

Fast Determination of Total Unbound Fat in Snack Foods Using Accelerated Solvent Extraction and the Rocket Evaporator

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Key Words

AOAC Methods 983.23 and 945.16, Dionex ASE 150/350 system, Dionex ASE Prep DE, Soxhlet Extraction NLEA and 21 CFR 101.9

Introduction

As a result of the Nutrition Labeling and Education Act (NLEA) enacted in 1990, the U.S. Food and Drug Administration (U.S. FDA) and the U.S. Department of Agriculture (U.S.D.A.) require that the nutritional content of processed and packaged food is declared on the product label. The mandatory labeling of total fat, defined by the NLEA as “total lipid fatty acids expressed as triglycerides”, was included in this regulation (21CFR101.9).¹ Additionally, food manufacturers need a reliable, fast and accurate process control method for the determination of fat content to maintain food product quality.

The traditional fat determination methods, AOAC Methods 983.23² and 945.16,³ use Soxhlet extraction, which determines fat gravimetrically following solvent extraction. These methods require a large volume of organic solvent (>200 mL per sample) and long extraction times (>2 h per sample). The accelerated solvent extraction technique provides many advantages over the traditional Soxhlet extraction methods, and is an automated extraction technique that significantly reduces extraction time (<0.5 h per sample) and solvent consumption (<25 mL per sample). This technique enables extraction of analytes from solid and semi-solid samples using common solvents (e.g. hexane, water, and isopropanol) at elevated pressures and temperatures to increase extraction efficiency.



The accelerated solvent extraction technique is a superior extraction technique to Soxhlet for the determination of fat in food products. More recently, the Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system was developed to replace the Dionex ASE 200 Accelerated Solvent Extractor system used in Thermo Scientific Application Note (AN) 321⁴: Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction⁴ and can automatically extract up to 24 samples unattended with flexibility accommodating 1, 5, 10, 22, 34, 66, and 100 mL cell sizes.

The Thermo Scientific™ Rocket™ Evaporator system is an automated centrifugal solvent evaporator that can concentrate or dry up to 18 ASE extractions unattended and was developed to simplify and speed up the process by eliminating the need for cumbersome nitrogen stream evaporation.

This application updates AN 321 and demonstrates the fast and simple gravimetric determinations of total unbound fat in snack foods using the Dionex ASE 350 system combined with the Rocket Evaporator.

Equipment

- Dionex ASE 350 Accelerated Solvent Extractor 120 V (P/N 083114) or 240 V (P/N 083146)
- Stainless Steel Extraction Cells 10 mL (P/N 060070)
- Dionex ASE 350 Collection Vials, Clear, 60 mL (P/N 048784)
- Cellulose Filters 27 mm Type D28 (P/N 068093)
- Rocket Evaporator 120 V (P/N 075904) or 240 V (P/N 082766)
- JULABO® Recirculating Cooler, Model FL601 (P/N 075905 (120 V) or 076364 (240 V))
- Pucks for ASE Vials (P/N 075910)
- Analytical balance (0.001 g or better)
- Commercial coffee grinder

Solvents and Reagents

- Hexane, HPLC grade (Fisher Scientific, P/N UN1208)
- Isopropanol, HPLC grade (Fisher Scientific, P/N UN1219)
- Dionex ASE Prep DE (diatomaceous earth) (Dionex, P/N 062819)
- Ottawa Sand (Fisher Scientific, Cat. No. S23-3)

Samples

- Potato chips
- Cheese-flavored snack
- Baked cheese-flavored snack
- Tortilla chips
- Baked crackers
- Reduced fat baked crackers

Sample Preparation and Extraction

To prepare the snack sample, weigh 10–20 g of sample, add it to the grinder and grind with equivalent amount of ASE Prep DE until it is a homogenous fine powder (particle diameters of < 2–3 mm). It is important to reduce the particle size of all samples by grinding or using another appropriate procedure to increase surface area of the sample and therefore the extraction efficiency. Dionex ASE Prep DE, which acts as a dispersant and drying agent, is added during the sample preparation at 1:1 ratio to prevent sample compaction and ensure efficient solvent contact.

To prepare the extraction cell, place a new cellulose filter in each extraction cell cap. Weigh the empty extraction cells, fill each cell with the previously ground sample/DE powder (1:1 mixture) to about 1mm from the top and weigh the cell again. Depending on the sample type, 2.5–5 g of sample/DE powder was loaded into each cell. After loading the sample, fill the remaining cell volume with clean Ottawa sand and hand tighten the cell cap (Figure 1).

Place the filled cells into the upper carousel of the Dionex ASE 350 system, and place the appropriate number of clean, pre-weighed, pre-labeled collection vials in the lower carousel at the same numbered positions. For the best precision, weigh each vial without its cap and septum before extraction and then again after solvent evaporation.

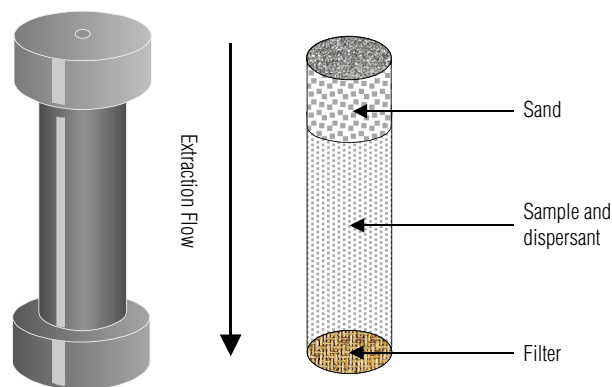


Figure 1. Illustration of a filled ASE cell.

Set the method conditions on the Dionex ASE 350 system according to method 1 or 2 and initiate the run. After extraction, take off the collection vial's cap, transfer the vials directly from the Dionex ASE system to the pucks for ASE vials, place the pucks in the Rocket Evaporator, select a method for medium boiling point solvent with the conditions specified below, and start. After each evaporation, the vials are reweighed. The evaporation is complete when the weight is stable despite the increased evaporation time. The solvent is evaporated completely by the Rocket Evaporator and the final weight of the residue is recorded as the unbound fat.



Figure 2. Dionex ASE Pucks are loaded into Rocket Evaporator.

Accelerated Solvent Extraction Conditions

	Method 1	Method 2
Solvent: Hexane/Isopropanol (v/v)	3:2	3:2
Extraction Cell Size (mL):	10	10
Temperature (°C):	125	125
Static Extraction Time (min):	5	15
Number of Static Cycles:	1	1
Purge Volume (%):	60	100
Purge Time (sec):	60	100
Total Extraction Time per Sample (min):	14	24
Total Solvent Volume per Sample (mL):	22	25

Method 2 is the modification of Method 1 to achieve complete extraction for all snack foods including baked snack foods.

The experiment starts with Method 1 conditions, the method used in AN 321. It is not the ideal method for baked snack foods (not included in original AN 321, but included in this update since this type of snack is healthier and became popular recently). Method 2 was modified from Method 1 and works well for baked snack food extraction. Further explained in the Results and Discussion section.

Rocket Evaporator Drying Conditions

Method:	Medium BP (boiling point)
Control Temperature:	60 °C*
To Final Stage:	Preset, ΔT
Final Stage Time:	30 min
Total Evaporation Time:	2 h 30 min for 18 samples

*The chamber temperature is increased to 60 degrees C for a medium boiling point method as this at the high end and within the range of medium BP method, to speed up the drying process.

Results and Discussion

Method Optimization

To evaluate the total unbound fat, snacks similar to those previously tested in AN 321 were selected for comparison in addition to two baked snack products that claimed lower fat content. The accelerated solvent extraction method steps and schematic are shown in Figure 3. The snack samples were initially extracted using Method 1 conditions, which was a method used in AN 321. The recovery of fat is expressed as % fat (wt/wt) and the precision of results are expressed as relative standard deviation (RSD).

The extraction results are listed in Table 1. For the cheese-flavored snack, the results were acceptable with the RSD < 1% and % fat (wt/wt) comparable (within 10%) to the one reported on the label. However, the baked cheese-flavored snacks had much lower % fat (wt/wt) than was stated on the label, and had more variation with the RSD > 3%. The results suggested inefficient extraction when Method 1 was used. To increase the extraction efficiency, the static extraction time was increased from 5 to 15 min; purge time and volume were increased from 60 to 100 sec and 60% to 100% in Method 2.

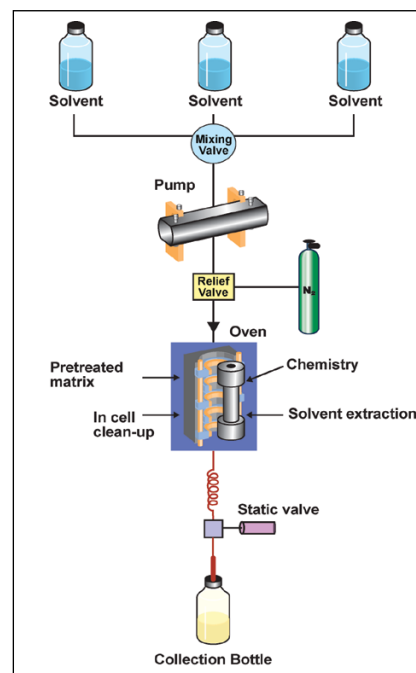
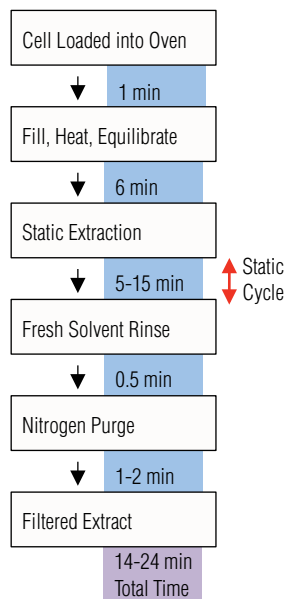


Figure 3. Accelerated solvent extraction method steps and schematic.

The results in Table 1 show that fat content of the baked cheese-flavored snack, which increased from 14.6 to 17.5% fat (wt/wt). The measurement precision improved from 3.3 to 1.2%, which was similar to the value reported on the label. Thus, for baked snack food samples, the additional extraction time, purge times and purge volumes were needed to achieve complete fat extraction. To ensure that all unbound fat had been removed using a single extraction, the snacks were extracted twice with Method 2 conditions. The results showed that the fat recoveries and precision were comparable for the single and double extractions. Therefore, a single accelerated solvent extraction, Method 2 extraction was applied for the analysis of all the snack foods.

Table 1. Comparison of % fat in two cheese-flavored snacks by two accelerated solvent extraction methods.

Sample	Label Fat (% wt/wt)	Accelerated Solvent Extraction Method	Measured (n=3)		
			Fat (% wt/wt)	Std. Dev	RSD
Cheese-Flavored Snack	35.3	1	32.1	0.26	0.8
		2	32.6	0.08	0.2
		2*	32.6	0.08	0.2
Baked Cheese-Flavored Snack	18.1	1	14.6	0.48	3.3
		2	17.5	0.21	1.2
		2*	17.5	0.04	0.2

*Extracted twice by accelerated solvent extraction Method 2 and all of the extracted material was combined

Table 2. Comparison of fat in snacks.

Sample	Label Fat (% wt/wt)	Measured (n=3)		
		Fat (% wt/wt)	Std. Dev	RSD
Cheese-Flavored Snack	35.3	32.6	0.08	0.2
Baked Cheese-Flavored Snack	18.1	17.5	0.21	1.2
Potato Chips	35.3	36.5	0.32	0.9
Tortilla Chips	28.3	25.6	0.17	0.6
Reduced Fat Baked Crackers	12.1	12.2	0.03	0.2
Baked Crackers	16.1	16.0	0.11	0.7

Reported Fat Content Comparison

Table 2 shows the extraction results of six snack foods using Method 2. In addition to recovery and precision, the fat content reported on the package label is included as an indication of accuracy.

The measured fat content in the six snack foods ranged from 12.2 to 36.5% fat (wt/wt). All results show precision, with RSDs from 0.2 to 1.2%. Although Method 2 measured only unbound fat (since no acid hydrolysis is applied before the extraction), the results are comparable to the reported label content range from 12.1 to 35.3% fat (wt/wt) showing the accelerated solvent extraction results were accurate for total fat estimate.

Results are similar to previous results highlighted in AN 321 where the accelerated solvent extraction method on a similar product (Dionex ASE 200 system) demonstrated comparable extractions to Soxhlet extractions but using considerably less solvent and time. The study used the Dionex ASE 350 to automatically extract 15 samples unattended and the Rocket Evaporator to dry 18 vials/run without manual sample transfer.

The Rocket Evaporator eliminates cumbersome nitrogen evaporation which requires constantly adjusting the needle position and additional long time (6 hr to dry 2 samples) to ensure the sample dried. Therefore, the accelerated solvent extraction method precisely determines fat in snack food in a short period of time. The total run time was approximately 35 min/sample with 25 min/sample for extraction and ~10 min/sample (2 h 30 min for 18 samples) to evaporate the extraction solutions. This method also saves solvent because it requires only ~25 mL per sample extraction when using a 10 mL ASE 350 cell, compared to more than 200 mL per sample using Soxhlet extraction. The methods used for each sample type yields quick and precise total fat determination while keeping solvent consumption to a minimum.

Conclusion

Method 2 demonstrates simple gravimetric determinations of unbound fat in snack food samples using the automated Dionex ASE 350 system to extract the samples and the automated Rocket Evaporator to evaporate the extraction solutions to dryness. The method is accurate, precise and fast (averaging ~35 min/sample from extraction to dryness) and minimized solvent use and waste (consuming ~25 mL/sample).

References

1. 21 CFR 101.9 – *Nutritional Labeling of Food*; U.S. Food and Drug Administration, Department of Health and Human Services. 2012. [Online] <http://www.gpo.gov/fdsys/pkg/CFR-2012-title21-vol2/xml/CFR-2012-title21-vol2-sec101-9.xml> (accessed March 12, 2014).
2. *AOAC Official Methods of Analysis, 15th ed.*; Method 983.23. Association of Official Analytical Chemists: Arlington, VA, 1990.
3. *AOAC Official Methods of Analysis, 17th ed.*; Method 945.16. Association of Official Analytical Chemists: Arlington, VA, 2002.
4. Dionex, now part of Thermo Scientific, Application Note 321. Determination of Unbound Fat in Various Food Matrices Using Accelerated Solvent Extraction. Sunnyvale, CA. [Online] <http://www.dionex.com/en-us/webdocs/4322-AN321-ASE-Fat-Food-28Apr2011-LPN0763-03.pdf> (accessed April 4, 2014).

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