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Methods of extraction composition and stability of vitamin A and other components in dogfish (*Squalus acanthias*) liver oil

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Oil was extracted from dog fish (*Squalus acanthias*) livers by the Bligh and Dyer, Soxhlet and steaming extraction methods. Oil yields using the steaming method (22% w/w) were lower than from the solvent extraction methods (68% w/w). The vitamin A content of the oil produced by steaming was 54 mg retinol/100 g oil, and slightly higher (62–68 mg/100 g) contents were recorded in the solvent extracted oils.

The Bligh and Dyer extraction produced an oil containing 12% phospholipid, much higher than the 4% via the Soxhlet and 1% in the steaming extractions. The level of squalene (40 mg/100 g) in the oil produced by steaming was higher than in the other oils (28 and 32 mg). Concentrations of vitamin E and cholesterol were similar in all three oils at approximately 25 mg tocopherol/100 g and 1.1 g cholesterol/100 g, respectively.

Oils were stored under air at room temperature in half filled dark coloured bottles. Although highest PVs were observed in the stored Bligh and Dyer-extracted oils, the highest apparent loss of vitamin A (30% in 3 weeks) occurred in the oil extracted by steaming.

INTRODUCTION

Vitamin A deficiency is the second most common form of malnutrition. A potentially valuable source of vitamin A is the livers of cartilaginous fish species (sharks, rays) and in Indonesia the total landings were 2278 tonnes in 1990 (FAO, 1990). Vitamin A contents of shark liver oil have been reported in the range 160 000–360 000 IU/g oil (48 000–108 000 μg/g oil: Brody, 1965; Kizevetter, 1973; Murray & Burt, 1983). However, assessment of the amount of oil extracted by various methods, its vitamin A content and the stability (of the vitamin A) is essential in order to evaluate the use of this potential source of vitamin A. In addition the method of extraction and the consequent oxidative stability of the oil in general was investigated.

This work investigated these factors using livers from dog fish (*Squalus acanthias*) since shark livers were not readily available in the UK.

MATERIALS AND METHODS

Materials

Livers from dog fish (*Squalus acanthias*) which had been caught in the North Sea between May and August, were obtained from Grimsby fish dock. The livers were chilled in ice prior to arrival in the laboratory. They were in good condition and did not show any visual trace of spoilage. After being chopped and mixed, they were stored at −60°C until required.

Methods

Direct steaming method

After being thawed at ambient temperature, the chopped livers were weighed (500–1000 g) and placed in a muslin bag on a metal screen over an aluminium pan. The pan containing the liver was cooked with steam in a retort at 70–80°C for 30 min. The liver in the muslin bag was then hand pressed and the oil–water released was combined with the oil–water released from the liver during steaming. The oil was separated from the water by centrifuging at 2000 rpm for 15 min, dried using anhydrous sodium sulphate and separated from the drying material by filtering under suction using a Buchner funnel.

Solvent (Soxhlet) method

After thawing at ambient temperature, the liver (300 g) was weighed and mixed with anhydrous sodium sulphate (1:1). The mixture was placed in thimbles in a large Soxhlet extractor and extracted continuously with

1500 ml of petroleum ether (40:60) for 5 h. The solvent containing the extracted oil was evaporated under vacuum on a rotary evaporator and the oil fraction was shaken under high vacuum to remove the last traces of petroleum ether with protection from the light by wrapping the flask with aluminium foil.

Bligh and Dyer method

After being thawed at ambient temperature, the liver (100 g) was weighed into a 2-litre beaker. To this, distilled water (100 ml) was added and mixed. Chloroform (200 ml) and methanol (400 ml) were added and the mixture was homogenized for 2 min whilst being cooled in ice. More chloroform (200 ml) was added and homogenized for 1 min, followed by the addition of distilled water (200 ml), and finally homogenized for 30 s. The mixture was then centrifuged at 2000 rpm for 20 min. The aqueous layer was removed by suction. The chloroform fraction was dried with anhydrous sodium sulphate, filtered and the chloroform evaporated under vacuum on a rotary evaporator. The oil fraction remaining was shaken under high vacuum to remove the last traces of chloroform with the flask being wrapped in aluminium foil to exclude light.

ANALYTICAL PROCEDURES

Peroxide value (PV) was measured by an adaption of the method of Wood and Aurand (1977), iodine value by Wij's method (Pearson, 1981), and the thiobarbituric acid value analysed using the non-distillation method of Sinnuhuber and Yu (1977).

Free fatty acid (FFA)

To a mixture of diethyl ether (25 ml) and absolute ethanol (25 ml), 1% phenolphthalein (1 ml) was added and the mixture carefully neutralized with 0.100 M sodium hydroxide. The lipid sample (approximately 1 g) was dissolved in the neutralized solvent and titrated with the sodium hydroxide (with shaking) until a pink colour persisted for 15 s. Free fatty acid was calculated as % oleic acid.

Fatty acid methyl ester (FAME)

Methyl esters were prepared based on the method of the British Standard of Analysis of Fats and Fatty Oils (1980) with slight modification (Smith *et al.*, 1990). These were analyzed using a polar packed column, active phase SP2330. Nitrogen was used as carrier gas with flow rate 20–23 ml/min. The temperature programme used was 80°C for the first 4 min, then increasing at 5°C/min to 230°C. A flame ionization detector was used with the signal automatically presented as a percentage of the total peak area (C14 upwards).

Vitamin A analysis

The extraction of retinol was carried out using a modification of the procedure in the methods of vitamin assay of the Association of Vitamin Chemist Inc. (Anon., 1966).

Squalene

Squalene was extracted from dog fish liver oil using the same procedure as for retinol extractions, but using chloroform to dissolve the unsaponifiable material. Quantification of squalene in the extract was carried out using HPLC based on the method of Holen (1985). The column used in this reverse phase method was a C18 0.5 mm × 25 cm column and acetonitrile/methanol (50/50) used as eluant with a flow rate of 2 ml/min and detection at 206 nm.

RESULTS AND DISCUSSION

Oil yield

The oil yields from the Bligh and Dyer and Soxhlet extraction methods were similar (67 ± 3% and 65 ± 3%, respectively) and higher than for the steaming method (22 ± 2%). Although the steaming method condition can result in the thermal rupture of the liver cells and so release of the oil, some is more closely held by the proteinaceous liver tissue and not released under these conditions. Bailey (1942) found that, after decanting the first portion of free oil from the steamed and centrifuged dog fish liver, the residue gave an oil yield from 2 to 10% of the total oil when it was solvent extracted.

The Bligh and Dyer and Soxhlet extraction gave results in agreement with data for the oil content of dog fish liver caught from many areas of the Pacific which have been cited as 50–70% (Brody, 1965; Kayama *et al.*, 1969; Kizevetter, 1973; Murray & Burt, 1983).

The yields in experiments using frozen livers were lower than experiments using fresh livers and this may be due to the loss of oil during freezing and thawing as mentioned by Brody (1965) or loss of free oil as mentioned by Bailey (1942) who reported only very small quantities of vitamin A in this free oil.

Chemical and nutritional composition of extracted oil

The chemical composition of the oils produced by the three different methods of extraction are tabulated in Table 1.

The quality of the dog fish liver was good with no visual or olfactory evidence of spoilage, and this was indicated by the low free fatty acid value (0.24–0.47) since spoiled liver contains a high level of free fatty acids (Shenjoy & Dey, 1984).

From Table 1 it can be seen that the oxidative rancidity parameters (PV and TBA values) of the oils produced by these three different methods were low (PV of 0.39–0.84 mg/kg oil and TBA of 1.20–1.80 mg MA/kg oil). However, the PV and TBA values for the oil produced by the steaming method were slightly lower

Table 1. Chemical composition of extracted dog fish liver oils

Analysis[a]	Bligh and Dyer extraction	Soxhlet extraction	Steaming extraction
Free fatty acid	0.41	0.47	0.94
(as % of oleic acid)	(4.8)	(6.3)	(4.2)
Peroxide value	0.84	0.66	0.39
(meq/kg oil)	(7.1)	(3.0)	(2.6)
TBA value	1.70	1.80	1.20
(mg MA/kg oil)	(5.9)	(5.6)	(0.0)
Iodine value	118	109	133
(g of iodine/100 g oil)	(1.9)	(0.9)	(0.1)
Saponification value	310[c]	123	139
(mg KOH/g oil)	(1.5)	(1.1)	(2.7)
Phospholipid (%)	12.21	4.23	1.26
	(3.2)	(2.4)	(0.0)
Unsaponifiable matter (%)	17.3	16.4	16.0
	(2.2)	(0.5)	(1.3)
Vitamin A[b]	61.9	66.8	54.6
(mg retinol/100 g oil)	(4.7)	(2.6)	(1.0)
Vitamin E	27.5	25.5	24.3
(mg tocopherol/100 g oil)	(6.1)	(9.8)	(14.3)
Squalene	28.3	23.1	40.1
(mg/100 g oil)	(3.2)	(4.1)	(2.9)
Cholesterol	1.15	1.14	1.13
(g/100 g oil)	(5.4)	(4.9)	(3.7)
Fatty acid profile			
C14:0 (percentage composition)	1.9–2.0	2.1–2.2	2.1
C16:0	15.3–15.4	15.8–15.9	15.1–15.3
C16:1	5.4–5.5	6.2–6.7	6.5–6.6
C18:1	23.7–24.0	29.3–27.7	27.5–27.9
C18:2	1.9–2.0	0.2–0.6	0.2–0.3
C20:1	12.1–12.5	12.5–12.8	12.7–12.8
C20:5	4.4–4.6	4.5–4.8	4.5–4.8
C22:1	14.7–15.3	14.7–15.1	14.5–14.6
C22:5	2.6–2.8	2.3–2.4	2.4–2.6
C22:6	11.1–11.7	10.0–10.5	9.9–10.2

[a]Values are means of triplicate determination; percentage coefficients of variation are given in parentheses; range only are shown for fatty acid composition (triplicate determination).
[b]HPLC analysis.
[c]See text.

than for the oils extracted by the Bligh and Dyer and Soxhlet extraction methods.

The iodine value of the oil produced by the steaming method was higher (133 g/100 g oil) than for the other two methods (109–117). Thus the oil produced by the steaming method has a higher average level of unsaturation. The unsaturated fatty acid profiles of the three samples are very similar, therefore the difference in iodine value cannot be due to differences in unsaturated fatty acids. It could be due to squalene, a long chain hydrocarbon with six double bonds which has been found in the liver of many species of shark and is responsible for the high iodine values observed (Kizevetter, 1973). The iodine value of squalene is calculated to be 371 and this was found experimentally (364.8–372.1 g iodine/100 g). From Table 1 it can be seen that the squalene content of oil extracted by the steaming method was only 40 mg/100 g of oil and this will only add an additional 1.5 g iodine value to the oil extracted by the steaming method.

It was found that the saponification value of oil extracted by the Bligh and Dyer method (310 mg KOH/g oil) was much higher than the others (123–139) and this corresponds with its higher phospholipid content (12.2%). The different phospholipid contents for the Bligh and Dyer (12.2%) and for the solvent method (4.2%) are due to the different polarities of the solvent used, the Bligh and Dyer used methanol–chloroform which is more polar than the petroleum ether used in the Soxhlet method.

The important factor in the Soxhlet method is not only the ratio of solvent volume to the material weight, but also the frequency of extraction. Thus in this experiment, probably the difference in solvent polarities is the most important factor in the different phospholipid content of oils extracted by the Bligh and Dyer and by the solvent method. The phospholipid content for oil produced by the steaming method is lowest (1.3%) and indicates that steaming cannot release the phospholipid which is usually present in the cell membranes.

The vitamin A contents, measured as retinol, of the three differently extracted oils, were slightly different, with the oil produced by the solvent extraction method having the highest concentration and the oil produced by the steaming method having the lowest (Table 1). This difference did not correspond with the amount of oil extracted and therefore was not caused by a dilution factor but more likely by a solubility factor in that vitamin A is readily soluble in the non-polar solvent used. Thus the use of solvents increased both the amount of oil extracted and the concentration of vitamin A is the extracted oil. HPLC of the oil without saponification showed only one peak with a retention time the same as that of retinyl palmitate. This result agrees with many reports that vitamin A in many biological sources, including fish, is in the ester form. Using reverse-phase HPLC for determination of retinol after saponification, two peaks were observed, corresponding to all *trans*-retinol and a *cis*-retinol. The formation of *cis*-retinol (8–15% of the total retinol) can result from isomerization during sample preparation, especially during saponification. A similar result was reported by Woollard & Indyk (1986), indicating that by far the major form occurring in the unsaponifiable matter of dog fish liver oil is the trans form. Anon. (1966) stated that fish oil and concentrate may contain 30–40% *cis*-vitamin A.

The vitamin A range of 54–67 mg/100 g oil (1 IU = 0.3 μg of retinol) is within the range of the vitamin A contents of shark liver oil reported by many authors, i.e. 160 000–360 000 IU/g oil (Brody, 1965; Kizevetter, 1973; Murray & Burt, 1983). The very wide range of vitamin A content in many shark liver oils is related to the season, species, size of fish and location. The other important factor is the method of analysis, especially when the vitamin A is analysed by the Carr–Price method. Different methods of analysis have been used [e.g. Carr–Price method (with or without modification), colorimetry based on the reaction with trichloracetic acid, spectrofluorimetry, UV absorption, HPLC], and

Table 2. Free fatty acid, peroxide value, TBA value, retinol content, iodine value and fatty acid composition of dog fish liver oil stored at 25°C under air with light excluded[a]

Storage temperature	25°C													
	Oil extracted by Bligh and Dyer method				Oil extracted by solvent method				Oil extracted by steaming method					
Storage time (days)	0	14	25	31	0	14	21	31	0	3	7	14	21	28
Free fatty acid	0.08	0.13	0.06	0.08	0.05	0.10	0.07	0.08	0.07	0.11	0.17	0.13	0.19	0.22
(% of oleic acid)	(20.5)	(10.6)	(4.9)	(12.7)	(5.7)	(12.5)	(10.2)	(7.3)	(28.5)	(9.1)	(5.8)	(7.7)	(15.7)	(4.5)
Peroxide value	0.71	1.80	3.05	5.20	0.48	15.2	31.4	33.5	1.80	2.85	2.53	4.63	9.52	17.87
(meq 0/kg oil)	(8.1)	(9.5)	(8.2)	(7.3)	(9.1)	(2.4)	(6.7)	(4.9)	(7.7)	(1.8)	(15.8)	(5.4)	(0.7)	(5.1)
TBA value	—	—	—	—	—	—	—	—	1.09	3.76	5.99	14.51	22.75	42.64
(mg MA/kg oil)									(22.0)	(7.9)	(3.4)	(14.2)	(2.0)	(5.4)
Retinol content	47.8	50.6	50.1	50.3	56.5	53.9	51.8	49.5	61.6	57.6	58.5	46.0	42.3	43.2
(mg/100 g oil)	(5.0)	(2.3)	(3.4)	(2.5)	(4.1)	(2.5)	(4.0)	(3.8)	(4.7)	(1.0)	(5.1)	(3.1)	(0.7)	(2.5)
Iodine value	116	118	117	117	121	121	120	121	132	132	133	132	132	131
(mg iodine/100 g oil)	(0.3)	(1.0)	(0.4)	(0.0)	(0.4)	(0.1)	(0.2)	(0.9)	(0.2)	(1.9)	(0.2)	(0.2)	(0.6)	(0.0)
Fatty acid composition (relative to 16:0 = 1)														
C18:1	1.52	—	—	1.48	1.42	—	—	1.41	1.58	1.51	—	—	1.58	1.65
	(8.0)			(7.1)	(9.3)			(10.2)	(8.8)	(0.6)			(5.7)	(0.6)
C18:2	0.10	—	—	0.16	0.14	—	—	017	0.09	0.14	—	—	0.10	0.06
	(20.5)			(10.5)	(15.7)			(10.0)	(66.6)	(0.0)			(40.0)	(16.6)
C20.1	0.82	—	—	0.80	0.78	—	—	0.81	0.84	0.82	—	—	0.83	0.85
	(4.1)			(5.7)	(8.1)			(9.5)	(2.3)	(3.7)			(0.0)	(1.2)
C20:5	0.21	—	—	0.27	0.19	—	—	0.21	0.29	0.29	—	—	0.30	0.32
	(11.7)			(9.1)	(10.5)			(11.5)	(10.3)	(3.4)			6.6	(3.1)
C22:1	1.10	—	—	1.11	1.00	—	—	1.11	1.01	1.00	—	—	0.99	1.00
	(1.5)			(2.9)	(3.5)			(4.7)	(1.0)	(1.0)			(5.1)	(1.0)
C22:5 +	0.82	—	—	0.84	0.79	—	—	0.81	0.72	0.77	—	—	0.76	0.77
C22:6	(5.6)			(4.1)	(5.7)			(4.9)	(4.2)	(3.9)			(0.26)	(2.5)

[a]Values are means of duplicate or triplicate determination: percentage coefficients of variation are given in parenthesis. Oil extracted by the Bligh and Dyer or by solvent methods are oils from the same batch of liver. Oil extracted by steaming methods was extracted from a different batch of liver.

can give different apparent values for vitamin A content.

It was found that the vitamin E content in the oil was from 20 to 30 mg/100 g oil, and was similar for all the differently extracted oils. Although the coefficient of variation was found to be high, especially for oil extracted by the steaming method (14.3%) these oils contain vitamin E in similar amounts to the 26 mg/100 g levels reported in cod liver oil by Sonntag (1979). However, no report of vitamin E content in shark liver oil could be found in the literature.

Cholesterol was found in similar amounts (1.1%) in the three differently extracted oils, and these results agree with reports by Kayama *et al.* (1969) that liver oil produced from dog fish landed in Japan contains 1.03% of sterols which is 90% cholesterol. In contrast to the varying squalene content in shark liver oils, sterol or cholesterol contents in six species of shark (including dog fish) were no different (Kayama *et al.*, 1969). Iwaski & Harada (1984) reported that, using GC analysis, blue shark liver tissue contains 1.16% sterol (which was 96% cholesterol). Although these figures were obtained after saponification of freeze-dried liver tissue, they are similar to those previously quoted for oils because blue shark usually contains high amounts of oil in the liver tissue.

From Table 1 it can be seen that the fatty acid profiles were similar for the three differently extracted oils, despite differences in phospholipid contents. As for most fish oils, the dog fish liver oil contained reasonable levels of C20:5 and C22:6 (about 4.6 and 10.5%, respectively) which are nutritionally important in the human diet. However, C20:1 and C22:1 were found in higher concentrations (12.5% and 14.5%, respectively), which is similar to those found in many species of shark liver or body oil (Peyronel *et al.*, 1984; Ackman, 1982; Gruger *et al.*, 1964). It is still under discussion whether erucic acid (C22:1 *n*-9) causes cardiac lipidosis, but Ackman (1982) reported that erucic acid is not present to any important degree (6.5% of total C22:1), but rather the dominant isomer is the *n*-11 in fish oils.

Storage in air

The oils produced by the Bligh and Dyer, solvent and steaming methods were placed under air in half-filled, dark coloured bottles (capacity 50 ml) and stored at 25 ± 1 and 5 ± 0.5°C for up to 40 days.

The changes in the iodine values of the three different oils during storage at 25 and 5°C were not significant ($P > 0.05$) (Tables 2 and 3). This result was supported by the fatty acid composition results which show no significant changes during these storage trials.

Retinol (vitamin A) contents for the storage trials at 25 and 5°C are tabulated in Tables 2 and 3. At 25°C, the changes in vitamin A concentration in the oils after

Table 3. Free fatty acid, perioxide value, TBA value, retinol content, iodine value and fatty acid composition of dog fish liver oil stored at 5°C under air with light excluded[a]

Storage temperature	5°C								
	Oil extracted by Bligh and Dyer method			Oil extracted by solvent method			Oil extracted by steaming method		
Storage time (days)	0	24	31	0	24	31	0	28	40
Free fatty acid	0.08	0.08	0.06	0.05	0.08	0.11	0.07	0.21	0.19
(% of oleric acid)	(20.5)	(14.1)	(10.5)	(5.7)	(12.5)	(9.4)	(23.5)	(14.3)	(20.5)
Peroxide value	0.71	0.81	0.79	0.48	0.52	0.49	1.80	4.82	3.55
(meq 0/kg oil)	(8.1)	(7.2)	(5.6)	(9.1)	(7.5)	(6.1)	(7.7)	(12.4)	(14.3)
TBA value	—	—	—	—	—	—	1.09	5.27	11.46
(mg MA/kg oil)							(22.0)	(2.1)	(11.6)
Retinol content	44.8	50.4	49.5	56.5	55.9	56.1	61.6	50.9	50.3
(mg/100 g oil)	(5.0)	(4.2)	(6.7)	(4.1)	(5.9)	(3.2)	(4.7)	(3.7)	(0.2)
Iodine value	116	117	117	121	121	121	132	132	132
(mg iodine/100 g oil)	(0.3)	(0.6)	(0.1)	(0.4)	(0.2)	(0.3)	(0.2)	(1.2)	(0.1)
Fatty acid composition (relative to 16:0 = 1)									
C18:1	1.52	—	1.32	1.42	—	1.41	1.58	—	1.67
	(8.0)		(9.4)	(9.3)		(5.7)	(8.8)		(0.6)
C18:2	0.10	—	0.11	0.14	—	0.13	0.09	—	0.05
	(20.5)		(30.5)	(15.7)		(12.4)	(6.6)		(0.0)
C20:1	0.82	—	0.81	0.78	—	0.80	0.84	—	0.85
	(4.1)		(7.5)	(8.1)		(10.1)	(2.3)		(0.0)
C20:5	0.21	—	0.20	0.19	—	0.19	0.29	—	0.39
	(11.7)		(10.5)	(10.5)		(12.1)	(10.3)		(3.2)
C22:1	1.10	—	1.00	1.00	—	1.04	1.01	—	1.00
	(1.5)		(1.5)	(3.5)		(6.2)	(1.0)		(0.0)
C22:5+	0.82	—	0.81	0.79	—	0.80	0.72	—	0.79
C22:6	(5.6)		(7.5)	(5.7)		(4.9)	(4.2)		(3.8)

[a]Values are means of duplicate or triplicate determination; percentage coefficients of variation are given in parethesis. Oil extracted by the Bligh and Dyer or by solvent methods are oils from the same batch of liver. Oil extracted by steaming method was extracted from a different batch of liver.

28 days were found to be 30% for the steaming method, 8–11% for the solvent extraction method and zero for the Bligh and Dyer procedure. However, at 5°C only the oil extracted by the steaming method showed a reduction in vitamin A, which was 18% after 28 days.

The PV of the solvent extracted oil increased faster than for the oil extracted by steaming. However, the decease in vitamin A content of oil extracted by the steaming method was faster than for the latter oil extracted by solvent. Thus the change of PV cannot directly reflect the loss of vitamin A in those oils.

Compared with the oils extracted by steaming or solvent methods, oil extracted by the Bligh and Dyer method seems to be the most stable, as indicated by the slower increase of PV and constant retinol content in this oil during the period of study. This correlates with the high phospholipid content of the Bligh and Dyer extracted oil. Yamaguchi & Toyomizu (1984) reported that the phospholipid may have a significant anti-oxidant effect in oils where the vitamin E contents are similar.

The steaming method is the extraction procedure most likely to be feasible on a commercial scale. Whilst the vitamin A in this oil is the least stable of the three, its stability can be enhanced by storing at 5°C rather than 25°C, using dark coloured storage containers, storing under nitrogen and addition of antioxidant.

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