A novel procedure for fat and oil extraction

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For over 100 years, procedures for the separation and quantitation of fat/oil have been based on solvent extraction using non-polar solvents such as diethyl or petroleum ether.

These procedures are long and arduous. The task of completely removing the oil embedded in complex plant matrices is particularly difficult due to the structural integrity of the cell wall and the limited opportunities for the relatively large lipid molecules to exit the matrix. Until recently, the solution has been to increase the time of extraction to ensure complete removal of the natural oils. The Soxhlet, Butt Type, or Goldfisch apparatus are commonly used for such extractions and are capable of carrying out extended extractions by refluxing the solvent. However, these methods have the disadvantage of limited sample throughput, limited solvent recovery and time-consuming (4-16 hours) technician involvement.

Certain instruments, including SoxTec™ Advanti (Foss Tecator AB, Hoeganaes, Sweden) and Soxtherm™ Extractors (C. Gerhardt GmbH, Königswinter, Germany) have been developed to reduce extraction times, and to provide for more convenient operation and automation of the extraction process. Based on the Randall modification of the Soxhlet process, extraction kinetics are increased and extraction times are decreased (2 –4 hours) by immersing the sample in the boiling solvent during the first part of the extraction. These instruments semi-automate part of the extraction process and attempt to provide greater control and reduce technician involvement.

Super Critical Fluid Extraction (SFE) utilizes super critical CO_2 as a solvent to extract the fat/oil. SFE can perform a primary extraction of one to three samples in about 45 minutes. The CO_2 is released to the atmosphere at the end of the analysis, during the recovery of the fat/oil. Secondary methods developed to reduce analysis time and eliminate solvent usage include Nuclear Magnetic Resonance (NMR) and Near Infra Red (NIR) spectroscopies. Both provide rapid solvent free extractions. The NMR procedure requires a pre-drying step to remove water from the sample.

With the exception of NIR, none of these alternatives has addressed the issue of significantly increasing throughput. When a high sample throughput is needed, extensive laboratory space and/or significant capital investment are required. Secondary methods are expensive, analyze one sample at a time and still require a primary method for calibration. There is a need for an automated instrument based on a primary method that can rapidly and efficiently analyze large volumes of samples utilizing limited laboratory space and low capital cost per sample. A primary method requires the use of a standard fat solvent and ideally, the instrument would automatically recycle the solvent in a closed system.

Beyond improvements in instrument set-up, convenience and ruggedness, ANKOM Technology based in Macedon, New York, saw an opportunity to develop an automated system that would accelerate the kinetics of the extraction by immersing the sample in solvent heated above its normal boiling point.

By providing a closed system that allows for batch extraction of numerous samples (10–20 at one time depending on the system) and solvent recovery, throughput has been increased while solvent usage, technician time and costs have been decreased. Studies have confirmed the accuracy and precision of this system.

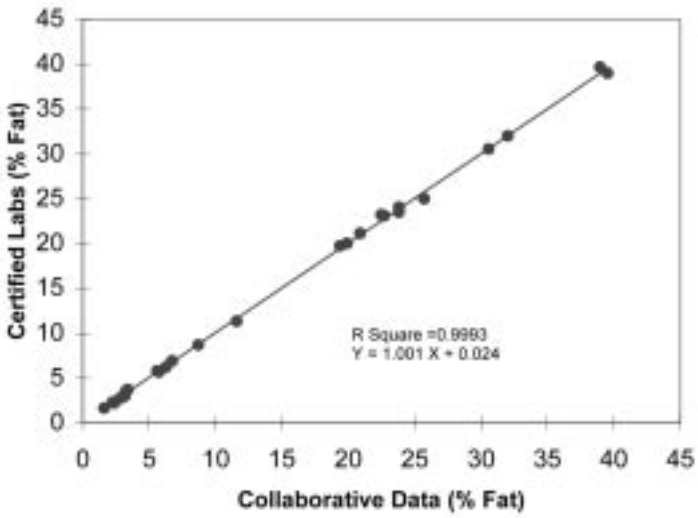

Figure 1. Regression Analysis of the accuracy of the analysis of fat/oil in 28 samples presented as 56 blind duplicates to collaborating laboratories using the FBT protocol relative to data from certified laboratories using official methods.

Accelerating the extraction kinetics

The high solvent temperatures needed to accelerate extraction kinetics with relatively low boiling solvents are achieved by conducting the extractions in a sealed chamber. Relatively moderate pressures of only 50–70 psi suppress phase change and keep the solvent in the liquid state at temperatures of 90º to 100°C. At these temperatures many samples can be completely extracted in 15 minutes. Conducting the extractions in robust stainless steel vessels with sealed and insulated heating elements with computer controls ensures safety.

Filter Bag Technique

Batch processing was accomplished by completely encapsulating the sample in a specialized filter media, formed in the shape of a bag. The filter bag permits rapid solvent exchange with the sample while preserving the quantitative identity of the sample during extraction. In addition, the media had to be solvent-resistant at high temperatures, capable of high volume manufacturing, convenient to handle and seal. Fat/oil is determined gravimetrically by the selective removal of fat/oil while retaining the sample. The filter bag and sample are weighed before and after the extraction; weight loss determines the fat/oil content. This process is known as the Filter Bag Technique (FBT).

Automating the process

To improve the ease and efficiency of the Filter Bag Technique three instruments were developed to support the diverse needs of laboratories around the world. Each is capable of processing large volumes ranging from 100 to >200 extractions per day and recycling of the solvent. Automation virtually eliminates technician

involvement during the extraction. Two innovations contributed to the successful development of these instruments. First was the development of an encapsulation process (FBT) that permits batch processing. This eliminated the need to replicate the extraction apparatus for each sample. The second was to further accelerate the kinetics of extraction by conducting the extractions at elevated pressures and temperatures over twice the boiling point of the solvent in a closed system. The result was that throughput was increased while solvent usage, technician time, costs and counter space requirements were decreased. The most advanced system, the ANKOM^{XT20} Fat Analyzer and XT Recovery System were introduced in 1999. Subsequent developments included the XT10 Extractor (2003) and the XT15 (2004). A system to support hydrolysis (total fat/oil) extraction was also introduced as a companion to any of the FBT solvent extractors.

Analytical Performance

The analytical method using these instruments for the analysis of the fat/oil content was extensively tested and culminated in an international collaborative study conducted under the auspices of AOCS. Data supporting accuracy, precision and ruggedness of the method together with the collaborative study were evaluated by AOCS and accepted as an approved procedure. The method, entitled *Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction*, is AOCS Approved Procedure Am 5-04.

The collaborative study included 28 samples representing foods, animal feeds, meat and oilseeds. The samples (56 blind duplicates) were extracted by laboratories in Italy, Belgium, England, Canada and the United States. The samples were also analyzed by three AOCS Certified laboratories and another commercial laboratory using officially recognized methods for the analysis of fat/oil. The statistical evaluation of the data indicated that the method accurately measured the fat/oil in the sample with excellent precision (Table 1). Regression analysis indicated a highly significant R Square of 0.9993 (Figure 1) when compared against the official methods.

The future

Numerous companies have forwarded advancements in laboratory instrumentation. Future successes will require the industry to further break paradigms and seek innovative solutions. ■

Table 1.
A summary of the statistical analysis of the international collaborative study of the Filter Bag Technique for the Approved Procedure, "Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction." 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	breakfast 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
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
repeatability														
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														
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