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RESEARCH REPORT

1983-1

INTERNATIONAL COLLABORATIVE TRIAL ON THE DETERMINATION OF SULPHUR IN FISH OIL

by

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The main conclusion to be drawn from two collaborative trials, involving four methods for determining sulphur in fish oil, is that the result depends on the method.

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

A modified Baltes' method gives values close to Dohrman's but lower, and Baltes' method gives values about 25% of the values estimated by Dohrman's method.

INTERNATIONAL COLLABORATIVE TRIAL ON THE DETERMINATION OF SULPHUR IN FISH OIL

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	Organization	Address
A. Brodin	Norwegian Herring Oil and Meal Industry Research Institute	5033 Fyllingsdalen Bergen Norway
N. Urdahl		
A. Verwey	Chemical Laboratory "Dr. A. Verwey"	32 Coolhaven 3024 AC Rotterdam The Netherlands
P. Olafsson	Icelandic Fisheries Laboratorium	Skúlagötu 4 Reykjavik Iceland
R. Merkle	Hobum	Hamburger Oelwerke Brinckman & Mergell Postfach 90 07 40 D-21 Hamburg 90 Germany
H.O. Sørensen	Andelssildeolie-fabrikken 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.

NORDIC METHOD DIFFERENCE

Modified Baltes'[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 hrs, 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, 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	FISH OIL LOT							
	1 Atlantic Menhaden	2 Capelin	3 Norwegian Sand-eel	4 Mexican Anchovy	5 Tuna	6 Atlantic Menhaden	7 Common Sprat	8 Mackerel
Dohrman Lab. 1.	9.0 9.0 9.0	15.8 15.8 15.0	127 127 126	14.8 14.8 14.2	43.2 42.8 42.2	186 185 182	17.8 17.3 17.0	22.7 22.0 22.0
Dohrman Lab. 2.	10 10 9	15 15 15	111 114 104	16 16 15	42 **) 42 39	152 **) 166 147	18 19 18	23 23 21
Modified Baltes Lab. 3.	10.9 10.3 10.5	15.4 15.7 15.8	109 103 113 108	15.1 15.1 15.4	26.5 a) 26.4 27.0	162 157 162	19.8 19.6 20.7	22.2 22.3 22.5
Modified Baltes Lab. 4.	10.1 9.2	16.6 16.0	100.7 101.1	14.5 15.0	31.7 31.6	168.4 180.2	17.2 18.2	19.7 20.9
Standard Test Method Lab. 5.	11.4 10.5 10.0	14.6 *) 12.5 12.9	124 120 121	12.5 14.0 13.8	36.5 37.0 37.2	-	24.6 **) 20.2 20.8	-

*) 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.

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
Modified Baltes Lab. 3.	8'	22.2 22.3 22.5	22.3	0.2	21.9 22.4 22.6	22.3	0.4
Modified Baltes 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
Modified Baltes 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 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

The modified Baltes' 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 presentes 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.

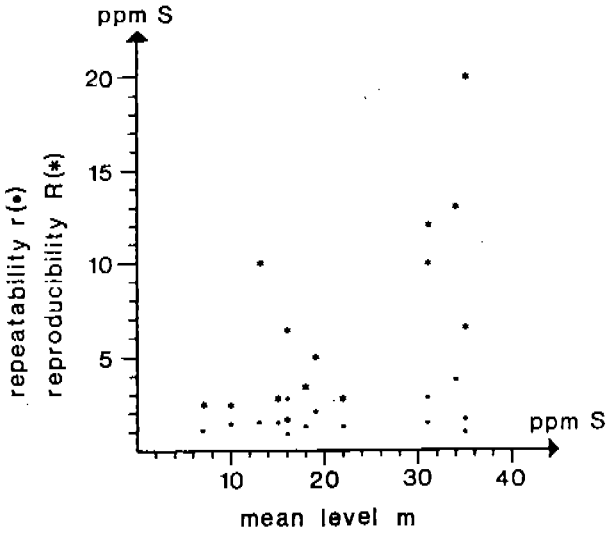

Fig. 1. Plot of r and R against m on each level. Two sets of data, (m=114,r= 9.5, R= 29) and (m= 171,r= 6.4, R=37), are not shown in the figure.

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 - 40 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.

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.

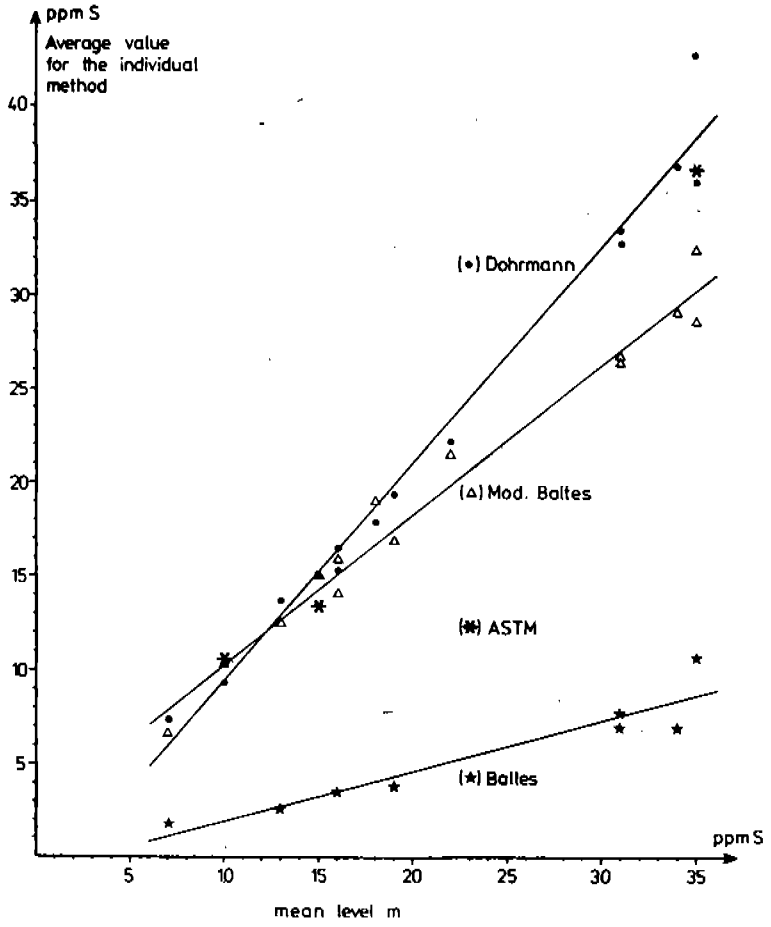

Fig. 2. Plot of the average values of the different samples of fish oil for all methods against the average values for the individual methods.

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 modified Baltes' 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 modified Baltes' 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.

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 produceres of fish oil are discussing 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 standardized

or

the methods should be standardized 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 coordination of the trials. Special thanks are offered to Mr. Gordon Smith of the Torry Research Station, Aberdeen, Scotland, for valuable advice.

7. References

1) Nilsen, Ø., E. Nygård & N. Urdahl: Instrumental Analysis of Sulphur in Fats and Oils. News Summary No 48, IAFMM, 1980,131.

2) Standard Test Method for Sulphur in Liquefied Petroleum Gases (Oxy-Hydrogen Burner or Lamp). American National Standard, ANSI/ASTM, D2784. (Reapproved, 1975).

3) Baltes, J.: Zur Kenntnis schwefelhaltiger Fette und ihres Härtungsverhaltens. Fette. Seifen. Anstrichmittel 69,1967, 512.

4) Determination of Sulphur Content in Fish Oil (Modified Baltes' Method). News Summary No 48, IAFMM, 1980, 139, + Corrigenda to the article by N.C. Jensen.

5) Precision of Test Methods - Determination of Repeatability and Reproducibility by Inter-Laboratory Test, International Standard ISO 5725 - 1981 (E).

6) Wallace,L.D., D.W. Kohlenberger, R.J. Joyce, R.T. Moore, M.E. Riddte, J.A. McNylty. Comparison of Oxidative and Reductive Methods for the Microcoulometric Determinations of Sulphur in Hydrocarbons, Analytical Chemistry, 42 (3), 1970,387.

SMB/RE 9th March 1983

Mr. Nils Chr. Jensen
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DENMARK

Dear Mr. Jensen

COLLABORATIVE TRIAL TO DETERMINE SULPHUR IN FISH OIL

Further to your letter of the 4th January 1983, I return the proof copy of your paper entitled "International Collaborative Trial on the Determination of Sulphur in Fish Oil". You will note that I have made a number of typographical changes in blue ink on the manuscript, together with a few notes for your consideration.

I took the opportunity of sending this paper to Mr. Gwedon Smith of Torry Research Station and he has raised a number of points which are as follows:

(1) In general the statistical analysis appears to have been carried out in a suitable manner.

(2) In the statistical analysis itself was only one of the hidden duplicates used? Although use of both would have unbalanced the design, it would have increased the degrees of freedom.

(3) On page 9, paragraph immediately following Figure 1, it is not true to say that the conclusions applied to all the analyses. For example, repeatability did not increase with mean level within the range of those samples included in the Figure, but was much higher for the two of high sulphur content which were not plotted.

I hope you find these comments of value.

Kind regards

P.T.O.

Yours sincerely
International Association of Fish Meal Manufacturers

S.M. Barlow
Director General

Encl.

GLS/AL

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Dr S M Barlow
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Your reference
SMB/RE
Our reference
TRS/108/7
Date
1 March 1983

Dear Stuart

INTERNATIONAL COLLABORATIVE TRIAL ON THE DETERMINATION OF SULPHUR IN FISH OIL

The following are my comments on Mr Jensen's paper, which you sent to me on 26 January, on the above subject.

1 In general, the statistical analysis appears to have been carried out in a suitable manner.

2 A similar point for discussion arises to that raised in relation to my analysis of the erucic acid trial. Is the repeatability calculated in the analysis a measure of analytical error through repeated determination of the same sample or does it measure sample-to-sample variation at the same laboratory at the same time? I am not sure of the answer, but I raise the question again. If the answer is "analytical error" then the only data in this trial allowing a measure of repeatability would be the small number of "hidden duplicates". I am sure you can answer the question to your own satisfaction before involving Mr Jensen.

3 In reply to your comment on page 5 - the stars indicate a group of triplicates whose variance is unusually high, they do not relate to single values. As such they are the correct conclusions from application of Cochran's test. What might then have been done was to investigate the reason for such large variance, eg (Lab 5, sample 7) one of the triplicates perhaps being in error.

4 I do not understand the paragraph on page 6, in particular the point about duplicate values being identical. What duplicate values? Your question is answered above under item 2.

5 In the statistical analysis itself, was only one of the hidden duplicates used? Although use of both would have unbalanced the design, it would have increased the degrees of freedom.

6 Your comment on page 7. As far as I can make out, labs 6 and 7 did not take part in the first test. It could also be considered that by presenting single values these labs did not conform to the protocol and did not really take part in the trial. The third reason on page 7 seems flimsy. An analysis can cope with one missing value.

7 On page 9, paragraph immediately following figure 1, it is not true to say that the conclusions apply to all the analyses. Repeatability did not increase with mean level within the range of those samples included in the figure, but was much higher for the two of high sulphur content which were not plotted.

8 Your comments on page 10. I would agree that graphs would help. When it comes to submission for publication, you would have to be careful that graphs and tables did not repeat themselves.

Best wishes

Yours sincerely

Gordon

G L Smith

SMB/RE 26th January 1983

Mr. Gordon Smith
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Dear Gordon

INTERNATIONAL COLLABORATIVE TRIAL ON THE DETERMINATION OF SULPHUR IN FISH OIL

I enclose a photocopy of a paper prepared by Mr. N.C. Jensen of Denmark on the above subject. The handwritten comments on the paper are my own, and I would appreciate your comments on them, particularly those addressed to you.

I would also very much appreciate your overall assessment of Mr. Jensen's statistical approach.

I will await your comments before combining them with my own and sending them off to Mr. Jensen.

Kind regards

Yours sincerely
International Association of Fish Meal Manufacturers

S.M. Barlow
Director General

Encl.

SMB/RE

10th January 1983

Mr. Nils Chr. Jensen
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Dear Mr. Jensen

COLLABORATIVE TRIAL TO DETERMINE SULPHUR IN FISH OIL

Thank you for your letter of the 4th January 1983. I look forward to reading the enclosures.

I am just about to depart for a two-week trip to the U.S.A. However, I shall be taking your letter and papers with me and I shall comment on my return.

Kind regards

Yours sincerely
International Association of Fish Meal Manufacturers

S.M. Barlow
Director General

FISKERIMINISTERIETS FORSØGSLABORATORIUM

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4 January 1983
NCJ/rn

Dear Dr. Barlow,

Collaborative trial to determine sulphur in fish oil.

./. Enclosed please find my paper concerning the two collaborative trials on the determination of sulphur in fish oil.

The paper includes the results and a statistical analysis of the results of both trials.

I would be glad if you would read the paper and make a critical and statistical comment on it.

The result of the Andelssildeoliefabrikken A.m.b.A., Esbjerg, in trial 1 (lab.No.1) differs from the results published at the Scientific Committee meeting in Copenhagen, 1981. The results have been divided by 1.25 in agreement with Mr. H.O. Sørensen, of the above factory.

If you can accept the paper, I shall send a copy (including the lab. code) to the participating laboratories for their comments. Afterwards, the paper can be published according to your decision.

Yours sincerely,

NC Jensen

Nils Chr. Jensen