Factors Affecting the Rapid Equilibrium Method for Analysis of Total Oil in Soybean Cultivars

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A one minute rapid equilibrium extraction for total oil in soybeans was developed and factors influencing the extraction were examined. Comparisons of the rapid equilibrium extraction with a Goldfisch extraction were made, using 100 mesh flour on ten cultivars over a three year period. The equilibrium extraction consists of slurrying 100 mesh flour in hexane until an equilibrium between oil content inside and outside of the soybean particle is achieved. One minute was required for equilibration of 98% of the oil in the soybean flour, and one hour was required for complete equilibration when compared with a five hour Goldfisch extraction. Accuracy and precision of the method were excellent when proper technique was followed. A comparison of the rapid equilibrium and the Goldfisch extractions showed similar changes in oil content due to changes in soybean flour moisture and in preheating the soybeans. The amounts of phospholipid extracted from fine flour were considerably less than usually extracted from flakes under commercial conditions. The correlation between the rapid equilibrium and Goldfisch extractions for ten cultivars was excellent (r=0.9956) when analyzing 100 mesh flour. Environmental conditions caused significant interactions between cultivars and years of production.

Soybeans contain approximately 20% oil and 40% protein which vary inversely (1). However, cultivars high in oil content and factors that influence oil content are not well defined. Since proposals have been made to buy and sell soybeans based on oil and protein content, information about cultivars and rapid methods of protein and oil determination have become important.

Sheu (2) investigated a new rapid equilibrium method for measuring total soybean oil in seeds. In this method, ground soybean tissue is slurried in hexane for 20 minutes. After 20 minutes the slurry is thought to have reached an equilibrium with equal concentrations of miscella both inside and outside the flour particles. The oil percentage is determined by taking a sample from the miscella, evaporating the hexane from the sample and then calculating the amount of oil from the weight of the sample. One percent less oil is extracted by the rapid equilibrium method compared to the official AOCS method of extraction. Further development indicated that the extraction was complete in one minute, and particles smaller than 150 μm should be extracted by either method for improved consistency (3).

Data is presented in this paper on the refinement of the rapid equilibrium method with comparisons to a Goldfisch extraction. Factors influencing the rapid equilibrium method were evaluated, and the differences between the rapid equilibrium extraction and the Goldfisch extraction were investigated. Ten soybean culivars were evaluated for oil content over three growing seasons.

MATERIALS AND METHODS

The soybeans, if not specified, were of the Forrest culivar. The soybean cultivars studied included Jeff, Epps, Leflore, Centennial, Narow, Tracy-m, Braxton, Forrest and Bragg. The soybean cultivars were grown under irrigation and in different locations for each of the three years.

Soybeans were prepared for extraction by heating them in a 70C oven for one hour. The soybeans were then cooled, dehulled in a blender, and the hulls were removed by aspiration. The soybeans were ground in a UDY Cyclone Sample Mill without a screen. Full fat flour was sieved with an Alpine Air-jet sieve fitted with a 100 mesh sieve. Appoximately 60-70% of the flour is 100 mesh using this procedure.

Rapid Equilibrium Extraction. A 1-2 g sample of 100 mesh (<150 μm) flour was extracted for one minute in a glass stoppered erlenmeyer flask with 20 ml of HPLC grade hexane. The flour-hexane slurry was stirred throughout the extraction, and then the flour was allowed to settle for five minutes. The miscella was poured into a 20 ml syringe and pushed through a 0.8 μm Millex-PF membrane filter into another erlenmeyer flask which was immediately stoppered. Aliquots of 5 ml were pipetted from this miscella into tared aluminum pans, and hexane was evaporated in an 80C oven for 20 minutes. The weight increase in the aluminum pan was used to calculate the percentage of oil extracted.

Due to the increase in volume that the oil contributed to the system, a correction in volume was made in the calculations. The increase in volume due to the oil was estimated by calculating the total weight of the oil and dividing by the density of soybean oil (density=0.9 g/ml).

Goldfisch Extraction. The AOCS official method Ac 3-44 (4) was used with the Goldfisch extractor, and the following adjustments were made: soybeans were preheated at 70C rather than at 130C, soybeans were dehulled, and flour that passed through a 100 mesh sieve was analyzed instead of a composite of particle sizes. Extractions were done in triplicate.

Evaporation Studies. Known amounts of oil were taken through the rapid equilibrium process and the percentage of oil was calculated as if one g of flour had been extracted. The contents of extraction vessels of various sizes and with varying amounts of solvent were checked for evaporation and compared.

Effect of Temperature on Pipetting Accuracy. Hexane and miscella were adjusted to various temperatures before pipetting during the extraction process. Known amounts of oil were used in the extraction process as in the evaporation studies.

Effect of Preheating Soybeans and Extraction Temperatures. The extractablities of oil and phospholipid from soybeans preheated at 70 and 130C were investigated. The soybeans were ground smaller than 150 μm and extracted using both the Goldfisch and rapid equilibrium methods. Two solvents were compared: petroleum ether, a low boiling point solvent (30-60C); and ligroine, a high boiling point solvent (90-110C).

The effect on the extractability of oil of preheating soybeans at 130C from unsieved flour was investigated. Soybeans were heated at 130C for one hour and then ground and extracted by the Goldfisch method. For comparison, soybeans were heated at 70C for one hour, ground completely to <150 μm, and extracted by the Goldfisch method.

Equilibration Time Study. 100 mesh flour was extracted by the rapid equilibrium extraction at time periods of 1, 15, 30, and 60 minutes to determine the length of time needed for equilibration.

Moisture Analysis. Moisture of the ground flour was determined according to the AOCS method Ac 3-44 d 5 (4).

Phospholipid Analysis. A known amount of extracted oil was wet-ashed and phosphorus-determined using the Bartlett (5) method of generating phosphomolybdate. Absorbance was measured at 750 nm on a double-beam spectrophotometer.

Effect of Flour Moisture. 100 mesh flour was adjusted to different moisture levels by incubating it in a closed desiccator over water for various times.

RESULTS AND DISCUSSION

In the rapid equilibrium method, a precise volume of hexane is slurried with the full fat soy flour. Any change in the volume of hexane during the extraction would lead to errors in the analysis. Therefore, evaporation of hexane was studied as a factor in the accuracy and precision of the method.

Evaporation with test tubes was significantly greater than with glass stoppered erlenmeyer flasks for 20 or 50 ml of hexane (Table 1). Overestimation of oil in the test tubes was 0.28% while in the erlenmeyer flasks, with 20 or 50 ml of solvent, over-estimation was reduced to 0.06%. The amount of headspace in the test tubes or flasks influenced the amount of evaporation only when using a 50 ml erlenmeyer flask and 10 ml of hexane. Evaporation when using the test tubes appeared to be related to the use of rubber or neoprene stoppers. During agitation of the slurry the stoppers became wet, which caused them to loosen. Hexane is also slightly soluble in these stoppers and this could reduce the total volume of miscella, resulting in an overestimation of oil. It was noted, when pipetting hexane into the extraction flask, that less evaporation occurred if one pipetted just above the surface rather than down the side of the flask. Also, taking sample volumes of 5 ml after filtering gave greater accuracy than 1 or 2 ml samples (data not shown).

When the temperature was kept constant throughout the extraction no significant difference from the true oil amount was found, regardless of the temperature. If hexane was pipetted at 29C and the miscella was pipetted at 24C the percentage of oil was overestimated. However, if the hexane was pipetted at 20C and the miscella at 24C the percentage of oil was underestimated (Table 2). Keeping the hexane at 24C and altering the temperature of the miscella gave opposite results. With the miscella at 29C the oil wasunderestimated, and at 20C the oil was overestimated (Table 2). This indicated that the second pipetting error compensated for the error made in the first pipetting. The net result was that if the temperature was kept constant throughout the process, the oil would not be influenced by small differences in extracting temperatures.

When the Goldfisch method (30-60C petroleum ether as solvent) was compared with the rapid equilibrium method (1 min.), approximately 1% more oil was extracted by the Goldfisch method (Table 3) at 130C and 70C preheating temperatures. One likely cause for the difference was the phospholipid content of the oil. However, phospholipid analysis showed that there was no statistically significant difference in phospholipid extraction by the Goldfisch or rapid equilibrium method for both preheating temperatures (Table 3).

A high preheat temperature and a high extraction temperature caused more oil and phospholipid to be extracted. The amount of oil extracted by petroleum ether (b.p. 90-110C), was unaffected by the preheat temperature. This solvent extracted more phospholipid with the 70C and 130C preheat temperatures than with either the rapid equilibrium method or the Goldfisch with low boiling point solvent. The flour moistures of the 130C and 70C preheated beans were 5.42% and 6.89%, respectively. Phospholipid extraction may be related to the moisture level of the flour, which will be discussed later.

Preheating at 130C has been in the recommended official methods of AOCS for most of its history. Many believe

TABLE 1

Effect of size and type of container and volume of solvent on overestimation of oil due to evaporation.

Container	Solvent volume 10 ml	20 ml	50 ml
	Percent overestimation of oil based on 1 g of flour.[a]		
30 ml test tube	0.28a		
15 ml test tube	0.28a		
25 ml erlenmeyer		0.04b	
50 ml erlenmeyer	0.19a		0.05b
125 ml erlenmeyer			0.06b

[a]Means within and between columns with the same letter are not significantly different at the 5% level (LSD=0.10).

TABLE 2

Effect of altering temperatures of miscella or hexane during the rapid extraction on oil recovery.

Temperature C	Miscella[a]	Hexane[b]
	Difference in percentage of oil based on 1 g of flour[c]	
29	-0.09c	+0.12a
24	0.00b	+0.01b
20	+0.09a	-0.15c

[a]Hexane was pipetted at 24C. Means with the same letter are not significantly different at the 5% level (LSD=0.07).
[b]Miscella was pipetted at 24C. Means with the same letter are not significantly differet at the 5% level (LSD=0.05).
[c]Known amounts of oil were extracted and recovered.

TABLE 3

Effect of extraction temperature and preheat temperature on oil and phospholipid extracted from dehulled 100 mesh soybean flour.

Extraction method Temperature	Preheating Temperature 130C	70C	130C	70C
	% Oil[a] (dry flour basis)		% Phospholipid[b] (dry flour basis)	
Goldfisch-Pet Ether 30-60C	25.39b	25.10c	.25c	.05d
Goldfisch-Ligroine 90-110C	25.74a	25.60a	.70b	.84a
Rapid Equilibrium 24C (1 min)	24.58d	24.09e	.19c	.04d

[a]Means within and between columns with the same letter are not significantly different at the 10% level (LSD=0.19).
[b]Means within and between columns with the same letter are not significantly different at the 5% level (LSD=0.08).

TABLE 4

Effect on the extractability of oil of preheating soybeans at 130C from soybean flour.

Preheat Temperature & Particle Size	Percent Oil[a] (dry flour basis)	Percent Phospholipid[b] (dry flour basis)
130C unsieved	21.63b	0.30a
70C[d] <150 μm	21.95a	0.02b

[a]Means with the same letter are not significantly different at the 5% level (LSD=0.16).
[b]Means with the same letter are not significantly different at the 5% level (LSD=0.04).
[c]Soybeans were not dehulled or sieved.
[d]Soybeans were not dehulled but ground completely to <150 μm particle size.

that the oil is more readily extracted with this high preheating. If this were true, the sieving to <150 μm particle size might be unnessary. Results in Table 4 show that preheating at 130C did not cause more oil to be extracted compared to <150 μm particles and did not replace the necessity of sieving. Significantly more neutral oil (0.61%) and significantly less phospholipid (0.28%) were extracted from <150 μm particles than from unsieved flours that had undergone preheating at 130C. This further substantiates the reason to extract particles ground smaller than 150 μm as indicated by Snyder *et al.* (3).

Neutral oil is mainly triglyceride which processors want to extract. Crude soybean oil commercially extracted from flakes typically contains 96% triglyceride and 2-3% phospholipids(6). In contrast, the oil extracted from the fine particles by the rapid equilibrium or Goldfisch methods contains 0.10-0.17% phospholipids (oil basis) when the soybeans are preheated at 70C. Phospholipids are removed in refining crude soybean oil, and cause a loss in neutral oil during degumming and alkali refining. It seems reasonable then that an extraction process which extracts small amounts of phospholipids would be desirable. Although one third of the total phospholipid extracted is used to produce lecithin, most lecithin is incorporated into animal feeds or sold to soap manufacturers (6).

TABLE 5

Effect of extraction time on the estimation of oil content by the rapid equilibrium method.

Time (min)	Percent oil[a,b]
1	24.40c
15	24.65b
30	24.72b
60	24.90a
5 hr Goldfisch	24.97a

[a]Dry flour basis.
[b]Means with the same letter are not significantly different at the 5% level (LSD=0.10).

The amount of oil extracted from <150 μm flour particles increased slowly but significantly over a one hour period (Table 5). A five hour Goldfisch extraction was not statistically different from the 60 minute rapid extraction in total oil yield. A phosphorus analysis of the oil extracted by the rapid equilibrium and Goldfisch extractions showed no increase in phosphorus content over time or difference between methods. Thus the rapid method was not in complete equilibrium in one minute although 98% of the oil was extracted.

This hard-to-extract oil (the oil unextracted in one minute) may be plasma membrane lipids. The plasma membrane of plant cells contains glycolipids, phospholipids, sterols, and neutral lipids (7). Half of the plasma membrane lipid content is a combination of sterols and neutral lipids, which may be slowly extracted over one hour.

We observe that the humidity of the lab altered the moisture of soybean flour and so the extractability of flour at various moisture levels was investigated. Results indicated that the percentage of oil extracted increased with increases in moisture (Table 6). Goldfisch extractions exhibited a significant increase of 0.67% oil from 4.35% to 8.85% moisture and no increase at the highest moisture level. This corresponds to the findings of Milner

TABLE 6

Effect of flour moisture on the amount of oil and phospholipid (dry flour basis) extracted.

% Moisture	% Oil		% Phospholipid[b]	
	Goldfisch	Rapid[c]	Goldfisch	Rapid[c]
4.35	24.86bc	23.75d	.02d	.02d
8.85	25.53a	24.66c	.21b	.13c
11.30	25.49a	24.96b	.24a	.24a

[a]Means within and between columns with the same letter are not significantly different at the 5% level (LSD=0.21).
[b]Means within and between columns with the same letter are not significantly different at the 5% level (LSD=0.02).
[c]One min extraction.

el al. (8) and Bull (9). The rapid equilibrium method showed significant increases in oil extracted with increases in moisture content, with a large increase (0.91%) from 4.35% to 8.85% moisture and a smaller increase (0.30%) from 8.85% to 11.30% moisture. The significant moisture x method interaction (5% level) indicated that moisture had a greater influence on the percentage of oil extracted by the rapid equilibrium method than by the Goldfisch method at the highest moisture level.

The increase in percentage of oil due to moisture was partly phospholipid. As with oil content, the phospholipid content increased significantly with increases in moisture content when extracted by the rapid method, and increased from the low to intermediate moisture levels with the Goldfisch extraction. The difference in methods at 8.85% moisture caused the method x moisture interaction for phospholipids to be significant at the 5% level. The increase in phospholipids from 4.35% to 11.30% moisture accounted for 0.2% oil increases for both methods. The Goldfisch and rapid equilibrium methods had total oil increases of 0.5% and 1.0%, respectively, that were not due to phospholipids. Since the moisture was altered by placing flour over water in a desiccator for 3 to 5 days, the length of time held at these moisture levels may have influenced results.

A factorial analysis of the oil content measured by the rapid equilibrium and Goldfisch methods for ten soybean cultivars grown in 1986 and 1987 exhibited a year × cultivar × method interaction (Fig. 1). A separation of the least squares means by the least significant difference test indicated that 752 out of 780 means comparisons were significantly different at the 5% level. The differences between methods for each variety averaged 1.14% oil, with a standard deviation of 0.22% oil. The correlation coefficient between methods was 0.9956 for the years 1986 and 1987. This high correlation was better than correlations found by Hymowitz *et al.* (10) between NIR readings and Soxlet extractions.

Environmental influences caused yearly differences between the varieties for each method. Nine out of ten cultivars produced more oil in 1986 than in 1987, but one variety, Bedford, yielded approximately 0.5% more oil in 1987. Differences between the years for each cultivar varied widely from 0.42% to 5.03% oil by the rapid equilibrium method and 0.56% to 4.66% oil by the Goldfisch method.

A three year analysis of the ten cultivars by the Goldfisch method save a year x cultivar interaction. Fig. 2 illustrates the year-to-year variations found in oil content with each cultivar, and exhibits the inconsistent changes between cultivars. These inconsistencies were not surprising since the soybeans were grown in different locations each year, but the inconsistencies make cultivar selection based on oil content difficult. Because the rapid equilibrium method had been improved since 1985, it was not included in this analysis.

The one minute rapid equilibrium method was found to be a fast and reproducible estimator of soybean oil content. When the proper technique was followed, oil determinations were accurate. From analyses of ten soybean cultivars, excellent correlations were found between the rapid equilibrium method and the Goldfisch extraction, with the rapid equilibrium method extracting about one

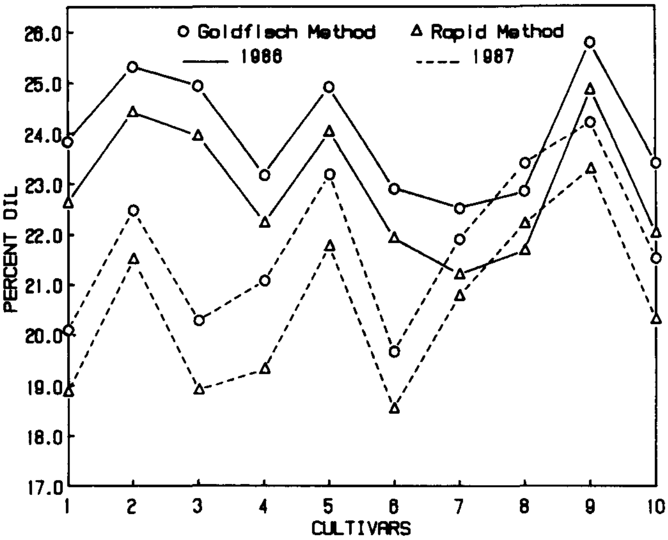

FIG. 1. Percentage of oil (dry basis) extracted from ten cultivars in 1986 and 1987 by the rapid equilibrium and Goldfisch methods. The key for cultivars is as follows: 1 Jeff, 2 Epps, 3 Leflore, 4 Centennial, 5 Narow, 6 Tracy-m, 7 Braxton, 8 Bedford, 9 Forrest and 10 Bragg.

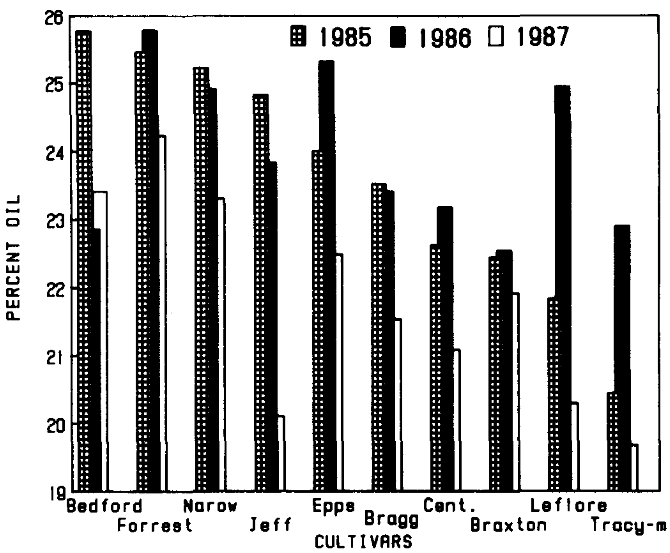

FIG. 2. Percentage of oil (dry basis) extracted by the Goldfisch method from ten cultivars over a three year period.

percent less oil. Important in both methods was the use of particles smaller than 150 μm.

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