IAFMM FISH OIL BULLETIN

international association of fish meal manufacturers

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No 16 October 1983

DETERMINATION OF SULPHUR CONTENT IN FISH OIL

- Nordic Method

1. GENERAL

Results obtained from two international collaborative trials involving four methods for determining sulphur levels in fish oil, indicate that the results obtained depend on the method and (see Appendix) Dohrman's method and the ASTM standard test method give the highest values. The Nordic method gives values close to the Dohrman method but lower. The Baltes method gives values about 25% of the values estimated by the Dohrman method.

Any contractual limits agreed on sulphur in fish oil, therefore, should be based on a specified analytical method. The Baltes method is not recommended for this purpose, and current scientific opinion suggests that the Nordic method might be the most appropriate.

2. PRINCIPLE

The oil is hardened catalytically by hydrogenation at 180°C and 1 atm. until an iodine value near 0 is reached.

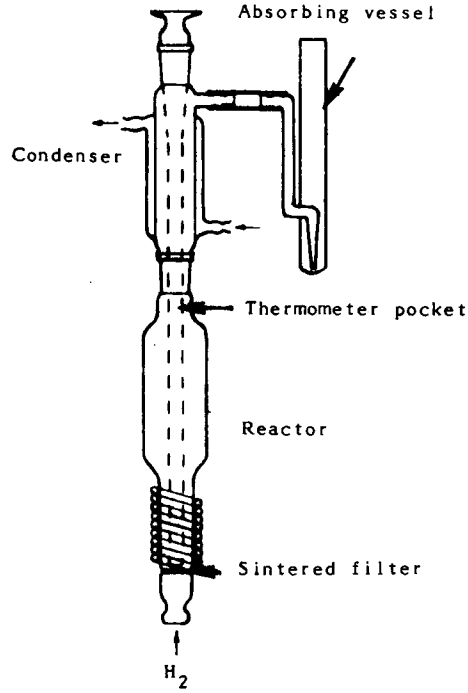
A commercial nickel catalyst is used. The poisoned part of the catalyst by the sulphur in the oil will precipitate as NiS. After hardening the NiS is released in isopropanol during admixture of hydrochloric acid, whereby H_2S is formed. The H_2S is absorbed in a 1 N NaOH-acetone mixture. The amount of HS^- is determined by continuous titration of the absorbing liquid with mercury acetate and dithizone as indicator.

3. APPARATUS

See figure

The upper 2/3 of the reactor has an inner diameter of 45 mm, the lower 1/3 has an inner diameter of 20 mm. The oil is held by a sintered filter (porosity G3), through which hydrogen is led.

The narrow part of the reactor is wound with 2 m heating coil taken from an ISOPAD ISOTAPE type ITH-150 (165 W). This heating coil is insulated with one layer of asbestos tape (same trademark as above). The heating coil is connected with a vario transformer.



The thermometer pocket is half filled with silicone oil.

4. REAGENTS

Nickel catalyst: A commercial Ni catalyst, e.g. G-53 from Süd-Chemie A.G., Girdler Katalysatoren, Lenbachplatz 6, 8000 München, Germany. This catalyst contains about 25% Ni. The amount of Ni should correspond to 1% of neutralised, bleached oil samples, and to 1.5 - 2% of unbleached oil samples.

Hydrogen: H_2, industrial grade, in cylinders.

Mercury acetate: Dissolve 1.352 g HgO (analytical grade) in 50 ml 2% acetic acid, dilute to 2 ℓ 1 ml mercury acetate solution ~ 100 µg sulphur.

Dithizone solution: Dissolve 0.01 g diphenylthiocarbazone in 10 ml analytical grade acetone. Keeps 1 week.

Hydrochloric acid: 1.5 vol. concentrated HCl + 1 vol. distilled water.

Sodium hydroxide: 1 N NaOH

Acetone, analytical grade.

Isopropanol, analytical grade.

5. DETERMINATION

Weigh the required amount of catalyst, transfer it to the reactor, start the hydrogen flow, and add the oil. The amount of oil depends on the sulphur content. It should correspond to a titration volume of 2-3 ml.

Mount the condenser and the thermometer pocket containing a thermometer on the reactor.

Start heating (100-125 Volt) and adjust the hydrogen flow to avoid too much foaming.

When the temperature has reached 150-160°C, lower the heating (to 75 Volt). Carry on hydrogenation at 180°C +/- 10°C(corresponding to 75-100 Volt). Watch the temperature during the first quarter of an hour. After that, only sporadic controlling is required.

Hydrogenation takes 2 hours. Hardening should be as complete as possible - iodine value should be less than 10.

Stop heating when hydrogenation has ended. Mount the absorption vessel on the condenser and start the water cooling. Cool the oil till under the boiling point of isopropanol (82.3°C) and add 25 ml isopropanol to the reactor. If the oil has hardened completely, it will be solidified. Add 10 ml diluted hydrocloric acid to the reactor; it will form, a third layer. Add immediately: 10 ml 1 N NaOH + 10 ml acetone + 5 drops of dithizone solution to the absorption vessel. Adjust the hydrogen flow to allow 1-2 bubbles to pass through the absorption vessel per second. Add 1-2 drops mercury acetate to the absorption liquid, which will be dark pink.

Start heating again (100 volt) till the boiling point of isopropanol is reached. The fat, containing the catalyst, will dissolve and the catalyst will come into contact with the hydrochloric acid. The hereby formed H_2S is absorbed in the absorption liquid, the colour of which changes to yellow. Titrate continuously until a constant pink colour appears. Now increase the hydrogen flow to remove the last H_2S.

Important: Keep isopropanol from passing into the absorption liquid. Keep absorption liquid from entering the tubing. It is possible for one person to run 3-4 hydrogenation units at a time. The standard deviation (s) is 0.7 ppm S.

6. DETERMINATION OF SULPHUR CONTENT OF CATALYST

The catalyst often contains some sulphur, which will be present in the analysis and some of the sulphur in the catalyst is deliberated by the hydrogenation and may be determined as HS^-. A blank oil (e.g. soybean oil free of sulphur) is hydrogenated in a similar way and deliberated sulphur determined to allow determination of K as shown on below.

Calculate K:

$$K = \frac{100 \times a}{c}$$

where

K = sulphur content of catalyst(p.p.m)
a = titration volume (ml)
c = amount of catalyst (g)

7. CALCULATION

$$\text{p.p.m. } S = \frac{100 \times a - K \times c}{0}$$

where

p.p.m. S = sulphur content in oil sample (p.p.m.)
a = titration volume (ml)
K = sulphur content of catalyst (p.p.m.)
c = amount of catalyst (g)
0 = amount of oil sample (g)

8. CLEANING OF APPARATUS

Remnants of the catalyst on the filter may be dissolved in concentrated hydrochloric acid.

Clean the filter of hardened oil by sucking chloroform or other solvent through the filter.

Rinse the apparatus with distilled water.

Dry the filter completely before next determination. (If the filter is not dry, the oil sample will splash).

9. REFERENCES

Baltes. J. (1967): Fette - Seifen - Anstrichmittel 69, 512.

Grimsvang, T., T. Hansen & O. Jordal (1971): 6. Nordiske Fettsymposium, Grenå, Denmark.

APPENDIX

INTERNATIONAL COLLABORATIVE TRIAL ON THE DETERMINATION OF SULPHUR IN FISH OIL

-by-

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1. Introduction

Sulphur is a catalyst poison, and the sulphur content in fish oil affects the hardening ability of the oil.

If the sulphur content is high, buyers will claim a reduction in price.

Therefore, it is necessary to estimate the sulphur content in fish oil as reliably as possible.

Today producers and buyers of fish oil use several and different methods for the determination of sulphur.

Bearing that in mind, the Scientific Committee of the International Association of Fish Meal Manufacturers (IAFMM) decided to run a collaborative trial to determine sulphur in fish oil.

The results of the two first trials are reported in this paper.

2. Experimental

Five laboratories participated in each trial (see table 1). The laboratories received 8 coded samples of fish oil for sulphur determination. In trial 1, the laboratories also received a hidden duplicate. The laboratories used their normal method for analysing sulphur, and the samples were analysed at least three times. The samples were not pretreated (centrifuged, filtered or otherwise cleaned) unless the method of analysis so demanded.

Table 1. List of participating laboratories.

Name of Collaborator	Organisation	Address
A. Brodin	Norwegian Herring Oil and Meal Industry Research Institute	5033 Fyllingsdalen Bergen Norway
A. Verway	Chemical Laboratory "Dr.A.Verway"	32 Coolhaven 3024 AC Rotterdam The Netherlands
P. Olafsson	Icelandic Fisheries Laboratories	Skudlagötu 4 Reykjavik Iceland
R. Merkle	Hobum	Hamburger Oelwerke Brinckman & Mergell Postfach 90 07 40 D-21 Hamburg 90 Germany
H.O. Sørensen	Andelssildeoliefabrikken A.m.b.A.	Ny Havn DK-6700 Esbjerg Denmark
M. Tudela	Pesca Peru	Av. Javier Prado Este 2465 PO.Box 2881(Lima 100) San Luis, Lima 30 Peru
N.C. Jensen	Technological Laboratory Ministry of Fisheries	Technical University Building 221 DK-2800 Lyngby Denmark

The following methods of determining sulphur were used in the trials:

1) Dohrman's [1] method was used by two laboratories in trials 1 and 2.

The method is based on combustion of the oil and determination of the sulphur dioxide formed.

2) The Standard Test method [2] was used by one laboratory in trial 1.

This method is also based on combustion of the oil. The sulphur was determined as sulphate.

3) Baltes' [3] method was used by two laboratories in trial 2.

The method is based on a hardening of the fish oil at 80°C for 30 min., using Raney - Nickel as a catalyst. The sulphur on the catalyst was determined as hydrogen sulphide.

4) Nordic [4] method was used by two laboratories in trial 1 and by one laboratory in trial 2. The oil was hardened at 180°C for 2 hours, using a commercial Nickel catalyst. The sulphur on the catalyst was determined as hydrogen sulphide.

The results in this report are given in ppm (µg S/g oil).The values of Cochran's and Dixon's tests, repeatability and reproducibility were calculated according to International Standard ISO-5725[5].

3. Results and statistical analyses

It was not possible to separate the method variance from the laboratory variance in the statistical analyses because the degrees of freedom were too low. Therefore the between laboratory variance (S_L^2) also included the variance between methods.

After the outliers on each level had been discarded, the mean value m, the repeatability r and the reproducibility R were preliminarily determined. The repeatability was defined[5] as $2.83\sqrt{S_r^2}$ where S_r^2 was the between-duplicate variance. The reproducibility R was defined[5] as $2.83\sqrt{S_r^2 + S_L^2}$.

The statistical analyses in trials 1 and 2 were of minor value because:

a) The reproducibility R included the variance between the methods

b) There was interaction between the laboratories/methods and the fish oil lot.

c) Some results were missing.

d) The degree of freedom was low and Cochran's and Dixon's tests for outliers were therefore superficial.

The individual results in trial 1 are presented in table 2.

Table 2. Collaborative results, trial 1, for the determination of sulphur (ppm) in fish oil.

Method / Laboratory	1 Atlantic Menhaden	2 Capelin	3 Norwegian Sand-eel	4 Mexican Anchovy	5 Tuna	6 Atlantic Menhaden	7 Danish Sprat	8 Mackerel
			FISH OIL LOT					
Dohrman	9.0	15.8	127	14.8	43.2	186	17.8	23.1
	9.0	15.8	127	14.8	42.8	185	17.3	22.0
Lab.1	9.0	15.0	126	14.2	42.2	182	17.0	22.0
					**)	**)		
Dohrman	10	15	111	16	42	152	18	22
	10	15	114	16	42	166	19	22
Lab.2	9	15	104	15	39	167	18	22
			109		a)			
Nordic	10.9	15.4	103	15.1	26.5	162	19.9	22.2
Method	10.8	15.7	113	15.1	26.4	157	19.6	22.3
Lab.3	10.5	15.8	108	15.4	27.0	162	20.1	22.5
Nordic	10.1	16.6	100.7	14.5	31.7	168.4	17.3	19.7
Method	9.3	16.0	101.1	15.0	31.6	168.2	18.2	20.9
Lab.4								
		*)					**)	
Standard	11.4	14.6	124	12.5	36.5		24.6	
Test Method	10.5	12.5	120	14.0	37.0	-	20.2	-
Lab.5	10.0	12.9	121	13.8	37.2		20.6	

*) Straggler - see text
**) Outliers - see text
a) Suspicious value - see text

Some results were missing but there were no obvious irregularities. The results of lab. 3, lot 5 ,were suspiciously low compared with the other results and especially with the results of lab. 4, which used the same method as lab. 3. The results of lab. 3, lot 5, were not discarded. Cochran's[5] test led to the identification of 1 straggler (*) and 3 outliers (**), which were discarded before computing m, r and R.

The values for m, r and R in trial 1, arranged in order of magnitude of m, are presented in table 3.

Table 3. The mean value m (ppm S), the repeatability r (ppm S) and the reproducibility R (ppm S) on each level in trial 1

	FISH OIL LOT							
	1	4	2	7	8	5	3	6
m mean	10	15	16	18	22	35	114	171
r repeatability	1.4	1.5	0.9	1.3	1.3	1.0	9.5	6.4
R reproducibility	2.4	2.8	1.6	3.4	2.8	20	29	37

The results will be discussed after the presentation of trial 2. The hidden duplicate values in trial 1 are presented in table 4. Since the duplicate values were identical random errors within laboratories can be excluded.

The sulphur content determined by Baltes' method (lab. 6 and lab. 7) were obviously too low. Only 25% of the sulphur was determined by this method as compared with Dohrman's method.

The results of lab. 6 and lab. 7 were discarded, because:

1) the results were too low
2) there were only single values, which did not conform to the protocol
3) some data were missing

Table 4. Results from duplicate samples, trial 1.

Method Laboratory	Lot	Sulphur content ppm. Values	mean	s.d.	Values	mean	s.d
Dohrman Lab. 1.	5	43.2 42.8 42.2	42.7	0.5	44.2 43.5 41.9	43.2	1.2
Dohrman Lab. 2.	8	22 22 22	22	0	22 23 22	22.3	0.6
Nordic Method Lab. 3.	8	22.2 22.3 22.5	22.3	0.2	21.9 22.4 22.6	22.3	0.4
Nordic Method Lab. 4.	7	17.3 18.2	17.8	0.6	17.5 17.9	17.7	0.3
Standard Test Method Lab. 5.	7	24.6 20.2 20.6	21.8	2.4	20.0 20.6 21.0	20.5	0.5

The individual results in trial 2 are presented in table 5.

Table 5. Collaborative results, trial 2, for the determination of sulphur (ppm) in fish oil.

Method Laboratory	FISH OIL LOT 1	2	3	4	5	6	7	8
Dohrman Lab. 1.	8.2 8.2 7.7	36.8 34.9 37.1	32.6 33.1 33.1	16.4 17.4 16.9	18.6 18.1 17.6	32.3 32.4 32.1	20.4 19.6 20.6	35.3 36.6 35.4
Dohrman Lab. 2.	6 7 7	39 37 37	35 33 34	10 10 9	15 15 15	33 34 33	19 18 19	37 37 36
Nordic Method Lab. 3.	6.8 6.4 6.6	30.6 27.3 29.3	27.2 27.1 24.8	12.5 12.0 12.9	12.3** 15.6 14.3	26.9 26.1 27.5	16.6 18.0 16.0	32.9 32.2 32.2
Baltes Lab. 6.	1	9	8.5	3	3.5	9	4.5	13.5
Baltes Lab. 7.	2.5	5.1	5.6	2.1	-	6.7	3.1	8.0

** Straggler- see text.

The Nordic method seemed to give lower results than Dohrman's method, but the results of lab. 3 were not discarded.

Cochran's test led to one straggler (**), caused by a zero variance in one of the three sets of data, but the test was too rough and the straggler was not discarded.

The values for m, r and R in trial 2 arranged in order of magnitude of m is presented in table 6.

Table 6. The mean value m (ppm S), the repeatability r (ppm S) and the reproducibility R (ppm S) on each level in trial 2.

	FISH OIL LOT							
	1	4	5	7	6	3	2	8
m mean	7	13	16	19	31	31	34	35
r repeatability	1.1	1.5	2.8	2.1	1.5	2.8	3.8	1.7
R reproducibility	2.5	10	6.4	5.0	10	12	13	6.5

4. Discussion

The results in table 3 and table 6 are shown in fig. 1, where the repeatability r and the reproducibility R were plotted as a function of the mean value m on each level.

Except for two sets of data, the sulphur content was lower than 40 ppm. The following conclusions are therefore based on the results of the analyses in the range of 0 - 400 ppm sulphur.

The repeatability r scatters with small differences, but seems to be independent of the sulphur level. On the contrary, the reproducibility R scatters with great differences and R seems to be dependent on the level. R increases with increasing sulphur content in the oil. In extreme cases R is 75% of the mean value.

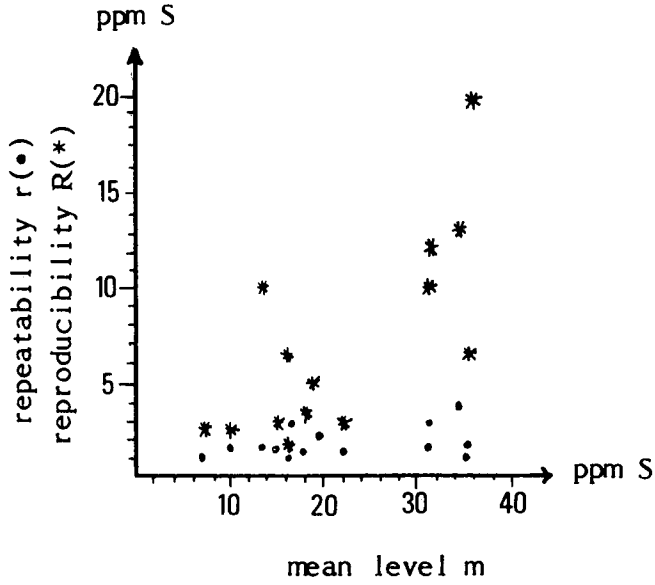

Fig. 1. Plot of r and R against m on each level. Two sets of data, (m=114, r= 9.5, R= 29) and (m= 171, r= 6.4, R=37), are not shown in the figure.

The only reasonable explanation of the variation and unacceptable magnitude of R is that R included the between - method variance.

In other words - **the result is dependent on the method,** and the differences between the methods increase with increasing sulphur content.

The results from table 2 and 5 are shown in fig. 2, where the average value (m) of the different samples of fish oil for all methods are plotted against the average values for the individual methods.

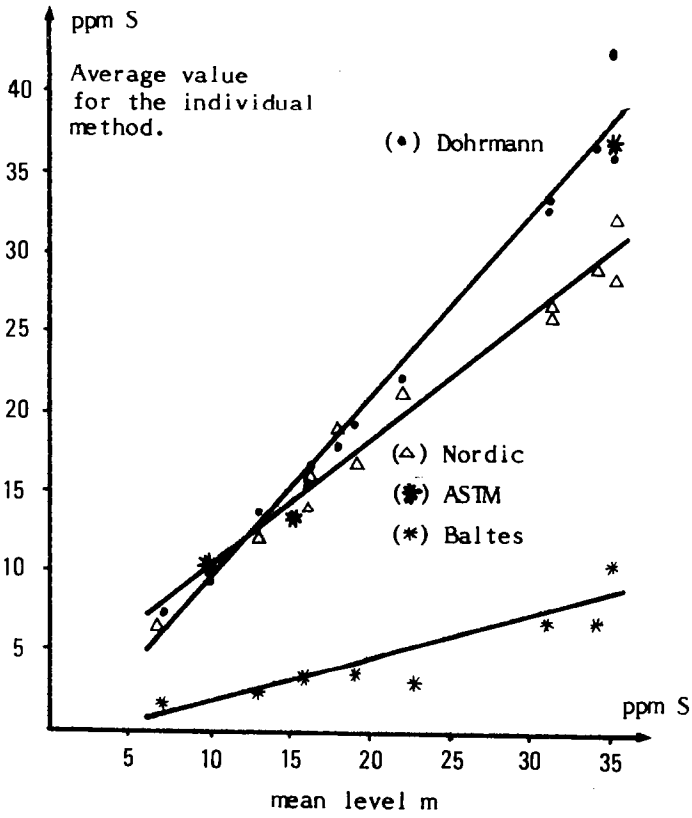

Fig.2 Plot of the average values of the different samples of fish oil for all methods against the average values for the individual methods.

The following conclusions can be drawn:

1) Dohrman's method and the ASTM Standard Test method give the highest values.

   Dohrman's method determines nearly the total sulphur content by combusting all the sulphur compounds to SO_2[6].

2) The Nordic method gives values close to Dohrman's but lower.

   The method determining that part of the sulphur which reacts with a commercial Nickel catalyst and precipitates as NiS.

3) Baltes' method gives the lowest result - values about 25% of what Dohrman's method gives.

   The method determines that part of the sulphur which reacts with Raney -Nickel and precipitates as NiS.

The Nordic method differs from the original method on the following points:

a) It gives higher results.

b) By applying a Nickel-catalyst which is used by the industry to harden fish oil.

c) It has a longer hydrogenation time, and

d) A higher hydrogenation temperature.

5. Conclusion

The main conclusion of this work is that the result of a sulphur analysis depends on the method used. Thus, when buyers and producers of fish oil are dicussion limit values of sulphur in fish oil, it is important that the method of analysis be known.

In the future standardization work on determination of sulphur in fish oil either one method should be chosen and standardised, or, the methods should be standardised one by one and then correlated with each other.

6. Acknowledgement

The author wishes to express his thanks to all the participating laboratories, to Dr. S.M. Barlow of the International Association of Fish Meal Manufacturers, to Mr. A. Bimbo of the Zapata Haynie Corporation, Reedville, U.S.A., and to Mr. W. Schmidtsdorff of the Technological Laboratory, Ministry of Fisheries, Lyngby, Denmark, for the sample collection, distribution of the samples and the co-ordination of the trials. Special thanks are offered to Mr. Gordon Smith of the Torry Research Station, Aberdeen, Scotland for valuable advice.

7. References

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