Total fat content by automated acid hydrolysis (HYDROTHERM) – ISO 8262-1

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Scientific article

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Introduction

Total fat is a crucial measurement in food analytics. Acid or alkaline hydrolysis of fatty acids bound to glycerides, sterol esters, and glycol and phospholipids is required. Hydrolysis disrupts cell wall, breaks up fat emulsions, and lipid-protein bonds. Multiple AOAC methodologies exist for various matrices that incorporate acid or alkaline hydrolysis to accomplish total release of fat from various products followed by total fat extraction by mixed ethers. These AOAC methodologies are labor intensive with variations in reproducibility across matrices.

The HYDROTHERM is an automated, fully enclosed acid hydrolysis system by the ISO 8262-1 Weibull-Berntrop gravametric method. Paired with the SOXTHERM® (rapid soxhlet extraction) the fat analysis of food products is fully automated with minimal labor. Presented in this validation study, HYDROTHERM data is compared to AOAC 922.06 method data which is a manual acid digestion utilizing Mojonnier tube extraction for acid hydrolysis. NIST 1546 Meat Homogenate (n=36) was analyzed using both methods in which the HYDROTHERM performed equivalent to the AOAC method. 21 products including cheese, soups/sauces, meats (raw/cooked, deli, breaded), pet treats, tortillas, and taco shells were analyzed in which the HYDROTHERM was found to have higher hydrolysis efficiency in comparison to the AOAC method. Utilizing the HYDROTHERM for automated acid hydrolysis, good repeatability, high productivity, precise results, and universal applicability is achievable.

Objectives

- Validate the use of the HYDROTHERM vs. AOAC 922.06 to accomplish fatty acid hydrolysis prior to SOXTHERM® fat extraction

- Improving safety due to acid exposure

- Saving real estate (hood space), labor, and supplies

- Demonstrate good repeatability, high productivity, and precise results

Methods

Weigh 2-3 grams of sample into HYDROTHERM weigh paper (C.Gerhardt #1004939). Place sample into HYDROTHERM beaker and add ½ teaspoon of celite. Place HYDROTHERM filters (C.Gerhardt #1004092) into corresponding collection funnel. Run HYDROTHERM program utilizing 15% hydrochloric acid as the hydrolysis reagent. After completion of the HYDROTHERM program, remove filters and dry for 30 minutes at 100°C. Place dried filters into cellulose thimble for extraction. Weigh initial beaker weights for the corresponding thimbles. Place thimbles into the corresponding beaker. Add 90-100 ml of Petroleum Ether to the beaker and extract on the SOXTHERM®. Following extraction, remove thimbles, and dry beakers for 30 minutes at 100°C. Cool and record final beaker weight. Total fat is calculated using the following calculation:

$$\text{Fat} = \frac{(\text{Final beaker weight - Initial beaker weight}) * 100}{\text{Sample Weight}}$$

HYDROTHERM

HYDROTHERM is the only system worldwide to do an automated acid hydrolysis for the traditional fat determination according to Weibull-Berntrop.

A minimum of 9 pairs of observations are included in this dataset. Twenty-one products were evaluated using both methods.

Data was statistically evaluated using a paired t test for the mean differences, while a general t test was utilized to evaluate the equality of the
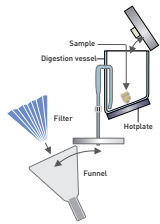
Results for matrix comparisons

Product	HYDROTHERM		Method 922.06	
	Mean	σ	Mean	σ
Alfredo (n =9)	22.6	7.14	22.9	7.48
Alfredo Base (n=9)	13.7	0.37[y]	13.1	1.56[z]
Alfredo Sauce (n=19)	23.3	5.40	23.7	5.76
Chips (n=10)	18.7	0.82	19.0	0.73
Ground Chicken Cartilage (n=12)	11.2	1.75	11.1	1.64
Pet Treats (n=45)	10.8[a]	4.31	7.7[b]	4.03
Roast Beef (n=9)	12.5[a]	1.20	10.6[b]	0.91
White Queso (n=17)	13.2[a]	1.23	12.4[b]	1.72
Breaded Chicken (n=25)	8.7	1.36	8.7	1.09
Chicken Cartilage (n=10)	7.9	1.99	7.2	1.46
Chicken Nugget (n=86)	13.7[a]	2.04	12.8[b]	2.34
Chicken Patties (n=45)	5.3[a]	1.70	4.3[b]	1.35
Chicken Tenders (n=45)	10.1[a]	1.66	9.3[b]	2.07
Meatball (n=69)	18.6[a]	4.24	18.1[b]	4.31
Popcorn Chicken (n=38)	14.2	2.11	14.3	2.20
Potato Soup (n=9)	8.3	0.56	7.9	0.82
Raw Chicken (n=21)	6.4[a]	5.05	5.7[b]	4.97
Sausage (n=9)	33.2	1.71	32.7	2.11
Taco Meat (n=10)	13.4	2.27	12.7	2.77
Taco Shell (n=36)	20.6[a]	1.49	21.2[b]	1.87
Tortilla (n=51)	7.6	1.25	7.4	1.52

[ab] Means within the same row with different superscripts differed ($P \leq 0.05$).
[yz] Standard deviations within the same row with different superscripts differed ($P \leq 0.05$).
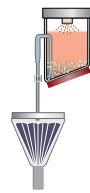
Hydrolysis

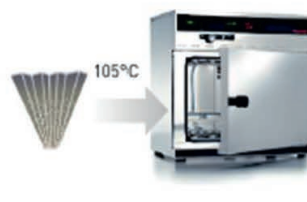| Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Insert filter and sample	**Addition hydrochloric acid**	**Hydrolysis**	**Dilution and rinsing**	**Filtration**	**Taking out of filter**
After putting the sample in the digestion tube, the filter is inserted into the funnel and the unit is closed. Funnel as well as the digestion tube are closed completely.	The process is initiated and a well defined amount of hydrochloric acid is added automatically. The entire process is run in a closed system.	The sample is hydrolysed in boiling hydrochloric acid. The digestion of the matrix is done in an ideally dimensioned digestion vessel.	When is digestion is finished, the sample is heavily diluted with hot water. Prior to the filtration, the filter is moisturised with hot water as well.	The diluted sample is quantitatively filtered through the humid filter. The digestion tube is rinsed with hot water till all fat particles are carried over and the filter is ph-neutral.	At the end the filter is taken out, dried and put into an extraction thimble for the fat extraction.
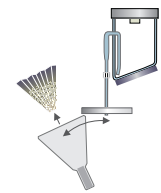
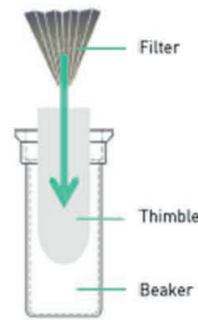**Time: appr. 90 min.**

Sample preparation for extraction

1. Weighing the beaker   2. Drying the filter   3. Insert filter in extraction beaker   4. Insert solvent

Time: appr. 30-60 min.

variance within each method, by product type. The largest difference was observed for the pet treats. In all instances, the Hydrotherm method had higher mean values than did the AOAC 922.06 method. Furthermore, in only 1 instance was the variation determined to be different within product type for the methods, Alfredo base.

SOXTHERM®

The SOXTHERM® principle simplifies and accelerates the traditional Soxhlet method significantly and observes international and national norms and extraction standards.
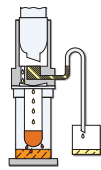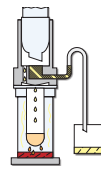
Extraction
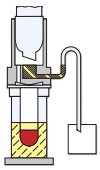
Stage 1
The sample is immersed in boiling solvent and the extractable material is liberated from the sample.
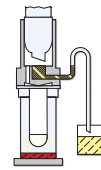
Stage 2
The level of the solvent is lowered below the extraction thimble. The excess solvent is collected in the rear solvent recovery tank.
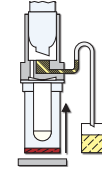
Stage 3
The material is extracted by the refluxed, condensed solvent and is collected in the solvent, below in the extraction beaker.

Stage 4
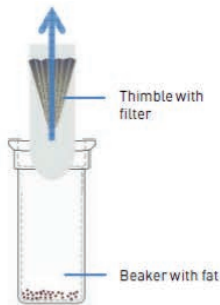The bulk of the solvent is distilled over into the rear storage tank for later recovery.

Stage 5
The extraction beakers are lifted from the hotplate automatically. Some of the residual solvent may be removed via convection heating.

Time: appr. 2 – 8 hours

Final preparation

Thimble with filter

Beaker with fat

1. Remove thimble

2. Drying the beaker in oven

3. Drying the beaker in exsiccator

Time: appr. 90 min.

Major Safety Improvements

- HYDROTHERM and SOXTHERM® are closed systems that do not require the use of fume hoods.

- The elimination of flammable hazardous waste allows the HYDROTHERM and SOXTHERM® combination to be greener and environmentally friendly.

- Hydrotherm allows for much less technician exposure to harmful acid/solvent fumes.

Discussion Topics

- What is the correct fat content

- Different methods of Acid Hydrolysis

- International methods Weibull-Stoldt and Weibull-Berntrop

Results for ISO: 17025 Validation

Matrix	n	Mean % (HT)	Std Dev (HT)	Mean % (AOAC)	Std Dev (AOAC)
NIST 1546 Meat Homogenate (COA value = 21%)	36	21.15	1.04	21.24	1.02
Internal QC Material	61	27.45	0.64	26.22	0.92
Chicken Breast	20	4.34	0.15	4.11	0.22
Tortilla	20	7.66	0.17	7.16	0.2
Raw Bacon	20	33.39	0.61	31.67	1.64

Proficiency Testing Results

Program/Sample ID	Lab Result	Mean	Std Dev	Z-score
AAFCO/Calf Starter	4.6	4.6253	0.46397	-0.05
AAFCO/Dry Dog Food	9.425	9.6576	0.41083	-0.57
AAFCO/Corn Gluten Meal	4.96	4.9295	0.39613	0.08
AAFCO/Calf Grower	4.555	4.5781	0.55513	-0.04
AAFCO/Cattle Grower	4.855	5.12	0.44344	-0.6
AAFCO/Swine Grower	4.495	4.4269	0.40048	0.17
AAFCO/Pelleted Beef Feed	6.32	6.4767	0.49843	-0.31
AAFCO/Show Pig Primer	5.995	6.4948	0.56559	-0.88
LGC/Cereal Based Sample	10.02	9.86	0.386	0.4
LGC/Meat Based Sample`	26.17	26.19	1.047	-0.02
LGC/Meat Based Sample`	14.59	14.76	0.59	-0.29

AAFCO – The Association of American Feed Control Officials
LGC – LGC Standards, ISO accredited PT provider

- Global trends – methods and labs

- Methods for different applications and sample types

- Röse-Gottlieb procedure; the Schmid Bondzynski-Ratzlaff procedure – see Introduction ISO 8262:20054

- Importance of sample preparation

Conclusion

Statistically, the HYDROTHERM method performed equivalent to the AOAC 922.06 method on the NIST 1546 Meat Homogenate known value sample.

Statistically, the HYDROTHERM performed equivalent or better than AOAC 922.06 on a low, mid, and high fat product (n=20), with better standard deviation and coefficient of variance on all three levels.

Based on the mean results of all products, the Hydrotherm method performed better on overall extraction of total fat than AOAC 922.06.

Total labor was reduced by 75% utilizing the HYDROTHERM/SOXTHERM® automated method versus the manual AOAC 922.06 method.

Total cost of materials and chemicals on a per sample basis was reduced 22% utilizing the HYDROTHERM/SOXTHERM® method.

References

1. AOAC Method 922.06, "Fat in Flour."

2. AOAC Method 991.36, "Crude Fats in Meats."

3. Gerhardt Application Note B.1.3.HT, "Total Fat in Meat and Meat Products."

4. ISO 8262:2005 and IDF 124-1:2005, milk products and milk based foods – determination of fat content by the Weibull-Berntrop gravimetric method (Reference Method); p. VI

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HYDROTHERM – automatic acid hydrolysis system for fat content determination as per the Weibull-Stoldt method. Together with SOXTHERM®, HYDROTHERM is an ideal system solution for determining total fat content.

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