Jaocs News Feature

Collaborative study of the determination of total oil in sunflower seed

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A collaborative study was made of two methods for determining the total oil content of sunflower seed. The seed of three sunflower varieties of low, medium, and high oil content were analyzed on different days by 12 collaborators. In one method, the sunflower seeds were ground with an equivalent wt of diatomaceous earth. In the second method, the seeds were ground with a high-speed grinder without diatomaceous earth. The method using diatomaceous earth in the grinding of the seed gave a significantly higher oil content for all three varieties. It was recommended that total oil on a per cent dry wt basis be calculated from the moisture determination of the whole seed and not from the moisture of ground sample, which was too variable.

INTRODUCTION

Because of the recent development of sunflowers as a commercial crop in the U.S. (1,2), the Sunflower Seed and Meal Analysis Subcommittee of AOCS has been concerned with developing a uniform method for determining the oil content of sunflower seed.

Existing official AOCS methods for oilseeds (3) are inapplicable to sunflower seed. A large proportion of the sunflower seed is a fibrous hull which has only a small amount of oil. The large seed sunflower varieties, used for confections and birdseed, contain 40-50% hull, whereas the new oilseed varieties contain ca. 25% hull. The kernels of both types of sunflowers are high in oil, ranging from ca. 50-65% (4).

The tough seed coat and the high oil kernel present problems in sample preparation. The conventional laboratory mills are not satisfactory for grinding sunflower seed, particularly the oilseed type. A simple solution to the problem is to grind the sunflower seed with an inert absorbent, such as diatomaceous earth. This reduces substantially the oil loss during grinding and results in a more uniform grind and representative sample.

COLLABORATIVE STUDY

The collaborative study presented here compares two methods patterned after the AOCS Method Ab 3-49 for oil in peanuts and the National Institute of Oilseed Product's method for oil in safflower seed in which sunflower seeds are ground with and without diatomaceous earth.

Twelve collaborators in 11 laboratories were sent 60 and 80 g samples of seeds of three sunflower varieties of low, medium, and high oil content for analysis by methods one and two, respectively. By the use of random numbers, each variety was analyzed on two different days by the two methods.

METHODS

Method One

Apparatus:

1. Butt type extraction apparatus, assembled as indicated in the illustration in AOCS Method Aa 4-38.
2. Filter paper, S&S no. 597, Reeve Angel no. 211, Whatman no. 2, or equivalent, 150 mm.
3. Absorbent cotton, free of petroleum ether extract.
4. High-speed grinder, Mikro-Samplmill, rated at 12,000 rpm and equipped with 0.035 x 1/2 in. herringbone screen (Mikro-Pul, 10 Chatham Road, Summit, N.J.) Other high-speed grinders, such as Stein Laboratory Mill or Blendex grinder, can be used but may not give as uniform grinding or mixing as the Mikro mill.

Reagents:

1. Petroleum ether (AOCS, Specification H 2-41).
2. Johns Manville Hyflo Super Cel, Fisher H-333 Super Cel, or equivalent. The Super Cel diatomaceous earth should be dried at 130 C overnight before use. The Official AOCS diatomaceous earth or Johns Manville Filter Cel are unsatisfactory, because they seem to absorb the oil too strongly for the petroleum ether to recover under practical extraction conditions.

Procedure:

1. Weigh ca. 50 g sunflower seed to the nearest 0.1 g into a large beaker. Add an equal wt Hyflo Super Cel. Mix well with a large spatula.
2. Grind the mixture immediately with Mikro-Samplmill into a 1-qt. container. After grinding is complete, remove the container from the mill and place a large rubber stopper (no. 7 or 8) into the container to aid in mixing. Cap and allow the sample to equilibrate at room temperature. This sample is used to determine oil and moisture in ground sample. Both weighings should be made at the same time. Mikro-Samplmill should be cleaned thoroughly between samples. Removal of feed screw for cleanout of lodged, heterogeneous material is necessary.
3. Thoroughly mix sample by inverting the container several times. The rubber stopper will dislodge any of the ground sample which may cake on the sides of the container.
4. Weigh accurately 4 g ground mixture to the nearest 0.001 g into a filter paper and enclose in a second filter paper folded in such a fashion to prevent escape of the meal (see illustration in AOCS Method Aa 4-38). The second paper is left open at the top like a thimble. A piece of absorbent cotton is placed in the top of the thimble to distribute the solvent as it drops on the sample.
5. Place in butt tube and extract with petroleum ether as directed in AOCS Method Aa 4-38, Section D, for 6 hr without interruption for grind with mortar and pestle.

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[1] ARS, USDA.

TABLE I

Collaborative Results for the Determination of Total Oil in Sunflower Seed[a]

	Peredovik		INRA 6501		Dahlgren 694	
	Method		Method		Method	
Collaborators	1 (% dry wt basis)	2 (% dry wt basis)	1 (% dry wt basis)	2 (% dry wt basis)	1 (% dry wt basis)	2 (% dry wt basis)
1	50.74 ± 0.37[b]	49.77 ± 0.08[b]	44.25 ± 0.80[c]	43.70 ± 0.83[c]	30.78 ± 0.39[b]	31.10 ± 0.23[b]
2	50.44 ± 1.51	50.25 ± 0.16	44.56 ± 1.29	44.04 ± 0.60	31.37 ± 1.22	30.67 ± 0.06
3	51.63 ± 0.22[b]	---	44.53 ± 0.45[b]	---	31.10 ± 0.46[b]	---
4	51.72 ± 0.92	50.33 ± 0.21	45.83 ± 0.35	44.01 ± 0.53	31.43 ± 0.47	31.29 ± 0.27
5	50.73 ± 0.16	---	44.73 ± 0.57	---	30.59 ± 0.69	---
6	50.85 ± 0.30	---	44.89 ± 0.12	---	32.04 ± 1.10	---
7	51.72 ± 0.33	49.33 ± 0.78	45.65 ± 0.32	43.49 ± 0.75	31.50 ± 0.70	30.93 ± 0.16
8	49.52 ± 0.11[b]	49.83 ± 0.05[b]	44.54 ± 0.08[b]	44.30 ± 0.05[b]	31.01 ± 0.21[b]	30.47 ± 0.07[b]
9	51.05 ± 0.07	49.90 ± 0.57	45.20 ± 0.57	45.45 ± 0.07	31.50 ± 0.00	30.75 ± 0.64
10	50.07 ± 0.30	49.50 ± 0.33	44.83 ± 0.16	44.24 ± 0.65	31.52 ± 0.35	30.83 ± 1.56
11	50.27 ± 0.11	49.53 ± 1.80	44.68 ± 0.24	44.11 ± 0.57	30.62 ± 0.53	30.41 ± 0.81
12	50.04 ± 0.26[b]	---	44.46 ± 0.32[b]	---	30.63 ± 0.05[b]	---
Mean ± standard deviation	50.73 ± 0.71	49.81 ± 0.35	44.85 ± 0.48	44.17 ± 0.58	31.17 ± 0.46	30.81 ± 0.30
Coefficient of variance, %	1.40	0.71	1.08	1.32	1.47	0.97

[a] Mean of two determinations unless otherwise noted.

[b] Mean of four determinations.

[c] Mean of six determinations.

6. Cool and disconnect the extraction flask. Evaporate the ether on a stream or water bath until no odor of ether remains. A gentle stream of nitrogen may be used to facilitate removal of the solvent. Cool to room temperature, carefully remove any moisture or dirt from outside of flask and weigh. Repeat heating until constant wt is obtained.
7. Determine the moisture in the ground sample as follows:
   a. Weigh 4 g to the nearest 0.001 g into a tared AOCS moisture dish.
   b. Slip the cover on the bottom of the dish and place the dish in a vacuum oven. Dry at 100 C for 1 hr at 30 in. mercury.
   c. Remove the dish from the oven and cover immediately. Cool in a desiccator containing an efficient desiccant to room temperature and weigh.

   $$\text{Moisture in ground sample, \%} = \frac{\text{Loss in wt x 200}}{\text{wt of sample}}$$

   d. Record the values obtained to the second decimal place.

Calculations:

$$\text{Oil in ground sample, \%} = \frac{\text{wt of oil x 200}}{\text{wt of sample}}$$

The percentage of oil is calculated to dry wt basis with the following formula:

$$\text{Oil, \% dry wt basis} = \frac{(\text{\% oil in ground sample})100}{100 - \text{\% moisture in ground sample}}$$

Record the values obtained to the second decimal place.

Method Two

Apparatus:

1. Butt type extraction apparatus, assembled as indicated in the illustration in AOCS Method Aa 4-38.
2. Filter paper, S&S no. 597, Reeve Angle no. 211, Whatman no. 2, or equivalent, 150 mm.
3. Absorbent cotton, free of petroleum ether extract.
4. High-speed grinder, Stein Laboratory Mill, rated at 17,500 rpm (Fred Stein Laboratory, Inc., Atchinson, Kansas); Blendex high-speed grinder (Blendex, Division of Cyclo-Science, Inc., 434 Bergen Blvd., Palisades Park, N.J.) or equivalent mill.

Reagents:

1. Petroleum ether AOCS Specification H 2-41.

Procedure:

1. Grind ca. 25 g seed in mill for ca. 30 sec, discard, and brush out cup. This coats inside of cup with a thin layer of oil which provides a more accurate analysis.
2. Weigh ca. 50 g sunflower seed into Stein mill cup.
3. Connect cup securely to grinder and grind 15-20 sec. Using a small spatula, redistribute the contents of the grinder, and grind for an additional 15-20 sec. Grinding should be timed so that there is little caking of sample in bottom of cup.
4. Thoroughly mix sample and weight accurately 2 g ground sample to the nearest 0.001 g into a filter paper and enclose in a second filter paper folded in such a fashion to prevent escape of the meal (see illustration in AOCS Method Aa-38). The second paper is left open at the top like a thimble. A piece of absorbent cotton is placed in the top of the thimble to distribute the solvent as it drops on the sample.
5. Place in butt tube and extract with petroleum ether as directed in AOCS Method Aa 4-38 Section D, for 3 hr, remove, add 0.5 g pumice, grind with mortar and pestle, and extract an additional 2 hr.
6. Cool and disconnect the extraction flask. Evaporate the ether on a stream or water bath until no odor of ether remains. A gentle steam of nitrogen may be used to facilitate removal of the solvent. Cool to room temperature, carefully remove any moisture or dirt from outside of flask, and weigh. Repeat heating until constant wt is obtained.
7. Determine the moisture in the ground sample as follows:
   a. Weigh 2 g to the nearest 0.001 g into a tared AOCS moisture dish.
   b. Slip the cover on the bottom of the dish and place the dish in a vacuum oven. Dry at 100 C for 1 hr at 30 in. Hg.
   c. Remove the dish from the oven and cover immediately. Cool in a desiccator containing an efficient desiccant to room temperature and weigh. Moisture in ground sample, %

   $$= \frac{\text{Loss in wt x 200}}{\text{wt of sample}}$$

   d. Record the values obtained to the second decimal place.

Calculations:

Oil in ground sample,

$$\% = \frac{\text{wt of oil x 200}}{\text{wt of Sample}}$$

The percentage of oil is calculated to dry wt basis with the following formula:

$$\text{Oil, \% dry wt basis} = \frac{\text{(\% oil in ground sample)100}}{\text{100} - \text{\% moisture in ground sample}}$$

Record the values obtained to the second decimal place.

RESULTS AND DISCUSSIONS

Results from the collaborators are given in Table I. Four collaborators did not submit results for method two because they did not have the specified high-speed grinder.

The overall mean and standard deviation for the total oil content of Peredovik, INRA 6501, and Dahlgren 694 for method one was 50.73 ± 0.71, 44.85 ± 0.48, and 31.17 ± 0.46, respectively, and for method two, 49.81 ± 0.35, 44.17 ± 0.58, and 30.81 ± 0.30, respectively. The coefficient of variation for the results from the 12 collaborators for method one was 1.40%, 1.08%, and 1.47%, respectively, for Peredovik, INRA 6501, and Dahlgren 694 sunflower varieties. The coefficient of variation for the results from the eight collaborators for method two was 0.71%, 1.32%, and 0.97%, respectively, for the three sunflower varieties.

The somewhat high deviations within the laboratories were expected with sunflower seed, due to the great difficulty in obtaining a homogeneous sample. This sampling problem was recognized by Wolff, et al., (5) who reported that the repeatibility of the determination of oil in sunflower seed by NMR was only ±0.60, whereas that of rapeseed, a homogeneous oilseed, was ±.225.

TABLE II

Analysis of Variance of the Determination of Total Oil in Sunflower Seed

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F value	Significant
Laboratory	7	8.238	1.176	3.023	*[a]
Method	1	11.613	11.613	29.839	**[b]
Variety	2	6249.971	3124.985	8029.362	**
Laboratory x method	7	4.993	.713	1.833	+[c]
Laboratory x variety	14	4.062	.290	.745	+
Method x variety	2	1.015	.507	1.304	+
Laboratory x method x variety	14	5.101	.364	.936	+
Error	48	18.681	.389		
Total	95				

[a]*=significant at the 5% level.

[b]**=significant at the 1% level.

[c]+=not significant at the 5% level.

The results of the analysis of variance of the determination of the total oil in sunflower seed by eight collaborators, using the two methods, are shown in Table II. There was a significant difference between laboratory, method, and variety. These differences were independent of each other, since there was no significant variance interactions. Method one, which used Hyflo Super Cel in the grinding of the seed, gave a significantly higher oil content for all three varieties. The varieties were chosen, because they were known to have different oil contents; thus, they would be expected to be significantly different.

Another factor which contributed to the difference between laboratories was the difficulty the collaborators had with both methods in the determination of the moisture of the ground samples. Five of the laboratories did not have a vacuum oven and determined the moisture with an air oven at 130 C for 2 hr. Four of these same laboratories did not analyze the samples by method two.

TABLE III

Determination of Moisture of Sunflower Seed[a]

	Peredovik		INRA 6501		Dahlgren 694	
	Method		Method		Method	
	1	2	1	2	1	2
Collaborators			Per cent			
1	5.80	5.59	4.54	4.44	6.55	6.76
2	6.08	4.23	4.37	3.94	6.80	5.77
3	7.31[b]	---	5.51[b]	---	6.93[b]	---
4	7.05[b]	5.60	4.85	3.85	6.00	6.70
5	5.17[b]	---	3.28	---	5.93	---
6	6.10[b]	---	4.20	---	6.15	---
7	5.78	6.02	4.46	4.28	6.75	6.51
8	5.26	5.08	4.40	4.10	6.23	6.28
9	5.55	4.95	4.00	6.10	6.30	4.25
10	5.12	5.83	4.61	4.97	6.58	6.81
11	6.82[b]	---	5.16	---	6.88	---
12	5.23	6.05	4.35	4.64	6.25	6.36
Mean	5.94	5.42	4.44	4.54	6.45	6.18
Standard deviations	0.760	0.626	0.553	0.731	0.347	0.849
c.v.[c]	12.80	11.54	12.46	16.09	5.39	13.74

[a]Average of two determinations.

[b]Moisture determined by air oven at 130 C for 2 hr.

[c]Coefficient of variation.

It has been the experience of our laboratory that accurate moisture determinations cannot be obtained on ground sunflower seed using a forced draft oven at 130 C, because of deterioration of oil in the sample. Results obtained with the vacuum oven also have been quite variable.

Since the sunflower seed samples were not sent to the collaborators in moisture proof containers, some variation in moisture content was to be expected; however, not as great as was obtained by the collaborators (Table III). Table III shows that the coefficient of variation ranged from 11.54-16.09% for the determination of moisture of sunflower seed by the two methods, except for method one of the Dahlgren sample which had variation of 5.39%. In view of these results, it was the recommendation of the Sunflower Seed and Meal Analysis Subcommittee that a moisture determination should not be made on ground sunflower seed. Total oil on a per cent dry basis would be calculated from the moisture determination of the whole seed. These analyses would be conducted simultaneously with the oil determination.

In addition, the Subcommittee recommended that another collaborative study be conducted on sunflower seed using method one for oil determination, but also including determinations for moisture of whole seed and protein content.

ACKNOWLEDGMENTS

The chairman of the Sunflower Seed and Meal Analysis Subcommittee acknowledges the following collaborators: L.V. Anderson, Minnesota Linseed Oil Co., Minneapolis, Minn.; C.M. Cater, Texas A&M University, College Station, Texas; D.L. Henry, Law and Co., Atlanta, Ga.; W.J. Johnson, The Buckeye Cellulose Corp., Memphis, Tenn.; H. Keith, Paymaster Oil Mill, Lubbock, Texas; A.J. Kuutti, Cargill, Inc., Minneapo-

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ery was satisfactory, evidently because of difficulties in quantification of the extraction procedure.

Several of the collaborating teams attempted preliminary tests involving an alternate approach to greatly increased sensitivities through the use of a graphite furnace with carbon rods or a carbon tube atomizer. Since these techniques do not involve extraction, they are more precise and rapid. If a sufficient number of collaborators possessing the required graphite furnace with carbon rods or a carbon tube atomizer can be found, a collaborative investigation of this technique will be conducted.

GAS CHROMATOGRAPHY SUBCOMMITTEE

S.F. Herb, Subcommittee chairman, has resigned, since his new work assignment will not be concerned with fat, oil, or lipid research. Also resigning were J.L. Iverson, chairman of a task group to investigate a procedure involving programed temperature analysis of fatty acid composition designed to modify or augment AOCS Tentative Method Ce 1-62 Rev. 1970, and B.D. Thomas who had taken over activities to investigate and evaluate gas liquid chromatographic (GLC) methods for determining resin acid in rosin and of turpentine. These resignations will require an almost complete reorganization and restructuring of the Gas Chromatography Subcommittee if the objectives (as outlined in the previous report (5), all involving collaborative effort, are to be achieved. See Table I. Major activities within this Subcommittee during the coming year will probably be confined to this reorganization and will include the appointment of a new Subcommittee chairman and new collaborative investigation or task group leaders.

NMR SPECTROSCOPY SUBCOMMITTEE

AOCS has not found a member willing to accept appointment as chairman of the NMR Spectroscopy Subcommittee. This vacancy has handicapped the progress of this group, particularly in establishing a collaborative task group to investigate published methods and select a preferred technique for the determination of total oil in oilseeds by wide-line NMR spectroscopy.

A.J. Haighton, Unilever Research, Vlaardingen, The Netherlands, has served as chairman of a task group established to study a method for the determination of solid/fat index by NMR. Despite the handicap of distance, he has made considerable progress with the task group. (See "Report of the Instrumental Techniques Committee 1971-72" for a summary of earlier collaborative tests [5]). This collaborative testing has been international with 2 collaborative teams from the USA, 3 from the UK, and 1 each from Sweden, Holland, Canada, and Switzerland.

In a report of latest collaborative investigation, Haighton stated that the instrumental precision was satisfactory and that the method could readily include procedures permitting the use of wide-line or pulsed-source instruments. A collaborator pointed out one "great advantage of the NMR instrument, namely that measurement can be made on texturized fat without altering the sample's crystalline state." These collaborative studies have demonstrated that the NMR procedures have advantages over the dilatometric techniques (AOCS tentative method Cd 10-57) in precision, scope, and time of analysis. However, the studies are revealing that precision is related to the method of tempering. Since tempering affects the solid/fat index, precision can be obtained among collaborators if, and only if, the method includes a precise, detailed description of a recommended method of tempering. Thus far there has been little, or no, agreement regarding the preferred method. An additional collaborative effort is being organized to test the method when a specific tempering procedure is followed. Collaborators will be asked to follow the method whether or not the tempering procedure agrees with that customarily used in their respective laboratories. Hopefully this method will reveal agreement among collaborators and can be recommended to the Uniform Methods Committee for inclusion as a standard or official method of the Society.

R.T. O'CONNOR, chairman
R.R. ALLEN, subcommittee chairman
K.M. BROBST, subcommittee chairman
S.F. HERB, subcommittee chairman

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lis, Minn.; P.L. Maiers, Doty-Wilhoit Labs, Inc., Minneapolis, Minn.; P.L. Maiers, Doty-Wilhoit Labs, Inc., Minneapolis, Minn.; D.C. Melear, Jr., Southwestern Labs, Fort Worth, Texas; J. Ridlehuber, Plains Cooperative Oil Mill, Lubbock, Texas; J.K. Thomas, USDA, ARS, Russell Research Center, Athens, Ga.; and B.D. Deacon, USDA, ARS, AMRI, Beltsville, Md.

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Call for Papers

AOCS 48TH ANNUAL FALL MEETING

DEADLINE: MAY 3, 1974

The Technical Program Committee has issued a call for papers to be presented at the AOCS Fall Meeting, September 29-October 2, 1974, in the Sheraton Hotel, Philadelphia, Pennsylvania. Papers on lipids, fats, and oils and all related areas are welcome. Submit three copies of a 100-300 word abstract with title, authors, and speaker to: Gerhard Maerker, Eastern Regional Research Center, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118. ■