AOCS Approved Procedure Am 5-04

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Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction

DEFINITION

This method determines the substances extracted by petroleum ether under conditions of the test. The compounds extracted, in most samples, are predominantly triacylglycerides. Small amounts of other lipids along with minor components having some solubility in petroleum ether are also extracted.

SCOPE

This method is applicable to extractions of oil seeds (see Numbered Notes, 1), meats, feeds, and foods and is equivalent to the petroleum ether extraction performed by the Butt and Soxhlet extraction apparatus.

APPARATUS

1. Analytical Balance, capable of weighing down to 0.1 mg.
2. An extraction instrument capable of performing extractions at elevated temperatures (90ºC-100°C) and pressures (40-80 psi) on multiple samples. The apparatus must also be capable of rinsing the sample and evaporating the residual solvent (ANKOM^{XT10} Extractor or ANKOM^{XT20} Fat Analyzer, ANKOM Technology Corp.).
3. Solvent and heat resistant filter bags, capable of being heat sealed closed, retaining one micron particles and permitting rapid solvent penetration (XT4 filter bags, ANKOM).
4. Impulse heat sealer
5. Oven, capable of maintaining a temperature of 100ºC ±2º. The oven should have sufficient convection flux to clear the water vapor.
6. Desiccator pouch (Moisture Stop weigh pouch, ANKOM).

REAGENTS

1. Petroleum ether—AOCS Specification H 2-41 (see Notes, Caution).

PREPARATION OF SAMPLE

1. Oilseed samples should be prepared according to Section A of AOCS Methods using the methods specified for each type of sample.
2. Meat samples should be ground to a uniform consistency with a food processor or similar device.
3. Feed samples should be ground to a particle size that will pass through a 1-mm sieve and mixed thoroughly (see Numbered Notes, 2).
4. Food samples should be processed with a food processor or grinder to produce a representative sample of uniform consistency.

PROCEDURE

1. Weigh filter bag
2. Weigh one gram (see Numbered Notes, 3) of prepared sample directly in filter bag and avoid placing the sample on the upper 4 mm of the bag (Wt. of Sample).
3. Completely seal the upper edge of the filter bag within 4mm of the top with the heat sealer encapsulating the sample.
4. Dry the samples in the bag at 100ºC for 3 hours (see Numbered Notes, 4).
5. Cool samples in a desiccator pouch to ambient temperature and weigh (Wt. of pre-dried sample).
6. Place up to 20 samples in the bag holder and extract in the instrument at temperatures between 90ºC-100ºC for 15 to 60 minutes (see Numbered Notes, 5).
7. At the completion of the process remove the samples from the instrument and dry at 100ºC for 30 minutes.
8. Cool samples in a desiccator pouch to ambient temperature and weigh (Wt. of extracted sample).

CALCULATIONS

$$\% \text{ Oil/Fat} = \frac{(\text{Wt. of Pre-dried Sample} - \text{Wt. of Extracted Sample})}{\text{Wt. of Sample}} \times 100$$

PRECISION

Results of the collaborative study in Table 1 indicates the precision (S_r, RSD_r, r) that the analyst should use as a benchmark for evaluation replication in the same laboratory.

NOTES

Caution

Petroleum ether is extremely flammable. Avoid static electricity. The explosive limits in air are 1-6%. A fume hood should be used at all times when venting petroleum ether.

NUMBERED NOTES

1. Specific data on the accuracy and precision of soybeans, canola, safflower and corn are found in the data from the collaborative study (Table 1) and similar results have been found with other oilseeds extracted with the Butt apparatus. Since this method does not regrind the sample after the original extraction and then re-extract, for reference purposes AOCS Method Am 2-93 is recommended.
2. If sample contains large amounts of H_2O soluble components such as carbohydrates, urea, glycerol, and lactic acid remove by a water extraction. If H_2O solubles

Table 1
A summary of the statistical analysis of the international collaborative study of the Filter Bag Technique, coordinated by Andrew Komarek and Ronald Komarek (ANKOM Technology Corp. Macedon, NY). Included in the summary is a comparison of the Filter Bag Technique with the results of analysis by AOCS certified Laboratories using official AOCS or AOAC methods.

Sample ID	oat meal	brownie mix	soybean A	canola	soybean meal	corn A	poultry starter	cattle feed	pig starter	alfalfa	cat food	cookies	bkft cereal	tortilla chips
Number of laboratories	12	12	11	9	12	12	11	10	11	11	12	11	12	12
Number of replicates	24	24	22	18	24	24	22	20	22	22	24	22	24	24
Collaborative Average, Oil/Fat %	5.8	8.7	20.9	39.0	1.6	3.3	3.3	3.2	5.6	2.4	6.3	22.7	2.3	19.9
Certified Labs Average[a]	5.7	8.7	21.1	39.7	1.6	3.6	3.5	3.0	5.5	2.2	6.2	23.1	2.3	20.0
AOCS Am 2-93 Avg. for Oilseeds			21.9	44.8		3.6								
repeatability														
s(r) = repeatability std dev	0.36	0.20	0.35	0.23	0.14	0.31	0.24	0.18	0.20	0.39	0.27	0.20	0.26	0.39
RSD(r) = repeatability rel. std. dev	6.2	2.3	1.7	0.6	8.5	9.5	7.3	5.6	3.6	16.1	4.2	0.9	11.4	2.0
r = repeatability value	0.99	0.56	0.98	0.65	0.39	0.88	0.68	0.51	0.56	1.08	0.75	0.56	0.72	1.09
Reproducibility														
s(R) = reproducibility std dev	0.54	0.31	0.63	0.68	0.27	0.42	0.42	0.20	0.28	0.50	0.30	0.20	0.36	0.48
RSD(R) = reproducibility rel std. dev	9.4	3.5	3.0	1.7	16.3	12.7	12.6	6.1	5.0	20.7	4.7	0.9	15.7	2.4
R = reproducibility value	1.52	0.86	1.76	1.90	0.75	1.18	1.16	0.55	0.78	1.39	0.83	0.56	1.00	1.35

Sample ID	dog food	crackers	turkey	ham	beef ground	chicken breast	soybean B	safflower	potato chips	hot dog	sausage	corn B	cheese curls	corn silage
Number of laboratories	12	10	12	9	11	11	11	9	11	12	11	12	12	12
Number of replicates	24	20	24	18	22	22	22	18	22	24	22	24	24	24
Collaborative Average	6.8	23.8	3.2	11.6	23.8	2.8	19.4	22.5	32.0	39.5	25.7	3.4	30.6	2.3
Certified Labs Average[a]	6.9	24.0	3.2	11.3	23.5	2.7	19.7	23.0	32.0	39.0	25.0	3.7	30.5	2.3
AOCS Am 2-93 Avg. for Oilseeds							20.1	24.5						
Repeatability (r)														
s(r) = repeatability std dev	0.35	0.23	0.21	0.30	0.24	0.33	0.38	0.53	0.48	0.35	0.34	0.39	0.48	0.23
RSD(r) = repeatability rel. std. dev	5.23	0.96	6.57	2.59	1.01	11.89	1.97	2.36	1.49	0.89	1.33	11.48	1.59	9.87
r = repeatability value	0.99	0.64	0.58	0.84	0.67	0.94	1.07	1.49	1.34	0.98	0.96	1.10	1.36	0.63
Reproducibility (R)														
s(R) = reproducibility std dev	0.35	0.23	0.34	0.30	0.36	0.33	0.62	0.83	0.52	0.59	0.51	0.41	0.69	0.51
RSD(R) = reproducibility rel std. dev	5.23	0.96	10.84	2.59	1.49	11.89	3.19	3.69	1.61	1.49	1.98	11.93	2.27	22.45
R = reproducibility value	0.99	0.64	0.96	0.84	0.99	0.94	1.73	2.33	1.45	1.65	1.43	1.14	1.94	1.44

[a]AOCS Official Methods Ba 3-38, AOAC 920.39 or equivalent

are present, weigh sample onto filter paper, extract with five 20 mL portions of H_2O allowing each portion to drain. Place the filter paper containing the washed sample into the filter bag and proceed with pre-drying step.

3. A range of sample size from about 0.25 g to 3 g can be analyzed with this method depending on the limitation of sample availability or the need for larger samples due to the lack of sample homogeneity.
4. Two hours or less should be used for samples that are highly susceptible to oxidation.
5. Extraction times will be dependent on the type of sample and the fineness of sample grind. Samples composed of animal tissue will require only 15 to 30 minutes. Samples containing plant tissue can range from 15 to 60 minutes.

REFERENCES

>>>>>INSERT BOOK REFERENCE HERE!