

Appraisal of Methods of Total Monoester Estimation in Commercial Monoglycerides

L. HARTMAN, Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand.

Analytical techniques of estimating total monoglycerides in industrial products and difficulties encountered in their estimation are critically discussed. A modification is suggested of a recently published procedure which combines the "Partition method" with the methods of Martin and of Brokaw *et al.* A tentative method of determining total monoglycerides based on simultaneous isomerization and periodate oxidation is described.

UNTIL RECENTLY 1-monoesters were regarded as the only active components of commercial monoglycerides and the periodate method in its various forms afforded convenient means of their estimation. The presence of 2-monoesters was generally ignored. However, Brokaw *et al.* (1) showed a few years ago that commercial monoglycerides contained the 2-isomer in quantities amounting to 5-8% of total monoglycerides and their findings have been repeatedly confirmed (2,3). Since 2-monoglycerides are efficient emulsifiers and behave like the 1-isomers in at least one baking application (1) it seems that they should be included in the evaluation of commercial monoglycerides. In addition it has been rightly pointed out (4) that the ignoring of their presence would lead to a wrong computation of diglyceride and triglyceride contents of commercial monoglycerides if based on the determination of acetyl values, free glycerol, and 1-monoesters only.

Admittedly Becker and Krull (5) have recently stated that 2-monoglycerides, while present in freshly prepared products, disappear on storage and that the usual oxidation with periodates measures therefore the total amount of monoesters. However, this could not be confirmed in this laboratory (2) since even after 5 years' storage appreciable amounts of 2-monoglycerides were detected in a number of commercial preparations. As may be seen from an interesting study by Brandner and Birkmeier (3) the equilibrium between 1- and 2-monoglycerides is a complex phenomenon and depends, among other factors, on the physical form of the products. Yet in all products examined by Brandner and Birkmeier 2-monoesters were present, in some 5-8% after 24 months' storage, which indicates that the shift of the equilibrium in favor of the 1-form of room temperature is, to say the least, a slow process.

Nevertheless, in view of the possible changes in the equilibrium, it seems that the content of total monoglycerides is a more stable characteristic of a given product than is its 1-monoester content, which is confirmed by the findings of Aylward and Wood (6). From the point of view of the process control the estimation of total monoglycerides is even more important because sometimes, especially in freshly distilled monoglycerides, the initial 1-monoester content is much lower than the value attained after several hours or days.

In the last few years several chromatographic methods have been suggested for the estimation of total monoglycerides (7,8,9). The gas-liquid chromatographic method of McInnes *et al.* (10) makes even possible a direct estimation of 2-monoglycerides on a micro-scale. All these methods are, however, for the

time being too laborious for routine purposes, and possibly not accurate enough in view of the comparatively small amounts of 2-monoesters present in industrial products. The present discussion will be therefore limited to methods based on the use of periodates.

Estimation of Total Monoglycerides According to Martin (11) and Brokaw *et al.* (1)

Martin's original technique is based on producing an equilibrium between 1- and 2-monoglycerides dissolved in chloroform with the aid of 56% perchloric acid in a 2-phase system (11). After the removal of the catalyst by washing with water the 1-monoester is estimated by the periodate method and the total monoester content is calculated by multiplying the result by an empirical factor of 1.15. An essential feature of the method is a careful purification of chloroform without which the isomerization is not complete. Brokaw *et al.* (1) replaced chloroform with a 2:1 mixture of acetic acid and chloroform in which perchloric acid is soluble, and dispensed with the removal of this acid after isomerization, the aqueous periodic acid reagent with a water content of 20% inactivating the catalyst. The procedure is accordingly simpler than the original technique, and does away with two of the three sources of error mentioned by Martin and namely, with the loss of fat and with the rearrangement of monoglyceride to diglyceride: there is no entrainment of fat due to washing, and glycerol produced during the above rearrangement does not escape estimation. However, the third possible error resulting from variations in the equilibrium composition remains. Further, the original monoglyceride must be washed free of glycerol in ether solution, the solvent evaporated, and the product dried *in vacuo*. The purity of acetic acid is critical, small amounts of water having an inhibiting effect on the degree of isomerization. Thus the procedure is rather time-consuming and requires rigorous control of analytical conditions.

Combined Partition Method—Martin—Brokaw *et al.* Procedure

The recently published monograph by Mehlenbacher (12) describes another procedure for total monoglyceride estimation. It combines the so-called Partition method (13) which is a modified version of the well-known Pohle and Mehlenbacher method (14) for the estimation of 1-monoglycerides, with certain features of both the Martin and Brokaw *et al.* techniques. According to this procedure a sample of monoglycerides is dissolved in 100 ml of chloroform and shaken with an equal volume of water or 5% aqueous acetic acid to remove free glycerol as in the Partition method. After the separation of the two layers 75 to 90 ml of the chloroform solution is isomerized with 0.08 ml of 56% perchloric acid by shaking for 1 min and allowing to stand for 9 additional min. The monoglycerides are estimated by the Pohle and Mehlenbacher method using a 50 ml aliquot of the chloroform solution. The

result multiplied by 1.15 gives the total monoglyceride content.

As may be seen the isomerization step is similar to that in the Martin technique, inasmuch as it is carried out in a 2-phase system. The inactivation of the catalyst is effected by the addition of the aqueous periodic acid reagent as in the Brokaw *et al.* procedure, except that the reagent contains in this case only 5% instead of 20% water which is, however, sufficient. The success of the method hinges on the quality of the chloroform employed. There is no stipulation that this solvent should be specially purified as stressed by Martin, and consequently there is a possibility of the isomerization being incomplete. This would as a rule produce results for total monoglycerides which are too high. According to the author's experience the purification of chloroform is essential, and even then some batches of chloroform may be found unsuitable. Water dissolved in chloroform during the washing step may also interfere. In view of these difficulties the modification below may be found useful.

The chloroform solution of monoglycerides is washed with water or 5% acetic acid as in the Partition method.

The chloroform layer is dried by shaking with about 5 w/v % of anhydrous sodium sulphate and decanted.

To 50 ml of the dried chloroform solution is added 0.21 ml of 56% perchloric acid dissolved in 20 ml of glacial acetic acid i.e. 0.003 ml perchloric acid per 1 ml of final mixture.

The mixture is shaken for 1 min and left standing for another 9 min.

Four ml of water is added to inactivate the catalyst followed by 50 ml of periodic acid reagent whereupon the monoglyceride content is estimated as in the Pohle and Mehlenbacher method.

Table I shows results obtained with this modification as compared with the procedure outlined in Mehlenbacher's monograph. The products used in these experiments were 1-monostearin prepared by a modified isopropylidene-glycerol method (15), 2-monostearin prepared essentially by Martin's method (16) and three commercial monoglyceride samples. It will be seen that the use of nonpurified chloroform resulted in incomplete isomerization. In particular the amount of total monoglycerides found in pure 1-monostearin was much too high and in 2-monostearin much too low. In linseed oil monoglycerides similar amounts of total monoglycerides were found by both methods since in this product the equilibrium existing between 1- and 2-monoglycerides approached that induced by perchloric acid isomerization, i.e., approximated the ratio of 88:12.

In practice it may be found convenient to estimate both total and 1-monoglycerides using aliquots of one weighed and washed sample. Accordingly 25 ml

aliquots of chloroform solution containing a double amount of material may be used instead of 50 ml, or a sample of suitable size may be dissolved in 250 ml instead of 100 ml of chloroform. Further, a periodic reagent containing 15% instead of 5% water may be prepared to ensure the inactivation of the catalyst.

A Tentative Method of Direct Estimation of Total Monoglycerides

Brokaw *et al.* (1) have tried various catalysts other than perchloric acid in the hope of achieving simultaneous isomerization and oxidation of monoglycerides. Sulphuric and hydrochloric acids were found unsuitable, and this was confirmed during the present work. The addition of perchloric acid simultaneously with, or subsequent to, the addition of the periodic acid reagent was considered by Brokaw *et al.* impracticable since this reagent is aqueous and inactivates the catalyst. However, there is no need to use water in the preparation of the periodic acid reagent. Periodic acid can be dissolved in glacial acetic acid either by warming or by shaking in the cold. Such a solution may be mixed with an equal volume of chloroform without precipitating periodic acid during the half to 1 hr period needed for the oxidation of monoglycerides. Moreover, an anhydrous reagent is not essential for a simultaneous isomerization and oxidation of monoglycerides since, owing to the continuous removal of the 1-monoester from the system, gradual isomerization results in the disappearance of the 2-form even in the presence of some water. Thus 50% aqueous periodic acid may be used for the preparation of the reagent. Such periodic acid may be diluted with glacial acetic acid to the required strength without formation of precipitates which appear only in the presence of some iodic acid or other impurities. (50% aqueous periodic acid is now available commercially at a relatively lower price than the crystalline product.) The reagent prepared as mentioned above and containing approximately 0.3% water was used for the direct estimation of total monoglycerides in the presence of perchloric acid, following otherwise the Pohle and Mehlenbacher procedure for 1-monoglycerides. It was found that the difficulty consisted not in effecting isomerization but in preventing side reactions, caused by perchloric acid when used in the amount of 0.003 ml of 56% acid per 1 ml of solution. These side reactions seem to consist in a hydrolysis of monoglycerides and their oxidation products. Several other catalysts have been tried. Aromatic sulfonic acids such as toluene-p-sulfonic acid mentioned recently as a possibility by Van Lohuizen and Verkade (17) proved to be almost as efficient as perchloric acid when used in double amounts on the molar basis. Unfortunately, their hydrolyzing effect was equally evident. The same applied to alkyl-aryl-sulfonic acids and to the co-called Twitchell reagents. The hydrolytic action of metallic perchlorates was less pronounced but so was also their isomerizing efficiency. Phosphoric acids and strong organic acids such as trichloroacetic acid were without any effect. The addition of 0.001 ml of 56% perchloric acid per 1 ml of solution proved successful but only in the case of saturated esters such as monopalmitates and monostearates, and if the amount of 2-monoglycerides did not exceed 20% absolute. In the presence of considerable amounts of unsaturated and low molecular monoglycerides, carbon tetrachloride had to be employed as solvent to suppress hydrolysis. The use of carbon tetrachloride for saturated monoglycerides was not feasible because

TABLE I
Estimation of Total Monoglycerides by the Method Described in Mehlenbacher's Monograph (12) Denoted as Method A and by Its Modification Denoted as Method B

Material	1-Monoglyceride content	Total monoglyceride content	
		Method A	Method B
	%	%	%
1-Monostearin (pure).....	100.2	111.4	99.9
2-Monostearin (synthetic).....	3.4	14.2	100.9
Distilled monoglyceride (commercial).....	87.3	98.0	92.3
Technical monostearate.....	38.5	43.9	39.9
Technical linseed oil monoglyceride.....	43.1	49.3	48.6

TABLE II
Direct Estimation of Total Monoglycerides with the Aid of Various Catalysts
Reaction Time: 30 min.

Material	Solvent	Catalyst	Amount of catalyst per 100 ml of solution (solvent + periodic acid reagent)	% Total monoglycerides found	% Total monoglycerides by Brokaw <i>et al.</i> technique
2-Monostearin.....	Chloroform	56% HClO ₄	0.2 ml	100.4	98.4
2-Monostearin.....	Chloroform	56% HClO ₄	0.1 ml	76.8
1-Monostearin.....	Chloroform	56% HClO ₄	0.1 ml	99.9	99.5
90% 1-Monostearin + 10% 2-monostearin.....	Chloroform	56% HClO ₄	0.1 ml	99.4	99.5
80% 1-Monostearin + 20% 2-monostearin.....	Chloroform	56% HClO ₄	0.1 ml	98.8	98.7
70% 1-Monostearin + 30% 2-monostearin.....	Chloroform	56% HClO ₄	0.1 ml	95.5	99.4
2-Monostearin.....	Chloroform	Toluene-p-sulfonic acid	0.5 g	98.5
2-Monostearin.....	Chloroform	Magnesium perchlorate	0.3 g	48.4
2-Monostearin.....	Chloroform	Aluminum perchlorate	0.3 g	49.6
2-Monostearin.....	Chloroform	Calcium perchlorate	0.3 g	45.7
Technical linseed oil monoglyceride.....	Chloroform	56% HClO ₄	0.1 ml	55.7	47.7
Technical linseed oil monoglyceride.....	Carbon tetrachloride	56% HClO ₄	0.1 ml	47.9

of their insufficient solubility in this solvent. Thus the solvent had to be varied according to circumstances.

If the technique is used in conjunction with the Partition method a 50 ml aliquot of the chloroform or carbon tetrachloride solution washed free from glycerol is mixed with 50 ml of the periodic acid reagent containing 0.3% water.

One tenth ml of 56% perchloric acid is added and after 30 min the monoglyceride content is determined as in the Pohle and Mehlenbacher method.

The percentage of total monoglycerides is calculated from the actual consumption of periodic acid without the use of the multiplication factor of 1.15.

Results obtained with some of the catalysts mentioned appear in Table II and the hydrolytic action of perchloric acid is shown in Table III. The isomerizing effect of metallic perchlorates is probably due to their acidic character in the solvent system employed. The continued consumption of periodic acid in the presence of perchloric acid after the oxidation of all 1-monoglycerides seems to indicate that the aldehydic oxidation products are subjected to further oxidation as such or, what is more likely, are hydrolyzed with the subsequent oxidation of the glycolic aldehyde formed.

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TABLE III
Consumption of Periodic Acid by 1-Monostearin in the Presence of Perchloric Acid

Time of reaction (hr)	Moles of periodic acid consumed by 1 mol. of 1-monostearin	
	Addition of 0.2 ml 56% HClO ₄ per 100 ml of solution	Control
1/2	1.02	1.00
1	1.06	1.00
24	1.38	1.01
30	1.63	1.01

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Sodium Salts of Alkyl Esters of α -Sulfo Fatty Acids. Wetting, Lime Soap Dispersion, and Related Properties¹

A. J. STIRTON, R. G. BISTLINE, Jr., J. K. WEIL, WALDO C. AULT, and E. W. MAURER, Eastern Regional Research Laboratory,² Philadelphia 18, Pennsylvania

A series of esters of the general formula RCH(SO₃Na)-CO₂R' of 14-19 carbon atoms prepared by the α -sulfonation of propionic, butyric, pelargonic, lauric, myristic, palmitic, and stearic acids and esterification with normal primary alcohols were compared for critical micelle concentration, surface and interfacial tension, Ca⁺⁺ stability, wetting

properties, foam height, detergency, and lime soap dispersing properties. Comparison of position isomers showed that as the hydrophilic portion moved from the center toward either end, cmc and wetting efficiency decreased, surface and interfacial tension increased, and Ca⁺⁺ stability and lime soap dispersing properties improved.

A coconut oil fatty acid forerun sulfonated with SO₃ vapor and esterified with 2-ethylhexanol gave a product with useful wetting properties in soft and hard water.

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² Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.