

peratures was slightly lower than that found after tempering (5). Once extensive precipitation of solids has occurred, it is unlikely that appreciable supersaturation would persist in the spread since the system then is seeded heavily. To account for the lower solids content, the solid obtained by rapid chilling must be presumed to be more soluble. This has been confirmed by solubility measurements. The 0.5-1.5% total weight increase in solids content reported previously to result from tempering global spread is somewhat higher than the difference in solubility at 30°C. caused by tempering as exhibited in Figure 1. The solubility data at that temperature however was obtained with precipitates from dilute solutions and hence may not be applicable quantitatively to precipitated systems with higher concentration of monoglycerides. The increase in solids content on tempering monoglyceride spreads is in agreement with the observations on cocoa butter by Jensen (8) and on artificial "hard butter" by Bailey (1) but is the reverse of findings on hydrogenated oils by Fulton (3) and on beef fat by Hofgaard (1, 6). Perhaps increased knowledge of oil solubility of triglycerides in various polymorphic forms or crystal states would clarify these apparent discrepancies. As previously reported (5), the paradox remains that tempering increases solids content and simultaneously decreases consistency of global spreads.

X-ray diffraction and microscopic observations reported here on dilute monoglyceride systems used for solubility determination confirm previous observations of crystal changes in spreads. In both dilute and concentrated systems, crystal edges became more sharply defined during tempering, and, in addition, an increase was noted in particle size. It therefore appears that conversion of a compact precipitate, containing poorly crystalline monoglyceride, into more perfect, larger, discrete crystals should be considered the dominant factor in causing softening on temper-

ing and may also impart flow properties which contribute to better mouthing qualities.

### Summary

Solubility data have been presented for mono-stearin, monopalmitin, and monoglycerides of triple-pressed stearic acid and hydrogenated lard in cottonseed oil. Monoglyceride precipitated from solution by quick chilling became less soluble after tempering 18 hrs. at 45°C. Decreased solubility was related to increased crystallinity as determined by X-ray diffraction. Solubility data thus confirmed conclusions previously reported that tempering operates to improve global spread primarily through recrystallization which is now shown to involve converting precipitates of low degree of molecular order into more perfect crystals.

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### REFERENCES

1. Bailey, A. E., "Melting and Solidification of Fats," p. 304, New York, Interscience Publishers Inc., 1950.
2. Bunn, C. W., "Chemical Crystallography," p. 367, Oxford (1946).
3. Fulton, N. D., Lutton, E. S., and Wille, R. L., J. Am. Oil Chemists' Soc., **31**, 98-103 (1954).
4. Harbury, L., J. Phys. Chem., **50**, 190-199 (1946).
5. Hellman, N. N., Zobel, H. F., and Senti, F. E., J. Am. Oil Chemists' Soc., **32**, 110-114 (1955).
6. Hofgaard, K., "Dilatometriske Fedstof-Undersogelser," J. Jorgensen and Co., Copenhagen, 1938.
7. Hulet, G. A., in "Colloid Chemistry" edited by J. Alexander, vol. 1, p. 637, New York, Chemical Catalog Co., 1926.
8. Jensen, H. R., "Chemistry, Flavoring, and Manufacture of Chocolate Confectionery and Cocoa," p. 214-8, Blakiston, Philadelphia, 1931.
9. Lancaster, E. B., et al., J. Am. Oil Chemists' Soc., **32**, 9-13 (1955).
10. Lutton, E. S., J. Am. Oil Chemists' Soc., **27**, 276-31 (1950).
11. Malkin, T., and el Shurbagy, M. R., J. Chem. Soc., 1628-1634 (1936).
12. Malkin, T., in "Progress in the Chemistry of Fats and Other Lipids," vol. 2, p. 1-50, New York, Academic Press Inc., 1954.
13. Ward, T. L., Gros, A. T., and Feuge, R. O., presented at American Oil Chemists' Society meeting, San Antonio, Tex., Apr. 12-14, 1954.

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## Component Fatty Acids in Body Fats of Some Marine Fishes

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THE FATTY ACID composition and complex nature of depot and body fats of many species of fish have been investigated. Though Lovern and his coworkers (1) have paid particular attention to depot fats, and the body fats of some fresh-water fish have been studied by Pathak and his collaborators (2), less attention seems to have been given to the composition of the body fats of marine species. The purpose of the investigations now reported was to study the composition of three species of marine fish caught along the coast near Bombay. These species were: white pomfret (*Stromateus cinerus*); black pomfret (*Stromateus niger*); and pala (*Hilsa ilsha*).

### Samples and Methods of Analyses

Muscles from the freshly caught fish were autoclaved at 15 lbs. pressure at 250°F. for 15 min. The lipides were then extracted with diethyl ether, obtaining about 100 g. of oil representing the body fat of each species. Some characteristics of the oils are given in Table I.

The oils were saponified, and the mixed fatty acids were prepared for analysis by the procedure of Hilditch (3). The acids were fractionated by low temperature crystallization in diethyl ether and acetone (10 ml./1 g.) to obtain fractions having different iodine values.

The fractions were methylated, and the methyl esters in each were fractionated by low pressure distillation (4). The yields, iodine values, and saponification equivalents of each fraction of the methyl esters were determined.

TABLE I  
Some Characteristics of Body Fats of Marine Fish

Fish	Oil		Mixed Fatty Acids	
	Iodine Value (Wijs)	Saponification Equivalent	Iodine Value (Wijs)	Saponification Equivalent
White pomfret.....	78.3	276.1	81.1	272.5
Black pomfret.....	105.3	233.7	113.7	278.8
Pala.....	77.4	277.4	81.4	271.0

The unsaturated fractions obtained by low temperature crystallization were isomerized, and the ultraviolet absorption spectra were studied to investigate the presence of unsaturated acids.

The compositions of the oils were calculated from the methyl ester fractionation and the analytical data according to the procedure of Hilditch (3). The results are presented in Table II.

TABLE II  
Composition of Body Fats of Some Marine Fish

Acids	White Pomfret		Black Pomfret		Pala	
	Wt.	MOL	Wt.	MOL	Wt.	MOL
	%	%	%	%	%	%
Saturated						
Myristic	4.75	5.68	4.37	5.3	5.32	6.34
Palmitic	20.57	21.92	13.29	13.56	23.48	24.81
Stearic	11.15	10.69	7.26	7.01	8.87	8.49
Arachidic	.....	.....	0.46	0.41	0.02	0.02
Unsaturated						
Tetradecenoic (-2H)	1.40	1.69	2.44	3.02	1.29	1.55
Hexadecenoic (-2H)	9.19	9.86	18.80	19.75	6.82	7.30
Octadecenoic (-2H)	33.16	32.08	33.16	31.98	32.88	31.68
Octadecadienoic (-4H)	3.56	3.46	0.44	1.23	1.68	1.63
Octadecatrienoic (-6H)	3.62	3.55	1.73	1.74	9.67	9.45
Eicosenoic (-2H)	7.54	6.63	4.53	4.09	9.01	7.90
Eicosatetraenoic (-8H)	5.06	4.54	6.74	6.21	0.48	0.43
Docosapentaenoic (-10H)	.....	.....	3.39	2.89	0.48	0.40
Docosenoic (-2H)	.....	.....	3.39	2.81	.....	.....

### Discussion

The calculated composition of the oils indicate their complex nature. The percentages of saturated acids in the body fats of white and black pomfret and of pala fish were found to be 36.5, 25.4, and 37.7, respectively. Palmitic and stearic were the major saturated acids present.

The higher percentage of unsaturated acids in the black pomfret oil was in conformity with its higher iodine value. It also contained elupanodonic and cetoleic acids. It is noted that it was abnormally high in palmitoleic acid. Oleic acid was present in about equal amounts in all three oils.

The totals of stearic and oleic acids were about the same in the black pomfret and pala oils. This may be

because of the result of biohydrogenation reaction as noted by Lovern (5). This reaction did not seem to have proceeded so markedly in white pomfret oil. The totals of palmitic and palmitoleic acids in all three oils were fairly constant, suggesting interconvertibility of one of them to the other (6).

Of the three oils the black pomfret contains the least linoleic and linolenic acid. The low content of linoleic may be attributed to its presence as a step in the hydrogenation of linolenic to oleic and then to stearic acid or to a rapid metabolism of it. Lovern (1) observed the absence of linoleic acid in halibut liver oil.

Gadoleic acid was found in all three oils; it was highest in pala oil. However arachidonic and elupanodonic acids were present in only small amounts in pala oil. These two acids were not found in white pomfret oil.

The black pomfret oil was the most typical of the three studied. It was not as highly unsaturated as the liver and depot fats studied by Lovern (1), in which more than 10% of C<sub>22</sub> acids occurred. The high temperatures of Indian waters may be a contributing factor to the presence of less polyunsaturated acids.

### Summary

The component fatty acids of the body fats of three typical species of Bombay marine fish were investigated. The saturated acid contents of pala and black pomfret oil were about the same. The black pomfret oil was abnormally high in palmitoleic acid. Linoleic acid was found to be remarkably low in pala and black pomfret oils. In white pomfret the amount of it present was about the same as that of linolenic. Polyunsaturated acids were present in black and white pomfret oils but only present in traces in pala oil.

### REFERENCES

1. Lovern, J. A., *Biochem. J.*, **26**, 1978-1984 (1932).
2. Pathak, S. P., Pande, G. D., and Mathur, S. S., *Biochem. J.*, **57**, 449-453 (1954).
3. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 2nd ed., Wiley, New York, 1947.
4. Longenecker, H. E., *J. Soc. Chem. Ind.*, **56**, 199-202 T (1937).
5. Lovern, J. A., *Biochem. J.*, **30**, 2023-2026 (1936).
6. Lovern, J. A., *Biochem. J.*, **26**, 1985-1988 (1932).

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## Determination of Butylated Hydroxy Anisole and Propyl Gallate in Food Antioxidants

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BUTYLATED hydroxy anisole and propyl gallate are used extensively, both alone and in combination, as antioxidants in food products. Many commercial preparations consist of one or both of these antioxidants and citric acid dissolved in propylene glycol. Analytical procedures for the determination of butylated hydroxy anisole and/or propyl gallate in such preparations should therefore be of value in controlling the addition of the antioxidants in their end-use.

Mahon and Chapman (2,4) have reported visible spectrophotometric methods for the determination of

propyl gallate and butylated hydroxy anisole in lard and shortening. After separation of the antioxidants by selective extraction from the fat, colors were developed, using a ferrous tartrate reagent for propyl gallate and a ferric chloride plus 2,2'-bipyridine reagent for butylated hydroxy anisole. These authors have reported (3,4) another colorimetric method, using 2,6-dichloroquinone chlorimide as the reagent, which is applicable to the determination of butylated hydroxy anisole in commercial antioxidant preparations. While the interference of propyl gallate is not serious in the latter procedure, no means are provided