Concerns for the Determination of Free Fatty Acid in Cottonseed

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ABSTRACT: The official AOCS method for the determination of free fatty acid (FFA) in cottonseed requires dehulling the seed, grinding the meats with a 12-blade food processor, and extracting the ground meats in a butt tube with three portions of room-temperature petroleum ether. The extracted oil, after desolventization, is then titrated with NaOH to the end point of phenolphthalein in a mixed solvent of isopropanol and hexane. Our study showed that this procedure tends to underestimate the amount of FFA present in the oil of cottonseed by as much as 11.5%. It was also found that to obtain consistent and accurate FFA content, a desirable particle size is smaller than 10 mesh (preferably <14 mesh), minimum extraction temperature should be no less than 40°C (preferably greater than 50°C), and the extraction time should be longer than 2 h in a Soxhlet extractor. *JAOCS 75,* 1321–1324 (1998).

KEY WORDS: Butt tube, cottonseed, free fatty acid, Soxhlet.

In 1996, more than 7.1 million tons of cottonseed were produced and traded in the United States (1). About half of the fuzzy, undelintered cottonseed is fed to livestock directly and the rest is processed at a cottonseed oil mill into protein meal and edible oil. Since cottonseed oil is considered a preferred frying oil for potato chips, it often demands a slight premium over soybean oil. The price of seed is determined by the market supply and demand and by the seed grade, which is defined in the Trading Rules published by the National Cottonseed Products Association (2). Prime Quality Cottonseed is seed containing no more than 1.0% foreign matter, not more than 12.0% moisture, and not more than 1.8% free fatty acids (FFA) in the oil of the seed. This seed is given a quality index of 100. Below Prime Quality, the seed quality index will be reduced by the following factors: 0.4 unit of the seed quality index for each 0.1% FFA above 1.8%; 0.1 unit for each 0.1% foreign matter in excess of 1.0%; and 0.1 unit for each 0.1% of moisture over 12.0%. When cottonseed has more than 12.5% FFA, more than 10% foreign matter, greater than 20% moisture, or more than 25% moisture and foreign matter combined, it is classified as Off Quality Cottonseed. Premium or discount of the seed price is, therefore, determined according to this seed quality index. As it is defined, seed quality index is weighted four times more for FFA than for moisture or foreign matter. Accurate determination of FFA will greatly impact the seed value, especially when the seed contains FFA near or higher than 1.8%. Cottonseed breeding companies select no more than 1% FFA as the prime quality for planting seed. It is also well known to the industry that seeds containing higher than 1% FFA may not germinate.

During the 1994–1995 processing season, several sets of cottonseed, mill-run meats, and collets before and after extraction were collected and analyzed by a commercial lab according to the American Oil Chemists' Society (AOCS) Official Method. One interesting observation from these data was that the FFA values in these samples were greatly affected by the thermal history of the samples during collection and handling. Thermal history includes whether samples were quenched with ice, dry ice, or liquid nitrogen; sample storage temperature; etc. The FFA contents of these samples determined by the AOCS Official Method were 15 to 80% lower than those of oil obtained by a 4-h Soxhlet exhaustive extraction. A series of experiments was performed to identify the possible cause(s) of the discrepancy between the AOCS Official Method and the true value of FFA in cottonseed as determined by a 4-h Soxhlet exhaustive extraction. A recommendation to determine the amount of FFA in cottonseed and cottonseed products more accurately is proposed.

EXPERIMENTAL PROCEDURES

Cottonseed samples. Cottonseed, mill-run meats, and raw flakes were obtained from an oil mill in Texas during the 1996 and 1998 crushing seasons. Clean meats, i.e., clean dehulled cottonseed kernels, were obtained by sieving the mill-run meats with a standard 7-mesh screen to remove hulls and fuzzy fibers and with a 14-mesh screen to eliminate fine kernel particles.

Storage of cottonseed samples. Clean meats and flakes were stored in screw-capped plastic containers and refrigerated at 5°C until used. White undelintered cottonseed samples were kept in a plastic bag at ambient temperature.

Preparation of samples for extraction. White cottonseeds were dehulled by using a Waring blender at high-speed setting. A 7-mesh screen was used to separate the kernels. Cottonseed meats or flakes were ground with a Bunn coffee

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grinder (Bunn-o-matic, Model G3, Springfield, IL) at a setting of "drip" to particle size smaller than 10 mesh (2 mm) U.S.A. Standard sieve.

Extraction of oil. AOCS Official Method Aa 6-38 (3) was followed to extract oil from the ground sample with petroleum ether at ambient temperature. The ground sample (40 ± 0.01 g) was packed properly in a Butt tube. Three portions of petroleum ether (50, 25, and 25 mL) were used to leach the oil at a flow rate of 150 drops per minute. In the Soxhlet extraction which follows the AOCS Official Method Aa 4-38 (3) for oil content determination in cottonseed, a ground sample (40 ± 0.01 g) was weighed into a paper thimble (43 mm × 123 mm, Whatman International Ltd., Maidstone, England) and covered with a piece of cotton before placing it in a Soxhlet extractor. Approximately 300 mL of commercial hexane was used for the extractions. The amount of oil extracted by the Soxhlet extraction is considered to be the total amount of oil available in the sample. A single-stage extractor similar to the one described in a previous extraction study (4) was also used for this work. It is basically a modified Soxhlet extractor linked with a pump to allow the miscella to be circulated through a heat exchanger to maintain the recycled miscella at a constant controlled temperature. A small miscella sample was taken periodically to determine the rate of cottonseed oil extraction. Oil content in the miscella was determined by a precision densitometer (Anton Paar, DMA58, Vienna, Austria). The flow rate of the recycled miscella was 20.9 gm/min/cm^2, which is comparable to the recycle rate of miscella in a full-scale extractor.

Free fatty acid determination. The oil sample extracted by either the AOCS or Soxhlet methods or by the single-stage extractor was desolventized with a vacuum rotary evaporator. Oil was then precisely weighed into an Erhlenmeyer flask. Isopropanol (75 mL) and *n*-hexane (15 mL) were introduced to the flask with volumetric dispensers. The mixture was titrated with 0.1 N sodium hydroxide to a steady pinkish end point of phenolphthalein. The percentage of free fatty acid in the oil sample was calculated in terms of oleic acid with the following equation:

$$\% \text{ FFA} = \frac{(2.82 \times \text{volume of 0.1 N NaOH in mL})}{\text{weight of oil in g}} \times 100 \qquad [1]$$

This titration procedure for free fatty acid content had a precision of ±0.1% FFA. This precision is improved by adjusting the weight of oil sample or varying the concentration of NaOH for titration. An intensely red oil sample, however, can make the determination of the end point difficult.

Fatty acid composition. Fatty acid profiles of some of the extracted oils were analyzed according to AOCS Official Method Ce 1-62 (3) by a commercial laboratory.

RESULTS AND DISCUSSION

Reproducibility of AOCS and Soxhlet methods for FFA. The reproducibility of the AOCS Official Method Aa 6-38 and the Soxhlet method for a single lot of cottonseed mill-run meats was determined (Table 1). Both methods are reproducible and are within the precision limit of the titration procedure. The FFA value determined by the Soxhlet procedure is higher than the FFA value determined by the AOCS Official Method for the same lot of cottonseed. Based on unpublished data from this lab, 4-h Soxhlet extraction of ground cottonseed kernels did not produce any detectable FFA rise. Therefore, the 4-h Soxhlet extraction was used as a method for comparison throughout this report.

TABLE 1
Reproducibility of AOCS and Soxhlet Method for FFA Determination in Cottonseed[a]

	Extraction method	
Run number	AOCS Aa 6-38	Soxhlet method
1	1.68	2.02
2	1.69	1.95
3	1.70	1.98
4		1.99
5		1.95
6		1.99
Mean	1.69	1.98
Standard deviation	0.01	0.02

[a]FFA, free fatty acids.

Correlation of FFA between AOCS and Soxhlet methods. Several lots of cottonseed were collected during the 1996 and 1998 crushing seasons and analyzed for FFA by both AOCS and Soxhlet extraction methods. A linear correlation is observed with a slope of 0.885, intercept of –0.138 and r^2 of 0.997 (Fig. 1). The AOCS method consistently underestimates the amount of FFA in cottonseed samples by about 11.5%. When seed is in good condition and contains less than 1% FFA, an 11.5% deviation will not impact greatly the value of the seed nor its germination performance. However, when the seed is less than prime quality and contains greater than 1.8% FFA, this 11.5% deviation can artificially inflate the seed price in the trade and increase the refining loss during the processing of the oil. This double loss situation can be significant for an industry which relies heavily on the volume of seed processed to maintain an acceptable profit margin.

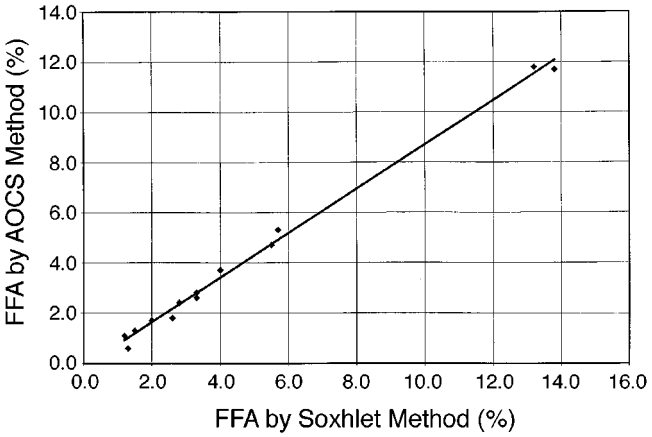

FIG. 1. Plot of free fatty acid (FFA) percentage of cottonseed samples determined by AOCS Official Method Aa 6-38 vs. Soxhlet method.

TABLE 2
Effect of Extraction Time Using Soxhlet Method on FFA (commercial hexane as solvent)[a]

Extraction time (h)	<14 Mesh FFA (%)	<14 Mesh (% oil extracted)
0.5	1.4	17.5
1.0	1.5	22.2
1.5	2.0	25.4
2.0	1.9	22.9
3.0	2.0	28.9
4.0	1.9	28.1

[a]For abbreviation see Table 1.

TABLE 3
Oil Yield and FFA in Cottonseed by AOCS and Soxhlet Methods[a]

Method:	AOCS Method Aa 6-38		Soxhlet method	
Solvent:	Petroleum ether		Commercial hexane	
Temperature:	Room temperature		~ Boiling temperature	
Sample	Yield of oil (%)	FFA (%)	Yield of oil (%)	FFA (%)
1	75	2.6	100	3.2
2	83	3.7	100	3.8
3	87	1.3	100	1.4
4	94	2.8	100	3.3
5	~99	2.4	100	2.8

[a]For abbreviation see Table 1.

Effect of Soxhlet extraction time on oil yield and FFA. To examine the effect of extraction time on oil yield and FFA, the Soxhlet extraction was stopped at predetermined time periods (Table 2). When particle size of the cottonseed meats was <14 mesh (1.4 mm), the amount of FFA reached a steady state value at around 90 min of extraction. But the total oil recovered did not reach a steady state until 3 h of extraction. A separate set of tests also indicated that the amount of FFA approached a steady state value after 90 min for particles <10 mesh (2 mm).

Oil yield and fractional extraction of the AOCS method. The oil yield obtained using the AOCS Official Method Aa 6-38 for five different lots of cottonseed samples ranged from 75 to 99% (Table 3). The fatty acid profile analysis on the oil extracted by the AOCS method and the oil extracted from the solid residue using the AOCS method with Soxhlet procedure were done on several sets of samples. A similar trend was observed: palmitic acid is lower and linoleic is higher in oils extracted by the AOCS method than in those extracted by the Soxhlet method. The fatty acid profile, oil recovered, and FFA content determined by the AOCS and Soxhlet methods, and by the Soxhlet-extracted solid residue from the AOCS method for one set of cottonseed samples are shown in Table 4. The computed values of FFA (13.6%), palmitic (25.5%), and linoleic acid (56.6%) contents based on the percentage of oil recovered (dry basis) by the AOCS method and by Soxhlet-extracted solid residue from the AOCS method checked reasonably well with those analyzed from Soxhlet-extracted seed oil (Table 4). These results suggest that the solid fat and solid fatty acid may not be equally extractable as the liquid oil at room temperature using petroleum ether as the solvent. This nonuniform extraction of FFA may be partially responsible for the discrepancies between the AOCS method and the Soxhlet extraction method.

Effect of oil extraction temperature on FFA determination. A dynamic extraction device is similar to a Soxhlet extractor, but with the addition of a pump that circulates the miscella. This extractor design permits the extraction of samples to be carried out at various temperatures within a ±0.5°C range. Extraction flow rate was controlled by a pump at 20.9 g/min/cm^2. The Soxhlet extractor can hold up to 60 g of ground cottonseed meats. Each extraction was continued for 50 min. The results shown in Table 5 indicate that the elevated temperature is crucial to achieve an accurate determination of FFA in cottonseed samples. The highest operable temperature for commercial hexane used in this study was 62°C, which yielded an FFA value of 3.2%, which is the same as 4-h Soxhlet-extracted oil from the same lot of cottonseed. Coincidently, the melting temperature of palmitic acid is 62.9°C (5).

From this study, it is important to note that the AOCS Official Method Aa 6-38 is likely to underestimate the amount

TABLE 4
Gas-Liquid Chromatographic Fatty Acid Profile, FFA Content, and Percentage Oil Recovered from Cottonseed Oil Extracted by AOCS and Soxhlet Methods

Extraction method	AOCS Aa 6-38	Soxhlet method	Soxhlet method solid residue from AOCS Aa 6-38
Fatty acid profile (%)[a]			
Myristic (14:0)	0.6	0.7	0.6
Palmitic (16:0)	24.6	26.8	27.6
Palmitoleic (16:1)	2.1	0.7	0.1
Stearic (18:0)	2.0	1.3	1.6
Oleic (18:1)	13.1	13.7	14.3
Linoleic (18:2)	57.4	56.5	55.3
Linolenic (18:3)	0.1	0.1	0.3
Arachidic (20:0)	0.1	0.2	0.2
FFA (%)	11.8	13.2	18.2
Oil recovered (% dry basis)	71.4	100.0	28.6

[a]Fatty acid name (carbon number:number of double bonds); fatty acid profiles of oils were analyzed according to AOCS Official Method Ce 1-62 (3). For abbreviation see Table 1.

TABLE 5
Effect of Extraction Temperature on FFA Determination in a Dynamic Soxhlet Extractor[a]

Temperature (°C)	FFA (%)
20	2.7
30	2.9
40	3.0
50	3.0
62	3.2

[a]Commercial hexane was used as the extraction solvent at a flow rate of 20.9 g/min-cm^2 with 20 g ground cottonseed for 50 min. FFA = 3.2% for a 4-h Soxhlet extraction of the same seed sample. For abbreviation see Table 1.

of FFA in cottonseed by about 11.5% when compared with the 4-h Soxhlet extraction method. This room-temperature extraction of ground cottonseed meats packed in a Butt tube apparently leaves more FFA in the cottonseed meats. This underestimation of FFA in cottonseed could translate into higher seed cost, due to erroneous higher seed grade, and to greater refining loss, due to the lower AOCS FFA than what is really present in the oil of the seed. To minimize the underestimate of FFA of the cottonseed oil, one can divide the AOCS FFA value by a factor of 0.885. However, this conversion factor should only be used to estimate the true value of FFA in cottonseed oil. Other approaches include: Soxhlet extraction for at least 2 h (Table 2); and extraction of oil with an instrument similar to the accelerated extraction system manufactured by Dionex (Salt Lake City, UT)for less than 20 min, followed by a titration procedure, to determine the amount of FFA present in the oil. Preliminary results indicate that the Dionex extractor operated at 130°C and 1000 psi with three 5-min soaking cycles yields an FFA content (13.9%) quite close to that of 4-h Soxhlet extraction from the same cottonseed sample (13.6%). Other cost-efficient replacement methods to the AOCS Official Method Aa 6-38 are currently being studied.

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REFERENCES

1. *Oilseeds Crop Yearbook,* Economic Research Service, United States Department of Agriculture, Washington, DC, 1997, p.7.
2. *Trading Rules,* National Cottonseed Products Association Incorporated, Memphis, 1998, pp. 50–54.
3. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th edn., AOCS Press, Champaign, 1993, pp. Aa 4-38, Aa 6-38, Ce 1-62.
4. Wan, P.J., D.R. Pakarinen, R.J. Hron, Sr., and E.J. Conkerton, Alternative Hydrocarbon Solvents for Cottonseed Extraction, *J. Am. Oil Chem. Soc. 72:*653–659 (1995).
5. Bailey, A.E., *Cottonseed and Cottonseed Products,* Interscience Publishers, New York, 1948, p. 376.

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