and C_{13} DBA were present only in a few particular species. Many show significant amounts of C_8 DBA. C_6 DBA is absent throughout while C_7 is present in traces in some instances. As a rule, three or more major (above 10%) components are present. C_8, C_9, C_{10} and C_{11} are the most favored positions for locating the first double bond in C_{18} unsaturated acids. (c) Wide variability of first double bond site distributions exists in fats from different biological species. Large variability exists in the case of varietal alterations in the same biological species, e.g., different varieties of peanut and sesamum, and also in the case of different specimens of fat from the same biological variety, e.g., fats from black seeded varieties of sesamum from different geographical areas. (d) In fats of palmitic-stearic-oleic-linoleic type, quantitative study of first double bond site distributions has so far been reported only for three specimens (6) and the results showed almost exclusive location of the first double bond site at 9:10 position just as in the case of purified esters examined earlier by acetone-permanganate oxidation (4,5). Permanganate oxidation at alkaline pH used in these earlier studies produces considerable overoxidation of mono and dibasic acids (3) and the number of samples studied is far too small for generalization. Apart from these there seems to be some possibility of double bond migrations also in some oxidation procedures. The reactions leading to the final rupture of double bonds in permanganate oxidations are not clearly known but the fact that overoxidation takes place mainly at the time of initial scission of double bonds in permanganate oxidations at alkaline pH (3) indicates that some of the intermediate stage complexes are more readily overoxidized than the final DBA produced. It is possible that some of these complexes are quite labile and may also produce double bond migration when favorable conditions exist. Migration of double bonds caused by alkali (high pH) and metallic catalysts at high temperatures are well known. In acetone-permanganate oxidation (4) the K_2O liberated when $KMnO_4$ is reduced can produce a high pH which may cause double bond migration in the intermediate complexes and the similarity of results in the case of periodate-permanganate oxidations (6) indicates the presence of the same or similar favorable factors in the latter procedure also. In acetic acid-acetone-permanganate, the K_2O is liberated into a medium containing excess acetic acid and hence no increase in pH can take place. Double bond migrations taking place at high pH will hence be eliminated and the DBA generated will correspond to the original position of the double bonds.

A. R. S. KARTHA
Y. SELVARAJ
Division of Chemistry
Indian Agricultural Research Institute
New Delhi, India

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A Standardized Procedure for Oxidative Determination of Fully Saturated Glycerides in Natural Fats

Abstract

Isolation of fully saturated glycerides from oxidation products of fats with K_2CO_3 solution is subject to error from hydrolysis of mono- and diazelains to di- and monoglycerides respectively. Treatment of azelaoglycerides with saturated sodium bicarbonate solution does not produce detectable hydrolysis of mono- and diazelains and the bicarbonate insoluble fractions do not contain detectable amounts of diazelains by the sensitive insoluble calcium salt test. Procedures are available for comparison of saturated acid distributions in fully saturated glyceride and monoazelain fractions. On the basis of the above the fully saturated glycerides present in oxidation products from fats can now be accurately estimated.

Errors from autoxidation and polymerization of unsaturated glycerides during processing are absent in oxidative determinations of glyceride structure. Fats are quantitatively converted by acetic acid-acetone-permanganate (1,2) to corresponding mixtures of GS_3, GS_2A, GSA_2 and GA_3 (G, glyceryl; S, U and A, saturated, unsaturated and azelaic acid radicals or acids, respectively; Sm for proportions of S present, molecules %). The usual procedure for isolation of GS_3 from the above mixture consists in washing a diethyl ether (ether) solution with 5% aqueous K_2CO_3 solution (3), but for reasons recorded earlier (1,4) this has been made use of only for lower saturated acid fats from various *Myristicacae* species (4–6) and from *Areca catechu* (7). The following standardizations show that the above procedure is subject to error from hydrolysis of GS_2A and GSA_2 to neutral products if contact with the carbonate solution is prolonged.

(a) Two grams of pure GS_2A prepared from *Garcinia cambogia* seed fat (8), are shaken in 1% ether solution with 120 ml of 5% K_2CO_3 (3 g K_2CO_3/g GS_2A) at 22–26 C for 20 hr. The reaction products are then recovered by extraction with ether, after acidification, and submitted to the azelaoglyceride separation (1,2) to remove dibasic acids liberated during the reaction. The azelaoglycerides recovered from the insoluble magnesium salts in the azelaoglyceride separation, (designated insoluble azelaoglycerides" or IAG) (1,2), amounted to only 97% against 100% recovery in blank runs in

which K_2CO_3 was omitted. The insoluble azelaoglycerides did not show the presence of any free higher saturated acids when tested by a procedure to be described in the sequel; the reaction hence consisted in selective removal of dibasic acids to give diglycerides.

(b) When an azelaoglyceride mixture consisting of approximately 28% GS_3, 56% GS_2A and 16% GSA_2 (obtained from a sheep depot fat) was submitted to the same procedure as above, the insoluble azelaoglycerides amounted to 94% only and no combined S was present in the filtrates of soluble magnesium salts in the azelaoglyceride separation. This showed that GSA_2 is hydrolyzed much more readily than GS_2A.

(c) When 1 g of pure GS_2A is heated with 60 ml of 5% K_2CO_3 solution at 90–95 C for 10 hr and products worked up as before, the insoluble azelaoglycerides amounted to 76% only as against 72% Bertram acids obtained after complete hydrolysis with alcoholic KOH (1).

In the procedure for quantitative removal of GSA_2 from insoluble azelaoglycerides by treatment with saturated sodium bicarbonate solution (8), the bicarbonate insoluble fraction of the insoluble azelaoglycerides (designated BIF IAG) will not contain any more than traces of GSA_2 at most. The proportions of GS_3 present in this fraction can be accurately calculated from its saturated acid content if it can be shown that: (a) removal of GSA_2 is complete; (b) there is no detectable hydrolysis of GS_2A and GSA_2 during processing; and (c) if a procedure is available for comparing the saturated acid distributions in GS_3 and GS_2A fractions.

Proof of absence of GSA_2 in the bicarbonate insoluble fraction of the insoluble azelaoglycerides has been obtained in the following way. It is believed that dry calcium salts of GS_2A, GSA_2, GA_3 as well as saturated fatty acids are insoluble in ethyl acetate (9). However investigations using high purity GS_2A have shown that calcium salts of pure GS_2A are fairly soluble in cold and freely soluble in boiling ethyl acetate. In contrast, the calcium salts of GSA_2, GA_3 and saturated fatty acids are entirely insoluble in the hot solvent. When pure GS_2A containing 2–3% of added GSA_2 or S is submitted to calcium salt-ethyl acetate separation, the soluble salts consist exclusively of GS_2A but the insoluble fraction (which may amount to 200–300% of added GSA_2 or S) is a mixture of GSA_2 or S with considerable amounts of GS_2A. This is obviously caused by double salt precipitation due to the divalency of calcium. Therefore if the bicarbonate insoluble fraction of insoluble azelaoglycerides gives calcium salts entirely soluble in boiling ethyl acetate, it cannot contain any GSA_2. The above fraction as obtained from depot fats of pig, cow, buffalo, sheep and goat, and also from seed fats from *Garcinia indica, Vateria indica, G. cambogia,* etc., earlier reported (8), were all submitted to this test and all of them gave dry calcium salts completely soluble in boiling ethyl acetate.

When the acids in the ethyl acetate insoluble fraction obtained in the calcium salt separation of saturated acid plus GS_2A mixture are regenerated and submitted to the same operation a second time, almost pure calcium soaps of saturated acids are obtained and as little as 1% of added saturated acids can be readily estimated by this procedure. This was the technique used for testing the presence of free saturated acids in the insoluble azelaoglycerides obtained in the standardization of GS_3 isolation with aqueous K_2CO_3 described earlier, and no ethyl acetate insoluble calcium salts could be obtained from the latter. This procedure was essential because it was found that when pure GS_2A in alcohol solution is titrated in the cold with aqueous or alcoholic KOH, the acid values observed are 10% or more higher than theoretically. Investigation of the titration products on lines similar to that used for standardization of GS_3 isolation showed that the reaction taking place was entirely limited to selective splitting off of the dibasic acid.

Absence of hydrolysis of GS_2A during bicarbonate separation (8) is proved as follows. One gram of pure GS_2A in 1% of ether solution was shaken with saturated, neutral to phenolphthalein, sodium bicarbonate solution (6 g bicarbonate in 75 ml water) in a separating funnel for 28 hr as against the 4–6 hr usually required for the bicarbonate separation of insoluble azelaoglycerides (8). The reaction products were worked up as earlier described for standardization of GS_3 isolation. The insoluble azelaoglycerides in this case amounted to 99.5% to 100% of starting material establishing absence of hydrolysis of GS_2A during processing.

The insoluble azelaoglycerides obtained from GS_3 nil vegetable fats containing above 40% of S with mean MW above 270 do not give any soluble azelaoglyceride fraction when resubmitted to the azelaoglyceride separation and such GS_2A + GSA_2 mixtures may contain up to 40% of GSA_2. They can be standardized for hydrolysis of GSA_2 during bicarbonate separation just like pure GS_2A now described. The insoluble azelaoglycerides from mowrah oil and *Sarcostigma kleinii* seed fat containing about 30–40% of GSA_2 were tested by this technique and were quantitatively recovered after 28 hr treatment establishing absence of hydrolysis of GSA_2 as well during processing.

Comparison of saturated acid distributions in GS_3 and GS_2A fractions in different types of natural fats may be carried out as follows. In C_{16}–C_{18} acid fats containing significant amounts of GS_3, the latter can be almost quantitatively isolated by single stage crystallization (10) and the S from this can be compared with the S in the bicarbonate insoluble fraction of insoluble azelaoglycerides which will be a mixture of GS_3 and GS_2A. In case of depot fats of cow, buffalo, goat and sheep the mean MW of the S from the two fractions did not differ by more than 1–2 units, the usual limits of experimental error. This technique is not applicable to lower saturated acid fats. However, in the case of these fats, a considerable amount of GS_2A can be obtained in a pure condition when the bicarbonate insoluble fraction of insoluble azelaoglycerides is washed in ether solution with large excess of dilute ammonia. The S in the pure GS_2A fraction can then be compared with the S in the balance GS_3 + GS_2A fraction. In the case of four specimens of arecanut fats and several of *Myristica malabarica* seed fats this comparison did not show any difference of S distributions.

The accuracy of GS_3 determination by the present technique is as follows. Duplicate GS_3 values on a buffalo depot fat (higher saturated acid fat) of Sm 68.9 were 28.8% and 27.7%, and on an arecanut fat (lower saturated acid fat), of Sm 81.2 were 58.2% and 58.7%. Addition of pure GS_3 (5–10%) in the form of peanut oil hydrogenated to zero iodine value, to the original fats, or to the oxidation products, or to the bicarbonate insoluble fraction of the insoluble azelaoglycerides could be estimated to $\pm 0.6\%$ by the present procedure. If nonglyceride matter

cannot be estimated according to known procedures (2,11) and is present in the final fraction analyzed for GS_3 content, then the GS_3 values may be subject to some error depending on the quantities of such materials present.

The glyceride structures of a large number of animal depot and lower saturated acid vegetable seed fats have been worked out by the present technique and the results are being communicated separately.

A. R. S. KARTHA
Division of Chemistry
Indian Agricultural
Research Institute
New Delhi, India

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