The Melting Points of Binary Mixtures of Oleic, Linoleic, and Linolenic Acids

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INTRODUCTION

THE MELTING POINT[2] and the solidification or setting point curves of mixtures of saturated fatty acids have been studied by a number of workers (1, 2, 3, 4, 5). Adjacent even-carbon acid pairs from 12 to 26 carbons have curves which indicate a 1:1 molecular compound, apparently with an incongruent melting point, and a eutectic at 25 to 30 per cent of the lower acid (4). Of the symptoms investigated, the myristic-palmitic pair appears to be the only one for which the complete phase-rule diagram has been determined with both solidus and liquidus lines (2, 3, 4). Jantzen considered it necessary to study this system by means of a dilatometer. Because of very slow equilibrium of the mixed crystals, he found it necessary to lower the warming rate to 0.2° per day in order to obtain the solidus lines.

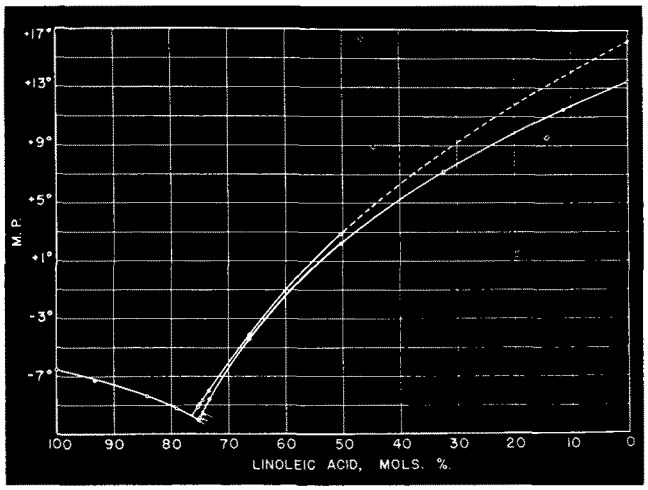

Figure 1.—Melting point diagram of oleic-linoleic acid mixtures. The upper curve is the β form of oleic acid.

Very little has been done on the melting point diagrams of mixed fatty acids containing unsaturated acids. Smith (6) has reported the melting point of mixtures of oleic acid with stearic and with palmitic acids, and of elaidic acid with the same two saturated acids. Oleic acid showed a eutectic with palmitic of about 94 per cent oleic acid at 12.1° C., while the eutectic with stearic was about 98 per cent oleic acid at 13.1° C. (23.8° for the β form). No evidence of compound formation was observed.

In connection with a study of the separation of the fatty acids of soybean oil by crystallization, it appeared desirable to determine whether mixtures of the unsaturated acids, oleic, linoleic, and linolenic, showed compound formation or eutectics, and if so, to locate their compositions and melting points.

This was accomplished by means of capillary tube melting point determinations on prepared mixtures of the pure acids. The capillary melting point is always somewhat higher than the freezing point obtained from the cooling curve, but it has been shown (3, 7) that the method is quite reliable in determining the general characteristics of binary systems. The melting point curves thus obtained correspond approximately to the freezing point curves or liquidus curves as applied to discussion of phase rule diagrams (8). They do not, of course, locate the solidus lines, and so permit nothing more than speculation as to the complete classification of the system.

EXPERIMENTAL

Oleic acid was obtained from methyl oleate prepared in a manner similar to that previously described (9). The acid had the following characteristics: Iodine value, 89.6[3] (89.9, theory); saturated acids, 0.3 per cent (Bertram method, using sintered glass filter); melting point, 13.4° C.[4] and 16.2° C. (capillary); clear point, 13.3° C. (thermometer in stirred liquid); and freezing point, 13.2° C. (from cooling curve, 5-gm. sample, 15 minute hold).

Linoleic acid was prepared directly from tetrabromostearic acid (melting point, 114.5-115.0° C.) by the method of Kaufmann (10). The acid had an iodine value of 181.2 (181.2, theory); melting point, —6.5° C. (capillary); and freezing point, —7.2° C. (cooling curve).

Linolenic acid was obtained from the methyl ester, prepared essentially by the method of Kimura (11) except that the amount of sulfuric acid used was only about 3 per cent by weight of the methanol, and 8 to 10 cc. of methanol per gram of hexabromide was used. The mixture was mechanically stirred and became clear in less than an hour of refluxing. Refluxing was continued for an hour. It is possible that the 25 cc. of sulfuric acid in 95 gm. of reaction mixture mentioned by Kimura is a misprint and should be 2.5 cc., since the former quantity is almost 50 per cent of sulfuric acid in the mixture [cf. Norris, Kass, and Burr, OIL & SOAP, *17*, 123 (1940)]. The ester was recovered, saponified, and the acid distilled at a pressure of less than 0.2 mm. The acid had an iodine value of 273.1 (273.7, theory); melting point, —12.8° C. (capillary); and freezing point, —13.4° C. (cooling curve). Several preparations of linolenic acid by the Kaufman method (10) gave products with iodine numbers from 267 to 270. These all gave a slight Beilstein test for halogen, while the samples prepared as above did not.

Melting points were determined in capillary tubes, 1.5 to 2 mm. in diameter, which had been filled to a height of 5 to 10 mm., evacuated to less than 0.5 mm., and sealed off with a micro flame. The mixtures in 1 gm. quantities were made by direct weighing of the pure acids and thorough mixing before placing in the capillary tube. The melting point bath was an unsilvered

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[2] The term "melting point" is used in this paper to mean the temperature of complete melting or disappearance of last trace of solid.

[3] The iodine values are averages of two or more determinations, with 30-minute reaction time, by the Wijs method.

[4] Temperatures were determined with calibrated thermometers permitting readings to the nearest 0.1°.

Dewar flask with alcohol as bath liquid. The bath was cooled and the temperature controlled by direct addition of dry ice. A motor driven stirrer afforded vigorous stirring. The calibrated thermometer was graduated at one-half degree intervals and permitted estimation to the nearest tenth of a degree.

On certain portions of the oleic acid curves, two forms appeared and double melting points could be obtained. This was observed only on the oleic acid side of the eutectic. (Compare Smith, loc. cit.)

Where only one form appeared, the melting point was determined as follows: The sample was rapidly frozen by immersion in an alcohol-carbon dioxide mixture and then placed near the thermometer bulb in the bath, which had been cooled below the expected melting point. The bath temperature was then raised 0.1° each 4 or 5 minutes. The temperature at which the last solid disappeared was taken as the melting point.

When two forms appeared, it was sometimes possible to get a complete melting of the lower melting or *a* form before change to the higher melting or *β* form occurred. In such cases, the bath was held at the desired temperature and the frozen sample immersed for 4 to 5 minutes. If it melted, the procedure was repeated at a lower temperature. If it did not melt, it was removed, melted, and the process repeated at a higher temperature until melting occurred. On certain other samples, the change to the *β* form occurred before complete melting of the *a* form. This change took place faster as the eutectic was approached from the oleic acid side. It also took place faster in the oleic-linolenic acid system than in the oleic-linoleic acid system. In these cases, the temperature recorded for the *a* form corresponds to a definite change in apparent structure of the crystallized material in the capillary tube. Such samples are marked in the following tables and indicated on the graphs by arrows. The melting point of the *β* form was obtained as usual by warming the bath at 0.1° each 4 or 5 minutes after the *β* form had appeared and observing the temperature of disappearance of the last particle of solid.

The data thus obtained for oleic-linoleic mixtures are presented in table I and figure 1. No compound formation is evident, but a eutectic appears at 75.2 mole per cent of linoleic acid for the *a* form and 76.3 mole per cent of linoleic for the *β* form at temperatures of —10.0° C. and —9.8° C., respectively. The *β* form was not observed below 50 per cent linoleic except on pure oleic acid.

TABLE I.—MELTING POINTS OF MIXTURES OF OLEIC AND LINOLEIC ACIDS.

Linoleic Mole per cent	Oleic Mole per cent	Melting point—capillary tube °C.	
100.00	0.00	—6.5	
93.42	6.58	—7.3	
84.12	15.88	—8.4	
79.18	20.82	—9.2	
		a form	*B form*
75.33	24.67	—10.0[1]	— 9.1
75.03	24.97	— 9.9[1]	— 8.9
74.50	25.50	— 9.5[1]	— 8.6
73.47	26.53	— 8.6	— 8.0
66.30	33.70	— 4.4	— 4.1
50.29	49.71	+ 2.2	+ 2.8
32.37	67.63	+ 7.2	.....
11.29	88.71	+11.5	.....
0.00	100.00	+13.4	+16.2

[1] Change in structure of crystallized material, without complete melting of *a* form.

In table II and figure 2 are shown the results on linoleic-linolenic mixtures. No compound formation is evident, and no eutectic is shown. The curve indicates a continuous series of solid solutions of intermediate melting points.

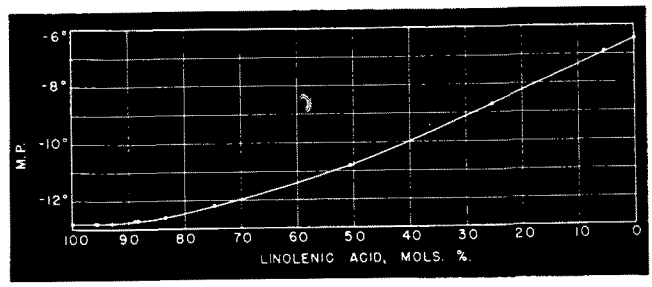

Figure 2.—Melting point diagram of linolenic acid mixtures.

TABLE II.—MELTING POINTS OF MIXTURES OF LINOLENIC AND LINOLEIC ACIDS

Linolenic Mole per cent	Linoleic Mole per cent	Melting point—capillary tube °C.
100.00	0.00	—12.8
95.70	4.30	—12.8
95.54	4.46	—12.8
93.00	7.00	—12.8
88.96	11.04	—12.7
88.43	11.57	—12.7
83.49	16.51	—12.6
74.82	25.18	—12.2
50.68	49.32	—10.8
25.32	74.68	— 8.7
5.34	94.66	— 6.9
0.00	100.00	— 6.5

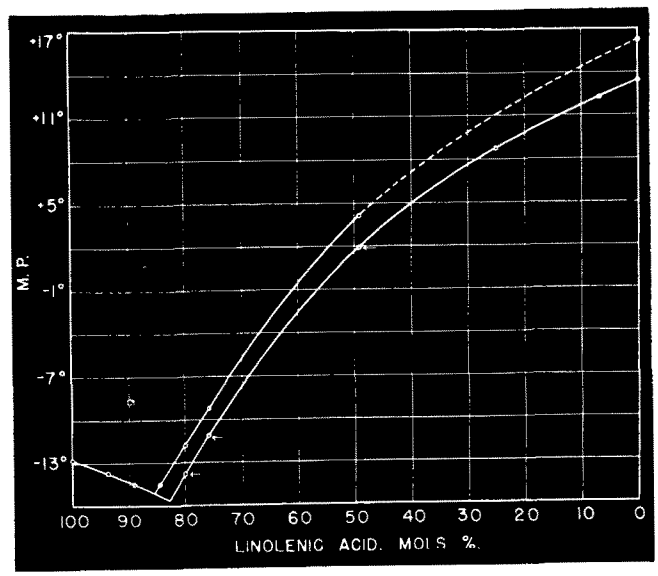

Figure 3.—Melting point diagram of oleic-linolenic acid mixtures. The upper curve is the *β* form of oleic acid.

Table III and figure 3 show the results with oleic and linolenic acids. No compound formation is evident, but again a eutectic for each form of oleic acid is indicated. That for the *a* form is 82.7 mole per cent linolenic acid at —15.7° C. while that for the *β* form is 85.5 mole per cent linolenic acid at—15.1° C.

TABLE III.—MELTING POINTS OF MIXTURES OF OLEIC AND LINOLENIC ACIDS

Linolenic Mole per cent	Oleic Mole per cent	Melting point—capillary tube °C.	
100.00	0.00	—12.8	
93.74	6.26	—13.7	
89.03	10.97	—14.5	
		a form	*B* form
84.62	15.38	.....	—14.5
79.97	20.03	—13.8[1]	—11.8
75.99	24.01	—11.1[1]	— 9.2
49.30	50.70	+ 1.9[1]	+ 4.1
24.99	75.01	+ 8.7	.....
6.96	93.04	+12.2	.....
0.00	100.00	+13.4	+16.2

[1] Change in structure of crystallized material, without complete melting of *a* form.

SUMMARY

1. The melting points of binary mixtures of oleic, linoleic, and linolenic acids have been reported.

2. The oleic-linoleic acid system has eutectics for the α and β forms of oleic acid of 75.2 and 76.3 mole per cent linoleic acid, at —10.0° and —9.8°, respectively.

3. Linoleic and linolenic acid mixtures show only melting points intermediate between the pure acids.

4. The oleic-linolenic acid system has eutectics for the α and β forms of oleic acid of 82.7 and 85.5 mole per cent linolenic acid, at —15.7° and —15.1°, respectively.

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A Convenient Method for the Rapid Estimation of Carotene in Butterfat

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Experimental

MOST colorimetric methods for the quantitative determination of carotene in biological material are based upon a comparison of the natural yellow color of this pigment in fat-solvents with that of known concentrations of carotene or other suitably prepared standards. These methods usually require saponification of the material and subsequent extraction with solvents which separate carotene from xanthophyll and associated carotenoid pigments of a similar color. This separation of pigments is especially important whenever the results of carotene determinations are to be interpreted in terms of vitamin A activity. Of the carotenoid pigments found in plant material, only carotene and cryptoxanthin are known to have value as a source of vitamin A.

In the case of butterfat it appears possible to make a reasonably accurate estimate of its carotene content from a determination of total yellow color. Palmer (7) first showed carotene to be the principal yellow pigment in butterfat. Baumann and Steenbock (2) concluded from spectrophotometric studies that about 95 per cent of the natural pigment in butterfat was carotene. Their work was substantially confirmed by Gillam (3) who found 94 per cent of the color in the unsaponifiable matter of butterfat to be due to carotene, the remaining 6 per cent being due to xanthophyll. Gillam has suggested that for routine work a spectrophotometer be used to determine the carotene content of butterfat. He suggests that the light absorption of the unsaponifiable matter be determined at 455-460 millimicrons and that the absorption due to carotene be taken as 94 per cent of this value.

Shrewsbury and Kraybill (8) found that the determination of carotene in butterfat by direct color comparison with potassium dichromate standards, as in the Willstätter and Stoll method used by Palmer (7), gave results which were much too high. Their high results were attributed to the greater color intensity of carotene in butterfat than in the usual solvents (4).

Barnett (1) proposed a colorimetric method which made allowance for the increased color of carotene in oil. This investigator determined the carotene of butterfat with the aid of a spectrophotometer, the results being taken as the true carotene content of the sample. In these determinations cocoanut oil was used to dilute the butterfat and also as a blank. The carotene content of the same samples was then determined colorimetrically. In the colorimetric determinations 0.2 per cent solution of potassium dichromate was used as a color standard and the concentration of carotene calculated from Palmer's chart (7). By dividing the concentrations obtained by the spectrophotometric method by those obtained by the colorimetric method an average correction factor of 0.28 was secured to be applied to the colorimetric determinations. This factor when applied to the colorimetric determinations corrected for the increased color intensity of the carotene in fat. Only a narrow range of carotene concentrations was covered in this work.

Baumann and Steenbock (2) determined the carotene content of butterfat spectrophotometrically, carotene dissolved in purified cottonseed oil being used for a standard. Shrewsbury and Kraybill (8) and Leuschen *et al.* (6) diluted butterfat with petroleum solvents previous to spectrophotometric determinations of carotene. Treichler, Grimes, and Fraps (9) deemed it advisable for routine work to devise a spectrophotometric method whereby the carotene content of butterfat could be determined without previous dilution with other oils or solvents and without the need of a blank to correct for the absorption of light by the fat itself. These investigators have prepared a table of conversion factors which when applied to spectrophotometric density readings at 470 and 480 millimicrons express the carotene content of butterfat in parts per million.

Hand and Sharp (5) devised a photoelectric colorimeter fitted with suitable glass filters for the rapid determination of carotene in milk fat. This instrument in the hands of a capable operator should prove very satisfactory.

The fact that equipment for spectrophotometric methods of analysis is not available in many laboratories often makes it necessary to rely upon the less direct methods for the determination of carotene. A method which is applicable to butterfat and which has been found to fit in well with the procedures ordinarily employed in determining butterfat constants, i.e., melting point, iodine number, etc., is herein described. The method is based upon the direct comparison of the color of butterfat samples contained in cylindrical bottles of uniform diameter with that of solutions of potassium dichromate of known concentration. Although not designed to replace the more accurate methods of analysis, the proposed procedure should be of practical value in grading butterfat samples for carotene content.