Determination of the Glyceride Structure of Fats

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A method has been described for the quantitative determination of the following six glyceride types in fats: SBS, SSU, SDS, SOT, USU, and UDU. The method involved a quantitative oxidation of the unsaturated acids in the whole fat to the corresponding dicarboxylic acids. The oxidized fat was separated on a liquid-liquid partition column into two fractions, the first containing glycerides having no dicarboxylic acid or one dicarboxylic acid and the second containing glycerides with two or three dicarboxylic acids. Analysis of these fractions by gas chromatography coupled with lipase hydrolysis allowed the calculation of the proportions of the above six glyceride types.

The oxidation, fractionation, lipase hydrolysis, gas chromatographic analysis, and the over-all method were checked on natural fats and mixtures of synthetic glycerides. The final glyceride composition appeared to be reliable to within plus or minus 2 unit per cent.

Analyses are given for five natural fats. The compositions found agree very well with those calculated by a distribution theory recently proposed by Vander Wal.
A small number of fatty acids gives rise to a comparatively large number of combinations as glycerides, and there is little difference in physical properties between adjacent glycerides of the series. As a result, the quantitative separation of the individual glycerides is extremely difficult, if not impossible. The problem can be simplified, at the expense of obtaining less information, by an oxidative cleavage of the unsaturated acids to form a free acid group at the position of the double bond. If the only distinction then made is between mono- and dicarboxylic acids, corresponding to saturated and unsaturated acids, the number of combinations as glycerides is limited to the following six: SSS, SSU, SUS, SUU, USU, and UUU. No distinction is made between the optical isomers. The oxidative cleavage also has the advantage of amplifying the difference between the various glyceride types, giving molecules with zero to three free acid groups corresponding to the four glyceride types S3, S2U, SU2, and U3.

This general method was first used by Hilditch and Lea (1), who determined the amount of fully saturated glycerides, S3, as the neutral fraction recovered after oxidation. Kartha (2,3) in a later study showed that the oxidation with potassium permanganate in acetone, used by Hilditch and Lea, resulted in partial hydrolysis of the glycerides. He avoided this hydrolysis by the addition of acetic acid to the acetone and extended the method by fractionating the oxidized glycerides as their magnesium salts. Since neither the oxidation nor fractionation were entirely quantitative by the addition of acetic acid to the acetone and results for a number of fats are summarized in Table I, and results for a number of fats are summarized in Table I. Peaks representing the unsaturated acids disaggregated completely on oxidation, and dicarboxylic acids were formed in corresponding molar amounts. Possible hydrolysis of the glycerides during oxidation was also checked by using gas chromatography. A known proportion of an acid, such as laurie, not present in a fat, was added to a sample of the oxidized fat, and the mixture was treated with diazomethane. The mixture was run on the chromatograph under conditions such that only the methyl esters

![Fig. 1. Gas chromatographic charts for original and oxidized lard.](image-url)