Further Studies on a Multisequential Method for Determination of Oil Content in Oilseeds[1]

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Abstract

The multisequential method for determination of oil content in oil seed developed at Svalöf has been used for ten years with good results.

The mean values obtained in analysis of comparative samples of rape, turnip rape and white mustard for seven years in five countries with official methods agree well with the mean values from the multi-sequential method as used in four laboratories in Sweden. However, the variation coefficient is considerably smaller for the multisequential method.

In addition to rape, turnip rape and white mustard, flaxseed, poppy seed, sunflower seed, safflower seed, soybeans and groundnuts can be successfully disintegrated in the steel tubes used in the multisequential method.

The free fatty acid content of a rapeseed sample can be determined on the oil used for the gravimetric analysis.

Introduction

SOME TIME AGO TROENG (15,16) described briefly the development of a multisequential method for the determination of oil content in some oilseeds. In this method use is made of stainless steel tubes in which the whole seeds are disintegrated and simultaneously extracted while shaken together with steel balls—as used in ball-bearings—and a petroleum fraction. After centrifugation of the homogenate an aliquot is evaporated and weighed. A series of specially designed aids such as tube racks, shaking boxes and shaking machines had to be constructed.

Since the method has been used for ten years with a daily analysis of 800 samples in duplicate during the peak season and 13,000–60,000 samples analyzed per year, much information about the usefulness of the method has been gathered. As increasing interest has been shown for this method, particularly in conjunction with plant breeding, it would appear timely to publish some details about the equipment used, together with the results of analysis for oil content in oilseed according to the Svalöf multisequential method compared with the results obtained on the same samples in other laboratories using the official method of their respective countries. Also, studies on the content of free fatty acids in the oil from rapeseed with the extraction step performed by the multisequential method compared to a conventional method will be reported, as well as the potential use of the method for seeds other than rape, mustard and flax.

Equipment

The stainless steel tubes in which the extraction is carried out are of a size and shape as shown in Fig. 1. These tubes are specially manufactured (AB Gävle Rostfria, Gävle, Sweden). The four steel balls which disintegrate the seed on shaking are also shown in Fig. 1 in a natural position in the tube. The tubes fit in Wifug centrifuges type, nr DX 3 (AB Winkelcentrifug, Stockholm). An improved ventilated drying cabinet is manufactured by Ingvar Svensson, Elvärmedetaljer, Skurup, Sweden. The temperature control of petroleum ether and oil solution has been improved and simplified by maintaining the standard temperature (at 25C, i.e., slightly above room temperature). The addition of petroleum ether into the tubes is now made easier by use of an automatic pipetting machine (Filamatic Vial Filler, Model AB).

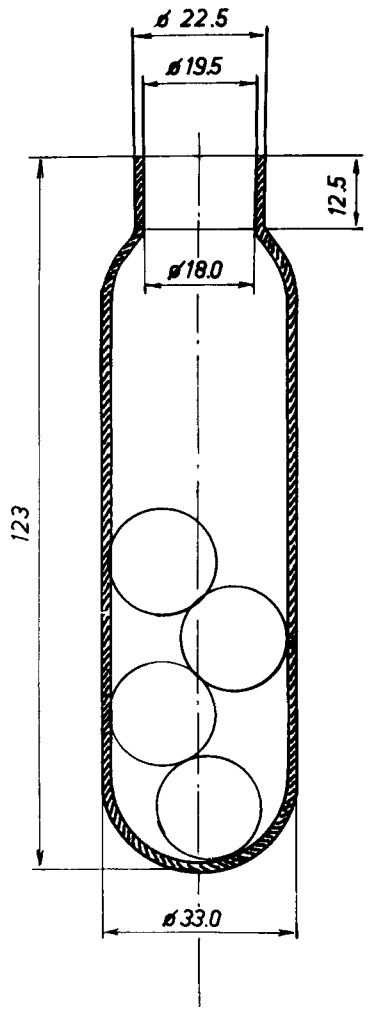

FIG. 1. A cross-section of a stainless steel extraction tube with four steel balls. Dimensions are shown in millimeters.

A report in Swedish with an English summary has been published by Lindberg et al. (9), in which many photographs and drawings illustrate the equipment used.

Comparison with Results from Other Laboratories

Each year since 1957 carefully selected samples of winter rape, winter turnip rape and white mustard have been freed from any dockage and then mixed before being packed in airtight metal tins. The seed samples had low moisture content (generally 6–7%). These metal tins have been sent to different laboratories in Sweden as well as in Denmark, Germany,

[1] Presented at the AOCS Meeting in Cincinnati, October 1965.

TABLE I

Results from Comparative Analyses of Winter Rape, Oil Content in Dry Matter

	Method																	
	1957		1958		1959		1960		1961		1962		1963		1964		1957–1964	
	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial
Number of laboratories	4	4	4	4	5	4	5	4	5	4	5	4	4	4	4	3	....	....
Number of duplicate analyses	8	8	8	17	9	12	10	10	10	9	10	5	8	6	8	4	71	71
Mean of all analyses	48.35	48.63	45.84	45.91	45.46	45.40	46.26	46.09	45.46	45.37	44.32	43.92	45.49	45.20	48.75	48.65	46.154	46.041
Variance between laboratories	0.1100	0.0100	0.1300	0.0567	0.0775	0.1333	0.5550	0.1167	0.1325	0.0933	0.9975	0.0067	0.3667	0.0200	0.3533	0.0250	....	....
Variance quotient	11.00^{x}		2.29^{o}			1.72^{o}	4.76^{o}		1.42^{o}		148.88^{xxx}		18.34^{x}		14.13^{o}			
Standard deviation between laboratories	0.332	0.100	0.361	0.238	0.278	0.365	0.745	0.342	0.364	0.306	0.999	0.082	0.606	0.141	0.594	0.158	0.535	0.217
Variation coefficient																	1.16%	0.47%

TABLE II

Results from Comparative Analyses of Winter Turnip Rape, Oil Content in Dry Matter

	Method															
	1958		1959		1960		1961		1962		1963		1964		1958–1964	
	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial
Number of laboratories	4	4	4	4	5	4	5	4	5	4	4	4	4	3	....	....
Number of duplicate analyses	8	17	8	12	5	5	10	9	10	5	8	6	8	4	57	58
Mean of all analyses	45.88	45.94	44.38	44.15	46.56	46.62	45.97	45.87	44.14	44.14	44.48	44.22	48.43	48.65	45.60	45.47
Variance between laboratories	0.0167	0.0433	0.0867	0.2833	0.6050	0.0033	0.2350	0.1700	0.6450	0.0167	0.1233	0.0033	0.1400	0.0400	....	....
Variance quotient	2.59^{o}		3.27^{o}			18.33^{xxx}		1.38^{o}		38.62^{xx}		37.36^{xx}		3.50^{o}	....	....
Standard deviation between laboratories	0.129	0.208	0.294	0.532	0.778	0.057	0.485	0.412	0.803	0.129	0.351	0.057	0.374	0.200	0.459	0.228
Variation coefficient															1.01%	0.50%

TABLE III

Results from Comparative Analyses of White Mustard, Oil Content in Dry Matter

	Method																	
	1957		1958		1959		1960		1961		1962		1963		1964		1957–1964	
	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial	Off.	Multi-sequen-tial
Number of laboratories	4	2	4	4	5	4	3	4	5	4	5	4	4	4	4	3	....	....
Number of duplicate analyses	4	2	8	17	9	12	3	5	10	9	10	5	8	6	8	4	60	60
Mean of analyses	30.73	30.35	30.86	31.14	30.60	30.45	29.93	30.46	28.85	28.82	31.40	31.52	32.98	32.63	33.41	33.18	31.14	30.89
Variance between laboratories	0.0967	.....	0.5567	0.0233	0.0250	0.0100	0.1450	0.0467	0.7400	0.0733	0.3650	0.0100	0.0900	0.0300	0.6333	0.0300	.....	.....
Variance quotient				23.89^{x}		2.50^{o}		3.10^{o}		10.10^{x}		36.50^{xx}		3.00^{o}		21.11^{x}		
Standard deviation between laboratories	0.311	.....	0.746	0.153	0.158	0.100	0.381	0.216	0.860	0.271	0.604	0.100	0.300	0.173	0.796	0.173	0.520	0.169
Variation coefficient																	1.67%	0.55%

Holland and England. Three other Swedish laboratories and our own have been analyzing the seeds according to the multisequential method. The laboratory at an edible oil industry in Sweden and three or four laboratories of more or less official character in the above-mentioned countries have been performing analyses, according to methods adopted as official in their respective countries. In certain years the samples have been sent twice to laboratories of which four have determined the moisture and oil content, according to the Svalöf multi-sequential method and four or five laboratories have been using the official methods (Butt-type or Soxhlet extractors). The figures for oil content reported on each sample represent the mean of two single determinations. Table I, II and III show the results obtained on winter rape, winter turnip rape and white mustard, respectively. In calculating the figures for the variation between laboratories, only the first result from each laboratory has been used, whereas the mean applies to all the results obtained a certain year on the seed in question. The figures presented clearly indicate that in all cases, where a statistically significant difference in "variance between laboratories" is obtained, the one between laboratories using the official methods is larger. The coefficient of variance indicates that the deviation is two to three times larger between the last-mentioned laboratories than between such ones using the Svalöf method. For laboratories using that method the coefficient of variance is about 0.5 for all the three seed species. For laboratories using the official methods on the other hand, the dispersion is quite obviously larger for white mustard than for rape and turnip rape.

Table IV contains the data from the first 60 duplicate determinations made at our laboratory with the Svalöf method on the same samples which were used for the analyses in Tables I, II and III. As in the other tables, the standard deviation has been calculated for each year's figures. The data cannot be statistically treated in all desired aspects since different standard samples have been used in different years and these samples differ in oil content as well as homogeneity. From the tables it is seen that there are statistically significant differences in deviation for different years, most likely due to the variation in homogeneity of the samples. This statement is supported by the fact that if the standard deviations for the three species are arranged in decreasing order, the order will be very different for the different species. Thus for rape it was largest in 1960 and smallest in 1962, giving $F = \frac{1960}{1962} = 5.74^{xxx}$; for turnip rape largest in 1959, smallest in 1958, giving $F = \frac{1959}{1958} = 2.95^{xxx}$ and finally for white mustard largest in 1961, smallest in 1958, giving $F = \frac{1961}{1958} = 3.62^{xxx}$. These facts indicate that the reason for the difference in deviation can hardly be the analytical technique as the analysis has been carried out in exactly the same way for all the three species.

It is of great interest to notice that the coefficient of variance is about 0.5 which means the same as between laboratories using the Svalöf method. Thus there is a similar deviation for the Svalöf method between laboratories as within one laboratory.

Determination of Free Fatty Acids in Rapeseed

The content of free fatty acids in oilseed is usually determined on oil extracted in a Soxhlet, Goldfish or Butt-type apparatus. In some preliminary trials, fairly large differences were found in free fatty acid content in a sample of oilseed determined by us after extraction according to the Svalöf method (Method I) and determined at an oil mill after extraction in a somewhat modified Butt-type apparatus (Method II). Doubtlessly it should be very valuable if the content of free fatty acid could be determined directly on the same sample of oil weighed for analysis in Method I, since the determination of the free fatty acid content in the oil is often desirable. Therefore 128 samples of rapeseed from a storage experiment were extracted both according to Method I and Method II. In our laboratory Method II was used as follows.

A Butt-type extraction apparatus as described in AOCS Official Method Aa 4–38 (1) was used, except that ground glass joints were used instead of the cork stoppers. After grinding predried samples in a mortar with sand, the samples were placed in an extraction thimble and extracted for 16 hr with petroleum ether bp 40–60C. The temperature in the thimble rose to about 37C.

TABLE IV

Oil Content, % in Dry Matter According to the Svalöf Method

	1958	1959	1960	1961	1962	1963	1964	1958–1964
Winter rape								
Number of duplicate analyses	60	60	60	60	60	60	60	420
Mean of all analyses	45.93	45.32	46.12	45.48	44.03	45.25	48.84	45.85
Variance within year	0.0290	0.0463	0.1429	0.0664	0.0249	0.0547	0.0459	.....
Standard deviation	0.170	0.215	0.378	0.258	0.158	0.234	0.214	0.232
Variation coefficient								0.51%
Order of increasing standard deviation	2	4	7	6	1	5	3	
Winter turnip rape								
Number of duplicate analyses	60	60	60	60	60	60	60	420
Mean of all analyses	46.04	44.06	46.60	46.07	44.27	44.29	48.78	45.73
Variance within year	0.0222	0.0656	0.0380	0.0597	0.0285	0.0564	0.0383	.....
Standard deviation	0.149	0.256	0.195	0.244	0.169	0.238	0.196	0.207
Variation coefficient								0.45%
Order of increasing standard deviation	1	7	3	6	2	5	4	
White mustard								
Number of duplicate analyses	60	60	....	60	60	60	60	360
Mean of all analyses	31.07	30.35	....	28.95	31.64	32.68	33.25	31.32
Variance within year	0.0163	0.0253	....	0.0590	0.0268	0.0322	0.0466	.....
Standard deviation	0.128	0.159	....	0.243	0.164	0.179	0.216	0.181
Variation coefficient								0.58%
Order of increasing standard deviation	1	2		6	3	4	5	

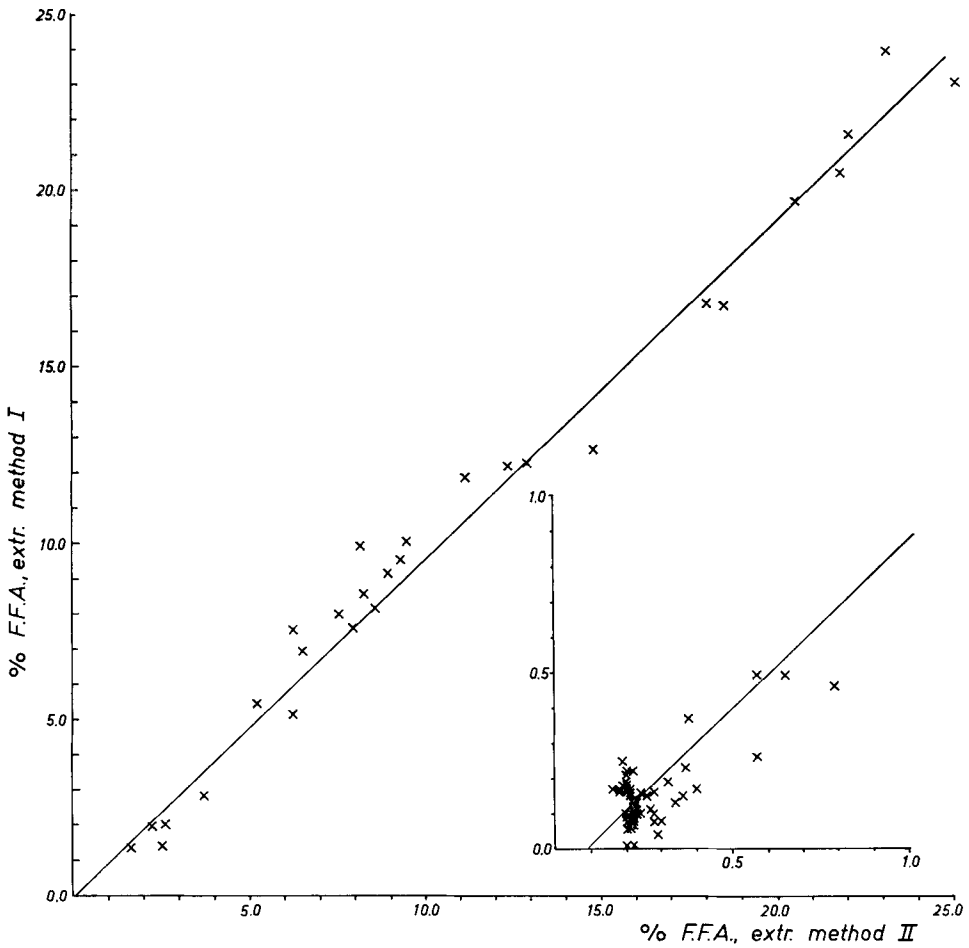

FIG. 2. The free fatty acid content of samples of rapeseed, extracted by two different methods. The small graph shows the data for FFA contents less than 1%.

The oils from Method I (y) and Method II (x) extractions were after weighing dissolved in a neutralized ethanol-ether (1:1) mixture and titrated with 0.1 N NaOH against phenolphthalein as indicator. An average molecular weight of 314 is used in the calculation of free fatty acid in rape oil as recommended in DGF-Einheitsmethoden C III 4–53 (4). The results obtained are plotted in Fig. 2. A statistical calculation shows a correlation coefficient of 0.997 and a regression coefficient of 0.9669.

The equation for the content of free fatty acid in the oil extracted according to Method I (y) and Method II (x) is

$$Y = 2.58 + 0.9669\ (x-2{,}74)$$

which can be transformed to

$$x = 1.03\ y + 0.08.$$

Whether the data obtained with Method I or Method II should be regarded as the "true" free fatty acid content of the oil in the seed remains to be investigated. Further studies into the effect of various seed pretreatments and extraction conditions upon the content of free fatty acid obtained are being undertaken.

In any case it is easy to transfer the figures obtained with Method I to those with Method II if necessary. Except when official contracts state the use of Method II, the content of free fatty acid in oilseeds is determined at our laboratory with the extraction step according to our multisequential method (Method I) and the value obtained is "corrected" in accordance with the equation above.

Potential Uses for Other Seeds and for Beans

Since rape, turnip rape and white mustard are the major oil crops in Sweden, the method was developed and tested for these crops as well as for flaxseed. As the comparative investigations between laboratories do not include flaxseed, being an unimportant oil crop in Sweden, comparisons were made in our laboratory on the oil content obtained in duplicates from 20 samples of flaxseed by the conventional Butt-type extractors and by our multisequential method. The results presented in Table V clearly show a smaller standard deviation with the multisequential method than with the Butt-type extraction. The means are somewhat higher for the multisequential method. A possible explanation for this is mentioned under "Discussion."

We also wished to test the possibility of extracting whole sunflower seed, safflower seed, poppy seed, soybeans and shelled as well as unshelled groundnuts in the tubes, since these seeds and beans are major products in other countries. Further, we included false flax (*Camelina sativa*) and Abyssinian kale (*Crambe abyssinica*) because they are oil crops of potential interest in Sweden and USA, respectively. The seeds and beans were extracted both "naturally dry" and after drying at 100C overnight.

The contents in the tubes, after being shaken for one hour, were filtered on Buchner funnels and washed with several portions of petroleum ether (bp, 40–60C). The material obtained was dried by continuing the air suction through the Buchner funnels. A sieving

TABLE V

Determination of Oil Content in 20 Samples of Flaxseed by the Multisequential Method and Conventional Extraction

Sample no.	Oil content determined by	
	Multisequential method	Butt extraction
216	40.0	39.8
217	40.1	39.7
218	39.4	39.2
219	39.6	39.1
220	38.9	38.7
221	40.6	40.1
222	40.3	40.0
223	43.4	43.5
224	45.6	45.2
225	44.7	44.4
226	45.8	45.3
227	45.7	45.5
228	44.6	44.4
229	44.5	44.0
230	45.0	44.9
231	44.9	45.0
232	46.0	45.1
233	45.3	45.2
234	46.4	45.9
235	44.5	44.0
Mean	43.27	42.95
Standard deviation	S=0.084	S=0.124
	F=2.160[x]	

analysis of the carefully recovered materials yielded the figures shown in Table VI. It is obvious that the nondried materials vary considerably with regard to degree of disintegration. The soybeans were, in fact, almost intact. After drying, however, all the samples studied were well disintegrated, most of them to a particle size smaller than 100 mesh.

The successful disintegration of dried sunflower seed, safflower seed, poppy seed as well as soybeans and groundnuts makes the multi-sequential method potentially useful materials, also for these although larger tubes should be used for soybeans and peanuts. In order to prevent larger sampling errors.

Discussion

The method developed at our laboratory after original ideas of Schwartze (13) seem to have some advantages when compared to other nonofficial as well as to the various official methods for determination of oil content in oil seeds. A larger number of nonofficial methods have been developed and these have been reviewed by Neustadt (11).

Our method can be used as a multisequential method, which means that the equipment is suitable for handling a large number of samples simultaneously, with most of the operations carried through by technically untrained personnel. As shown by Hougen (7) the tubes can also be used, on a small scale, without access to some of the special aids necessary when used in our multisequential method provided minor changes in procedure are made.

Compared to the dielectric method developed for soybeans, flax, sunflower and safflower (5,6,8), which method also has been tested on a larger scale (11,12,17), it seems to compete favorably, e.g., regarding precision. Further, the latter method requires individual handling of the samples. The smaller sample size used—generally 5 g compared to 50–80 g—which may be considered a disadvantage when analyzing commercial samples, does not seem to be too important when analyzing smaller oilseeds, as judged from the precision obtained. As pointed out earlier, larger tubes, take racks and centrifuges are to be used if the Svalöf method should be applied to whole soybeans or groundnuts, otherwise sampling errors could be serious. On the other hand, the small sample size needed is a great advantage when the method is used in plant breeding investigations, as single determinations on 2–3g samples have been made without introducing important analytical errors. Thus numerous seed samples from individual plants of rape, turnip rape and white mustard have been analyzed during the past ten years in efforts to increase the oil content of these seeds.

When compared to official methods it shows a precision as good as, or even better than, that of such methods. The reason it yields a higher oil percentage of flaxseed than that obtained by extraction in a Butt-type apparatus, may be due to the fact that highly unsaturated lipids, after being oxidized, react with proteins during the grinding process. Such an error may be serious if regrinding after a certain extraction time is practiced, as the aggregates of protein and oxidized lipids are not split by hexane.

Compared to some screening methods used in plant breeding research (3,14,18) it is obviously much more precise. Depending upon the nature of the problem, in certain cases the lower cost per sample favors the screening methods, whereas in other cases the higher precision favors the multisequential method.

It has also been utilized in large-scale nutritional studies on the feed value of cereal grains for the analysis of the fat content of the carcass of mice (10).

The mild extraction conditions, 1 hr at 30C, are in favor of using the oil received for further studies on its composition, provided necessary changes in procedure are made in certain cases, e.g. in the determination of the content of peroxides in undamaged and heat-damaged seeds of rape and white mustard (2). The tubes have also been used for extracting seed oil for studies on its fatty acid composition and triglyceride structure as will be reported elsewhere.

TABLE VI

Sieving of the Defatted Materials Obtained after Shaking in the Steel Tubes and Suction Filtration

Seed sample	Moisture content %	Recovery %	Sifted fractions, % weight of recovered material			
			<35 mesh	35–60 mesh	60–100 mesh	>100 mesh
Rape	6.9	96	8.5	12.8	13.4	65.3
Rape	0	93	0.9	2.1	3.8	93.2
White mustard	7.4	94	12.0	9.7	11.9	66.4
White mustard	0	95	0.3	0.7	1.8	97.2
False flax	7.5	94	32.3	10.3	11.4	46.0
False flax	0	94	0.7	4.3	7.5	87.5
Flax	7.4	95	5.7	13.4	15.8	65.1
Flax	0	93	1.0	2.2	3.8	93.0
Abyssinian kale[a]	7.1	94	3.0	7.7	8.1	81.2
Abyssinian kale[a]	0	95	0.2	2.0	4.7	93.1
Poppy	7.4	93	17.3	23.8	19.2	39.7
Poppy	0	91	3.1	7.3	13.4	76.2
Safflower[a]	5.3	94	14.2	23.7	19.1	43.0
Safflower[a]	0	94	3.3	14.2	20.1	62.4
Sunflower[a]	7.6	97	9.9	23.7	16.6	49.8
Sunflower[a]	0	101	0.6	7.4	14.0	78.0
Soybeans	8.0	100	97.6	0.4	0.5	1.5
Soybeans	0	94	3.8	2.5	3.0	90.8
Peanuts, shelled	6.0	88	3.5	5.9	7.4	83.2
Peanuts, shelled	0	89	0.7	1.3	1.8	96.2
Peanuts, unshelled	....	95	8.9	16.4	14.7	61.0
Peanuts, unshelled	0	94	0.3	3.3	9.9	86.5

[a] Not dehulled.

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