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Alpha-Tocopherol in Some Marine Organisms and Fish Oils

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INTRODUCTION

Although it is recognized that the tocopherols are extensively distributed in Nature, many details with regard to the pattern of distribution are still unknown. As for most vitamins, the early determinations of vitamin E were based on bioassays. Marine organisms contain highly unsaturated fats, and the subsequent oxidation of the tocopherols in the experimental rations made bioassays unsuccessful. The introduction of chemical methods made great advances possible. They were, however mainly applied to analysis of common foods and to detailed studies of previously recognized rich sources such as grains and vegetable oils. Marine sources have been little studied, and only a few values are reported in the literature.

BOCCHI (1938) claimed the presence of vitamin E in tunny liver oil besides the vitamins A and D. The first direct evidence for the occurrence of tocopherols in fish liver oil was given by QUAKENBUSH et al. (1941), who determined tocopherols in oils by distillation and found 260 μg per g cod liver oil. The following years scattered analyses were reported for marine oils (JANSON & KRINGSTAD, 1942; ROBESON & BAXTER, 1943) and for turbot liver and herring fillets (LIECK & WILLSTAEDT, 1945). With the introduction of chromatographic analysis for the separation of tocopherols, further values for marine products and organisms were reported. Thus tocopherols were analysed in seaweed (BROWN, 1953a) and in fish oils and some fish products (BROWN, 1953b; LAMBERTSEN & BRÆKKAN, 1959).

In fish oils the tocopherols undergo rapid oxidation, causing losses which may make the reported values unreliable. ROBESON & BAXTER (1943), studying the protection of vitamin A in thin films of mangena shark liver oil, claimed that *a*-tocopherol is the chief antioxidant present in this oil. Few investigators have been able to work with absolutely fresh samples, in which no oxidation had taken place. The present study was mainly carried out with such samples to obtain more reliable informations with regard to the distribution of tocopherols in marine organisms.

METHODS

The samples were collected fresh. The fish were bought, mostly alive, at the fish market in Bergen. The invertebrates were collected alive and put on dry ice in Dewar jars. If not analysed at once, the samples were stored at — 20° C until analysis.

All samples were saponified directly in ethanolic potassium hydroxide solution, with pyrogallol and ascorbic acid added for protection against oxidation. If necessary the percentages of fat or dry matter were determined on aliquots. The unsaponifiable matter was extracted mainly as described in U.S.P. 16 Ed. (1960) for vitamin A determination. The extract was evaporated to dryness and the residue at once taken up in hexane. These steps were all carried out as quickly as possible to avoid prolonged contact with water and alkali. The chromatographic separation of *a*-tocopherol from the unsaponifiable matter was conducted as previously described (LAMBERTSEN & BRÆKKAN, 1959). The unsaponifiable matter was sent through a column of alumina (Merck "nach BROCKMANN") softened with 7 per cent water. The weight relation between unsaponifiable matter and alumina did not exceed 1:100, and the elution was carried out slowly using pure hexane followed by 5 per cent ethyl ether in hexane. The collection of the *a*-tocopherol fractions was controlled by the ferric chloride-dipyridyl reaction (EMMERIE & ENGEL, 1938). The next step was a chromatographic separation on horizontal paper strips (WHATMAN 3 MM) impregnated with squalane (2:6:10:15:19:23 — hexamethyltetracosane). The strips were developed by 85% ethanol in water, and the *a*-tocopherol fraction cut out and extracted with cyclohexane. This solution was measured spectrophotometrically at the wavelengths 285, 298 and 303 mμ. Calculations were based on the following formula, which corrects for irrelevant absorption according to the principle of MORTON & STUBBS (1946):

$$E_{corr} = 3.831 \cdot E_{298} - (1.064 \cdot E_{285} + 2.767 \cdot E_{303})$$

The *a*-tocopherol values were calculated using the extinction value $E^{1\%}_{1\ cm\ (298\ m\mu)} = 93.5$. The procedure was standardized using d-*a*-tocopheryl hydrogen succinate (DPI) as a reference.

In our hands this method had a recovery and reproducibility of 95 $\pm$ 5%. Colorimetric methods based on the EMMERIE—ENGEL reaction has been found to give better values, but such methods lack the advantage of a spectrophotometric identity control. The present method seemed quite sufficient in relation to the biological variance.

For some samples a further check on the identity of the *a*-tocopherol or the presence of other tocopherols were carried out, using thin layer

chromatography on glas plates. Adsorption as well as partition methods were used, employing the systems of silica gel — 10 per cent ethyl ether in hexane and squalane impregnated celite — ethanol: water (85:15), respectively. The plates were developed with a dipyridyl — ferric chloride spray.

RESULTS AND DISCUSSION

The results are reported in Tables 1—5. In Table 1 are given values for α-tocopherol in commercial fish oils, in Tables 2—4 in fish organs and in Table 5 in some marine invertebrates.

The α-tocopherol contents in commercial marine oils (Table 1) were of the same order as those in vegetable oils. Some typical vegetable oils show the following values: Olive oil 200, soybean oil 310, corn oil 100, arachis oil 95 and cotton seed oil 590 μg α-tocopherol per g (LANGE, 1950; GREEN et al., 1955). In addition the seed oils contain other tocopherols, whereas only α-tocopherol was present in marine sources as ascertained by thin layer chromatography. The discrepancy expressed between report of α-tocopherol in fish oils and earlier denials of its existence in these oils, was explained by MOORE et al. (1959). They found that even high amounts of α-tocopherol did not remove avitaminosis E when given in highly unsaturated fish oils.

The values for cod liver oil, 150—225 μg α-tocopherol per g, are lower than the values reported by QUAKENBUSH et al. (1941) and by BROWN (1953b), who found respectively 260 and 256—322 μg per g, but higher than the values of 102 μg per g reported by MOORE et al.

Table 1. *α-Tocopherol in commercial fish oils.*

Origin	Number samples	μg α-tocopherol per g	
		Liver oil	Body oil
Cod (*Gadus morrhua*)	10	150—125	
Norway pout (*Gadus esmarkii*)	1		70
Ling (*Molva molva*)	2	35—65	
Capelin (*Mallotus villosus*)	4		50—230
Menhaden (*Brevoortia tyrannus*)	1		75
Herring (*Clupea harengus*)	4		20—60
Anchovies (*Engraulis ringens*)	1		25
Greenland shark (*Somnius microcephalus*)	3	300—700	
Dogfish (*Squalus acanthias*)	5	150—350	
Whale (*Baleanoptera sp.*)	2		50—80
Fish oils (Unspecified)*	8		40—70

* The unspecified oils are probably herring or whale oil, or a mixture of these oils.

(1959). The values for ling liver oil, 35 and 65 μg per g are much lower than the value reported by BROWN (1953b), who found 272 μg per g in his sample. Our oils were apparently of good quality and not rancid, thus this discrepancy is difficult to explain. The values for Greenland shark liver oil deserves some comments. This oil showed high values for α-tocopherol, 300—700 μg per g. As a fish liver oil of low unsaturation (iodine value 100—130), it may be placed as a vitamin E source. Among the body oils studied, capelin oil showed fairly high values, 50—230 μg α-tocopherol per g. The values found for herring oil, 20—60 μg per g, are lower than those reported by JANSEN & KRINGSTAD (1942) who found 33—130 μg per g. The same authors, however, found only 23 μg α-tocopherol per g whale oil, compared with 50—80 μg per g found in the present study. In our experience α-tocopherol values of fish body oils fall off quickly with increasing rancidity of the oils.

In Table 2 are reported the α-tocopherol contents in the liver of different fish, and the estimated content in the liver fat. The fat livers usually showed higher α-tocopherol values per g fresh weight than the lean livers, but calculated on the fat the latter had the highest and in some samples extraordinarily high values. Thus catfish liver oil and wrasse liver oil were estimated to contain 1540 and 3100 μg α-tocopherol per g, respectively. This called for a special investigation, and the fats were extracted from fresh samples of these livers. One such sample of catfish liver oil was found to contain 1855 μg α-tocopherol per g, whereas three similar samples of wrasse liver oils contained 3820, 1650 and 2050 μg per g. These values are to our knowledge the highest ever reported

Table 2. *α-Tocopherol in the liver of different fish.*

Species	% fat	α-tocopherol	
		μg per g fresh weight	μg per g fat (estimated)
Cod (*Gadus morrhua*)	60.3	90—224	210
Haddock (*G. aeglefinus*)	62.5	125	200
Coalfish (*G. virens*)	75.0	41—150	126
Pollack (*G. pollachius*)		55	
Tunny (*Thunnus thynnus*)	25.4	50	200
Plaice (*Pleuronectes platessa*)		45	
Turbot (*Rhombus lupus*)		30	
Redfish (*Sebastus marinus*)	25.0	165	660
Catfish (*Anarrhichas lupus*)	18.8	290—300	1540*
Wrasse (*Labrus berggylta*)	4.8	108—180	3100*
Porbeagle (*Lamma cornubica*)	25.4	140	165

* See text on values for extracted oil from these species.

in Nature. Thin layer chromatography before and after saponification proved that α-tocopherol of wrasse liver was in the free alcohol form. It should be recalled that both these species feed mainly on molluscs, which were found to be very rich sources of α-tocopherol (*v.i.*). LIECK & WILLSTAEDT (1945) reported a value of 18 μg per g turbot liver, compared with 30 μg per g in the present sample. The values reported by LAMBERTSEN & BRÆKKAN (1959) for livers from cod, haddock, coalfish and catfish have been used in the present tables together with further material.

In Table 3 are reported the α-tocopherol contents of the fillets of several species, and the estimated contents per g fat. The α-tocopherol contents of the muscle seemed to be related to the fat content. The lean species (*Gadidae*) generally showed low values, whereas the fat species showed high values. The calculated values for the fats from fish muscle showed values in the order of 300—500 μg α-tocopherol per g. These values are fairly high compared with those in vegetable oils, and were checked on fat extracted from the fillets. The fat species offered the best opportunity for quantitative extraction, and catfish and redfish muscle were chosen as suitable samples. Two oils extracted from catfish muscle had 345 and 365 μg per g, whereas one sample of redfish muscle oil contained 650 μg per g, corresponding well with the estimated values. LIECK & WILLSTAEDT (1945) reported fillets from Baltic herring to contain 10.8 μg α-tocopherol per g, compared with 14—16 μg per g in the present study. LAMBERTSEN & BRÆKKAN (1959) analysed one sample of herring fillets, and found only 3 μg per g. Probably this sample was not as fresh as the present samples.

In Table 4 are reported analyses of the distribution of α-tocopherol

Table 3. *α-Tocopherol in the muscle of different fish.*

Species	% fat	α-tocopherol	
		μg per g fresh weight	μg per g fat (estimated)
Cod (*Gadus morrhua*)	0.34	1.5—2.1	560
Coalfish (*G. virens*)	0.70	3.6	507
Pollack (*G. pollachius*)	0.60	2.6	435
Torsk (*Brosmius brosme*)	0.25	1.0	400
Ling (*Molva molva*)	0.37	3.0	810
Herring (*Clupea harengus*)	14.7	14—16	100
Halibut (*H. hippoglossus*)	4.6	4—13	178
Catfish (*Anarrhichas lupus*)	4.3	21	495*
Redfish (*Sebastes marinus*)	3.5	12.5	360*
Wrasse (*Labrus berggylta*		6.0	

* See text on values for extracted oil from these species.

in different organs of fish. The samples were in all instances quite fresh and free of rancidity. Whereas the livers and muscles in Tables 2 and 3 referred to different samples of the species, the samples in Table 4 refer to mixed samples of organs from 5 fishes of the species reported. The most striking finding was the difference between the livers from mature male and female herrings. Thus the estimated value in the male was 7000 μg α-tocopherol per g liver oil, compared to only 700 μg per g in the female. The former value is higher than found in any other source. The values for the muscles have been commented on in connection with Table 3. As the pyloric caeca is the fat absorbing part of the intestine, analyses of this organ have been included. The values were fairly low, 8—18 μg α-tocopherol per g, in herring as well as in the Gadus species. The ripe gonads from herring showed fairly high values, with 32 and 41 μg α-tocopherol per g fresh weight for testis ans ovaries, respectively.

In Table 5 are reported the α-tocopherol contents of some echinoderms and molluscs. Each sample of echinoderms contained 5 individuals whereas each sample of molluscs contained 20—100 individuals. The echinoderms and the common mussel, cockle and whelk, all showed contents of the same order, 4—15 μg α-tocopherol per g fresh weight. A frozen sample of common mussel had 25 μg per g, which could be explained by losses of water during thawing. The contents calculated on dry weight were in the order 50—150 μg per g, with the exception of *Asterias glacialis*, which showed only 16 μg per g. Two of the littoral snails, the limpet and the periwincle, showed high α-tocopherol values,

Table 4. *The distribution of α-tocopherol in different organs of fish.*

Species	Organ	% fat	α-tocopherol	
			μg per g fresh weight	μg per g fat (estimated)
Herring (*Clupea harengus*)				
Male	Muscle	14	16	114
	Pyloric caeca		18	
	Liver	1.8	115	7000
	Testis (ripe)	5.1	32	630
Female	Muscle	15.5	14	90
	Pyloric caeca		16	
	Liver	2.0	14	700
	Ovaries (ripe)	4.0	41	1010
Cod (*Gadus morrhua*)	Muscle	0.35	1.5	430
	Pyloric caeca	2.1	17	810
	Liver	66.7	224	340
Coalfish (*Gadus virens*)	Muscle	0.7	3.6	507
	Pyloric caeca	3.7	8.0	216
	Liver	78.3	41	52

Table 5. *α-Tocopherol in some marine invertebrates.**

Species	% dry matter	μg/g fresh weight	μg/g dry weight
Starfish (*Asterias glacialis*)	25	4	16
— (*Asterias rubens*)	15	9	60
Sea urchin (*Echinus esculentus*)	9	10	110
Common mussel (*Mytilus edulis*)			
Small	6	9	150
Full size	8	10	125
—	—	15	—
Frozen	25	25	100
Cockle (*Cardium edule*)	12	7	58
Whelk (*Buccinum undatum*)	20	8	40
Periwinkle (*Littorina littorea*)	20	45	225
—»—	20	40	200
Limpet (*Patella vulgata*)	20	150	750**
—»—	10	130	1300
Squid (*Ommatostrephes todarus*)			
Muscle	—	12	—
Liver	—	110	**

* Samples of echinoderms consisted of 5 individuals, and samples of mollusc consisted of 20—100 individuals.

** Samples of oils, see text.

with respectively approx. 40 and 140 μg per g fresh weight, corresponding to approx. 200 and 1000 μg per g dry weight. A lipid extract from the limpet showed 1500 μg α-tocopherol per g. The values may reflect that the limpet and the periwincle are algea eaters, whereas the whelk, with only 18 μg α-tocopherol per g fresh weight, is a predatory snail. The samples from the squid showed fairly high values for the liver, 110 μg per g. The fat content was found to be 25%, and thus a liver oil should be expected with 440 μg α-tocopherol per g. A commercial squid oil contained 210 μg per g. To gain some informations on α-tocopherol in crustaceans, a fat extract was made from the deep sea prawn (*Pandalus borealis*). It contained 950 μg α-tocopherol per g, corresponding to an estimated 28.5 μg per g fresh weight.

The present study give α-tocopherol values for a wide range of marine sources, ranging from invertebrates to fish, from muscle, liver and other organs to commercial marine oils. None of the samples were devoid of α-tocopherol, and in some instances values among the highest reported in Nature were found. Considering the normally very high unsaturation of marine fat, with high contents of hexanoic and pentanoic acids, the present findings may support the importance of α-tocopherol as an biological anti-oxidant in these species.

SUMMARY

A study has been made of α-tocopherol in some marine sources. Apart from commercial oils, which were of normal quality, care was taken to ensure fresh material.

The α-tocopherol contents have been determined in eleven marine oils, four liver oils and seven body oils. The liver oils showed values from 35—700 μg per g. Cod liver oil ranged from 150—225, whereas Greenland shark liver oil showed very high contents, 300—700 μg α-tocopherol per g. The body oils normally contained 20—80 μg per g, thus eight samples of unspecified fish oils for hydrogenation showed 40—70 μg per g. Capelin oil showed high values, 50—230 μg per g.

Fish livers varied greatly, the highest values were found in wrasse and catfish with respectively 108—180 and 290—300 μg α-tocopherol per g. Estimated values for the liver oils were in the order of 200 μg per g for most species studied, exceptions were again wrasse and catfish with 1540 and 3100 μg per g, respectively. Values of this extremely high order were confirmed in additional analysis on extracted oils from these species.

The muscle of ten species of fish were analysed. The α-tocopherol contents varied from 1.0 to 16 μg per g, with a clear tendency for fat fillets to show the highest values. Estimation of the contents in the fats showed values generally in the order 300—500 μg α-tocopherol per g. Some values were confirmed on extracted oils.

The distribution of α-tocopherol in different organs were studied on male and female herring, cod and coalfish. There was a striking difference between the content in the liver of the male and female herring. Both were in the spawning state and the male contained ten times more than the female, the estimated values for the liver fat being respectively 7000 and 700 μg per g. No difference of importance could be observed between the testis (soft roe) and ovaries (hard roe), with 32 and 41 μg per g, respectively. The pyloric caeca showed similar values for all species, 8—18 μg per g.

The α-tocopherol content of ten species of echinoderms and molluscs were investigated. The analysis refer to the whole animal except for the squid. The values were in the order 4—15 μg per g fresh weight, and approx. 100 μg per g dry weight. Exceptions were the littoral snails periwinckle and limpet, with approx. 40 and 150 μg per g fresh weight, or approx. 200 and 1000 μg per g dry weight, respectively. The high values in the limpet was confirmed in extracted oils. Fat extracted from the deep sea prawn showed 950 μg α-tocopherol per g.

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