manner of procuring and preparing the sample, and it should therefore be the policy of those best informed to bring its needs clearly before parties concerned as a means of eliminating disputes and as a basis of correcting errors." Any of those present who have had experience in buying or selling crude soybean oil, where price is based on refining loss, will appreciate the significance of this statement, particularly in view of the example. In this connection we have found that the use of a divider or riffle is quite effective in reducing the gross sample taken from a car of soybean oil to the required three 1-gal. samples specified in trading rules.

Conclusion

It is hoped that the foregoing general remarks on sampling procedures will point up the necessity for more care in this very important operation. In too many instances sampling has been delegated to persons not too well aware of the purpose and importance of the samples which they are taking, and possibly not too well qualified to handle the job. This situation could be improved markedly by better training and instruction of persons delegated to take samples, or preferably by selecting persons better qualified and more interested in this essential but rather non-glamorous operation. There is also the need for better equipment specifically designed to handle the more difficult and troublesome sampling situations, some of which have been mentioned previously. Much unnecessary work on the part of the analyst could be eliminated, and at the same time his prestige could be raised materially, if the samples he received for analysis were always true and representative parts or segments of the entire lot of material on which his evaluation is based.

Determination of Impurities in Fats and Oils

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IMPURITIES present in fats and oils and fatty acid products are mainly moisture, volatile compounds, insoluble matter, unsaponifiable matter, trace metals and their soaps. The term M.I.U. (moisture, insoluble, unsaponifiable) is a frequently used group designation for the determination of the non-fatty constituents of crude oils and other fatty acid products where settlement is on the basis of oil or acid content. It also figures predominantly in the trading rules of the various oil trading organizations, such as the National Soybean Processors Association, National Cottonseed Products Association, New York Produce Exchange, and the National Institute of Oilseed Products.

A. A. Rodeghier

Moisture and Its Determination

Moisture is such a universal constituent of all materials analyzed in the laboratory that its determination is probably the most common of all those conducted.

One of the most common methods of determining moisture in a fat or oil is that by the "hot plate" method (1). In this method approximately 10 g. of a representative sample of the oil is weighed into a clean, dried tared beaker. This is then heated on a hot plate, applying gentle rotation by hand. As the end-point is approached, the absence of foaming and steam vapor is apparent, and heating is continued until incipient smoking of the sample occurs. The beaker is then cooled in a desiccator and weighed; percentage loss is calculated as moisture and volatile matter.

The "oven" method (2) is more time-consuming than the "hot plate" method, but results are more accurate and reliable. This is even more true when using the vacuum oven. In the "oven" method for moisture a representative sample of approximately 5 g. of the oil is weighed into a dried, tared moisture dish. It is then set into the oven for 30 min. at 101 ± 1°C., cooled in a desiccator, and weighed. Continued heatings and weighings are necessary to obtain a constant weight. Loss in weight is again calculated as moisture and volatile matter.

A forced draft oven reduces the drying time by the sweeping away of the moisture molecules by diluting the vapor with warm air so that more of the water molecules can escape from the liquid phase. Drying is usually about four times faster in this type of mechanical convection oven than in a gravity convection oven. A forced draft oven also tends to maintain a more uniform temperature throughout the oven drying chamber.

The "vacuum" oven method (3) is applicable to all fats and oils and for various other materials where moisture is deep-seated and must diffuse largely through the capillaries. A decided advantage may be gained by using the vacuum oven. The same technique is followed for the oven method except that the sample is dried in a vacuum not exceeding 100 mm. of mercury at a temperature not less than 20°C. and not more than 25°C. above the boiling point of water at the operating pressure.

Today there are various kinds of moisture testers available for the determination of moisture in all kinds of materials. One device measures moisture content on a modified conductivity principle where no sample weighings are required and results are obtained on a calibrated dial. Another rapid drying moisture teller uses a stream of air at a controlled temperature and velocity to flow over or through the sample, giving results in a few minutes. Another unique method for determining moisture is one called a "moisture balance." This apparatus has a built-in torsion balance, the sample is placed on the pan, and infrared heat is applied until all the moisture is driven off. The percentage of moisture is read directly from a dial scale.

The "distillation" method (4) for moisture is applicable to fats and oils and other materials that do not contain water-miscible substances. The distillation apparatus in its simplest form consists of a boiling flask to hold the sample and liquid connected to a condenser and a trap. All joints of the apparatus are standard tapered glass type. The trap is so made that the condensate flows back into the flask and the trap collects and measures the water liberated. The trap commonly used is that of Bidwell-Sterling design, and the feature of this is a small reservoir above the calibrated tube which enables the water to fall in the measuring tube.

Essentially the distillation method involves the formation of an azeotrope of an immiscible organic liquid with water with distillation of the azeotrope and separation of water as a separate phase where it is measured volumetrically. Cleanliness and dryness of the apparatus is of extreme importance, and all glass joints should be lubricated with pencil graphite and moistened when assembled with a few drops of the distilling liquid. From 100–200 g. of a representative sample under test are weighed into the boiling flask, and an equal amount of solvent (toluene) is added. The trap or moisture receiver is filled with additional solvent by pouring it through the condenser top until it overflows into the flask. The top opening of the condenser should be plugged loosely with cotton to prevent atmospheric moisture from condensing in the condenser tube.

The boiling flask is then heated so that a distillation rate of not over 200 drops per minute is exceeded, and distillation is continued until the water level in the trap is at a constant reading. Heating is discontinued, and any drops of water adhering to the condenser tube are dislodged by thrusting a wire down the condenser and flushing the condenser with a few ml. of the toluene. The trap or receiver is cooled in water to 25°C., and the volume of water is read.

$$\text{Moisture } (\%) = \frac{\text{vol. of water} \times \text{density of water @ } 25^\circ\text{C.} \times 100}{\text{wt. sample used}}$$

Perhaps the most exploited method of determining moisture, chemically, is that by the "Karl Fischer Method," which was introduced in 1935 by the German chemist, Karl Fischer (5). Since introduced, it has been recognized as an extremely valuable analytical tool, offering many advantages in speed and versatility. This method is adaptable for determining moisture in a wide variety of materials, and it has been adopted by many laboratories as a standard test procedure for moisture.

The Karl Fischer volumetric method for moisture may be determined by an ordinary visual titration or by an electrometric method (6). The reagent used is composed of iodine, sulphur dioxide, pyridine, and anhydrous methanol. It is usually split into two parts, namely, reagent A and reagent B. Reagent A contains anhydrous methanol, pyridine, and sulphur dioxide, and Reagent B contains iodine in anhydrous methanol. They are usually mixed prior to use in order to maintain better stability. It is now possible to buy this reagent in a stable form. Since the Fischer reagent has an affinity for water, the reaction goes stoichiometrically according to the equation $H_2O + SO_2 + I_2 = SO_3 + 2\,HI$.

The reagents must be standardized against a methanol-water standard solution or with sodium tartrate dihydrate; also the anhydrous methanol used must be titrated with the reagent to determine its water content. When this is accomplished, a titer of the reagent is known and titration of a sample for moisture can be made. The extreme sensitivity of the reagent to moisture presents a problem in maintaining a completely anhydrous system. Therefore all apparatus must be clean and dry. The sample is weighed into an Erlenmeyer flask; anhydrous methanol is added and titrated immediately to the end-point.

When one is familiar with the technique involved, titrations can be made of various moisture-bearing materials. Accuracy of the titration end-point is limited on the basis of the sample size used, and this can be overcome by experimentation.

The end-point is usually a color change from yellow to brown, and an experienced operator working with relatively clear solutions can make an accurate titration. For colored samples or suspension of solids the end-point is very difficult; also if the reagent is decomposing, fading end-points will occur.

$$\text{Moisture } (\%) = \frac{(\text{ml. sample} - \text{ml. blank}) \times H_2O \text{ equiv.} \times 100}{\text{wt. of sample used}}$$

The electrometric method of titration for moisture is invaluable when dark solutions are encountered. For laboratories that run several moistures daily the automatic titrating apparatus is the ideal equipment for this test.

Two platinum electrodes appear as a high resistance in the presence of water. At the end-point, with moisture gone, the electrodes depolarize, and the resistance is decreased. This changes a balance of a bridge in the circuit, causing the reagent to stop flowing into the sample beaker. Certain substances require a "back titration" because either they contain high moisture or the moisture does not react readily with the reagent. In this case an excess of Fischer reagent is added beyond the end-point and then brought back to the end-point with the methanol water standard.

Insoluble Impurities

The insoluble matter found in fats and oils is meal, dirt, and other substances insoluble in kerosene and petroleum ether (7).

The same sample can be used from the moisture determination by the hot plate or oven method. About 50 ml. of kerosene are added to the sample and then filtered through a dried, tared Gooch crucible. After several washings with warm kerosene a final wash of petroleum ether is used, and the crucible is dried to constant weight.

Gardner Heat Test

The determination of this characteristic break (8) in a crude soybean oil gives an estimate of the amount of mucilaginous material, phosphatides, and pigments present. This value indicates how well the oil is degummed or clarified, and it is of importance to know this in paint and resin formulation and in steam refining.

Unsaponifiable Matter

Unsaponifiable matter is found dissolved in fats or fatty acids, and it is that material which cannot be saponified by KOH. The unsaponifiable is sterols, higher alcohols, and some hydrocarbons. Refining of the oils removes most of this substance. The determination is made by weighing a 5-g. sample into a a 250-ml. Erlenmeyer flask (9). Then 30 ml. of alcohol and 5 ml. of 50% KOH are added, and the mixture is refluxed until saponified.

The saponified material is then transferred to an extraction cylinder, and the flask is rinsed with alcohol and washed into the cylinder to the 40-ml. mark. The transfer is completed by washing with water until the cylinder is at the 80-ml. mark. The flask is then again rinsed with a little petroleum ether added to the cylinder, and the entire contents are cooled. A 50-ml. portion of petroleum ether is added to the cylinder, stoppered, shaken vigorously, and allowed to settle.

The ether layer is siphoned off into a 500-ml. separatory funnel, and the extraction is continued this way for at least six times. The combined extracts are washed three times with 25 ml. of 10% alcohol, and the ether extract is then transferred to a tared beaker in portions where it is evaporated to dryness. The residue (unsaponifiable) is then weighed. Any free fatty acids present should be titrated with 0.02N-NaOH to give corrected results.

The modified Kerr-Sorber method is normally used for oils containing a high percentage of unsaponifiable. The same procedure is followed for refluxing. At the end of the saponification period the flask is cooled; and 50 ml. of ethyl ether are added and the contents are transferred to a 500-ml. separatory funnel. Two more ether rinses are required before the flask is rinsed with 100 ml. of dilute KOH. The funnel is then rotated vigorously, and the soap solution is drawn off upon settling. An additional two washes are required, followed by water washing until the washes are free from alkali. This method is completed by distillation of the ether extract after transfer to an Erlenmeyer flask, followed by drying in an oven and weighing.

The official A.O.C.S. method for the above determinations involves a series of extractions, using extraction cylinders and separatory funnels.

A modified method by C. R. Buerki and K. E. Holt (10) is a liquid-liquid extraction apparatus. A magnetic stirrer is used for agitation of the soap-ether mixture with a constant refluxing of the solvent, dispersed through an orifice of an inner tube within the special extraction cylinder. This method is now being used by several laboratories, and experimentation is necessary with the apparatus to maintain the optimum conditions.

The summation of the M.I.U. is considered as valueless material except for those who may recover sterols and tocopherols present in the unsaponifiable fraction of the fatty acids split from the soapstock of soybean oils.

Lauric type acid oils are bought and sold on a 98% saponifiable matter basis, the difference being the M.I.U. group. Oleic type acid oils from corn, soya, and cottonseed oils are bought and sold on a 95% total fatty acid basis.

Generally moisture and volatile of most crude oils do not exceed 0.3%, and some other oils like crude coconut may run as high as 1%. Refined, bleached, or bleachable grade oils should run under 0.1% moisture. Finished products such as salad oils, margarine oils, or shortenings should be moisture-free, but usually specifications are given as a 0.05% maximum.

A visual inspection of a crude oil is usually sufficient to determine how much sediment or suspended material is present for an insoluble matter test. The present-day methods of handling seeds and beans by the farmer, improved storage conditions, and modern expression and extraction of the oils are a big improvement over the methods used 15 years ago.

The National Soybean Processors Association have in the trading rules a 0.6% maximum Gardner heat test. While most crude soybean oils are of the extracted non-degummed type, a 0.6% break test is reasonable although some of the expeller non-degummed oils exceed this figure. Crude oil in prolonged storage will also show a high break test. A well degummed crude oil will show a negative break test.

Trace Metals

Many publications have been cited in the past dealing with the serious effect which trace metal contamination has upon the stability of soybean oil. Various procedures have been used for determining trace quantities of iron and copper in edible oil products. Some of these procedures include colorimetric determinations, spectrophotometric determinations, and more recently spectrographic analysis.

In all three analyses the fat or oil is ashed prior to the determination. In the case of the colorimetric and spectrophotometric method visual colors are developed with the trace element and some organic reagent. In this method the color developed conforms to Beer's Law and is directly proportional to the trace element being determined.

More recently the spectrographic analysis has been utilized to determine trace quantities of iron and copper in vegetable oils. As the amount of ash of any of the metallic elements present in fats and oils is very small, a preliminary preparation of the sample by special ashing methods is essential in order that the full amount may be recovered and at the same time the organic matter may be eliminated by combustion. The spectra of the ash with the internal standard utilized in the ashing technique is then determined and is indicative of the concentration of the trace elements involved.

In dealing with soybean oil the following apply to the trace elements, iron and copper:

1. The native content of iron in soybean oil is approximately 0.6 to 1.5 parts per million, and the copper content usually ranges from 0.4 to 0.8 p.p.m.
2. The commercial extraction of soybean oil usually increases the iron content of the crude oil 4 to 8 times. No significant increase was noted in the copper content at any stage of the processing.
3. The normal caustic refining removes over 95% of the iron and copper contamination indigenous to the oil.
4. Deodorization is the most critical and sometimes a very damaging process in the stability and quality of soybean oil, and in many cases the deleterious effect can be attributed to the iron contamination. Experiments in the laboratory have shown that the presence of 0.3 parts per million of iron is very detrimental to the quality and stability of soybean oil.
5. Deodorization is the processing step in which improvements most logically could be made on both the quality and stability of soybean oil. A reduction of the metal contamination and the use of a metal scavenger will improve both the quality and stability of soybean oil.

Soap in Refined Oil

Fatty acids react readily with alkali to produce soaps, and it is during the refining operation that the the soaps are introduced as a contaminant. Traces of soap in a refined oil are detrimental to the quality of the final product, and their adsorption during hydrogenation accounts for off-quality hardened oils.

A low soap content is best insured by water-washing the neutral oil, followed by a thorough drying before bleaching with an adsorbent. The analysis of the refined oil for soap, which should run not over 5 parts per million, is rather uncertain in this low concentration, and there are several methods for this determination.

Essentially the determination involves the extraction of the soap from the oil, followed by the quantitative determination of the sodium ion. Extraction is made by using alcohol and burning off the alcohol in a platinum crucible followed by ignition. The ash is then dissolved in water and titrated with 0.02N-HCl using methyl orange indicator. Extraction is also done with 1:1-hydrochloric acid, followed by evaporation of the extract. Water is added to the residue, and the mixture is titrated with 0.01N-$AgNO_3$ with potassium chromate indicator added.

Although more time-consuming, the oil may be burned off and ignited in a platinum crucible without extraction. The more modern method of determining trace soaps is by the conductivity method where soap is determined against standard soap solutions.

In conclusion, the methods given herein and their implications to trading rules are of concern to all who process, or buy and sell fats and fatty acid products.

The impurities, except for sterols used in hormone and pharmaceutical preparations, are of no commercial value but may indicate to the refiner how best to refine for the maximum recovery of neutral oil.

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The Determination of Refining Loss

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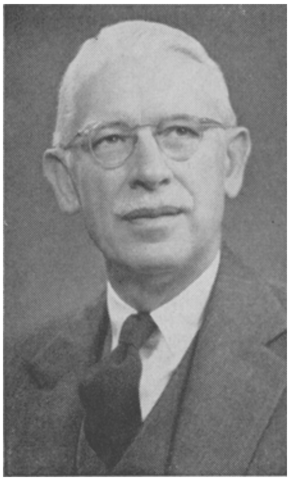
THE ANALYTICAL chemist is required to determine refining losses on crude oils for two distinct purposes: as a basis for settlement of crude oil contracts under the trading rules of the National Cottonseed Producers Association and the National Soybean Processors Association; and as a yardstick for the efficiency of refinery operation.



E. M. James

The standard method of determining refining losses is known as the A.O.C.S. Cup Method. It is described in every detail in the standard methods of the A.O.C.S. and in the manuals of trading rules issued by the two associations mentioned above. Without going into detailed discussion of the cup method, I believe however that you will find of interest the historical background which led up to its development.

Thirty-five years ago the principal oils traded in on the basis of refining loss were cottonseed and peanut oils. Soybean oil, except as imported from Manchuria, was practically unknown in this country. A strong group of mills and refiners, operating under the name of the Interstate Cottonseed Crushers' Association, had formulated a series of specifications for the various grades of crude cottonseed oil, refined oils, and soapstock, and penalties upon the seller were provided for deliveries which were not up to specified quality.

At that time all cottonseed oil was refined in the kettle, and the average loss in the refinery was estimated to be approximately 9.0%. All oil having a loss of 9.0% or less was defined as a prime crude oil. Other requirements were that the oil be sweet in flavor and odor, not musty or sour, and must refine to a color not higher than the combined Lovibond glasses of 35.0 yellow, 7.6 red. Penalties were levied for off-flavor and odor and for colors darker than the specified 7.6. But the principal penalty was for loss and amounted to ¾ of 1% of the purchase price of the oil for every per cent in excess of 9.0%. (With peanut oil, the prime loss was 5.0%.) Limits were also set on the free fatty acid of the crude, which was 2.5% for prime oil. Crude cottonseed oil was sold on "a basis prime" contract, and upper limits were set for the loss (16.0%) and the color of the refined oil (35 yellow, 12 red). No premiums were allowed for losses better than prime.

Now it is obvious that a seller of crude oil could not afford to wait for payment until the refinery had refined his oil and determined whether it met the specifications under which it had been bought. Therefore it became necessary to find a method for refining a small lot of crude oil in the laboratory in a manner which would simulate plant operations to the extent of giving approximate plant lossess and color. A complicating factor was the tendency of some refiners to underrefine oil in the plant, thus obtaining lower losses at the expense of color. So the method adopted had to employ enough caustic solution to give at least a prime color on prime oils.