scribed, with a diamond-point, with twenty parallel and closely-spaced lines forming a strip 2.5 mm wide. The amount of material separated was minute (about half a microgram), and examination with a magnifier and coloured filters was necessary to observe the coloured zones. Activation of the glass was carried out in the same way as that of the sintered layers.

Sintered layer plates might also be used as support for a stationary phase in partition separations, with some advantages over analogous paper chromatography.

Commonwealth Laboratory, Customs House,
11 William Street, Melbourne 3000 (Australia)

IAN S. TAYLOR

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Quantitative determination of saturated triglycerides in fats

During the partial catalytic hydrogenation of edible oils, trisaturated glycerides are formed in varying amounts. The extent of formation of these high melting glycerides is not rules by chance, but governed by the hydrogenation conditions. In order to study the parameters which influence their formation there was needed a practically suitable and quantitatively reliable method for the determination of these glycerides. The method described below, based on a TLC–GLC procedure, has been shown to fulfil these requirements.

The classical method is based on a wet oxidation procedure with $KMnO_4$ originally described by HILDITCH AND LEA[1] and later modified by others[2,3] and recently reviewed by CHAKRABARTHY AND GAYEN[4]. The procedure is rather time consuming and the precision and accuracy may, no doubt, be questioned depending on the quantitatively unreliable reactions and manipulations involved. ESHELMAN AND HAMMOND[5] have critically studied the method and conclude that it does not give satisfactory results. Recently another method has been published[6], based upon mercury adduct formation, separation of the non-adduct-forming saturated glycerides and subsequent gravimetric determination.

The present procedure is based upon thin-layer separation of the saturated glycerides on silver nitrate–silica gel coated plates followed by gas chromatographic analysis of the saturated triglyceride fraction after its conversion to methyl esters. The quantification is accomplished with the aid of an internal standard consisting of a suitable saturated triglyceride, which is added to the sample *before* the thin-layer chromatographic procedure. The standard thus accompanies the sample throughout the analysis.

EXPERIMENTAL

Thin-layer chromatography. Glass plates, 20 × 20 cm, coated with Silica Gel G (Merck AG, Darmstadt, Germany) containing 12.5% $AgNO_3$, 0.5 mm in thickness. About 25–50 mg of the sample was dissolved in a chloroform solution containing a suitable (see below) and known amount of the standard (glycerol triheptadecanoate, Hormel Institute, Austin, Minn., U.S.A.). The solution was applied on the starting line of the plate as a streak together with two spots of the reference mixture. The plate was eluted with chloroform until the solvent front traversed about 15cm. After drying, the chromatoplate was visualized with 2',7'-dichlorofluorescein. The saturated triglyceride zone was scraped off the plate and extracted with *n*-hexane–diethyl ether (1:1). The solvent was driven off with the aid of nitrogen, and the remaining glycerides transesterified according to a modified method originally described by GLASS AND JENNESS[7].

Gas chromatography:

Instrument: Varian Aerograph 1200.

Column: 6 ft. × $^1/_8$ in. stainless steel, packed with 10% DEGS on Anakrom ABS 70–80 mesh.

Temperature: injector, 270°; column, 175°; detector, 210°.

Area measurement: disc integration.

Calculation. From the percentage of the methyl esters the ratio methyl heptadecanoate/Σ(saturated methyl esters) was calculated. Based upon the known amount of glycerol triheptadecanoate added to the sample, the total amount and percentage of saturated triglycerides originally present in sample could then be calculated.

Results and discussion

From a number of determinations of hydrogenated vegetable oil (softening point*, 38–40°) a mean value of 16.7% saturated triglycerides was found with a standard deviation of 1.7%. By allowing the standard to accompany the sample during the whole procedure, the quantitative reproducibility of the thin-layer procedure is not critical in this respect as long as the ratio of the standard and the sum of saturated glycerides remains constant. The accuracy was further investigated in recovery tests by adding known amounts of a secondary standard. The determining factor for the reliability of the whole analysis is thus primarily the GLC procedure. Taking into consideration the generally accepted precision of the gas chromatographic analysis reported by HORNING *et al.*[8], among others, the above mentioned deviation seems reasonable, since the individual GLC percentage error is roughly doubled in the following ratio calculation. In order to achieve the highest possible precision and accuracy the amount of standard must be chosen in such a way that the ratio falls in the 0.25–4 range, since low percentages of either standard or sample increase the relative error of the GLC analysis, which very considerably influences the ratio and subsequently the precision and accuracy of the total analysis.

Owing to the presence of the standard the quantitative removal of the saturated triglyceride zone is not critical. The contamination of unsaturated glycerides should, however, not be overlooked. Such complications are obviously more frequent in the case of hydrogenated fats, owing to the presence of positional and geometrical isomers. *Trans* double bonds for instance are not so firmly bound to $AgNO_3$ compared to the

corresponding *cis* bonds. In those cases where contamination occurred, which could be seen by the presence of unsaturated fatty methyl esters, the percentage of the sum of saturated fatty esters was corrected by substracting twice the percentage of the unsaturated esters from the above mentioned sum with the assumption that the contaminating glycerides were mono-unsaturated. The empirically corrected values were in agreement with those values obtained without any observed contamination. The general validity of the correction has, however, not been investigated.

Research Laboratory, U. PERSMARK
AB Karlshamns Oljefabriker, B. TÖREGÅRD
Karlshamn (Sweden)

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Estimation of trisaturated glycerides in fats by argentation thin-layer chromatography

The classical methods for the estimation of trisaturated glycerides(GS_3) in fats originally developed by HILDITCH[1] and later modified by KARTHA[2] and VON RUDLOFF[3] were essentially based on the oxidation of the unsaturated portion(GU) of the mixed glycerides. In recent years these methods have largely been replaced by modern chromatographic techniques such as column[4], thin-layer[5] and gas–liquid[6] chromatography, by which rapid and accurate determination of triglyceride composition has been achieved.

For the evaluation of mixed glycerides the application of argentation thin-layer chromatography, initially developed by DE VRIES[5], has been well established. This method is based on the separation of mixed glycerides into components according to the degree of unsaturation, for example GS_3, GS_2U, GSU_2 and GU_3, and also according to the isomeric configuration of unsaturated glyceride molecules. Taking advantage of this principle, quantitative methods for the analyses of both natural and synthetic triglycerides have been developed. Thus BARRETT *et al.*[7] have separated mixed glycerides of a number of natural fats on a silver nitrate-impregnated silica gel plate using chloroform–acetic acid–ethanol as solvents. They subsequently estimated the