sticky, gummy, gluey, or dry, depending upon personal reaction. A structure which is too coarse-grained and thick-walled gives a harsh, sandy sensation in the mouth.

The baking performance test enables the consistent control of product uniformity. It serves to detect variations or errors in any of the manufacturing procedures, such as the compounding of shortenings, processing of other ingredients, formulation of mixes, etc. For example, Figure 4 shows the effect of variations in the amount of shortening in a white layer cake formula, and of deviations in the monoglyceride content of the shortening. It will be noted that, to some extent at least, the effects of abnormal amounts of emulsifier can be corrected by appropriate variation in the amount of shortening incorporated in the mix.

In addition to serving as a check on the uniformity of manufacturing procedures, baking tests are also useful as a research tool for determining proper formulation of ingredients. For example, Figures 5 and 6 illustrate the results of tests on emulsifier composition. By varying the amounts of hard and soft emulsifiers, *i.e.*, those of relatively lower and higher iodine values, layer cakes of different volumes and textures were obtained. Baking performance tests permitted the selection of the proper blend of hard and soft emulsifiers possessing an average iodine value which produced the desired results. (A single emulsifier with the same iodine value as the blend average would not work as well.)

Another interesting phenomenon demonstrated by baking performance tests is shown in Figure 7. This series of photographs illustrates the interrelated effects of mono- and diglyceride emulsifier composition on layer cake quality. It can be seen that neither the mono- nor the diglyceride alone yield the proper degree of emulsification. Tests such as these have demonstrated that a certain proportion of each is necessary to produce the desired cake volume and texture.

X-Ray Spectroscopy

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SPECTROSCOPY is a means of determining certain properties of materials by utilizing the effects produced by applied energy of various wavelengths. Based upon the wavelength of the energy employed, spectroscopy is divided into several fields. One of these main divisions is x-ray spectroscopy, in which x-rays are employed as the source of energy for bombarding the material under analysis. X-ray spectroscopy can be subdivided in the same manner as is ultraviolet or infrared spectroscopy. Thus x-ray absorption is possible because some materials absorb x-rays more strongly than do other materials. In x-ray fluorescence, matter which is excited by x-rays will alter the primary wavelength. In x-ray diffraction the direction of the primary wave is changed by the material, but its wavelength is unchanged. The techniques of these subdivisions of x-ray spectroscopy may be applied at one time or another to numerous classes of materials. However when applied to the field of fats and oils, x-ray diffraction is by far the most utilized application. The wavelength of the primary wave remains unchanged in diffraction. By means of diffraction it is possible to determine crystalline structure, and the molecular dimensions of crystalline substances. Crystal structure in general will be treated briefly in order to lend understanding to the usual habit of long chain compounds under normal conditions. The major concern of this paper is that phase of crystal structure having to do with polymorphism, and it reports the development, application, and interpretation of x-ray diffraction patterns as an analytical method of determining polymorphism and changes in polymorphic phases in fats, fatty acids, and derivatives.

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The economic aspects of polymorphism in fats and products derived therefrom should be mentioned briefly. The manipulation of fatty materials to obtain the desired polymorphic forms and to prevent subsequent changes to undesirable forms requires considerable effort on the part of manufacturers. In the processing of shortening and margarine, equipment is designed and operated so as carefully to control the temperature levels and rate of cooling during plasticization, packaging, and subsequent tempering. Some of these operations also serve, of course, to control crystal size. In candy-making the temperature and rate of cooling of chocolates and fat-containing coatings is carefully controlled.

In certain instances the need of a given polymorphic form of a product is functional. For example, the melting point of a given fat can vary many degrees depending upon its polymorphic form. In soap-making precautions are taken to obtain certain polymorphic forms as polymorphism has a decided influence upon the hardness and the lathering properties of soap.

In other instances polymorphism is important because of its effect on appearance. Partial melting of a low-melting form in shortening or margarine followed by resolidification to a higher melting form destroys the smooth texture of the product and produces instead a grainy appearance. Polymorphic changes in candy fats produce "bloom," dullness, and possibly other faults, which can harm the reputation and competitive position of the manufacturer.

Equipment for X-ray Diffraction

The source of x-rays for obtaining x-ray diffraction patterns will not be discussed in detail since excellent

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treatises on this subject are available by the manufacturers who produce this equipment. Suffice it to say that the wavelengths employed in diffraction work are generally 0.7–2 Å. All diffraction equipment has a system of slits or pinholes to define the x-ray beam; a means for holding the specimen in the beam; and a photographic plate or film in a suitable camera to record the diffraction pattern.

Methods of X-ray Diffraction

Development. The most obvious characteristic of a crystal is its geometric form, which is the result of plane faces forming boundaries. The external form is one of the results of the internal pattern, which is a regular arrangement in space of certain units. In any one crystal face (two-dimensional), a unit of pattern is repeated at regular intervals. Likewise, in the interior of the crystal (three-dimensional), there is no difference in pattern repetition in any direction. This three-dimensional system is the space lattice and is divided into identical unit cells which by repetition build up the crystal form.

Fatty acids and other organic crystals are molecular in structure rather than atomic. The points of a space lattice, occupied by these molecules, can be arranged in parallel equidistant sheets or planes which act as gratings, thus giving rise to x-ray diffractions (8).

The method by which x-ray diffraction patterns as discussed here are obtained and interpreted was developed by Bragg (2, 3). The development of Bragg's method is illustrated in Figure 1. Monochromatic

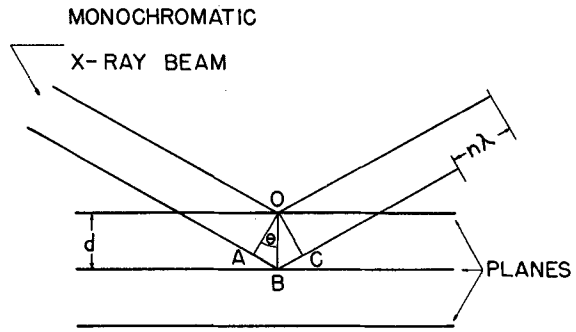

FIG. 1. Schematic diagram of development of Bragg equation.

waves strike a series of parallel planes or points of the space lattice of a crystal; reflections from the uppermost plane will have a shorter optical path than those from a lower plane spaced an unknown distance d apart. The difference in the lengths of the optical paths is

$$AB + BC$$
$$AB = BC = OB \sin\theta$$

The difference in the lengths of path is equal to

$$2\,OB \sin\theta$$

In order to obtain a reflection on a photographic plate, the diffracted beams must be in phase, or reinforced, and therefore their paths must differ by an integral number of wavelengths. Since

$$OB = d,$$

then

$$n\lambda = 2\,d \sin\theta$$

for waves to reach the photographic plate in phase. By putting n equal to 1, 2, 3, etc., at known angles of θ which produce reflections, d can be calculated.

The technique of obtaining an x-ray diffraction pattern of fatty materials according to Bragg's principles may be found in many publications (19, 16, 7). Figure 2 represents a simplified sketch of the proce-

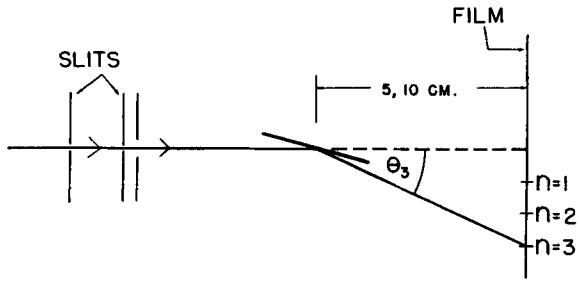

FIG. 2. Schematic diagram of x-ray diffraction method.

dure employing a single crystal. X-rays, passing through slits, strike the mounted specimen which is aligned to get different angles of θ. For an arbitrary θ angle, the reflection from successive planes might be out of phase, resulting in interference. Varying θ by proper alignment gives path differences between reflections of successive planes which are an integral number of wavelengths, n, overcoming interference and producing spots. The reflected radiation is recorded on the film by means of curved or pinhole cameras. Reflection occurs at angles θ_1, θ_2, θ_3, etc., and calculations are made according to Bragg's equation. By means of this technique, patterns may be obtained of either single crystals or of powdered material.

Single Crystal Method. X-ray diffraction patterns of single crystals give the maximum information as to molecular structure. Single crystal patterns of fatty materials are not plentiful, because of the difficulty of obtaining fats in the form of a crystal of sufficient size. The classic example of a single crystal diffraction pattern is that of stearic acid, obtained by Muller (14). Muller determined the unit cell structure and dimensions of this acid. Four molecules are contained in the unit cell, whose axial dimensions are $a = 5.546$ Å, $b = 7.381$ Å, and $c = 48.84$ Å. The c-axis is at an angle with the two parallel planes. The four molecules of the unit cell are composed of a zigzag chain of carbon atoms, about 1.54 Å apart. The unit cells are stacked in parallel arrangement so that the CH_3 end groups form the layers or sheets from which the x-ray beam is reflected by repetitive units. These reflections give rise to the long or interplanar spacings. In an aliphatic acid the spacing, d, of these planes is double the dimension of a paraffin (15) containing the same number of carbon atoms. The —COOH groups of the acid cannot account for the difference in molecular dimension therefore the acid unit cells are two molecules high, with the —COOH groups together at the ends of two oppositely turned molecules.

Powder Method. By far the most work in x-ray diffraction of fatty materials has been done by the powder method. Whereas the single crystal method requires orientation of the crystal to obtain angle θ, the powder method is based on a multitude of small crystals oriented at random, and various angles of θ obtained by oscillating or rotating the specimen dur-

ing exposure. In such a vast number of small crystals there will always be some which are oriented in the correct position to give reflection. The powder method gives information as to identity of materials and polymorphic phases but not much information as to molecular structure.

Preparation of Sample

Samples for x-ray diffraction patterns by the powder method may be mounted on a glass slide or prepared in the form of rods. If the glass slide technique is used (19), the sample may be dissolved in acetone and a few drops of the solution placed on the slide and the solvent evaporated. Placing a drop of the molten sample on a slide and allowing it to solidify is a second way of mounting the sample. A third way is merely to press a flaked sample onto a slide, upon which some will adhere. However where a polymorphic change might be involved, samples prepared in the form of a rod seem preferable (9). A thin-wall pyrex capillary, diameter 0.5–1.0 mm., is packed with the powdered sample. Another means of forming a rod is to pack the sample in a thick-wall capillary and extrude the formed rod by means of a small plunger.

Interpretation of Patterns

Patterns obtained by x-ray diffraction, with the x-ray beam defined by slits or pinholes, are reproduced in many publications, among which are those by Muller (13), Lutton (9), and Bailey *et al.* (1). The pattern obtained on the photographic film by diffraction consists of a central image due to the undiffracted beam or beam stop, and reflections repeated through several orders, n. A discussion of the somewhat dissimilar diffraction patterns of fatty acids and of triglycerides will serve as a means of interpreting the x-ray patterns of all fatty substances.

Fatty Acids. The repeated orders, n, in diffraction patterns of aliphatic acids are due to reflections from parallel planes and represent the long axial dimension (long spacings). This dimension varies with the length of the carbon atom chain. Since d in Bragg's equation is inversely proportional to θ, lines of various orders are closer together for the longer chain compounds than for the shorter.

Odd orders of n are strong, and even orders weak at the lower angles of θ. This alternation of intensity occurs because the molecules are linked with their —COOH groups together and the CH_3 groups at opposite ends of the unit cell, resulting in density differences and hence in intensity of reflections. The distribution of density is higher where —COOH groups meet, and lower where CH_3 groups are in contact (18). Continuity is disturbed where chains begin and end.

In addition to the prominent long spacings observed on the film pattern, there are also diffuse reflections, farthest removed from the center of the pattern (13). These diffuse lines indicate the side or short spacings and are independent of the chain length. These side spacings are 2–5 Å and in aliphatic acids are not affected greatly by polymorphic changes.

Triglycerides. The diffraction patterns of triglycerides display side spacing reflections of prominence, in addition to long spacing reflections. Molecules between the planes of a crystal are parallel and are either perpendicular or inclined to the surface of the reflecting planes. The side or short spacings indicate the distance between the parallel molecules. In glycerides, unlike fatty acids, the side spacing reflections are very prominent and are the most important spacings utilized in the determination of polymorphism of such materials. This will be discussed later.

Calculation of Interplanar Spacings. The interplanar spacings, d, are calculated from known dimensions of specimen to film distance, wavelength of the beam employed, and measurements taken from the film (19). On the developed film the diameters of the various diffraction interferences (rings) are accurately measured. This can be accomplished by means of a known reference distance. The diffraction pattern of a known material, for example NaCl, is obtained, using the same conditions. The angle θ for the first order reflection of NaCl is known and closely corresponds to the third order reflection of fatty materials. Various angles of θ for the n orders of the sample are found by conversion of the linear diameter measurements, and d is calculated. The interplanar distances of some saturated fatty acids, determined by Slagle and Ott (19) using three different methods of mounting the samples, are summarized in Table I.

TABLE I

Interplanar Distances (001 planes) of Some Saturated Fatty Acids

Acid	Method of mounting		
	Melted	Acetone	Pressed
	Å	Å	Å
C_{12}	27.18	27.18	27.31
C_{14}	31.44	31.39	31.26
C_{16}	35.52	35.47	35.53
C_{18}	30.83	39.62	39.92

It is obvious that the spacings of a series of compounds once measured will serve as identification for later determinations. X-ray analysis alone, however, is not sufficient for identification unless other properties are determined also.

Diffraction Patterns in Polymorphism

As mentioned previously, this discussion presents the viewpoint of the determination of polymorphic phases, and the change in these phases as the principal use of x-ray diffraction patterns as an applied analytical technique to fats and oils. This technique has been applied to innumerable fatty materials, of which a few general examples will suffice to indicate its value.

In general, the long spacings of x-ray diffraction patterns are mainly a measure of carbon chain length and change but little with various polymorphic changes. In the aliphatic acids however it is this small change which indicates polymorphic changes; the side spacings remain almost constant. The reason for this is that the crystalline packing in the acids is relatively unaltered, but the mode of juncture of the —COOH groups changes, giving different angles of tilt, hence differences in chain length. An example of the change in long spacings of some fatty acids is illustrated by Table II, using some of the data of Slagle and Ott (19). The side spacings remain constant, at values of about 3.8 and 4.2 Å for both forms (17).

In contrast to the fatty acids the glycerides exhibit well-defined short spacings which differ markedly with polymorphic changes. These differences reflect changes in molecular packing, which produce the various crystalline properties of glycerides in relation to their polymorphic form. Some of the characteristic

TABLE II

Long Spacings of Some Saturated Fatty Acids in Two Polymorphic Forms

Acid	β form	β′ form
	Å	Å
C_{10}	23.02	.....
C_{12}	27.18	30.6
C_{14}	31.39	35.0
C_{16}	35.47	39.4
C_{18}	39.62	44.1

short spacings and the single long spacing of the polymorphic forms of the even numbered triglycerides from trilaurin through tristearin are listed in Table III. The data are those of Lutton (9).

It is important to note that the characteristic short spacings of a homologous series are about the same. After the short spacings for the polymorphic phases of one homolog have been determined, a particular polymorphic phase of the other homologues may be determined by comparison of patterns, knowing sample to film distance, and enlargement.

TABLE III

Short and Long Spacings of the Polymorphic Forms of Trilaurin, Trimyristin, Tripalmitin, and Tristearin

Tryglycerides	α form		β′ form		β form	
	Long	Short	Long	Short	Long	Short
Trilaurin through Tristearin		4.14 VS[a] 2.40 W		4.18 VS 3.78 S 3.42 VW 3.05 W 2.81 W 2.53 M . . .		5.24 M 4.61 VS 3.84 S 3.68 S 2.85 W 2.57 M 2.38 W 2.22 W 2.20 W 2.12 W . . .
Trilaurin	35.5		32.85		31.15	
Trimyristin	41.4		37.65		35.45	
Tripalmitin	46.3		42.3		40.9	
Tristearin	50.6		46.8		45.15	

[a] The intensities are indicated as VS very strong, S strong, M medium, and W weak.

The x-ray diffraction patterns of the polymorphic forms of many fatty materials have been obtained and the short spacings calculated. A brief summary of the characteristic short spacings of some of the more common materials are included in Table IV.

In order to obtain information as to the exact crystal spacing and structure of the unit cell of a material, exact calculations have to be made. However x-ray diffraction patterns offer a very reliable means of determining phase changes in materials without extensive calculations. As indicated by Ferguson and Lutton (5), if a characteristic ring pattern can be recognized as pertaining to a definite polymorphic phase, then nothing need be known as to the calculated spacings or unit cell dimensions to follow the subsequent change of that phase. This type of analysis is used extensively in polymorphism and is of extreme practical importance in fat products. For example, an excellent comparison of the diffraction patterns of the polymorphic phases of soap is presented by Ferguson *et al.* (7). The various orders, as measured in centimeters on the film, are given together with the calculated interplanar spacings which identify the phases. By simple measurement of orders on a subsequent diffraction pattern of an unknown phase of the soap being processed, phase identification can be made, provided sample-to-film distances are the same.

TABLE IV

Characteristic Short Spacings of Fatty Compounds

Material	Sub α form	Short spacings, Å α form	β′ form	β form
Unsaturated acids[b]	.....	.....	4.19 S[a] 3.80 M 3.59 M	4.65 VS 4.37 M 3.83 M 3.67 VS
Saturated glycerides[c]	.....	4.15 S	4.2 VS 3.8 M	4.6 VS 3.85 M 3.7 M
Unsaturated glycerides[d]	.....	4.36 VS	5.22 W 4.35 S 4.09 W	4.57 VS 3.97 M 3.84 M
Diglycerides[e]	.....	.....	.....	4.73 VS 3.82 W 3.59 W
Monoglycerides[f]	4.14 VS 3.92 M 3.74 M 3.54 M	4.65 W 4.18 VS 3.80 W	4.15 VS 3.69 W 3.29 W	4.55 S 4.37 S 3.86 S

[a] The intensities are indicated as VS very strong, S strong, M medium, and W weak.
[b] (10)
[c] (9)
[d] (6)
[e] (4)
[f] (11)

Additional Applications of X-ray Diffraction

Crystalline structure, molecular structure, and chain length have been mentioned as being derivable by the diffraction technique but will not be discussed in detail in the present article.

The polymorphism of fats, shortening, fatty materials, such as soaps, etc., is important economically since the correct polymorphic form is important for imparting desired characteristics to finished products. This has already been discussed in detail. Some other possibilities of using x-ray diffraction for analytical determinations include: molecular weight determinations by interpolation of crystal spacing in graphs of known spacing-carbon atom chain length plot; isomerism, by means of the intensities of the various orders of reflection; chemical analysis, by comparison of spacings with those of known materials; some chemical reactions, such as soap formation, oxygen absorption, etc.; surfaces and interfaces, by orientation of monomolecular films and the film structure of greases; and solid solutions, for the determination of mixed crystals.

In conclusion, it might be mentioned that the interplanar spacings and length and diameter of the molecule of liquids can be determined by x-ray (12). The spacings are not the same in the liquid and solid states.

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