Determining Total Fat Content by Automated Acid Hydrolysis (HYDROTHERM)

B.J. Bench[1,2], Wade Whittington[1,2], Markus Kranz[3], Frank Guerrera[4], Owen Brown[4]
1. Tyson Foods, Inc., Springdale, AR, USA; 2. WBA Analytical Laboratories, Springdale, AR, USA;
3. C. Gerhardt, Konigswinter, Germany; 4. OI Analytical, College Station, TX, USA

Abstract

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 the 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 gravimetric 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. Twenty-one 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
- Improve safety due to acid exposure
- Save 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:

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

HYDROTHERM

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

Figure 1.

Figure 2. Hydrolysis

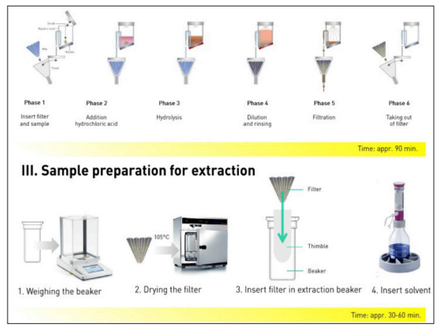

Table 1. Results for matrix comparisons

	HYDROTHERM		Method 922.06	
Product	**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 Nuggets (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=21)	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 < 0.05).
[yz] Standard deviations within the same row with different superscripts differed (P < 0.05).

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 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.

Figure 3.

Figure 4. Extraction

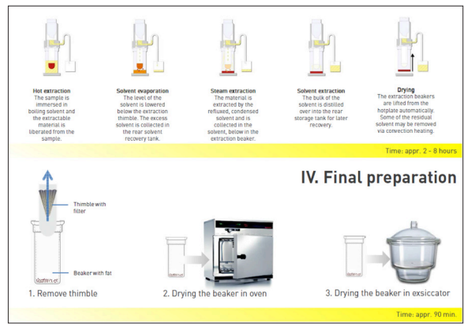

Table 2. Results for ISO: 17025 Validation

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	0.20
Raw Bacon	20	33.39	0.61	31.67	1.64

Table 3. 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	-0.60
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	0.40
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

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
- Global trends – methods and labs
- Methods for different applications and sample types
- Röse-Gottlieb procedure; the Schmid Bondzynski-Ratzlaff procedure – see Introduction ISO 8262:2005[4]
- 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.