Letters to the Editor

Glyceride Analysis of Commercial Fats by Lipase Hydrolysis

Sir: The fatty acid distribution theory proposed almost simultaneously by R. Van der Waal (1) and by Coleman and Fulton (2) has enabled convenient and reasonably accurate estimations of the triglyceride compositions of most natural vegetable fats. The theory's utility lies mainly in the fact that it can be used to interpret data on the composition of the fatty acids at the glyceride 2-position, which is easily obtained by the lipase hydrolysis technique also developed in the early 1960's (3,4). Determination of the fatty acids at the glyceride 2-position, deduction of the composition of the acids at the 1- and 3-positions, and use of the 1,3-random-2-random distribution theory to calculate the triglyceride composition of a natural oil has led to a much greater understanding of oil compositions, and the validity of the approach has been confirmed in numerous papers and publications (5, 6, 7, 8, 9).

Unfortunately, these papers deal exclusively with whole, natural oils and fats, and scarcely mention commercial fats. In few of the papers is the term "natural fat" explained, and readers may at times be led to think that the theory should apply to all oils and fats of natural origin, that is, not only to pure fats, but also to blends, fractions, or even hydrogenated natural fats. Unfortunately, however, the theory cannot be applied to fat blends or fractions, but views within the industry have led us to think that some misunderstandings do occur.

The lipase hydrolysis technique may, of course, be applied to all fats, and the results obtained for the composition of the fatty acids at the glyceride 2-position are truly accurate within the limits of the experimental procedure and the lipase specificity. It is also quite valid to compare these results with the overall fatty acid composition, and to so deduce the composition of the acids distributed at the 1- and 3-positions in the normal manner (2). It is the application of the 1,3-random-2-random distribution law to these results in order to estimate the triglyceride composition which can lead to errors. The nature of these errors may be demonstrated by reference to a few simple examples. A mid-fraction may be separated from palm oil, e.g., by solvent fractionation, which would contain the glycerides POP and PPO. If suitably prepared it would be entirely free of any trisaturated glyceride such as tripalmitin. Lipase hydrolysis would reveal the existence of, say, 10-20% of palmitic acid at the glyceride 2-position, while comparison with the overall fatty acid composition would show that the composition at the 1-, and 3-positions comprised over 60% palmitic acid. Application of the Van de Waal theory would, therefore, show the presence of 5 to 10% tripalmatin, a result quite at variance with the facts.

Fully hydrogenated linseed oil, containing mainly tristearine, may be blended with olive oil and would be shown to contain glycerides such as SOS, SSO, or SOO by the invalid application of the theory. Alternatively, a blend of high erucic rapeseed oil, which contains erucic acid (Er) exclusively at the 1-, and 3-positions, with, say, lard, which has palmitic acid (P) at the 2-position, would be shown to contain the glyceride ErPEr, again at variance with the facts.

Reasons for potential confusion are to be found in the literature. For instance, the early paper by Coleman and Fulton (2) reports work on the positional specificity of pancreatic lipase and the application of the 1,3-random-2-random distribution theory to several vegetable and animal fats of natural origin. These fats include cocoa butter, palm oil, lard, etc. The triglyceride compositions were determined by an alternative procedure, and the validity of the rule was confirmed. Where the paper may confuse is that it goes on to discuss the application of the law to *fractions* separated from shea oil, and to interesterified lard. It is reported that the theory gives acceptable results in these two cases. Careful consideration will show that the law will, in fact, give accurate results for an interesterified, or truly random fat, as has been reported again quite recently (10). However, it is purely fortuitous that acceptable results were obtained for the sheanut oil *fractions*, a coincidence arising from the very simple glyceride structure of the oil where the glycerides SOS, SOO and OOO accounted for 87% of the total in the analysis (2) of the whole oil.

Other early papers (5, 6, 7) discuss the validity of the theory to "natural" fats, but do not explain exactly what is meant by "natural". A paper by Jurriens (8) describes the substantiation of the theory by experiments in which the fats are separated into fractions by TLC over $SiO_2/AgNO_3$. The fractions are then split by the lipase hydrolysis technique. The composition of the 2-position acids and the overall fatty acid compositions of the fractions are used to deduce the triglyceride composition of each fraction, and thus the oil. The authors do not, however, make it clear that the 1,3-random-2-random distribution theory is inapplicable to these fractions.

Carter Litchfield in his excellent book on triglyceride analysis (11) discusses the validity of the 1,3-random-2-random hypothesis and draws attention to the fact that it is only applicable to natural fats of "homogeneous origin." He exemplifies potential wrong application by reference to palm fruit, the oil from the whole of which would be a blend of palm oil and palm kernel oil. The hypothesis would give totally wrong results. However, there is no mention of fat fractions or hydrogenated fats, and a reader is left to ponder the validity of the theory to fractionated or hydrogenated versions of natural fats of homogeneous origin.

The problems of incorrect use of the Van der Waal theory are at the moment becoming more serious with current moves towards harmonization of national laws concerning the composition of commercial chocolate fats. Many more laboratories are becoming involved in the chemical analysis of fats intended for use in chocolate, and results are being discussed and quoted in a variety of meetings.

Commercial fats sold for use in chocolate are normally blends of natural fats, and many contain triglyceride fractions derived from natural fats. The fats are often, quite properly, described as "fats of natural origin." We would, however, like to emphasize that the 1,3-random-2-random distribution theory should not be used in the evaluation of lipase hydrolysis results with these fats, as this will almost certainly lead to the reporting of incorrect triglyceride compositions. Discussion of any such results in international meetings could lead delegates to draw inaccurate conclusions and make inappropriate recommendations. This could have unfortunate long term effects on the understanding of

the problem, and ultimately on world trade.

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Glyceride Composition by Lipolysis

Sir: A recent article in the Journal (A. Sungupta and S.P. Basu, 55:533 1978) prompts me to draw the attention of your readers to a common error in the interpretation of results obtained by lipolysis of glycerides using pancreatic lipase.

By careful experimentation it is possible to determine the component acids present in natural mixture of triglycerides and in the 2-monoacylglycerols derived from them by lipolysis. By simple calculation these provide the composition of fatty acids in the 2-position and in the combined 1- and 3-positions, but how can they yield further information about glyceride composition? The method suggested independently by Vander Wal (1) and by Coleman (2) is generally employed. This involves two assumptions: (a.) that the fatty acids attached to the 1- and 3-positions are the same, and (b.) that the fatty acids occupying the 1-, 2-, and 3-positions are associated with each other in a random manner. These assumptions may or may not be valid for natural glyceride mixtures but *the second cannot be true for natural glyceride mixtures which have been segregated into fractions on the basis of some physical property.*

The many seed oils with saturated (S), oleic (O), and linoleic (L) acids, for example, could contain SSS, SSO, SOO, SSL, OOO, SOL, OOL, SLL, OLL, and LLL triacylglycerols where these symbols indicate merely the acyl groups present and not their position. Silver ion chromatography produces bands in this order with SSS having the highest R_f and LLL the lowest (3). Whilst it is difficult to get complete separation leading to glycerides of a single type, it is to be expected that there will be useful concentration of each glyceride category, and separated fractions will no longer contain all the triglycerides present in the original mixture. The Vander Wal-Coleman assumptions are then no longer valid.

Low temperature crystallization is based on the solubility of glycerides and indirectly on their degree of unsaturation. Even though we know less about the relative solubility of the different glyceride categories, it is still true that glycerides of a particular kind are concentrated in each fraction, that each fraction is more homogeneous (less random) than the original mixture, and that the Vander Wal-Coleman procedure cannot be validly applied to the segregated fractions.

The results of Sengupta and Basu are complicated by the wide range of saturated acids (C_{14}-C_{24}). Consider, however, fraction B (11.8% mol) which contain 14:0 (5.4%), 16.0 (19.6%), 18.0 (2.9%), 22:0 (1.2%). 18:1 (32.7%), 18:2 (35.3%), and 18:3 (2.9%) and has the highest iodine value of all fractions. This fraction is likely to concentrate the SU_2 glycerides along with U_3 glycerides and to contain less of the S_2U and S_3 glycerides which will predominate in the less soluble fractions of lower iondine value. Since crystallization should produce fractions of increased homogeneity, this fraction probably contains SOL as its major conponent rather than mixtures of SOO and SLL or of S_2U and U_3, each of which should have been separable. The calculations of Sengupta and Basu, using the Vander Wal-Coleman procedure, indicate the presence of SSS (1.2%), SSO and SSL (18.1%), SOO, SOL, and SLL (47.1%), and OOO, OOL, OLL, and LLL (33.6%). It seems likely that the content of S_3, S_2U, and U_3 glycerides will be lower than this and that of SU_2 will be correspondingly higher.

I hope, sir, that your authors and referees will be alerted to this error and that we shall see less of it in the future.

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