Determination of Oil in Sunflowerseeds

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Oil content measurement in sunflowerseeds on an "as is" basis by current official methods is often associated with poor reproducibility. This study shows that the main factor contributing to this poor agreement is the particle size to which seeds are ground. This invariably influences the homogeneity of the bulk ground sample from which subsequent subsamples are taken. It is therefore suggested that oil content determinations on sunflowerseeds should be carried out on seed samples that have been evenly and finely ground, to a particle size not greater than 2.0 mm, in a mechanical mill such as the Ultra-Centrifugal mill. Other factors investigated were seed composition (free husk, empty husk, crude fiber and seed meats) and structural differences in the seeds by light microscopy.

The Federation of Oils, Seeds and Fats Associations Ltd. (FOSFA International) is a contract-issuing body whose contracts are used for the bulk of world trade in oils, seeds, fats and edible peanuts. Quality aspects of the various cargoes are determined by appropriate analysis of relevant samples, and FOSFA accordingly maintains a list of 24 approved analysts in 11 countries on all 5 continents. To ensure that approved analysts maintain the standards required by the trade, they must participate in compulsory ring tests. From time to time, unacceptably large variations in the results returned by the laboratories show that a particular method may be defective. This was the case with the determination of oil content in sunflowerseed on an "as is" basis (i.e., the seeds are analyzed without removal of impurities).

In the experience of the FOSFA/Food R.A. laboratory and of many other analysts, there was often poor agreement between replicate analyses on portions taken from the same ground sample and occasionally differences in the oil contents of different subsamples from the same bulk seed. The official FOSFA method (1) routinely used for oil extraction specifies that the difference between duplicate analyses should be no greater than 0.6%. The American Oil Chemists' Society (AOCS) official method (2) for sunflowerseeds, however, allows the oil content of duplicates to differ by up to 1.93% for two single tests carried out in any one laboratory. In contrast, the AOCS figure for cottonseeds or peanuts is 0.42% (2). This suggests that the AOCS collaborative study on sunflowerseed analysis also revealed a wider than normal variation of oil content results.

It is known that sunflowerseeds have tough, fibrous hulls or husks that make it very difficult to obtain a uniform, finely ground sample (3). A significant negative correlation between percentage of oil content and hull thickness has been observed by Anand and Chandra (4). Position of seed in the sunflower head and the size and shape of the head have been reported to influence the oil content and fatty acid composition of oil (5,6). Fick and Zimmerman (7) have noticed that the oil content is highest for seeds located in a band halfway between the center and the circumference of the flower head. Anderson (8) has defined physiological maturity for sunflower as the point at which seed yield and oil content are maximum. Several workers (9–11) have reported that dry weight and oil content are maximum at 35 days after initiation of flowering. Moreover, the climate, temperature and genetic factors are well known to have significant influence on the oil content and fatty acid composition of sunflowerseed oil (12,13). Some of these variations in sunflowerseeds may also affect the duplicate determination of oil content.

It has been maintained in the trade that one cause of the poor reproducibility may be a large number of empty hulls present in some samples of sunflowerseed. The distribution of these empty hulls throughout the bulk sample and, in consequence, throughout successive subsamples may be extremely nonuniform. Another factor that is probably important is the grinding technique, since the hulls of some samples appear to be harder, making it more difficult to produce uniform ground material by the Christy-Norris mill (CNM) routinely used in the Food R.A. laboratory.

TABLE 1

Characteristics and Composition of Five Clean Sunflowerseed Samples

Sample	Wt ratio, free hulls to free kernels	Wt ratio, hulls to kernels in full hulls	% Empty hulls ("as is")	% Free hulls + % free kernels ("as is")	Oil content (%)[a]				Moisture content (%)				Admixture content (%)
					"As is"	Clean seeds	Hulls	Kernels	"As is"	Clean seeds	Hulls	Kernels	
A	1:4.32	1:2.08	0.26	1.27	41.57	42.14	12.48	57.20	5.37	4.97	8.04	3.92	3.97
B	1:2.67	1:2.29	Nil	1.64	40.99	41.94	8.94	57.41	5.42	4.96	8.04	3.73	3.59
C	1:2.23	1:2.42	0.63	1.22	41.53	40.87	6.52	56.05	6.39	5.56	8.19	3.98	3.50
D	1:3.46	1:2.13	0.26	5.46	40.33	41.84	13.69	57.42	5.41	4.97	6.42	3.31	3.83
E	1:1.14	1:1.89	Nil	1.21	39.57	41.53	17.24	57.34	5.55	5.35	8.88	4.59	4.10

[a]Mean of two or three determinations, where results were within the permitted range (±0.6) of the FOSFA method.

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The purpose of the present study was to investigate the effects of seed composition (free husk, empty husk, crude fiber and seed meats), moisture, grinding technique and particle size distribution on the replicate analyses of oil content and thus ascertain why analysis should be more difficult with sunflowerseeds than with other seeds. In addition, the seed samples were examined by light microscopy for any structural differences that might have an effect on reproducibility.

EXPERIMENTAL PROCEDURES

Materials. Sixteen sunflowerseed batches, obtained through contacts of FOSFA, were used in the work. Subsamples, representative of the different batches, were drawn for analysis on a rotating conical divider. Grinding of each representative sample (300 g) was carried out using (i) the Christy-Norris mill (CNM) with a 3-mm bar screen (used routinely in the Food R.A. laboratory) and (ii) the Ultra-Centrifugal mill (UCM) with 1-mm screen and a cyclone attachment (on loan from Glen Creston Ltd., London).

Methods. The oil content, moisture content, admixture (amount of foreign matter) and crude fiber of various samples were determined according to FOSFA/Food R.A. (1), ISO 665 (14), ISO 658 (15) and the AOCS (16) methods, respectively.

The particle size distributions of six samples, each

TABLE 2

Oil, Moisture and Admixture Contents of Four Sunflowerseed Samples and Microscopic Examination of Individual Seeds

Sample	Oil content (%)[a]	Moisture content (%)	Admixture content (%)	Microscopy comments
F	42.63	6.49	ND[b]	No obvious differences between seeds
G	42.76	5.80	3.08	
H	41.75	5.23	3.14	
J[c]	43.45	7.39	4.20	

[a]Dry weight basis; mean of two or three determinations where results were within the permitted range (±0.6) of the FOSFA method.
[b]ND, not determined.
[c]One seed out of eight examined had considerably thicker cell walls.

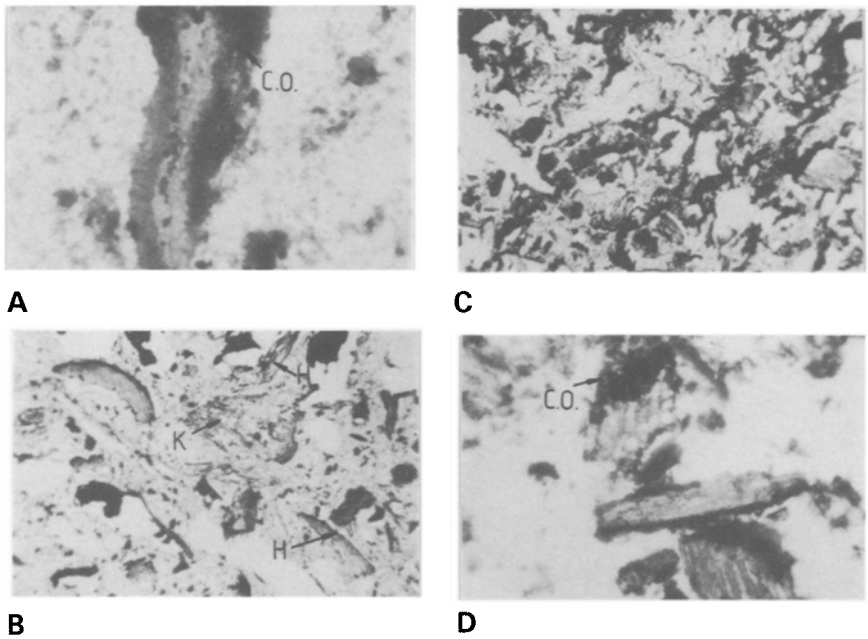

FIG. 1. Light micrographs of sample P. C.O., cellular oil; K, kernel; H, husk. (A) Sample ground using Christy-Norris mill (CNM), before extraction; some of the cells still holding oil (×100). (B) Sample ground using CNM, before extraction; large particles of kernel and husk (×10). (C) Sample ground using Ultra-Centrifugal mill, before extraction; small cell groups with broken-down cell material (×10). (D) Sample ground using CNM; extraction residue showing oil still trapped within cell groups and slight loss of defined structure within the cell walls (×100).

ground using the two mills, were determined by sieving (2.00-mm, 1.18-mm and 0.50-mm sieves). To avoid blinding of the 0.50-mm sieve, the sample was passed through manually, while an electric shaker was used with other sieve sizes.

Microscopic examination of 12-μm-thick sections of several individual seeds and of three selected samples (ground separately using both mills) was carried out. The stains used were (i) 0.2% Toluidine Blue in 30% glycerol plus 0.66% phenol and (ii) 1% osmium tetroxide, 1-min wash; 1% periodic acid, 10-min wash; Schiffs reagent, 30-min wash; 0.125% light green, 5-min wash; mount in glycerol.

RESULTS AND DISCUSSION

Empty hull and crude fiber. The percentage levels of empty hulls and of free (broken) hulls plus free kernels in five sunflowerseed samples (A to E) are listed in Table 1. The weight ratios of free hulls to free kernels and of hulls to kernels (in full hulls, i.e., whole seeds) are also presented along with the admixture content of these seed samples and oil and moisture contents of hull, kernel and whole seeds. Three seed samples had a few empty hulls while two had none. Sample D had an appreciable amount of free hulls and free kernels, while sample C had most empty hulls. Nevertheless, the poor reproducibility in oil content was experienced in all of these samples when ground using the CNM. The admixture content of all the subsamples is about 4% (see Table 1). This study on the limited number of samples indicated that the presence of small amounts of empty hulls in the seeds probably is not the primary cause of poor oil content replications.

The oil content of the kernels is between three and eight times that in the hulls, while the moisture level of the hulls is about twice that of the kernels. Calculations from these results, together with oil and moisture values for whole seed samples, show that as little as 0.4 g extra kernel, or 0.2 g extra hull, per 10 g analytical subsample is sufficient to take an analytical result outside the range of permitted tolerance (difference in oil content of not more than 0.6% between two duplicates). These small weights represent a very small amount of sunflowerseed and suggest that better duplication would be achieved by a finer grind of the subsample, followed by thorough mixing, to give a completely homogeneous mixture and more representative subsamples for analysis. The CNM with a 3-mm bar screen, which is routinely used for milling most oilseeds, leaves the sunflowerseed hulls in rather large pieces. Some batches may have harder hulls than others, leading to more heterogeneity in the ground bulk sample, thus exacerbating the subsampling problem. This aspect may be part of the reason for the trade view that analytical problems are associated with the empty hull content, and this could also magnify the grinding and subsampling problems in some cases.

Oil and crude fiber contents were very variable. For example, one sample was analyzed six times for oil content, and in each case the residue or meal was examined for crude fiber content, giving mean values (and standard deviations) of 41.58% (0.407) and 17.48% (1.487), respectively. There was little correlation (correlation coefficient = −0.25) between the oil content and the amount of crude fiber in the aliquots of meal.

Microscopic examination. Sections of several individual seeds of four sunflowerseed samples (F, G, H and J) were examined by light microscopy for cell size, cell wall thickness and extent and distribution of vascular tissue. The microscopy results and the oil, moisture and admixture findings on these samples are presented in Table 2. In all the sample seeds examined, the cell walls were largest at the outside of the cotyledons and smaller towards the centers, but the seeds had widely varying amounts of vascular tissue. When the sections were stained for protein, the distribution of protein bodies was found to be very uneven. All batches showed the same seed-to-seed variation, with similar variations between individual seeds, and no particular trends. Two batches (H and J) seldom gave acceptable duplicate oil determinations (±0.6). One seed (out of eight examined) of subsample J had considerably thicker cell walls than the others, and the staining was generally poor with seeds of this batch. It is, therefore,

TABLE 3

Oil Extraction (%) and Moisture and Admixture Contents of Sunflowerseed Samples Ground Using Christy-Norris Mill (CNM) and Ultra-Centrifugal Mill (UCM)

Sample	Oil content (% dry weight basis)		Moisture content (%)	Admixture content (%)
	Ground using CNM	Ground using UCM		
K	45.99, 45.69[b], 46.35[b]	47.32[a]	6.22	1.92
L[c]	46.6[a]	46.94[a]	5.92	2.45
M	42.58, 42.27[b], 43.00[b]	42.20[a]	5.78	3.40
N[c]	45.42[b], 42.11[b], 42.53	45.11[a]	6.50	3.80
P[c]	49.25, 49.11[b], 48.47[b]	48.11[a]	5.86	2.15
Q	47.41, 47.22[b], 46.43[b]	45.20[a]	6.96	3.64

[a]Mean of three or four determinations; all results were within the permitted range (±0.6).
[b]Results within a wider range than that permitted (±0.6).
[c]One g anhydrous sodium sulphate was added to the extraction thimble prior to the sample addition to minimize any water carryover into the extracted oil.

plausible that the problem of poor sunflowerseed oil content reproducibility is caused by a small but heterogeneously distributed proportion of seeds with thick cell walls present in the batch.

Three selected sunflowerseed subsamples (L, N and P), ground separately using the two mills, were also examined microscopically, (i) to assess the extent of cell damage and (ii) to see whether any oil could be located in the residue after extraction. The section of milled samples (prior to extraction) showed oil inside and outside cell groups. In the CNM-ground sample L, most of the oil was free, i.e., outside the cell structure, while some of the oil in the other CNM-ground samples (N and P; see Fig. 1a) was trapped inside the cell groups. Moreover, the kernels of these two batches were in medium and large pieces, with some large pieces of hulls (Fig. 1b). This shows that the size of fragments produced by CNM grinding of P and N samples was not even. On the other hand, the particle size of the UCM-ground samples was small, and they were mixed evenly (Fig. 1c). The CNM-ground sample L was an even mixture of medium-sized kernel and hull particles, with only a few large pieces. After extraction, the particle size of the residues had decreased, and all the samples (both the CNM- and UCM-ground) were made up of small groups of cells and cell fragments. Very little oil was present after extraction, except that when N and P samples were ground with the CNM they still retained some oil (Fig. 1d). However, no correlation could be found between the amount of oil seen microscopically and the extracted oil value within the subsamples (replicate analyses).

Grinding technique and particle size distribution. The effect of the grinding mill on the reproducibility of oil content in six sunflowerseed samples can be seen from Table 3. The content of admixture and moisture analyzed in these samples is also listed. These values are normal for average quality sunflowerseeds traded worldwide. The seed samples ground using UCM always gave replicate oil content values lying within 0.6% of one another, as permitted by the FOSFA method. The majority of the samples ground using the CNM normally gave results outside the acceptable range of the method. These findings show clearly that the Christy-Norris milling technique is the main contribution to the poor reproducibility in oil content analysis of sunflowerseeds. Furthermore, on the bases of some collaborative testing of the AOCS method (2), it was apparently established (C. Burton Smith, Zone Devices Co., PO Box 714, Mill Valley, CA 94942, USA, personal communication) that the poor reproducibility of this method is due to heterogeneity of the bulk ground sample.

The particle size distribution of the six samples ground using the two different milling techniques is shown in Table 4. The CNM-ground samples contained 20-35% seed particles of size greater than 1.18 mm, while only 2-12% of the UCM-ground samples were in this particle size range. Of these UCM-ground samples, sample N had the highest level (12.4%) of fragments of sizes between 1.18 and 2.00 mm. This seed sample (admixture content 3.8%) had a substantial quantity of large stalks. Visual inspection of the ground samples also showed that, on milling with the UCM, the seed kernels and hulls were ground to a much finer particle size than with the CNM. In other words, UCM grinding of sunflowerseeds provides a more homogeneous bulk sample (for subsequent subsampling) than can be achieved using the CNM. These observations are in full agreement with the results of microscopic examination at the cell level. A sample of the seed ground using the CNM was reground, using the same milling conditions, a second and third time. This increased the clogging problem of the mill but did not alleviate the problem of poor reproducibility of oil content results. The actual amounts of oil extracted appeared to decrease after subsequent grinding, perhaps due to loss of oil in the mill itself. Circular screens of different sizes (1-4 mm) were also used, but these were subject to extensive clogging, resulting in no ground sample being available for analysis. Therefore, a compromise has to be made between a finely ground sample and an oil and/or moisture loss due, for example, to overheating during grinding. It is therefore recommended that oil content determinations on sunflowerseeds be carried out on seed samples that have been evenly and finely ground in the mechanical mill, such as the Ultra-Centrifugal mill. The ground material should have no particles bigger than 2.00 mm, and less than 12% of the particles should be bigger than 1.18 mm. The grinding should be carried out so that there is no overheating in the mill and no loss of moisture or of liberated free oil inside the

TABLE 4

Particle Size Distribution (% wt) of Ground Samples

Particle size (mm)	Sample											
	K		L		M		N		P		Q	
	CNM[a]	UCM[b]	CNM	UCM	CNM	UCM	CNM	UCM	CNM	UCM	CNM	UCM
>2.00	3.5	Nil	3.4	Nil	4.3	Nil	3.7	Nil	3.6	Nil	4.6	Nil
1.18–2.00	16.9	7.1	32.3	1.7	23.1	4.0	20.8	12.4	21.1	1.9	22.6	3.3
0.50–1.18	34.2	42.3	20.4	38.7	37.2	35.6	37.8	52.8	34.8	37.7	32.0	44.6
<0.50	45.4	50.6	43.9	59.6	35.4	60.4	37.7	34.8	40.5	60.4	40.8	52.1

[a]Christy-Norris mill.
[b]Ultra-Centrifugal mill.

mill during grinding. Subsamples of this finely ground, homogeneous bulk sample should then be analyzed for oil content in the normal way.

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