

Fatty Acid Analysis

In the last decades there has been an increasing focus on marine omega-3 fatty acids due to the positive health effects both in pets, livestock, farmed fish and humans.

The supply of omega-3, and especially EPA and DHA, has become short. As a result, optimizing of food/feed recipes regarding omega-3 content is of great importance, and the focus on new sources of omega-3 fatty acids has increased both for the food and feed industry.

When there is short of a product in the market, the prices rise, and possibilities for purchasing poor quality products and fraud increase. In the omega-3 market, there is then important to have reliable and correct analysis of product quality, total content of omega-3 and especially EPA and DHA.

Regarding optimizing of food, feed and healthcare products it is also important to know the amount of omega-3 in the recipe and the finished product.



Project objectives

Due to the demanding omega-3 market and thus the need for a reliable, quantitative analysis of fatty acids, Eurofins Food & Feed Testing Norway AS started a project aiming to optimize the quantitative analysis of omega-3 fatty acids. The project was made in cooperation with the accredited laboratories Eurofins Steins Laboratorium in Denmark (Eurofins DK) and Eurofins Food Testing Rotterdam B.V. in the Netherlands (Eurofins NL). The project had a special focus on marine matrixes containing omega-3 fatty acids.

Analytical methods

Based on gas chromatography, there are different methods for analysing fatty acids, but most analysis is based on two different principles.

1) By area percent

The most common method for analysis of fatty acids has been to analyze and report fatty acid composition by *area percent* (A%). This method measures and reports the content of different fatty acids based on the sum of identified and unidentified fatty acids (results given in % of fatty acids). In the laboratory business many labs use a fixed fatty acid factor like 0,956 to calculate fatty acids in g/100g sample from A% and fat content of the sample. This factor will, however, vary in different products depending on the amount of fatty acids in the lipid phase. From experience, a factor like 0,956 can overestimate the amount of fatty acids by 10-30 %, depending on matrix.

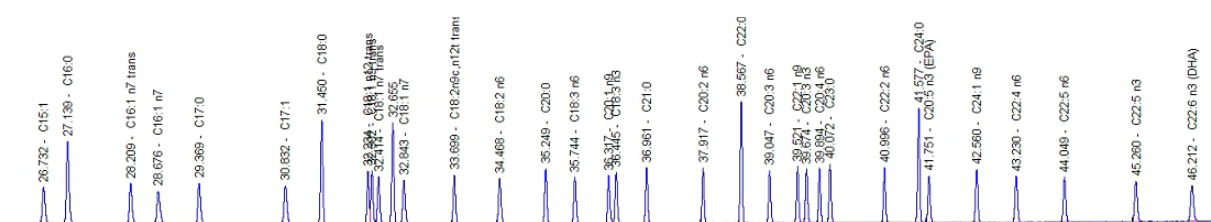
2) Internal Standard

The other method is based on quantitative analysis of fatty acids in the sample (g/100g) by use of *internal standard* (IS).

Because fat also contains other lipid components than only fatty acids, the two methods described above will give different results depending on the lipid composition of the fat.

Materials and methods

Both Eurofins DK and Eurofins NL were already experienced in the analysis of fatty acids using internal standard, and the two laboratories use similar methods based on the same standard, ISO 12966-2 and -4. In the present project, their methods have been compared and optimized with special focus on the quantitative analysis of marine omega-3 fatty acids. For comparative purposes, samples have also been analysed by an external lab approved by AOCS.



Samples

In total 24 samples were included in the project:

- Fish oil (5 samples)
- Fish feed (5 samples)
- Fish meal from herring (5 samples)
- Salmon muscle (4 samples)
- Rape seed oil (3 samples)
- Linseed oil (1 sample)
- Soy oil (1 sample)

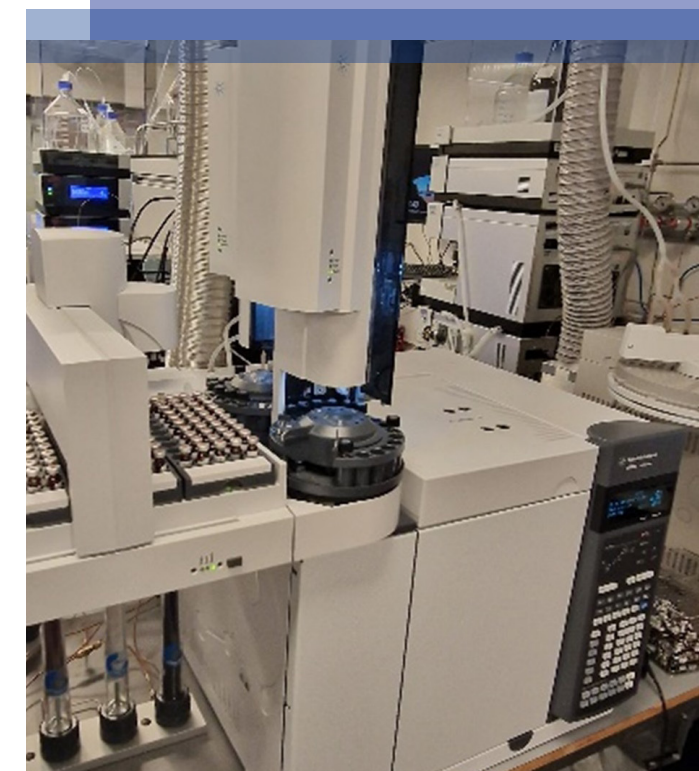
Samples were split in three by Eurofins Norway, and sent to Eurofins DK, Eurofins NL and external lab, respectively, for analysis.

Fatty acid analysis

For solid samples, that are not pure fats or oils, extraction of fat was performed prior to the fatty acid analysis and an internal standard is added to the extracted oil before transmethylation.

Fatty acids are analyzed by gas chromatography (GC) after conversion to fatty acid methyl esters (FAMES) which are more easily separated and quantified than either triglycerides or free fatty acids. The fatty acids are trans-esterified to form FAMES. FAMES are then separated on the GC and quantified using a flame ionization detector (FID). Separations are performed with a polar wax type capillary column where fatty acids are separated based on chain length and saturation. The FID burns the FAMES producing ions who generates an electrical current which is measured and plotted as the response in the chromatogram. Peaks from the chromatogram are integrated for further calculation.

The results are calculated on product or in the fat by use of an internal standard and the total fat content of the sample.



Gas chromatograph equipped with autosampler and flame ionization detector (FID)

Results and discussion

Overall, the results from different laboratories appear to correlate well. Figures 1-4 show comparisons among the different laboratories regarding analysis of total omega-3 and DHA/EPA.

Table 1 provides an overall comparison of results from Eurofins labs and the reference lab for total omega-3, EPA, and DHA in the matrixes tested. The most significant difference between the labs is in total omega-3 for fish oil, calculated at 8%. Such variations are considered normal when comparing analyses and laboratories of this nature.

Table 1: Correlation between Eurofins results and reference lab for all samples tested

	Fish oil	Fish meal	Fish feed	Salmon muscle
EPA	1,04	0,98	0,99	1,00
DHA	1,04	1,00	0,99	1,02
Omega-3	1,08	0,99	0,99	0,99

Marine oils

Figure 1 shows average results for 5 fish oils. Eurofins DK reports slightly lower values than the other laboratories, both for omega-3, EPA and DHA. The results are within 8 % difference from the reference lab.

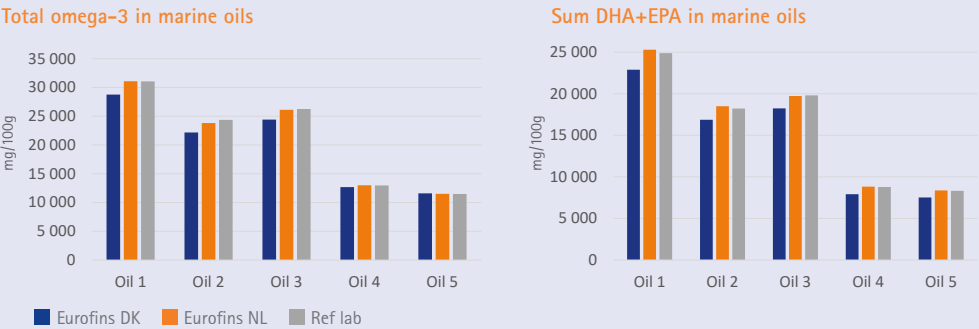


Figure 1: Results marine oils

Fish meal

Figure 2 shows average results for 5 fish meals. Results from all laboratories show similar levels of omega-3, EPA and DHA, varying in both positive and negative direction. The results are within 6 % difference from the reference lab.

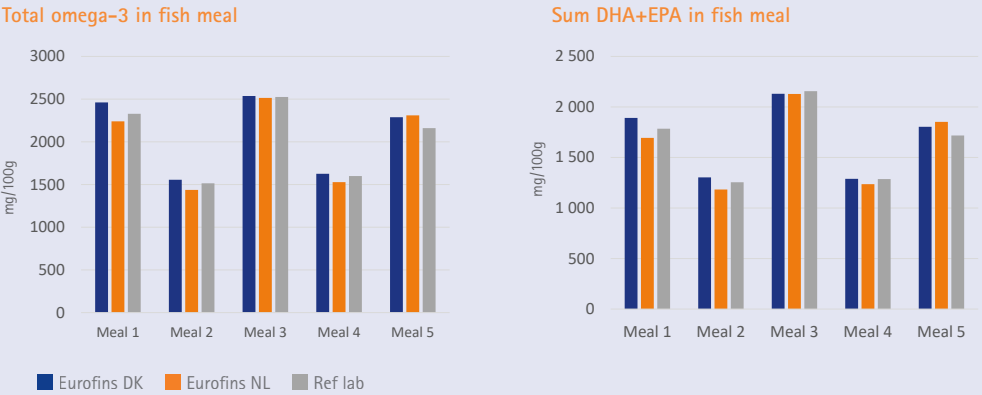


Figure 2: Results fish meal

Fish feed

Figure 3 shows average results for 5 fish feeds. The results are within 9 % difference from the reference lab.

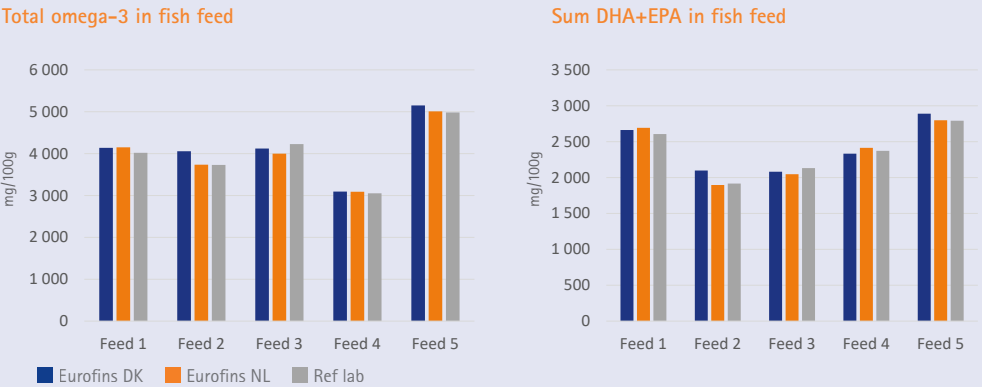


Figure 3: Results fish feed

Fish meat

Figure 4 shows average results for 4 fish meat samples. The results are within 14 % difference from the reference lab.

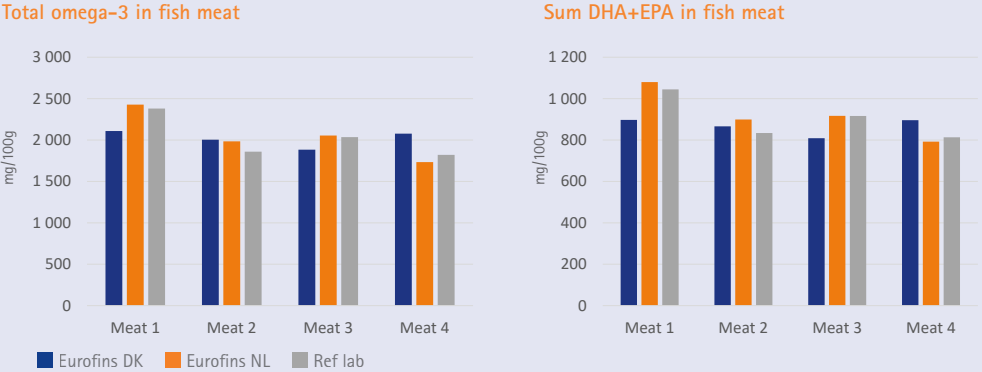
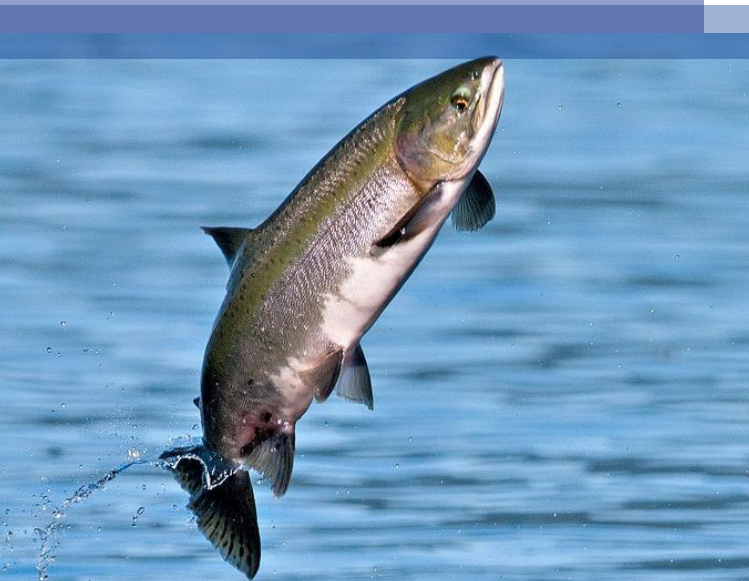
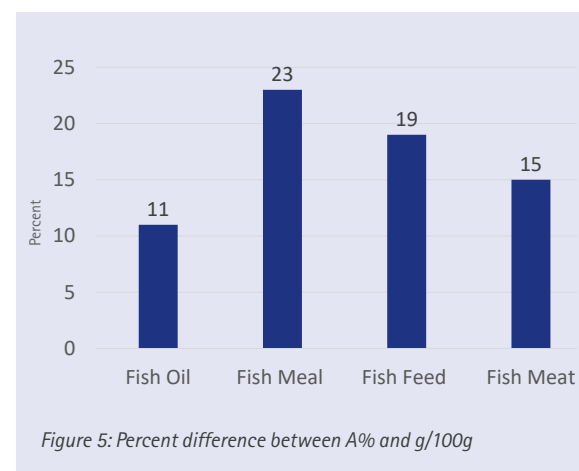


Figure 4: Results fish meat



Area % vs g/100g for omega-3 fatty acids

Figure 5 indicates difference between A% and quantitative analysis. Furthermore, the figure illustrates percent overestimation of results when using A% instead of quantitative analysis. Results shown in the figure below are average difference of all samples within one matrix for the omega-3 fatty acids. The results are collected from the analysis performed at Eurofins DK.



We see from the figure that if we are using A% instead of g/100 g, A% overestimates the results in the range between 11-23 %. The highest overestimation is for fish meal. For example in fish oil, we see that only 89% of the oil is fatty acids. If we had used the standard conversion factor of 0,956 from A% to g/100 g, the analysis had overestimated the fatty acid content with nearly 7%.

Conclusions

Two Eurofins laboratories have been optimizing their methods for fatty acids determination in marine matrixes, to correlate with the results from the reference lab. The results presented show that the results are comparable within normal distribution of fatty acid analysis. The quantitative analysis give similar results as the reference lab on the matrixes tested. This is not surprising since this analysis is well known from an analytical point of view.

Use of A% for estimation of amount of fatty acids overestimates in this trial the fatty acid content between 11 and 23% depending on the matrix.

A% is recommended if you only want the % fatty acid distribution of sum of detected fatty acids.

Eurofins recommend use of internal standard for quantitative fatty acid analysis for applications where concentration of fatty acids in a matrix is of importance. Use of A% and conversion factors for calculation of fatty acid concentration is not recommended.



Test codes and packages

Please contact us for further information regarding this analysis. The new packages will express the results both in A% and in g/100 g.



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