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Newer Analytical Methods for the Fat and Oil Industry

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THE NEWER METHODS for the analysis of fats and oils fall for the most part into two groups, that is, those relating to physical characteristics and those used for the estimation of composition. This is altogether fitting because certainly the functional properties of fats and oils are intimately related to their composition and to their physical properties. Major advances in analysis in the last several years have occurred in the following specific areas: chromatography, dilatometry, nuclear magnetic resonance, urea fractionation, and spectroscopy—with a few miscellaneous methods in other areas.

Chromatography certainly heads the list of important advances in fat analysis. It has probably been the single most active area of exploration in the field in the last several years and still remains very active. The word chromatography which identifies this technique stems from the work of Tswett and probably is not a good choice since it does not denote the action or the process. However we do not need to be concerned here with nomenclature.

Chromatography does provide a means of obtaining quantitative separations of certain mixtures, and if you will permit a prediction, the applications will undoubtedly be greatly expanded in years to come.

Chromatography can for our purpose be very simply defined as a technique which utilizes such phenomena as surface adsorption, partition between solvents, and ion exchange to bring about separations of simple and complex mixtures into their various components. It is recognized that the definition may not be altogether adequate or complete but it will furnish a basis for our discussion.

The materials commonly involved in chromatography as applied to the analysis of fats include a) a solid support, such as diatomaceous earth, silicic acid, various chemical salts, paper, and other substances, b) a liquid stationary phase, usually adsorbed to the surface of the support, of varying composition depending upon the specific application, and c) a mobile phase which passes over or by the stationary phase. The mobile phase may be a liquid or a gas and in the case of the former may be designated as solvent, eluant, etc.

A good though elementary example of column adsorption chromatography is the A.O.C.S. method for the estimation of total neutral oil. Briefly this procedure involves pouring the sample, dissolved in a solvent, onto a column of aluminum oxide, and allowing the solution to percolate through the column. The eluate, *i.e.* the portion that passes through the column, is collected and the solvent is evaporated. The weighed residue represents neutral triglyceride.

The reason for being able to separate neutral oil from free fatty acids under the prescribed conditions for this method is that the less strongly held neutral triglycerides pass through the column with the solvent and the more polar, free fatty acids are adsorbed on the surface of the aluminum oxide and thus do not pass through the column.

In the case of partition chromatography separation is attained by distribution of the components of the mixture between the mobile and stationary phases based on partition coefficients.

Broadly speaking then, the fact that the different components of a mixture can be retained on or can be made to pass over or through a column at different rates by suitably adjusting the conditions and by properly selecting the solvents and other materials is the basis for the technique of chromatography. The separation may involve adsorption as in the procedure just mentioned, or partition between liquids as is applied to the fractionation of fatty acids. Ion exchange is not to my knowledge applied in many areas of fat analysis.

Separations employing column adsorption or partition chromatography have been successfully applied to the fractionation of fatty acids, to the determination of individual fatty acids such as butyric acid in butterfat and others, and to the determination of saturated fatty acids. It obviously is a good technique but when the mixture becomes complex the labor involved is not inconsiderable. Therefore in such instances paper chromatography and gas chromatography are more practical.

PAPER chromatography is very widely used for all sorts of separations. The literature contains voluminous references to its applications and even in the limited area of fats and oils this technique has received considerable attention. In paper chromatography, the paper (cellulose) serves as the support and it is impregnated with the stationary phase required for the specific analysis. The appropriate mobile phase is caused to flow downward (descending technique), upward (ascending technique), horizontally, or radially as in circular paper chromatography. Many shapes and forms of the solid support are employed as may best fit the circumstance.

Following is a description of a very simple one-dimensional ascending paper chromatographic technique which is probably the most common type.

A drop of the sample is placed near one end of a strip of suitable paper. This end of the paper is dipped into an appropriate solvent and allowed to remain in this position for some specified time for development of the chromatogram. Due to capillary attraction, the solvent ascends the paper, the sample advancing with the solvent but at a slower rate. If the sample is a mixture, the individual constituents of the mixture advance at different rates and thus become separated into discrete areas or spots on the paper. Again differences in the rate of advancement of different substances in different solvents determines the efficacy of chromatographic separation.

The separation procedure described here yields a one-dimensional chromatogram. For more complex mixtures, two dimensional chromatography may be employed by running a second solvent through the paper at right angles to the direction of the first chromatogram. Also there are a variety of modifications that can be applied.

One of the problems connected with the application of paper chromatography to fats and fatty acids has been the visualization of the spots after development of the chromatograms. This is more often than not accomplished by spraying the paper with selective indicators after development of the chromatogram.

The R_f value is a term associated with paper chromatography. It designates the ratio of the rate of advance of the sample to the rate of advance of the solvent front along the paper. For a given set of conditions and a given substance it is constant. Therefore the R_f value is a useful qualitative guide but it is advisable always to run standards or samples of known composition for definite confirmation.

Quantitative estimates are derived in several ways including a) measuring the area of the spot, b) cutting out the area and determining the particular constituent, and others.

Paper chromatography has been applied to the determination of tocopherol, the separation of phosphatides, the estimation of antioxidants, and the fractionation of fatty acids. It is widely used for the determination of pesticide residues and it is the only method I know of for the determination of free gossypol.

GAS chromatography includes those chromatographic techniques in which the mobile phase is a moving gas. It is certainly obvious that gas chromatography is one of the most valuable analytical tools ever to become available to the fat and oil industry. This technique provides the most accurate, most precise, and most rapid means of estimating fatty acid content yet devised. In general the procedure involves passing a portion of the mixture to be analyzed through a heated column by means of a carrier gas such as helium or nitrogen. The stationary phase, usually Aziepon or the polyesters of adipic or succinic acid is distributed over an inert substance such as diatomaceous earth. The components of the mixture are eluted with the gas and detected and measured at the exit end of the column by a suitable means. The retention time is the time required for a given compound to pass through the column. It is designated as an R_f value and is related to the specific compound and the stationary phase but it is also dependent upon other factors. The fatty acid esters determined with Aziepon as the stationary phase exit in the order of boiling point, whereas when separated on the polyesters they come out of the column in the order of saturation. The retention line is indicated on the horizontal axis of the chart and it is a qualitative index of the substance and the area under the curve is in each case a quantitative measure of the component. This area, of course, may be determined by triangulation or with a planograph.

The esters are ordinarily employed for this separation because of their relatively lower boiling points. Columns of various diameters and lengths have been employed and capillary dimensions are becoming more common. The capillary types seem to aid in certain difficult separations, but usually they require more time for completion of the analysis. Figure 1 depicts a separation of lard stearine. Figure 2 is a chromatogram of the methyl esters of a hardened vegetable oil in a capillary column using succinic acid-diethylene glycol polyesters. Figure 3 is a chromatogram of the methyl esters of the same hardened vegetable oil with an ordinary column using Apiezon L.

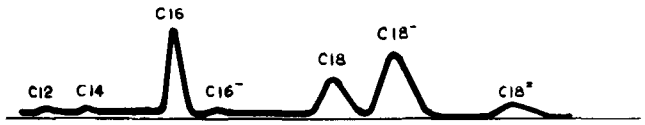

FIG. 1. Gas chromatographic separation of lard stearine.

One caution to be observed with chromatographic methods is the necessity for employing reliable standards. The conditions and materials for specific meth-

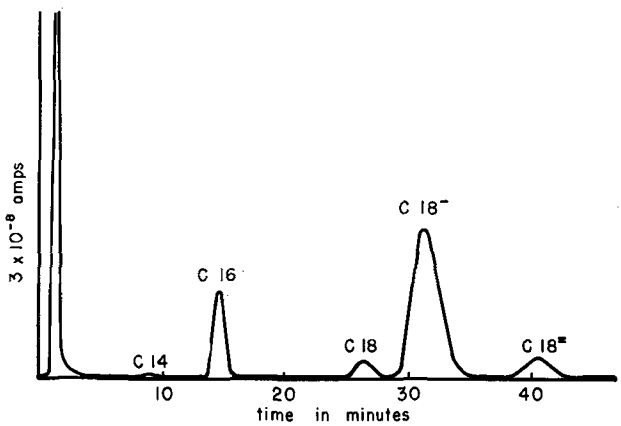

FIG. 2. Gas chromatographic separation of hardened vegetable oil in a capillary column.

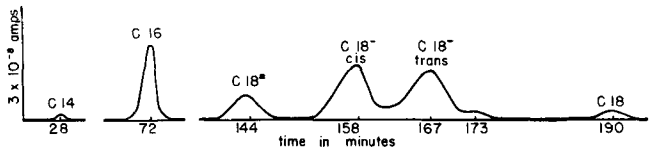

FIG. 3. Separation of hardened vegetable oil in an ordinary column.

ods are of necessity established experimentally and they are to a degree empirical.

The determination of soild fat content (SFI) at any given temperature is no longer in reality a new method in the fat and oil industry even though there are still many who do not employ it. For this reason and because previous reports have dealt primarily with methodology it was deemed proper to include some discussion of the significance of this determination at this time. To begin with, we must recognize that the results of the SFI determination are arbitrary, for in the determination we make some assumptions and take some liberties insofar as precise measurement is concerned.

These liberties and assumptions are: 1. The use of volumetric instead of gravimetric measurements. 2. The use of solutions other than mercury as the confining liquid. 3. The assumption that the slope of the liquid and solid lines are parallel. 4. The assumption that the slope of the liquid line is the same for all fats. 5. The assumption that the melting dilation is 0.1. Some of these assumptions are of necessity and others are for convenience. However even with the assumptions that we make and the liberties that we take for practical reasons, the results have relative significance and they are also related to other properties of fat which in turn are important with respect to performance and use.

Several factors contribute to the hardness of a given fat under a given set of conditions but one of the most important contributors is the solid fat content. The fact that the solid fat content is closely related to hardness or consistency is probably the single most important application of this procedure, although it has also been employed in studies of polymorphic changes.

Generally speaking, the procedure consists of determining the volume of a given quantity of fat at a given temperature. By a series of such determinations at different temperatures, in the temperature range over which the fat passes from a solid to a liquid state, and then plotting and connecting the results on graph paper, we get a curve which shows the change in volume over the temperature range selected. From such an SFI curve, that is, a plot of melting dilation *vs.* temperature, we can glean considerable information. Figure 4 represents an ideal melting dilation curve, since an actual one consists of a series of short straight lines joined together and not a smooth line such as this. The point at which the melting curve joins the liquid line approximates the melting point of the fat as determined by the closed capillary proved method. Since the solid fat content at any given temperature determines in part the hardness of the fat at that temperature, the SFI is of course an index of hardness. However a single relationship between SFI and hardness does not hold for all fats or all blends of fats. Figure 5 shows the SFI–consistency relationships of two common varieties of fats and it is obvious that these are different. Therefore anyone choosing to use SFI as a basis for controlling consistency is obliged to establish the specific relationship that holds for his product or products.

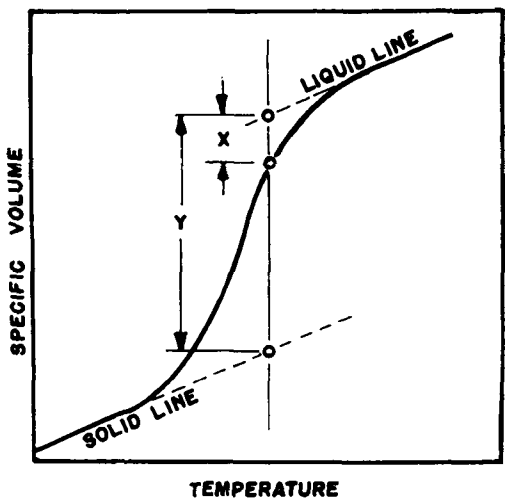

FIG. 4. Melting dilation curve.

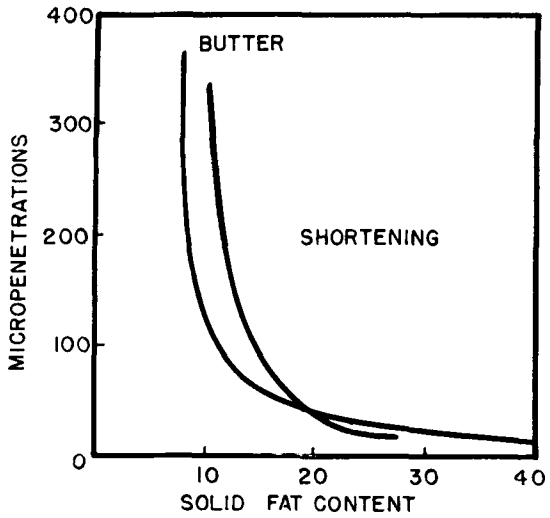

FIG. 5. Consistency *vs.* penetration.

Plastic range, in the fat and oil industry, usually refers to the temperature range over which the fatty products remain plastic or workable. This is of course with particular reference to shortenings. The shape and extent of the SFI curve provides an index of the so-called plastic range. When SFI–temperature data are plotted as shown in Figure 6, this feature shows up more distinctly. These are natural products about which we have a good idea of their plastic range and melting characteristics. In this graph the shape of the curve over a substantial part of the melting range is exaggerated and thus provides a better picture of the area we are interested in. The sharp melting characteristics of butter and cocoa butter are evident. Also the harder characteristic of cocoa butter is obvious. Likewise the more plastic nature of some other fats is indicated.

Jasperson and McHenry plotted results somewhat differently, Figure 7, *i.e.*, they plotted differential cooling curves relating the rate of expansion to

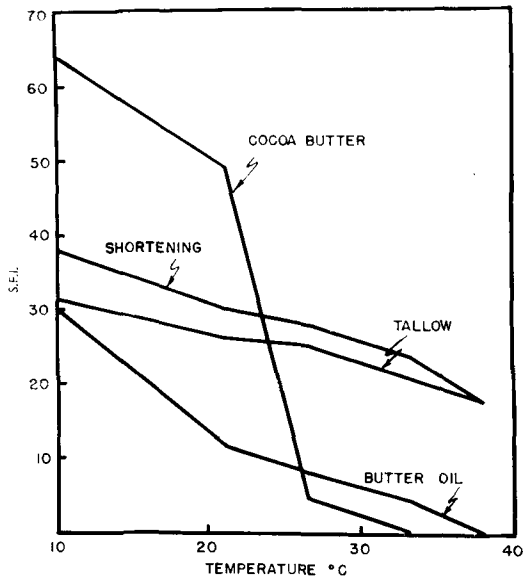

FIG. 6. SFI curves for various fats.

temperature. When a specific type of glyceride predominates, it is indicated by a sharp maximum in a narrow temperature range.

SPECTROSCOPY was a notable contribution to the technology of fats and oils when it came into being many years ago. Certain spectral methods are now well established and well standardized and very satisfactory within their limitations. There have been continuous advances in the broad area of this field. A method for determining the so-called *trans* acids based on infrared adsorption spectroscopy is now widely used even though the method has not yet been accepted as a standard method. The near infrared adsorption has been applied to the estimation of *cis*-unsaturation, and to the characterization of fatty acid. X-ray diffraction is being applied to the elucidation of structure and to the determination of certain types of polymorphism. Flame photometry provides a rather simple procedure for the estimation of certain metals and particularly for sodium soaps through the measurement of sodium.

One of the most interesting developments in the area of spectroscopy is nuclear magnetic resonance. Historically speaking the first NMR signals were observed in 1945 in two independent physics laboratories located on opposite sides of the United States. The co-discoverers Felix Bloch at Stanford and Edward M. Purcell at Harvard received the Nobel Prize in November 1952. However it is doubtful if even they envisioned the full potential of their discovery.

NMR spectroscopy is based on measurement of the adsorption of radio-frequency energy which takes place when some materials are placed in a magnetic field and simultaneously exposed to an R_f field of the right frequency. Approximately 100 isotopes of the chemical elements can be detected in this way. The magnetic properties of the nucleus of the atom and the value of the applied magnetic field determine the frequency at which energy is absorbed. In a magnetic field of given strength no two isotopes absorb energy at the same frequency, which makes this a highly selective technique.

Low resolution (broad line) NMR has successfully replaced chemical analyses in certain instances and applications are being extended. For example the rapid, nondestructive determination of moisture in a variety of products is an accomplished fact and recent publications have indicated that the determination of liquid-solid content of fats can be accomplished by NMR as is now done by dilatometry. These applications are possible because the hydrogen contained in the sample can be measured as a function of its mobility in the compound. This permits differentiation on the basis of chemical combination and physical state.

In addition so-called high resolution (narrow line) NMR has been used as a supplement to other spectral methods in the elucidation of molecular structure problems. While NMR spectroscopy is still comparatively new it bids fair to become an ever more valuable addition to the fields of physical and analytical chemistry.

There seems to be some possibility of fractionating by means of urea inclusion compounds. This depends on the ability of urea to form adducts with the naturally occurring fatty acids. The adducts formed contain the constituents in definite proportions depending upon the character of the fatty acid. The long chain acids form adducts more readily than the short chain acids and the saturated acids form adducts more readily than unsaturated acids. Urea-adduct formation has been applied analytically several times and the reports have appeared in the literature. It has been employed to detect rapeseed oil in olive oil, to detect linseed oil in mustard seed oil, and to fractionate the fatty acids of various fats and fatty products. While for the time being it does not approach other fractionating techniques, it may have some specific, even though limited, applications. So far as I know, only one specific and detailed method has been published and this pertains to the detection of rapeseed oil in olive oil.

A rapid dielectrometric method for the estimation of fat content is now available. This procedure has been applied to a variety of source materials and appears to work quite well. This method depends on the fact that when a fat-solvent solution is placed between the plates of a high-frequency oscillator, the characteristics of the oscillator are determined in part by the concentration and type of fat in the solvent. The apparatus is designed so that the changes induced in

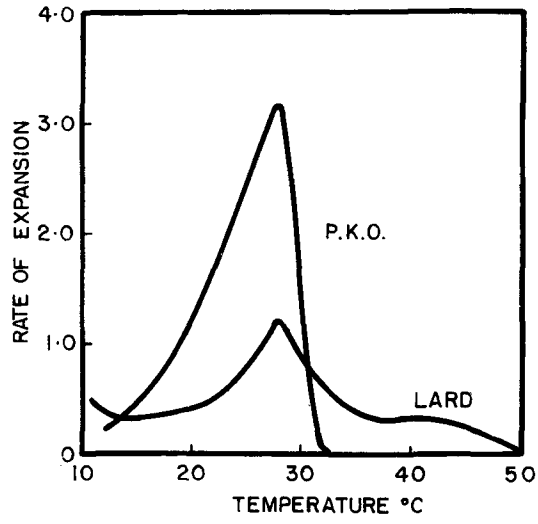

FIG. 7. Differential dilatometric curves.

the radio frequency impedance of a condenser by altering the characteristics of the dielectric medium between the plates of the condenser are related to frequency and indicated by a frequency meter. When oil is added to a standardized solvent, there is an increase in frequency which can be related to the concentration of the oil or fat in the solvent. This method provides the distinct advantage of rapidity which is sometimes of paramount importance.

The measurement of fat stability continues to be a major problem. Oxygen absorption methods appear to be gaining in popularity and they possess the distinct advantage of being a direct measure. The TBA test (thiobarbituric acid) has been investigated by many people. This is a colorimetric procedure, but whether or not it proves to be any better than other colorimetric tests remains to be seen.

Undoubtedly improvement in methods for the analysis of fats deserves a share of the credit for the advancement of fat and oil technology of recent years. Some of the areas mentioned herein will be improved and broadened in the future. New techniques and methods will appear as the needs of the industry dictate.

The problems connected with providing complete and objective methods with which to characterize and determine the ultimate composition of fats and oils has not been easy because of the complexity of these materials, but progress is in the air.

Acknowledgment

The author wishes to express his appreciation to F. L. Kauffman, Swift and Company, for Figures 1, 2, and 3.

Economics of World Supply of Edible Fats and Oils

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IT IS DIFFICULT to separate the economics of fats and oils supply from the demand for fats and oils. As with all goods and services, production is a function of demand. Any comments about the prospects for supply of the various fats and oils must be conditioned by considerations of use.

Similarly it is difficult to separate edible from nonedible fats. There are several fats and oils that go into two categories or more of use so that the whole structure is interrelated. Soybean oil, for example, is an edible oil but is also used extensively for industrial purposes. Tallow is primarily a soap fat but is extensively used for edible purposes. Most tallow is strictly inedible, but substantial quantities can go either way.

The edible fats and oils do not make up an homogeneous unit. They have different characteristics and different uses. At the same time several of them overlap the various uses so that they are competitively interlinked, but they are not widely interchangeable over the total usage of the several fats and oils.

Nearly all of the fats and oils are either by-products or joint products. Their production is a part of the production process of something else. Thus supply is partially a function of the demand for some other product. There is a wide range in the "other" product importance of the various fats and oils, ranging from, say, coconut oil for which oil is almost all of the value, to cottonseed oil which makes up a very small proportion of the value of cotton.

Resource Use

In a capitalistic, competitive, economic system productive resources are allocated on the basis of the value of the marginal product compared to the value of the marginal product of the resources when employed in alternative uses. This is the essence of the economics of supply. When a given set of resources, land, labor, and capital can be employed in the production of oil-bearing crops more profitably than in some other use, say grain, they will be used for oil-bearing crops. The converse is, of course, true.

However the whole of the world is not ordered in this fashion. There are extensive governmental programs and activities throughout the world that distort the economics of production. The extent of the distortion varies widely among the many countries involved. In some countries, like the United States, there is nearly complete freedom of employment of agricultural resources while in others, particularly those socialist countries where agriculture is ordered along delivery quota lines, resource use in the short run is very little responsive to relative profitability.

In looking at the long-run supply considerations, we must assume that resource use is responsive to relative profitability so that the basic economics of supply will prevail. This is a valid assumption. Even in those situations in which agriculture is ordered by government fiat, the relative profitabilities are the same as in less controlled areas. Misallocation results in a decrease in total production and so is not likely to be indefinitely continued. While there are limitations to the free flow of fats, oils, and oil-bearing materials among nations, they are not sufficiently great to avoid competition. These restrictions result in distortions, but in the final analysis nations will either produce or purchase fats and oils on the basis of the cheapest source. For example, the production and use of rapeseed is fostered in north Europe by governmental activity even though it is uneconomic. While the production and trade of total fats and oils is distorted, the total effect is small and should be expected finally to disappear. Similarly the production of peanuts in Africa is a part of the colonial scheme and is fostered by various devices. My argument here is that in the final analysis peanut production will increase or decrease, depending upon the economics of its competitive position relative to other oilseeds.