. With some high moisture samples, it may be necessary to dry the vials in an oven at 40 °C to achieve constant vial weights.

## DISCUSSION AND RESULTS

Samples were provided by a number of different food companies. In all cases, the Soxhlet results shown were determined by the food company supplying the sample. Table 1 shows the results of performing extractions of various snack foods by ASE and determining the fat content. The recoveries were equivalent to Soxhlet, and the precision, expressed as relative standard deviation (RSD), was extremely good.

Tables 2 through 6 show the results of the comparison of ASE with Soxhlet for the determination of fat in the indicated foods. In most cases, close agreement is reached between Soxhlet and ASE using the same extraction solvent. Similar agreement can also be reached if a different solvent scheme is desired. In Table 5, the Soxhlet extraction uses a methanol/chloroform mixture. In this case, the laboratory wanted to remove the methanol from the production line and eliminate the use of chlorinated solvents (i.e., chloroform). By changing the solvent to hexane/isopropanol (3:2) for the ASE extraction, equivalent data were generated.

In summary, ASE exhibits equivalent recovery and good precision in a faster extraction that uses much less solvent than Soxhlet.

## REFERENCES

- Clemmitt, M. *Scientist* **1991**, 8, 120.

**Table 4. Extraction of Fat from Snack Crackers: Comparison of Results by Soxhlet and ASE**

Sample	Method	Avg. %Fat (wt.%)	Std. Dev.	RSD (%)
Cracker 1	Soxhlet*	15.4	N/A	N/A
Cracker 1	ASE**, n = 3	14.6	0.09	0.65
Cracker 2	Soxhlet*	28-30	N/A	N/A
Cracker 2	ASE**, n = 3	28.1	0.20	0.70

\*After acid hydrolysis

\*\*Conditions: 5-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 25-min static, 60% flush, 60-s purge, 1 static cycle, hexane/isopropanol (3:2).

**Table 5. Extraction of Fat from Sweet Cereal: Comparison of Results by Soxhlet and ASE**

Method	Solvent	%Fat (wt.%)	Std. Dev.	RSD (%)
Soxhlet	Methanol/ chloroform (2:1)	10.0-12.0	N/A	N/A
ASE*	Hexane/ isopropanol (3:2)	11.6	0.09	0.73

\*Conditions: 3-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 25-min static, 60% flush, 60-s purge, 1 static cycle.

**Table 6. Extraction of Fat from Low-Fat Snack Crackers: Comparison of Results by Soxhlet and ASE**

Method	%Fat (wt.%)	Std. Dev.	RSD (%)
Soxhlet	1.40	N/A	N/A
ASE*	1.43	0.03	2.1

\*Conditions: 5-g samples, 125 °C, 6.7 MPa (1000 psi), 6-min heatup, 25-min static, 60% flush, 60-s purge, 1 static cycle, hexane/isopropanol (3:2).



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