A Modified Method for Determining Free Fatty Acids from Small Soybean Oil Sample Sizes

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ABSTRACT: A modification of the AOCS Official Method Ca 5a-40 for determination of free fatty acids (FFA) in 0.3 to 6.0-g samples of refined and crude soybean oil is described. The modified method uses only about 10% of the weight of oil sample, alcohol volume, and alkali strength recommended in the Official Method. Standard solutions of refined and crude soybean oil with FFA concentrations between 0.01 and 75% were prepared by adding known weights of oleic acid. The FFA concentrations, determined from small sample sizes with the modified method, were compared with FFA percentages determined from larger sample sizes with the Official Method. Relationships among determinations obtained by the modified and official methods, for both refined and crude oils, were described by linear functions. The relationship for refined soybean oil had an R^2 value of 0.997 and a slope of 0.99 ± 0.031. The values for crude soybean oil are defined by a line with $R^2 = 0.9996$ and a slope of 1.01 ± 0.013.

JAOCS 75, 563–568 (1998).

KEY WORDS: Crude soybean oil, free fatty acid, Official Method, refined soybean oil, titration.

In the soybean industry, the presence and development of any amount of free fatty acid (FFA) in soybean oil is undesirable. The FFA content of oil in soybeans indicates how well the beans have been treated during the period between harvest and processing. The eventual consequence of high FFA content is monetary loss because processing soybeans with high FFA content results in more refining loss. Specifically, refining loss is the sum of oil weight lost during removal of FFA and other impurities plus the weight of entrained neutral oil in soapstock during the normal alkali-refining process. The percentage of oil lost during refining can be estimated as a multiple of the FFA percentage. Norris (1) estimates the potential loss at about three times the FFA content. Because of the economic impact of high FFA, it is important to determine the initial level of FFA in soybean oil and also to monitor its concentration during processing.

The standard method for FFA determination in extracted crude and refined soybean oil samples is based on an acid-base titration technique in a nonaqueous system. The method commonly used is AOCS Official Method Ca 5a-40 (2). The procedure prescribes an oil sample of maximum 56.4 g and minimum 3.53 g for the titration, depending on the level of FFA in the oil sample. When oil quantities are limited, as in some storage studies or when experimental use of oilseed crops is evaluated, sample sizes of this magnitude are not possible. A simple and inexpensive method is needed that accurately quantifies FFA content from a smaller sample size.

The use of smaller amounts of chemicals will have an impact, especially in developing countries, where chemicals are scarce and expensive. Also, it is prudent to limit the use of resources, particularly those hazardous to the environment. For example, hexane, a solvent used in the titration, is a hazardous, highly flammable compound, and its disposal can be harmful to the environment. A smaller sample size also would result in savings of storage space in laboratories that handle large numbers of samples, or if the protocol warrants that oil samples be kept after analysis.

Lanser *et al.* (3) developed a user-interactive computer-assisted Fourier transform infrared (FTIR) method to estimate FFA content in soybean oil samples. The method requires only one drop of soybean oil. The limitation of the method, and other similar spectrometric methods, such as that described by Canham and Pacey (4), is the cost of the instruments: it is prohibitive for most laboratories. Lanser *et al.* (3) also evaluated a modified version of the Official Method in which between 6 and 7 g of oil is used, but no further description of the method was given. The coefficient of determination (R^2) of the modified method was 0.999. The high correlation indicates that the officially recommended sample size can be scaled down without losing accuracy of FFA determination.

The objective of this research was to develop and evaluate a method for determining FFA concentration within the range of 0.01 to 75% in soybean oil by using an oil sample of about 10% (between 0.3 and 6.0 g) of the weight recommended in the AOCS Official Method.

MATERIALS AND METHODS

Different amounts of oleic acid were added to refined-bleached-deodorized (RBD) and crude soybean oil samples

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to make up the desired FFA concentrations in standard soybean oil solutions. Levels of FFA in known oil standards were then determined by using both the proposed modified method and the AOCS Official Method.

Refined soybean oil sample. An RBD soybean oil (Hy-Vee Food Stores, Des Moines, IA) was purchased from a local grocery store. FFA content of the original RBD soybean oil was determined to be 0.035 ± 0.005% by using AOCS Official Method Ca 5a-40. The value is a mean from three replicate determinations.

Crude soybean oil sample. Crude soybean oil was obtained from Archer Daniels Midland Company (Decatur, IL). Mean FFA content of the crude oil, as determined by three determinations with AOCS Official Method Ca 5a-40, was 0.33 ± 0.037%.

Oleic acid. Oleic acid (Fisher Scientific, Fairlawn, NJ) of National Formulary (NF) and Food Chemicals Codex (FCC) grades was used to make up known FFA concentrations in refined and crude soybean oil samples. Oleic acid purity was not tested but was assumed to be 100%, as described in the vendor's specifications.

AOCS Official Method Ca 5a-40. FFA content of soybean oil is usually determined by titration with a standard alkali, NaOH, of specific strength or normality (N). For AOCS Official Method Ca 5a-40 (2), recommended oil sample size, volume of alcohol (ethyl alcohol), and NaOH strength are 3.53 to 56.4 g, 50 to 100 mL, and 0.1 to 1.0 N, respectively. In our experiments, NaOH solutions of various normalities were standardized according to AOCS Official Method H 12-52 (5).

FFA concentration in fats and oils is calculated as percentage oleic acid. The expression as given in AOCS Official Method Ca 5a-40 (2) is:

$$\text{\%FFA as oleic acid} = \frac{\text{alkali volume (mL)} \times \text{alkali normality} \times 28.2}{\text{sample weight (g)}} \quad [1]$$

Modified method. In the modified method, oil sample and reagent quantities used were about 10% of amounts recommended for the standard procedure (Table 1). The titration procedure, however, remained the same for both methods.

Preparation of standard soybean oil solutions for titration. Refined and crude soybean oil samples of known initial FFA concentrations were each divided into five and four lots, respectively, in Erlenmeyer flasks. Five FFA concentrations for refined soybean oil and four levels for crude soybean oil were used, corresponding to approximately the average FFA percentages for ranges of FFA in the AOCS Official Method (Table 2).

Oleic acid weights were calculated based on the estimated total weight of soybean oil to be used in the titration for a particular FFA level. The calculated weights of oleic acid were added to the respective soybean oil lots to give the predetermined FFA concentrations. Solutions were stirred for 3 to 5 min with magnetic stirrers. All standard oil solutions were stored at 2 to 5°C in stoppered flasks. Flask headspaces were flushed with nitrogen prior to closure.

TABLE 1
Sample Size and Reagent Concentrations Used in the Modified Method

FFA range[a] (%)	Weight of oil sample (g)	Volume of ethyl alcohol (mL)	Normality of NaOH (N)
0.01–0.2	5.64	5.0	0.013
0.20–1.0	2.82	5.0	0.013
1.00–30.0	0.70	7.5	0.031
30.00–50.0	0.70	10.0	0.13
50.00–100.0	0.35	10.0	0.125

[a] FFA, free fatty acid.

TABLE 2
Refined and Crude Soybean Oil Levels for Titration[a]

Ranges of FFA from AOCS Official Method (%)	Estimated FFA content prepared from refined soybean oil (%)	Estimated FFA content prepared from crude soybean oil (%)
0.01–0.20	0.1	—
0.20–1.00	0.6	0.6
1.00–30.00	15.0	15.0
30.00–50.00	50.0	50.0
50.00–100.00	70.0	70.0

[a] For abbreviation see Table 1.

Experimental design. The experiment was divided into two parts. Part one dealt with determination of FFA levels in refined soybean oil. Ten treatment combinations were used, consisting of two methods and five FFA levels (2 × 5 factorial). Part two involved use of crude soybean oil samples and included two methods and four levels of FFA (2 × 4 factorial), giving eight treatments. Each treatment was replicated three times, resulting in a total of 30 and 24 observations, respectively, in the first and second parts. The experiment was a completely randomized block design (CRBD), with replications serving as blocks.

Statistical Analysis Software (6) was used for analysis of data. Analysis of variance (ANOVA), general linear model (GLM), and regression through the origin procedures were used. Coefficients of determination (R^2) and coefficients of variation (square root of the means square error divided by the mean of the measured values) were determined to evaluate suitability of the method. Hypothesis tests were conducted on regression results to find the best-fit model. Significance was established at $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Modified method. Preliminary determination of FFA in refined soybean oil samples of 2.82 g (10% of the AOCS Official Method weight) showed that the NaOH concentration needed to detect and quantify FFA content was about 0.0125 N. Based on this estimation, NaOH concentrations for the modified method at different ranges of FFA were adjusted accordingly. Alcohol volume used in the modified method also was reduced to 10% of that used in the AOCS Official

Method (Table 1). Although oil sample weights and alcohol volumes were one-tenth of those recommended by the Official Method, NaOH normalities were slightly more than 10% of the recommended strengths. Table values served only as guidelines in preparing samples and reagents.

In the modified method, burette and Erlenmeyer flask sizes were rationalized with expected titer volume. The graduation of the commonly used burette (50-mL capacity) was not always small enough to capture the reading accurately. For lower FFA ranges, smaller burettes with capacities between 5 and 25 mL were used, along with 150-mL Erlenmeyer flasks.

The procedure used in the modified method was the same as for the Official Method, but in the modified method only about 10% of the amount of sample and reagent specified by the Official Method was used. These smaller quantities can result in savings in reagent costs and in time spent if the oil being tested needs to be extracted first. Use of weaker strengths of NaOH, as in the modified method, allows a more accurate end point determination. Caution should be used, however, during titration of crude soybean oil, which has higher FFA levels than refined oil, because it consistently produced turbid solutions. Under such conditions, end point determination could not be made with great precision.

FFA determination of refined soybean oil. Table 3 shows FFA contents determined by the AOCS Official Method and by the modified method. In assuming the AOCS method as a reference, the modified method slightly underestimated FFA content in refined oil samples that contained 0.1 to 50.0% FFA. The magnitude of underestimation ranged from 3.9 to 12.1%. But for samples with 0.01 to 0.2% FFA, the normal range of FFA content allowed under the standard definition of refined soybean oil and trading values of soybean oil (7), the deviations were small. Adoption of the modified method for determination of FFA content in refined soybean oil destined for consumer markets would amount to a 3.9% variation from the AOCS Official Method. This amount can be considered small enough to risk any possible marked variation in FFA values between the two methods during FFA determination based on the current refining capability. Differences in FFA content are known to be a function of the processing steps. Typical variations are shown in Table 4 (8); each processing step resulted in removal of a fraction of FFA. For the data shown in Table 4, FFA content was about 0.05% after refining and 0.03% after hydrogenation and deodorization.

ANOVA showed that there was no significant difference between the mean FFA values produced by the two methods ($P = 0.54$). There was a significant interaction effect between FFA content and the methods ($P < 0.0014$). This observation could be explained by underestimation of FFA in the lower ranges of FFA (0 to 50%) and overestimation of FFA content by the modified method at the higher concentrations (greater than 50% FFA content). The amount of underestimation increased for samples that contained 0.01 to 30.0% FFA, then decreased for samples with 30.0 to 50.0% FFA. The modified method, however, overestimated FFA content in samples with 50.0 to 100.0% FFA.

Crude soybean oil. FFA in crude soybean oil samples also was determined by both methods. Table 3 shows FFA contents in the four standard crude soybean oil solutions. The modified method underestimated FFA values at all concentrations examined. Magnitude of underestimation from the AOCS method results was larger (11.2%) at a lower range of FFA. Difference in FFA content measured by the modified method therefore became less when the FFA content in the soybean was greater. Unlike the results from the refined soybean oil, there was no interaction between concentration and method with crude soybean oil. There was no significant difference between the FFA mean values for the two methods ($P < 0.11$).

Generally, the modified method did better in estimating FFA content in crude soybean oil [coefficient of variation (CV) = 2.1%] than in refined oil (CV = 3.1%).

Linear regression analysis: refined soybean oil. Figure 1 shows a plot of the relationship between values obtained with the Official Method and those obtained with the modified method. Simple linear regression was conducted to correlate the two methods. Statistical analysis, based upon ANOVA ta-

TABLE 3
FFA Values Determined by AOCS and Modified Methods in Evaluating Refined, Bleached, and Deodorized (RBD) and Crude Soybean Oils[a]

	FFA concentrations (%)[b]					Overall mean	
Method	0.01–0.2	0.2–1.0	1.0–30.0	30.0–50.0	50.0–100.0	(%)	CV (%)
RBD oil							
AOCS	0.103	0.603	15.60	51.45	70.95	27.74	3.64
Modified	0.099	0.57	13.71	49.10	72.10	27.51	3.67
% Difference from AOCS[c]	−3.90	−5.00	−12.10	−4.60	1.60		
Crude soybean oil							
AOCS	na	0.89	15.53	50.90	71.20	27.76	2.05
Modified	na	0.79	14.20	49.80	70.90	27.28	2.09
% Difference from AOCS[c]	na	11.20	−8.60	−2.20	−0.40		

[a] Standard error of means: RBD soybean oil = 0.58; crude soybean oil = 0.33.
[b] Average from three replications.
[c] Negative values indicate underestimation, positive values indicate overestimation; na = not available. CV, coefficient of variation; for other abbreviation see Table 1.

TABLE 4
Relationship of FFA Content as a Function of Processing Steps[a]

	FFA from two different runs	
Processing step	I (%)	II (%)
Crude	0.61	0.53
Degummed	0.31	0.44
Refined	0.05	0.05
Deodorized	0.02	0.03
Hydrogenated and deodorized	0.025	0.03

[a]Data from Reference 8. For abbreviation see Table 1.

bles generated by the GLM procedure, showed the first-order regression model gave a good description of the relationship between results of the two methods over a wide range of FFA contents (0.01 to 75%). Approximately 99.5% of the variation in FFA values obtained from the AOCS Official Method was accounted for by the linear regression with the modified method ($R = 0.995$). The linear relationship can be described as:

$$\text{OM} = \beta_0 + \beta_1 * \text{MM; Model 1} \qquad [2]$$

where OM = Official Method, % FFA; MM = modified method, % FFA; $\beta_0 = 1.04$; and $\beta_1 = 0.97 \pm 0.04$.

The y intercept, β_0, of 1.04 is the mean FFA value when the measured FFA value MM equals zero. The value can be quite misleading because MM = 0 was not within the range of the experimental data, and the predicted value cannot be 1.04 when the measured value is zero. Estimates produced by linear Model 1 may be in error, especially when samples contain low amounts of FFA. For example, if the measured FFA content is 0.1, the predicted FFA is 1.11%. For the relationship of the modified method and Official Method to be as desired, the slope and the intercept of the regression line should be 1.0 and 0.0, respectively.

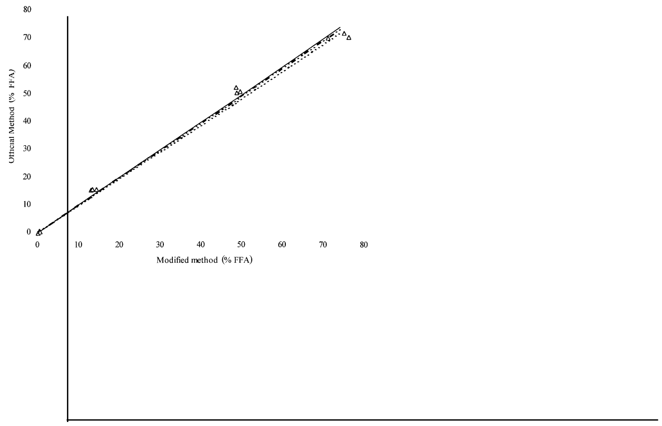

FIG. 1. Relationship between the AOCS Official Method and the modified method based on refined soybean oil. (△), Experimental data; (----), Model 1; (– -- – --), Model 2; and (——), line of equal values. FFA, free fatty acid.

Alternatively, a linear regression analysis "through the origin" was performed on the data. Under this procedure, the regression will set the y intercept at zero. The regression procedure would therefore meet, in part, the expectation of the results. The estimated slope given by Model 2 (0.99 ± 0.031) is close to the ideal value of 1 (Table 5). The R^2 value (0.997) is slightly greater than that obtained from Model 1 (Fig. 1). The full model is:

$$\text{OM} = \beta_1 * \text{MM; Model 2} \qquad [3]$$

where $\beta_1 = 0.99 \pm 0.031$.

Hypothesis testing was therefore made for the slope of the line (β_1) with a null hypothesis, H_o: $\beta_1 = 1$. The t-statistic is calculated from the expression:

$$t = \frac{\text{estimate} - \text{hypothesized value}}{\text{standard error of estimate}} \qquad [4]$$

The "estimate" equals the slope from the regression analysis, the "hypothesized value" equals 1, and "standard error of estimate" is obtained from SAS output. Table 5 summarizes results of the regression analysis. The calculated t of 0.7 infers an acceptance of the null hypothesis that the slope is 1.

Figure 1 shows the plot of Model 2 and the line of equal values drawn to indicate how far off the models are from an ideal relationship. Model 2 is superior to Model 1 in defining the relationship between the modified method and the Official Method.

Crude soybean oil. FFA values for crude soybean oil samples, as determined by the two methods, are shown in Figure 2. Again, the correlation between the two methods can be best described by a linear function.

By following the same argument for the intercept, a regression analysis was also conducted, setting the intercept = 0 as default. Statistics from the regression analysis are shown in Table 5. The model yields a slope value of 1.01 ± 0.013 with an R^2 value of 0.9996. The t-statistics of 1.7 (Table 5) infer acceptance of the null hypothesis that the slope value β_1 for the crude oil also is 1. The plot of the regression line for crude soybean oil FFA values ($R^2 = 0.9995$) indicates better correlation among data values than was observed for refined soybean oil FFA values ($R^2 = 0.997$). For crude soybean oil, correlation between performance of the modified method and that of the Official Method is described by the model:

$$\text{OM} = \beta 1 \ * \ \text{MM; Model 3} \qquad [5]$$

where $\beta_1 = 1.01 \pm 0.013$.

Figure 2 shows the best-fit linear model that relates results from the modified method with results from the Official Method for crude soybean oils. Linear Model 2 for refined soybean oil and an ideal model are superimposed on the plot for comparison.

Lanser's revised procedure vs. modified method from the study. Lanser *et al.* (3) published FFA percentages for crude soybean oil samples as established by the AOCS Official Method and a modified procedure. Regression analysis,

TABLE 5
Linear Regression Statistics Between Official Method and Modified Method for RBD and Crude Soybean Oils

Model	Slope β_1	Standard error of estimate, $S_{\beta 1}$	t for $H_0: \beta_1 = 1$[a]	Status of H_0	R^2
Refined soybean oil					
2[b]	0.99 ± 0.031	0.0143	0.7	Accept H_0 $\beta_1 = 1$	0.997
Crude soybean oil					
3[c]	1.01 ± 0.013	0.006	1.7	Accept H_0 $\beta_1 = 1$	0.9996

[a] Null hypothesis (H_0) is rejected if $t > t_{\text{table}}$ at $\alpha = 0.05$.
[b] Model 2: $t_{0.025,14} = 2.145$.
[c] Model 3: $t_{0.025,11} = 2.201$. For abbreviation see Table 3.

through the origin, of FFA values from the two methods was carried out to compare the performance of Lanser's procedure with that of our modified method. Lanser's data yielded a line with an R^2 of 0.999 and a slope of 1.09 ± 0.025, as compared to a slope of 1.01 from our Model 3. The regression line for Lanser's model is plotted in Figure 3, along with lines for Model 3 and the line of equal values. Deviations from the ideal line were 9 and 1% for Lanser's model and Model 3, respectively. Variation was not unexpected because, in any titration, resulting values are a function not only of FFA content but also of all other components that will react with alkali. Sample variation may have contributed to the difference. In addition, a titration end point usually is subjective and not distinct. Also, Lanser examined a small range of FFA values (0.03 to 4.98%). In our study, the greatest variation between the Official Method and the modified method occurred at the lowest range of FFA content.

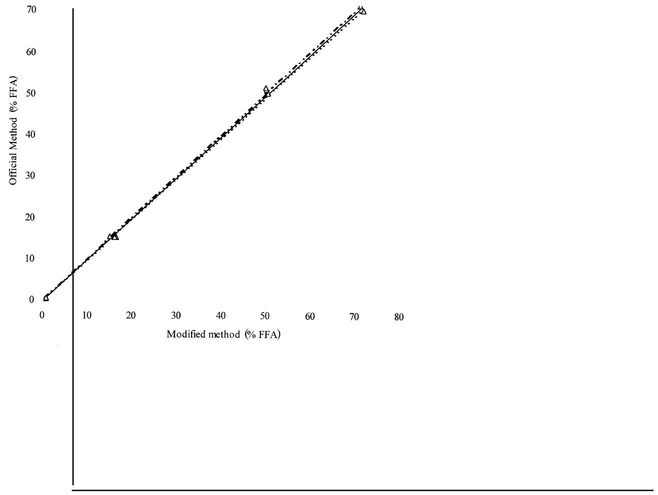

FIG. 2. Relationship between models for crude (Model 3) and refined (Model 2) soybean oils and line of equal values. (△), Experimental data; (-----), Model 2; (– -- – --), Model 3; and (——), line of equal values. For abbreviation see Figure 1.

Findings of the current study indicate that the modified method for determining FFA in refined and crude soybean oils is reliable and can be used as an alternative to the AOCS Official Method. This method is specific for FFA determinations in soybean oil and may be modified for other types of oils. The method may be applicable to industries and laboratories that currently use the AOCS Official Method.

Results obtained with the modified method agree closely with those obtained by the AOCS Method. The model that best describes the relationship between results for the modified and official methods for refined and crude soybean oil is a regression line that passes through the origin with slopes equal to 0.99 ± 0.031 (refined soybean oil) and 1.01 ± 0.013 (crude soybean oil), respectively. Acceptance of the null hypothesis for the slopes indicates that FFA values, determined by the modified method, do not require correction of the data to the Official Method for either RBD or crude soybean oils. The modified method estimates FFA content better in crude soybean oil than in refined soybean oil. Use of the modified method reduces the constraint associated with preparation of

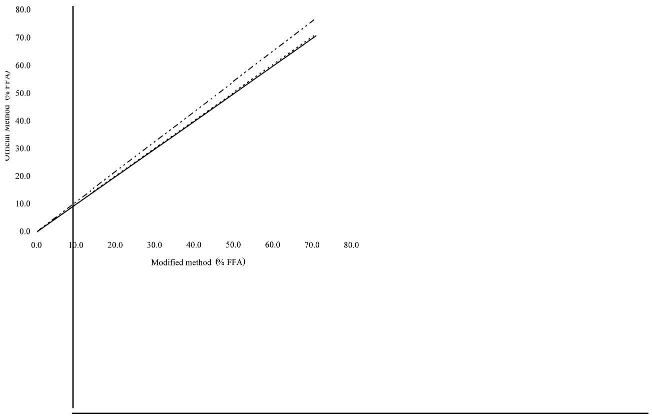

FIG. 3. Relationship between model 3 and Lanser's model for crude soybean oil. (-----), Model 3; (– -- – --), Lanser's model; and (——), line of equal values. For abbreviations see Figure 1.

larger oil samples and greatly lowers consumption of organic solvent and other reagents used in the procedure.

ACKNOWLEDGMENTS

This work was supported by the Iowa Soybean Promotion Board and the Iowa Agricultural and Home Economics Experiment Station. Journal Paper No. J-17445 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; Project No. 3163.

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[Received July 10, 1997; accepted December 9, 1997]