

Extraction of Fat from Fresh Meat Products by Pressurized Solvent Extraction (PSE)

Introduction

Pressurized solvent extraction is a new technique that reduces both solvent consumption and sample preparation time. Solvent, pumped into an extraction vessel containing the sample, is heated and pressurized. The pressurized solvent at high temperature then accelerates the extraction process by increasing the solubility of the analyte in the solvent and also increasing the kinetic rate of desorption of the analyte from the sample matrix.

Fat is traditionally removed from canned meat products using tested methods such as the Association of Official Analytical Chemists (AOAC) Method 960.39 (16th edition). This is a solvent-based method that employs a Soxhlet apparatus to extract fat from meat products. Depending on the reflux rate used, the method takes 4 – 6 hours, not including the 1 – 1.5 hour pre-extraction heating period.

The *one* PSE method described herein can extract a fresh meat sample in less than 30 minutes. The method is simple in design and easy to employ: the meat product sample is ground together with *Spe-ed* Matrix (diatomaceous earth), and poured into an extraction vessel. The vessel then is inserted into the *one* PSE, the run program is selected, the START button is pressed, and the extract containing the fat is collected in a vial. No further operator involvement is required.





Equipment

- ✓ Applied Separations' *one* PSE Pressurized Solvent Extractor
- ✓ 11 mL Extraction Vessel-Cat.#10625
- ✓ Microwave Oven – for sample drying
- ✓ Mortars (90mm o.d. Coors #60316) and pestles (Coors #60317)

Solvents and Materials

- ✓ Petroleum ether (b.p. 35 - 60°C) or Hexane (ACS grade)
- ✓ Nitrogen-high purity grade to purge the extraction vessel.
- ✓ S/S Frits (10 micron)- Cat. #10710
- ✓ Collection Vials (60mL for extract collection)-Cat.#10650
- ✓ *Spe-ed*TM Matrix-Cat.#7950
- ✓ Cellulose Filter Disk-Cat. #10711
- ✓ Ottawa Sand-Cat. #10548

Summary of Method

 <p>1. Prepare Sample</p>	 <p>2. Load Sample</p>
 <p>3. Run Sample</p>	 <p>4. Collect Extract</p>

Procedure

Sample Preparation

Ensure that the meat product sample is homogeneous before proceeding with this method. Failure to prepare a totally uniform sample may result in inconsistent results. Place 2 g of *Spe-ed* Matrix in a weighing dish. Accurately weigh a 1 – 1.5 (to 0.1 mg) sample onto the *Spe-ed* Matrix already in the weighing dish. If the sample has a very low fat content (less than 2%), it may be advisable to adjust the ratio as follows: 4 g *Spe-ed* Matrix and 2-3 g meat product sample using a 22 mL instead of an 11 mL extraction vessel. Pour the weighed material carefully into the mortar and grind with the pestle until uniform. Scrape all particles from pestle into the mortar and set the pestle aside. Place only the mortar in a microwave oven and heat for four minutes at a high setting. When mortar cools, regrind mixture with pestle until smooth.

Load Sample

Prepare the extraction vessel(s) for analysis by placing a filter disk in the bottom opening followed by a 10 μ m frit, and secure them in place with the retaining nut. Place adaptor and funnel in top opening. Pour the sample mixture through a funnel into the extraction vessel. Weigh out and add an additional gram of *Spe-ed* Matrix to the mortar. Swirl the material around in the mortar with the pestle to sweep up residual sample. Transfer this additional material to the extraction vessel and tap the vessel on the bench top to settle and compact the contents.

Add Ottawa sand to within 1 cm of the top of the vessel's interior flange (see illustration on page 4-3 of User's Manual). Transfer vessel to oven well of *one* PSE and close cover. Ensure that the pump is primed and that the extraction solvent is petroleum ether or hexane. Attach tared collection vial to needle port and program the instrument using the following parameters:

Extraction Conditions

Program the following extraction parameters on the one PSE

Program B Mode – 11 mL vessel

Solvent:	Petroleum Ether
Temperature:	125 ° C
Pressure:	100 Bar
Refills:	Refills/solvent volume: 7/2
Static:	1 minute
Pause:	N=0
Flush:	Solvent/gas/repeat flush:25 sec/2min/0

At the end of the extraction sequence, remove collection vial from the needle port and place vial in an evaporation apparatus. Remove solvent using low heat under a gentle stream of nitrogen. Dry vial in oven set at 100 °C for 1 hour. Weigh dried vial, calculate the percent recovery, and report result.

Example:

Extraction of Fat from Fresh Meat Products

Several types of fresh meat products were selected for analysis by the *one* PSE method and for comparison with an official method. The fat content of these samples ranged from 0.85 - 29%. The 11mL vessels were used for most of the samples and the sample sizes ranged from 1.0 – 3.0 g. (In one set of samples the 22 mL vessels were used, and in this case the sample size was increased to 3 g.) The samples extracted in the Soxhlet apparatus ranged from 3.0 – 3.5 g. The data obtained by the *one* PSE method were compared directly with AOAC Method 960.39. This comparison of the two methods is shown in the following table:

Percent Fat Recovery from Fresh Meat Products
one PSE vs. Soxhlet

Mean ± s.d.

Sample	<i>one</i> PSE	n	AOAC 960.39	n
Frankfurter	29.59 ± 0.15 (day 1)	3	29.46 ± 0.26	3
	29.68 ± 0.21 (day 2)	3		
Sausage	26.18 ± 0.48	5	26.12 ± 0.48	3
Ground Beef	24.63 ± 0.32 (11mL)	3	23.42 ± 0.42	3
	24.87 ± 0.12 (22mL)	3		
#1 Turkey Breast	1.54 ± 0.09	3	1.49 ± 0.39	3
#2 Turkey Breast	1.44 ± 0.06	5	1.07 ± 0.10	3
#3 Turkey Breast	0.85 ± 0.05	6	0.65 ± 0.03	3

In the above table, note that 3 – 6 determinations were made for each set of samples extracted on the *one* PSE while only sets of 3 samples were extracted on the Soxhlet apparatus, yet for every type of meat sample analyzed, the means for the *one* PSE sets were higher and the standard deviations (s.d.) lower than those obtained by the official method, indicating the repeatability possible with the *one* PSE technique. The amount of solvent used for each *one* PSE extraction was about 20 mL in 11 mL vessels compared with 200 – 220 mL for the Soxhlet technique.

To determine day-to-day method variability, a frankfurter sample was analyzed on subsequent days on the *one* PSE. Note the agreement obtained between these two sets. A test also was performed to determine the agreement between sets of ground beef samples analyzed on the same day in both 11 mL and 22 mL extraction vessels. The results for both sets were in close agreement with each other, although the sample sizes and total solvent volumes varied considerably.

The above *one* PSE method for fresh meats employs the same solvent, petroleum ether or hexane, as the Soxhlet method; however it differs in notable ways, such as faster sample preparation, shorter extraction times, and significantly lower solvent consumption.

Safety

The use of organic solvents, elevated temperatures, and high pressures present potential safety concerns in the laboratory. Common sense laboratory practices can be employed to minimize these concerns.

Extraction vessels in the *one* PSE oven are hot enough upon removal to burn unprotected skin. Allow the vessels to cool before removing them from the oven or use appropriate protective equipment (e.g., insulated gloves or tongs), as recommended by the manufacturer.

During the gas purge step, some solvent vapors may exit through a vent port in the instrument. Connect this port to a fume hood or other means to prevent release of solvent vapors to the laboratory atmosphere. This precaution also applies to the removal of post extraction solvent from the collected extract.