Report of the Cooperative Work of the International Fat Commission 1938-1939†

Translated by
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GENERAL ANALYTICAL METHODS

(Part A of the work program)

I. Determination of Volatile Soluble and Insoluble Fatty Acids in Edible Fats. (Reichert-Meissl and Polenske Numbers.)

In the test the procedures were carried out in accordance with proposal of the French delegation, the text of which is rendered below:

Definition: The index of the soluble volatile fatty acids (standard Reichert-Meissl-Number) is the number of milligrams of KOH necessary to neutralize the soluble volatile fatty acids set free (under definite conditions) from 1 g. of fat; the index of the insoluble volatile acids (standard Polenske-Number) is the number of milligrams of KOH necessary to neutralize the insoluble volatile fatty acids set free under definite conditions from 1 g. of fat.

Method: The (*Polenske*) apparatus is used (see illustration).* Previously there is added to the flask: 5 g. fat, 20 g. neutralized glycerine, and 2 cc. of 50% sodium hydroxide. With constant rotation the flask and contents are heated with a small flame until the fat is completely saponified. After 5 minutes the water has been evaporated and the mixture becomes clear. The flask is heated a moment more, cooled down to 80° and 90 g. of freshly boiled water, also cooled down to 80°, is added. The soap dissolves in the water. Then 50 cc. of dilute sulfuric acid (50 g. in 1,000 cc.) and a little pumice are added. (The flask is quickly attached to the apparatus and heated by an open flame in such a way that in 20 minutes 110 cc. distills over. The flask is then allowed to stand, is rotated, and the contents filtered through a dry filter of 8 cm. diameter.) One hundred cc. is caught and titrated with N/10 NaOH in the presence of phenolphthalein. (A blank determination is necessary!) If n is the volume of sodium hydroxide used, best expressed in cc., and P is the weight of fat used, then the standard Reichert-Meissl-Number is:

$$RMN = \frac{5.6 \cdot n \cdot 1.1}{P}$$

The insoluble acids remaining on the walls of the condenser, the receiver and on the filter are taken up three times with 15 cc. of 95% alcohol (well neutralized). The entire amount of liquid is titrated with N/10 NaOH. If n′ is the volume of N/10 alkali used, expressed in cc., then the standard Polenske-Number is:

$$PN = \frac{5.6 \cdot n'}{p}$$

† This translated version of the International Commission report is published here because of its particular interest to the Fat Analysis committee in its work on analytical methods on fats and oils, and because of its general interest to the industry.

* Translators' note. For similar illustration see figure 4 on page 38 of "Official and Tentative Methods of the American Oil Chemists' Society."

In the cooperative investigation for which samples composed of butter and coconut oil were sent by Professor Maragaillan, Marseille, the following investigators participated:

A. DENMARK
A. Johansen, Aarhus Oliefabrik A/S, Aarhus

B. GERMANY
1. *Dr. H. Finken* und *H. Hölters*, Chemische Fabrik Stockhausen & Cie., Krefeld
2. *Dr. E. Delvaux*, Louvain-Belgien
3. *Dr. Heinz*, Henkel & Cie., GmbH, Düsseldorf
4. *Dipl.—Ing. H. Nowack*, Institut für Nahrungsmittlechemie und landwirtschaftl. Gewerbe der techn. Hochschule Danzig, Danzig-Langfuhr
5. *Dr. Wagner*, Staatl. chem. Untersuchungsamt f. d. A. Duisburg, Duisburg
6. *Dr. Ehrbächer*, Sunlicht Gesellschaft, A. G., Mannheim-Rheinau

C. FRANCE
1. *Prof. Dr. Margaillan*, Marseille
2. Laboratorium *Vizern & Guillot*, Marseille

D. HOLLAND
Dr. Voerman, 'S-Gravenhage, Laboratorium des Reich büros für Untersuchung von Handelswaren und Vereinigung der holländischen ölfabriken

E. ITALY
1. *Dr. Martinenghi* und *Dr. Ardissone*, R. Stazione Sperimentale per le Industrie degli Olii e dei Grassi, Milano
2. *Dr. Anselmi*, Instituto Sanita Publica, Roma
3. *Dr. Loew*, S. A. Spremitura Triestina, Trieste

F. NORWAY
Staatswardein *Dr. W. Holwech*, Dr. Holwechs chem. Laboratorium

G. SWITZERLAND
1. *Dr. Sturm*, Zürich
2. *Dr. Jaag*

The results obtained (average values) by the separate investigators are collected in Table I.

Attitudes taken:

A. DENMARK

1. Why must neutralized glycerine be used? Its use prevents an easy detection by the appearance of

TABLE I

	Reichert-Meissl No.	Polenske No.
A. Denmark		
1.	16.4	12.7
2.	16.5*	11.6*
B. Germany		
1.	16.0	13.6
2.	17.0	10.7
3.	15.7	12.2
4.	16.8	13.3
5.	17.4	10.5
6.	14.9	11.0
C. France		
1.	14.4	11.4
2.	15.2	11.7
D. Holland	16.3	13.8 12.6†
E. Italy		
1.	15.4	11.2
2.	15.3	10.9
3.	14.9	11.2
F. Norway	15.1	11.5
G. Switzerland		
1.	16.3	8.8
2.	15.6	10.4

* By consideration of the proposed modification.
† See text.

color of possible overheating. I suggest the use of pure glycerine.

2. After 5 minutes' boiling the mixture is seldom entirely clear. It would be better to change the directions so that they read, the saponification should be carried out sufficiently long, until the solution just shows a complete slight turbidity. For this seldom more than 15 minutes will be needed.

3. After the dilutions with water it would be perhaps better to add the pumice before the sulfuric acid, and certainly in the powdered state.

4. Distillation, filtration and titration would be more suitably performed according to "Directions of the Uniform Methods of the D.G.F." With the Polenske Number it is noticed that it is still generally customary to wash out the portion of the water soluble fatty acids still adhering with water, before one washes the water insoluble fatty acids with alcohol. Also here it would be more suitable if one proceeded according to the stated directions of the "Uniform Methods of the D.G.F." It is clear that the Polenske Number obtained by the "Directions of the Uniform Methods of the D.G.F." is lower since here a portion of water soluble acids will be titrated together with the insoluble acids.

B. GERMANY

The working directions proposed by the French delegation are in themselves good, but they omit the details of procedure, which is plainly very necessary with these conventional methods. They are contained in the *German Uniform Methods.*

C. FRANCE

It must be admitted that determinations repeated after several weeks give significantly higher values, which probably can be traced back to ageing of the fat.

D. HOLLAND

On using the French directions for the determination of the Polenske Number, the condenser, funnel and receiver are not washed with water before the addition of 95% alcohol. On this basis the Polenske Number will be found too high because a portion of soluble acids, which still adheres to the walls, will be dissolved by the alcohol. If washed three times with 15 cc. water before the addition of alcohol, the Polenske Number will be found to be not 13.8 but 12.6.

E. ITALY

It is recommended, that the section: "The flask is quickly attached to the apparatus and heated by an open flame in such a way that in 20 minutes 110 cc. distills over. The flask is then allowed to stand, rotated and the contents filtered through a dry filter of 8 cm. diameter," be changed as follows: "The flask is quickly attached to the apparatus and heated by means of an open flame in such a way that in 20 minutes (19-20 minutes) 110 cc. distills over. The flask is then allowed to stand about 15 minutes at a temperature of 12-16° and rotated and filtered . . . (as above)." The last named modification is necessary, in order that the insoluble fatty acids conglomerate better. Further it is necessary to describe more accurately the determination of volatile insoluble acids. It is above all necessary to exclude the possibility that owing to the succeeding washing with alcohol the residue will still retain volatile water soluble acids. It is recommended to select the following wording: "The filter, flask and receiver are washed with cold water (about 15°) long enough that the wash water, after the addition of phenolphthalein, is turned red by a single drop of N/10 potassium hydroxide. The insoluble acids on the walls of the condenser . . . etc. (see text)."

F. SWITZERLAND

The use of 2 cc. of 50% sodium hydroxide is impractical. One can just as well use 3 cc. of a 35% alkali, which always remains fluid and is convenient to handle. The heating has to be continued until the saponification is complete, which is easy to detect. But 5 minutes is usually insufficient for this. The quantity of pumice added is specified in isolated methods (0.7 g.). Perhaps this specification is not unimportant, in order to assure a fixed distillation velocity. It was called to our attention (Pritzker) afterward, that a large amount of material has been collected in the literature concerning Reichert- Meissl and Polenske numbers computed on the basis of the cc. of alkali used up. Also the official food control method is still computed on the basis of the cc. of alkali consumed, and the question is raised, if on these grounds a change in the method of calculation is timely.

II. Investigation of Sterols

The testing of the procedure of A. Bomer was suggested by the Holland delegation, the text of this procedure is rendered below:

Preparation of Fatty Acids:

About 50 g. of fat is saponified by heating on a water bath with 100 cc. of alcoholic potassium hydroxide (200 g. KOH in 1,000 cc. of 70% alcohol) with occasional shaking. After about 15 minutes the solution is homogenous and the saponification complete. To the soap solution is then added 150 cc. warm water and 50 cc. of 25% hydrochloric acid. For a clean separation of the fatty acids the solution is left on the water bath 15 to 30 minutes longer and then filtered through a large previously moistened filter paper. The watery portion runs relatively quickly through the filter, while the fatty acids remain on the filter. After the running off of the watery portion the filter is pierced and the fatty acids collected on a large folded filter, on which the fatty acids remain dry and clear (if the fatty acids have a high melting point, it is expedient to use a hot water funnel).

Preparation of the Digitonides:

The fatty acids are heated in a small beaker to about 80° and mixed with 20 cc. of a 1% solution of digitonin in alcohol (96%) (about 10 cc. of solution is used to 20 g. of fatty acids). The mixture is heated at about 80° for 15-30 minutes stirring occasionally with a glass rod. During this period the digitonides precipitate. Then 20 cc. of warm carbon tetrachloride is added, the precipitate filtered with suction on a small filter paper and washed twice with ether (if the fatty acids have a high melting point, it is expedient to use a hot water suction filter). The digitonides remain in a thin layer on the filter, from which they can be easily removed.

Preparation of Sterol Acetates:

The digitonides are heated about 15 minutes with 3 cc. of acetic anhydride under a reflux condenser. The solution obtained is then evaporated to dryness

on a water bath. The residue is taken up with 10 cc. of warm 96% alcohol, the solution filtered after several minutes and concentrated to 2-3 cc. The crystals formed are filtered off with suction and recrystallized twice from alcohol. After the third crystallization and drying the melting point is determined. A melting point of 117° or higher indicated the presence of phytosterol (the melting point of cholesterol acetate is 113-115°; that of phytosterol acetate is 126-137°).

Preparation of Sterols:

The sterol acetates are saponified with a little (about 3 cc.) N/2 alcoholic potassium hydroxide. After dilution with water, the sterols which split off are taken up in ether. The ether solution is washed once or twice with a little water, the ether evaporated and the sterols thus obtained taken up with 1 or 2 cc. of 96% alcohol. The sterols are allowed to crystallize out on a slide, and the crystal form and behavior in polarized light observed microscopically. The phytosterol crystals are oblong and longer than they are broad. Their extinction in polarized light is parallel. The cholesterol crystals are much wider and show an inclined extinction in polarized light.

For the comparative investigation two samples were sent out by Professor Margaillan, Marseille:

a) a mixture of lard and palm fat
b) a mixture of tallow and shea butter.

The following investigators reported:

A. DENMARK
I. A. Broge, Aarhus Oliefabrik A/S, Aarhus, Kemisk Laboratorium (Knud Helholt)

B. GERMANY
1. *Dr. H. Finken* und *H. Hölters*, Chemische Fabrik Stockhausen & Cie., Krefeld
2. *Arno Eichler* und *Edith Voss*, Hansa-Mühle, A. G., Hamburg
3. *Dr. H. J. Heinz*, Henkel & Cie., GmbH, Düsseldorf
4. *Dr. Wagner*, Staatl. Untersuchungsamt f. d. A. Duisburg, Duisburg
5. *Dr. Rosenbaum*, Van den Bergh's Margarine GmbH, Kleve, Rhld.

C. FRANCE
1. *Prof. Margaillan*, Marseille
2. Laboratorium *Vizern & Guillot*, Marseille

D. HOLLAND
Dr. Voerman, 'S-Gravenhage, Laboratorium des Reichsbüros für Untersuchungen von Handelswaren und Vereinigung der holländischen Ölfabriken

E. NORWAY
Amanuensis *Alf Klem* und *Dipl. Ing. Ivar Poulsson*, Biologisches Institut und staatl. Walforschungs-institut der Universität Oslo, Oslo

F. SWITZERLAND
1. *Dr. Fellenberg*
2. *Dr. Villier*

TABLE II

SAMPLE A: LARD AND PALM FAT

A. Denmark	122.5°	After two recrystallizations.
B. Germany		
1.		Even after standing 24 hours and after further addition of digitonide solution no precipitate was obtained.
2.	116.0-117.5°	Unsaponifiable = 0.6%; digitonide precipitable sterols = 0.07.
3.	123.5°	The digitonides precipitate only after 45 minutes.
4.	124.2-124.4°	After three recrystallizations. Because of the small quantity of sterols no final melting point of the sterol acetates could be obtained.
C. France	117-125°	The crystallization was difficult to carry out, also no clear melting point was obtained.
D. Holland	122.8; 123.6°	After three recrystallizations (Phytosterol positive).
E. Norway	117.3; 114.8°	After three recrystallizations. After repeated acetylation of the filtrate and three recrystallizations:
F. Switzerland	122.5°	
1.	122.5°	
2.	118.5°	

The melting points found for the sterol acetates by the individual investigators are collected in Tables II and III.

Attitudes:

A. DENMARK

By following the proposed method impure sterol acetates are obtained from the samples investigated. Therefore the melting point is not sharp. In both samples the acetates begin to melt at about 100°; at 125-126° the mass suddenly becomes liquid. With fat mixtures of a high unsaponifiable content or with hard fats a purification of the digitonides is necessary. By microscopic means phytosterol as well as cholesterol crystals are detected in both samples.

Proposed Change:

Necessary Reagents:

1. Potassium hydroxide; 200 g. potassium hydroxide in 1000 cc. of 70% alcohol
2. Hydrochloric acid; 25%
3. Digitonin solution; 1 g. digitonin in 100 cc. of 96% alcohol

TABLE III

SAMPLE B: TALLOW AND SHEA BUTTER

A. Denmark		
1.	110.0°	After two recrystallizations.
2.	112.8°	After three recrystallizations.
3.	117.5°	After four recrystallizations.
4.	119.6°	After five recrystallizations.
B. Germany		
1.		Sintered at 110°, largest part melted at 130° and melted clear at 145°.
2.	100-102°	After one recrystallization, unsaponifiable = 1.65%; digitonin precipitable sterols = 0.145%. Melting point of free sterols 132-134°.
3.	121.5°	The digitonides precipitate only after one hour.
4.	116.6; 119.2°	After repeated purification.
5.	117.1°	
C. France		
1.	119-120°	
2.	117-118°	
D. Holland	114°; 116°	No clear melting point.
E. Norway		
1.	115.1°	
2.	114.5-115° (corr.)	
F. Switzerland		
1.	113.5°	
2.	108.5°	

4. Carbon tetrachloride
5. Acetic anhydride
6. Alcohol
7. Ether

Preparation of Fatty Acids:

About 100 g. fat are saponified under a reflux condenser with potassium hydroxide; in general, about 15 cc. of solution is used to 10 g. of fat. The soap solution is diluted with 150 cc. of warm water. The clear warm solution is mixed with an excess of hydrochloric acid. When the fatty acids float on top as a clear layer they are separated in a separatory funnel. The acid water is discarded and the fatty acids washed with hot water, in order to remove the remaining alcohol. The washed fatty acids are filtered through a dry folded filter paper. With solid fatty acids the filtration is carried out in an oven or through a hot water funnel.

Preparation of Digitonide:

The entire quantity of fatty acids, or a suitable small quantity, if the sterol content is high, is warmed to about 70° in a beaker and mixed with digitonin solution, using 10 cc. of solution to 20 g. of fatty acids. The digitonin solution is added at one time and with stirring. The mixture is warmed for an hour at 70°, being occasionally stirred with a glass rod. Then 25 cc. of carbon tetrachloride or chloroform is added and the contents filtered with suction on a warmed suction filter. The filtrate should give no more precipitate with digitonin solution. The digitonin crystals are washed three times with warm carbon tetrachloride and five times with ether. The crystals are then dried for ten minutes at 100° on a watch glass. After drying, the precipitate is triturated with ether in a small dish and then filtered in order to remove the last trace of fatty acids. The digitonides remain on the filter in a thin layer and can be easily removed from the filter.

Preparation of Sterol Acetates:

The digitonides are heated for about 15 minutes with 3-5 cc. of acetic anhydride in a flask with a ground glass connection to a reflux condenser. The heating is continued until the contents of the flask becomes clear. While still hot the mixture is treated with 4 times its volume of 50% alcohol. After an hour's cooling in cold water the separated sterol acetates can be filtered with suction and are washed with 50% alcohol. The crystals are dried for 10 minutes at 100° on a watch glass and then recrystallized repeatedly in a refrigerator from 1-2 cc. of absolute alcohol. A porous clay plate serves for pressing out the fraction. After a second recrystallization and drying the melting point is determined.

Preparation of Sterols:

The sterol acetates are saponified with a little (about 3 cc.) N/2 alcoholic potassium hydroxide. After diluting with water, the separated sterols are taken up with ether. The ether solution is washed 1 or 2 times with water, the ether evaporated, and the sterols so obtained taken up with 1 or 2 cc. of 96% alcohol. The sterols are allowed to crystallize out on a slide and the crystal form is then observed.

B. GERMANY

The procedure developed by A. Bömer is not applicable in all cases. The many years' experience of German fat analysts has shown that it is more expedient to undertake the determination of sterols in the unsaponifiable matter. This procedure is universally applied in the recent literature. Also with small quantities of sterols a quantitative determination is thus possible. The following text is recommended as an analytical procedure (see *Schramme*, Fette und Seifen *46*, 443 [1939]).

C. HOLLAND

It was shown that the sterols of shea butter form acetates which are soluble in alcohol; in this case the procedure is thus inaccurate, since by recrystallization in alcohol the sterols of shea butter are washed out.

D. NORWAY

Preparation of Fatty Acids:

The saponification was finished after less than 15 minutes. In both samples the two layers which result after the addition of water and hydrochloric acid were not entirely clear even after remaining constantly on the water bath. For the filtration a hot water funnel was used. The water layer was not entirely clear after running through twice. The fatty acids do not remain on the second filter, as is stated in the directions, but run through dry and clear.

Preparation of Digitonides:

For filtering a hot water suction funnel G_3 was used. The filtration was finished after about 20 minutes without crystallization of the fatty acids when using a weak suction. The precipitation was not entirely completed for any test after a half hour. The filtrate (without wash ether) was still turbid after a new addition of 10 cc. of carbon tetrachloride and further heating on the water bath. From the filtrate of Sample b 0.02 g. (total quantity 0.20 g.) of digitonide was isolated after a half hour. Longer heating on the water bath is therefore recommended. By the washing with ether, after the principal quantity of filtrate was decanted, there resulted in the filter flask a white turbidity along with the small residue. This may be explained by assuming that the digitonin is likely precipitated in a solution of alcohol and/or carbon tetrachloride. By washing with ether the small quantity of excess digitonin can therefore be washed onto the filter. Another washing agent may therefore be more suitable.

Yield: Sample a (45 g. weighed in): 0.07, 0.04, 0.03 g.
Sample b (47 g. weighed in): 0.20, 0.20 g.

Preparation of Sterol Acetates:

The crystallization was carried out in miniature beakers. For the filtration the smallest possible glass frit was used. The crystals were dissolved from the filter each time with a little ether, sucked directly into a new beaker, the ether evaporated, and the residue again crystallized from a little alcohol. If ice is used for cooling, a recrystallization can be carried out in this way in 5-10 minutes. For the melting point determination the crystals are removed from the filter plate with a spatula and dried over night in a vacuum desiccator.

F. SWITZERLAND

On the basis of the melting points Sample a contains plant fat, Sample b none. The microscopic examination shows no clear result, since also with b, beside dense plates of cholesterol, tuftlike, oblong

crystals of the higher melting fatty acids make identification unsatisfactory.

The following supplement was attached (Fellenberg):

In the precipitation the digitonides must be vigorously stirred for some time, otherwise the digitonides may precipitate incompletely and only with difficulty. It should be mentioned, further, that the digitonides after filtering off and separation from the filter should be treated once more with ether, in order to remove the last traces of fatty acids. The double washing out with ether described above is not sufficient. One can proceed as follows: The digitonide is put in a test tube, a few cc. of ether added to it, the solution boiled a short time and then centrifuged out. The residue is again extracted in the same way, freed from ether by immersing the test tube in hot water and then acetylated in the same vessel. The description of the procedure after the acetylation can be completed as follows: The evaporation of acetic anhydride is done in a small crystallizing dish. The residue is dissolved in about 2 cc. of hot 96% alcohol, filtered through a 3 cm. diameter filter into a similar crystallizing dish and washed twice with a little alcohol. The solution is evaporated to about 2 cc. and allowed to crystallize. After 10 to 15 minutes, the solution is filtered through a small filter stick according to the method of Emich, the precipitate is put back into a dish, dissolved in a very little alcohol, evaporated only so far that, while hot, no crystals have separated, and allowed to crystallize again. This time the crystal mash is poured on a clay plate and the crystallization repeated. After the mother liquor is removed, the process is carried out a third time in the same way. Now the melting point is determined. Concerning the preparation of the free sterols the length of time the acetates are saponified is added. Fifteen minutes is here sufficient. For the crystallization of the sterols on the slide it is necessary first to bring the alcoholic solution to the proper concentration. The solution is concentrated in a test tube in stages and a drop poured from time to time on the slide and allowed to crystallize.

III. Oil Determination in Oil Seeds, Oil Cakes, Grist, Etc.

To test

a) a French procedure
b) an Italian procedure
c) a German procedure

The text of the first two working directions is reviewed below:

a) French Procedure:

The solvent used should be given in the analyst's report. It would be desirable to have this fixed in the contract. The International Commission permits ethyl ether, petroleum ether, and carbon disulfide to be used as solvents.

The seed is ground in a mill in such a way that a homogeneous product is obtained and the sample is truly representative. In case the seed contains normal moisture content, it is not necessary to dry it.

Twenty-five grams of this product are placed in a paper shell and exhaustively extracted for six hours in a Soxhlet or similar apparatus, whose flask has been weighed. After this time the shell is removed from the apparatus. The greater part of the adhering solvent is evaporated in air and the meal is ground in a mortar with washed and dried sand, in order to thoroughly divide the material. It is then returned to the apparatus and extracted again for six hours. The flask is then removed, the greater part of the solvent distilled in the boiling water bath, and the last trace removed at moderate temperatures on a sand bath. For this a stream of air or CO_2 may be blown through with a small jet, if necessary. (This is not continued until the occurrence of fatty acid vapors, but only until the weight is constant within approximately .01 g.) The flask is allowed to cool and is weighed. In case the oil is not completely clear the impurities are determined and subtracted.

b) Italian Procedure:

Introduction: The method described below can be used on all kinds of oil seeds. It is especially suitable for such oil seeds which still contain a considerable amount of moisture, perhaps after a technical drying. In general, it can be said that a complete and quick extraction is possible with solvents such as carbon disulfide, trichlorethylene, ethyl ether, and acetone. On the other hand, it has been frequently observed that petroleum ether is not suited for a direct and quick extraction of oil seeds, since it does not have the same penetration and solvent powers as the above-named solvents. This disadvantage is increased by the presence of moisture in the seeds to be studied. Because fat extracted with carbon disulfide, ethyl ether, trichlorethylene, or acetone is always contaminated with other components, the use of petroleum ether is to be employed in a second step of the procedure on account of its specific solvent properties for fat. The following method is based on these observations:

Methods of Analysis: About 30 g. of oil seed is finely ground (about to the fineness of ground coffee; in any case, the grinding must be of such a nature that sufficient penetration with solvent is possible; it cannot be so fine, however, that the normal Soxhlet extraction is disturbed) and accurately weighed into an extraction shell. On the seed is placed a cotton plug, then a small round piece of filter paper. This prepared extraction shell is then placed in a Soxhlet apparatus whose flask has been previously weighed. The Soxhlet apparatus should be equipped with a ground stopcock which regulates the drainage. The use of apparatus with standard joints is recommended. Through the turning of the stopcock the flow is stopped. The necessary amount of acetone pro analysi is added for the extraction and the extractor allowed to stand overnight (14-15 hours) with the condenser running. The next morning the drainage cock is opened and the extraction begun on a boiling water bath. In one hour the solvent should run through 6 to 8 times (with a Soxhlet of about 200 cc. content). After 4 hours the extraction is interrupted and the extraction shell removed from the apparatus. The oil seed, soaked with solvent, is well spread out to evaporate the solvent. In order to divide the seed still more, it is ground once again in an ordinary coffee mill. After it has been quantitatively returned to the extraction shell, it is reextracted 2 or 3 hours. After this time the flask containing the fat solution is removed, the solvent distilled off completely, and the meal reextracted with 80 to 100 cc. of petroleum ether (b.p. 35-55°). The solution is then allowed to stand for one-half hour and is filtered through a filter paper into a

weighed flask. A slight turbidity of the filtrate has no noticeable influence on the accuracy of determination of fat content. The petroleum ether is then distilled off, the flask and contents are dried for one hour in a drying oven at 105°, weighed, and the drying repeated to constant weight.

c) German Procedure:

On the basis of the investigation carried out by the German delegation, the German working procedure has been subjected to an exhaustive working over. All of the ascertained results have been considered which should support the wording quoted later. The firms given below in the text participated, that is, the investigators, respectively, of the firms of *Noblee und Thörl*, Hamburg-Harburg, and *F. Thörl's Vereinigte Harburger Ölfabriken A. G.*, Hamburg-Harburg. On the basis of expediency repetition of the original procedure is not made, as it is found in these collected reports.

For the cooperative investigation, the following oil seeds or oil cakes were sent in behalf of the D.G.F. by the firm *F. Thörl's Vereinigte Harburger Ölfabriken A. G.*, Hamburg-Harburg.

1. Linseed
2. Soy beans
3. Palm kernels
4. Peanuts
5. Copra
6. Peanut cake

The following investigators participated:

A. DENMARK
S. B. Holmsteen, Aarhus Oliefabrik A/S, Aarhus

B. GERMANY
1. *Frl. Dr. Wittern*, Harburger Ölwerke Brinckman & Mergell, Hamburg-Harburg
2. *Dr. Detert*, F. Thorl's Vereinigte Ölfabriken A. G., Hamburg-Harburg
3. *Dr. Delvaux*, Louvain-Belgien
4. *Dr. Rosenbaum*, Van den Bergh's Margarine GmbH, Kleve/Rhld.

C. ENGLAND
International Association of seed crushers, London. (*Dr. A. B. Shepherd*)

D. FRANCE
1. *Prof. Margaillan*, Marseille
2. Laboratorium *Vizern & Guillot*, Marseille

TABLE IV

1. OIL CONTENT FOUND FOR LINSEED (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	36.9	5.7
B. Germany						
1.	36.8	.....	.....	35.4	36.8	.....
2.	.....	.....	.....	.....	36.8	8.2
3.	35.4	.....	.....	35.0	35.7	.....
4.	37.4	36.1	38.1	35.8	35.8	7.4
C. England (I.A.S.C.)	38.2	37.1	.....	37.4	37.5	.....
D. France						
1.	.....	37.5	.....	37.8	37.7	.....
2.	.....	37.8	38.0	37.6	37.3	.....
E. Holland	.....	38.0	.....	38.4	37.6	7.4
F. Italy						
1.	.....	36.5	.....	36.2	36.3	.....
2.	36.4	.....	.....	33.2	37.0	.....
3.	35.4	36.3	.....	36.3	34.4	.....
4.	.....	36.7	.....	.....	36.2; 36.4	5.6
G. Norway	37.5	.....	.....	36.1	36.3	6.7
H. Switzerland						
1.	.....	33.3	.....	.....	33.6	7.9
2.	.....	.....	.....	.....	36.4	6.3

TABLE V

2. OIL CONTENT FOUND FOR SOYBEANS (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	17.1	12.8
B. Germany						
1.	17.1	.....	.....	17.4	17.6	.....
2.	.....	.....	.....	.....	17.4	13.9
3.	17.2	.....	.....	17.8	17.3	.....
4.	17.9	17.0	18.0	17.1	17.0	12.7
C. England (I.A.S.C.)	18.2	17.4	.....	18.4	17.3	.....
D. France						
1.	17.4	.....	.....	17.7	17.3	.....
2.	.....	.....	18.0	17.9	.....	.....
E. Holland	.....	17.4	.....	17.7	17.3	13.4
F. Italy						
1.	.....	17.0	.....	17.3	17.4	.....
2.	17.9	.....	.....	18.1	16.9	.....
3.	17.9	.....	.....	18.7	17.7	.....
4.	.....	16.4; 17.1	.....	.....	17.5; 17.4	10.8 11.1
G. Norway	17.9	.....	.....	17.9	17.4	13.3
H. Switzerland						
1.	.....	17.4	.....	.....	17.5	11.7
2.	.....	.....	.....	.....	17.8	12.9

E. HOLLAND
Dr. Voerman, 'S-Gravenhage, Laboratorium des Reichsbüros für Untersuchungen von Handelswaren und Vereinigung der holländischen Ölfabriken

F. ITALY
1. *Dr. Martinenghi* und *Dr. Rossi*, R. Stazione Sperimentale per le Industrie degli Olii e dei Grassi, Milano
2. *Dr. Paleni*, S. A. Gaslini, Genova
3. *Dr. Loew*, S. A. Spremitura Triestina, Trieste
4. The investigation with the German and French methods *Dr. Rossi* carried out at the Pharmazeutischen Institut in Münster (Germany)

G. NORWAY
Frl. Dipl. Ing. Turid Wik, Zentrallaboratorium der A/S Lilleberg Fabriker, Oslo

H. SWITZERLAND
1. *Dr. Viollier*
2. *Dr. Weder*, St. Gallen

The results obtained (average values) by the individual investigators are collected in Tables IV to IX.

TABLE VI

3. OIL CONTENT FOUND FOR PALM KERNELS (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	51.5	6.1
B. Germany						
1.	49.8	.....	.....	49.3	50.6	.....
2.	.....	.....	.....	.....	50.4	6.3
3.	49.0	.....	.....	49.3	49.4	.....
4.	51.2	50.4	50.9	50.5	50.6	6.9
C. England (I.A.S.C.)	53.0	52.3	.....	52.2	52.7	.....
D. France						
1.	.....	50.3	.....	50.6	.....	.....
2.	.....	50.3	50.5	.....	.....	.....
E. Holland	.....	51.1	.....	51.4	50.7	6.1
F. Italy						
1.	.....	49.9	.....	50.1	50.1	.....
2.	51.0	.....	.....	51.2	51.2	.....
3.	50.2	.....	.....	50.0	49.9	.....
4.	.....	50.0	.....	.....	50.2	5.5
G. Norway	50.5	.....	.....	50.0	50.2	6.1
H. Switzerland						
1.	.....	50.1	.....	.....	49.4	6.0
2.	.....	.....	.....	.....	48.7	5.7

Attitudes:

a) On the French Method:

B. GERMANY

The German delegation declines to allow the choice of three separate solvents for extraction. The extraction should be done with *petroleum ether*. Consequently, regarding the differences between seeds, a detailed description is necessary.

C. ENGLAND (I.A.S.C.)

It must be positively stated in the procedure which solvent should be used. Insofar as the seeds exhibit a normal water content, drying before extraction is not necessary. The sample weight of 25 g. is too large. It means a large extraction apparatus, a long extraction time, and makes the grinding of the seed after the first extraction many times more difficult. Also, the drying time of the larger amount of oil derived by the extraction is much longer. The results are without doubt accurate if a weight of only 10 g. is chosen.

D. FRANCE

The results obtained by the different methods vary only a little from each other. They lie mostly within the limit of error. Consistently higher values were obtained by the use of carbon disulfide as a solvent, moreover, it is difficult to extract the oil completely from copra and even palm kernels. The French and German procedure are almost identical in their details. The French delegation takes a lower sample weight as being more convenient, and omits a preliminary drying of the seeds.

E. HOLLAND

The French method gives a choice of three solvents. Petroleum ether (40-60°) should be used. The designation in the procedure of a definite solvent is recommended. Since the sample weighed is very large, the drying and also the whole determination consumes too much time.

F. ITALY

The French method is equal to the German (vide) in general usefulness.

G. NORWAY

Ethyl ether is used as a solvent in all investigations. Consequently, the drying is conducted in air. There was obtained for example cloudy oil samples from peanuts (dissolved phosphatides and similar substances). In our experience there is always obtained from undried peanuts an oil content 0.1 to 0.5 per cent higher than from peanuts which were dried for an hour at 100-103°. This fact is especially noticeable in partly damaged peanuts with a high fatty acid content. According to our experience, four

TABLE VII

4. OIL CONTENT FOUND FOR PEANUTS (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	47.9	5.7
B. Germany						
1.	47.1	.....	.....	46.4	46.8	.....
2.	.....	.....	.....	.....	47.0	6.0
3.	47.3	.....	.....	47.1	47.3	.....
4.	47.5	47.1	47.2	46.5	47.0	5.4
C. England (I.A.S.C.)	47.8	47.6	.....	47.1	47.6	.....
D. France						
1.	.....	47.2	.....	47.6	47.2	.....
2.	.....	47.7	48.0	47.3	47.9	.....
E. Holland	.....	47.4	.....	45.6	47.1	5.2
F. Italy						
1.	.....	47.6	.....	47.7	47.8	.....
2.	47.6	.....	.....	47.6	47.3	.....
3.	48.4	.....	.....	48.0	48.2	.....
4.	.....	47.8	.....	.....	47.8; 47.4	4.7
G. Norway	48.1	.....	.....	47.6	47.4	5.6
H. Switzerland						
1.	.....	47.5	.....	.....	48.2	5.6
2.	.....	.....	.....	.....	46.7	8.2

TABLE VIII

5. OIL CONTENT FOUND FOR COPRA (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	66.1	4.6
B. Germany						
1.	66.9	.....	.....	64.8	66.6	.....
2.	.....	.....	.....	.....	66.6	4.7
3.	63.6	.....	.....	63.5	63.6	.....
4.	66.6	65.7	66.0	64.5	65.5	4.6
C. England (I.A.S.C.)	68.2	67.8	.....	68.0	67.9	.....
D. France						
1.	.....	67.2	.....	.....	.....	.....
2.	.....	.....	67.4	.....	.....	.....
E. Holland	.....	66.8	.....	66.3	66.1	4.4
F. Italy						
1.	.....	60.0	.....	65.4	65.4	.....
2.	66.2	.....	.....	64.8	67.4	.....
3.	68.0	.....	.....	67.0	68.0	.....
4.	.....	66.1	.....	.....	65.5	4.2
G. Norway	65.3	.....	.....	64.1	64.7	4.6
H. Switzerland						
1.	.....	63.4	.....	.....	65.3	4.8
2.	.....	.....	.....	.....	65.7	4.4

TABLE IX

6. OIL CONTENT FOUND FOR PEANUT CAKE (IN PER CENT)

	French Procedure Ether	French Procedure Petroleum Ether	French Procedure CS_2	Italian Procedure	German Procedure Oil and Water Content	
A. Denmark	.....	.....	.....	.....	6.5	.....
B. Germany						
1.	6.3	.....	.....	5.6	6.6	.....
2.	.....	.....	.....	.....	6.2	9.8
3.	6.0	.....	.....	.....	6.0	.....
4.	6.2	6.1	6.5	6.2	6.2	9.6
					Protein content: 48.5%	
C. England (I.A.S.C.)	.....	.....	.....	.....	.....	.....
D. France						
1.	.....	6.9	.....	7.1	7.0	.....
2.	.....	.....	7.0	7.0	.....	.....
E. Holland	.....	6.3	.....	6.4	6.3	8.8
F. Italy						
1.	.....	6.3	.....	6.3	6.3	.....
2.	6.6	.....	.....	6.5	6.2	.....
3.	6.2	.....	.....	6.8	6.1	.....
4.	.....	6.2	.....	.....	6.3	6.4
G. Norway	6.6	.....	.....	6.6	6.5	9.2
					Protein content: 51.0%	
H. Switzerland						
1.	.....	.....	.....	.....	5.8	9.6
					Protein content: 49.7%	
2.	.....	.....	.....	.....	6.6	7.9

hours' extraction with ethyl ether in a Soxhlet is sufficient. Five grams of seeds are weighed out, which have been dried previously for an hour, at 100-103°. In certain cases we have found up to 1 per cent more oil in cake which had not been previously dried. We view 25 g. as an unnecessarily large sample, especially when the seeds are previously well mixed and ground. Such large samples need large extraction flasks and thimbles and consequently entail much loss of solvent.

b) On the Italian Method:

A. DENMARK

The disadvantage noted in the Italian method is that at high water content (e.g. for olives) the acetone is greatly diluted, so that the extraction is not with pure acetone, but is carried out with a water-acetone solution.

B. GERMANY

The Italian method can give accurate results when carefully done. It is, however, too detailed for commercial use.

C. ENGLAND (I.A.S.C.)

A weight of 30 g. is too great; it needs an especially large extractor. The method requires too much time and it is difficult to dry the large amount of oil obtained to constant weight.

D. FRANCE

The preliminary extraction with acetone and the additional extraction with petroleum ether by no means show especial advantage but, on the contrary, it must be understood to be a disadvantage. It might be that the seeds contain a large amount of soluble impurities which produce no fat; in this case the subsequent extraction by petroleum ether is, of course, unavoidable.

E. HOLLAND

The weight used in this method is also too large. The whole determination, especially the drying, takes very much time. The second grinding of the seed described after the first extraction cannot be done in a coffee mill, since in this case the seed residue can be removed quantitatively only with great difficulty but must be done in a mortar with the aid of sand. The extract obtained by the Italian method from peanuts, soy beans, and linseed was not clear. Consequently a further solution in petroleum ether and subsequent filtration was necessary.

F. ITALY

The Italian method has the advantage that it should be useful where the extraction must be carried out in the presence of water, large amounts of fatty acids in the oil, and considerable amounts of impurities such as oxidized and polymerized fatty acids, components which occasionally hinder a normal extraction. This is the case, for example, in grape seeds containing old or partly rancid oil. Below are given the results which were obtained by using three different kinds of grape seeds. The seeds were first extracted with ethyl ether or acetone and the extract obtained treated with petroleum ether to remove the impurities present. The seeds were already dried by the industrial process of recovery. It is therefore proposed to use the Italian acetone method for the analysis of certain seeds, such as old grape seeds and the like. For all usual determinations of oil content, the German method shall be used (vide).

Sample No.	Water	Oil Content by Extraction With Ether	Oil Content by Extraction With Acetone	Petroleum Ether Extract of Ether	Petroleum Ether Extract of Acetone
1.	10.93	13.80	14.45	12.13	12.39
2.	8.27	16.30	16.51	16.19	16.23
3.	9.10	14.86	15.03	14.66	14.88

G. NORWAY

This method is viewed as not unobjectionable. Like the French method, the Italian method prescribes altogether too much sample, and much acetone is lost by drying the meal. The second part of the method appears hazy to us. Since a Soxhlet is used, which contains 200 cc. and in another place the amount of petroleum ether used is given as 80 to 100 cc., one has to assume that the petroleum ether should be used to dissolve the extracted oil in the flask. Washing of the filter claims much time and one loses much solvent through evaporation.

c) On the German Method:

A. DENMARK

The extraction is not yet completed after 4 + 2 hours because by several more 2-hour periods a considerable amount of fat can be extracted; this can be traced back to an insufficient extraction or to an insufficient grinding of the seeds. Modifications: to section 16: use 10 g. samples; to section 22: extract for 12 hours, paying attention to vigorous boiling and free percolation of the petroleum ether, then dry the thimble and extracted material for one-half hour at 105° C. For drying and semi-drying oils, drying is done in an atmosphere of carbon dioxide. Next grind the residue in a dish with pumice or equivalent. A coffee mill can also be used. After repeated grinding, the residue is extracted for 3 more hours. For the second extraction the same thimble, the same cotton wad, and the same flask are used. After a lapse of 3 hours the thimble is dried as before, the contents ground once more (in a coffee mill, if so desired) until all passes a 1-mm. sieve. Then the residue is extracted for three more hours as a control. This last period should extract none or very little material.

B. GERMANY

On the basis of the researches carried out on the part of the German delegation, the German method, as previously explained, contains the following *new wording:*

I. Oil Seeds and Oil Fruits

(1) *Taking the Sample:*

a) *Preliminary:* For taking the sample the oil seeds and fruits are suitably divided into three groups:

1. Copra; Babussa, Palm and Tukuman nuts
2. Unshelled peanuts
3. Shelled peanuts, cottonseed, sunflower seeds, soy beans, linseed, rapeseed, turnip seed, sesame, gold-of-pleasure (Camelina Sativa), poppy seed, etc.

(2) b) *Procedure:*

1. *Taking a sample by sampling the lot (Thief tests).*

Here is included sample taking from open cargo, tanks, silos, etc., or from sacks. Accordingly we will carry out the process for the seed groups named above as follows:

(3) *For a 1:* For each lot 10 sacks are selected out of every 100 tons by the representatives of the buyer and seller. The total contents of the sacks

are shaken into a heap and thoroughly mixed with a shovel. The heap is then quartered by the usual method as used in ore, coal, etc., and the diagonal quarters taken. The opposite quarters and their accompanying dust are discarded, and the remaining quarters remixed and redivided the same way as before. This quartering is carried on until a sample of at least 25 kg. remains, which is divided into 5-kg. tin cans with tight covers. The can lid and label are to be furnished and secured with suitable seals. The remainder of the sample serves both parties as a reserve sample.

(4) *For a 2:* For unshelled peanuts the sample taking is carried out according to international provision (Bordeaux usage).

(5) *For a 3:* The samples are taken from sacks with a thief. From open holds, tanks, silos, etc., the sample is taken from separate places as done for Group 1.

(6) 2) *Sampling in the factory.*
This is done by the previous method when unloading the seeds. Should samples be taken of seeds going through the processes (works or factory sampling), this must be done so that a true average of the processed seed is taken. Consequently the sample must be taken over a sufficiently long time, at least 8 hours.

(7) *For a 1:* The sample shall be taken every half hour just after the preliminary grinder, stored in a tightly covered can and well mixed to give an average over the shift or over the day. Also in this case the quantity of the sample shall be at least 5 kg.

(8) *For a 2 and a 3:* The sample shall be taken from a feed pipe perpendicular to a cleaner or sheller and shall be treated according to a 3.

(9) 3) *Packing the sample.*
All samples are to be packed in tightly closed tin or glass containers. (By the use of the jute bags, customary in times past, loss of dust and water could take place.) The manner of packing is given in the report of the investigation.

General Analytical Method

(10) *Fat determination:* All seeds and fruits with normal water content are ground, extracted, reground and reextracted.

(11) For an extraordinarily high water content (occurring in moist, fresh seeds, like rape and turnip seed, cereal germs, and oily fruits which have suffered through dampness, heating or sea water in storage) the seeds are dried before grinding.

(12) b) *Water determination:* This is done for normal water content by drying the material previously ground for extraction.

(13) For an extraordinarily high water content (section 11), the material is dried before grinding, ground and then the residual water determined.

(14) *Grinding:* At least 2 kg. are ground into pieces of 2 mm. diameter. For extraordinarily high content of dirt, the material is sieved before grinding and examined separately. The following machines are used:

a) For copra,[2] Babassu nuts and shelled peanuts use a rasp mill.[1]
b) For palm nuts,[2] Tukuman nuts, unshelled peanuts, soy beans, sunflower seeds, etc., use a disc mill.
c) For cottonseed, rape, turnip seed, linseed, poppy, hemp, sesame, etc., use a disc or coffee mill.

If machine grinding is difficult, about 25 g. from the well-mixed, larger sample is thoroughly crushed in a mortar.

(15) *Fat determination:*
Explanation: The determination is carried out with petroleum ether. The extract consists chiefly of fats (glycerides and free fatty acids) to which is added the natural unsaponifiables and unimportant amounts of other petroleum ether soluble substances (lipoids, etc.).

(16) a) *Treatment of normal seeds and fruits* (Section 10).
Depending on fat content, 5-10 g. of substance is weighed into a thimble immediately after grinding and covered with a disc of filter paper, the thimble is then closed with a cotton plug.[3] A Soxhlet extractor is used. Petroleum ether, boiling between 45 and 55° is tested according to the following directions:
One hundred cc. of petroleum ether is distilled from 3 g. of a water-free, neutral, stripped, non-drying oil and the oil dried for two hours at 105° C. The increase in weight must not be more than 5 mg.
After 4 hours of vigorous extraction the thimble is freed of solvent, the extraction residue ground to a fine dust with sea sand [4] and extracted further for 2 hours in the same thimble. The thimble is closed with the same cotton wad as previously used and which has also been used to transfer traces of fat and dust particles from the mortar.

(17) After extraction the petroleum ether is driven off, the residue dried at 105° or at 60 to 70° in vacuum (this drying takes at most 2 to 3 hours or one-half to 1 hour, respectively). For drying oils, either vacuum is used or an atmosphere of carbon dioxide. The weight of petroleum ether extract is considered constant if its percentage content does not change by more than 0.1 after successive quarter-hour drying periods.

(18) b) *Treatment of wet seeds and fruits* (Section 11).
For materials of section 14a and b the extraction thimble is placed in glass container after determining the weight of the ground material in it, and dried for one hour at 105°. Some seeping fat is taken up with a cotton wad wet with petroleum ether, with which the thimble is closed. Then it is extracted and treated as for normal seeds.

(19) For materials of section 14c, about 25 g. of the well-mixed, large-size test material is weighed

[1] Excessive grinding and crushing is to be avoided.
[2] When the sample is sufficiently large, pre-grinding with a small breaker is recommended.
[3] It is important that the cotton be free of extractables; this is often not the case.
[4] The sand was prepared as follows: boiled with concentrated hydrochloric acid, washed free of acid, and ignited.

into a dish and dried for 1 hour at 105°, after which the loss of water is determined and the material ground. It is then treated as with ordinary seeds. The fat content is calculated on the basis of the weight of undried material.

(20) For drying oils, the preliminary drying of sections 18 and 19 takes place in vacuum at 60-70°, or in carbon dioxide at 105°.

(21) *Determination of water.*

a) *Procedure with normal seeds and fruits.* Immediately after grinding about 5 g. are weighed into a stoppered flask and dried with the stopper out at 105° (in carbon dioxide for drying oils) or at 60-70° in vacuum. The weight is taken as constant if the percentage content does not change more than 0.1 after repeated quarter-hour drying periods.

(22) b) *Procedure with moist seeds and fruits* (Section 11).

About 25 g. are weighed into a dish and dried for one hour at 105°. The loss of water is determined. It is then ground and the residual water in about 5 g. is determined as for normal seeds (Section 21). After both dryings, total water content is calculated.

II. Oil Cake and Meal

(1) *Pretreatment:*

The sample is so ground with an appropriate mill, that the whole amount will pass a 1-mm. sieve. Immediately after screening the sample is thoroughly mixed and divided by the quartering method (see Investigation of Oil Seeds, Section 3) so that finally the mixing of two quarters gives a sufficient amount for weighing. In case the weighing can not follow immediately, the material is transferred to a tightly closed vessel to avoid loss of water.

(2) For cake and meal which have suffered damage and consequently show an inordinately large water content, the prescribed grinding is possible only after drying, which accordingly follows as described in Section 19 on oil seeds.

(3) *Determination of water:*

About 5 g. of sample is dried in a weighing bottle for 3 hours at 105°. After cooling in a desiccator it is weighed.

(4) *Determination of fat:*

The determination is carried out with ethyl ether. About 10 g. of material is weighed into an extraction thimble and dried at 80° for 1½ hours. The thimble is closed with a wad of extract-free cotton. The extraction follows in the customary apparatus.[1] After a 4-hour extraction the residue is dried as described for oil seeds in Section 16.[2] In contrast to oil seeds, cake and meal are extracted with ethyl ether because in this case besides pure oil also the nutritionally important lipoids and similar substances are to be obtained.

(5) *Protein determination by the Kjeldahl method.*

One g. of finely ground substance is weighed into a Kjeldahl flask and decomposed with 20 cc. of nitrogen-free concentrated sulfuric acid, 1 g. of mercury or mercuric oxide and about 20 g. of potassium sulfate. This mixture is decomposed at first by a small flame. Towards the end a larger flame is used until the mixture becomes colorless.

After an additional heating of from 10-20 minutes, the solution is allowed to cool, then there is added 250 cc. of distilled water, 1 g. zinc dust and a lye mixture which consists of 80 cc. of sodium hydroxide solution (D = 1.38) and 20 cc. of saturated potassium sulfide solution (40 g. potassium sulfide in 1000 cc. of water).[3] Previously exactly 75 cc. of a standard $\frac{n}{10}$ sulfuric acid solution[4] has been placed in a receiver to which a drop of methyl orange has been added. The free ammonia is now distilled into the receiver, which takes about 20 minutes. The unchanged sulfuric acid is back-titrated with standard $\frac{n}{10}$ sodium hydroxide.

Calculations: if e = weight of material
a = acid added
b = acid back titrated
a − b = c = alkali used

then % nitrogen = $\frac{(c)(0.0014)(100)}{e}$

and % Protein = % nitrogen × 6.25.

(6) The recent decomposition method using selenium, as proposed by Wieninger, may be mentioned at this point. This method can of course be used, but it is recommended that the certificate state which method has been used.

(7) The literature reference is: König, "Untersuchungen landwirtschaftlicher und gewerblich wichtiger Stoffe," p. 172 ff. & 248 ff. Publisher: Paul Parey. Berlin. 1929.

G. England (I.A.S.C.)

A weight of 5-10 g. (according to oil content) is thought correct. Also it should be emphasized, that the extraction should be undertaken as soon as possible after taking the sample, however it it not considered necessary to dry beforehand seeds with a normal water content, but insofar as drying is necessary, a drying temperature of 100° is thought sufficient. The following statements of the German method should be recognized as being especially important:

1. That for unusually high contents of impurities, an attempt should be made to remove and identify them.
2. That the cotton wad be fat-free.
3. That an especially quick sampling and a relatively slow determination of extraction value should take place when the content of free fatty acids in the oil makes it necessary.
4. That a previous washing and ignition of the sand used is necessary.

For water determination in seeds, oil cake, and meal a drying temperature of 102° is considered adequate. With oil cakes and meal it is generally necessary to dry material before extracting the oil. Inasmuch as oil cake and meal is not again pulverized and extracted, a corresponding grinding of the material (fine as dust) is undertaken before the ex-

[1] The ether must be peroxide free, else explosions may occur.
[2] After drying, ether soluble lipoids can be extracted.
[3] Strong foaming can be decreased by addition of about one-half g. of paraffin.
[4] Hydrochloric acid is permissible in the receiver, but not in the Kjeldahl.

traction. For protein determination it is considered expedient to use 2 g. of substance and $\frac{N}{5}$ solutions because in this case experimental errors are considerably lessened. If a weight of 2 g. is chosen, 25 cc. of concentrated sulfuric acid should be used, also it is considered better to use 10 g. potassium sulfate instead of 20 g. since otherwise the residue is very hard and can be dissolved only with difficulty.

D. FRANCE

See the French method.

E. HOLLAND

Since no vacuum drying apparatus was available, the material was dried at 80° to constant weight. The extraction of the cake with ethyl ether and the extraction of the seeds with petroleum ether made calculation of the yield difficult as ether dissolves other substances in addition to fat.*

F. ITALY

The German method is considered generally useful. The following changes in the method are recommended:

a) To Section 10: Instead of 2 kg. only 500 g. of seed should be ground in the preparation of the sample for analysis.

b) To Section 18: It is not deemed necessary in every case to dry the seeds before extraction. The French method is selected as being better here; inasmuch as the seeds contain normal moisture, a preliminary drying is unnecessary.

c) To Section 21: The boiling range given for petroleum ether as 45-55° should be changed to 40-60°.

d) To Section 22: An extraction of 4 + 2 hours is perhaps not always sufficient for a complete extraction, whereas the French method, 6+ 6 hours is thought too long. It is suggested to investigate the method devised by the Italian delegation: Allow the ground material to stand in the solvent in a Soxhlet overnight (14-15 hours) and then extract for 4 + 2 hours. By this means considerable time is saved.

e) To Section 16: A weight of 5-10 g. is thought too small; it has the danger that no correct average sample is subject to analysis, also by using a small weight an accurate determination of acid number is difficult. A weight of 10-30 g. is therefore recommended.

G. NORWAY

The German method is simple and clear and requires no longer an extraction time than is necessary. Yet it is a disadvantage that it requires different solvents for cake and for seeds, especially in the case where the analysis might be applied in trade agreements and as a basis of horticultural calculation, if the differences between samples determined in this way are not large.*

General

A. DENMARK

The German procedure appears to be the most appropriate of the methods proposed.

* See footnote to the table entitled: "Per Cent of Oil by the Method of France, Italy, Germany, and I.A.S.C."

C. ENGLAND

In this connection the method used by the International Association of Seed Crushers (I.A.S.C.), London, should be mentioned:

The preparation of the material used for analysis is especially important; also attention must be paid so that the weighing takes place on a true cross-sectional sample.

Five g. (or 10 g. with seeds of low oil content) are weighed in an extraction thimble made of filter paper and covered with a wad of cotton.

The extraction thimble is placed in a Soxhlet or similar apparatus and extracted for 4 hours with petroleum ether (b. p. 40-60°; this petroleum ether is in general use in Great Britain as a solvent).

The residue is ground in a mortar and again extracted for 4 hours with petroleum ether. This second grinding must be carried out very carefully. For this object the grinding must be carried out for many minutes until the seed meal becomes fine as dust. Fine sand may be used to aid the grinding. If the material is not fine enough, the petroleum ether may not be able to extract the last 0.5% of fat. At the end of the second extraction, the solvent is distilled off and the oil dried at 100-103° to constant weight.

Comparative tests carried out by the I.A.C.S., London, give the following results (average value) (Table X):

TABLE X

PER CENT OF OIL BY THE METHOD OF

Seed	France Pet. Ether*	France Ether*	Italy	Germany	I.A.S.C.
Linseed	37.1	38.2	37.4	37.5	37.7
Soybeans	17.4	18.2	18.4	17.3	17.4
Palm kernels	52.3	53.0	52.2	52.7	52.3
Peanuts	47.6	47.8	47.1	47.6	47.6
Copra	67.8	68.2	68.0	67.9	67.8

* In this connection there was emphasized for the German part in section 4, paragraph 2, of the German investigation procedure for oil cake and meal, based on the observation reported, that the necessity of an ethyl ether extraction is sufficiently established.

Dr. Shepherd made the following remarks. A heating or drying of seed meal before extraction is not advisable since low results can very easily be obtained. If the test shows an especially high moisture content or danger of loss of moisture taking place during grinding, the moisture content is determined both on the original sample and on the sample ground for test and consideration given to the difference so obtained. A weight of 5 g. is in almost all cases sufficient; it is small enough to undergo a quick and complete extraction and is sufficient to give a good average sample. The simple through dropping apparatus * serves as the extractor; this makes possible a complete saturation of the seed with the solvent vapor and allows the condensed solvent to flow through freely. In this manner the extraction is completed in a short time. Erlenmeyers are used as extraction flasks. These have a particularly large bottom which facilitates boiling the solvent. They also use a small stopper and the solvent does not spurt on the upper part of the flask wall.

In some laboratories it is customary to warm or heat the seed meal. To this end, the extraction thimble containing the ground seeds is warmed for 15-45 min. in an oven heated by water. This pre-treatment

* Translators' note: This is probably a Butt extractor.

should cause a partial drying of the seeds and a coagulation of proteinaceous material. This pre-treatment is not considered necessary (it may be if the seeds are especially damp) since hereby it would be very easy to obtain low values. These low values are found because the fat residue is very difficult to extract after this treatment, especially when petroleum ether is used as solvent. The extraction should be carried out for 4 + 4 hours. In most cases 3 + 3 hours is sufficient, however for standard procedure an extraction of 4 + 4 hours is to be used. For oil cake and meal which contain only a small amount of fat, a first extraction of three hours and a second of one hour following the usual grinding is sufficient.

F. ITALY

(1) It is necessary to differentiate between an *"oil seed analysis"* and a *"determination of oil content in oil seeds."* In the first case we propose the German wording with the omission previously stated, for the determination of oil content of oil seeds, the following is proposed:

1. The solvent used must be stated in the investigation report. It is desirable that the solvent be stated in the separate contracts. As solvent, the following may be used: Petroleum ether, ethyl ether, and carbon disulfide. If the kind of solvent is not stated in the investigation report, petroleum ether is to be used. If the seeds contain important amounts of surface moisture (unnatural moisture), they are dried before crushing. The seeds are then ground in a mill which gives sufficiently fine grinding; 20-30 g. of the material so prepared is weighed into an extraction thimble and placed in a Soxhlet. The Soxhlet shall be equipped with a stop cock which regulates the speed of flow, also Soxhlets with standard taper joints may be used. The cock is then closed, an amount of solvent sufficient for extraction poured into the Soxhlet and the ground material allowed to stand in the solvent over night (14 to 15 hours). The following morning the stop cock is opened and the extraction begun. The distillation and reflux shall be so regulated that the solvent should run through 6-8 times per hour in a 200 cc. Soxhlet. After four hours the extraction is stopped, and the thimble removed from the apparatus. The residue is spread on a surface which is as large as possible, and the solvent driven off. The residue is ground in a mill to pass a sieve of 256 meshes per square centimeter. The residue is again placed in the thimble and extracted 2-3 hours more. After driving off the solvent, the residue is dried to constant weight at 105°. The constant weight is reached when two consecutive weighings differ by no more than 5 mg.

2. It is recognized as necessary to measure the fineness of the ground seed by means of a sieve. Since this is not always possible in seeds containing oil, the measurement should be made after the first extraction, as specified above. There are seeds, for example, grape seeds, whose strong woody cell walls are penetrated by the solvent with difficulty. The determination of fineness assumes importance on this account. The desired fineness is reached when the seed meal will totally pass a sieve of 256 meshes per square centimeter.

The Effect of Glyceryl Monostearate on the Baking Properties of Cakes

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Within the last few years various substances known as "addition agents" have been patented and used by certain commercial firms for the improvement of shortening. The role of these agents in baked products, for the most part, appears to be that of a stabilizer which, by virtue of its emulsifying properties, produces an unusually smooth, well-combined batter with a capacity for carrying relatively large amounts of sugar and moisture and with a marked resistance to variable conditions of baking and handling.

In cakes, especially those made with a high proportion of sugar and/or liquid to flour, the main beneficial effect of addition agents is to decrease markedly the customary tendency of such cakes to shrink during baking and cooling (2, 4, 7). Thus, successful high-sugar, high-moisture cakes, which are of particular commercial interest because they are described as having advantages over ordinary cakes not only in volume but also in color, texture, flavor, grain, keeping quality, and cost, have been made possible by the inclusion of suitable addition substances in the formulae (1, 2, 4). As far as characteristics other than volume are concerned in cakes made from conventional proportions of ingredients, at least one worker (4) claims to have obtained better color, texture, grain, and keeping qualities with an addition agent than without one. Of course, it should be borne in mind that the specific effects produced in a baked product by an improving substance may vary widely with the type and quantity of the substance used as well as with the baking formula and method of combining ingredients.

In view of the fact that most of the work found in the literature on addition agents seemed to emphasize their use with formulae of the high-sugar, high-moisture type, the study about to be reported was undertaken to determine the effect of such a substance, a glyceryl monostearate preparation, with several basic types of fats on the characteristics of a plain cake made from a conventional recipe.

The experimental work of the study included the baking and testing of cakes made both with and without a monostearate addition agent; using as shortenings butter, a type of hydrogenated vegetable oil, a modified lard which was partially hydrogenated, and an oleomargarine consisting of 100 per cent hydrogenated cottonseed oil with a saponification value of 190 to 192. As a matter of interest, a commercial preparation of monoglycerides whose exact composition was not available was employed as the improving substance for one additional group of butter cakes.