International Chemical Union

Minutes of Meeting of International Commission on Fats and Oils. Part II.

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THIS translation gives the minutes of the meeting in London during July 1947, incorporating the decisions made on the unified international methods and the plans for collaborative work to be done before the next meeting of the Commission. Methods under discussion included those for the sampling and analysis for moisture and oil specified for several oil-bearing seeds. They also included those for alkalis and rosin in soaps and those for soluble and insoluble volatile acids and sterols in fats and oil, also for the determination of thiocyanogen and peroxide numbers in fats and oil. The organization of and representation on the present International Commission on Fats and Oils are also given.

SUPPLEMENT I_1

Analysis of Oleaginous Seeds

a) *Recommendations Concerning the Sample.* To permit a correct analysis the sample of large seeds such as coconut, shea, etc., and of medium size seeds such as palm kernel, peanut, and soya should weigh from 2 to 5 kilos, and of small seed such as flax, rape, and sesame the sample should weigh at least 200 grams.

b) *Conditions of Analyses.* The moisture and oil contents shall be expressed as a percentage of the original sample containing any impurities present in it. To satisfy the demands of some parties these values may be indicated for the pure seed sample. Depending on the circumstances, the chemist will conduct the determinations on the original sample or on the pure seed sample after separating the impurities by screening or sorting. In the latter case he will calculate the percentages of moisture and oil in the original seed sample by taking into account the percentages of impurities. Separate determinations will be conducted on the impurities for moisture and oil that they may contain and take the results into account in the final calculation of the results for original sample. When the sample contains foreign oleaginous seed, the analyst will, according to the provisions of the contract, determine oil on these seeds separately.

When acidity of the oil is requested, the analyst who extracts the oil from the different fractions (pure seed, impurities, and foreign seed) should take into account the different acidities observed on these fractions in making the final calculations on the original sample.

Whether the impurities have been separated or not, the analyst will mix the sample carefully in order to make it homogenous, and after successive reductions will retain and weigh exactly the reduced sample. The weights of the reduced sample shall be 1 kilo for copra and olives, 500 grams for all other seeds except small seeds, and approximately 100 grams for small seeds such as flax, sesame, and those of the cruciferous plants such as poppy and tomato.

c) *Separation of Impurities.* The operations shall be conducted under such conditions that there is no appreciable loss of moisture. When a separation is judged necessary, the sample is reduced, the dust separated by screening, and then the foreign bodies remaining with the seed after screening are removed by sorting. The very laborious sorting of foreign bodies of approximately the dimensions of the seed in small seed samples may be effected on an aliquot which should not weigh less than 10 grams. The weights of the fractions of impurities are obtained and reported.

The chemist may indicate the percentage of inert impurities according to the specifications of the contracts. In this case the proportion obtained above should be diminished by the quantity of seed dust and particles it contains. This quantity will be calculated by taking into account the oil contents of the impurities and the pure seed. It will be subtracted from the quantity of the total impurities. Letting "P" be the percentage of total impurities, "h" their oil content, and "H" the oil content of the pure seed, the percentage, "I," of total inert impurities will be

$$I = P\ (1 - h/H)$$

The chemist may separate and analyze and study separately the foreign oleaginous seeds, if any are present, according to specifications of the contracts.

d) *Grinding.* The reduced sample, either original or after separation of impurities according to the choice of the analyst, is ground to a fine meal in a mill appropriate to the nature of the seed. The grinding will be done without heating and under conditions which do not cause sensible loss of moisture.

For such materials as copra, for which grinding is always impossible without loss of oil, recourse must be made to grating, either by hand or by use of a mechanical rasp which allows the entire sample to be reduced. If the grating is done by hand, which does not permit the entire sample to be grated, care must be taken to obtain a sub-sample which is as representative as possible. The dimensions and colors of the different pieces of copra should be taken into account in sub-sampling.

e) *Determination of Moisture.* The determination is made on the well mixed or grated sample, except for small seeds containing drying or semi-drying oils. These latter are dried without previously grinding.

Five grams ($\pm$ 0.5 g.) are weighed into a tared metal crystallizing dish (preferably of aluminum), 7 cm. in diameter and 3 to 4 cm. high. The sample should be weighed to 1 milligram.

The use of a vacuum oven is recommended. If one is not available, the dish and sample are placed in an air oven regulated to 103°C. $\pm$ 2° and in which the temperature is as uniform as possible. After 3 hours in the oven the dish is allowed to cool in a desiccator and is then weighed. The heating, cooling, and weighing are repeated with one-hour heating periods until the loss observed between two weighings is 5 mg. or less. The percentage of moisture is based on

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the lowest dry weight observed and referred to the original sample containing the impurities.

Until the industry is able to furnish regularly a pure hydrocarbon, such as pentane or normal hexane, petroleum ether distilling between 40° and 60°C. and having a bromine number less than one will be used as the extraction solvent. As soon as possible after the sample is ground, 10 grams (within 1 g.) weighed to 1 mg. is placed in the thimble of an appropriate extraction apparatus. Continuous extractors are recommended. The extraction flask (A) receiving the extract is previously dried and weighed. Extract for 4 hours. Remove the solvent and weigh the flask and contents to 1 mg. The thimble is removed from the apparatus and placed in a current of air until the major part of the solvent is lost from the meal. The contents of the thimble are emptied into an iron or bronze mortar and ground as fine as possible after adding a dozen grains of fine silicious sand. The reground meal is replaced in the thimble and extracted for an additional 2 hours, receiving the extract in the same extraction flask (A). Following the same procedure, the meal is again ground in the mortar and extracted for another 2-hour period. However, the extract is received in a fresh dry and tared flask (B). The major part of the solvent is removed from flasks A and B by distillation on a boiling water bath.

The last traces are eliminated by heating at a temperature of approximately 100°C., but not exceeding 105°C. The removal of the solvent may be aided by blowing air into the flasks at intervals or by submitting the flasks to vacuum. The heating must not be prolonged for more than 20 minutes. The flask is allowed to cool in a desiccator and weighed. The heating and weighing is repeated in the same manner until two successive weights do not differ more than 10 mg.

The last weight obtained is used to determine the weight of the oil extracted. If the weight of the oil in flask B is not more than 10 mg. the operation is terminated. The weight of the oil in the analytical sample is the sum of the weights of the oil in flasks A and B. If the weight of oil in flask B exceeds 10 mg., new extractions preceded by fresh grinding should be made until a weight of oil extracted during a 2-hour period does not exceed 10 mg.

It should be verified that the oil extracted is quite clear. If not, the impurities are determined with the aid of petroleum ether and corrections made accordingly. When the determination of oil is made on a very wet seed, the filled thimble is submitted to partial drying to reduce the moisture content to approximately 10% before placing it in the extractor.

When oil is determined in the dust separated from the seed by screening, the procedure is limited to a single extraction of 4 hours. The effect of the small error thereby introduced on the oil content of the original sample is negligible because of the usual small percentage of dust.

SUPPLEMENT I_2

Determination of Rosin

Note: As we do not know the accurate mean molecular weights of the rosin and the nature of the fatty acids used, the results of the determination of rosin are only approximate.

Reagents:

a) Methyl alcohol, 98-99%, containing 10 g. per liter of 66°Bé. sulfuric acid.

(b) N/5 alcoholic solution of potassium hydroxide.

Method: Approximately 2 g. (called P) of the mixture of fatty acid and rosin to be analyzed are weighed exactly in a 150-ml. flask, which can be attached to a reflux condenser. Add exactly 20 ml. of the methyl alcohol solution of sulfuric acid. Bring to gentle boil under reflux and continue boiling for 30 minutes. Allow to cool and titrate the liberated acid with the N/5 alcoholic potassium hydroxide, using phenolphthalein as the indicator. Let "N" be the ml. of alkali necessary.

At the same time make an identical test in which only exactly 20 ml. of the methyl alcohol solution of sulfuric acid is added to the flask. Let "N'" be the ml. of alcoholic potassium hydroxide necessary for the blank run. The percentage of rosin will be

$$\frac{(N - N') \times 6.6}{P} - 1.6$$

SUPPLEMENT II_1

Determination of Free Caustic Alkali in Soaps

The analytical sample of soap is 10 grams.

Into a 250-ml. flat-bottomed flask pour enough absolute alcohol so that after dissolving the soap the alcohol titer will be at least 95%, taking into account the moisture in the soap. In all cases the amount of alcohol will be at least 100 ml. The flask is attached to a reflux condenser and the alcohol brought to a boil for 10 minutes in order to eliminate dissolved gases. The flask is detached from the condenser, phenolphthalein added, and when the temperature is reduced to approximately 70°C., the contents are exactly neutralized with N/10 alcoholic potassium solution.

The analytical sample of soap is then rapidly introduced into the flask. The flask is attached to the condenser and brought to boil to facilitate solution of the soap. When solution is complete, the flask is detached from the condenser. As soon as the temperature is reduced to approximately 70°C., the contents are titrated with N/10 alcoholic solution of sulfuric acid for soaps containing small amounts of free alkali (less than 0.10% as NaOH) or N/2 solution for those of higher contents of free alkali.

SUPPLEMENT II_2

Determination of Free Carbonate Alkali in Soaps

Make a preliminary test by dissolving approximately 5 grams of soap in 95% alcohol.

First Case: The solution is clear or presents but little trouble. The carbonate alkali is determined by the method given in Supplement II_1 for the determination of free caustic alkali. As soon as the titration for free caustic alkali is completed, add a volume of water (previously freed of carbonic acid by boiling and cooling to 70°C.) equal to the volume of alcohol used. The solution is titrated with N/10 aqueous sulfuric acid solution. Let "N" be the ml. of the N/10 acid used. The content of carbonate alkali in the analytical sample expressed as Na_2CO_3 is:

$$2\,N \times 0.0053$$

Second Case: The solution in alcohol is incomplete or leaves an appreciable residue. The quantitative analysis is made on 5 grams of the sample. The soap is dissolved in such a volume of absolute alcohol that the alcoholic titer of the solution will be at least 95%, taking into account the moisture in the soap. The volume will be at least 100 ml. After dissolving the soap, the insolubles are filtered and thoroughly washed with hot 95% alcohol. The carbonates in the insoluble substances are determined by the methods of mineral analysis: a) dissolve in water and titrate alkalimetrically, if only water soluble alkaline products are carbonates; b) determine CO_2 in other cases, avoiding the causes of error due to the possible presence of insoluble carbonates.

SUPPLEMENT II_3

Qualitative Research on Rosin

Reagents:

Solution A: One part by weight of phenol and 4 parts by weight of carbon tetrachloride.

Solution B: One part by volume of bromine and 4 parts by volume of carbon tetrachloride.

Method: Place approximately 20 mg. of soap in a porcelain capsule, 11 cm. in diameter. Add several drops of hydrochloric acid and heat over a small flame in a manner to assure the release of the fatty acids. Allow to cool to ambient temperature. Add approximately 2 ml. of solution A. Dissolve the fatty acids and wet the walls of the capsule by rotating it at an inclined angle. Allow to stand for several seconds in order that the walls of the capsule remain covered with a very thin film of the solution. Bring the mouth of the bottle of solution B to the capsule so the bromine vapors flow along the walls into the capsule. A violet coloration and a characteristic blue coloration develop in a few seconds when rosin is present. The test is made in the same manner for oil and fatty acid samples except that only several centigrams (about one drop) of the material is used and the previous treatment with hydrochloric acid is unnecessary.

SUPPLEMENT II_4

Indices of Soluble and Insoluble Volatile Acids

Reagents:

Sodium hydroxide	50% solution
Dilute sulfuric acid	5% solution
Sodium hydroxide	N/10
Phenolphthalein	1% in alcohol

Apparatus: The arrangement of Polenski is used.

Method: Place accurately weighed sample of fat, weighing about 5 grams, in the flask. Add 20 grams of pure glycerol and 3 grams of the 50% sodium hydroxide solution. Heat over a small flame with constant agitation until the saponification is complete. The saponification is generally complete in 5-15 minutes and the mixture has become clear.[1] The mixture is cooled to 80°C. and then 90 ml. of recently boiled water, cooled to 80°C., is added.

When the soap has dissolved add several grains of pumice stone and 50 ml. of dilute sulfuric acid. Attach the flask to the apparatus immediately and heat with a naked flame in such a manner as to distill 110 ml. in 19-21 minutes. When 110 ml. of distillate has been collected, extinguish the flame and replace the receiving flask with a 25-ml. cylinder. Allow the distillate to stand for 15 minutes at a temperature of 15°C. and then mix. Filter through an 8-cm. dry filter and save 100 ml.

Titrate the 100 ml. distillate with N/10 sodium hydroxide in the presence of 5 drops of phenolphthalein indicator solution. Conduct a blank determination to provide the essential correction. The insoluble acids which remain on the sides of the condenser, the receiver, and the filter are washed three times with 15 ml. of recently boiled water which has been cooled to 15°C. Then the pure insoluble acids are recovered by three washings with 15 ml. of 95% neutralized alcohol and the combined alcoholic solution is titrated with N/10 sodium hydroxide solution.

[1] If the fat contains much unsaponifiable matter (for example cacao butter) or much highly hydrogenated oil, the mixture does not become clear. It may remain slightly turbid.

SUPPLEMENT II_5

Characterization of Sterols

Preparation of the Fatty Acids: Approximately 50 grams of oil or fat are saponified with 100 ml. of alcoholic solution of potassium hydroxide (200 g. KOH in one liter of 70% alcohol) by heating on a water bath and stirring from time to time. After approximately a quarter of an hour the liquid becomes homogeneous and the saponification is complete. Then add 50 ml. of 25% hydrochloric acid. Allow to remain on the water bath for 15-30 minutes until the fatty acids become sufficiently clear. Pour on a large and previously moistened filter paper. The aqueous liquid filters rapidly while the acids remain on the filter. As soon as the aqueous liquid has passed through the filter, the filter is pierced and the fatty acids are received on a large, dry folded filter paper, through which they filter sufficiently dry and clear. In the case of fats with high melting points it may be necessary to use a heated filter.

Preparation of the Digitonides: The prepared acids are heated to approximately 80°C. in a small beaker and mixed with 20 ml. of a solution of 1% digitonin in 96% alcohol (approximately 20 g. of fatty acids for 10 ml. of the reagent). The mixture is stirred from time to time with a small glass rod and maintained at approximately 80°C. After 15 to 30 minutes a precipitate of the digitonides is formed. Add 20 ml. of hot carbon tetrachloride and filter with suction on a small disk of filter paper. The precipitate is washed twice with 10 ml. of hot carbon tetrachloride and once with ethyl ether. In the case of fatty acids of high melting points it may be necessary to use a heated filter. The digitonides are found in a thin layer on the filter paper, from which they can be removed easily.

Preparation of the Sterol Acetates: The digitonides are heated for about 15 minutes with 3 ml. of acetic anhydride under a reflux condenser. The solution is evaporated to dryness on a water bath. The hot residue is dissolved in 10 ml. of 96% alcohol and the solution filtered after several minutes. It is then concentrated to 2 to 3 ml. The crystals formed are filtered with suction. They are twice recrystallized from alcohol. After the third crystallization and drying the melting point of the crystals is determined. A melting point of 117°C. or higher indicates the presence of phytosterol. The melting point of the acetates of cholesterol is 113° to 115°C. and that of the acetates of phytosterol is 126° to 137°C.

Preparation of the Sterols: The sterol acetates are saponified with the aid of a little (about 3 ml.) half normal alcoholic potassium hydroxide. After dilution with water the sterols are extracted with ethyl ether. The solution is washed once or twice with a little water and then the ether is evaporated. The sterols obtained are dissolved in 1 or 2 ml. of 96% alcohol.

The sterols are crystallized on a microscope slide. The form of the crystals, as well as their extinction of polarized light, is observed under the microscope. Crystals of phytosterol are oblong and longer than wide and show parallel extinction while those of cholesterol are wider and show inclined extinction.

SUPPLEMENT II_{5A}

Characterization of Sterols

Reagents:

1. Alcoholic potassium hydroxide, 200 g. KOH per liter of 70% alcohol.
2. Hydrochloric acid, 25% solution.
3. Digitonin solution, 1 g. in 100 ml. 96% alcohol.
4. Chloroform.
5. Acetic anhydride.
6. Alcohol.
7. Ethyl ether.
8. Alcoholic potassium hydroxide, approximately N/2.

Preparation of Fatty Acids: One hundred g. of fat are saponified with 200 ml. of the alcoholic potassium hydroxide solution in a flask under reflux. When the saponification is complete, dilute the soap solution with 300 ml. of hot water. Add an excess of hydrochloric acid. Heat until the fatty acids form a clear film on the surface. Pour the mixture on a previously moistened filter paper. When the aqueous liquid has filtered, pierce the filter. The fatty acids are received in a beaker. Add 30 ml. of chloroform and filter the solution through a large dry folded paper.

Preparation of the Digitonides: The fatty acids are heated in a beaker to 70°C. and, while stirring, add at once 50 ml. of the solution of digitonin. The mixture is reheated to 70°C. and stirred at that temperature for 15-20 minutes with a glass rod. Add another 30 ml. of chloroform and filter the mixture on a small filter with suction. The crystals of digitonide are washed three times with 20-ml. portions of hot chloroform and five times with 15-ml. portions of ethyl ether. Afterwards they are dried on a watch glass at 100°C. for 10 minutes.

Purification of the Crystals: Dissolve the crystals in 100 ml. of absolute alcohol, heat, and add 10 ml. of water. Allow the mixture to stand overnight and then add 15 ml. of water. Let stand for an hour and filter on a small suction filter. The crystals are dried at 100°C. for 10 minutes.

Preparation of Sterol Acetates: Digest the crystals for 10 minutes with 7 ml. of acetic anhydride in a flask under reflux until the solution becomes clear. Add 28 ml. of 50% alcohol. Place the flask in cold water for an hour. Filter on a small suction filter. Wash the crystals with 50% alcohol.

Dry the crystals on a watch glass at 100°C. for 10 minutes. Recrystallize several times from absolute alcohol. A porous porcelain plate will serve to absorb the alcohol. After the second crystallization determine the melting point.

Preparation of the Sterols: The sterol acetates are saponified with 2-3 ml. of N/2 alcoholic potassium hydroxide solution. The sterols so produced are collected on a porous porcelain plate. They are recrystallized from absolute alcohol and microscopically examined under both normal and polarized light.

Observations on the Mechanism of the Autoxidation of Methyl Linoleate[1,2]

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Introduction

ALTHOUGH a number of people have studied extensively the autoxidation of methyl linoleate and other simple esters of linoleic acid, the structures of the peroxides that are formed during the initial stages of the autoxidation have never been completely established. It is the purpose of this paper to present a few preliminary observations obtained in a study of the autoxidation of methyl linoleate that was undertaken to gain further evidence concerning the structures of the initial peroxides.

Before presenting the data, however, it is advisable to review briefly some of the findings that have been reported by other investigators. It appears to have been well established by Farmer and others (1,2,3,4) that the autoxidation of olefins occurs by a free radical mechanism which, in some cases at least, results ultimately in the formation of hydroperoxides in which the peroxidic group is attached to a carbon atom adjacent to an unsaturated center. Kass *et al.* (5), Mitchell *et al.* (6), Farmer *et al.* (7), and others (8,9) have also observed by spectrophotometric methods that, in polyolefins whose double bonds are separated by methylenic groups, treatment with alkali at higher temperatures or autoxidation at ordinary temperatures apparently causes some double bonds to shift, thereby producing some conjugation of the double bond systems.

Bolland and Koch (10), Bergström (8), and also several people in this country have devoted considerable attention to the conjugation that develops during the autoxidation of linoleates. Bolland and Koch, in particular, on the basis of studies of the autoxidation of ethyl linoleate, postulated the formation of a

[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 231 in the series of papers approved for publication. The views and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army. (Hormel Institute Publication No. 35.)

[2] Presented at the meeting of the American Oil Chemists' Society, New York, November 15-17, 1948.