NITROGEN / PROTEIN DETERMINATION IN FISH MEAL BY FLASH COMBUSTION DUMAS METHOD IN COMPARISON WITH KJELDAHL DIGESTION METHOD

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Introduction

High quality fishmeal is recognized by animal nutritionists as an excellent source of protein, energy, minerals and vitamins. Worldwide, millions of tons of fishmeal are produced annually. Fish Meal is used as an ingredient in feedstuffs in aquaculture, livestock and poultry industries. The majority of the fishmeal produced is included in commercial diets for poultry, swine, dairy cattle, mink, pigs, ruminants, farmed fish and very important in the diet of younger animals.

As Fish Meal is a thick powder obtained from cooking, drying, and grinding raw fish, the freshness of raw material is important in its effect on the quality of the protein in the end product. Process control in the factory is necessary for the manufacture of high quality fish meal. The evaluation of the protein content is through the determination of the nitrogen concentration. The analysis of nitrogen in fish meal is critical for daily quality control of production and for specification in contracts. All fish meal is traded on its protein content whether through pricing on a unit-of-protein basis or by guarantee of a minimum quantity of protein content.

For this reason the use of an accurate instrumental analytical technique for nitrogen determination is required. An alternative to the classical Kjeldahl method, based on Dumas (combustion) method, has been developed and approved by different associations (AOAC, AACC, AOCS, ASBC, ISO and IFFO). IFFO (International Fishmeal and Fish Oil Organisation) has recommended that its members adopt the Dumas method as an Official Method for Nitrogen and Crude Protein determination. An additional benefit is that the use of toxic chemicals can be avoided.

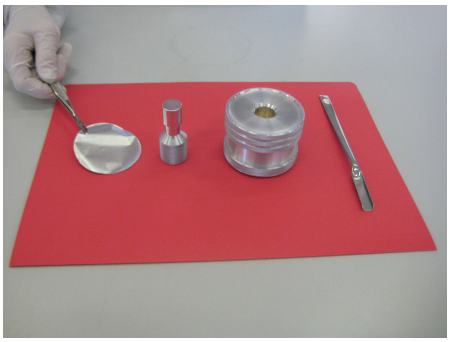
As the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming every day more notable, it is very important to have a simple and automated technique which allows short analytical runs providing results with an excellent reproducibility and accuracy. The Thermo Scientific FLASH 4000 Elemental Analyzer (Figure 1), based on the dynamic combustion of the sample, requires no sample digestion or usage of toxic chemicals, while providing important advantages in terms of time, automation and quantitative determination of nitrogen over a large range of concentration.

FIGURE 1 – FLASH 4000 N/Protein Analyzer

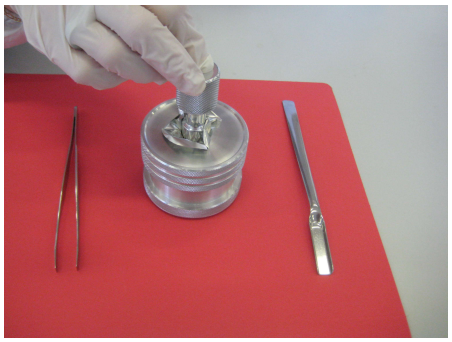
Analytical Configuration

The sample is weighed in a tin capsule (Figure 2) and introduced into the combustion reactor of the analyzer via the MAS 4000 autosampler. The complete flash combustion of each sample is ensured by simultaneously introducing a proper amount of oxygen by the OxyTune® function. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper wires for the reduction of the contained nitrous gases to elemental nitrogen. The water is trapped through a water condensation drainage device while the CO2 is adsorbed on the NoStop Twin Traps. Then the nitrogen is focused on a short GC column and finally detected by a thermal conductivity detector (Figure 3).

Figure 2 – Sample weighing technique

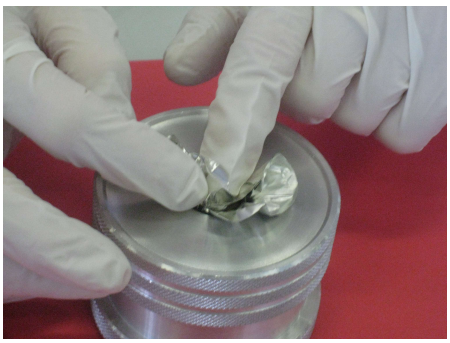


A tin disk is rested on the capsulator.

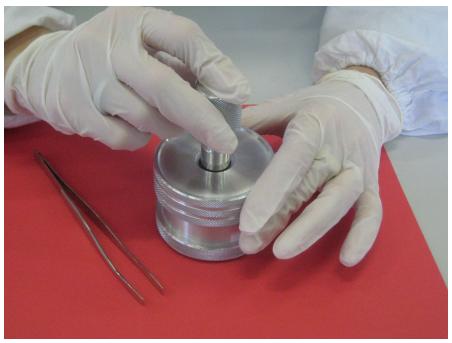


The tin disk is pressed through the cylindrical tool.

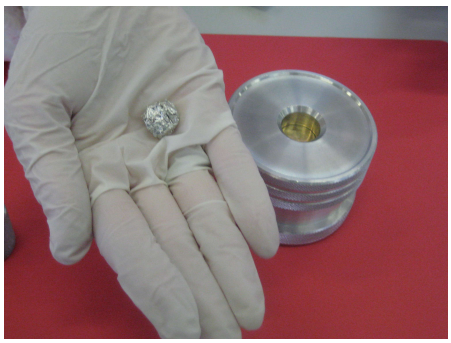
Using a spatula, introduce the sample into the capsule.



The tin disk included the sample is closed by hand.



Using the cylindrical tool, press the disk and make it enter the cavity.

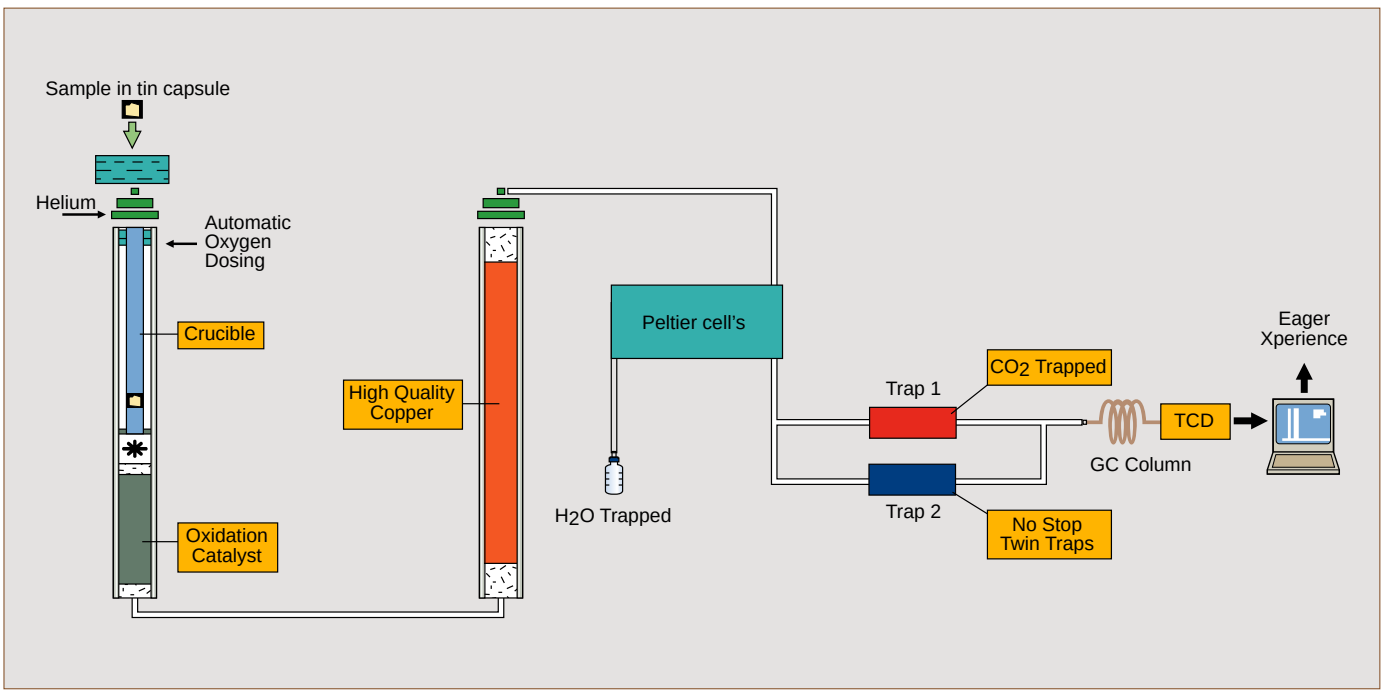


Press the top of the device downwards to have the capsule come out of the cavity.

Analytical conditions:
Temperature left furnace: 950°C
Temperature right furnace: 840°C
Temperature oven: 50°C
Standard: EDTA (9.59 %N)
EDTA: EthyleneDiamineTetraAcetic acid
Standard weight: 400-700 mg
Sample weight: 400-700 mg

***Note**: The Oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune® function present in the Eager Xperience software.*

Figure 3 – FLASH 4000 Nitrogen/Protein configuration



Results

Several fish meal samples were chosen with respect to different countries of origin and different nutritional composition (content of fat, salt, moisture and ashes). The obtained data demonstrate the robustness of the method in the determination of nitrogen in different fish meal matrices, indicating complete combustion for all types of samples. The calibration of the system was performed with EDTA (9.59 %N) using K factor as calibration method. The protein factor used to calculate the protein content was the default value 6.25 present in the Eager Xperience software.

The performance of the Thermo Scientific FLASH 4000 Analyzer was evaluated in the following terms:

- Reproducibility (RSD%) obtained analyzing the samples in triplicate
- Evaluation of results using different weight of sample.
- Comparison with results of the Kjeldahl method

Table 1 shows the N/Protein reproducibility analyzing several fish meal samples in triplicate. In all cases the RSD% obtained was excellent.

Table 1 – N/Protein reproducibility of fish meal (combustion method)

Sample	N %	Protein %	RSD %
A	11.06 11.10 11.08	69.12 69.35 69.23	0.17
B	9.59 9.63 9.65	59.93 60.18 60.32	0.33
C	9.56 9.53 9.59	59.78 59.54 59.92	0.32
D	9.75 9.77 9.69	60.92 61.07 60.56	0.43
E	9.93 9.90 9.93	62.06 61.90 62.03	0.14
F	9.77 9.80 9.74	61.04 61.27 60.89	0.31
G	10.60 10.57 10.54	66.24 66.09 65.88	0.27
H	11.01 10.95 11.03	68.84 68.41 68.96	0.42
I	10.35 10.38 10.40	64.67 64.90 64.99	0.25
J	10.93 10.99 10.88	68.34 68.68 68.01	0.49
K	10.77 10.73 10.65	67.28 67.08 66.58	0.53
L	10.64 10.54 10.52	66.47 65.85 65.73	0.60

Table 2 shows two fish meal samples analyzed at different weight to evaluate the influence on the results. No memory effect was observed changing the amount of sample, indicating a complete combustion of the sample. No significative difference in Nitrogen and Protein content was observed.

Table 2 – N/Protein determination of fish meal at different weight (combustion method)

Sample	Weight (mg)	N %	Average N%	Protein %	Average Protein %	RSD %
B	400	9.70 9.61 9.62	9.64	60.60 60.04 60.15	60.26	0.49
	500	9.59 9.63 9.65	9.62	59.93 60.18 60.32	60.14	0.39
I	500	10.42 10.37 10.40	10.40	65.16 64.81 64.99	64.99	0.27
	700	10.35 10.38 10.40	10.38	64.67 64.90 64.99	64.85	0.25

Table 3 shows a comparison between FLASH 4000 results (same samples reported on Table 1 indicating the average of the results) and Kjeldahl method data. The values demonstrate a high correlation between both methods

Table 3 – Results comparison FLASH 4000 combustion method vs Kjeldahl digestion method

Sample	FLASH 4000 Analyzer		Kjeldahl Method		Difference
	N %	Protein %	N %	Protein %	in Protein %
A	11.08	69.23	11.09	69.30	-0.07
B	9.62	60.14	9.60	60.00	+0.14
C	9.56	59.75	9.54	59.60	+0.15
D	9.74	60.85	9.73	60.80	+0.05
E	9.92	62.00	9.92	62.00	+0.00
F	9.77	61.07	9.78	61.10	-0.03
G	10.57	66.07	10.66	66.60	-0.53
H	11.00	68.74	10.94	68.40	+0.34
I	10.38	64.85	10.35	64.70	+0.15
J	10.93	68.34	10.92	68.20	+0.14
K	10.72	66.97	10.64	66.50	+0.47
L	10.57	66.02	10.48	65.50	+0.52

Conclusion

The obtained results demonstrate that the Dumas combustion method performed with the Thermo Scientific FLASH 4000 Analyzer is superior to the conventional Kjeldahl method due to the following reasons:

- ***The FLASH 4000 Analyzer is a complete automated system (only sample weigh-in needed, thus higher sample throughput, less manual lab work and lower costs per sample compared to the Kjeldahl method).***
- ***Excellent reproducibility and accuracy (equal or better than Kjeldahl)***
- ***The FLASH 4000 Analyzer is able to analyze sample with high nitrogen without matrix effects.***
- ***The FLASH 4000 Analyzer allows large sample weigh-in without matrix effect any memory effects.***
- ***The data obtained by FLASH 4000 show excellent comparability with those of the Kjeldahl method, so that can be used to substitute the well established Kjeldahl method without any limitations.***
- ***The flash combustion method is already approved by official organizations (ASBC, AOAC, AACC, AOCS, ISO, IFFO) and should become the reference method for fishmeal.***