familiar quotation that "every scientific advance is an advance in method" and with the prediction that the countercurrent-distribution method will aid fat chemists of the future in making many scientific advances in their field.

REFERENCES

1. Ahrens, E. H. Jr., and Craig, L. C., J. Biol. Chem., *195*, 299 (1952).
2. Cannon, J. A., Zilch, K. T., Burket, S. C., and Dutton, H. J., J. Am. Oil Chemists' Soc., *29*, 447 (1952).
3. Craig, L. C., Anal. Chem., *22*, 1346 (1950).
4. Craig, L. C., J. Biol. Chem., *155*, 519 (1944).
5. Craig, L. C., Hausmann, W., Ahrens, E. H. Jr., and Harfenist, E. J., Anal. Chem., *23*, 1236 (1951).
6. Craig, L. C., and Post, O., Anal. Chem., *21*, 500 (1949).
7. Curl, A. L., and Bailey, G. F., J. Agr. Food Chem., *2*, 685 (1954).
8. Dutton, H. J., "Progress in the Chemistry of Fats and Oils," vol. 2, "Countercurrent Fraction of Lipids," pp. 292–325, London, Pergamon Press Ltd., 1954.
9. Dutton, H. J., and Cannon, J. A., "Glyceride Structure of Vegetable Oils by Countercurrent Distribution. I. Linseed Oil," presented at fall meeting of American Oil Chemists' Society, Oct. 11-13, 1954, in Minneapolis, Minn.
10. Granick, S., and Bogarod, L., J. Biol. Chem., *202*, 781 (1953).
11. Lancaster, C. R., Lancaster, E. B., and Dutton, H. J., J. Am. Oil Chemists' Soc., *27*, 386 (1950.)
12. Scholfield, C. R., Dutton, H. J., Tanner, F. W. Jr., and Cowan, J. C., J. Am. Oil Chemists' Soc., *25*, 368 (1948).
13. Swern, Daniel, and Parker, W. E., J. Am. Oil Chemists' Soc., *29*, 431 (1952).
14. Williamson, B., and Craig, L. C., J. Biol. Chem., *168*, 687 (1947).
15. Willstätter, R., and Stoll, A., Trans. by Schertz and Merz, "Investigations on Chlorophyll," Science Press Printing Co., 1928.

Microscopy of Fats and Oils

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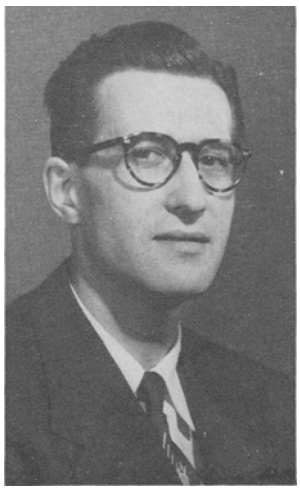
IT HAS BEEN SAID that there is not "a single great industry today where microscopic methods, intelligently applied, will not lead to more or less marked improvements" (1). The fat and oil industry is no exception. With the aid of the microscope much can be learned about the physical states in which fats and oils occur naturally and the various forms in which they are manufactured by man.



C. W. Hoerr

Remarkably few instances of microscopic examination of fats and oils are reported in the literature. The pioneering microscopy of Carlin (2) helped to explain the role of shortenings in air-leavened baked goods. Bailey (3) published a few photomicrographs of various fat crystals. The microscopic investigations of King (4) demonstrated the globular structure of butter. The photomicrographic studies of Quimby (5) have helped to unravel the various polymorphic forms of the triglycerides. The author (6) has utilized polarized light microscopy in characterizing the crystals of lard and rearranged lard. Although these references report applications of microscopy to various phases of fat and oil research, they refer only briefly, if at all, to the techniques and equipment involved.

Of the many books which have been written about the microscope and its uses, either of two relatively simple manuals on photomicrography (7) will acquaint the beginner with the general principles involved. Typical of almost the entire literature on the subject, these books refer to the microscopic examination of biological and mineralogical specimens for it has been in the various fields of biology and geology that the microscope has been most widely utilized. The equipment and techniques used in these areas can however be applied directly to the examination of fats and oils.

Compared with other modern laboratory instrumentation, photomicrographic equipment is relatively simple and need not necessarily be expensive. The optical instrument makers, chiefly Bausch and Lomb Optical Company, E. Leitz Inc., and Carl Zeiss Inc., have developed a wide assortment of microscopes and cameras suitable for fat and oil investigations. Since they have printed many excellent booklets describing the various specifications of their equipment, there is no need for a detailed review here.

The basic instrument for fat and oil applications is a microscope with appropriate lens combinations which possess good resolution at magnifications of 50–250 X. Suitable illumination must, of course, be provided. It is virtually essential that the microscope be provided with light polarizing equipment. All modern polarizing microscopes are equipped with a pair of nicol prisms, one of which is capable of being rotated 90° with respect to the other. A practical necessity with polarizing equipment is a revolving stage capable of being rotated 360°.

For purposes of recording observations, a suitable camera must be provided. Although some 35 mm. cameras can be adapted to the microscope, this negative size has not generally been considered satisfactory for the purposes which will be discussed. Most photomicrographic cameras utilize 3¼ x 4¼, 4 x 5, or 5 x 7-in. films. The first two sizes are most commonly used. Any of the relatively fine-grained films, such as one of the contrast process films, is satisfactory. Extremely fast shutter speeds are unnecessary since exposures of the order of several seconds are generally of little consequence in this work.

For purposes of accurately determining the dimensions of physical structures, such as crystals, water droplets, air bubbles, etc., some form of measuring device must be provided. In general use is the ocular net reticule consisting of a glass disc on which is ruled a rectilinear grid of accurately-spaced fine lines. This disc is placed at the appropriate position between the ocular lens components. The grid can be calibrated by means of a stage micrometer consisting of a glass slide on which is ruled an accurate scale usually 2 mm. in length, graduated in 0.01-mm. intervals. By viewing the stage micrometer through the ocular reticule, the apparent spacings of the grid lines can readily be determined at various magnifications.

There are no hard-and-fast rules regarding the preparation and viewing of fat and oil samples. Except for certain exceptional instances, such as investi-

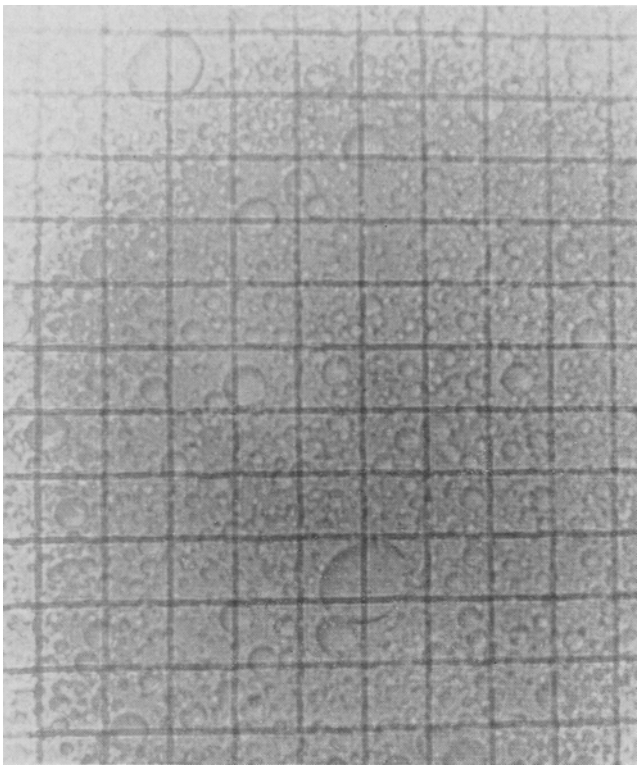


FIG. 1. Oil-in-water emulsion. Grid represents 9 microns between lines.

gation of polymorphic modifications (5), only a few obvious precautions need be observed. Viewed in ordinary incandescent light, physical structures such as globules of fat in emulsion, bubbles of air in shortening, and starch granules in cake batter can readily be distinguished because of the differences of their refractive indices from those of their environments. Fat crystals, on the other hand, are quite transparent to ordinary light but are readily visible when viewed in polarized light. The introduction of the polarizing microscope has generally outmoded the practice of staining specimens with oil-soluble dyes, except in special circumstances.

The usual polarizing equipment consists of two nicol prisms, one located below the substage condensing lens and the other between the objective and ocular lenses of the microscope. The lower nicol prism, known as the "polarizer," alters the character of ordinary light to produce plane-polarized light rays. When the upper nicol, the "analyzer," is parallel with the lower, the polarized light passes through and appears normal to the eye. When the prisms are crossed, *i.e.,* when the analyzer is rotated 90°, the light from the polarizer is completely absorbed and the field appears black and empty to the eye. Noncrystalline substances such as liquid fat, water, and air do not alter the path of plane polarized light and are therefore invisible when viewed through crossed nicols. Crystalline materials, on the other hand, by virtue of their molecular orientation possess the ability to refract polarized light. Fat crystals thus alter the angle of incidence on the analyzer and the light rays are not completely absorbed, the crystals being clearly visible against a dark background.

It must be remembered that the "depth of focus" of the microscope lens system is relatively narrow and decreases markedly with increased magnification. Quite thin specimens therefore are desirable to obtain the most sharply focussed images. All microscopes have a Vernier scale on the fine-focussing adjustment. By focussing from top to bottom of the specimen, the thickness of the sample can be read from the scale directly in microns. Since a sample thickness of no more than 50 microns is desired for the best viewing conditions at magnifications of 100–250 X, it is imperative that a cover glass be placed over the specimen on the microscope slide to obtain the proper sample thickness.

Fat emulsions generally require dilution with a relatively large volume of the appropriate continuous phase to separate the droplets of dispersed phase so that they can be seen distinctly. A small drop of diluted emulsion placed on a slide by means of a capillary pipette is sufficient for microscopic examination.

A small mass of plastic fat or cake batter, approximately one cubic millimeter, can be put on a microscope slide by means of a microspatula. Minimum distortion of the structure of the sample is obtained if, after placing the cover glass on the specimen, a second slide is placed over the cover glass and pressed straight down, gently but firmly, with no sidewise movement. Sample thickness can be governed quite simply by putting narrow strips of ordinary gummed

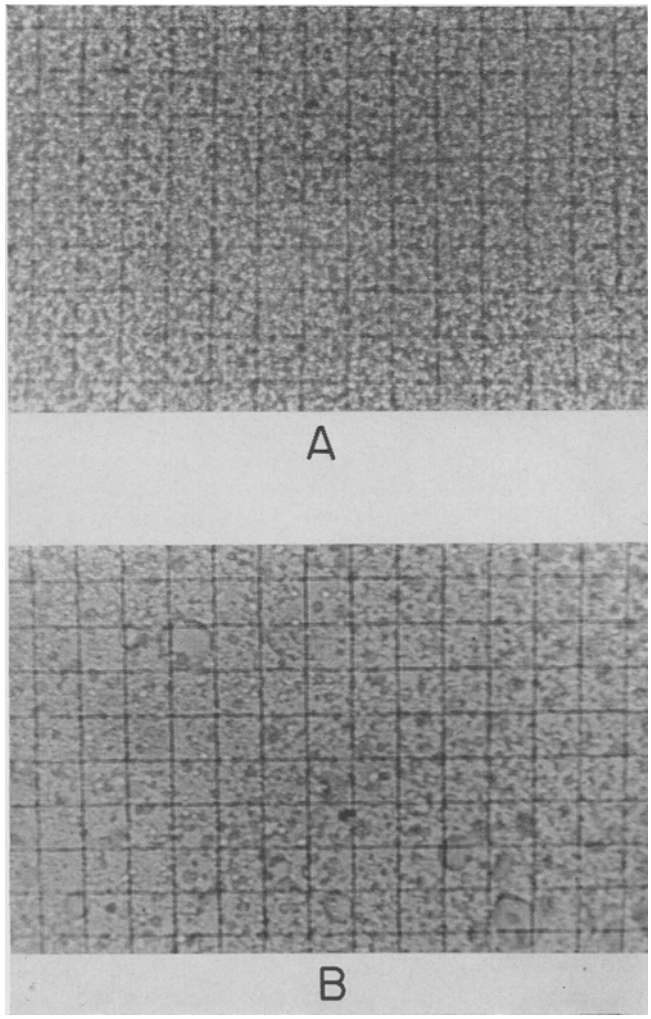


FIG. 2. Fat globules in margarines. Grid represents 28 microns between lines.

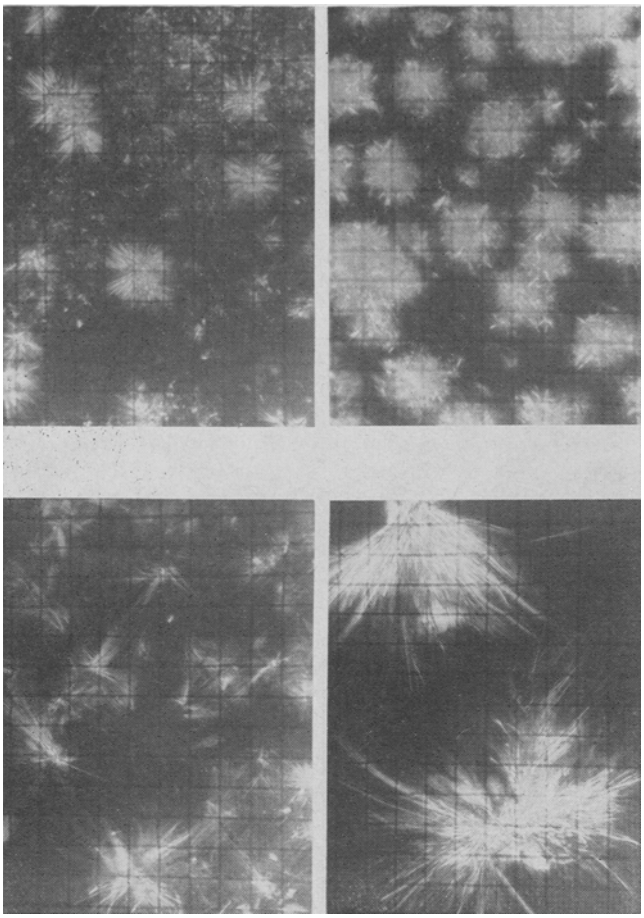


FIG. 3. Crystals of ordinary lard in polarized light. Grid represents 18 microns between lines.

paper at both sides of the specimen between the slide and cover glass.

Fat crystals can readily be precipitated by placing a small mass of fat between a slide and cover glass, melting the fat by means of the heat of a small flame (as that of a match), and allowing the slide to cool. Polarized light observations of crystals growing from their melt can be a source of unending novelty. Electrically heated microscope stages are available whereby the behavior of fats can be observed during heating, and melting points and transition temperatures can be determined accurately by means of thermometers or thermocouples.

Some of the areas in which microscopy has been found useful in the fat and oil field have been mentioned above and are illustrated by the accompanying photomicrographs. These areas can be summarized by the following general categories:

a) measurement of the particle size of oil droplets in aqueous emulsions;

b) determination of the size of fat globules in butter and margarine;

c) investigation of the crystal habits of unplasticized fats;

d) examination of the air bubble distribution in plasticized fats;

e) study of the incorporation of air in cake batters; and

f) characterization of the polymorphic modifications of triglyceride crystals.

Droplets of oil in an aqueous emulsion are shown in Figure 1. The grid in this instance represents 9 microns between lines. The larger droplets therefore ranged from about 7 to 12 microns in diameter, with the smaller droplets approximating one micron.

Figure 2 shows the globular structure of two margarines. Since the grid in these pictures represents 18 microns between lines, the globules of margarine 2A ranged from about 3 to 5 microns, and those of sample 2B up to about 20 microns.

Some of the variations in the crystal habits of lard are shown by the photomicrographs in Figure 3. These pictures were taken with polarized light, and the grid represents 18 microns between lines in each case. The variations in crystal sizes illustrate the influence of the rate of cooling. In general, the most gradual rate of cooling produces the largest crystals.

Figure 4 illustrates the influence of the method of processing on the distribution of air in the product. Figure 4A is a picture of ordinary lard produced by the chill roll and picker box procedure; 4B represents the same type of lard processed in a Votator; and 4C is a representative hydrogenated vegetable shortening also processed by Votator. The more irregular air bubbles are characteristic of Votated lards (6).

Variation in the air distribution in cake batters is

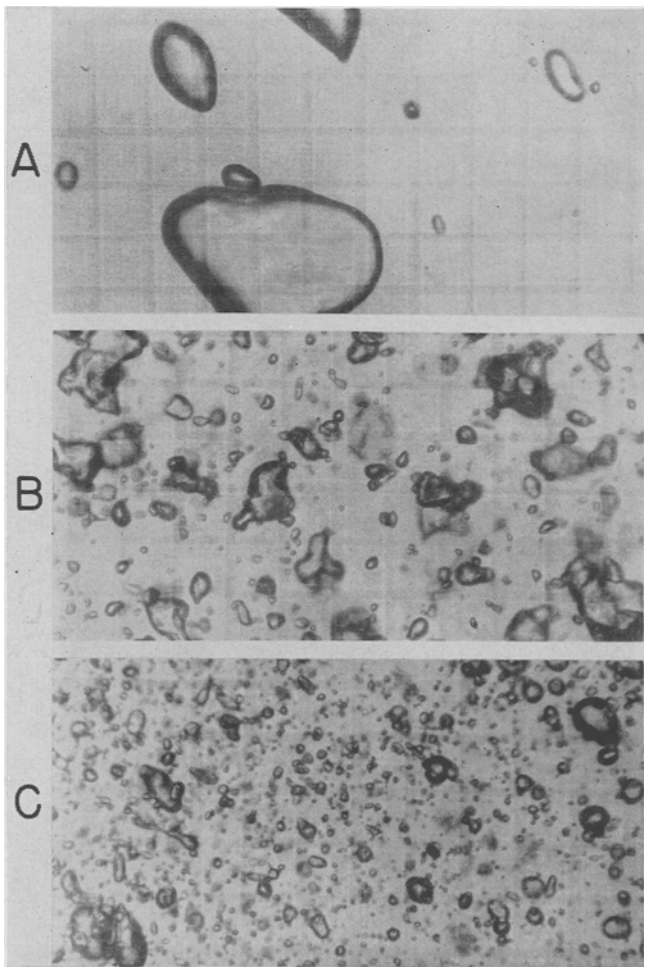


FIG. 4. Air bubbles in shortenings. Grid represents 18 microns between lines.

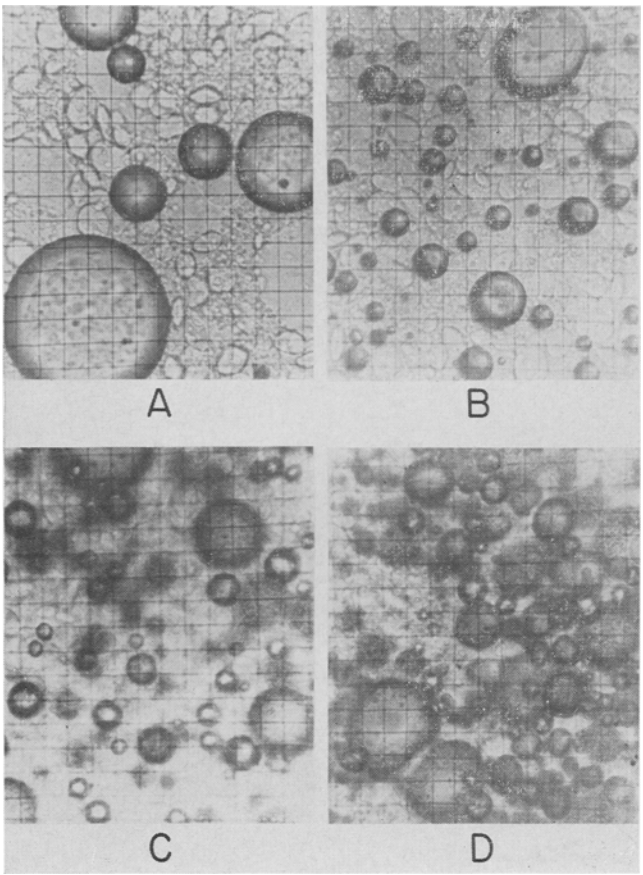


FIG. 5. Air bubbles in cake batters. Grid represents 9 microns between lines.

shown by Figure 5. In general, the more numerous the air bubbles in the batter, the higher will be the final volume of the cake, and the smaller the bubbles, the finer will be the texture of the cake (2). The batters shown in pictures 5A and 5B produced cakes of substandard volumes. Batter 5C produced a cake of excellent volume and texture. Batter 5D, on the other hand, was far too frothy and yielded a cake which rose too rapidly during baking and subsequently "fell" upon cooling.

Familiarization with general microscopy and examination of the accompanying illustrations will suggest many variations of the above applications. For example, with a little practice, quantitative approximations of the relative proportions of various constituents can be estimated with a reasonable degree of accuracy. From the average crystal size and the total volume of sample, the ratio of solid to liquid can be judged (3). By simple calculation of the volumes of the air bubbles in a given volume of cake batter, the percentage of incorporated air can be determined.

REFERENCES

1. Slogan of The State Microscopical Society of Illinois attributed to Prof. D. E. M. Chamot of Cornell University, reputed to be the "father of chemical microscopy."
2. Carlin, G. T., Cereal Chem., *21*, 189 (1944).
3. Bailey, A. E., "Melting and Solidification of Fats," Interscience Publishers Inc., New York, 1950; "Industrial Oil and Fat Products," 2nd Ed., Interscience Publishers Inc., New York, 1951; Proceedings of the Fifth Research Conference, Am. Meat Inst., Chicago, Ill., March 26, 1953.
4. King, N., Netherlands Milk and Dairy J., *4*, 30 (1950), containing numerous references to previous work in this field.
5. Quimby, O. T., J. Am. Chem. Soc., *72*, 5064 (1950).
6. Hoerr, C. W., and Waugh, D. F., J. Am. Oil Chemists' Soc., *32*, 37 (1955).
7. Allen, R. M., "Photomicrography," D. Van Nostrand Company Inc., New York, 1941; Shillaber, C. P., "Photomicrography," John Wiley and Sons Inc., New York, 1944.

Automation – Instrumental Analysis

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TODAY with the ever increasing demand on industry to provide more product, better product of more uniform quality—the role of instrumentation, control, and now automation takes on a new significance. Automation has various meanings for various people. This paper will be concerned with automation as the development of new types of sensing elements that have emerged from theory into practical laboratory instruments and then finally into the plant for instantaneous control.

Lewis Malter

The modern system of instrumentation has produced improvements in plant process control in many industries. The need is apparent for a general review of some of the present laboratory instruments and methods to determine if further savings of money, time, and manpower can be accomplished in other industries. Instrumentation has outstripped the conventional measurements of temperature, pressure, flow, etc., and is being extended to measure other important process variables used in the petroleum, chemical, and food industries: electrochemical, color and turbidity, and chemical composition.

It has become necessary for the plant instrument engineers to transfer the useful analytical instruments and methods from the laboratory and make them work under the more difficult plant conditions. One of the early analytical tools to be moved from the laboratory to the process plant was that for the measurement of pH. During the early development of pH instrumentation very delicate electrometers were used, and for industrial applications these were out of the question. Attempts were then made to use galvanometers with large thin-walled electrodes, but the extreme fragility of the electrodes and the unstable galvanometers again would not stand up in the plant. Later with the development of stable low current amplifiers the standard glass electrode was made practical for industrial application.

Applications of pH control can be found in practically every industrial process where aqueous solutions are involved. pH will affect the quality of the product as well as the speed and yield of reactions. Emulsification, electrolysis, neutralization, biochemical processing, bleaching and dyeing, coagulation and precipitation, and hydrolysis all require some degree of pH control. The most obvious applications, of