South African Pilchard Oil

2. CONCENTRATES OF HIGHLY UNSATURATED FATTY ACIDS AND ALCOHOLS DERIVED FROM SOUTH AFRICAN PILCHARD OIL*

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The saponification of marine oils affords a complex mixture of fatty acids of widely differing chain length and degree of unsaturation. Acids of from 12 to 28 carbon atoms in chain length have been reported, the higher unsaturated members containing as many as six or seven double bonds per molecule (Lovern, 1942).

Analysis of such mixtures is greatly facilitated if preceded by a fractionation into groups of acids of largely similar properties. Preliminary segregation of a concentrate of highly unsaturated acids from the total hydrolysate has been achieved by several methods, among which are adsorption chromatography (Kaufmann, 1939, 1940), low temperature crystallization (Hilditch & Riley, 1945) and methods based on the relative solubility of the metallic salts of saturated and unsaturated acids in various solvents (Twitchell, 1921; Farnsteiner, 1903; Voorhies & Bauer, 1943). Of such solubility methods, the lithium salt–acetone procedure of Tsujimoto (1920) (cf. Tsujimoto & Kimura, 1923) has been claimed to be the most efficient in application to marine oils.

More recently, a new segregation procedure has been suggested by the fact that fatty acids of a high degree of unsaturation form complexes with urea less readily than do acids of lower unsaturation (cf. Schlenk & Holman, 1950). The efficiency of the urea complex method has been contrasted with that of the low temperature crystallization technique by Swern & Parker (1952), and in the present communication a comparison is made between the efficiency of the urea method and that of the lithium soap–acetone procedure for segregation of unsaturated acid concentrates.

As a basis for study, the lithium soap–acetone and urea complex procedures have been applied to the total acids from the body oil of the South African pilchard (*Sardina ocellata* Jenyns). Urea complex fractionation has also been applied to the unsaturated acid concentrate obtained by the lithium soap–acetone method, and the concentrates resulting from all procedures have been compared.

The alcohols obtained by lithium aluminium hydride reduction of pilchard glycerides have also been fractionated by the urea complex method. A concentrate of the more highly unsaturated alcohols has been compared with the corresponding unsaturated acids segregated by the urea and lithium soap–acetone procedures.

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EXPERIMENTAL

Unsaturated materials were sealed under high vacuum and stored at 0°. Wherever possible, operations with these materials were carried out under N_2.

Analytical methods

Iodine values were determined by the Wijs method as described in 'British Standards Specification (1950), Appendix E, p. 38', and a reaction time of 3 hr. was allowed.

Concentrates of unsaturated acids were completely hydrogenated over Adams's PtO_2 catalyst.

The molar percentages of the chain lengths present in the saturated material were determined by quantitative reversed-phase partition chromatography (Silk & Hahn, 1954).

Total pilchard fatty acids. Saponification of crude bleached pilchard oil gave the mixed fatty acids of mean equiv. wt. 282, and average number of double bonds/mol., 2·40 (H_2 uptake over Pd–$BaSO_4$).

Total pilchard fatty alcohols. A dry ethereal solution of pilchard oil (50 g.) was reduced by slow addition to an ethereal solution of $LiAlH_4$ (5 g.) (cf. Ligthelm, von Rudloff & Sutton, 1950). The total fatty alcohols (46 g., 99% yield) had an iodine value of 218.

Oxidation of pilchard alcohols to acids. To facilitate analysis, the concentrate of highly unsaturated alcohols was completely hydrogenated over Adams's PtO_2 catalyst and quantitatively oxidized to the corresponding mixture of acids with CrO_3 in glacial acetic acid (cf. Pollard, Chibnall & Piper, 1931).

Concentration of unsaturated acids by lithium soap–acetone procedure

Total pilchard acids (100 g.) in acetone (250 ml.) were neutralized at 15° with saturated aqueous LiOH solution (50 ml.). Acetone (700 ml.) was added to adjust the solvent concentration to 95%. The mixture was thoroughly shaken

and allowed to equilibrate for 4 hr. at 30° and then overnight at 15°. The precipitated lithium soaps were filtered off and discarded. The more highly unsaturated acids were recovered from the filtrate by acidification followed by extraction with *n*-pentane. The procedure was repeated once more with 95% aqueous acetone and finally with 97% aqueous acetone. Results are shown in Table 1.

Urea complex fractionation

The procedure was the same in general application to acids or alcohols.

Table 1. *Lithium soap–acetone segregation of pilchard unsaturated acids*

No. of treatments	Aqueous acetone (% acetone)	Weight (g.)	Equiv. wt.	No. of double* bonds/mol.
0	—	100	282	2·40
1	95	38	295	4·23
2	95	31	304	4·52
3	97	29·5	311	4·62

* H_2 uptake over Pd–$BaSO_4$.

General method. Total pilchard alcohols (45·4 g.) were dissolved in purified absolute ethanol (1200 ml.). Pure urea (50 g.) was added to the solution and dissolved by vigorous stirring and warming to 40°. After cooling slowly overnight, the crystalline adduct was filtered off and washed with cold ethanol (2 × 10 ml.).

Urea (20 g.) was added to the combined filtrate and washings and dissolved as before. After cooling overnight, the second crop of complexes was removed and washed. The process was repeated until the fourth crop of complexes had been obtained. The filtrates and washings were then evaporated to two-thirds of their volume under reduced pressure at 30°, and left to deposit the fifth crop on cooling. Thereafter the solution was concentrated somewhat at each step so that 5–10 g. of urea complexes were obtained on cooling. The mother liquors from the final precipitation were evaporated to yield a concentrate of the more highly unsaturated alcohols. The complexes precipitated at each stage were decomposed with water and the fatty material was recovered by ether extraction.

The method could be adjusted to afford a greater or lesser number of fractions as required, while by exhaustive precipitation with urea in the case of the alcohols, it was possible

Table 2. *Urea complex fractionation of total pilchard fatty acids*

Fraction	Weight (g.)	Weight (As % of starting material)	Equiv. wt.	No. of double* bonds/mol.	Iodine value
Total fatty acids	95·0	100·0	282	2·40	201
1	5·0	5·6	278	0·20	12·0
2	13·5	15·0	276	0·35	20·7
3	13·5	15·0	271	0·46	46·7
4	17·0	19·0	280	1·49	135
5	12·0	13·3	298	3·65	300
6	2·0	2·2	305	4·54	—
7	0·5	0·6	310	—	—
8	4·0	4·5	304	4·55	368
9	1·0	1·1	302	—	368
10	1·0	1·1	305	4·65	—
Non-complex forming residue	20·0	22·0	304	4·89	385·7

* H_2 uptake over Adams's catalyst.

Table 3. *Urea complex fractionation of unsaturated acids segregated by the three-stage lithium soap–acetone procedure*

Fraction	Weight (g.)	Weight (As % of starting material)	Equiv. wt.	No. of double* bonds/mol.
Li soap–acetone concentrate	93·0	100·0	307	4·55
1	4·8	5·16	315	3·00
2	2·48	2·67	304	3·38
3	1·85	1·99	300	3·39
4	4·40	4·74	300	4·01
5	2·58	2·78	302	4·21
6	1·50	1·61	304	4·40
7	3·95	4·25	304	4·54
8	1·90	2·04	303	4·60
9	4·05	4·36	301	4·69
10	2·24	2·41	305	4·82
11	0·82	0·88	303	4·71
Non-complex forming residue	55·0	59·1	308	4·85

* H_2 uptake over Pd–$BaSO_4$.

to obtain a non-complex forming fraction representing approximately 12% of the total.

Total acids. The urea complex fractionation of total pilchard fatty acids is illustrated in Table 2.

Lithium soap–acetone concentrate of unsaturated acids. Further subdivision by means of the urea method of the lithium soap–acetone concentrate prepared as described in Table 1 is illustrated in Table 3.

Total alcohols. The urea complex fractionation of total pilchard alcohols is illustrated in Table 4.

Chain length analysis and properties of the concentrates obtained by the lithium soap–acetone and urea complex fractionation procedures

Concentrates were quantitatively chromatographed after saturation, the alcohols being converted into the corresponding acids. The results are summarized in Table 5.

DISCUSSION

Four concentrates of highly unsaturated material have been prepared from total pilchard fatty acids and alcohols.

Table 4. *Urea complex fractionation of total pilchard fatty alcohols*

Fraction	Weight (g.)	Weight (As % of starting material)	Iodine value
Total fatty alcohols	45·4	100·0	218
1	3·10	7·5	16·6
2	6·75	16·3	25·5
3	3·30	8·0	52·2
4	4·10	9·9	96·9
5	2·00	4·8	147
6	4·35	10·5	228
7	1·60	3·9	297
8	1·80	4·3	344
9	0·95	2·3	358
10	1·05	2·5	379
Non-complex forming residue	12·45	30·0	365

(*A*) A concentrate of highly unsaturated acids prepared by application of the lithium soap–acetone procedure to total pilchard fatty acids.

(*B*) A concentrate of highly unsaturated acids prepared by application of urea complex fractionation to total pilchard fatty acids.

(*C*) A concentrate of unsaturated acids prepared by urea complex fractionation of concentrate *A*.

(*D*) A concentrate of unsaturated alcohols prepared by urea complex fractionation of total pilchard fatty alcohols.

Direct comparison of concentrates *A* and *B* shows that the latter is of greater average unsaturation and, moreover, contains proportionately less of the longer-chain C_{20} and C_{22} acids. It is thus apparent that the urea procedure is more efficient than the lithium soap for removal of long-chain acids of low unsaturation. For the purpose of preparing a concentrate rich in shorter-chain highly unsaturated acids, the urea method is therefore to be preferred. The better stepwise fractionation obtainable with the urea method would also commend its use in preference to the lithium soap procedure for this purpose.

Comparison of concentrates *B* and *C* shows that both have the same average unsaturation, but that the latter is proportionately richer in the longer-chain C_{20} and C_{22} unsaturated acids. Thus if a preliminary lithium soap–acetone segregation is carried out before application of the urea method, then the final unsaturated concentrate is richer in C_{20} and C_{22} unsaturated acids than the concentrate obtained by direct application of the urea method to total pilchard acids. The lithium soap–acetone procedure is thus effective in removing an appreciable quantity of C_{16} and C_{18} acids of relatively low unsaturation which are not easily removed by the urea method. This is consistent with the first observation that the lithium soap–acetone procedure, in direct application to total pilchard acids, affords a concentrate

Table 5. *Comparison of concentrates after saturation*

Material	Yield (as % of total)	Equiv. wt. (unsaturated)	No. of double bonds/mol.	Molar % C_{16}	Molar % C_{18}	Molar % C_{20}	Molar % C_{22}
Total pilchard fatty acids	100	282	2·40*	18·25	23·9	39·6	18·25
A. Li soap–acetone segregated acids	32	307	4·55*	23·5	12·0	40·8	23·6
B. Urea complex segregated acids	22	304	4·88†	29·6	18·6	31·9	19·9
C. Urea complex segregated acids ex Li soap–acetone concentrate	19·7	308	4·85*	19·9	13·4	38·6	28·1
D. Urea complex segregated alcohols	37·6	—	‡	29·0	11·9	43·5	15·6

* H_2 uptake over Pd–$BaSO_4$.
† H_2 uptake over Adams's catalyst: corresponding iodine value, 386.
‡ Iodine value, 371.

richer in longer chain acids than does the urea method.

Application of the urea procedure to total pilchard fatty alcohols affords a satisfactory stepwise fractionation, resulting in a concentrate rich in C_{16} and C_{20} unsaturated alcohols with a relatively small proportion of the C_{18} and C_{22} chain lengths.

Each of the four procedures investigated would appear to have its respective merits depending upon the nature of the unsaturated concentrate required. Material relatively rich in any desired chain length can be prepared for subsequent isolation of individual acids or alcohols by choice of the appropriate procedure. For convenience in analysis, fractionations involving the acids rather than the alcohols are to be preferred.

Urea fractionation of acid esters was not undertaken, although this has been claimed by Abu Nasr & Holman (1951–2) to result in the removal of material more highly unsaturated than is the case when the free acids are fractionated. The reduced tendency of the esters to associate is offered as an explanation of this behaviour.

SUMMARY

1. The urea complex fractionation and lithium soap–acetone techniques have been applied to the segregation of a concentrate of highly unsaturated fatty acids from the body oil of the South African pilchard. For comparison, the alcohols resulting from lithium aluminium hydride reduction of pilchard glycerides have also been fractionated by the urea complex method.

2. A comparison has been made between the unsaturated concentrates prepared by each procedure. The urea complex method affords an excellent stepwise fractionation resulting in a concentrate of high average unsaturation rich in the shorter C_{16} and C_{18} chain lengths. The lithium soap–acetone procedure affords a concentrate of lower average unsaturation than does the urea method, but the segregate is relatively richer in the longer C_{20} and C_{22} chain lengths. Urea fractionation of total pilchard fatty alcohols affords a concentrate of high average unsaturation rich in the C_{16} and C_{20} chain lengths.

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3. THE FATTY ACID COMPOSITION OF SOUTH AFRICAN PILCHARD OIL

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The complete and unambiguous analysis of the fatty acid composition of marine oils is a matter of extreme complexity owing to the diverse nature of the component acids. Not only are acids of differing chain length and unsaturation encountered, but also acids which have the same chain length and a varying degree of unsaturation. Acids of from 12 to 28 carbon atoms in chain length have been reported; the higher members containing as many as six or seven double bonds per molecule (Lovern, 1942).

A large number of the more highly unsaturated acids of marine oils have been isolated and character-

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