X-ray Diffraction of Fats

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X-RAY DIFFRACTION is among the oldest of what we can consider the modern instrumental tools for investigating the nature of fats and oils. Barely 12 years have elapsed since Martin and James published their classic paper on the separation of fatty acids by gas chromatography in 1952. It is less than 15 years ago that Binkered and Harwood presented their brief descriptive paper on the application of infrared spectroscopy to fat and oil chemistry at the Fall Meeting of the AOCS in 1949. In contrast, 30 years have gone by since Malkin and his collaborators in 1934 published the first of their series of papers on X-ray studies of glycerides.

Once they were introduced, both gas chromatography and infrared spectroscopy received relatively rapid universal acceptance by the fat and oil field. Consequently, in these areas there has resulted a veritable deluge of thousands of publications from laboratories throughout the world. On the other hand, the application of X-ray diffraction has developed very slowly in the fat and oil field. It is doubtful that 100 papers can be found in the literature relating to the crystal structure of glycerides, and most of these have been published by only two investigators, Malkin in England and Lutton in this country. In fact, the number of laboratories that have published in this area can almost be counted on the fingers of one hand.

Both gas chromatography and infrared spectroscopy seem to have been applied to fats and oils in such a straightforward, systematic manner that they are quite readily understood by everyone. As a result, the literature on these subjects is remarkably consistent; each investigator can easily duplicate the data and substantiate the results of others. In contrast, the systematic correlations have not been so evident in the application of X-ray diffraction to fats. Each successive investigator in this field has published his own theories, each of which differs in some respect from those of the others. During the years before Malkin's death in 1958, a long-standing controversy ensued over the interpretation of the crystal structures and polymorphic transformations of glycerides. This has left the literature confused by conflicting explanations, inconsistent nomenclature and outright misconceptions which have not been conclusively resolved to this day.

I should mention at this point that I happen to be part of the controversy. In an AOCS Short Course lecture on "Morphology of Fats, Oils, and Shortening" [*JAOCS 37,* 539 (1960)], I attempted to correlate Malkin's and Lutton's divergent interpretations of the various crystal forms of the triglycerides and tried to apply their ideas in an effort to explain the polymorphic behavior of commercial fat products. Instead, I found that I could not accept some of the interpretations of these investigators, and I succeeded only in introducing yet another viewpoint with an additional system of nomenclature which differs from the others. Thus, the reader must bear in mind that the interpretations, explanations and comments which appear in this article are largely my own and, plausible as they may seem to be, they may not necessarily be accepted wholeheartedly by other investigators.

For those who may not be familiar with the application of X-ray diffraction in the investigation of fats and oils, I shall review the background very briefly without dwelling in detail on the more embarrassing aspects of the historical developments. I shall limit my discussion in this article to the crystal structure of glycerides, particularly to the work on triglycerides, with only passing reference to the mono- and diglycerides. Consideration of the studies on fatty acids, soaps and other derivatives will be deferred to possibly another article.

Long before it was demonstrated by X-ray diffraction that fats exist in several distinctly different, but inter-related, crystal forms, it was known that the triglycerides exhibit more than one melting point. As far back as 1849, Heintz reported that tristearin, after being quickly solidified and reheated, melted at about 52C, but with less rapid solidifying and reheating it melted at about 63C. In 1853, Duffy found three melting points for tristearin at about 52, 64 and 70C. During the next 60 years, Berthelot (1854), Guth (1902) and Lautz (1913) reported only two melting points for tristearin, as well as for tripalmitin and trimyristin. Then, in 1915, Othmer rediscovered three melting points for tristearin and, in 1928, Loskit again found three melting points for tripalmitin. However, in 1930, Jogtekar confused the issue by reporting only two melting points for a number of triglycerides thought to be of high purity.

Up to this point in history, it seems to have been assumed that the multiple melting points of the triglycerides were attributable to some sort of isomerism. In 1932, Weygand and Grüntzig suggested that the phenomenon may be related to differences in crystal form and, on the basis of cooling and heating curves, they deduced that tristearin existed in seven different modifications. It has since been demonstrated that thermal measurement alone can be quite misleading without thorough consideration of the entire sequence of physical events occurring during such treatments.

Credit must be given to Malkin for greatly clarifying the situation as it existed at that time. As a result of his pioneering experiments which correlated melting point determinations with X-ray diffraction patterns, he proved definitely that the glycerides actually exhibit polymorphism and not isomerism by showing that they transform systematically through a series of successive crystalline forms. In making his correlations, he identified the lowest melting form, the form which first solidifies from the liquid state, as a non-crystalline, vitreous solid that he called the *gamma* form. According to his interpretations, this transformed to the crystalline *alpha* form which, in turn, transformed to the *beta prime* form, and then to the highest melting, stable *beta* form. Thus, he identified four distinct solid forms of the triglycerides, three crystalline and one noncrystalline.

In his series of papers on glyceride crystal structure which began in 1946, Lutton denied that the glycerides exist in the noncrystalline, vitreous form and claimed that they exhibit only the three crystalline forms. He identified the lowest melting form as the *alpha* form. According to his interpretations, this transforms successively to the *beta prime* form, corresponding to Malkin's *alpha,* and then to the *beta* form identical to Malkin's *beta.* Lutton has insisted that the form which Malkin called *beta prime* does not exist. Subsequently, Daubert and Bailey independently confirmed Lutton's interpretations. This led Malkin to refer to the latter as the American school of thought as distinguished from the European interpretations fostered by himself. Incidentally, while Daubert used Lutton's Greek-letter designations, Bailey identified the respective triglyceride forms by Roman numerals I, II and III (highest to lowest melting).

At the 1957 Spring AOCS Meeting, I presented a paper on the crystalline forms of commercial fats and oils which served as the basis of the Short Course lecture mentioned above. As a result of my investigations, I presented experimental evidence that the triglycerides can exist in four crystalline forms. Of these, the *alpha, beta prime* and *beta* forms are identical to the corresponding forms postulated by Lutton. In addition, I have proposed the existence of the *intermediate* form which occurs between the *beta prime* and *beta* forms and corresponds to Malkin's *beta prime* form.

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X-ray Diffraction . . . .

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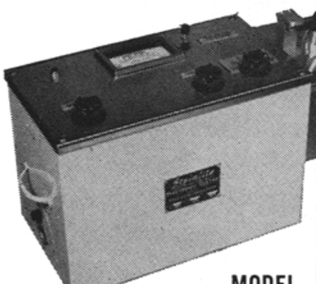
In 1961, Giddey reported that a number of triglycerides in cocoa butter exist in four crystalline forms. Within the past year or two, Giddey's results on cocoa butter have been repeated and confirmed independently by Vaeck, Chapman and Andersson. Although the four crystal forms found by these investigators correspond to the four crystal forms that I postulated earlier, they chose to introduce yet another set of names for the crystal forms, calling them *alpha, beta double-prime, beta prime* and *beta* (lowest to highest melting, respectively). As a consequence, the five different existing systems of nomenclature require considerable mental gymnastics in order to determine the correlation between the respective crystal forms. Fortunately, except for Bailey, all investigators from Malkin to the present have agreed to call the highest melting form *beta.*

Doubtless, many reasons could be given for the state of confusion that exists in the literature on this subject today. However, I submit that all of the diverse data that have been published can ultimately be explained on the basis of the degree of purity of the materials. The basis for this opinion has been presented in my Short Course lecture. I showed that, as triglycerides are more highly purified, their rate of transformation to the stable *beta* form is accelerated to such an extent that, in some cases, the less stable forms are not observed, or are confirmed only with considerable experimental difficulty. Thus, the more homogeneous fats, i.e., those which consist of relatively few closely related homologs, tend to transform rapidly to the stable *beta* form. On the other hand, the more heterogeneous fats, i.e., those containing a diverse assortment of different triglyceride molecules, tend to transform relatively more slowly. In fact, certain fats consisting of completely randomized assortments of triglycerides appear to exist indefinitely in the *beta prime* form and transform further only under extremely unusual conditions. There is, therefore, a direct correlation between molecular composition and polymorphic behavior. I suggest that, contrary to current opinion, the apparent stability of individual crystal forms cannot be related to specific molecular configurations but is a function of the degree of impurity of the material.



It must be realized that practically all the published work on crystal structure of glycerides was done on materials which were prepared before the modern chromatographic techniques were available for accurately defining their compositions. With the application of these newer methods, we are becoming increasingly aware of the difficulty in preparing really pure triglycerides. We now know, for example, that a one-degree depression in the melting point of tristearin can be brought about by the presence of 15% or more of certain homologous impurities, whose presence can be demonstrated only by extensive analytical detective work, but which nevertheless produce a profound influence on the polymorphic behavior. Only 2–3% of certain impurities that can barely be detected even by precise thermal measurements may markedly affect the polymorphic behavior. Consequently, we are now convinced that compounds which were once considered to be of reasonably high purity were actually rather impure.

Thus, some of Malkin's observations which have been thought by others to be erroneous must be re-interpreted in the light of our knowledge of the effect of impurities. Although his ideas cannot be regarded as explanations of the true polymorphism of pure triglycerides, they do substantiate what we know about the crystal transformations of fats, which are really only very impure triglycerides. Although a great deal of evidence has been arrayed against the existence of the vitreous form [see Chapman's summary of glyceride polymorphism in *Chem. Reviews, 62,* 443 (1962)], we are not so sure that Malkin was entirely mistaken about this form. We now have indications that certain mixtures of glycerides may, on occasion, exist in a metastable (liquid crystalline) state, indicating only very limited molecular orientation. We might yet rediscover that glycerides can under certain conditions exist in a noncrystalline form.

Having thus reviewed the background of the subject, we may well wonder whether there is any justification for further concern over the crystal structure of fats, other than merely to straighten out the record. Rest assured that the problem is becoming increasingly more important to commercial operations with the passage of time. Although very few laboratories have published reports relating to their use of X-ray diffraction, we know that there is an indeterminately larger number of X-ray diffraction installations scattered throughout the fat and oil industry in this country and in Europe. Since these machines are not inexpensive, we can be certain that they are not being purchased for decoration.

Reference has been made to the relation between crystal form and melting points. This relation has a bearing on all other physical properties of the fats. Each crystal form exerts a specific effect on the plasticity, hardness, solubility, texture, mouth-feel and other properties of the product, as well as profoundly influencing the performance of fat products in their various applications. Because of their specific physical structure, certain crystal forms enhance the aerating properties of fats, others are deleterious; certain forms are desired in confectionery coatings, others are not. Crystal structure is, therefore, of concern in many bakery operations, in the production of certain dairy products, in confectionery coating products, in cosmetic preparations and, indeed, in every application of fats in which any solid triglyceride component is involved. Thus, it is obvious that determination of the crystal form in which a fat exists and the knowledge of the treatment required to produce the desired form have increasingly important commercial implications. In fact, the utilization of fats has attained a degree of sophistication such that, on occasion, X-ray characteristics are being included in product specifications.

Perhaps a better understanding of X-ray diffraction can be gained by a brief explanation of the principles involved. An X-ray diffraction unit is primarily a large transformer connected to a special type of electron tube, the X-ray tube. The purpose of this machine is simply to produce high-speed

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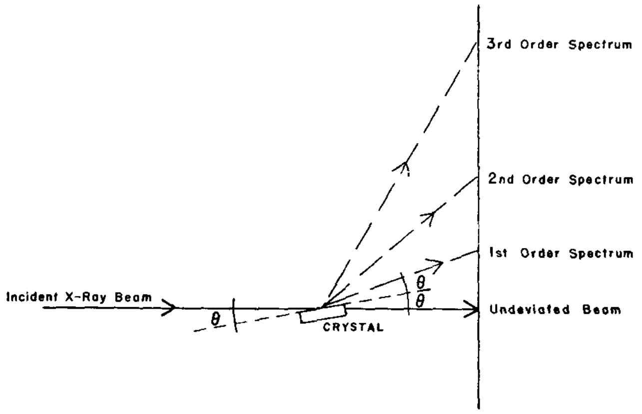
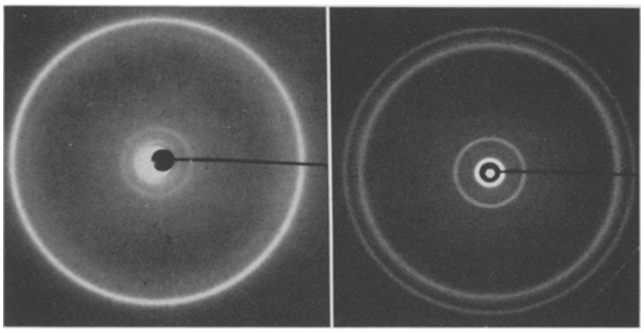


Fig. 1. Schematic diagram of the optics of X-ray diffraction.

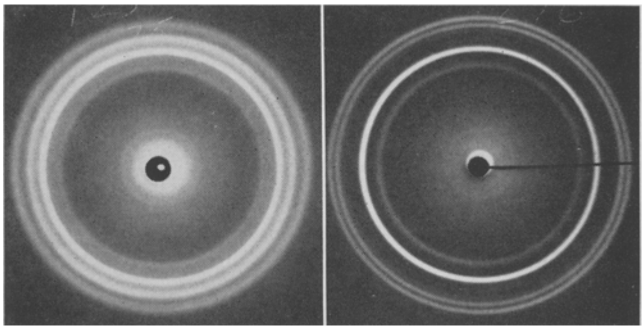
electrons which are impinged on a target of specific elemental material (copper, iron, molybdenum, etc.) in the X-ray tube. Interaction of the high-speed electrons with the atoms of the target produces X-rays of characteristic wavelength according to the atomic composition of the target. When these X-rays pass through any crystalline material, i.e., a solid in which there is a repetitive orderly arrangement of atoms, they are diffracted in much the way that visible light is refracted when it passes, for example, through a prism. The optics of the system are shown in Figure 1.

If the crystal in the X-ray beam happens to be a fat, and if we place a photographic film in the path of the diffracted X-rays, we get patterns like those in Figure 2. By measurement of the diameter of the diffraction rings, and by calculations using the Bragg equation, $n\lambda = 2d \sin\theta$, we obtain numbers called lattice spacings which relate to various distances between the respective atoms in the crystal, measured in Angstrom units. These lattice spacings thus characterize the molecular orientation in the various crystal forms.

We now need no longer to go through the relatively complicated photographic procedure. With automatic-recording equipment, a Geiger counter is moved mechanically through the path of diffracted X-rays, the electrical impulses from the counter are amplified and recorded on strip-charts like those shown in Figure 3. No calculations are required; the $2\,\theta$-angle is merely read from a dial and the lattice spacing is read from a chart. These spectra are related to those in the photographs in that the Geiger counter, in effect, swings from the center of the photograph to the outer edge, and the peaks in the strip-charts, therefore, correspond to the respective diffraction rings in the photographs.



Alpha Beta Prime



Intermediate Beta

Fig. 2. X-ray diffraction patterns of glyceride crystal forms obtained by photographic-film technique.

The spectra shown in Figures 2 and 3 cover the range of lattice spacings that relate to the lateral orientations (short spacings) of the triglyceride molecules. At smaller $2\,\theta$-angles are found other spacings that relate to the longitudinal organization (long spacings) of the molecules. By comparison of both the long and short spacings with the criteria that we have accepted, we can determine the crystal form of the sample under investigation.

Granted, the above description is not intended to instruct the beginner in determination of the crystal structure of fats. Because of the inherent danger in X-rays, certain precautions must be rigidly observed in using the equipment. The X-rays used for crystal structure determinations, unlike those used for medical and dental purposes, are of wavelengths that severely damage living tissue after exposure of only a few seconds. Therefore, only trained personnel can be permitted to operate the equipment. The major manufacturers of X-ray units in this country regularly offer intensive short-courses in the operation of their equipment, including a review of the theory of X-ray diffraction. In addition, many states require that a film-badge radiation monitoring service be provided for all persons who operate X-ray equipment, as well as for those who regularly go near it.

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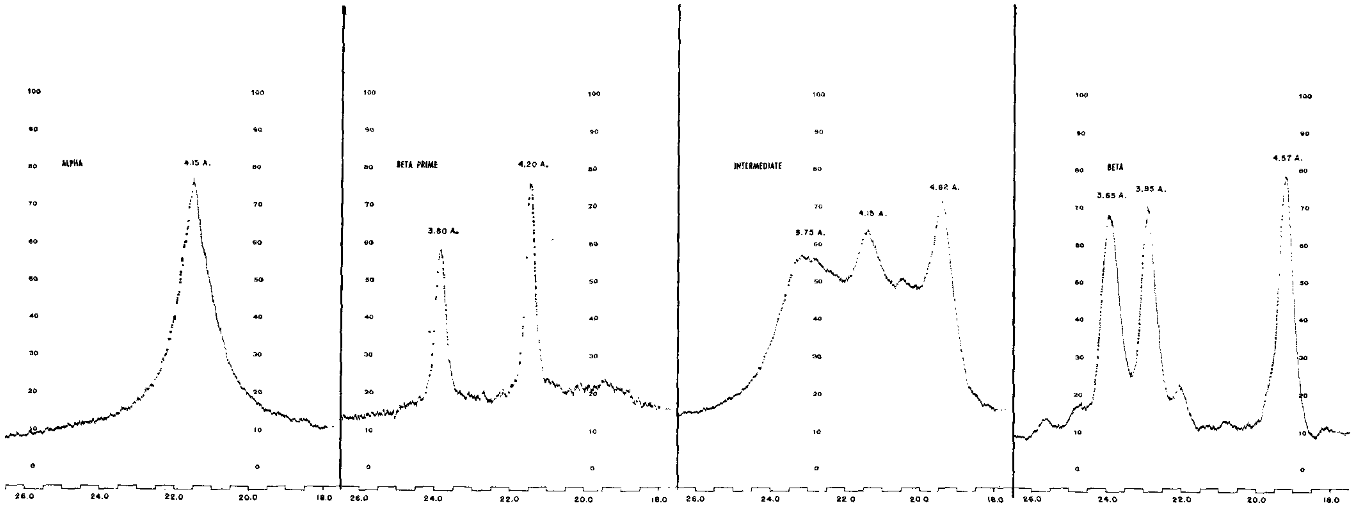

Fig. 3. X-ray diffraction spectra of glyceride crystal forms obtained with automatic-recording equipment.

New Products . . . .

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Beckman Instruments, Inc., Fullerton, Calif., has introduced three newly-designed IR analyzers which make rapid and continuous determinations of minor or even trace components in a gas stream or environmental atmosphere. They are Models IR 215, a single unit for laboratory or pilot plant use; and IR 315 and 415, of two units, an analyzer unit and an amplifier control.

Brinkman Instruments, Inc., Westbury, N.Y., announces a new Radiochromatogram Scanner for TLC plates in sizes up to 400 x 200 mm, featuring a transporting mechanism, special detector head and mechanical alignment.

New Brunswick Scientific Co., Inc., New Brunswick, N. J., now has available a bench-top fermentor (Micro-Ferm) designed for making a wide range of microbial investigations in the laboratory under controlled conditions of temperature, agitation, aeration and illumination.

Colab Laboratories, Inc., Chicago Heights, Ill., announces a new advance in TLC with novel Unoplan Leveller (No. 2810) for uniform layer thickness, aligning all the chromaplate surfaces in the same plane.

Scientific Kit Co., Washington, Pa., has developed a line of miniature pipettes for injecting small liquid samples into a chromatograph—constructed of stiff, fine bore stainless steel tubing but having a blind hole.

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X-ray diffraction thus provides us with a tool for learning much about the physical behavior of the fats. We can determine whether a given fat is in the crystal form that we desire, or whether it requires some kind of treatment to transform it to the form that we want. We can re-examine a sample at intervals over a period of time to determine whether it is remaining in a given form or is transforming to another modification. We can determine the effect of storage at various temperatures on crystal stability or on the rate of crystal transformation.

Transformation from form to form can be followed quite easily by means of X-ray diffraction, since the spectra always exhibit all the characteristic bands of each of the crystal forms present in the sample. For example, if more than one diffraction band is found, we know immediately that the sample has transformed beyond the *alpha* form. Although some of the bands of the various forms tend to overlap to some extent, it will be noted that the *beta prime* form has a band at 3.8 Angstroms that does not occur in the *alpha* form; the two higher melting forms have a band around 4.6 Angstroms which does not appear in either of the lower melting forms. By measuring the intensity of one or another of these bands (height above base line in the strip-charts), we can determine the approximate proportion of these forms in the sample.

X-ray diffraction can be used for determining the compatibility of glycerides. Mixtures containing molecules which are closely related structurally tend to be miscible in the solid state and their individual crystals will, therefore, contain molecules of all the components in the proportion in which they occur in the total mixture. The X-ray spectra of such mixtures exhibit a single set of diffraction bands. On the other hand, mixtures containing molecules differing markedly in structure tend toward more limited solubility in the solid state and their components crystallize separately in eutectic systems. X-ray spectra of such mixtures exhibit separate sets of diffraction bands for the various components present in the mixture.

Despite the increasing usefulness of X-ray diffraction in the field of fats and oils, we are faced with certain inherent limitations. Because fats simply do not crystallize in large single crystals (greater than 0.1 mm in shortest dimension), we are restricted to the X-ray powder technique that we have described in this article. While the lattice spacings obtained in this manner are extremely useful for identifying different crystal forms, they cannot in themselves be related to specific planes in the crystal lattices. Hence, the exact orientation of the molecules in specific lattice planes cannot be deduced with certainty by this method. Also, by this method, the spectra may contain the bands of several orders of diffraction, as indicated in Figure 1. Therefore, when certain bands are found to be simple integral multiples of each other, it cannot be determined with certainty whether they are of the same order or of different orders.

For those who may be interested, F. R. Paulicka and I have elaborated on the theoretical aspects of the molecular packing in the respective crystal forms in an article on triglyceride structure in *Clark's Encyclopedia of X-Rays and Gamma Rays* (Reinhold Publishing Co., New York, 1963). By consideration of the various ways in which the molecules could possibly align themselves, we have deduced certain three-dimensional arrangements that seem to fit quite closely the lattice spacings obtained experimentally.

Having been directly involved in X-ray diffraction for many years, I feel a little ashamed that I cannot present a more clear-cut picture of a field that has had 30 years in which to develop. Obviously, it is high time that something be done to bring about some degree of order out of the confusion that exists. Therefore, I propose that the AOCS organize an X-ray Diffraction Sub-Committee of the Instrumental Methods Committee and that this sub-committee be assigned to establish criteria for the identification of glyceride crystal forms and a uniform system for naming them. Perhaps in this way we can, in time, attain some coordination of the divergent viewpoints that now exist.

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