# **Empirical Relationships Between Iodine Value and Polyunsaturated Fatty Acid Content in Marine Oils and Lipids**

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### **Abstract**

From data obtained in this laboratory two empirical formulas have been developed which correlate polyunsaturated fatty acids indicated by GLC analyses with iodine values of marine oils or their fatty acid methyl esters. These formulas have been applied to data from the literature with good agreement. It is suggested that these formulas function only with fats having the basic composition of marine lipids, which consist principally of saturated, monounsaturated and very highly unsaturated fatty acids. The presence of modest amounts of dienoie and trienoie fatty acids such as are found in freshwater aquatic life and in land animals makes the formulas inapplicable, suggesting their use to distinguish marine fish oils and lipids from other types. The formulas could be particularly useful in technological applications of marine oils where a rapid and approximate knowledge of amount of polyunsaturated fatty acids is desirable.

## **Introduction**

**M** ARINE LIPIDS are distinguished from most anisaturated fatty acids. Until the advent of gas-liquid chromatography (GLC) studies on proportions of particular fatty acids were limited to saturated fatty acids, since for the unsaturated fatty acids only chain length and average unsaturation could be determined. More recently knowledge of particular mono- and polyunsaturated fatty acids has been extended as reviewed elsewhere (1-4). Accumulated GLC data have shown that there are broadly similar relationships among fatty acids found in depot fats of various species, with species variations in iodine values reflecting changes in the proportions of monoethylenie and polyethylenie fatty acids, since the saturated fatty acid contents are rather similar and moderately independent of species  $(5-7)$ .

A study of cod liver oils showed that some fatty acid composition changes, probably due to feeding habits and metabolic changes connected with the reproductive cycle, could be correlated with iodine value. As the iodine value rose the per cent of total monoethylenic fatty acids declined linearly and correspondingly total polyunsaturated acids rose (8). In a detailed study of Atlantic herring oils (9) it was observed that, despite moderately large variations in individual fatty acid components, the total of both saturated and monounsaturated fatty acids declined smoothly with increasing iodine values, and per cent of total polyunsaturates rose accordingly. This observation stimulated a survey of the relationship between polyunsaturated fatty acids and iodine value and two empirical formulas were developed graphically from analyses carried out in this laboratory. The first formula (No. 1) is

Per cent polyunsaturates $=$ 

 $13.3 + 0.317$  (Iodine value esters  $-100$ ),

and applies to methyl esters. For reasons discussed below this formula is most accurate when the iodine value employed is that actually calculated from GLC analyses. The second formula (No. 2) is

Per cent polyunsaturates =  $10.7 + 0.337$  (Iodine value oil  $-100$ ).

As discussed below, this gives the total polyunsaturates as indicated by GLC but is based on the actual iodine value (normally Wijs) of the oil. These formulas were developed from data obtained in this laboratory on a few species (Table I). It seemed desirable to test their applicability to other analyses. The results of this survey are given in the Tables. Quite good correlations were found with fatty acids from the extracted lipids from marine and freshwater fish and shellfish (Table II), some commercial fish oils (Table III), extracted lipids, including diaeyl glyeerides and phosphoplipids (Table IV), and particular phospholipids (Table V). Correlations with spectrophotometric data on alkali-isomerized fatty acids were less satisfactory (Table VI), but the same formulas could be applied to marine animal depot fats with reasonable results (Table VII).

In these tables lipids have been largely grouped by laboratory and class rather than by species. Common names have been used; for complete species identifications references should be made to original publications.

# **Discussion**

Owing to the rendering or extraction procedures employed in producing commercial marine oils there





a Ester sample known to be oxidized.



is a strong possibility that they will contain some polymeric material. The probabilities are that the most highly unsaturated fatty acids would be chiefly involved (36). Such polymers would normally remain as derivatives and their unreacted double bonds would contribute to chemically determined iodine values not only of oils but also of fatty acids and esters prepared from such oils unless distillation purification (37) were employed. The gas-liquid chromatograph determines only the volatile fatty acid esters and therefore the fatty acids involved in polymers are not included. For this reason iodine values calculated from GLC data are usually lower than those determined on the original oil (see Tables I, III, VI, VII) even if the nonsaponifiables  $(1-3\%$  for teleost fish depot fats) are removed. During isolation, saponification and esterification some solubility losses, possibly coupled with some inadvertent oxidation  $(12,24)$  may occur. Powerful catalytic agents such as boron trifluoride must be used with caution

TABLE III Examination of Some Commercial Oils by Application of Formulas 1 and 2

			% Polyunsaturates				
Oil and reference		Iodine values	From	From			
	Oil exp.	GLC calc.	exp. oil I.V.	$\>$ ester calc. I.V.	$\mathbf{B} \mathbf{v}$ GLC analysis		
Sardine (5)	156	149	29.6	28.9	30.1		
Cuttlefish (5)	180	173	37.6	36.5	34.2		
Flatfish (5)	109	101	13.6	13.3	12.2		
Goby(5)	208	192	47.0	42.5	44.3		
Tunny $(5)$	164	164	32.5	33.3	32.1		
Swordfish (5)	124	122	18.7	20.3	18.4		
Cod liver (5)	147	148	26.7	28.5	27.5		
Shark liver (5)	146	145	26.2	27.6	26.5		
Mackerel pike (14)		161		32.7	33.5		
Skipper $(15)$	170	161	31.4	32.7	33.5		
Salmon oil (16)	154	144	32.3	27.3	29		
Cod liver oil.							
White Sea (8)	148	132	26.1	23.4	23.0		
Cod liver oil.							
White Sea (8)	168	158	33.6	31.7	31.9		
Cod liver oil.							
Norway (17)	169	169	33.8	35.3	34.8		

TABLE IV Examination of Some Particular Extracted Lipids by Formula 1

% Polvunsaturates			Calc.	$\%$ Polyunsaturates		
From ester calc. I.V.	By GLC analysis	Lipid and reference	I.V. of esters	From ester calc. I.V.	By GLC analysis	
		Dogfish (Pacific) (18)				
39.9	42.3	Acids from flesh diacyl glyceryl ethers	165	33.8	32.6	
32.3	31.5	Acids from liver diacyl glyceryl ethers	77	6.2	8.0	
25.9	30.0	Acids from flesh triglycerides	119	19.3	19.9	
29.3	31.7	Acids from liver triglycerides	97	12.2	13.2	
28.5	27.7	Tuna (Pacific) (19)				
32.3	37.0	(GLC data from area $\%$ )				
25.2	30.3	Acids from Albacore light meat				
		$(7.5\%$ lipid)	149	28.8	31.0	
		Acids from Albacore dark meat				
26.8	27.6	$(4.3\%$ lipid)	170	35.6	36.2	
18.9	19.1	Acids from Bluefin light meat				
		$(5.0\%$ lipid)	151	29.6	30.4	
28.2	27.7	Acids from Bluefin dark meat				
22.5	23.9	$(5.0\%$ lipid)	156	31.2	33.4	
		Acids from Yellowfin light meat				
28.5	27.7	$(0.6\%$ lipid)	167	34.4	36.7	
27.5	27.4	Acids from Yellowfin dark meat				
		$(0.7\% \text{ lipid})$	167	34.6	36.9	
21.2	22.7	Acids from Skipjack light meat				
		$(0.9\%$ lipid)	180	38.7	40.9	
32.8	33.1	Acids from Skipjack dark meat				
		$(1.0\%$ lipid)	222	52.1	52.4	
35.5	35.8					
		Fresh fish neutral lipids (20)				
45.4	44.8	Sole (June)	169	35.2	37.4	
51.5	49.8	Sole (December)	163	33.3	35.7	
		Halibut (July)	248	60.3	58.8	
		Halibut (December)	182	39.2	37.9	
33.3	38.3	Dogfish flesh (December)	184	40.1	39.8	
39.9	42.9	Dogfish liver (December)	136	24.6	24.6	
31.6	36.3					
		Fresh fish phospholipids (20)				
		$_{\rm Cod}$	255	62.6	59.5	
34.4 33.8	37.6	Sole (June)	235	56.2	56.3	
52.7	36.5	Halibut (July)	265	65.5	61.1	
56.2	53.8 58.2	Halibut (December)	232	55.2	51.4	
		Dogfish flesh (December)	210	48.3	47.6	
		Dogfish flesh (July)	241	57.9	54.2	

(12,38). The net effect is normally a small further lowering of calculated iodine values.

In part the lower iodine values obtained by GLC may reflect the possibility of oxidation during the actual analysis (39,40) and the nonrecognition of trace amounts of polyunsaturates such as the oddnumbered polyunsaturated fatty acids (30,41). There are numerous minor even-chain polyunsaturated fatty acids which are not reported by some authors. This aspect of marine oil analyses as carried out by GLC has recently been reviewed by Lambertson and Braekkan (17). Many of these acids may have been included in homologues and adjacent peaks of unsaturated fatty acids, thus to some extent correcting the calculated iodine values. The major contributions to the iodine values are, however, due to  $20:5\omega 3$ ,  $22:5\omega 3$  and  $22:6\omega 3$ .<sup>1</sup> This is particularly true in phospholipids, where  $22:4\text{\tiny e}6$  may also be prominent  $(20,21)$ . The data, however sketchy, will therefore usually include the really significant components. The empirical formulas are based on reasonably complete analyses of whole oils. Other comparisons will necessarily be with the available GLC data. The iodine values calculated from GLC data are not normally significant to more than two figures. In the tabulated data three figures have been retained to allow three figures in the calculations of per cent polyunsaturates.

Gunstone and Russell (42) were able to obtain expressions giving good correlations for the polyethenoic fatty acids (considered as C18) and iodine values with low-iodine value animal fats. It was, however, necessary to use different formulas for iodine value ranges of 30-60 and 60-90. In the present survey of marine lipids almost all iodine values are in the range 110-250, with values in excess

<sup>1</sup> Notation for chain length: number of double bonds and position of ultimate double bond.





of 200 derived from phospholipids. The formulas appear adequate to cover this range but the largest errors are at the extremes where analytical difficulties are the greatest.

The evaluation of the relationship between the per cent polyunsaturates indicated by GLC and the figure obtained by applying formula No. 1 to the iodine value calculated from the *same* data is not statistically meaningful. Assessment of this data is therefore restricted to the algebraieal mean error (M.E.). Where a statistically significant number of actual oil iodine values can be compared with gas-liquid chromatographic data standard deviations  $(\sigma)$  may also be calculated.<sup>2</sup> In Table I (omitting the cod liver oil iodine results, see below)  $\sigma = 1.61$  for per cent polyunsaturates from the oil iodine values and 1.11 for per cent polyunsaturates from the ester calc. iodine values. The data from the laboratory of Ito and Fukuzumi (5,14,15), Table III, give respective values of 1.82 and 1.48.

The data in Table I include one analysis (cod liver oil) in which it was recognized that oxidation and loss of polyunsaturates had taken place during esterification (12). The figure derived from the oil formula is therefore markedly higher than given by the



TABLE VI Comparisons of GLC and Alkali Isomerization Analyses with Application of Formula 1 to Both Exp. and Calc. Iodine Values

			% Polyunsaturates					% Polyunsaturates			
	Iodine value		From	$\mathbf{B}\mathbf{y}$		Iodine values		From	From		Found by
$_{\rm Ester}$ exp.	GLC calc.	From ester I.V.	ester calc. I.V.	<b>GLC</b> anal- ysis	Fish and lipid	$_{\rm Ester}$ exp.	Ester GLC	ехр. ester I.V.	ester calc. I.V.	GLO	alkali isom.
248 200 .	261 205 201	60.3 45.0 .	64.4 46.6 45.3	60.4 42.5 44.7	Body oils (6) Sardine Herring Bonito	199 119 187	175 111 180	44.7 19.3 40.9	37.0 16.8 38.5	41 17 40	54.8 17.2 45.7
171 208 178	189 248 229	35.8 47.6 38.1	41.5 60.1 64.2	40.6 55.7 51.6	Whale (mammal) Liver lipids (6) Cod Haddock	133 159 172	106 172 174	23.8 32.0 36.1	15.0 36.1 36.9	17 34 35	24.1 28.7 29.3
133 . 145	100 51 115	23.8 1.1.1.1 27.6	13.3 . 18.0	20.1 9.0 21.9	Whiting Ling Anglerfish Dab Turbot	176 152 163 171 140	163 150 157 188 131	27.5 29.7 33.2 35.8 26.0	33.2 29.1 31.3 40.6 23.2	33 27 33 43 23	27.9 21.4 27.9 30.8 17.9
. . 	191 242 220	. . .	42.0 58.4 51.2	56.1 56.2 50.2	Porbeagle (shark) Cod liver oil (29) Cod liver oil	122 152 <sup>a</sup>	101 .	20.3 31.6	13.6 .	14 .	10.4 29.5
.	192	.	42.4	42.5 ---	esters $(29)$ 3.03.1.2.33	.	140		26.1	28.3	

a Oil iodine value.

GLC results. The oil I.V. calculations for the other analyses in this Table have a M.E.  $= -0.42$  and the ester figures a M.E.  $= +0.21$ . Data obtained in another laboratory on extracted lipids is given in Table II. The agreement for the Pacific species of fish is very good  $(M.E. = -0.08)$ , but less satisfactory in the case of the Atlantic fish  $(M.E.=-2.44)$ . The reason for this is not known, although it should be mentioned that the calculated iodine value for the cod flesh fatty acids is markedly lower than values obtained in other laboratories (Tables IV and V) and that the mullet oil may contain unreported amounts of polyunsaturated odd-numbered fatty acids (41). Since only three freshwater species have been listed (see also trout data, Table V) it is not known if the low values given by the formula  $(M.E. = -4.2)$ are due solely to the high levels of  $\dot{C}_{18}$  acids with two and three double bonds (see below). It is significant that the shellfish data (including the crab) are in more reasonable agreement  $(M.E. = -2.05)$ since these are marine species.

A survey of commerical oils from a third laboratory (5,14) forms the basis of Table III, with some additional data. The agreement obtained with both oil and ester calculations is satisfactory. These particular calculations  $(5,14)$  give respective M.E. values of  $+0.73$  and  $+0.40$  for oil and ester iodine val-

TABLE VII

Examination of Some Marine Animal Oils by Formulas 1 and 2										
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a Methyl esters, formula No. 1 *employed~* 

ues. The cod liver oil data derived from a fourth laboratory (8) reports only the highest and lowest iodine value oils since cod liver oil results are available from other laboratories (Tables I, II and VI). The particularly detailed study of cod liver oil from Norway (17) gives good agreement with both formulas.

Table IV is particularly interesting since the fatty acids from dogfish lipids, studied in two different laboratories, provide very satisfactory agreement  $(M.E. = +0.31)$  with the formula based on esters (for other selachian data see Tables II, III and VI). The difficulties in isolating and analyzing fish phospholipids (24) may cause some loss of accuracy as shown in both Tables IV and V. The differences in the tuna data (19) are all negative  $(M.E. -1.61)$ suggesting a systematic error possibly associated with the use of GLC composition reported as area per cent. The data from another laboratory (20) gives algebraically random differences for the neutral lipids  $(M.E. -0.22)$  but a positive bias in all the phospholipid figures except one  $(M.E. +3.11)$ .

More detailed studies of fish phospholipids (Table V) are based on somewhat limited data (24-27), and the possibility of inadequate GLC data due to sample oxidation (24) is suggested by the failures of some experimental iodine values to agree with the calculated values, as well as from poor agreement from the formula calculations. There are, however, indications that the formula may apply not only to specific phospholipids but also to perhaps at least one specific glyeeride ester position in the phospholipid. Thus in the lecithins examined in detail  $(26)$ agreement of the cale. polyunsaturates with the GLC data is good in the two significant a-position analyses. The principal difference in analyses of the two positions is the low saturated acid content in the  $\beta$ -positions. Further data is required to evaluate these points. The neutral lipids in the tuna analyses (24) agree well when the iodine values are high. In menhaden flesh (27) iodine values calculated from the GLC composition for the neutral lipids appear somewhat low when compared with typical menhaden oil (whole fish) values of up to 175. However, these particular fish were very lean. The fact that the two menhaden phospholipid fractions of high iodine value (cephalin II and lecithin I) are in good agreement with the formula in contrast to the fractions of lower iodine value suggests analytical difficulties as noted by the authors (27).

The data in Table VI includes some alkali isomerization determinations of polyunsaturated acids. Unfortunately certain ealcuIations have to be based on the assumption that a fractionation step (6) provided a clear-cut separation of these acids, and the omission of one or more U.V. absorption values in most of the analyses makes interpretation of the results difficult. The majority of the polyunsaturates indicated by experimental iodine values obtained with esters and by iodine values calculated from GLC analyses are in agreement with totals indicated by GLC analysis. Data from another laboratory (29) gives reasonable agreement if allowance is made for the low calc. iodine value for the esters.

In animals the only common high-iodine value lipids are the marine animal depot fats. The oil and ester formulas apply to these fats nearly as accurately as to fish lipids (Table VII). In view of the certain differences (see below) between fish oils and marine animal oils, including fulmer oil (35), it is perhaps surprising that there is reasonable and useful agreement. For the oil calculations the M.E. was  $+0.49$ (omitting the three values where esters were involved) and for the ester calculations the M.E. was -0.43. It will be noted that finwhale liver lipids and blue whale visceral oil give particularly low values when the results from the calc. ester iodine values are compared with GLC analyses. This appears to be the distinguishing feature of animal lipids in general. Evaluation of a number of animal lipids of high iodine value from the literature invariably gave markedly lower results when the GLC-ester formula was applied. However, the only depot fats with high iodine values reported are those of animals fed on diets rich in polyunsaturated fatty acids, and the alternative high iodine value lipids, phosphotipids etc., usually had GLC results based on very few fatty acids. There may, however, be some alternative relationship applicable to animal lipids of high iodine value.

The oil and GLC-ester formulas are different empirical approaches allowing for differences in iodine value due to polymers, removal of nonsaponifiables, losses, etc. as discussed above. Marine lipids differ in a number of ways from other animal lipids. The preservation of dietary triglyceride structure (retention of the  $\beta$ -monoglyceride bond) in marine life, other than marine mammals, has been established (43,44). Moreover it is known that the basic structure is established by phytoplankton and retained in zooplankton, these forming the basis of the marine food chain (45,46). Thus polyunsaturated fatty acids are found preferentially in the  $\beta$ -position in marine triglycerides and phospholipids (excepting marine mammals). In other relationships palmitie acid is a key metabolite (47) occurring in nearly constant proportions of the total saturates in fish depot fats (48) and together with myristie acid accounting for most of the saturated acids. It seems probable that monounsaturated  $C_{20}$  and  $C_{22}$  fatty acids provide the basic depot fat acids for metabolism as needed (22,33).

The two formulas fortuitously accommodate a smooth partial replacement of various monounsaturated fatty acids (average iodine value about 80) with the  $\dot{C}_{20}$  and  $C_{22}$  highly unsaturated fatty acids (5 and 6 double bonds, average iodine value about 400). In most marine lipids the amounts of dienoic and trienoic fatty acids (chiefly  $C_{16}$  and  $C_{18}$ ) are small and relatively constant, although as suggested by the results with trout and other fresh-water species (Tables II and V) the slightly higher levels of  $C_{18}$  dienoic and trienoic acids may give low values with the formulas. This can be demonstrated with application of formula No. 1 to model mixtures, and thus accounts for the low calculated polyunsaturate values obtained with land animal fats. Since marine animals deposit depot fats substantially similar to typical fish fats in composition the formulas work reasonably well in these cases. Although not investigated in detail it appears that the formulas may be inapplicable, to marine life forms such as phytoplankton (46), but agreement through formula No. 1 was excellent in the case of zooplankton analyses (49).

The empirical formulas are therefore limited to typical marine life fatty acid composition, although the range covered from marine animal depot fats to fish phospholipids is surprisingly wide. For this reason their use in corroborating the validity of GLC

results is very limited as only gross errors in analytical identification and quantitation could be detected. Checking of experimental iodine values and polyunsaturate contents from the formulas against GLC analyses would however probably reveal adul-<br>teration of fish oils with other types of fats and distinguish fresh-water fish oils from marine fish oils. More obvious applications of these formulas lie in the technological applications of marine oils. From the oil iodine value alone it is possible to calculate with reasonable accuracy the total polyunsaturates, information of interest in selecting oils for extraction of these materials (50), in hydrogenations, and in employment as drying oils. Since the level of saturated fatty acids is nominally  $20-25\%$  depending on the type of oil, the monounsaturates may be estimated by difference. In lightly polymerized marine oils the presence of polymers not indicated by GLC could be checked from experimental iodine values even if the composition of the raw material was not known.

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