Analysis of Total Oil in Soybeans: A New Equilibrium Extraction Method and the Effect of Particle Size

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A new method of total oil analysis is proposed in which the solvent was equilibrated with dissolved oil inside and outside of soybean particles rather than exhaustively removing all oil. By filtering, evaporating, weighing and multiplying by a factor (based on total miscella volume/sample volume) a satisfactory analysis could be done. Particle size was found to have a profound effect on amounts of oil in soybeans extracted by a conventional procedure. Sieving ground, dehulled soybeans into three particle sizes gave 15.3, 21.9 and 24.8% oil for >40 mesh, 40-100 mesh and <100 mesh, respectively, and 23.1% oil for the unsieved sample. Evidence is presented to support the idea that the amount of oil found in the smallest particle size was the true oil content of the soybeans analyzed. Using the equilibrium method to analyze the <100 mesh particles led to a rapid and economical analysis procedure. A comparison of the equilibrium and exhaustive extraction methods showed the exhaustive extraction gave a consistently larger oil content but less than 1% larger. The difference could be attributed to phospholipid.

We started with the objective of obtaining a representative sample of oil from soybeans that could be analyzed for minor components such as pigments, free fatty acids and phospholipids. To obtain the oil sample, hexane was mixed with ground soybeans, and the miscella was recovered by filtering. After experimenting with this method, we realized that if the volume of hexane were known and if an equilibrium were achieved between miscella concentrations inside and outside the soybean particles, then we had a quantitative method for measuring oil content.

This equilibrium method was tested by comparing it with a traditional exhaustive extraction (Goldfisch), and the equilibrium method was applied to several soybean varieties.

In a previous paper we had indicated that particle size had an effect on the amount of oil extracted (1). There is evidence in the literature of the effect of flake thickness on the rate of soybean oil extraction (2), and the influence of particle size has also been investigated (3), but we are unaware of any studies on the effect of particle size on the total oil analyses. In the 1950s it was realized that oil content extracted from soybeans varied considerably between laboratories, and the variation was in sample preparation rather than in the extraction procedure (4). The first issue of the precursor to the *Journal of the American Oil Chemists' Society* in 1924 cautioned that samples for oil extraction should be ground fine but not too fine (5).

In this paper we report the results of oil analysis using the equilibrium method of extraction, the effect of particle size on exhaustive oil extraction, and a comparison of the exhaustive and the equilibrium extraction methods.

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MATERIALS AND METHODS

Soybeans of specified cultivars were used. If cultivar is not specified, it was Forrest.

HPLC-grade hexane was used for the extractions, but reclaimed hexane, petroleum ether and ligroine were also used with no apparent differences in amounts of oil extracted.

Dehulling was done by breaking up soybeans in a blender and aspirating off the hulls.

Grinding to produce full fat flours was done with a Udy Cyclone mill with no screen at the outlet.

Sieving was done with an Alpine Air Jet sieve (40 and 100 mesh) in which air pressure from a revolving nozzle under the sieve combines with vacuum to give an efficient sieving action. It is impossible to simply shake full fat soybean flours through fine sieves, because the sieves plug immediately.

Two extraction procedures were used in this study, a new equilibrium method and a traditional exhaustive extraction.

Equilibrium method. A 10-g sample of ground soybean was stirred for 20 min with 100 ml of HPLC hexane. The mixture was allowed to settle and sufficient sample of the miscella was pulled through a filter (Millex-PF 0.8 μm) with a syringe to provide two 10-ml samples. The 10-ml samples were pipetted into tared aluminum dishes, the solvent was evaporated, and the remaining oil was weighed. In later studies different proportions of full fat soybean flour to solvent were used. Also, it was found to be a better procedure to pour the miscella after settling into a syringe and to force the miscella through the filter using the plunger of the syringe.

Exhaustive extraction. Exhaustive extraction of soybean oil was done by the Goldfisch apparatus for four hr using petroleum ether and one- or two-g samples (8).

Phosphorus analysis was by the method of Bartlett (6).

Moisture content was determined by drying at 100 C in a forced air oven for two hr.

RESULTS AND DISCUSSION

The equilibrium method was compared with a Goldfisch extraction of the same soybeans and found to give about 1% less oil. The equilibrium method gave 19.33% oil with a standard deviation of 0.32 for nine replications, and the Goldfisch method gave 20.48% oil for 12 replications with a standard deviation of 0.46. These results supported the idea that a rapid quantitative analysis could be achieved. Table 1 shows data obtained by the equilibrium method for 13 cultivars of soybeans.

TABLE 1.

Oil and Moisture Contents of 13 Cultivars of Soybeans[a]

Cultivar	% Oil (dry basis)	% Moisture
Mack	21.71a[b]	5.84
Jeff	21.31b	6.63
Narow	20.84c	6.40
Bedford	20.81c	5.71
Bragg	20.57d	5.80
Epps	20.55d	6.52
Forrest	20.47d	5.92
Davis	19.73e	7.26
Lee 74	19.57e	6.92
LeFlore	19.27f	6.35
Centennial	19.20f	7.42
Braxton	19.12f	6.66
Tracy-m	18.09g	5.47

[a]Oil by the equilibrium method.
[b]The same letter indicates no significant difference at the 5% level.

At the same time we were working with the equilibrium method, we became aware that particle size of the full fat flour could influence quantitative results (1). Consequently, we began to gather data on the effect of particle size on the quantity of oil extracted. Although experiments were done by both a traditional exhaustive extraction procedure (Goldfisch) and the equilibrium procedure, here we report only data obtained by the Goldfisch extraction.

Table 2 shows the oil content measured for Forrest soybeans. A sample was analyzed as ground (composite) and then for each of three fractions obtained by sieving (>40 mesh, 40-100 mesh, and <100 mesh). The composite sample had 20.99% oil, and the three sieve sizes ranged from 9.64% to 23.61% as particle size decreased. To try to better understand such a wide variation in oil content due to particle size, we looked for several possible explanations.

It was conceivable that moisture content was less for the smaller particles and that this was responsible for increased oil with decreasing particle size. As can be seen from Table 2, moisture content did decrease as particle size decreased. The loss of moisture in the small particles must have occurred during sieving because the composite sample had the highest moisture content. The amount of moisture difference could not account for the oil differences, as can be seen for samples calculated on a dry basis.

TABLE 2.

Influence of Particle Size on Oil, Moisture and Phosphorus Contents of Ground and Sieved Soybeans

Particle size	% Oil (as is)	% Moisture	% Oil (dry basis)	Phosphorus (ppm)
Composite	19.75 (0.23)[a]	6.27	20.99	250
>40 mesh	9.09 (0.64)	6.03	9.64	256
40-100 mesh	16.36 (0.03)	5.63	17.28	174
<100 mesh	22.49 (0.60)	4.97	23.61	261

[a]Figures in parentheses are standard deviations.

Another possible reason for the increased oil obtained from small particles was that more phospholipid was being extracted from the smaller particles. A phosphorus analysis showed that phospholipid could not account for the difference in amounts of oil extracted from the different particle sizes (Table 2). The amount of phosphorus found indicated a phospholipid content of about 0.75%. This is less phospholipid than is generally extracted from soybeans. Two possible reasons for the decreased amount of phospholipid are that pure solvent rather than miscella is the extracting solvent in the Goldfisch procedure and that particles rather than flakes are being extracted.

Another factor that we had not accounted for was the presence of hulls low in oil content. Since hulls had not been removed from these soybeans, it was conceivable that the hulls had concentrated in the larger particle sizes and were causing the differences in oil content. An analysis of ground and sieved dehulled soybeans is shown in Table 3. Because the oil content was considerably greater for dehulled soybeans than for soybeans with hulls (in the two larger particle sizes), we concluded that hulls were concentrated in the two larger particle sizes. However, for dehulled soybeans, particle size still was a variable that caused different amounts of oil to be extracted.

Because hulls appeared to concentrate in the larger particle sizes, we thought it necessary to see if other components might be segregated by grinding and sieving to cause an oil-rich fraction to concentrate in the smallest particles. To investigate this possibility the 40-100 mesh fraction (dehulled soybeans) was reground and sieved to give two fractions, 40-100 mesh and <100 mesh. If segregation was causing the 40-100 mesh fraction to have less oil, then regrinding should still give the same oil content regardless of particle size. Data in Table 3 show that regrinding caused the new small particles to have about 2.6% more oil (21.70 vs 24.36%).

Our interpretation of these results is that intact soybean tissue is difficult to penetrate by hexane as Othmer and Agarwal (7) proposed, and that the oil amount of the small particles was the true amount of oil in soybeans. Furthermore, we suggest that studies on ground soybeans that have particle sizes larger than 100 mesh are likely to give incorrect results, particularly if extraction times are of the order of four to six hr. With longer extraction times it may be possible to come closer to a complete extraction.

Evidence in the literature indicated that difficulty with oil extraction existed because of sample preparation rather than the extraction technique. Collins in 1953 (4) commented on the high variability in oil content of soybeans due to sample preparation. He investigated the Bauer mill (operating it hot or cold) and found approximately 0.5% more oil when the mill was operated hot (with close plate settings and high feed rates) than when it was operated cold. The main variable here may have been particle size distribution obtained by the two operating conditions. Also, comments were made over 60 years ago that soybeans

TABLE 3.

Effect of Hulls and Regrinding on Percent Oil Content (dry basis) of Ground and Sieved Soybeans

Particle size	Soybeans	Dehulled soybeans	Reground 40-100 mesh from dehulled soybeans
Composite	20.99	23.07 (0.05)*	
>40 mesh	9.64	15.31 (1.11)	
40-100 mesh	17.28	21.88 (0.29)	21.70 (0.53)[a]
<100 mesh	23.61	24.81 (0.17)	24.36 (0.13)

[a]Figures in parentheses are standard deviations.

should be ground finely for oil extraction but not too finely (5). Fine particles have a tendency to pack, and the solvent tends to channel so that a less than complete extraction might result. This leads us back to the advantages of the equilibrium method.

We investigated the time needed for maximum extraction and found extraction was almost instantaneous with the <100 mesh flour. Using one g of full fat flour (<100 mesh) and five ml of hexane in a test tube, one can do a rapid extraction by shaking the mixture thoroughly for one min, allowing particles to settle, and filtering. Taking one-ml samples, evaporating the hexane and multiplying the weight of oil by five gave 22.57% oil (standard deviation of 0.11 for 10 replications). In contemplating this analysis, we realized that when the oil is fairly concentrated as in this extraction, the oil contributes to the total volume in the system. Therefore, instead of multiplying by a factor of five to get the final oil content, one should multiply by 5.26 (5.26 ml would be the hexane plus oil volume if 0.23 g of oil is present). Using 5.26 as a factor rather than five, the oil content by this rapid equilibrium method was 23.74%.

Hence, the rapid equilibrium method does not suffer from the defects of channeling or packing, no special equipment is needed, and analyses can be completed in ca. 15 min. Our results from the equilibrium method were encouraging for simplifying analyses compared to exhaustive oil extraction, and our results from particle size investigations showed promise for improving the accuracy of total oil analyses.

To compare the new equilibrium extraction directly with the exhaustive extraction, the cultivars of Table 1 were analyzed for total oil content. A flour of <100 mesh was analyzed rather than a composite flour, and the flour was from dehulled soybeans. The results are shown in Table 4. The equilibrium extraction method (1 min equilibrium time, 5 ml of solvent and 1 g of sample) gave consistently less oil than the exhaustive extraction, but the difference was usually less than 1%. The exhaustive extraction removed about 0.6-0.7% phospholipid from ground soybeans, while the equilibrium method removed less than 0.1% (data not shown). Hence the phospholipid difference could account for most of the difference in oil content shown by the two methods.

We have presented evidence which forces thinking about how the current analytical procedure should be changed to take into account the effects of particle size. It seems there are two options, either select a grinding method that would insure a sufficiently small particle size, or grind by any method but sieve the sample to obtain the appropriate particle size. The presence of hulls can be a problem because, depending on the grinding procedure, they may concentrate in different size fractions. Probably the best recommendation with respect to hulls is to remove them first.

All of our reported results have been with soybeans, but we have done enough work with cottonseed to think that the same considerations about particle size apply to cottonseed.

TABLE 4.

Oil Content of 13 Cultivars of Soybeans Using Dehulled Beans on a Dry Weight Basis[a]

Cultivar	Goldfisch	One min Equilibrium
Mack	24.89 (0.09)*	24.43 (0.09)[b]
Jeff	24.83 (0.09)	24.29 (0.01)
Narow	25.23 (0.22)	23.56 (0.08)
Bedford	25.45 (0.59)	23.91 (0.19)
Bragg	23.51 (0.06)	22.93 (0.01)
Epps	24.01 (0.15)	23.39 (0.13)
Forrest	25.47 (0.08)	23.97 (0.07)
Davis	23.87 (0.57)	23.20 (0.22)
Lee 74	22.83 (0.11)	22.60 (0.22)
LeFlore	21.83 (0.26)	20.87 (0.10)
Centennial	22.62 (0.07)	22.40 (0.07)
Braxton	22.44 (0.12)	21.05 (0.16)
Tracy-m	20.44 (0.02)	20.33 (0.16)

[a]All samples were ground and sieved to <100 mesh.
[b]Figures in parentheses are standard deviations.

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