Impact of Sample Preparation on the Determination of Crude Fat Content in Corn

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ABSTRACT: Sample preparation is an important step in any analysis as it directly affects the assay results. The effect of sample preparation on the extraction of crude fat from 10 corn samples with varying fat content was studied. Samples were ground on seven different grinders, namely, the Mega-grinder, Knifetec, Cyclotec, Cemotec, Mikro-mill, UDY, and Brinkmann-Retsch mill. The crude fat was extracted from ground samples using Soxtec and/or Butt-tube extraction procedures and was determined gravimetrically after evaporation of the extraction solvent. The lowest amount of crude fat was extracted from samples ground on a Cemotec grinder. However, similar yields of crude fat were obtained with the other six sample preparation mills by using the proper screen size and grinding conditions. We observed an increase of 0.3% in the grand mean of crude fat extracted from samples ground on a Cyclotec grinder fitted with a 1.0-mm screen as compared with a 2.0-mm screen. Modifying the grinding time from 5 to 10 s/cycle and increasing the number of cycles from 2 to 3 resulted an increase of 0.4% in the grand mean of crude fat extracted from 10 corn samples. No significant differences were observed in crude fat content when samples were ground with a Monsanto-built Mega-grinder, located at two different sites. The efficiency of crude fat extracted from ground samples was found to increase with the decrease in particle size.

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KEY WORDS: Brinkmann-Retsch mill, corn, Cemotec, Cyclotec, crude fat, Knifetec, Mega-grinder, Mikro-mill, particle size, Soxtec and Butt-tube extractions, UDY.

Accurate and precise analysis of crude fat (oil) is important for research programs related to nutritional labeling and to biotechnology research focused on the development of new value-added products. To facilitate improved analysis and global trade of value-added oilseed products, several international societies such as the American Oil Chemists' Society (AOCS), the Federation of Oil Seeds and Fat Association Ltd. (FOSFA), the German Fat Science Society (Deutsche Gesellschaft für Fettwissenschaft, DGF), the International Organization of Standardization (ISO), the American Association of Cereal Chemists (AACC), and the Association of Official Analytical Chemists (AOAC) have developed standard reference methods to assay crude fat in a variety of matrices (1,2). Nevertheless, analytical problems exist due to inherent variations in sample preparations and the methods practiced.

The common approach for total crude fat determination is based on the solubility of lipids in nonpolar organic solvents such as hexanes or petroleum ether (3). Diethyl ether or supercritical fluid extraction using carbon dioxide with and without a solvent modifier has also been used for crude fat extraction (4,5). The volatile solvents are removed by evaporation, and the nonvolatile residue (crude fat) is measured gravimetrically. The nonvolatile fraction consists of TAG and trace amounts of other components, namely, FFA and their alkyl esters, sterols, sterol esters, long-chain aldehydes and alcohols, fat-soluble vitamins, and other nonpolar natural products. Most of the current research is focused on development of new high-throughput, nondestructive secondary spectroscopic procedures (NIR and low-resolution pulsed NMR) for the assay of crude fat (6–9). The impact of sample preparation on crude fat assay has often been overlooked. Reports estimate that approximately 30% of errors in analytical measurements come from faulty sample preparation techniques (10). The term "sample preparation" encompasses multiple steps such as sample collection, drying, grinding, and extraction.

Several different grinders/sample preparation mills are commercially available. Mikro-mill is a hammer mill that grinds samples with rotating hammers (2). Knifetec uses high-speed rotor blades, whereas the Cemotec grinder uses two discs for grinding samples, one stationary and one rotating (2). Cyclotec, UDY, and Brinkmann-Retsch sample preparation mills are cyclone-type grinders (2). The Monsanto-built Mega-grinder is a ball-mill type grinder (2). The Mega-grinder consists of a 2-horsepower electric motor that drives a crankshaft *via* a belt. The crankshaft drives a piston that holds the sample trays. The piston is moved in an up–down motion. Approximately 9.0 ± 0.5 g of sample was loaded in a Delrin® tube (length 90.0 mm, internal diameter 39.0 mm). One steel ball (32.0 mm diameter, weight 130 ± 1.0 g) was placed inside each tube. The tube was closed tightly with a Delrin® lid. Eight tubes were placed on each rack that was loaded on a Mega-grinder. This grinder utilizes extremely rapid shaking (1200 rpm) to impact the steel balls into intact seeds, causing the seeds to become pulverized in 30 s to 2 min.

Several papers in the literature describe the influence of moisture content determination and comparison of different

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The experimental work was carried out at the two Monsanto locations.

extraction conditions and technologies on crude fat assay (11–13). In this paper, we have evaluated the impact of particle size, sieve dimensions, and grinding conditions on the assay of crude fat extracted from 10 corn samples ground with seven different grinders/sample preparation mills.

EXPERIMENTAL PROCEDURES

Sample preparation. Ten corn samples with varying oil content ranging from 3 to 21% were obtained from corn breeders (Monsanto, St. Louis, MO). Approximately 150 ± 1 g of each sample was ground by seven sample preparation mills. Grinding with the Cemotec (Foss, Eden Prairie, MN), Cyclotec (Foss), and Mega-grinder (Monsanto, St. Louis, MO) were done in-house (Monsanto, Ankeny, IA). Samples for the Cyclotec mill were ground with 1.0 and 2.0 mm screens. Samples were submitted to outside contract laboratories for grinding on other mills: the USDA Lab (Kansas City, MO) for the UDY grinder (Seedburo Equipment Co., Chicago, IL), Foss North America (Eden Prairie, MN) for the Knifetec grinder (Foss), and Woodson-Tenent Labs (Des Moines, Iowa) for the Mikro-mill (Hosokawa Micron Ltd., Cheshire, United Kingdom), respectively. Samples were ground for two time durations, 30 or 90 s, on a Knifetec mill. The data in Table 1 summarize the grinding conditions, screen size, grinding time, sample preparation time, space utilization, and cost of seven grinders. Samples were ground with a Mega-grinder at two sites (Monsanto, St. Louis, MO; Monsanto, Ankeny, IA) to evaluate instrument-to-instrument variations.

Crude fat extraction. Three blind replicates of all ground samples were extracted for the crude fat assay by a Soxtec extraction procedure with a Soxtec™ Avanti 2050 automated extraction system (Foss). Approximately 2.0 ± 0.1 g of ground corn sample was weighed into tared cellulose thimbles (Foss). A defatted cotton plug (Foss) was placed on top of each sample to keep the material immersed during the boiling step and to prevent any sample loss from the top of the thimble. Samples were extracted with hexane (boiling range 68–70°C, EMD Chemicals Inc., Gibbstown, NJ, or equivalent) solvent. The extraction thimble was set into the weighed aluminum cup (Foss) and approximately 80 ± 1 mL of hexane was added to each cup. Crude fat was extracted by immersing the sample in the boiling solvent under reflux for 20 min. The sample thimble was raised and rinsed with condensed solvent for an additional 40 min. The reflux rate was adjusted to approximately 3–5 drops/s during the extraction and rinse steps. Afterward, the extraction solvent was removed by a final evaporation step (8 min). The sample cups were lifted about 1 cm during the evaporation cycle to avoid excessive sample heating. After completion of the extraction process, sample cups were dried at 105 ± 5°C for at least 30 min and transferred to a desiccator and cooled to ambient temperature. Weight of the crude fat extracted was determined on an as-is and dry-matter basis (DMB). Moisture analysis on the ground samples was performed by the AOCS oven drying procedure Ac 2-41 (1). The AOCS Official Butt-tube Method Ac 3-44 (1) was also used for crude fat extraction to compare crude fat extraction to verify and confirm results obtained by the Soxtec procedure.

Particle size distribution. The influence of particle size distribution on the crude fat assay was determined by sieving ground sample through multiple stacked sieves (2.4, 2.0, 1.0, and 0.6 mm) by two methods. In the first procedure, approximately 20 ± 1 g of ground sample was placed on the top of 2.4-mm mesh size sieve. The stacked sieves were then placed on a shaker for 20 min, and fractions were collected from the top of each mesh sieve and the bottom of the lowest mesh sieve. Each fraction was weighed separately to determine the particle size distribution.

In the second approach, a single corn sample (approximately 250 g) was ground separately on a Cemotec grinder and a Mega-grinder to provide a wide particle size distribution. The combined ground sample was sieved using multiple stacked sieves (1.4, 1.0, 0.6, and 0.3 mm). Approximately 30 g of ground sample was placed on the top of the 1.4-mm mesh size sieve. The sample was brushed through each sieve to eliminate/reduce the impact of sample caking. After brushing, fractions were collected from the top of each mesh sieve and the bottom tray below the finest mesh sieve. Each fraction was weighed separately to determine the particle size distribution. Each particle size fraction was analyzed individually for the crude fat assay.

TABLE 1
Comparison of Operation Performance of Different Grinders

Grinder type	Screen size	Grinding time	Sample prep time[a]	Dimensions[b] (W × D × H) (ft)	Cost[b]
Mega-grinder	N/A	2 min	45 min	44 × 51 × 55	<$10,000
Cyclotec	1.0 and 2.0 mm	30 min	75 min	15 × 8 × 16	$4,200
Knifetec	N/A	30/90 s (3 × 2 × 5)[c] (3 × 3 × 10)[c]	5 min	9 × 7.5 × 9.5	$3,995
Mikro-mill	1.0 mm	30 s	3 min	30 × 30 × 57	$9,110
UDY	2.0 mm	15 min	30 min	10.5 × 10.5 × 18.5	$3,400
Brinkmann-Retsch mill	0.75 mm/1.0 mm	2 min	5 min	18 × 16 × 18	$6,205
Cemotec	Setting #1	2 min	5 min	14 × 8.5 × 15	$4,200

[a]Sample preparation time is the average time required to grind 150 g of each sample.
[b]Approximate estimate of cost and dimensions.
[c](# of 50 g sample/load × # of cycles × time in s/grind cycle).

TABLE 2
Mean and Grand Mean Average of Percent Crude Fat Extracted (DMB) by the Soxtec Procedure from 10 Corn Samples Ground on Seven Sample Preparation Mills

	Corn 1			Corn 2			Corn 3			Corn 4			Corn 5			Corn 6		
Grinder	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range
Brinkman-Retsch (0.75 mm)	9.1	0.04	0.08	14.9	0.29	0.54	6.7	0.11	0.21	4.3	0.11	0.21	7.9	0.03	0.05	7.6	0.02	0.04
Cemotec (# 1)	7.0	0.09	0.18	12.3	0.22	0.42	6.1	0.06	0.12	3.4	0.08	0.13	7.0	0.21	0.41	6.0	0.16	0.31
Cyclotec (1.0 mm)	9.3	0.03	0.06	15.7	0.08	0.16	7.4	0.28	0.54	4.3	0.25	0.47	8.0	0.05	0.10	8.0	0.07	0.13
Cyclotec (2.0 mm)	9.2	0.15	0.30	14.9	0.22	0.40	6.9	0.09	0.18	4.2	0.05	0.09	7.8	0.13	0.24	8.0	0.11	0.20
Knifetec (30 s)	8.6	0.19	0.34	14.5	0.01	0.03	6.6	0.23	0.40	4.1	0.12	0.23	7.4	0.34	0.68	7.4	0.19	0.37
Knifetec (90 s)	9.0	0.09	0.18	15.0	0.20	0.36	6.9	0.10	0.17	4.3	0.14	0.28	7.8	0.02	0.05	7.7	0.12	0.21
Mega-grinder: ANK[a]	9.1	0.03	0.07	15.5	0.04	0.09	6.8	0.14	0.27	4.2	0.07	0.13	7.7	0.10	0.19	7.7	0.03	0.07
Mega-grinder: STL[b]	9.5	0.25	0.45	15.3	0.15	0.27	6.9	0.11	0.22	4.3	0.20	0.35	7.8	0.14	0.26	7.8	0.07	0.13
Mikro-mill (1.0 mm)	9.1	0.31	0.54	14.8	0.27	0.48	6.8	0.10	0.19	3.9	0.07	0.14	8.1	0.50	0.90	7.6	0.08	0.14
UDY (2.0 mm)	9.2	0.14	0.28	15.2	0.07	0.14	6.9	0.31	0.55	4.4	0.07	0.13	7.9	0.07	0.13	7.8	0.07	0.13

	Corn 7			Corn 8			Corn 9			Corn 10			Grand		
Grinder	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 3)	SD (n = 3)	Range	Mean (n = 30)	SD (n = 30)	Range
Brinkman-Retsch (0.75 mm)	3.6	0.05	0.11	14.5	0.12	0.23	12.7	0.23	0.43	20.5	0.46	0.91	10.2	0.15	0.28
Cemotec (# 1)	2.6	0.19	0.38	12.0	0.48	0.96	9.6	0.37	0.74	18.2	0.70	1.38	8.4	0.26	0.50
Cyclotec (1.0 mm)	3.8	0.08	0.14	15.0	0.13	0.24	13.7	0.22	0.41	21.5	0.02	0.04	10.7	0.12	0.23
Cyclotec (2.0 mm)	3.8	0.17	0.31	14.9	0.33	0.65	13.3	0.20	0.37	20.6	0.19	0.37	10.4	0.16	0.31
Knifetec (30 s)	3.4	0.15	0.29	13.7	0.28	0.52	12.8	0.08	0.15	20.2	0.18	0.32	9.9	0.18	0.33
Knifetec (90 s)	3.8	0.09	0.17	14.3	0.32	0.57	12.8	0.13	0.26	20.9	0.43	0.85	10.3	0.16	0.31
Mega-grinder: ANK[a]	4.1	0.15	0.28	15.0	0.12	0.24	13.4	0.23	0.45	20.9	0.16	0.27	10.5	0.11	0.21
Mega-grinder: STL[b]	3.8	0.08	0.16	14.6	0.14	0.25	13.3	0.10	0.18	20.7	0.27	0.54	10.4	0.15	0.28
Mikro-mill (1.0 mm)	3.7	0.06	0.12	14.2	0.14	0.27	13.1	0.06	0.12	20.1	0.22	0.43	10.1	0.18	0.33
UDY (2.0 mm)	3.9	0.16	0.32	14.3	0.15	0.31	13.0	0.09	0.17	20.3	0.10	0.20	10.3	0.12	0.24

[a]Mega-grinder: ANK—Grinding done on a Mega-grinder at the Ankeny site.
[b]Mega-grinder: STL—Grinding done on a Mega-grinder at the St. Louis site. DMB, dry matter basis.

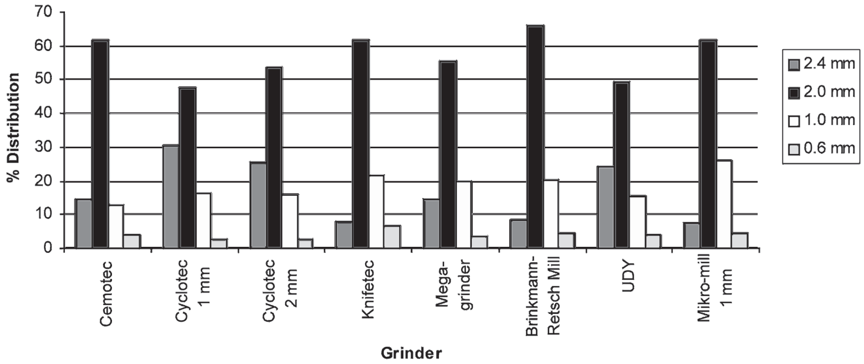

FIG. 1. Particle size distribution of corn sample ground by seven different grinders.

RESULTS AND DISCUSSION

The data in Table 2 show the mean percent crude fat and grand mean percent crude fat extracted from 10 different corn samples ground with seven different sample preparation mills using the Soxtec extraction procedure. The grand mean (n = 30, triplicate analyses per sample for 10 samples) of crude fat extracted from different samples varied from 8.4 to 10.7%. The Cemotec grinder (coarsest grinder) provided the lowest crude fat yields with the highest SD between replicate analyses, resulting from inefficient and incomplete extraction of crude fat from larger particles. The highest crude fat yields were found in samples ground with the Cyclotec mill with a 1.0-mm screen (grand mean = 10.7%). The crude fat yields of samples ground with the other grinders varied between 9.9 and 10.5%. The differences in crude fat extraction yield with different grinders may be due to differences in particle size distribution obtained with various grinders. The attempt to analyze particle size distribution by placing multiple stacked sieves on a shaker (procedure 1) was not successful, as we observed particle sizes greater than the sieve dimensions fitted on the sample preparation mills. The Cyclotec and Udy grinders fitted with 1.0-mm screens showed over 24.0% of particle sizes greater than 2.4 mm. Similar results were obtained with other sample preparation mills (Fig. 1). These observations were attributable to sample caking with high-oil and/or high-moisture samples. However, a more accurate means to evaluate the effect of particle size on the crude fat assay was achieved by brushing one corn sample through four screens, thus eliminating the problem of sample caking. Assessment utilizing four-screen particle size separation showed that the efficiency of crude fat extraction from ground corn samples increased with the decrease in particle size. The mean crude fat extracted from a corn sample with particle size <1.0 mm was 2.7 times greater than the mean crude fat extracted from a corn sample with particle size >1.4 mm. Lower crude fat extraction efficiency from coarse particle size samples (>1.4 mm) stems from inefficient extraction of crude fat from large particles (Table 3) by the current method and conditions. However, increasing extraction time and varying extraction conditions may result in an improvement of the total crude fat content extracted from larger particles.

Crude fat extraction varied with changes in grinding conditions. For example, the grand mean crude fat yield for the Knifetec grinder varied with the changes in grinding conditions. There was a 0.4% increase in grand mean crude fat yield when the grinding time was changed from 30 to 90 s. This is due to the higher proportion of finer particles, which increased the crude fat extraction efficiency. This illustrates that it is essential to carry out sample grinding with the same grinder and with identical conditions to get consistent results. An increase of 0.3% in the crude fat grand mean average was obtained with samples ground on a Cyclotec grinder when the screen size was reduced from 2.0 to 1.0 mm. This is also due to an increase in crude fat extraction efficiency with a finer particle size. Insignificant differences (<0.1%) in grand mean averages were observed with samples ground with the Megagrinder when grinding was done at two different sites with two different instruments. One type of grinder can give consistent results regardless of operator or location when operated with the same standard operating procedure.

All statistical analyses were carried out using Systat software, version 10.2, from Systat Software Inc. (Richmond, CA). Tukey's method was used for statistical comparison of crude fat extracted from corn samples ground with different grinders. Tukey's method considers all possible pairwise differences of means at the same time. Tukey's multiple comparison test is one of several multiple comparison tests that can be used to determine the means among a set of means that differ from the rest (14). The results presented in Table 4 show that the values for crude fat extracted from samples ground with a Cemotec grinder were different from the crude

TABLE 3
Impact of Particle Size on Percent Crude Fat Extracted (DMB) from a Single Corn Sample by the Soxtec Procedure[a]

Sample particle size, corn	% Average crude fat extracted (DMB) (n = 5)	SD (n = 5)
>1.4 mm	1.19	0.08
<1.4 and >1.0 mm	1.89	0.09
<1.0 and >0.6 mm	3.17	0.21
<0.6 and >0.3 mm	3.53	0.26
<0.3 mm	4.10	0.02

[a]For abbreviation see Table 2.

TABLE 4
Least Squares Mean Differences, Tukey Highly Significant Differences, for Corn Samples

Level					Least sq. mean
Corn (α = 0.050, *Q* = 3.25352)					
Cyclotec (1.0 mm)	A				9.71
UDY (2.0 mm)	A	B			9.41
Cyclotec (2.0 mm)	A	B			9.38
Mega-grinder: ANK[a]	A	B			9.27
Mega grinder: STL[b]	A	B			9.22
Knifetec (90 s)		B	C		9.06
Brinkman-Retsch mill (0.75 mm)		B	C		9.06
Mikro-mill (1.0 mm)		B	C		9.04
Knifetec (30 s)			C		8.72
Cemotec (setting #1)				D	7.46

[a]Mega-grinder: ANK—Grinding done on a Mega-grinder at the Ankeny site.
[b]Mega-grinder: STL—Grinding done on a Mega-grinder at the St. Louis site.

fat extracted with samples ground with other grinders. The crude fat extraction yields from Knifetec samples ground for 30 s were also statistically different from the Mega-grinder, UDY, and Cyclotec grinders. The values for crude fat extracted from samples ground with a Mega-grinder at two sites were not statistically different. The designations of A, B, C, D shown in Table 4 represent the performance differences among the various sample grinders. Overlapping performance was assigned as AB and BC in some cases.

Owing to resource limitations and to our business interest in sample preparation mills extracting higher oil yields, only samples ground on six grinders were extracted by the Butt-tube method. This experiment was intended to reconfirm the oil yields extracted from 10 corn samples by the Soxtec procedure. Samples from the Knifetec (30 s) and Cemotec sample preparation mills were not included for extractions with the Butt-tube procedure because of unsatisfactory performance in terms of overall crude fat recovery with the Soxtec procedure (Table 2). As both Mega-grinders at two sites, Ankeny and St. Louis, yielded similar oil yields with the Soxtex procedure (Table 2), only samples ground on the Mega-grinder at Ankeny were extracted with the Butt-tube method. The results in Table 5 confirmed the percent crude fat extracted from 10 corn samples by the Soxtec method. Detailed statistical analysis on oil extracted from corn samples with the Butt-tube procedure was not performed, as limited sample preparation mills and grinding conditions were included in this experiment.

The Monsanto-designed and -built Mega-grinder provides significant advantages over the commercially available grinders, as it is the only grinder capable of grinding multiple samples (from 1 to 96 samples) with no cross-contamination. The Mega-grinder is the best grinder for small sample sizes (200 mg–10 g) because of the minimal loss during the grinding process resulting from the sample being ground in a single closed container. For very large sample sizes, the Mega-grinder is limited by the loading capacity of each individual tube. However, using multiple tubes can circumvent this problem. The Mega-grinder requires significant space as compared with other commercial grinders; however, improving the engineering design will eliminate this issue. The Mikro-mill and Knifetec grinders could be better grinders for bulk samples because the grinding and sample preparation time is significantly lower for larger sample sizes. The grinding time was significantly increased with high-oil and high-moisture samples for the UDY, Cyclotec, and Brinkmann grinders owing to frequent clogging of the 0.75-, 1.0-, and 2.0-mm screens. A Cemotec grinder may be used for preliminary grinding of bulk samples; these samples can be ground further on a cyclone-type sample preparation mill. This combined process may reduce some of the clogging problems observed with high-oil and high-moisture samples on cyclone mills. However, several other factors, such as space, cost, sample quantity, grinding time, and sample throughput needed, should be considered before selecting a sample preparation mill for grinding samples.

The results presented in this study indicate that both grinding technology and conditions (number of cycles, particle size, sieve dimensions, and grinding time) influence the percent crude fat extracted from corn samples. However, several grinders provide comparable oil extraction yields. Statistical analysis of the data suggested that similar oil extraction yields were obtained with samples ground on the Mega-grinder, UDY, Mikro, and Cyclotec mills. Thus, the sample preparation technique plays a critical role in extraction and determination of the total crude fat content in oilseeds.

TABLE 5
Comparison of Grand Mean of Percent Crude Fat Extracted (DMB) from 10 Corn Samples by the Soxtec and Butt-tube Methods

	Soxtec		Butt-tube		
	Grand mean (%)	SD	Grand mean (%)	SD	Difference Soxtec – Butt-tube (%)
Corn					
Cyclotec (Screen 1.0 mm)	10.7	0.12	10.6	0.17	0.08
Knifetec (90 sec)	10.3	0.16	10.3	0.14	–0.02
Mega-grinder: ANK[a]	10.5	0.11	10.4	0.09	0.05
Brinkmann-Retsch mill	10.2	0.15	10.4	0.1	–0.18
UDY (Screen 2.0 mm)	10.3	0.12	10.3	0.11	0.03
Mikro-mill (Screen 1.0 mm)	10.1	0.18	10.3	0.11	–0.16
Average	10.3	0.14	10.4	0.12	–0.03

[a]Mega-grinder: ANK—Grinding done on a Mega-grinder at the Ankeny site.

REFERENCES

1. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 5th edn., edited by D. Firestone, AOCS Press, Champaign, 1997.
2. Luthria, D.L., K. Noel, and D. Vinjamoori, Effect of Moisture Content, Grinding and Extraction Technologies on Crude Fat Assay, in *Oil Extraction and Analysis: Critical Issues and Comparative Studies*, edited by D.L. Luthria, AOCS Press, Champaign, 2004, pp. 118–136.
3. Thiex, N., S. Anderson, and B. Gildemeister, Crude Fat, Hexanes Extraction, in Feed, Cereal Grain, & Forage (Randall/Soxtec/Submersion Method): A Collaborative Study, *J. AOAC Int. 86*:888–898 (2003).
4. Taylor, S.L., F.J. Eller, and J.W. King, A Comparison of Oil and Fat Content in Oilseeds by Analytical Supercritical Fluid Extraction and Related Analytical Techniques, *Food Res. Int. 30*:365–370 (1997).
5. Moreau, R.A., M.J. Powell, and V. Singh, Pressurized Liquid Extractions of Polar and Nonpolar Lipids in Corn and Oats with Hexane, Methylene Chloride, Isopropanol, and Ethanol, *J. Am. Oil Chem. Soc. 80*:1063–1067 (2003).
6. Tseng, C.-H., K. Ma, and G. Wang, Internet-Enabled Near-Infrared Analysis of Oilseeds, in *Oil Extraction and Analysis: Critical Issues and Comparative Studies*, edited by D.L. Luthria, AOCS Press, Champaign, 2004, pp. 166–192.
7. Barthet, V.J., T. Chornick, and J.K. Daun, Comparison of Methods to Measure the Oil Contents in Oilseeds, *J. Oleo Sci. 51*:589–597 (2002).
8. Baianu, I.C., T. You, D.M. Costescu, P.R. Lozano, V. Prisecaru, and R.L. Nelson, High-Resolution Nuclear Magnetic Resonance and Near-Infrared Determination of Soybean Oil, Protein, and Amino Acid Residues in Soybean Seeds, in *Oil Extraction and Analysis: Critical Issues and Comparative Studies*, edited by D.L. Luthria, AOCS Press, Champaign, 2004, pp. 193–340.
9. Gambhir, P.N., Applications of Low-Resolution Pulsed NMR to the Determination of Oil and Moisture in Oilseeds, *Trends Food Sci. Technol. 3*:191–196 (1992).
10. Majors, R.E., Trends in Sample Preparation and Automation—What the Experts Are Saying, *LC-GC 13*:742–749 (1995).
11. Hill, L.D., and K.L. Blender, Evaluation of Changing the Moisture Reference Method, *Cereal Foods World 39*:19–27 (1994).
12. Matthaus, B., and L. Bru, Comparison of Different Methods for the Determination of the Oil Content in Oilseeds*, J. Am. Oil Chem. Soc*. *78*:95–102 (2001).
13. Luthria, D.L., and R. Cantrill, Evaluation of Five Different Methodologies for Determination of Oil Content in Ground Corn and Soybean Seeds, *INFORM 13*:893–894 (2002).
14. Box, G.E.P., W.G. Hunter, and J.S. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building*, Wiley-Interscience, 1978, pp. 203–207.

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