# A TENTATIVE METHOD FOR THE ESTIMATION OF MONO-UNSATURATED AND DI-UNSATURATED GLYCERIDES

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A QUANTITATIVE estimation of the four classes of glycerides (trisaturated, disaturated-monounsaturated, monosaturated-diunsaturated and triunsaturated) present in any fat or oil can be arrived at if any two of these two classes can be analytically determined. The trisaturated glycerides ($GS_3$) are capable of exact determination by permanganate oxidation in acetone, developed by Hilditch and Lea[1] arising out of Armstrong's and Hilditch's method[2] for preparing saturated esters from a mixture. The estimation of the disaturated-monounsaturated glycerides ($GS_2U$) has been attempted by the separation of the monoazelao-glycerides ($GS_2A$) formed by oxidation, as their metallic salts, by Lea[3] and others. A comparison of published data shows that when a fat yielding 25% of mono-, and 75% of diazelao-glycerides is oxidised, the separation of the monoazelao-glyceride by crystallisation of its salt leaves much to be desired from the point of accuracy. For determining the completely unsaturated glycerides, Hilditch and co-workers[4] have developed two methods. From a purely experimental standpoint the estimation of the trisaturated glycerides is the most satisfactory of all the methods so far developed.

In the most recent publication to hand Hilditch and Meara[5] discuss how far it is possible by empirical calculations, based on the proportions of the component fatty acids in a fat, to predict the general proportions of the chief mixed glycerides present. About a year back we had worked out an experimental method based on saponification values for determining $GS_2U$-, and $GSU_2$-glycerides and had incorporated the same in a thesis.[6] It is our intention to pursue the subject but in view of Hilditch's recent paper we have decided to publish the work so far accomplished.

The acidic products of oxidation in the determination of $GS_3$-glycerides have been utilised by us in developing our method which is really an extension of Lea's[3] attempt to estimate $GS_2U$-glycerides. Hilditch[7] has observed that from the ethereal solution of the products of oxidation bicarbonate removes

the aliphatic acids, triazelain, and a portion of the diazelao-glycerides, the whole of the monoazelao and some of the diazelo remaining behind in the ethereal layer. For the determination of $GS_2U$ and $GSU_2$ we decided to make use of the saponification equivalents of the corresponding azelao-glycerides, $GS_2A$ and $GSA_2$. If we determine the saponification equivalents of the mixture of the two and that of the diazelao-compound, which can be obtained free from admixture, we can calculate the amount of each. This is possible since we know that the saturated acids occur in the same ratio in these two types of glycerides. The equality of ratio can be proved by calculating the $C_{16}$- and $C_{18}$- acids in the two types from published results. Hilditch and Stainsby[8] separated cacao butter into three fractions by systematic crystallisation from acetone and therefrom deduced that the fat is approximately composed of 2% saturated-, 77% disaturated-, and 21% mono-saturated glycerides. This fat contains 24·3 and 35·5 respectively of $C_{16}$- and $C_{18}$-acids calculated as molar percentages. By apportioning these two acids in the disaturated glycerides we get the following values:—

Palmitic acid $77 \times 24 \cdot 3/59 \cdot 8 \quad 31 \cdot 29$.

Stearic acid $77 \times 35 \cdot 5/59 \cdot 8 \quad 45 \cdot 71$.

It has actually been found that these two acids are distributed in $GS_2U$ in the ratio of 32 to 45, thereby confirming the equality of ratio.[12] We have made this calculation with the data available for a large number of fats[9] and in no case did we observe any deviation which is not within experimental error. It is possible to calculate the saponification equivalents of the glycerides with this equality of distribution of saturated acids in $GSU_2$ and $GS_2U$. The saponification equivalents of the corresponding azelao-glycerides will be a measure of the glycerides themselves.

In carrying out the estimation according to our method the following steps are involved:

(1) The fat or oil is oxidised by acetone-permanganate and $GS_3$ separated.

(2) The mixture of mono-, di-, and tri-azelao-glycerides is taken up in pure ether and washed alternately with bicarbonate solution (5%, five or six times) and the extract and washings rejected. This removes the whole of the monobasic acids, triazelain and a portion of the diazelo-glycerides.

(3) The ethereal solution is now cautiously extracted alternately with 10% aqueous potassium carbonate and distilled water to extract mono- and di-azelao-glycerides. The combined extract and washings are acidified to congo-red with dilute sulphuric acid and then thoroughly extracted with ether and dried over anhydrous magnesium sulphate.

(4) The ether is removed and the residue completely freed from traces of ether under reduced pressure at a very low water-bath temperature.

(5) The saponification value of a portion of the residue is determined, this value corresponding to the S.V. of the mixture $GS_2A$ and $GSA_2$.

(6) The remainder of the residue is again taken up in ether and washed alternately with bicarbonate and water. The combined washings and extract acidified with dilute sulphuric acid and the liberated pure diazelao-glycerides taken up in ether and dried.

(7) The residue obtained from this ethereal solution is used for the determination of the saponification value which is that of $GSA_2$.

(8) From the values from (5) and (7) and knowing the amount of saturated acids present, the values of $GS_2A$ and $GSA_2$ can be determined.

That only diazelao-glycerides are removed by bicarbonate washings (see 6 above), first established by Hilditch and Salatore,[10] has been confirmed by us.

By this method we have estimated $GS_2A$ and $GSA_2$ in the acidic oxidation products obtained in the trisaturated glyceride estimations[11] of the oil of *Mimusops elangi* and *Jatropha curcas*. We claim for our tentative method an accuracy within experimental errors and facility of estimation within a reasonable time.

## *Experimental*

*I. Oil of Mimusops elangi.*—80 Grams of the neutral oil were oxidised. The ethereal solution of mono-, and di-azelao-glycerides was washed with bicarbonate and water. It was then dried over anhydrous magnesium sulphate and the ether completely removed, yielding 22·8 g. of residue. 1·6396 gs. of this required 20·85 c.c. of seminormal KOH corresponding to a saponification value of 356·8.

Elangi oil contains[11] 44·25 of stearic, 54·05 of palmitic and 1·7 of behenic acid (in mols.), as its saturated acid components. The theoretical S.V. for dipalmitoazelain is 304·2 and the value when the palmitic acid content is 54·05 will be $304·2 \times 54·05/100$ or 164·4. Similarly the values for distearoazelain and dibehenoazelain are 125·1 and 4·2 respectively. The sum of the three values is 293·7 and on a similar basis the sum for the diazelains is 410·4. A saponification value of 356·8 corresponds to a mixture of 46·17% of monoazelao- and 53·83% of diazelao-compounds from which follows that the weight of the monoazelao-glyceride is $22·8 \times 46·17/100$ or 10·53 g.

From the theoretical values (for S.V.) we can calculate the ratio of mono- and diazelao-glycerides corresponding to the experimental value in a different manner. The difference between the theoretical values for the two types of glycerides is $116 \cdot 7$ and the difference between the theoretical value for the sum of the mono-azelains and experimental value for the mixture is ($356 \cdot 8$ less $293 \cdot 7$) $63 \cdot 1$. Hence the value for the diazelao-glyceride in the sample is $63 \cdot 1 \times 100 \, 116 \cdot 7$ or $54 \cdot 06$ and for the mono-azelao-glyceride $45 \cdot 94$. The residue of $22 \cdot 8$ g. is therefore composed of $10 \cdot 48$ g. of monoazelao and $12 \cdot 32$ g. of diazelao-glyceride.

A portion of the mono-, and diazelao-glyceride mixture was dissolved in ether, washed alternately with bicarbonate and water, the extract and washings combined, exhausted with ether, and the aqueous portion acidified with dilute sulphuric acid to congo-red and the liberated diazelao-glyceride, taken up in ether, dried and the ether removed. $1 \cdot 5016$ g. of the residue obtained required $22 \cdot 0$ c.c. of seminormal KOH giving a saponification value of $410 \cdot 9$ as against $410 \cdot 4$ calculated on the basis of the distribution of the saturated acids.

The whole of the above computation is based on the non-extractability of monoazelao-glycerides by bicarbonate. This was established as follows:—

21 Grams of the mixture of the two glycerides were taken up in ether and repeatedly shaken with bicarbonate and water. The combined alkaline solution was re-extracted with ether, added to the main ether solution, dried and the ether completely removed, yielding $15 \cdot 8$ g. as residue. $4 \cdot 0246$ Grams of this required $48 \cdot 9$ c.c. of seminormal KOH corresponding to a saponification value of $338 \cdot 2$. This corresponds to a mixture of $61 \cdot 95$ of mono-, and $38 \cdot 05$ of diazelao-glycerides, the weight of the monoazelao-glyceride in the mixture being $10 \cdot 49$ as against $10 \cdot 53$ obtained previously. This proves that no monoazelao-glyceride was extracted with bicarbonate.

*II. Oil of Jatropha curcas.* — 120 Grams of the neutral oil were oxidised yielding a residue of $39 \cdot 15$ g. of mono-, and diazelao-glycerides.

The saturated acids of *Jatropha curcas*[11] consists of a mixture of $5 \cdot 07 \%$ myristic, $57 \cdot 74 \%$ palmitic, $35 \cdot 88 \%$ stearic and $1 \cdot 31 \%$ arachidic acids. The saponification value for the corresponding monoazelao-glycerides is $297 \cdot 54$ and for diazelao-glycerides $413 \cdot 22$.

$12 \cdot 73$ Grams of the mixture were taken up in ether and a portion of the diazelao-glyceride extracted with bicarbonate, regenerated by acidification and re-extracted with ether yielding $2 \cdot 48$ g. of a residue. $2 \cdot 2494$ Grams

of this residue required 33·10 c.c. of seminormal KOH giving a saponification value of 413·0 as against the theoretical value 413·22.

The ethereal solution containing the mixture of glycerides was worked up yielding a residue of 10·25 g. 3·7994 G. of this required 48·7 c.c. seminormal KOH corresponding to 359·6 as its S.V. The ratio of the two glycerides would then be :—

The difference between the theoretical values for the two types of glycerides is 115·68 and that between the theoretical for the mono-azelao and experimental for the mixture is 62·06. From this the value for the diazelao-glyceride is 62·06 × 100/115·68 or 53·64 and its weight is 39·15 × 53·64/100 or 21·00 g. The weight of the monoazelao-glycerides is 18·15 g.

### Summary

The acidic portion of the oxidation products derived in the estimation of the trisaturated glycerides is made use of in experimental determination of the mono-, and diazelao-glycerides. The method consists in determining the saponification value of the mixture of the two and that of the diazelao-glyceride which can be extracted free from the monazelao-glyceride.

### REFERENCES

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12. Calculated from the molar composition of the glycerides as given in Ref. 8.