Chemical Microscopy of Fats and Waxes

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I. Preliminary Communication

SOME years ago the term "chemical microscopy" was suggested by Chamot to apply to all chemical investigations utilizing the microscope. This is distinguished from "microchemistry" or the chemical manipulation of very small quantities of material by the modified quantitative procedures developed by Pregl and others. In Europe, however, microchemistry is applied to all reactions involving minute quantities, including qualitative reactions under the microscope. The writer considers Chamot's term the most appropriate for the work described here and in subsequent papers. Before we leave the question of terminology it may be well to mention that the word "fat" is used in its strict chemical sense, regardless of whether the material is solid or liquid at the ordinary temperature.

Although a large amount of work has been carried on in recent years concerning the chemical microscopy of technical products, the fats and waxes have not shared in this progress. A glance at the reference works on the subject and the indices of the various abstract journals will at once indicate the need for a systematic investigation along this line. The fact that very little work has been done may possibly be explained by the difficulties to be expected in the development of procedures for the identification of these products and the detection of their adulteration. In the first place the fats and waxes are not chemical individuals but complex mixtures of high molecular weight and as a consequence simple reactions for their identification are not easy to find. Secondly, their composition varies as to source, methods of production, age, conditions of storage and other factors. They are apt to be contaminated, either by natural or artificial means, with other fats or waxes or with extraneous matter. Obviously the only satisfactory approach to an ultimate solution of the problem lies in the investigation of the largest possible number of authentic specimens of known history. The task of collecting these specimens is now under way and the writer wishes to take this opportunity to thank Dr. G. S. Jamieson of the U. S. Bureau of Chemistry and Soils, Mr. H. P. Trevithick of the New York Produce Exchange, Dr. Arthur D. Holmes of the E. L. Patch Company and many others for their kind cooperation.

The need for micro methods applicable to the fats and waxes will be apparent when we consider that only minute quantities of materials are required and, as a rule, decisions can be made with an economy of time and equipment. After almost a year's work on this problem it is possible to anticipate procedures for identifying the individual fats and waxes, to detect adulteration, and to arrive at some idea as to the composition of the specimen—all on less than a gram of material. The identification of oil seeds and seed cakes by identifying the fat contained therein would be useful and some work in this direction has already been reported in the literature.[11] The investigations of Maclennan[7] on the microscopic structure of soaps in polarized light point to a wealth of information concerning the whole soap making process which may be obtained only with the microscope. His technic might possibly be applied to the identification of the various commercial soaps, and further to the identification of fats by the structure of their alkali soaps as seen in polarized light.

The purpose in indicating the practical advantages which may be reasonably expected from a study of the chemical microscopy of fats and waxes is two-fold. It will serve as an introduction to the papers which will follow from time to time and, it is hoped, will interest other workers to pursue similar studies.

Staining Methods

As mentioned above, very little work on this subject has been reported in the literature and no effort will be made at this time to give a complete bibliography of such papers as do exist. For years it has been known that certain dyes (Sudan III, Scarlet Red, Indophenol, Oil Red A, etc.) will stain fats as a group and thus aid in establishing their presence in tissue sections. Unfortunately essential oils and some resins are similarly stained but elaborate procedures have been developed to distinguish these from the fats. Stain procedures involving the use of dyes and osmic acid are also known for differentiating between the true fats, sterols, phosphatides and cerebrosides in tissues. No reference has been found so far to dyes which are specific for the individual fats and a number of experiments in this direction met with failure.

Reaction Methods

Since many fats yield crystalline soaps with the alkali and alkaline earth metals under certain conditions the saponification reactions have been used by biochemists to recognize fats as a group. Eckerson,[3] following the method of Molisch, employed a concentrated aqueous solution of KOH and 20% NH_4OH in equal parts to identify tissue fat by the formation of crystalline needles of the potassium soap. Crystalline soaps of sodium, potassium, calcium and magnesium have been found in various secretions of the human body under pathological conditions by Hausmann,[6] Müller,[8] Nothnagel,[9] Oesterlein,[10] Stadelmann[12] and others. These soaps have been called "fat crystals" and "margarine needles."

Thirty years ago Hartwich and Uhlmann[5] used the above KOH—NH_4OH solution for the identification of the individual fats, but the method had the draw-back that a considerable length of time was required before the desired crystal forms developed. To overcome this Rosenthaler[11] employed saturated alcoholic solutions of NaOH and KOH in absolute ethyl alcohol and obtained characteristic crystals almost at once with a number of fats. In some cases there was considerable difference in the types of crystals produced by the two alkalies and he found that cod liver oil was unique in that it gave no crystals with KOH but did yield them with NaOH. The writer has observed that the use of alcohols other than ethyl for dissolving the alkalies aid in the differentiation of fats. Particular attention has been paid to the saturated solution of KOH in n-butyl alcohol. Microscopic soap crystals, many of them apparently characteristic, have been obtained with this reagent and the following fats:

Almond, Apricot Kernel, Brazil Nut, Beef Tallow, Castor, Chinese Vegetable Tallow, Corn, Crude, Cottonseed, Refined, Gorli Seed, Hazel Nut, Lard, Lard Oil, Neatsfoot, Olive, Perilla,

Safflower Seed, Sesame, Shark Liver, Soy Bean, Sunflower Seed, Tea Seed, Tomato Seed.

No crystalline soaps resulted with the following:

Carpotroche, Chaulmoogra, Cherry Kernel, Chia Seed, Coconut, Cod Liver, Cohune Palm, Cottonseed, Crude, Grapefruit Seed, Ergot, Hempseed, Horse, Menhaden, Mustard Seed, Linseed, Palm Kernel, Parsley Seed, Peanut, Pimento Seed, Rapeseed, Rapeseed—Blown, Rice, Rubberseed, Sardine, Seal, Tung, Wheat Germ.

The history of these specimens, details of technic and photomicrographs of crystal forms will be presented at an early date. In some instances a number of specimens of the same fat have been examined while other observations have been made on only one specimen each. The study of additional specimens may show, of course, a number of changes in the above lists.

Another type of reaction which may prove of value for micro purposes is the formation of crystalline halogen addition products when some fats or their mixed fatty acids are treated with solutions containing bromine or iodine. A little progress has been made with the mixed acids of such fats as menhaden, coconut, linseed and horse.

The most promising reagent investigated to date is phenylhydrazine. In 1895 Curtius[2] reported that hydrazine hydrate ($NH_2NH_2 \cdot H_2O$) reacts on esters of fatty acids to give hydrazides and bihydrazides. Later the action of hydrazine hydrate on fats was studied by Falciola[4] who showed that the saturated acids yielded hydrazides while the unsaturated acids, due to the reducing action of hydrazine, became saturated. Then van Alphen[13] repeated Falciola's work but employed solutions of hydrazine in absolute methyl alcohol. He found that the unsaturated acids of linseed oil were first hydrogenated to stearic acid and the latter combined with additional hydrazine to form stearic acid hydrazide. He further reported that by heating phenylhydrazine with fats to 130-150° no hydrogenation took place but in every instance phenylhydrazides were formed.

It was decided to investigate the latter reaction to learn if it could be employed as a basis for micro identification of the various fats and waxes. Of the few specimens so far tried crystals differing markedly in structure were obtained with the following:

Almond, Carpotroche, Chaulmoogra, Cod Liver, Linseed, Menhaden, Palm, Rapeseed, Sardine, Tea Seed.

Apricot kernel, olive and castor oils gave no crystals in the same length of time. The mixed fatty acids of linseed and sardine oils gave different types of crystal structure than those produced by the parent fats. The waxes so far studied gave no crystal reaction products. With a few of the fats listed above two, and sometimes three, distinct crystal types were produced in a single preparation, probably due to the phenylhydrazides of the predominant acids in the fat. This seems to indicate that the reagent may give some insight into the composition of the fat.

The technic when using phenylhydrazine is simple: A drop of the specimen (solid specimens are melted by warming) and a drop of the reagent are intimately mixed on a slide with a glass rod and the preparation covered with a cover glass. It is now heated until a temperature of 150° is reached and then allowed to stand. A magnification of 100 diameters is sufficient to resolve the crystals and in some instances only 30 diameters are required. The only disadvantage in the method is that two or three days must elapse in some cases before all the crystals have matured, although with one or two specimens crystals formed after standing over night. Longer heating at the temperature indicated would undoubtedly speed the reaction to completion and work is now under way with this in view.

We have seen that both hydrazine and phenylhydrazine have the power to split fats and it is not unreasonable to expect that the other hydrazines together with their derivatives may prove of value as micro reagents. Since phenylhydrazine is a useful reagent for aldehydes and ketones it might serve in procedures for the micro detection of rancidity.

Although other substances react with fats (such as the urethanes) to give crystalline products the above will be sufficient to indicate some of the most promising leads which have developed in this investigation.

Other Methods

In addition to reaction methods the normal appearance of the fats and waxes under the microscope sometimes offers evidence which assists in identification. Considerable difference in structure has been observed between normal and deodorized coconut oil and this statement covers the examination of a number of specimens. Many fats when chilled yield crystalline structures which are occasionally characteristic. Gorli seed oil, for example, shows up at 100 diameters as a peculiar mass of overlapping globules; a structure which has not been met with in any other specimen examined to date.

The fats and waxes, and their mixtures, sometimes produce characteristic forms when crystallized under standard conditions from an appropriate solvent. This fact was made the basis of micro procedures for the detection of beef and mutton tallows as adulterants of lard by a number of investigators and a general summary of the work will be found in Volume II of Lewkowitsch (6th. ed., pp. 733-49). The methods which have been proposed are not entirely reliable but do, in some cases, yield useful information.

The detection of small quantities of carnauba wax as an adulterant of beeswax has been made possible by observing the characteristics of crystals deposited from solutions in n-butyl alcohol. A small amount of carnauba wax decidedly alters the characteristic crystals given by authentic specimens of beeswax crystallized from this solvent. The method was first proposed by Watson[14] using dark field illumination and modified by Baughman and Keenan[1] for examination in ordinary transmitted light. The latter investigators showed that it is possible to detect by this means as little as 0.3% of carnauba wax admixed with beeswax.

It will be noted that investigators have occasionally utilized the unique powers of the microscope for the examination of the fats and waxes. Much remains to be done before the advantages of microscopical technic can be fully employed in the analysis of these substances and products derived from them.

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