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Sample preparation

# Fast determination of total unbound fat in snack foods using a new fully automated parallel extraction and concentration system

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## Keywords

Nutrition Facts label, Nutrition Labeling and Education Act (NLEA), AOAC Method 983.23, AOAC Method 945.16, pressurized fluid extraction, EXTREVA ASE Accelerated Solvent Extractor, Dionex ASE 350 Accelerated Solvent Extractor, Soxhlet

## Goal

To demonstrate a method for the determination of total unbound fat in snack foods using a Thermo Scientific™ EXTREVA™ ASE™ Accelerated Solvent Extractor, a newly developed and fully automated parallel extraction and evaporation system.

## Introduction

The analysis of food lipids presents significant challenges due to the wide variety of sample matrices, large range of total fat contents, and complex compositions of fatty acids. Food lipids provide a major source of energy in the diet. From the consumer's perspective, information related to differences in the content and composition of total fat and fatty acids in foods and food ingredients is important for making educated, healthful food choices. Greater fat intake is indeed a major cause of obesity and hypertension, diabetes, and gallbladder disease. Specific saturated fatty acids raise blood cholesterol levels and, thereby, increase the risk of atherosclerosis. The Nutrition Labeling and Education Act (NLEA) of 1990 amended the Federal Food, Drug and Cosmetic Act (FD&C Act) to require mandatory nutrition labeling for packaged foods regulated by the FDA and the US Department of Agriculture (USDA).[1,2] The NLEA also gave the FDA authority to regulate health claims on food labels and in food labeling.[3] Under provisions of the NLEA, declarations for the content of total fat are to be expressed in triacylglycerol (TAG) equivalents, whereas those for saturated fat are expressed as free fatty acid equivalents.[1,2] In particular, the contents of *cis*-monounsaturated and *cis*-polyunsaturated fatty acids are also permitted as voluntary declarations on product labels.

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A wide range of analytical techniques are currently available for the determination of total fat and fatty acids in foods and food ingredients. FDA regulations do not specify methods of analysis, but the Agency accepts those that yield accurate results with satisfactory precision and are considered appropriate for the analysis of specified nutrients and other food components. The traditional fat determination methods are the AOAC Methods 983.23[4] and 945.16[5]. They both use Soxhlet extraction, which determines fat gravimetrically following solvent extraction and evaporation to dryness. These methods typically require a large volume of organic solvent (over 200 mL per sample) and long extraction times (over 2 h per sample). It has been previously reported that replacing a Soxhlet system with a pressurized fluid extraction system (Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor) significantly saves solvent and time,[6] especially when the accelerated solvent extraction is combined with the Thermo Scientific™ Rocket Synergy™ 2 Evaporator System.[7]

The EXTREVA ASE system (Figure 1) is based on many proprietary technologies including gas assisted solvent extraction technology[8] and parallel accelerated solvent extraction[9]. This fully automated system combines the extraction and evaporation capabilities in one instrument, and it can be conveniently used for extracting and concentrating/drying compounds from up to 16 solid and semisolid samples.

The method reported here is applicable for the fast determination of total unbound fat in snack foods using the EXTREVA ASE system. For comparison purposes, the same snack food samples have been extracted with the Dionex ASE 350 system and concentrated to dryness with the Rocket Synergy 2 Evaporator system.



**Figure 1. EXTREVA ASE Accelerated Solvent Extractor**

## Experimental

### Samples

Four different snack foods were purchased from local stores.

### Equipment and consumables

- EXTREVA ASE Accelerated Solvent Extractor (P/N 22184-60101)

- Dionex ASE 350 Accelerated Solvent Extractor 120 V (P/N 083114) or 240 V (P/N 083146)

- Rocket Synergy 2 Evaporator (P/N C0960-01-00092)

- Thermo Scientific™ Dionex™ ASE™ Collection Vials, 60 mL (P/N 048784)

- Thermo Scientific™ Dionex™ Extraction Cell Filters, Cellulose, 27 mm (P/N 068093)

- Stainless steel extraction cells 10 mL (P/N 060070)

- Analytical balance

- Commercially available coffee grinder

### Solvents and chemicals

- Fisher Chemical™ Hexanes, Optima™ for HPLC and GC (P/N H303-4)

- Fisher Chemical™ Isopropanol (IPA), Optima™ for HPLC and GC (P/N A464-4)

- Thermo Scientific™ Dionex™ ASE™ Prep Diatomaceous Earth (P/N 062819)

- Fisher Chemical™ Ottawa Sand (P/N S23-3)

### Extraction and concentration

The samples and the extraction cells were prepared using the same procedures for both the EXTREVA ASE system and Dionex ASE 350 system. A sample of 10–20 g was weighted, added to the coffee grinder, and ground with an equivalent amount of diatomaceous earth (ASE Prep DE) until a homogenous fine powder was obtained (particle diameters of <2–3 mm). The ASE Prep DE, acting as a dispersant, plays a key role in preventing sample compaction during the compression phase and in ensuring efficient solvent contact with the sample. A cellulose filter was placed in the cell end cap, followed by hand tightening it to the corresponding body. A known amount (between 2 and 2.5 g) of the previously prepared mixture was carefully poured into the extraction cell while lightly tapping the cell. Any empty volume was filled with ASE Prep DE while lightly tapping the cell. After placing another cellulose filter on top of the cell body, the second end cap was hand tightened. Before proceeding to the extraction of the samples, both systems were rinsed with a mixture of hexane and isopropanol (60/40%). The two accelerated

solvent extractors were programmed according to the conditions reported in Tables 1 (EXTREVA ASE system) and 2 (Dionex ASE 350 system). The extracts from the Dionex ASE 350 system were manually transferred to the pucks of the Rocket Evaporator system and concentrated to dryness, while the extracts produced from EXTREVA ASE system were directly concentrated to dryness without user intervention. The evaporation parameters are reported on Tables 1 and 2. Upon completion of the evaporation, the lipid residue was weighted and expressed as a percent of the original sample weight.

Table 1. Extraction and evaporation conditions for the EXTREVA ASE system

| Extraction | |
| --- | --- |
| Cell type | Stainless steel |
| Cell size | 10 mL |
| Oven temperature | 125 °C |
| Purge time | 90 s |
| Nitrogen flow (gas assisted extraction) | 10 mL/min per channel |
| Cell fill volume | 70% |
| Solvent flow rate | 0.6 mL/min |
| Collection vial | 60 mL |
| Extraction solvent | Hexane-Isopropanol (60%/40%) |
| Extraction volume | ~24 mL per sample |
| Extraction time | ~20 min for 4 samples |
| Rinse solvent | Pre-run, 10 mL, Hexane-Isopropanol (60/40%) |
| Evaporation | |
| Mode | Evaporation to dryness |
| Collection vial | 60 mL |
| Evaporation temperature | 70 °C |
| Nitrogen flow rate | 50 mL/min per channel |
| Evaporation time | 60 min for 4 samples |
| Vacuum | 2 psi (100 torr/138 mbar) |

Table 2. Extraction and evaporation conditions for the Dionex ASE 350 and the Rocket Evaporator systems

| Extraction | |
| --- | --- |
| Cell type | Stainless steel |
| Cell size | 10 mL |
| Oven temperature | 125 °C |
| Purge time | 100 s |
| Rinse volume | 100% |
| Static extraction time | 15 min |
| Static cycles | 1 |
| Extraction solvent | Hexane-Isopropanol (60%/40%) |
| Collection vial | 60 mL |
| Total solvent volume per sample | ~25 mL |
| Total extraction time per sample | ~30 min (for a total of 120 min) |
| Evaporation | |
| Method | Medium boiling point |
| Vial | 60 mL |
| Evaporation temperature | 60 °C |
| To final stage | Preset, ΔT |
| Final stage time | 30 min |
| Total evaporation time | ~30 min |

## Results and discussion

The EXTREVA ASE system is a fully automated sample preparation platform, designed for extracting and concentrating organic compounds from a variety of solid and semi-solid matrices. The system can use up to six different extraction solvents (or mixtures of them) and extract up to four cells in parallel. The newly developed gas-assisted solvent extraction basically consists of the addition of the hot extraction solvents and nitrogen gas to the stainless-steel cell to reach the working pressure of 200 psi (~14 bar). The combined effect of temperature and pressure greatly increase the efficiency of the extraction process, significantly reducing the amount of time and solvent required for extraction when compared to traditional techniques such as Soxhlet. The evaporation process starts immediately after the completion of the extraction step without any user interaction. The extracts can be evaporated to dryness or concentrated in 2 mL vials, with the final volume controlled by artificial intelligence machine vision. A schematic diagram of the EXTREVA ASE system is shown on Figure 2.
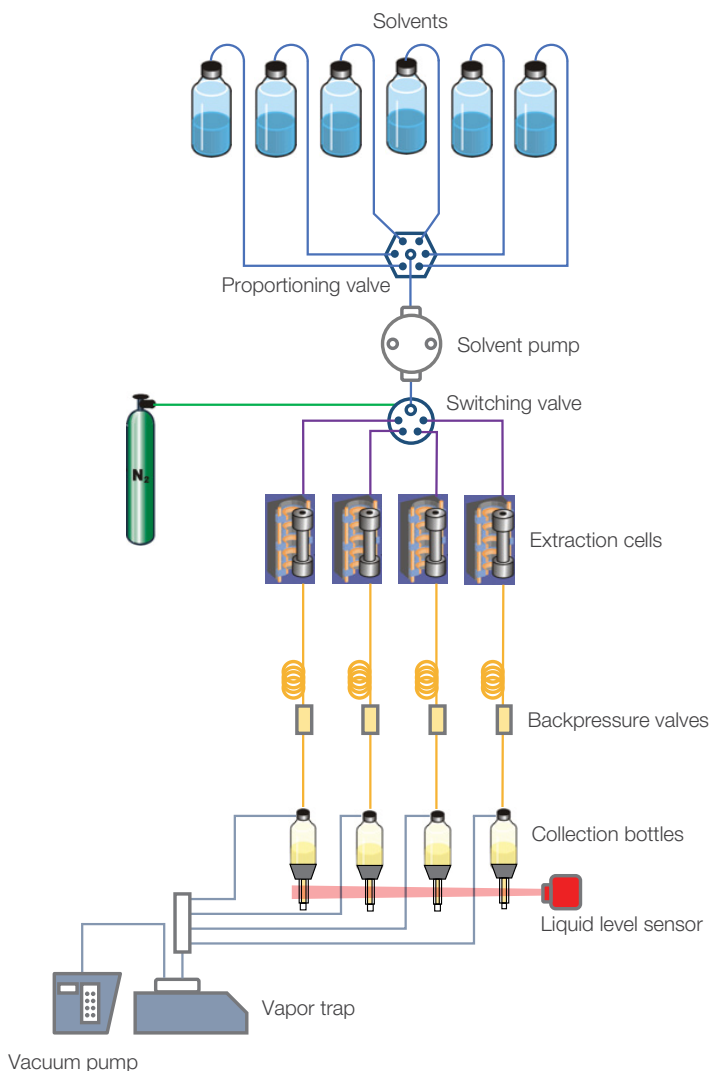
Figure 2. Schematic diagram of the EXTREVA ASE system

The extraction results of four commercially available snack foods using the EXTREVA ASE system and the combination of the Dionex ASE 350 system and Rocket Evaporator system are reported on Table 3. 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 four snack foods ranged from 16.1 to 35.3% fat (w/w). All results show precision, with RSDs from 0.3 to 1.6%. Although both extraction methods measured only unbound fat (since no acid hydrolysis is applied before the extraction), the results are comparable to the reported label content range from 14.3 to 35.7% fat (w/w) showing the accelerated solvent extraction results were accurate for total fat estimation. Remarkable is the minimal difference in the results obtained with the two pressurized fluid extraction systems, showing exhaustive extraction and the suitability of the EXTREVA ASE system for the determination of unbound fat in snack food.

The EXTREVA ASE system also shows a significant increase in throughput and productivity compared to the classical combination of the Dionex ASE 350 system and Rocket Evaporator system. Based on the extraction and evaporation times reported on Tables 1 and 2, a set of 16 snack food samples (corresponding to a full batch on the Rocket Evaporator system) would require on average approximately 40–45 min per sample. The same 16 samples on the EXTREVA ASE system would require on average only 20 min/sample, with a remarkable 50% time saving compared to the Dionex ASE 350 system and Rocket Evaporator system while keeping solvent consumption to a minimum. Considering the non-volatility of the extracted lipids, the time savings on the EXTREVA ASE system could be possibly optimized by decreasing the vacuum during the evaporation phase.

Table 3. Comparison of fat extraction from snacks with the EXTREVA ASE system and Dionex ASE 350 system + Rocket Evaporator system

| Sample | Declared fat content (% w/w) | Instrument | % Fat (%w/w) | RSD | Standard deviation (%) |
|---|---|---|---|---|---|
| Cheese snack | 28.6% | EXTREVA ASE system | 26.8 | 1.1 | 0.3 |
| | | Dionex ASE 350 system and Rocket Evaporator system | 26.8 | 1.0 | 0.3 |
| Popcorn snack | 14.3% | EXTREVA ASE system | 16.5 | 0.7 | 0.1 |
| | | Dionex ASE 350 system and Rocket Evaporator system | 16.3 | 1.2 | 0.2 |
| Potato chip | 35.7% | EXTREVA ASE system | 35.2 | 0.3 | 0.1 |
| | | Dionex ASE 350 system and Rocket Evaporator system | 35.3 | 0.6 | 0.2 |
| Baked snack #1 | 17.9% | EXTREVA ASE system | 16.1 | 1.6 | 0.3 |
| | | Dionex ASE 350 system and Rocket Evaporator system | 16.2 | 1.1 | 0.2 |
| Baked snack #2 | 17.9% | EXTREVA ASE system | 18.6 | 0.5 | 0.1 |
| | | Dionex ASE 350 system and Rocket Evaporator system | 18.3 | 1.2 | 0.2 |

## Conclusion

This application note demonstrates the successful use of the fully automated EXTREVA ASE system for the fast determination of total unbound fat in snack foods. With a 50% time saving compared to the combination of the Dionex ASE 350 system and Rocket Evaporator system, and the even higher time and solvent savings compared to the traditional Soxhlet extraction, the EXTREVA ASE system is the perfect solution for the modern analytical laboratory seeking increased sample throughput with cost reduction and environmental sustainability.

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