TABLE V.

F-Values for Physical and Chemical Analyses. Data From Four Varieties of Sunflower Seeds Grown at Seven Stations in 1943

	Bu. test weight	Weight per 1000 kernels	Proportion hulled seed	
Whole seed (air-dry basis):				
Varieties	9.84[1]	108.82[1]		9.60[1]
Stations	2.89[2]	6.24[1]		8.99[1]
Hulls (moisture-free basis):	Ash	Nitrogen	Oil	
Varieties	1.02	7.25[1]	1.85	
Stations	6.06[1]	10.26[1]	1.85	
Hulled seed (moisture-free basis):	Ash	Nitrogen	Sugar	Oil
Varieties	.80	2.13	3.70[2]	5.81[1]
Stations	6.35[1]	7.01[1]	3.02[2]	5.78[1]
Oil from hulled seed:	Iodine No.	Oleic acid	Linoleic acid	
Varieties	13.00[1]	11.93[1]	12.62[1]	
Stations	5.27[1]	7.16[1]	6.42[1]	

[1] Significant, 1% point.
[2] Significant, 5% point.

four varieties grown at seven locations, contained an average of 29% oil which was composed chiefly (51 to 68%) of linoleic acid glycerides. The eight varieties of safflower seeds grown at Huntley, Montana, contained an average of 33% oil, with an average content of 78% linoleic glycerides. Data on the amounts of ash, nitrogen, sugar, as well as oil, are presented for the whole seeds and their fractions.

Acknowledgments

The preparation of the samples and the extensive analyses summarized in this paper required a large amount of painstaking work. The authors are indebted particularly to J. J. Curtis, Carrie L. Brown, and J. C. Warner, of the Commodity Development Division, and to A. J. Ernst, R. W. Von Korff, Dorothy R. Frazer, T. A. Scott, and F. R. Earle, of the Analytical and Physical Chemical Division, Northern Regional Research Laboratory.

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Some Factors Which Affect the Determination of Oil in Soybeans[1]

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ACCURATE determination of the percentage of oil in soybean seed is of great importance to the plant breeder in the development of high oil producing strains. It is equally important that cooperating laboratories be able to secure comparable analyses. Commercial processors of soybeans are also vitally interested in improving the accuracy and reproducibility of their analyses. The present official A.O.C.S. method (1, 2) is still subject to considerable variation as shown by the fact that in the analysis of seven samples by 79 collaborating laboratories the standard deviation was about 0.34% oil. Some of the variations in results may be caused by differences in fineness of initial grinding, moisture content of the meal, relative humidity, and different techniques at regrind time. It was the purpose of this investigation to study the effect of these factors on the determination of oil in soybean seed.

Materials and Methods

The soybeans studied comprise lots of four varieties (Chief, Dunfield, Lincoln, and Illini) each of which was carefully cleaned and divided with a Boerner sampler into portions of several hundred grams each. Some of these samples were predried, using the official A.O.C.S. method (1), and immediately placed in one quart metal containers with tightly fitting lids. Other portions were predried under vacuum over Drierite for more than a month. These soybeans were ground with the following mills: Wiley mill, with 2 mm, 1 mm, and 35 mesh screens, Mikro-Pulverizer, Bauer, and Arcade Mills. The samples were ground in laboratories using these mills in their regular work and by operators familiar with their best operation. All the samples were kept as dry as possible during the initial grinding and subsequent analysis. Two ranges of relative humidity (determined with Friez Hygro-Thermograph) were used, the lower range being 30-34% and the higher range 60-65%. Condensers were capped with lead foil during the oil extraction to prevent possible condensation of atmospheric moisture inside of the condensers. Moistures were determined by the official method (1) except that 2 grams of the sample were used instead of 5 grams.

Moistures were determined in partially extracted samples by means of a modified Karl Fischer reagent as prepared by Krober and Collins (3). Samples for this moisture determination were placed in 200 ml centrifuge bottles, and 50 or 100 ml of methanol added. The bottles were tightly stoppered and allowed to stand overnight. These samples were centrifuged 30 minutes, and a suitable aliquot of the clear solution was taken for analysis. The aliquot was treated with excess modified Karl Fischer reagent and then back titrated with standard water methanol solution using an electrometric titrimeter.

Iodine numbers were determined by the official A.O.C.S. Wijs method and were also calculated from the refractive index of the oil according to the equation developed by Majors and Milner (4) from 1938 Butt extracted soybean oil.

[1] A publication by the U. S. Regional Soybean Laboratory, a cooperative organization participated in by the Division of Forage Crops & Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. D. A., and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin.

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Comparison of Grinding Mills and Effect of Regrinding

It is the standard procedure of this laboratory to grind soybean samples for analysis with an intermediate model Wiley mill using a 1 mm screen and to use 200 strokes for regrinding instead of the 100 strokes specified in the official A.O.C.S. method (1). Previous work in this laboratory has shown conclusively that a regrind of 100 strokes is insufficient to get closely checking results on soybeans ground through a 1 mm screen. Results of studies on the effect of regrind technique and of fineness of initial grind are shown in Table I.

TABLE 1

Effect of Methods of Sample Preparation and Regrind Treatment on the Determination of Oil in Soybeans. Percentage of Oil Calculated on 14% Moisture Basis

(A) Comparison of Grinding Mills and Effect of Regrinding

Mill	Treatment	Strokes of regrind		5 hours continuous extraction not reground
		200	100	
		% oil	% oil	% oil
Wiley (2 mm)	Predried	18.99	18.82	15.46*
Wiley (1 mm)	Predried	18.90	18.78	16.61*
Wiley (35 mesh)	Predried	19.02	18.92	17.50*
Mikro-Pulverizer	Predried	19.08	19.04	18.87
Bauer	Predried	19.06	19.02	18.88
Arcade	Predried	19.12	19.11	19.10
Average		19.03	18.95	17.74

(B) Comparison of Predried, Vacuum Predried, and not Predried

Mill	Treatment	Moist. %	Strokes of regrind		5 hours continuous extraction not reground
			200	100	
			% oil	% oil	% oil
Wiley (1 mm)	Oven Dried	2.99	18.90	18.78	16.61*
Wiley (1 mm)	Vac. Dried	4.66	18.78	18.60	15.67*
Wiley (1 mm)	Not Dried	7.25	18.96	18.76	16.34*
Bauer	Oven Dried	2.72	19.06	19.02	18.88
Bauer	Vac. Dried	3.50	19.00	18.97	18.89*

*Based on four analyses. Remainder of data based on 16 analyses.

Most of the data shown in the table represent the average of 16 oil determinations since duplicate analyses of each of the four varieties were made on different days. The data in Table 1 were obtained from samples exposed for 75 minutes at regrind time to air of 40-50% relative humidity. During this period the samples were left wrapped except for the two minutes' time required for regrinding. The moisture content of the oven predried samples varied from 1.2 to 3.5% at the time the samples were weighed for oil analyses.

In Table 1 the average difference of about 0.13% oil which may be attributed to initial grinding of the 2 mm, 1 mm. and 35 mesh Wiley mill compared with the Mikro-Pulverizer, Bauer, and Arcade mills is probably significant as is the average difference of about 0.15% oil found between 100 and 200 strokes of regrinding of the 2 mm and 1 mm grinds.

The percentage of oil remaining in the samples after two hours of extraction with petroleum ether varies with the fineness of initial grind. For example, the Dunfield variety when ground in different mills contained at the time of regrinding these approximate percentages of oil: *Wiley mill* 2 mm screen 6.9%, 1 mm screen 4.3%, 35 mesh 2.4%, 1 mm screen (beans not predried) 3.7%; *Mikro-Pulverizer mill* 0.5%; *Bauer mill* 0.3%; *Arcade mill* 0.1%. When samples containing such greatly different amounts of oil were reground with the same mortar and pestle, one coarse grind immediately following a fine grind, the film of oil left on the mortar and pestle caused errors of 0.1 to 0.3% of oil in these analyses. This residual oil was washed from the regrinding apparatus and weighed to determine these losses. The very fine initial grinds gave abnormally high oil percentages when reground alternately with coarser initial grinds and the results for the coarser initial grinds were correspondingly low. However, when all of the samples which were reground were of the same initial grind, no errors of this magnitude were observed.

The use of heat to predry whole soybeans before grinding probably makes possible a finer grind than vacuum predrying. Response of the resulting meal to changes in relative humidity was probably different. Oven predried samples gave slightly higher average percentages of oil probably due to fineness of grinding, as predrying the soybeans seems to be essential to very fine initial grinding. Vacuum drying is less convenient and seems to have no particular advantage over oven drying.

Since moisture changes may occur during the regrind period, modifying or omitting the regrind may have caused a part of the differences in results in Table 1. The greater percentages of oil found when using a regrind, compared with 5 hours of continuous extraction of the Mikro-Pulverizer, Bauer, and Arcade mill meal, could easily be the result of an increase of the moisture content of these samples. However, the very fine initial grinds all gave higher percentages of oil than 100 stroke regrinding of the 2 mm and 1 mm Wiley meal.

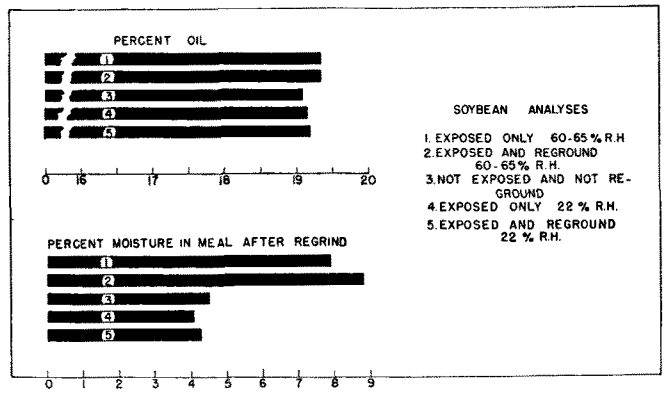

Fig. 1. Effect of treatment at regrind time on the moisture content of soybean meal and on the total percentage of oil extracted. Each bar for percentage of oil represents the average of 18 determinations and each bar for percentage of moisture represents the average of nine determinations.

In Figure 1 each bar showing percentage of oil represents the average of 18 analyses of three varieties (Chief, Dunfield, and Lincoln) since duplicate samples of Mikro-Pulverizer, Bauer, and Arcade mill grinds were analyzed. Each bar for percentage of moisture of the samples (taken after 30-50 minutes of exposure during the regrind period) represents the average of nine of each of these initial grindings. Samples taken for percentage of moisture after five hours of continuous extraction were transferred directly to the titration flask with minimum exposure. Percentages of moisture on all of these samples were determined using the modified Karl Fischer reagent.

All of the reground samples and the exposed but not reground samples were unwrapped and spread on their filter paper wrappings while the samples were

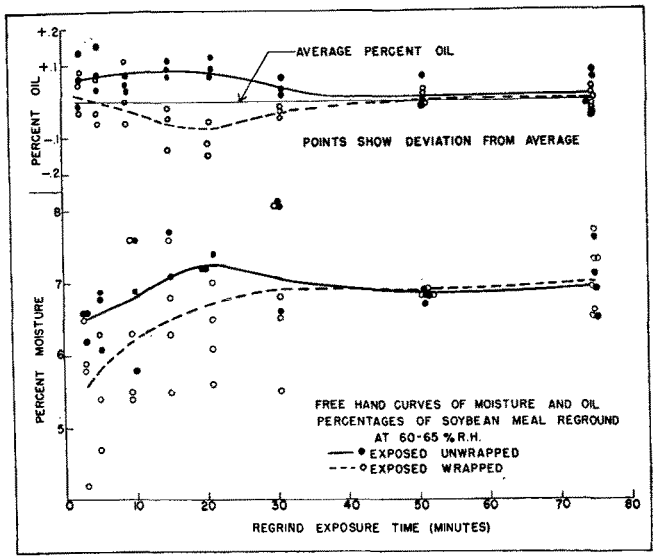

FIG. 2. Effect of technique of exposure under conditions of high humidity on the moisture content of soybean meal and on the total percentage of oil extracted. The average percentage of oil represents 58 analyses with 30-75 minutes exposure at regrind.

saturated with petroleum ether. Figure 1 shows that the samples exposed during the regrind period to 60-65% relative humidity gained 3-4% more moisture and gave an average of 0.14% more oil than samples similarly treated under 22% relative humidity. Regrinding was only slightly more effective in obtaining a higher percentage of oil and moisture than was exposure of the sample for the same length of time without regrinding.

At 22% relative humidity the reground samples and the exposed but not reground samples gave slightly higher average percentages of oil than were obtained by five hours of continuous extraction. Moisture differences could not account for this slight increase in percentage of oil since the percentages of moisture in the exposed samples were slightly less than those of the samples which were extracted five hours continuously. Perhaps the disturbing of the arrangement of the particles of meal in the unwrapping and rewrapping process may account for this slight increase in the percentage of oil.

The writers (3) in a previous study found that 1 mm ground soybean meal (not predried), containing from 3-4% of oil at regrind time, was more responsive to changes in relative humidity as shown by the percentages of oil obtained than were these predried Mikro-Pulverizer, Bauer, and Arcade mill samples, which contain 0.5% or less of oil at the time of regrinding.

To measure the oil remaining in the finely ground meal after the initial two-hour extraction, 22 samples of oven predried Bauer mill ground soybean meal were analyzed for oil content using relative humidities ranging from 38-86% during the regrind period and with a fresh extraction flask used for the final two hours of extraction. Extraction of the sample was interrupted only momentarily while the receiving flasks were changed. The average percentage of oil obtained in the last two hours of extraction was less than 0.01% with a maximum of 0.02%.

Some analysts expose their samples to atmospheric conditions at regrind time wrapped as for extraction until they are free of solvent while others unwrap the samples and expose the meal while it is still saturated with solvent. The time interval of exposure of the samples for regrinding varies from less than five minutes in some laboratories to over an hour in others. Figure 2 shows the effect of the two techniques under conditions of high relative humidities for time intervals from three to 75 minutes.

Moisture determinations, using modified Karl Fischer reagent, were made on duplicates of each oil analysis sample immediately following its regrinding.

Samples reground under conditions of high relative humidity using the unwrapped exposure technique gained 2-4% in moisture content during the first few minutes of exposure, and analytical results were erratic during the first 20 or 30 minutes. Using the wrapped exposure technique, moisture changes occurred more slowly but when using either technique analytical results were more consistent if the time of exposure of the samples was over 30 minutes, especially under conditions of high humidity.

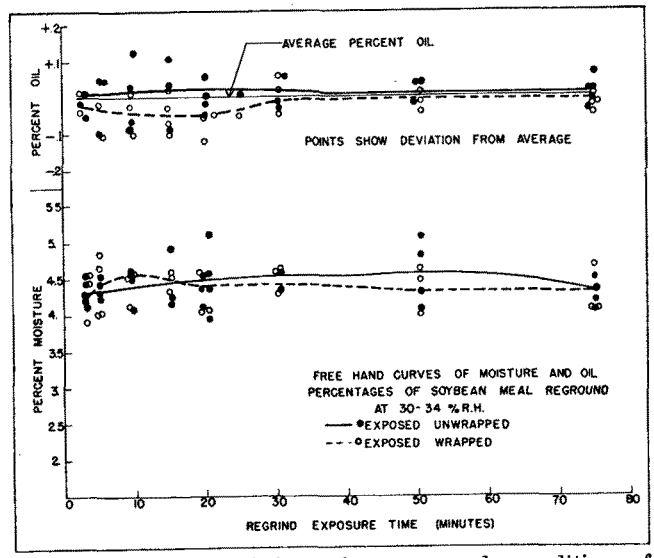

FIG. 3. Effect of technique of exposure under conditions of low humidity on the moisture content of soybean meal and on the total percentage of oil extracted.

Under conditions of 30-34% relative humidity, time and technique of exposure were of slight importance but exposures of over 20 minutes gave better checks between duplicate analyses.

Effect of Condenser Water Temperature

Butt extraction apparatus for the analysis of soybeans for oil content under conditions of high relative humidity or when the water for cooling the condensers is cooler than 20° C., often condenses atmospheric moisture in troublesome amounts. This condensate of atmospheric moisture may get into the samples and spoil the analytical results. It was arranged to control the temperature of the condenser water stepwise from 18-40° C. by adding hot water to the cold water supplied to the condensers. Under ordinary laboratory conditions, condenser water of 30° C. prevented condensing of atmospheric moisture on the apparatus at relative humidity conditions below 90%. Within the range of 18-30° C. no differences in the percentages of oil obtained could be ascribed to the temperature of the condenser water.

TABLE 2

Effect of Method of Wrapping the Sample of Soybeans on the Percentage of Oil Obtained, as Determined by the Percentage of Oil Recovered Through an Additional Two Hours of Extraction

Grind	Oil percentages calculated to 14% moisture basis			
	Spread thin and tightly wrapped		Not spread and loosely wrapped*	
	Official method	Two hrs. additional extraction	Official method	Two hrs. additional extraction
	% oil	% oil	% oil	% oil
Wiley 1 mm	18.21	0.03	18.13	0.08
Mikro-Pulverizer	18.28	0.03	18.16	0.12
Average	18.25	0.03	18.15	0.10

*The loosely wrapped samples were spread on the filter paper and tightly wrapped before extracting them for the additional two hours.

Table 2 shows the average results of oil analyses of triplicate samples of Wiley mill 1 mm and Mikro-Pulverizer mill grinds with two varieties of soybeans. Samples of this meal which were thinly spread on the filter paper and tightly wrapped gave an average of about 0.1% more oil than samples which were not spread on the filter papers and were loosely wrapped during the extraction of the oil. After completing the regular five hours of extraction as specified in the official A.O.C.S. method, these samples were removed from the Butt tubes, left wrapped as they were when extracted, and exposed overnight to 40-50% relative humidity. Then all of the samples were spread thinly on the filter paper, tightly wrapped, and extracted for an extra two hours. Those samples which had been loosely wrapped during the regular extraction yielded about 0.1% oil during the extra two hours of extraction while those samples which had been tightly wrapped throughout their extraction yielded only about 0.03% oil during the same extraction period. The greater amount of oil obtained during the extra two-hour period from the samples which had been loosely wrapped during the regular extraction makes the total percentage of petroleum ether extract obtained from these samples almost equivalent to that obtained from the tightly wrapped samples. It was evident that the loosely wrapped samples were less completely extracted during the regular oil analysis than were the tightly wrapped samples. Increased moisture content of these samples due to the overnight exposure to the atmosphere probably caused the percentage of extract obtained in the extra two hours of extraction to be higher than might be expected. However, since all of the samples were exposed for the same time interval, conclusions drawn from the comparative data should be valid.

Thin spreading of the meal and loose wrapping of the samples for oil analyses caused results to vary as much as 2%. Most of these samples contained so much visible meal that the reason for abnormally high percentages of oil and the lack of close checking between duplicates was obvious.

Effect of Predrying Soybean on the Iodine Number of the Oil

The effect of oven predrying of soybeans before grinding and fineness of initial grind on the Wijs iodine number and on the iodine number calculated from the refractive index of the oil are shown in Table 3. Data given in the table are averages of results of analyses of the four varieties used in this study. Although oven predrying probably changed many of the physical properties of the meal, it did not change seriously the Wijs iodine number or the refractive index of the oil, under the conditions of this experiment.

Summary and Conclusions

Initial very fine grinding, such as is obtained by using the Mikro-Pulverizer, Bauer, and Arcade mills, is essential to more complete extraction of the oil in the official A.O.C.S. method of analysis. Much of the increase of 0.2-0.4% of oil obtained by regrinding very finely ground soybean meals which has been attributed to reduction in particle size is probably caused by the gain of 3-5% in moisture content of the meal due to high humidity conditions at the regrind period. The results obtained in this study make it appear that, using a very fine initial grind, better reproducibility of analyses might be obtained if the regrind were omitted or the samples exposed for 30 to 50 minutes at controlled relative humidity during the regrind period. Further study of the moisture level in these very finely ground meals which will give maximum oil extraction with the regrind omitted would seem essential. Time and technique of exposure of the samples at their regrind may have caused variations as great as 0.2% in oil analyses. The percentage of oil obtained in the last two hours using the official A.O.C.S. method of oil analysis on finely ground samples was seldom greater than 0.02%. Control of the temperature up to 30° C. of the cold water which was supplied to the condensers of the oil extraction apparatus prevented condensing of atmospheric moisture without affecting the percentages of oil obtained in the analyses. The heat treatment of soybeans to predry them before their initial fine grinding did not affect appreciably either the Wijs iodine number or the refractive index of the oil which is extracted with petroleum ether.

TABLE 3

The Effect of Predrying Soybeans and Fineness of Initial Grinding on the Iodine Number of the Oil. Iodine Numbers for Each Treatment Represent Averages of Four Determinations

Mill	Treatment	Iodine number	
		A.O.C.S. Wijs	Calculated from refractive index
Bauer	Oven predried	133.9	133.4
Bauer	Not oven predried	134.0	133.4
Wiley 1 mm	Oven predried	133.9	133.6
Wiley 1 mm	Not oven predried	133.9	133.4
Average		133.9	133.5

Acknowledgment

The authors are indebted to E. R. Barrow, G. W. Agee, and C. H. Cox of Barrow-Agee Laboratory, Memphis, Tennessee; to S. O. Sorensen and R. E. Anderson of Archer-Daniels Midland Company, Minneapolis, Minnesota; to Frank Hasbrouck of Allied Mills, Peoria, Illinois, for grinding samples; and to Dr. R. T. Milner and F. R. Earle of the Northern Regional Research Laboratory, Peoria, Illinois, for advice and assistance in making this study.

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