

FISH OIL BULLETIN

No. 21 May 1987

Recommended Method for the Determination of the Free Fatty Acid Content in Fish Oil

1. General

The method described below is that published by the American Oil Chemists' Society (A.O.C.S. Official Method Ca 5a-40). The repeatability and reproducibility values have been determined with commercial fish oils with varying levels of Free Fatty Acids (FFA) during two international collaboration trials. (see appendix for details).

2. Principle

The sample is mixed with hot, neutralized alcohol and indicator and titrated with aqueous alkali.

3. Reagents

1. Ethyl alcohol, 95% (U.S.S.D. Formulas 30 and 3A are permitted). The alcohol must give a definite, distinct and sharp end-point with phenolphthalein and must be neutralized with alkali to a faint but permanent pink colour just before using.

2. Phenolphthalein indicator soln., 1% in 95% alcohol.
3. Sodium hydroxide solns., accurately standardized to 0.25 N.

4. Apparatus

1. Oil sample bottles, 115 or 230 ml. (4 or 8 oz.) or 250-ml. Erlenmeyer flasks.

5. Method

1. Samples must be well mixed and entirely liquid before weighing.
2. Weigh 7.05 + 0.05 g of samples into an oil-sample bottle or Erlenmeyer flask. Cap bottle and shake vigorously for one minute if oil has been blanketed with carbon dioxide gas.
3. Add 75 ml of hot, neutralized alcohol and 2 ml. of indicator.
4. Titrate with alkali shaking vigorously to the appearance of the first permanent pink colour of the same intensity as that of the neutralized alcohol before addition of the sample. The colour must persist for 30 seconds.

6. Calculations

1. The percentage of free fatty acids is calculated as oleic acid.

a. Free fatty acids as oleic, % =

$$\frac{\text{Ml. of alkali} \times 0.25 \times 28.2}{\text{Weight of sample}}$$

2. The free fatty acids are frequently expressed in terms of acid value instead of % free fatty acids. The acid value is defined as the number of mg. of KOH necessary to neutralize 1g. of sample. To convert % free fatty acids (as oleic) to acid value, multiply the former by 1.99.

7. Repeatability

The difference between the results of two parallel determinations carried out on simultaneous samples or in rapid succession by the same analyst should not exceed 6% of the mean FFA value.

lab 9 and consequently the estimate for variance between laboratories was artificially negative. The estimates came in line with those for the other samples when lab 9 was excluded.

As is shown in Figure 1, the repeatability and reproducibility (in absolute terms) showed a tendency to increase with mean value. The values are plotted against the means obtained from the Smalley trial, since a combined mean was considered unsuitable because of the change in values between the two experiments, and more data were taken from that trial than from the IAFMM one. The repeatability (in relative terms) varied from 2.0% to 6.6% of the Smalley means (excluding the effect of lab 9 on sample 5) and the reproducibility ranged from 6.4% to 11.3% of the means.

CONCLUSIONS AND RECOMMENDATIONS

1. Mean FFA values differed between the two trials, being higher in the IAFMM one.
2. Variation between replicates, and estimates of repeatability, could be meaningfully combined using the data from both trials.
3. Because of the shift in mean values, it was not meaningful to obtain an aggregated value for variance between all laboratories but it was possible only to strengthen the estimates of variance between laboratories within the same trial by pooling. This also applied to reproducibility.
4. Measures of variation in absolute terms increased with mean value.
5. Assuming that determinations in these trials were performed by some of the most experienced users of the method, it is possible to give recommendations on repeatability and reproducibility.

The difference between the results of two parallel determinations carried out on simultaneous samples or in rapid succession by the same analyst should not exceed 6% of the mean FFA value; for determinations, carried out on two samples by different analysts the difference should not exceed 11% of the FFA value.

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APPENDIX

REPEATABILITY AND REPRODUCIBILITY IN THE DETERMINATION OF FREE FATTY ACID CONTENT OF FISH OILS

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INTRODUCTION

Two collaborative experiments had been carried out on the determination of free fatty acids and iodine in eight fish oils. The first of these, organised in conjunction with the American Oil Chemists' Society (AOCS) Smalley Committee, involved 15 collaborators, and also included determination of moisture. It took place in 1981-82, and the methods were AOCS recommended. The second, involving the International Association of Fish Meal Manufacturers (IAFMM) Scientific Committee, took place later in 1982 using the samples of the same eight fish oils. There were also 15 participants, but they were not the same as for the other study. Each laboratory was given the freedom to use the method of its choice.

The present report examines both sets of data to give combined estimates of the repeatability and reproducibility of free fatty acid determinations.

RESULTS AND DISCUSSION

The separate analyses, previously reported, had indicated some sets of values which were outliers or stragglers. It is the present writer's belief that where such values are detected, they should not be discarded unless, after discussion with the organisers of the experiment, there is good reason to do so.

After such discussion, it was decided that from the Smalley trial, all the results from one of the laboratories (number 13), which were for only three samples, should be omitted, as should four of the sets of triplicate values. They are shown as asterisks in the table of means (Table 1). A dash indicates a sample for which a laboratory did not submit data.

It was decided that for the combined analysis eight laboratories in the IAFMM trial (numbers 1B, 2, 4, 6, 7, 8, 14 and 15) should not be

considered as they had not used the AOCs method of determination. No single remaining values were excluded.

The mean values are listed in Table 1. There is a clear and statistically significant difference between the values obtained in the two trials for each sample with a relative increase of between 5% and 17% from the mean values in the Smalley trial to those in the IAFMM trial. This would suggest that over the time between the trials, the samples had changed in some way.

The following measures of variation are shown in Table 2: variance between replicates, variance between laboratories, repeatability and reproducibility.

Repeatability and reproducibility are used here in accordance with the definitions in ISO 5725, being the values which the absolute difference between two single test results obtained with the same method on identical test material under (respectively) the same or different conditions may be expected to lie with 95% probability.

Mathematically, they are defined by

$$\text{Repeatability} = 2.83 s_r^2$$

$$\text{Reproducibility} = 2.83 \sqrt{s_r^2 + s_L^2}$$

where s_r^2 is the variance between replicates and

s_L^2 is the variance between laboratories

Although it was not agreed to discard any single sets of results from the IAFMM trial, it has been considered appropriate to indicate the effect on the measures of variation of one set with abnormally high variation.

Because of the change in mean values from one trial to the other it is not appropriate to treat the two trials as one to obtain combined figures for variance between labs or reproducibility. Instead these measures of variation from the two trials have been pooled to express variation between labs within a trial. This is not a problem for the variance between replicates and hence repeatability as the required combined figures are, in any case, obtained by pooling.

There was good agreement between the two trials on the values obtained for the measures of variation. In the one exceptional case, sample 5, the variance between replicates was distorted by the contribution from

8. Reproducibility

For determinations carried out on two samples by different analysts, the difference between the results should not exceed 11% of the FFA value.

FIGURE 1 PLOTS OF REPEATABILITY AND REPRODUCIBILITY AGAINST SMALLLEY MEAN VALUE

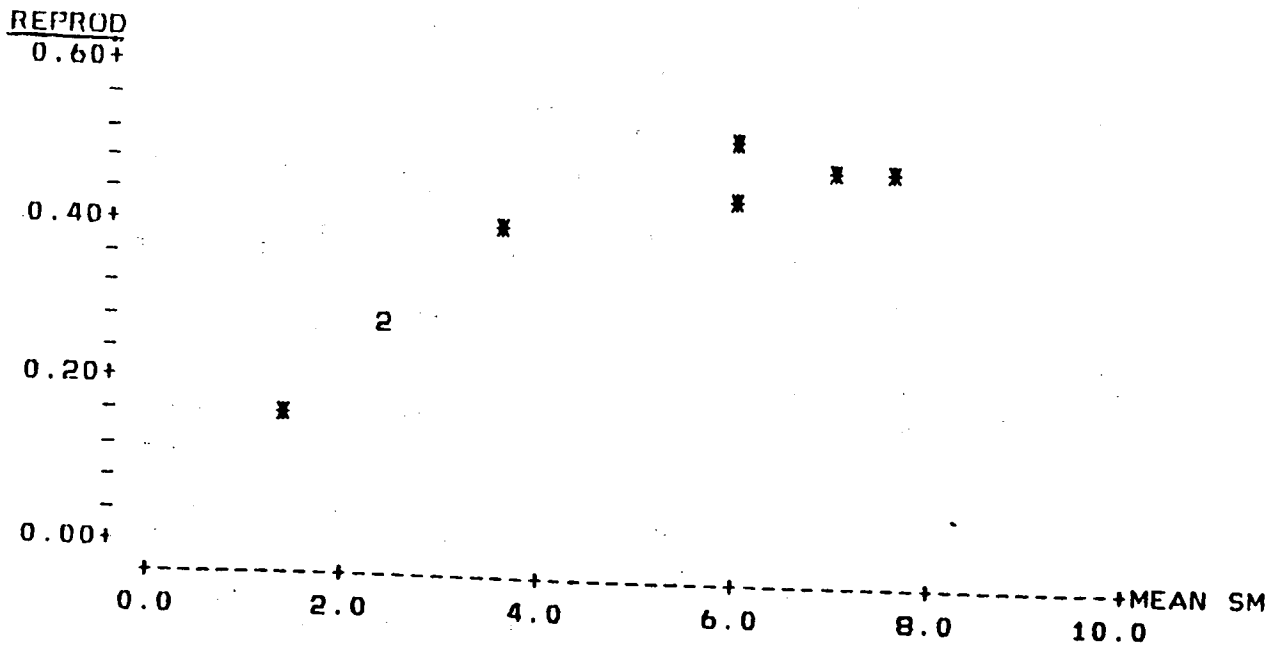
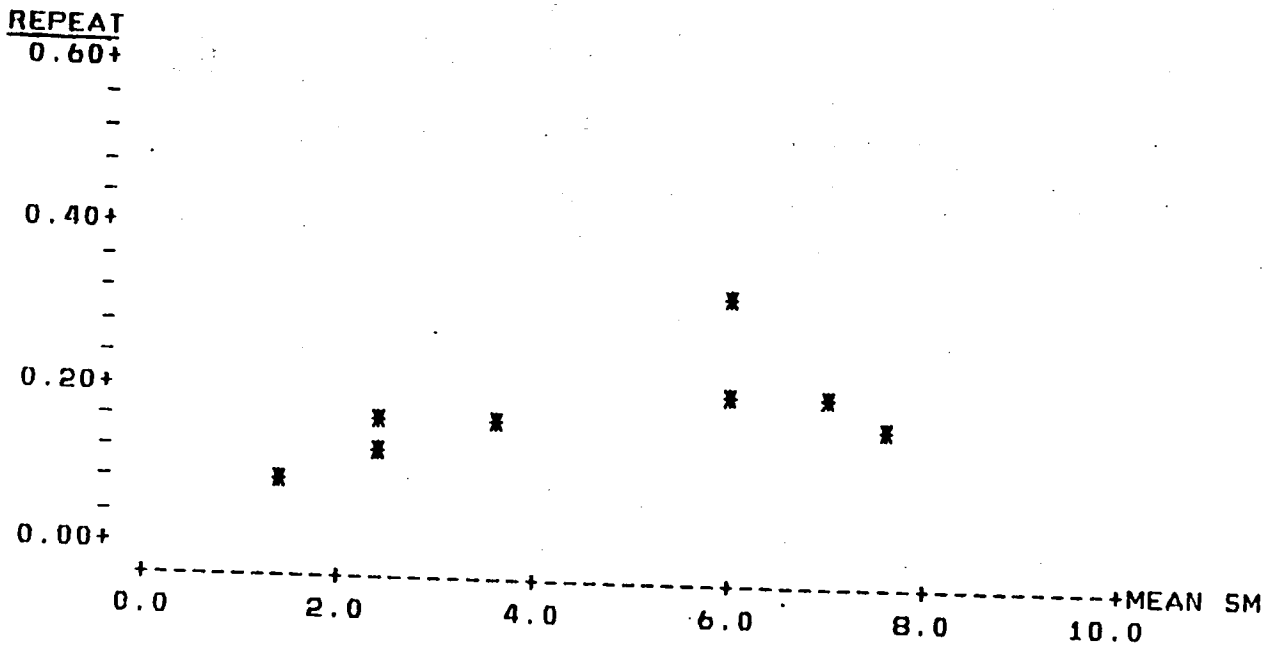


TABLE 1		COMBINED COLLABORATIVE STUDIES				MEAN FFA VALUES			
SAMPLES - LABS	1	2	3	4	5	6	7	8	
SMALLEY									
1	1.29	7.11	2.30	6.10	5.90	2.50	3.57	7.57	
3	1.35	7.14	2.45	6.23	5.83	2.44	3.57	7.62	
4	1.38	7.10	2.42	6.20	6.00	2.50	3.60	7.60	
5	1.34	7.06	2.42	6.21	5.96	2.46	3.55	7.60	
6	1.40	7.09	2.39	6.19	5.89	2.49	3.59	7.58	
7	1.31	7.09	2.40	6.19	5.94	2.50	3.54	7.57	
8	1.27	6.87	2.41	#	5.74	2.32	3.27	7.19	
9	1.30	7.24	2.46	6.12	5.78	#	3.42	7.59	
10	1.26	7.13	2.40	5.98	6.27	2.31	3.50	7.69	
11	1.24	7.46	2.41	6.01	5.91	2.38	3.66	7.54	
12	-	7.14	2.42	6.28	5.95	2.50	3.47	7.57	
14	1.26	6.86	2.13	5.70	5.78	2.28	3.39	#	
15	1.33	7.06	2.42	6.16	5.88	2.49	3.51	7.61	
16	1.26	#	2.39	5.83	5.99	2.47	3.54	7.29	
MEAN	1.31	7.10	2.39	6.09	5.92	2.43	3.51	7.54	
IAFMM									
3	1.55	7.90	2.64	6.69	6.43	2.70	3.86	7.90	
5	1.49	7.64	2.53	6.40	6.18	2.52	3.63	7.65	
9	1.52	8.05	2.42	6.63	6.54	2.46	3.63	8.29	
10	1.55	7.86	2.64	6.59	6.35	2.67	3.75	7.87	
11	1.59	7.53	2.56	6.37	6.03	2.67	3.77	7.79	
12	1.50	7.82	2.62	6.58	6.18	2.58	3.80	7.82	
13	1.50	7.90	2.66	6.55	6.30	2.74	4.12	7.87	
MEAN	1.53	7.81	2.58	6.54	6.29	2.62	3.79	7.88	
INCREASE PERCENT	0.22	0.71	0.19	0.45	0.37	0.19	0.28	0.34	
	17	10	8	7	6	8	8	5	

TABLE 2		COMBINED COLLABORATIVE STUDIES				MEASURES OF VARIATION IN FFA VALUES			
	SAMPLE -	1	2	3	4	5	6	7	8
VARIANCE BETWEEN REPLICATES	SMALLEY	0.0008	0.0058	0.0023	0.0098	0.0055	0.0014	0.0026	0.0024
	IAFMM	0.0011	0.0015	0.0027	0.0151	0.1235	0.0038	0.0033	0.0035
	POOLED	0.0009	0.0043	0.0024	0.0117	(0.0054) 0.0448	0.0022	0.0028	0.0028
VARIANCE BETWEEN LABORATORIES	SMALLEY	0.0022	0.0201	0.0058	0.0257	0.0152	0.0060	0.0092	0.0189
	IAFMM	0.0008	0.0312	0.0067	0.0096	(0.0125) 0.0093	0.0093	0.0278	0.0398
	POOLED	0.0017	0.0240	0.0061	0.0201	(0.0198) 0.0060	0.0072	0.0154	0.0262
REPEATABILITY	SMALLEY	0.082	0.215	0.136	0.281	0.210	0.107	0.145	0.138
	IAFMM	0.094	0.110	0.147	0.347	0.994	0.175	0.164	0.168
	POOLED	0.086	0.185	0.140	0.306	(0.209) 0.599	0.135	0.152	0.149
	PERCENT	6.6	2.6	5.9	5.0	(0.210) 10.1	8.6	4.3	2.0
REPRODUCIBILITY	SMALLEY	0.157	0.456	0.256	0.533	0.407	0.244	0.307	0.413
	IAFMM	0.124	0.512	0.274	0.444	0.994	0.325	0.499	0.589
	POOLED	0.146	0.476	0.262	0.504	(0.450) 0.663	0.275	0.382	0.482
	PERCENT	11.1	6.7	11.0	8.3	(0.420) 11.2	11.3	10.9	6.4
						(7.1)			

N.B. Figures in brackets are recalculated values after exclusion of the triplicate determinations (7.46, 6.44, 5.71) by Lab 9 on sample 5.