concluded, on the basis of the data on this lot of prime cottonseed, that none of the experimental solvents compare favorably with hexane as extractants for cottonseed.

Acknowledgment

The authors are indebted to Carroll F. Hoffpauir, Mack F. Stansbury, Robert T. O'Connor, and Evald F. Skau for their valuable suggestions. They also wish to express appreciation to Walter A. Pons Jr., Frank C. Magne, I. W. Lohmann, Hilton G. Damare, Claire Lesslie, Vidabelle O. Cirino, Elizabeth R. McCall, Alva F. Cucullu, Mildred D. Murray, S. M. Stark Jr., and Lloyd G. Burkenstock for analytical and physical determinations.

REFERENCES

1. MacGee, A. E., Oil Mill Gazetteer, *52*, No. 2, 17-43 (1947).
2. Sievers, A. F., McIntyre, J. D., Cotton Oil Press, *4*, No. 10, 44-8 (1944).
3. Shrader, J. H., Cotton Oil Press, *5*, No. 12, 29-32 (1922).
4. Boatner, C. H., Hall, C. M., O'Conner, R. T., Castillon, L. E., Curet, M. C., J. Am. Oil Chem. Soc., *24*, 276-283 (1947).
5. Harris, W. D., Oil Mill Gazetteer, *50*, No. 5, 14-21 (1945).
6. Boatner, C. H., Hall, C. M., Oil and Soap, *23*, No. 4, 123-138 (1946).
7. Fischer, Karl, Angew. Chem., *48*, 394-396 (1935).
8. Stansbury, M. F., Guthrie, J. D., J. Agr. Research, *75*, No. 2, 49-61 (1947).
9. Pons, W. A. Jr., Murray, M. D., O'Connor, R. T., Guthrie, J. D., J. Am. *Oil Chem.* Soc., *25*, 308-313 (1948).
10. Pominski, J., Molaison, L. J., Crovetto, A. J., Westbrook, R. D., D'Aquin, E. L., Guilbeau, W. F., Oil Mill Gazetteer, *51*, No. 12, 33-39 (1947).
11. Olcott, H. S., Ind. Eng. Chem., *33*, 611 (1941).
12. "Official and Tentative Methods of the American Oil Chemists' Society," ed. by V. C. Mehlenbacher, 2nd Ed., Chicago (Rev. to 1950).
13. "Handbook of Colorimetry," Massachusetts Inst. Tech., Cambridge, Mass. (1936).
14. Vix, H. L. E., Pollard, E. F., Spadaro, J. J., Gastrock, E. A., Ind. Eng. Chem., *38*, 635-642 (1946).
15. Olcott, H. S., Fontaine, T. D., J. Am. Chem. Soc., *61*, 2037-2040 (1939).
16. Pons, W. A. Jr., Guthrie, J. D., J. Am. Oil Chem. Soc., *26*, 671-676 (1949).
17. Pons, W. A. Jr., Hoffpauir, C. L., O'Connor, R. T., J. Am. Oil Chem. Soc., *27*, 390-393 (1950).
18. Pons, W. A. Jr., Hoffpauir, C. L., O'Connor, R. T., J. Am. Oil Chem. Soc., *28*, 8-12 (1951).
19. Conner, R. T., Straub, G. I., Ind. Eng. Chem., Anal. Ed., *13*, 380 (1941).
20. "Methods of Vitamin Assay," The Association of Vitamin Chemists, Interscience Publishers Inc., N. Y. (1947).
21. Stansbury, M. F., Guthrie, J. D., J. Agr. Research, *75*, 49 (1947).
22. Cheldelin, V. H., Lane, R. L., Soc. Expt. Biol. and Med. Proc., *54*, 53-55 (1943).
23. Fraps, G. S., Tex. Agr. Expt. Sta. Bull. 128 (1910).
24. Rewald, B., Biochem. J., *36*, 822-824 (1942).
25. Thurber, F. H., private communication.
26. Goldovskii, A. M., Podol'skaya, M. Z., Vsesoyuz. Nauch.-Issledovatel. Inst. Zhirov, Cottonseed-Oil Production *1936*, 55-61 (in English 61).
27. Goldovskii, A. M., Podol'skaya, M. Z., Mirer, E. A., *ibid.*, 71-77 (in English 77).
28. "Cottonseed and Cottonseed Products," ed. by Bailey, A. E., Interscience Publishers Inc., N. Y., 1948, p. 343.
29. *Ibid.*, p. 239.
30. Withers, W. A., Carruth, F. E., J. Agr. Research, *5*, No. 7, 261-288 (1915).
31. Hale, F., Texas Agr. Expt. Sta. Bull., 410 (1930).
32. Lyman, C. M., Holland, B. R., Hale, F., Ind. Eng. Chem., Anal. Ed., *15*, 489-491 (1943).
33. Eagle, E., Hall, C. M., Castillon, L. E., Boatner, C. H., J. Am. Oil Chem. Soc., *27*, 300-303 (1950).
34. Ambrose, A. M., Robbins, D. J., J. Nutrition, *43*, No. 3, 357-370 (1951).
35. Grimm, F. V., Patrick, W. A., J. Am. Chem. Soc., *45*, 2794-2802 (1923).
36. "Solubility of Non-electrolytes," Hildebrand, Joel H., Reinhold Publishing Corp., N. Y., 2nd Ed. (1936).
37. Jamieson, G. S., "Vegetable Fats and Oils," Monograph Series No. 58, The Chemical Catalog Co., N. Y. (1932), pp. 400-403.
38. Boatner, C. H., Oil and Soap, *21*, 10-15 (1944).
39. Boatner, C. H., Samuels, C. S., Hall, C. M., Curet, M. C., J. Am. Chem. Soc., *69*, 668 (1947).
40. *Ibid.*, p. 1268.
41. Boatner, C. H., Botan. Gaz., *108*, 484 (1947).

[Received October 17, 1951]

Melting Points and Solubilities of Ternary Mixtures Containing Oleic Acid

ERNEST SCHLENKER,[1] Research Laboratory, Fournier-Ferrier, Marseille, France

THE literature contains very few references to the melting or setting points of ternary mixtures of the naturally occurring fatty acids. Heintz (4) almost 100 years ago prepared a few of them and determined their melting points. His data appear to be in good agreement with the facts. Recently Paquot (5) has reopened the subject. Shriner *et al.* (8) have studied the behavior of a ternary mixture containing a component margaric acid, which is not found in fatty oils.

It may be concluded from the few publications cited above that the melting-point behavior of fatty acid mixtures is complicated and unpredictable. This is not surprising inasmuch as irregularities due to eutectics are to be expected. This circumstance is of still greater importance in this case than in that affecting binary mixtures. It is well known (1, 3, 7) that, in the latter case, characteristic depressions appear in the respective curves but that their positions are not a matter of prediction. There is one exception however, and that pertains to binary mixtures containing oleic acid. In this case the irregularities are confined to the extremities of the curves probably because of the fact that oleic acid has a low melting point. The curve is therefore on the whole regular, and values obtained by interpolation agree well with those determined experimentally. The general character of such a curve up to about 50% oleic content is illustrated by the line for mixtures of palmitic-oleic acid, designated "0% stearic" in Figure 1. The eutectic existing for very low contents of oleic acid (9) has been neglected as being of no importance for our purposes.

Parallel to the curve marked "0% stearic acid," there have been plotted others for various mixtures of this acid with palmitic acid, using as starting points the melting points of binary mixtures of these two. It can be seen that this procedure leads to a very complete presentation of the melting points of the ternary system palmitic-stearic-oleic acids. They are accurate within the range where the oleic acid content is of commercial importance, or 10 to 50%.

Each line therein (Fig. 1) refers to a well defined palmitic-stearic acid mixture containing increasing quantities of oleic acid, the amount of which is revealed on the abscissa of the graph. For example, the melting point of a ternary mixture of 27 parts stearic acid, 63 parts palmitic acids, and 10 parts oleic acid can be determined by assuming that the saturated part of the mixture contains 30% stearic acid. In that case the reference curve bearing this name will furnish the needed information.

The data of Carlinfanti and Levi-Malvano (1) were found valuable in checking the accuracy of our graphic method (Fig. 1). The authors reported the

---

[1] Present address: St. Barnabé-Marseilles (France).

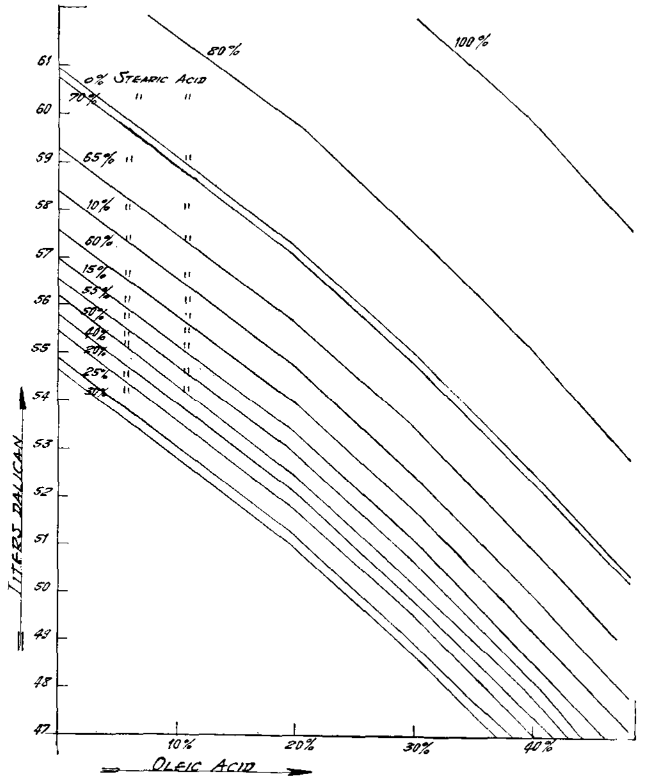

Fig. 1. Titers of mixtures of stearic-palmitic-oleic acid.

melting points of a series of ternary mixtures characterized by the presence of an unsaturated fatty acid. Their communication is the only published work on this subject. For purposes of comparison with our graphic method it was necessary to convert their values in the manner explained above, i.e., to express each ternary system in terms of two components, one of which is saturated and characterized by a value stating its stearic acid content. The other component is oleic acid. The values of Carlinfanti (1), reproduced in bold-face type in Table I, agree very well with those taken from the graph (Fig. 1). Our own values (regular type) cover the simple proportions 10, 20%, etc., oleic acid which are not given in the Carlinfanti (1) communication.

Solubilities. Striking correlations exist between melting points and solubilities in the fatty acid series. The higher the melting point, the lower is the solubility and vice versa. Ralston and Hoerr (6) have clearly illustrated this fact in a series of curves for mixtures of palmitic and stearic acids. It is indeed true that the minimum of the curve representing the melting points of that mixture as a function of its composition has its counterpart in the solubility curve as a maximum at the same molecular composition. The same is true also of the other solid fatty acids. It may be assumed that the irregularities of the curves disappear under the same circumstances as stated above, viz., the presence of oleic acid in the mixture. Practically nothing however has been published in proof of this point. Pertinent to it are the investigations of Van Beneden (10) on the corresponding solubilities of a mixture of fatty acids, one of whose components is oleic acid. The proportions chosen by him however preclude the relevance of his data to the problem in hand.

Experiments were made with ternary mixtures containing oleic acid for the purpose of studying the recovery of acids by solvent separation. To that end each mixture was dissolved in methanol, and then turbidity points were determined at various temperatures upon slowly cooling the whole in a thermostatically controlled bath. The data obtained in those experiments were plotted (Fig. 2) as isotherms showing dissolved matter against oleic acid at a given temperature. Three of these mixtures are described in Table II. They are graphically presented in the upper left-hand corner of Figure 2. The same unit has been used for both coordinates, and each unit represents 0.1 g. The three mixtures given are typical of a large number of cases. Except for the content of oleic acid, the exact composition of the mixtures is of no consequence insofar as it affects our conclusion; therefore it has been omitted.

The data (Table II) show that the first analogy to the melting-point behavior of ternary mixtures lies in the fact that when the concentration of the oleic acid exceeds 15%, the solubility of the others is proportional to its quantity. The second analogy arises from the fact that the pertinent solubility lines are practically parallel for mixtures in which the proportions of stearic and palmitic acids are different.

Both characteristics are also demonstrated in the case of high concentrations of oleic acid. This is shown by lines 1-3 (Fig. 2), which are pertinent to the composition of the liquid fraction obtained by solvent separation of the acids of fatty oils of technological interest. These acids had been obtained by hydrolyzing the parent oils in an autoclave under

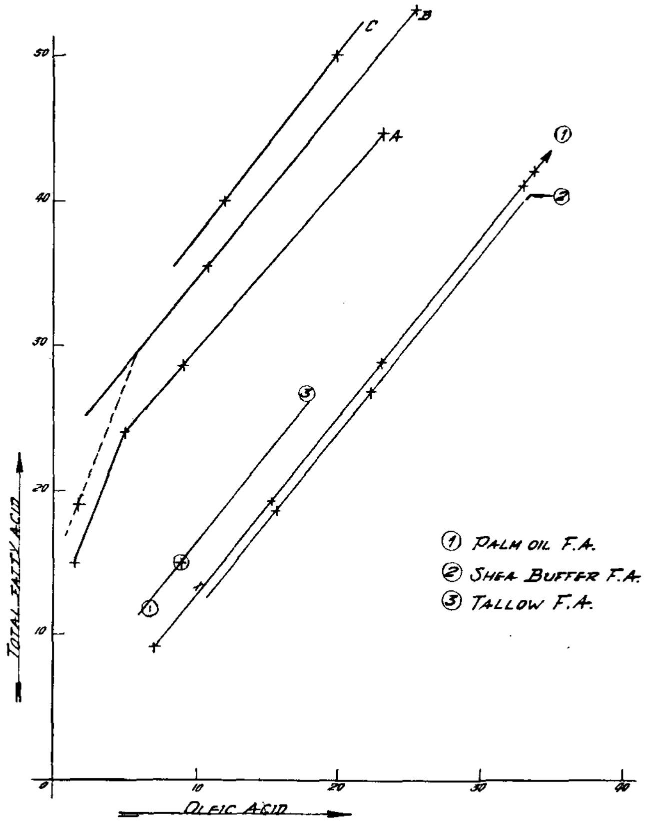

Fig. 2. Solubilities of ternary mixtures (A-C) and technical fatty acids (1-3).

TABLE I

Melting Points of Mixtures of Palmitic, Stearic, and Oleic Acids

Oleic acid content, %	Stearic Acid Content of the Saturated Component of the Mixture per cent														
	0	10	11	13	20	21	25	30	31	35	37	40	42	44	45
68															
67			38.9						36.9					38.2	
60	46.3	43.7			41.1			40.1				41.2			
51															
50	49.8	47.2			44.6			43.6				44.7			
46		47.3							44.7				45.4[a]		
44						45.2									
41															
40	52.6	50.0			47.4			46.4				47.5			
36															
30	55.1	52.5			49.9			48.9				50.0			
27															
26			52.4												
23															
22					50.7		50.1	50.3		50.9		51.3			51.7
20	57.3	54.7			52.1			51.1				52.2			
10	59.2	56.1			54.0				53 0			54.1			
9							52.7								
7											53.8				
6															
3															
1	61.0	58.4			55.8			54.8				55.9			
0															
	49	50	53	54	60	61	70	75	80	85	87	90	91	95	100
68						40.9									
67								45.0							
60		41.6			43.0		46.1		48.8			51.2			
51										53.3					
50		45.1			46.5		49.6		52.3			54.3			
46		46.1		46.5	48.2			52.6		55.1					
44															
41											56.9				
40		47.9			49.3		52.4		55.1			57.5			
36												58.7			
30		50.4			51.8		54.9		57.6			60.0			
27														61.7	
26															
23			52.0												
22					53.7					58.1				62.8	
20		52.6			54.0		57.1		59.8			62.2			
10		54.5			55.9		59.0		61.7			64.1			
9															
7					56.3										
6	54.6														
3							60.1			63.0[b]					
1													65.6		
0		56.3			57.7		60.8		63.5			65.9			68.2

[a] 47.0% oleic acid. [b] 82.0% stearic acid.

such conditions that at most only 3% of the latter remained unhydrolyzed. The reaction mixture was then dissolved; the solution was maintained at constant temperature until the state of equilibrium which answered our purposes had been reached and the two phases separated. True equilibrium is not reached even after 10 hours (2). It was not deemed necessary, for technical reasons, that this point be reached. The liquid phase was analyzed for its content of total and unsaturated fatty acids which, in this case, did not consist entirely of oleic but contained small quantities of linoleic acid. Summarized in Table III are the values from which curves 1 to 3

TABLE II

Solubility of Mixtures of Stearic, Palmitic, and Oleic Acids in Methanol

(Isotherms at 20°)

Mixture	Oleic acid in mixture	Dissolved in methanol	
		Total	Oleic acid
	%	*g./100 g.*	*g./100 g.*
A	10	1.5	0.15
A	20	2.4	0.48
A	30	2.9	0.87
A	50	4.56	2.28
B	10	1.9	0.19
B	30	3.55	1.06
B	50	5.4	2.7
C	10	2.4	0.24
C	30	4.0	1.2
C	40	5.0	2.0
C	50	6.6	3.5

TABLE III

Solubility of Palm Oil, Shea Butter, and Tallow Fatty Acids in Methanol

Fatty acid mixture	Methanol solution		% of total
	Total acids	Liquid acids	
	g./100 g.	*g./100 g.*	
Palm oil	9.2	7.1	77
Palm oil	19.2	15.4	80
Palm oil	29.0	22.9	79
Palm oil	41.0	32.8	80
Palm oil	42.1	33.7	80
Shea butter	13.0	10.4	80
Shea butter	18.6	14.8	80
Shea butter	27.0	21.8	80
Tallow	12.0	6.6	55
Tallow	15.0	9.0	60

(Fig. 2) were constructed. Herein each unit of the coordinates is equal to 1 g.; for the curves A, B, and C it is however, as mentioned above, 0.1 g.

Summary

The presence of oleic acid in mixtures containing palmitic and stearic acids has the effect, within certain limits, of lowering the melting point without the irregularities observed in those cases where the former is absent. Experiments have been made which show that this behavior of oleic acid has a counterpart, insofar as solubilities are concerned, in its influence on the solubility of the same ternary system in methanol. Thus the quantities of the solid fatty acids dissolved are, within commercially important ranges, proportional to the amount of oleic acid present in the mixture.

REFERENCES

1. Carlinfanti, E., and Levi-Malvano, M. Gaz. Chim. ital., *39* II, 353 (1909).
2. Foreman, H. D., and Brown, J. B., Oil and Soap, *21*, 183 (1944).
3. Francis, F., Collins, F. J. E., and Piper, S. H., Proc. Roy, Soc. (London), *A 158*, 619 (1937).
4. Heintz, W., Ann. Physik, *92*, 588 (1854).
5. Paquot, C., Bull. soc. Chim., *1950*, 400.
6. Ralston, A. W., and Hoerr, C. W., J. Org. Chem., *10*, 170 (1945).
7. Schuette, H. A., and Vogel, H. A., Oil and Soap, *16*, 209 (1939).
8. Shriner, R. L., Fulton, J. M., and Burks, D., Jr., J. Am. Chem. Soc., *55*, 1494 (1933).
9. Smith, J. C., J. Chem. Soc., *1939*, 976.
10. Van Beneden, J. pharm. Belg., *16*, 78 (1934).

[Received December 12, 1950]

A Procedure for the Determination of Total Unsaturation in the Products of in Vitro Oxidation of Fatty Acids in Biological Systems

S. MUKHERJEE, Department of Applied Chemistry, Calcutta University, Calcutta, India

NUMEROUS halogen absorption methods are available for measuring the unsaturation of fats and fatty acids and the majority of customary methods are satisfactory when the fat is available in an anhydrous state. The estimation of the absolute amount of unsaturation produced in the course of the biological oxidation of fatty acids *in vitro* in the presence of mold suspensions presents a problem of considerable difficulty. The prevalent methods for estimating unsaturation of fatty substances, viz., those of Wijs, Hanus, and Hubl, that have been used by previous investigators in the field of fat metabolism entail the laborious process of extraction of the fatty acids from the reaction mixture by the ether-alcohol mixture, evaporation of the solvent, and the determination of the iodine absorption of the residual fat, which must be present in an absolute anhydrous state. The difficulty experienced in complete extraction of the fatty matter and the consequent inaccuracy of the results obtained has been mentioned again and again, and so far absolutely no attempt has been made to devise a method for the determination of unsaturation produced in biological systems without involving the extraction methods. The interest in evolving a suitable method for the estimation of the unsaturation of the reaction fluid obtained in the biochemical oxidation of the fatty acids in *in vitro* experiments lies in the fact that the absolute quantities of the fatty acids employed in such experiments are exceedingly small to effect an efficient extraction by the prevalent technique with ether-alcohol mixture and that for easy manipulation such estimation of unsaturation should only be made in aqueous solutions of salts of the fatty acids. Goswami and Basu (1) employed hypochlorous acid reagent for determining the unsaturation of oils and fats, and their method consisted in first preparing the soap and then conducting the absorption of the hypochlorous acid for effecting the saturation of the double bond.

The method originally employed by them has however been modified greatly by the present author, and an extensive investigation regarding the concentration of the acid, the relative amounts of reagents, and the time of reaction has resulted in a more simplified method.

Experimental

The acid is weighed directly into a 250-ml. reagent bottle, provided with a ground glass stopper, and treated with 5 ml. of glacial acetic acid (A. R.); 10 ml. of sodium hypochlorite solution (approximately 0.14 N) is added by a pipette and the bottle stoppered immediately. The stopper is sealed by moistening it with potassium iodide solution. A definite reaction time is allowed for the absorption of HOCl, after which 10 ml. of a 15-p.c. solution of potassium iodide is added and the flask is kept in the dark for 5 minutes. Distilled water (100 ml.) is added and the liberated iodine titrated with 0.1 N thiosulfate. The amount of HOCl absorbed is determined by carrying out a blank experiment. The results can be expressed in terms of iodine equivalents of the HOCl absorbed by the fatty acid, and the absolute amount of acid can be calculated directly from the volume of thiosulfate consumed.

A study of the relative effect of time, reaction temperature, sample size, and excess of reagents, factors which are of primary importance in the addition of halogen to double bonds, has been made, using pure oleic acid (I.V—89.86) as a substrate. Table I indicates that halogen absorption is almost complete within 5-10 minutes at 37°C. when 0.1-0.12 gm. of the sample are used.

The amount of reagent used in excess varied from 210 to 236%. As a safe limit a 15-minute reaction

TABLE I

Effect of Time and Temperature on the Determination of Unsaturation of Oleic Acid by Use of Hypochlorous Acid Reagent

Temp. (°C.)	5 min.	10 min.	15 min.	30 min.	60 min.	120 min.	24 hr.
5±1	89.40	89.60	89.60	89.64	89.80	89.81	90.0
16±1	89.43	89.65	89.80	89.86	89.90	89.90	89.96
37±1	89.72	89.87	89.87	89.89	89.90	89.90	89.94