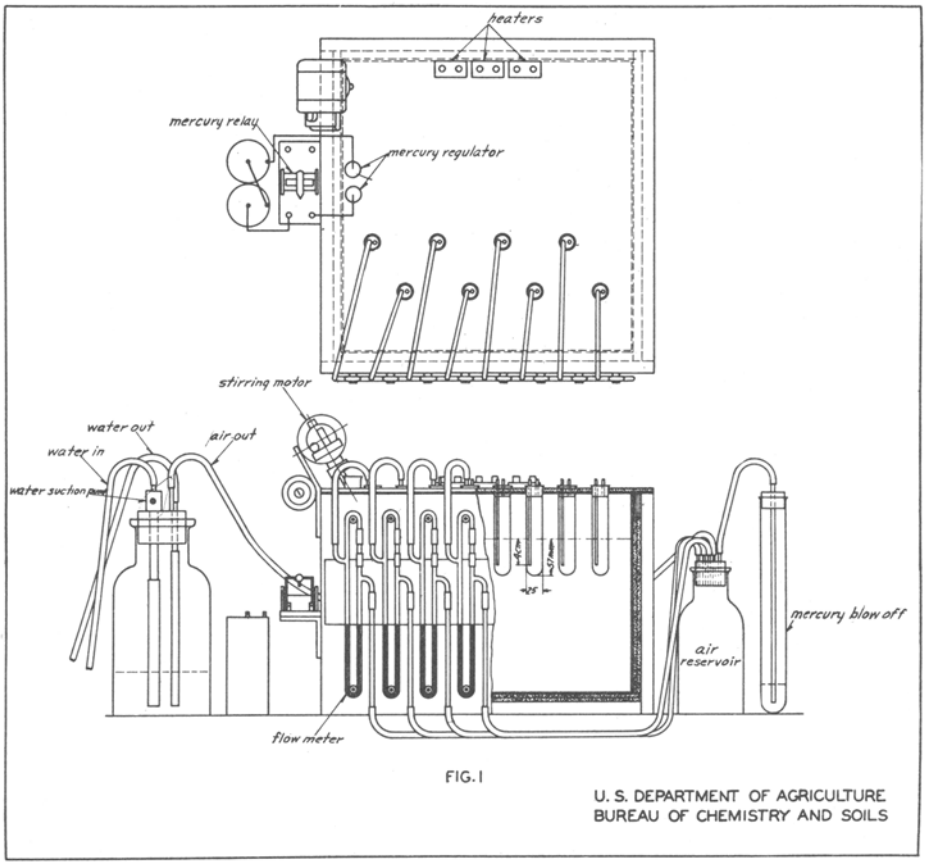

FIG. 1

U. S. DEPARTMENT OF AGRICULTURE
BUREAU OF CHEMISTRY AND SOILS

Fats—and Their Examination

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Bureau of Chemistry and Soils
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IN biological and chemical investigations the importance of knowing the source and history of fat samples cannot be overrated. Whenever possible they should be prepared under the personal direction of the investigator. Fats are usually obtained by expression, solvent extraction or rendering, the last process being used for the recovery of those from animal sources. In such cases where the fat can be expressed, it has the advantage over the other methods in that the samples are obtained ready for study with less expenditure of time and effort; besides expressed fats not infrequently contain less of the non-oil constituents. Whether a fat is obtained by expression or other process attention should be given to the prompt removal of all soluble substances which may affect it adversely in a very short time.

For the solvent extraction of fats, petroleum ether of good quality is preferable to most, if not all other available solvents. It can be entirely removed from the fat about as readily as any other solvent and has the advantage, in addition to that of cost, of generally extracting less foreign substances along with the fat. The removal of the last of the solvent can be conveniently accomplished with samples of fat weighing up to about 50 grams by heating them in an oven at 115° in an atmosphere of carbon dioxide in order to prevent their possible oxidation. But with larger quantities it is best to remove the last portion of the solvent by heating them under a pressure of 50 mm. or less. The time required for the removal of the solvent can be considerably reduced by bubbling carbon dioxide or other inert gas through the fat.

In order to obtain animal fats in good condi-

tion, they should be rendered at as low a temperature as possible and the time used for heating should also be kept to the minimum. Then, after the extraction of the fat as previously mentioned, attention is to be given to the prompt removal of all foreign matter. All fat samples should be placed in clean, dry, tight containers and kept away from direct sunlight. Otherwise rapid deterioration can be expected. When necessary to defer the investigation it is preferable in most cases to keep the samples at 10° or lower.

It may be noted that the neglect of any or all of the subjects so far discussed, together with the unsuitable selection or use of investigational methods, largely accounts for the very considerable mass of published information that has been found to be of little or no value.

In connection with the examination of fats, it is important to know the type or class to which they belong. Frequently, the selection of a method or methods to be used depends upon this information. There is now available a considerable amount of information in regard to the peculiarities and similarities in the composition of fats from the members of various families or groups of species of a family of organisms whether of animal or vegetable origin. For example, palm kernel fats, including that of the coconut, are characterized by containing caprylic and capric acids, as well as large quantities of lauric and myristic acids, along with

Before undertaking the investigation of the glycerides, it is necessary to determine the kind and quantity of both the saturated and unsaturated fatty acids that are present.

small quantities of unsaturated acids. The only other group that resembles this one is that of the milk fats. The seed fats of the plants belonging to the Cruciferae are characterized by containing a large proportion of erucic acid and the fats from those belonging to the Umbellferae, by petroselinic acid. On the other hand, the known fats of the seeds from the plants which belong to the Flacourtiaceae can be divided into two distinct groups, depending upon whether or not the fats are optically active; the optical activity being due to the presence of acids which are known as chaulmoogric, gorlic, and hydrocarpic acids. There are many other

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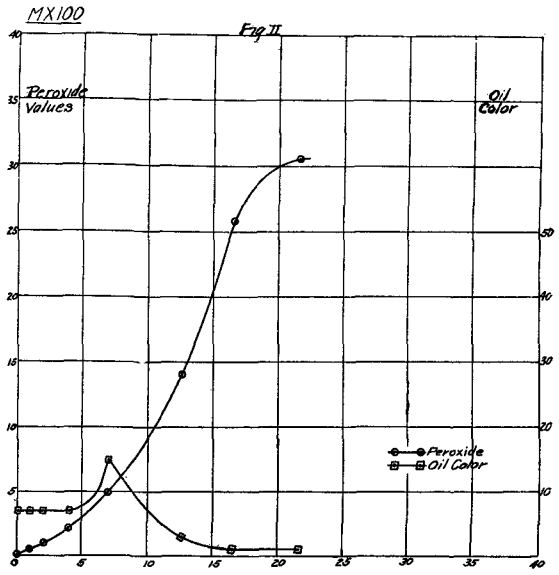
plant and animal groups, the fats of which are distinctive in one way or another.

Fats of either animal or vegetable origin are not entirely composed of tri-glycerides of the fatty acids, but in all cases contain other constituents, the quantity of which may vary from a few tenths to several per cent or more. These include various phosphatides, sterols, hydrocarbons, pigments, vitamins and possibly other substances in very small quantities. After extraction, they also contain more or less non-oil sub-



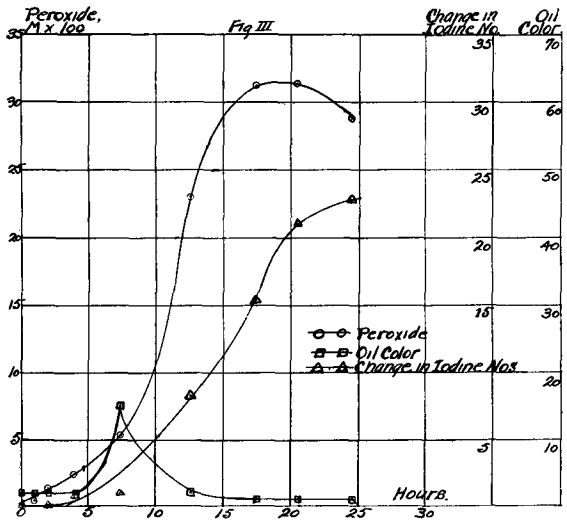
stances, the quantity that may be present depending upon the nature and the condition of the oleaginous product and the method employed for the extraction of the fat. These substances may partially separate from the fat upon standing or they may remain in solution almost indefinitely. In our study of the non-oil constituents of crude cottonseed oil (1), raffinose, pentosans, phytosterolines, proteoses, peptones and resins were found. It is interest-

ing to note that when a crude fat of any kind is refined by the caustic soda process, the non-oil substances are removed, as well as a considerable proportion of the phosphatides, such as lecithins, cephalins, etc.

Depending upon their sources, vegetable fats extracted by any means may also contain essential or volatile oil in small or large quantities. Generally, when desirable, they can be removed by means of steam distillation.

Before attempting the examination of a fat, consideration should be given to the selection of the methods best adapted for the purpose, keeping in mind, if necessary, their limitations when applied to certain types of fat. In connection with the determination of the characteristics where there are two or more usable methods as, for example, the iodine number, the method used should always be given, particularly as the different procedures do not give identical results. With some fats such, for example, as those from oiticica and tung nuts, the methods as ordinarily used give widely different results.

As quite a number of procedures have been proposed for the determination of unsaponifiable substances in fats and as many of them



for one reason or another are unsatisfactory, it appears desirable to call attention to the Modified Kerr-Sorber Method (2) which can be applied to the examination of all types of fats with equally good results, whether small or large quantities of unsaponifiable substances are present.

In connection with the estimation of linoleic and linolenic acids by means of their insoluble bromide compounds, attention is called again to the fact that the petroleum ether insoluble tetrabrom linoleic acid and the ether insoluble hexabrom linolenic acid represent only a portion of the respective acids actually present. All fats so far examined contain more or less insomeric acids, the bromine derivatives of which, on ac-

In connection with the examination of fats, it is important to know the type or class to which they belong. Frequently, the selection of a method or methods to be used depends upon this information.

count of their solubility, do not separate along with those of the so-called alpha acids. However, by means of the Kaufmann thiocyanogen value (3) and the iodine value, it is now possible to determine approximately the proportions of oleic, linoleic and linolenic acids present in a fat. Kaufmann has shown that the thiocyanogen radical combined in the ratio of one molecule to one of the following acids: Elaeostearic, erucic, linoleic, ricinoleic and other oleic acids, whereas linolenic acid reacts with two thiocyanogen radicals.

Although various methods have been described for the separation and determination of saturated and unsaturated acids, only three will be mentioned. These are the modified lead-salt-ether method of Gusserow and Varrentrap (4), the Twitchell lead-salt-alcohol method (5), and the Bertram oxidation method (6). For those not especially acquainted with these methods, it should be observed that they are not applicable to the examination of the whole range of natural fats; consequently the type of fat under investigation must be known. For example, in applying the lead-salt methods to the analysis of fats which contain chaulmoogric, hydnocarpic, elaeostearic, erucic, petroselinic and the so-called isooleic (hydrogenation) acids, their lead salts separate more or less completely with those of the saturated acids. Also, these methods are not adapted to the determination of saturated acids in those fats which contain notable quantities of myristic or any of the acids of

lower molecular weight, such as lauric, capric, etc., on account of the solubility of their lead salts in alcohol or ether. Consequently, these

It is interesting to note that when a crude fat of any kind is refined by the caustic soda process, the non-oil substances are removed, as well as a considerable portion of the phosphatides, such as lecithins, cephalins, etc.

methods are not applicable to palm kernel, myristica and milk fats.

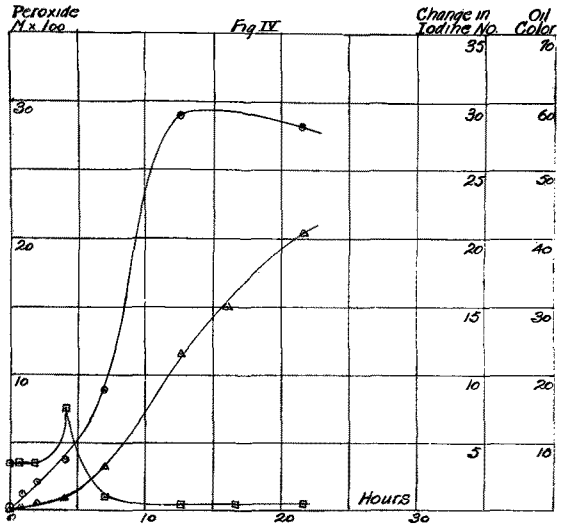
The Bertram procedure which is only used for the determination of saturated acids is also restricted to those fats which do not contain lauric or other saturated acids lower in the series, or those which contain unsaturated acids that yield lauric or similar acids upon oxidation with permanganate. Unlike the lead-salt methods, this one, from the few experiments made in the author's laboratory, apparently can be applied to the approximate determination of the saturated acids in chaulmoogra and related fats.

Recently Hilditch and Priestman (7) completed an extensive investigation regarding the limits of accuracy of the Twitchell and Bertram methods when applied to the determination of saturated acids. Also, they have emphasized

An original mixture of fully saturated and mixed saturated and unmixed saturated glycerides, after oxidation, becomes a corresponding mixture, respectively, of neutral and acidic compounds. These are separated and the composition of the acids in each fraction is determined in order to get an insight into the structure of the original fat.

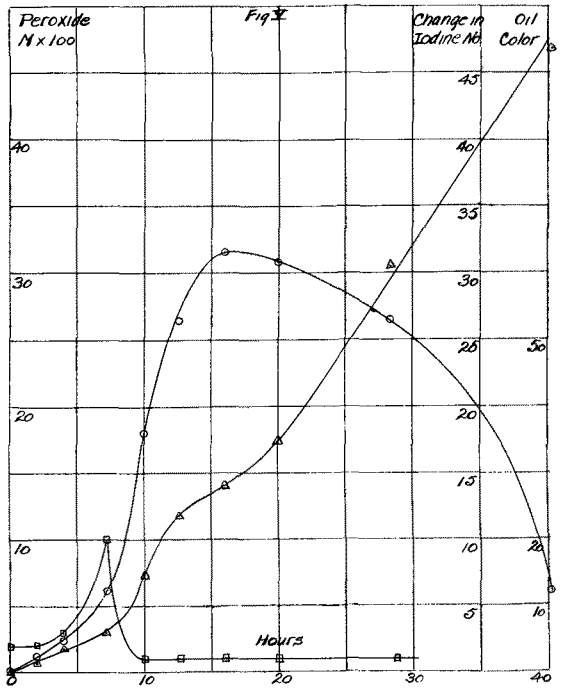
certain but necessary precautions that must be observed in conducting these methods and they have suggested some improvements on the original methods. At the same time the paper by Cocks, Christian and Harding (8) appeared, which discusses the accuracy of the Twitchell method when applied to the determination of solid unsaturated acids and describes a new modification which gives distinctly higher yields of solid unsaturated acids than does the original procedure. Both of these outstanding investigations should be consulted.

Up to comparatively recent years there was but little reliable information in regard to the kinds and proportions of the fatty acids occurring in the fats, and even less information about their glycerides. Methods which have been developed during the past fifteen years have made it possible to approximately determine the quantity of the individual fatty acids in many types of fats, with the result that a considerable amount of such data has already been obtained. Formerly, attempts to get some information as to the naturally occurring glycerides were confined to the fractional crystallization of the fats from various solvents. The most extensive investigations, using this method, were those of Amberger, Bomer and Klimont which were made between the years 1902 and 1924. Although quite a number of solid glycerides were at one time separated and identified through the application of this laborious frac-

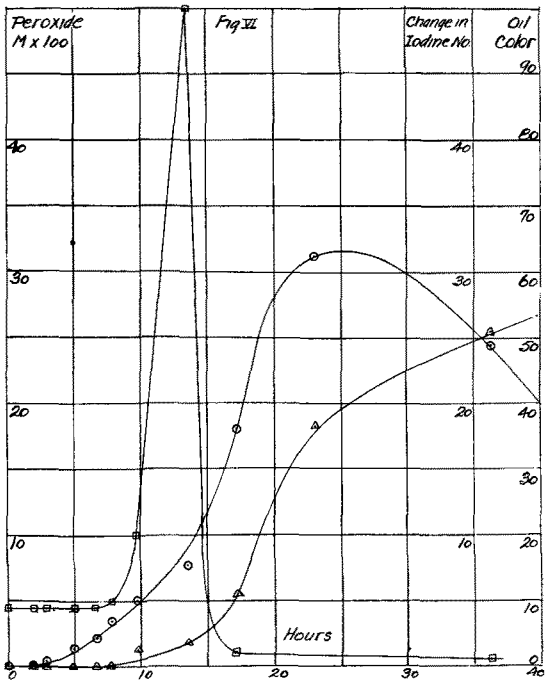


tionation process, it was in most cases only possible to obtain but few of those present in a given fat. Nor was it possible, with the exception of one or two instances, to get any idea as to their quantity. Consequently, any further advance in obtaining a more intimate knowledge of the distribution of the fatty acids among the glycerides of an individual fat was dependent upon the development of a radically different method of attack. Such a method was first de-

scribed by E. F. Armstrong and T. P. Hilditch (9) in connection with the determination of the position of the ethylenic linkage in acids of the oleic series. This method was based upon the identification of the oxidation products formed by the action of powdered potassium permanganate on the esters of the acids being investigated, and dissolved in acetone. Subsequently the method was applied to glycerides, and since then it has been used extensively by Hilditch and his associates at the University of Liverpool for the determination of the component glycerides in animal and vegetable fats. Before undertaking the investigation of the glycerides it is necessary to determine the kind and quantity of both the saturated and unsaturated fatty acids that are present. As the character of these investigations does not lend itself to an adequate discussion at this time of either the methods used or the results obtained the original papers must be consulted. As indicated by Hilditch (10), this method is best suited for the investigation of those fats which contain considerable proportions of both saturated and unsaturated acids in combination, and this includes practicaly all of the edible fats besides many of those used for technical purposes. Briefly, the method consists of oxidizing the neutral fat previously dissolved in acetone

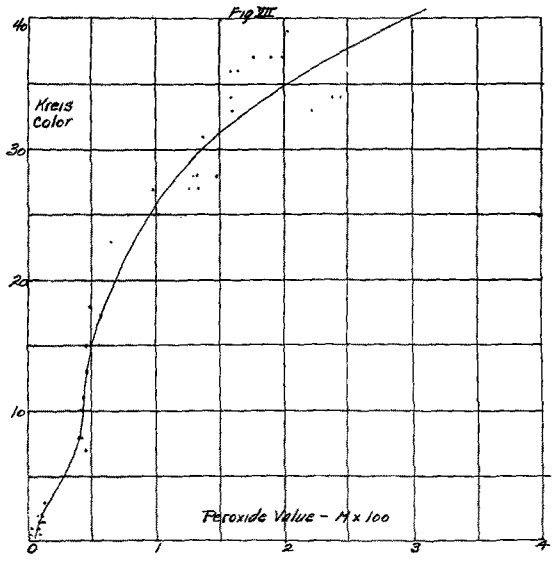


with powdered anhydrous potassium permanganate. For example, an original mixture of



fully saturated and mixed saturated and unsaturated glycerides, after oxidation, becomes a corresponding mixture, respectively, of neutral and acidic compounds. These are separated and the composition of the acids in each fraction is determined in order to get an insight into the structure of the original fat. Such an examination of a single fat naturally means much work and time. As early as 1929 Collin and Hilditch (11) stated that a sufficient number of natural fats had already been examined with reference to their glyceride structures to justify the general conclusion that animal fats are constructed upon more heterogeneous lines than vegetable seed fats, whereas it is beginning to appear that vegetable fats from other parts of the plant resemble animal fats rather than the kernel fats in so far as the distribution of the fatty acids amongs the glycerides is concerned. It is interesting to note that the synthetic glycerides prepared by Bhattacharya and Hilditch (12) from mixtures containing various but known quantities of the higher saturated and unsaturated acids were similar to those which have been found in animal and those vegetable fats not from the seeds, but as a rule the animal fats contain somewhat more and these vegetable fats somewhat less of the fully saturated glycerides than the synthetic fats having the same ratio of saturated to unsaturated acids.

In the case of seed fats, the determining factor according to Hilditch in the formation of glycerides is the marked tendency towards the even distribution of saturated and unsaturated acids amongst the glyceride molecules. Appreciable quantities of fully saturated glycerides are usually not found unless the molar proportion of saturated acids is about 60 per cent of the mixed fatty acids. As a result of these and some other studies, evidence has been obtained which indicates that triglycerides containing but a single acid are only formed in any quantity in a fat of either animal or vegetable origin when no other method of combination of the fatty acids is possible. These conclusions are further supported by the investigations of Eibner (13), Suznki and Yokoyana (14). For many years palm oil,



for example, was believed to consist chiefly of tri-olein and tri-palmitin, but recent investigations indicate that it contains only 6 or 7 per cent of palmitin. Also, various milk fats and mutton tallow were found to contain but small quantities of simple tri-glycerides such as palmitin and stearin. On the other hand, stillingia or Chinese vegetable tallow contains up to about 25 per cent of tri-palmitin, nutmeg butter about 50 per cent of tri-myristin, and laurel kernel fat about 25 per cent of tri-laurin. In view of the number of individual fats of various classes that have been examined by modern methods, it can be concluded that relatively few fats will be found which contain notable quantities of simple triglycerides of either saturated or unsaturated fatty acids.

As biological studies, in which the fats are concerned, obviously, should necessitate the consideration of their composition, it appears desirable at this time not only to indicate those things to be avoided in their preparation and examination but to offer some helpful suggestions, as well as to call attention to recent investigations on the glyceride structure of the fats.

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*The reference Annalen 27, 153 (1828) so universally quoted is incorrect. The first volume of the Annalen appeared in 1832.

Simplified Practice Ruling on Mayonnaise Containers

SIMPLIFIED practice recommendation No. R131-32, covering glass containers for mayonnaise and kindred products, which was approved at a general conference of representatives of the industry on June 30, 1931, may now be considered as in effect, according to an announcement by the division of simplified practice of the Bureau of Standards.

This announcement is prompted by the division's receipt of sufficient acceptances to insure the general adoption of the program by the industry.

Formerly 25 varieties of glass containers were used for packing mayonnaise and kindred products. This recommendation provides for 5 stock sizes of glass containers which are based on liquid capacity. They are: the 3 fluid ounce, the one-half pint or 8 fluid ounce, the pint, the quart, and the gallon.