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Fractionation of Sesame and Safflower Oil Fatty Acids with Urea

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THE FORMATION of urea complexes with straight-chain organic compounds was first discovered by Bengen (3). The stability of these complexes varies greatly according to the type of compound complexed. This phenomenon makes it possible to achieve separation of certain organic compounds by varying the temperature, urea concentration, and solvent concentration.

Straight-chain compounds form much more stable adducts with urea than do cyclic or branched-chain compounds. Bengen (3), Rudloff (11), and Mehta and Sharma (9) were able to obtain fairly satisfactory separations of straight-chain compounds from cyclic and branched-chain compounds.

Recent investigations (1, 8, 10, 12, 13, 14) have shown that it is possible to separate mixtures of fatty acids or methyl esters of fatty acids on the basis of their degree of unsaturation. These studies have shown that the stabilities of urea adducts decrease as their degree of unsaturation increases.

This investigation was conducted to study the fractionation and separation of fatty acids according to their degree of unsaturation. Both the methyl esters and mixed fatty acids of sesame and safflower oils have been used. A comparison is made between two different methods of fractionation. In one method increasing increments of urea are added to the fatty acid-solvent systems to obtain increasingly unstable urea adducts. In the other method the mixed fatty acids are completely converted into urea adducts and eluted with varying amounts of solvent. By this method the most unstable components are eluted first while additional increments of solvent allow the more stable components to be progressively eluted.

Experimental

Preparation of Methyl Esters. Methyl esters of sesame (I.V. −106.4, S.V. −188.6) and safflower (I.V. −146.0, S.V. −190.4) oils were prepared by interesterification of the oils and anhydrous methanol by the method of Bradley and Johnston (4), using potassium hydroxide as the catalyst. The esterified product was then washed with acidulated ice-cold distilled water and extracted with ethyl ether. The extract was washed free of mineral acid and dried over anhydrous sodium sulfate, and the ether was removed under vacuum.

Preparation of Mixed Fatty Acids. The mixed fatty acids of sesame and safflower oils were prepared by the method of Hilditch (7).

Fractionation by the Urea Adduct Elution Method. The general procedure followed for this method was to dissolve the methyl esters or fatty acids in a mixture of powdered urea and 95% ethanol. The mixture was heated and kept over-night at room temperature. The adduct obtained was filtered through a sintered glass funnel by suction. An additional amount of ethanol was added to the adduct, and the filtration procedure was repeated. This elution technique was repeated a number of times. Each of the filtrates thereby obtained was concentrated by removing the ethanol under reduced pressure. The residue was treated with acidulated distilled water, washed free of mineral acids, and extracted with ether. The ether extracts were dried over anhydrous sodium sulfate and evaporated to remove the ether. Each of these fractions was analyzed for iodine and saponification values.

The fatty acid composition of each fraction was calculated from the iodine and saponification values by assuming the fractions to be binary mixtures. This assumption has also been made by other investigators (2, 5, 6, 7) in calculating the fatty acid composition of various fats. The mean molecular weight of the

TABLE I

Fractionation of Methyl Esters of Safflower Oil Fatty Acids with Urea
(Starting Materials: 50 g. of Methyl Esters, 100 g. of Urea, 250 ml. of Ethyl Alcohol)

Fraction No.	1	2	3	4	5	6	7	Total
Ml. of ethanol used	250	200	200	200	150	150	Residue	....
% eluted	33.94	17.90	11.40	10.16	10.16	6.12	6.58	96.26
I.V. of fraction	159.7	153.0	145.5	130.4	106.9	72.7	14.1	....
S.V. of fraction	190.6	....	190.8	....	198.4	191.3	199.5	....
S.V. of saturated esters	....	....	....	....	....	201.5	201.4	....
Saturated C_{18} (%)	....	....	....	....	....	0.29	1.72	2.01
Saturated C_{18} (%)	....	....	....	....	....	0.64	3.78	4.42
Oleic ester (%)	5.00	4.02	3.54	4.93	7.69	5.19	1.08	31.45
Linoleic ester (%)	28.94	13.88	7.86	5.23	2.47	....	....	58.38

saturated acids in each fraction can be calculated from the saponification values after correcting for the oleic acid portion. A typical set of data obtained by this method is shown in Table I.

Fractionation by the Cumulative Method

Approximately 25 g. of mixed fatty acids were diluted to a volume of 500 ml. with methanol. 50-ml. portions of this solution were pipetted into different flasks containing increasing amounts of urea. After warming and standing over-night, the adducts were filtered by suction. Each of the filtrates was made up to a volume of 100 ml. with methanol. The titratable acidity of 25-ml. aliquots of each of the filtrates was determined. The adducts were decomposed in a manner like that of the elution method, and the fatty acids obtained were analyzed for iodine and neutralization values.

The amount of fatty acids in the filtrates was calculated from the titratable acidity readings by assuming the mixed fatty acids to have similar molecular weights. This method minimizes the errors on account of losses during extraction. If X be the titration reading for aliquot not treated with urea and X_1, X_2, X_3, etc., be the titration readings for the filtrates, then the weight of fatty acids in the filtrates would be X_1f/X, X_2f/X, X_3f/X, etc., where "f" is the total weight of fatty acids in each experiment. The weight of fatty acids in the adducts is the difference between the total fatty acids and the amount in the filtrates.

The additional amount of fatty acids complexed by an additional increment of urea can be termed the "differential" fraction. If a_1, a_2, a_3, etc., represent the fatty acids in each of the adducts, then their differences in weight ($a_2 - a_1$, $a_3 - a_2$, etc.) represent the weights of the differential fractions. The iodine values of the differential fractions can be calculated as follows:

$$\text{I.V. differential fraction } 1 = \frac{(\text{I.V. } a_2)(\text{wt } a_2) - (\text{I.V. } a_1)(\text{wt } a_1)}{\text{wt } a_2 - \text{wt } a_1}$$

The fatty acid composition can then be calculated from the iodine values of the differential fractions, assuming each fraction to be only a binary mixture of fatty acids. A typical set of data obtained by this method is shown in Table II.

Discussion

Separation of Fatty Acids by Urea Adduct Elution. The extent of adduct elution is primarily dependent on its stability. Linoleate component appears to the maximum extent in the first fraction (Table I). The linoleate concentration decreases in the successive fractions with a corresponding increase in the oleate concentration. A maximum oleate concentration is reached after which it decreases with a corresponding increase in the saturated components.

Separation of Fatty Acids by the Single-Dose Method. This is a new approach to the fractionation of fatty acids by urea. It has the advantage of minimizing errors because of extraction losses. Like the elution method, composition curves can be drawn to determine the composition of various intermediate fractions. The ratios of fatty material to urea and solvent can be adjusted to complex a desired proportion of the fatty material. This will allow the composition of the filtrate or adduct to be altered to the desired fatty acid composition. Thus this method provides great flexibility in concentrating certain fatty acid components.

Comparison with Other Fractionation Methods

Urea fractionation methods appear to have advantages over other fractionation procedures. Lead salt-alcohol methods are rather tedious and fail to give precise separations of the fatty acid components.

Low temperature crystallization procedures have been compared with urea methods by Swern and Parker (12). The flexibility and efficiency of these two methods are similar. Low-temperature crystallization has the disadvantage however of requiring facilities to produce and maintain subzero tempera-

TABLE II

Fractionation of Fatty Acids of Sesame Oil by Cumulative Method

Observation No.	1	2	3	4	5	6	7	8	Mixed acids	Total
Urea added (g.)	6	7	8	9	10	11	13	16	....	....
Alc. KOH titration for 25-ml. of raffinate	28.5	26.3	23.0	20.0	13.6	12.0	9.10	3.30	34.1	....
Wt. of fatty acids in raffinate (W)	2.1048	1.9360	1.6828	1.4720	1.0008	0.8980	0.6696	0.2428	2.5096	....
I.V. of adduct acids	28.50	36.56	47.20	55.84	73.19	77.40	86.30	104.5	113.5	....
N.V. of adduct acids	....	....	202.0	201.1	200.4	200.4	200.4	200.3	....	....
Wt. of fatty acids in adduct (2.5096—W) (g.)	0.4048	0.5736	0.8268	1.0376	1.5088	1.6116	1.8400	2.2668	2.5096	....
Wt. of differential fraction (g.)	0.4048	0.1688	0.2532	0.2103	0.4712	0.1028	0.2284	0.4268	0.2428	....
Differential fraction (%)	16.15	6.71	10.09	8.38	18.77	4.10	9.10	17.00	9.68	100.0
Calculated I.V. of differential fraction	28.5	55.9	71.3	90.0	113.4	140.1	148.9	183.0	197.7	....
Unsaturated acids (%)	5.11	4.17	8.63	8.38	18.77	4.10	9.10	17.00	9.68	84.94
Saturated acids (%)	11.04	2.54	1.46	....	....	....	....	....	....	15.04
Oleic acid (%)	5.11	4.17	8.63	8.38	13.92	1.84	3.21	....	....	45.26
Linoleic acid (%)	....	....	....	....	4.85	2.26	5.89	16.62	7.93	37.55
Linolenic acid (%)	....	....	....	....	....	....	....	0.38	1.75	2.13

TABLE III
Composition of Sesame and Safflower Oils

Reference	Safflower oil			Sesame oil		
	(a)	(b)	(c)	(a)	(b)	(c)
Arachidic	0.02	....	....	....	0.02	....
Stearic	6.06	6.36	....	2.01	2.19	....
Palmitic	6.54	5.45	....	4.42	3.83	....
Myristic	....	....	....	....	....	....
Total sat'd	12.62	11.81	15.04	6.43	9.95	6.71
Oleic	52.68	54.96	45.26	31.45	28.36	22.48
Linoleic	31.41	28.39	37.55	58.38	61.07	67.21
Linolenic	....	....	2.13	....	....	3.62

(a) Composition by urea adduct elution of methyl esters.
(b) Composition by urea adduct elution of mixed fatty acids.
(c) Composition from cumulative fractionation data.

tures while the urea method can be used at room temperatures.

Chromatographic and countercurrent distribution methods yield fairly good separations but require unwieldy equipment for large-scale separations.

Summary

The fatty acid compositions of sesame and safflower oils have been determined by two urea fractionation procedures. A simple cumulative urea fractionation procedure has been found to give results similar to those obtained by an urea adduct elution method.

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The Effect of Diet on the Fatty Acid Composition of Several Species of Fresh Water Fish[1]

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IN A RECENT REPORT from this laboratory (6) it was shown that the nature of the body fatty acids of the common mullet, a marine teleost fish, was markedly influenced by the dietary fatty acids. It was noted that the polyunsaturated acids which remained in these animals after 104 days on a fat-free diet could have been residual pre-experimental dietary acids. That they could also have been the result of synthesis by the fish was recognized as a possibility. Certainly however the fish on the fat-free diet did not synthesize polyunsaturated fatty acids to the degree as found in their tissues immediately after removal from their natural habitat.

In a continuation of the study of the origin of the aquatic type of fats a number of fresh-water fish were fed low-fat, 10% cottonseed oil and 10% menhaden oil diets. After various periods on these rations the relative amounts of the polyunsaturated fatty acids of the whole fish were determined. These were then compared with a group analyzed immediately after capture and with the marine mullet of the earlier study (6).

Experimental

Four species of fresh water fish were maintained on a series of synthetic diets. These diets were: a low fat diet; a simulated terrestrial fat type of diet, incorporating a commercially refined and winterized cottonseed oil; and a simulated marine fat diet, incorporating menhaden oil, a typical marine oil. The diets, the same as used in the previous study (6), are based on egg albumen and starch plus generous amounts of minerals and water and fat-soluble vitamins. The starch utilized in formulating the diets contained a maximum of 0.32% fat. It was considered that this fatty material was not enough to influence the study and would prevent possible fatty acid deficiency on the low fat diet. The fats were incorporated at 10% of the dry weight of the diet.

The fish employed in these experiments were the yellow bullhead (*Ameiurus natilis natalis* LeSueur), the common bluegill (*Lepomis macrochirus macrochirus* Rufinesque), the rockbass (*Ambloplites rupestris ariommus*), the small-mouth buffalo (*Ictiobus bubalus* Rufinesque), and the rainbow trout (*Salmo gairdnerii irideus* Gibbons). The fish were kept in aged, aerated, tap water in a 45-gal. aquaria. The water was constantly filtered through glass wool and charcoal. They were fed once daily; the amount varied with the size of the fish but approximated about a twenty-fifth of the mass of the fish. Uneaten food was removed. The bluegills and rockbass were between 3 and 6 in. in length, and no growth was observed in the course of the experiment. The buffalo grew 1 in. to a final length of 3 in., and bullheads doubled their length to 6 in. from their original 3 in. The trout developed from the eyed egg stage to fishes 1 to 1¼ in. in length. The rockbass ingested less food than all others except the trout.

In the experiment with the bullheads the fish were maintained on the fat-free diet for 51 days. They were then separated into three groups. One group was continued on the fat-free diet, and the others were placed on the marine and terrestrial fat diets

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