# First Double Bond Site Distributions in 16 Vegetable and Six Animal Reserve Fats

**Abstract**

The dibasic acids corresponding to the first double bond site in 16 vegetable and 6 animal fats were obtained by standardized procedures, fractionated by liquid-liquid countercurrent extraction and individual fractions analyzed. Results show that the site of the first double bond is not restricted exclusively or even predominantly to any single position but is spread over three or more positions in major proportions. First double bond site distributions show large differences in different biological species, different varieties from the same species and also different specimens from the same variety. Possible migration of double bonds in permanganate oxidation at alkaline pH is discussed.

Acetic acid-acetone-permanganate oxidation (1) does not produce overoxidation of monobasic acids (2), half esters of dibasic acids or azelaoglycerides (3). Dibasic acids (DBA) corresponding to the first double bond site in natural fats (3) can hence be isolated unchanged by oxidation of fats or mixed acid esters, but not of the free mixed acids, by this procedure. A preliminary report of the first double bond site distributions in 16 vegetable and 6 animal fats by this technique is presented.

The experimental procedure is given in some detail since the results now obtained are somewhat different from those reported in the literature using other techniques (4–6). The fats (5–7 g) were oxidized (1) using 10 g permanganate per gram, the products isolated with diethyl ether and heated in an open dish on a water bath to remove volatile acids untill loss per hour was reduced to 2–3 mg. Residue was hydrolyzed with alcoholic KOH and the liberated mixture of mono and dibasic acids exhaustively extracted with ether and submitted to hexane water partitioning (7) to isolate the DBA. The filtered aqueous solution when evaporated to dryness gave the total DBA. With *Brassica campestris* seed fat, some of the higher DBA was left in the hexane; this was recovered by removing the solvent and separating the mono and dibasic acids with boiling water. The additional fraction thus obtained was added to the DBA obtained in the usual way.

The dibasic acids were fractionated by the classical macro liquid-liquid countercurrent extraction using ether and water (double distilled, from pyrex glass). Seven separating funnels (I to VII) were used and the volume of ether was kept constant at 25 ml in I and II, 50 ml in III, 100 ml in IV and V, and 200 ml in VI and VII. DBA (200 mg) was introduced into I and, after addition of a known portion of water, was equilibrated by brisk shaking for 2 min, settled, the aqueous portion drawn into II, equilibrated as before, settled and drawn into III and so on till it was drawn out from VII. For palmitic-stearic-oleic-linoleic type of fats the first three 25 ml portions of aqueous extract formed water soluble fraction 1($W_1$); the next three 150 ml portions formed $W_2$ and the last five 150 ml portions formed $W_3$. The ether solutions left in the separating funnels formed the different ether fractions $E_1$ to $E_7$. The solvents were removed to constant weight from all 10 fractions and the acids titrated against KOH (0.01 N). Fraction $W_1$ usually contained some water soluble neutral matter and the acids in this fraction were taken to be the same as in $W_2$. Fraction $E_1$ is likely to contain water-insoluble nondibasic acid impurities and was therefore submitted to a second hexane water partitioning and weight and equivalent weight of the water soluble fraction were used for the calculations. Boiling water separation was used for $E_1$ from *Brassica campestris* seed fat. All fractions were calculated as binary mixtures and the rounded figures are given in Table I. The total weight of the final fractions was lower than the weight of DBA started by 3% to 6%. Figures for individual acids are reproducible to ±2% as in the example of *Bassia longifolia* seed fat (Table I). Figures for highest and lowest components are minimal; those for intermediate members are subject to some more fluctuations.

The results show the following general trends: (a) If only a single dibasic acid is produced, analysis should show at least 95% of the acid concerned and there should be little difference in the equivalent weight of the 10 different fractions. This feature was not shown by any fat studied so far. (b) Azelaic acid was present in considerable proportions in all cases and in predominant amounts in a few cases. $C_{10}$ or $C_{11}$ DBA or both were shown in considerable, but highly variable proportions, by most of the fats. $C_{12}$

TABLE I

Composition by Weight of Dibasic Acids Corresponding to First Double Bond Site Isolated From Some Natural Fats

| No. | Fat | Chainlength of dibasic acid | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $C_7$ | $C_8$ | $C_9$ | $C_{10}$ | $C_{11}$ | $C_{12}$ | $C_{13}$ |
| Vegetable fats | | | | | | | | |
| 1 | *Bassia longifolia* (a) | 4 | 24 | 63 | 9 | .... | .... | .... |
| | (b) | 5 | 20 | 66 | 9 | .... | .... | .... |
| | (mean) | 5 | 22 | 64 | 9 | .... | .... | .... |
| 2 | *Carthamus tinctorius* | 2 | 48 | 41 | 9 | .... | .... | .... |
| 3 | *Azadirachta indica* | 1 | 28 | 56 | 15 | .... | .... | .... |
| 4 | *Olea europea* | 1 | 32 | 44 | 13 | 10 | .... | .... |
| 5 | *Linum usitattissimum* | .. | 7 | 54 | 26 | 13 | .... | .... |
| 6 | *Sarcostigma kleinii* | .. | 13 | 33 | 25 | 3 | 19 | 7 |
| 7 | *Brassica campestris* (a tetra ploid variety) | .. | 5 | 20 | 9 | 11 | 7 | 48 |
| 8 | Peanut var. TMV 1 | .. | 15 | 59 | 12 | 13 | 1 | .... |
| 9 | Peanut var. TMV 2 | 2 | 32 | 51 | 6 | 7 | 2 | .... |
| 10 | Peanut var. TMV 3 | 4 | 44 | 33 | 18 | 1 | .... | .... |
| 11 | Peanut var. TMV 6 | 1 | 13 | 55 | 23 | 8 | .... | .... |
| 12 | *Sesamum indicum*, black, Delhi | .. | 23 | 48 | 15 | 11 | 3 | .... |
| 13 | *Sesamum indicum*, black, Coimbatore | .. | 3 | 43 | 33 | 18 | 3 | .... |
| 14 | *Sesamum indicum*, black, Trichur | .. | 3 | 53 | 21 | 22 | 1 | .... |
| 15 | *Sesamum indicum*, TMV 1 (red) | .. | 15 | 35 | 25 | 24 | 1 | .... |
| 16 | *Sesamum indicum*, TMV 2 (white) | .. | .... | 27 | 52 | 21 | .... | .... |
| Animal fats | | | | | | | | |
| 17 | Beef tallow, South Indian | .. | 1 | 33 | 37 | 21 | 8 | .... |
| 18 | Pig depot fat, Indian | .. | 23 | 38 | 15 | 21 | 3 | .... |
| 19 | Goat tallow, Delhi | 1 | 32 | 39 | 19 | 9 | .... | .... |
| 20 | Chicken fat, Delhi | .. | 26 | 52 | 15 | 7 | .... | .... |
| 21 | Seengala, fresh water fish of *Mystus* species, Delhi | 6 | 12 | 35 | 14 | 23 | 10 | .... |
| 22 | Shark liver oil (Salt water fish) | 1 | 23 | 23 | 41 | 12 | .... | .... |

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.

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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