A TENTATIVE METHOD FOR THE DETERMINATION OF MIXED GLYCERIDES PRESENT IN NATURAL FATS BY ESTIMATING THE SATURATED ACIDS PRESENT IN AZELAO GLYCERIDES OBTAINED BY PERMANGANATE OXIDATION IN ACETONE

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WE have recently developed a method[1] for the estimation of GS_2U and GSU_2 in natural fats and in continuation of our investigation with the ultimate object of perfecting a simple and reliable method applicable to any glyceride mixture, we have started a series of investigations. The maximum possible proportions of GS_2U and GSU_2 can, of course, be calculated from the GS_3 value and the saturated acid content of the specimen. But it must be remembered that these maximum proportions represent only limiting values and definite knowledge about the saturated-unsaturated glyceride constituents cannot be obtained from these values alone. Several attempts have been made to determine the actual proportions of GS_2U and GSU_2 in natural fats. Hydrogenation studies with the object of determining GU_3 did not achieve the hoped for reliability as revealed by the more accurate crystallisation studies.[2,3] Crystallisation studies have not yielded any more detailed information than a minimum limiting value of GS_2U.[4,5,6,7] Fractional crystallisation studies of elaidinised fat[8] is very tedious and also quite restricted in its usefulness especially in view of the observation that at least in some natural vegetable fats, linoleic acid may be preferentially combined in GS_2U[9] and the linoleic glycerides undergo undesirable changes under the drastic conditions of elaidination.

It is well established that when the mixture of acidic products obtained in the oxidation of a fat is taken up in ether and washed with sodium bicarbonate, only a part of the diazelao-glyceride is extracted along with the aliphatic acids and triazelain. Thus after a few washings, it is possible to get the mixture of GS_3, GS_2A and GSA_2 in a pure condition from which by further extraction with bicarbonate, GSA_2 can be obtained in a pure condition. Investigations on some vegetable fats like cocoa butter,[10] mowrah oil,[4] etc., had indicated the possibility that the saturated acids may be distributed in the same proportions in GS_2U and GSU_2 types in many

other vegetable fats and oils also. A certain degree of confirmation of this argument was revealed during an examination of pure GSA_2 obtained by the oxidation of the oil of *Mimusops elangi*.[1] In order to obtain further confirmation we have examined the GS_2A-GSA_2 mixture obtained in the oxidation of a number of fats and oils and in every case we obtained results substantiating the even distribution. The method we adopted was to fractionally extract the mixture of GS_2A and GSA_2 obtained in the oxidation with bicarbonate and determine the S.V. (S.E.) of the fractions obtained. The S.V. was in every case quite in agreement with the value calculated on the basis of even distribution, and this fact as well as the constancy of the S.V. for the different fractions established that there was no preferential removal of the diazelao-glycerides of lower saturated acids by the bicarbonate. We have now proceeded a step further in the same direction. We isolated the saturated acids of the GS_2A-GSA_2 mixture and also that of the pure GSA_2 obtained from the mixture by bicarbonate extraction. In each case we obtained identically the same acid mixture as proved by the melting point and mean molecular weights.

It must be emphasised that the method developed by us, although accurate and simple, is applicable only in the case of those fats where the distribution of the saturated acids is the same in both GS_2U and GSU_2 and also where the unsaturated acids are all of the type which leave azelaic acid residues on oxidation. Mixtures of glycerides can exist which do not conform to these two conditions. Hydrogenated fats, which are very important from an industrial standpoint, do not have the saturated acids distributed in the same ratio in GS_2U and GSU_2. A number of natural fats are known to contain unsaturated acids with the first double bond in positions other than the 9 : 10-position and consequently not yielding azelaic acid residue on oxidation. The following combinations are possible:—

1. Saturated acid distribution the same in GS_2U and GSU_2 and the dibasic acid residue attached to the glycerol molecule (after oxidation) being the same in all the molecules.

2. Saturated acid distribution same in GS_2U and GSU_2. The dibasic acid residue left attached different giving rise to the following:

(*a*) Mean molecular weight of dibasic acid same in GS_2A and GSA_2.

(*b*) Mean molecular weight of dibasic acid different in the two.

3. Saturated acid distribution different in GS_2U and GSU_2 with the same dibasic acid left attached.

4. Saturated acid distribution and dibasic acid residues different:

(*a*) Mean molecular weight of dibasic acid same in both.

(*b*) Mean molecular weight of dibasic acid different in both.

Of these different types the first is relatively the simplest and fortunately the great majority of natural fats seem to belong to this group. Hydrogenated fats approximate to the third type.

The azelaic acid generated by the saponification of the azelao-glycerides can be quantitatively separated from the higher saturated fatty acids according to Bertram's method and from the data thus obtained a better insight into the complicated question of glyceride structure expected.   Let us say that " B " stands for the GS_2A-GSA_2 mixture and " C " for GSA_2, and the following respective symbols for the various values :—

	" B "	" C "
S.V.	A_1	A_2
Percentage of saturated acids/100	S_1	S_2
A.V. of acids (saturated)	a_1	a_2
Percentage of dibasic acids/100	D_1	D_2
Mean molecular weights of acids	$k_1/a_1, k_2/d_2$	$k_1/a_2, k_2/d_2$
	where k_1 and k_2 are constants (the corresponding equivalents)	
Acid value of the azelao-glycerides	x_1	x_2
A.V. of dibasic acids	d_1	d_2

Then the following relationships follow :—

$$A_1 = S_1 a_1 + D_1 d_1$$
$$A_2 = S_2 a_2 + D_2 d_2$$
$$x_1 = \tfrac{1}{2} D_1 d_1$$
$$x_2 = \tfrac{1}{2} D_2 d_2.$$

If A_1, A_2; S_1, S_2; and a_1, a_2 are all determined, then the percentage and the mean molecular weight of the dibasic acids can be calculated.   Thus for D_2 and d_1 we have :—

$$A_2 = S_2 a_2 + D_2 d_2$$
$$D_2 d_2 = A_2 - S_2 a_2$$
$$D_2 = 1 - S_2 - \left[\frac{S_2 a_2 + \tfrac{1}{2}(A_2 - S_2 a_2)}{3 \times 56 \cdot 11 \times 1000} \cdot 38 \right]^{*}$$
$$= 1 - S_2 - \left[\frac{(\tfrac{1}{2} A_2 + \tfrac{1}{2} S_2 a_2) \times 38}{3 \times 56 \cdot 11 \times 1000} \right] \text{ say } P.$$

Hence $d_2 = A_2 - S_2 a_2 / P.$

Similarly in the other case also.

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* Please see appendix for details of this derivation.

If k_1/a_1 is equal to k_1/a_2, *i.e.*, when the mean molecular weights are equal, then the distribution of saturated acids is the same in GS_2U and GSU_2. Similarly if k_2/d_1 is equal to k_2/d_2, then the distribution of the unsaturated acids (with respect to the double bond nearest the carboxyl group) is the same in both glyceride types. The simple animal and vegetable fats will thus be characterised by the equations

$$k_1/a_1 = k_1/a_2$$
$$k_2/d_1 = k_2/d_2 = 188 \text{ (M. wt. of azelaic acid).}$$

If the unsaturated acids contain the first double bond in a position other than the 9 : 10-position, then k_2/d_1 will not be equal to 188, except in cases where a balanced mixture of isomers exist.

If k_1/a_1 is not equal to k_1/a_2, then the variation is due to the saturated acids in GS_2U glyceride type alone and hence the actual proportion of GS_2A can be calculated as follows:—

Let us assume that k_1/a_1 is higher than k_1/a_2. The higher value of k_1/a_1 is due to the presence in GS_2U (GS_2A) of acids of mean molecular weight higher than k_1/a_2. For calculation the increase in mean molecular weight is assumed to be due to the presence of a specific acid of a higher mean molecular weight and the quantity of this necessary to give the observed difference can be easily calculated. This amount is present in " B " as GS_2A alone and hence the balance weight of GS_2A and GSA_2 and its S.V. can be calculated. This portion is distributed as GS_2A and GSA_2, of saturated acids having mean molecular weight k_1/a_2 and the amount of GS_2A added to the previous value gives the total of GS_2A. Similarly when k_1/a_1 is lower than k_1/a_2 the difference is assumed to be due to the presence of a specific acid of lower mean molecular weight than k_1/a_2 and the calculation of the total GS_2A is effected exactly as before.

If on the other hand k_1/a_1 is equal to k_1/a_2 but k_2/d_1 is not equal to k_2/d_2 (due to the difference in the distribution of the different unsaturated acids in GS_2U and GSU_2), then the dibasic acids in " B " are to be partitioned into a mixture of mean molecular weight k_2/d_2 and another one of lower or higher value as the case may be. If the nature of the different unsaturated acids is known, then this difference can be directly attributed to the specific dibasic acid left attached to the glyceryl residue. Moreover, in such cases, the different amounts of the different unsaturated acids in GS_2U, GSU_2 and GU_3 can be directly calculated, thus throwing much further light on the distribution of the unsaturated acids.

When k_1/a_1 and k_2/d_1 are different from k_1/a_2 and k_2/d_2 respectively, then the distribution will be different both with regard to the saturated and

unsaturated acids. In such cases the dibasic acids are first partitioned and the fraction of mean molecular weight different from k_2/d_2 is combined as GS_2A, where S is K_1/a_2. The weight, S.V., etc., of the remainder can then be calculated and this then represents the case where k_2/d_1 is equal to k_2/d_2 but k_1/a_1 is not equal to k_1/a_2 and hence is partitioned again. Or the procedure can be adopted in the reverse way, the saturated acids being first partitioned (calculated to be in combination as GS_2A where the dibasic acid have the mean molecular weight k_2/d_2) and then the dibasic acids partitioned in the resulting mixture. Proceeding in this manner not only is the total amount of GS_2A obtained, but some more knowledge about the distribution of the different acids among the glycerol molecules is simultaneously available.

In cases where considerable amount of C_{14} and lower saturated acids occur, the quantitative separation of saturated acids is not possible according to Bertram's method. In such cases, if it is known that the same dibasic acid (k_2/d_1 is equal to k_2/d_2) occurs in both GS_2A-GSA_2 mixture and in GSA_2, then by determining the free acid value carefully and substituting in the equation $x_1 = \frac{1}{2} D_1 d_1$, D_1 can be determined and from this S_1, k_1/a_1, etc., can be easily calculated. This leads to a knowledge of the glyceride composition. The question as to whether all the unsaturated acids leave the same dicarboxylic acid residue on oxidation has to be settled by careful analysis of the unsaturated acids obtained by the hydrolysis of the oil. In those cases it is not so, appropriate procedures can be worked out for the determination of the dibasic acids, saturated acids leading to the determination of the glyceride composition.

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APPENDIX

D_2 is the weight of dibasic acid in one gram of the Di-azelao-glyceride mixture and can be obtained by subtracting the combined weights of the monobasic acids and the glycerol residue from one gram, *i.e.*,

$$1 - [S_2 + \text{glycerol residue}].$$

Glycerol residue is evaluated from the saponification value of the glyceride mixture (A_2) and the weight in one gram and acid value of the monobasic acids (S_2 and a_2 respectively). The acid value is the weight of KOH in milligrams required to neutralise one gram of the acid. The value obtained by multiplying the acid value with the weight of the mono basic acids in one gram of the glyceride mixture (S_2a_2) will be the weight of KOH required to neutralise the whole of the monobasic acids in the mixture. This value is proportional to the weight of glyceryl residue combined with the monobasic acids. The equivalent of glyceryl residue is 38/3 and so its weight will be $S_2a_2 \times 38/56 \cdot 11 \times 1000 \times 3$. The dibasic acids are combined with the glyceryl radical so that half the product of the saponification value and the weight of the dibasic acids corresponding to this combination will be proportional to the weight of glyceryl radical involved in this type of combination. This value (D_2d_2) is obtained by substracting the product of the saponification value corresponding to the monobasic acids and its weight from that of the saponification value of the mixture, *i.e.*, $A_2 - S_2a_2/2$. The weight of the glyceryl radical corresponding to this will be $A_2 - S_2a_2 \times 38/2 \times 56 \cdot 11 \times 1000 \times 3$.

Hence $1 - (S_2 + \text{glyceryl residue})$ becomes

$$1 - S_2 - \left[\left(\frac{S_2a_2 \times 38}{3 \times 56 \cdot 11 \times 1000} \right) + \left(\frac{A_2 - S_2a_2 \times 38}{2 \times 3 \times 56 \cdot 11 \times 1000} \right) \right]$$

$$= 1 - S_2 - [S_2a_2 + \tfrac{1}{2}(A_2 - S_2a_2) \times 38/3 \times 56 \cdot 11 \times 1000).]$$

A2