



Nitrogen/Protein determination in dairy products using the Thermo Scientific FlashSmart Elemental Analyzer

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Goal

Demonstrate the performance of the Thermo Scientific FlashSmart Elemental Analyzer for food quality and labeling purposes, while showing compliance to international standards requirements.

Introduction

The determination of Nitrogen/Protein for food quality control and R&D purposes is very important in the dairy industry. Food market globalization demands that accurate quality control of product characteristics is carried out, in order to protect commercial value, to safeguard consumer health and manufacturer reputation. Official regulations establish protein content and labeling requirements which enable consumers to compare price and quality. For this reason the use of accurate instrumental analytical techniques avoiding the use of toxic chemicals is required, and an alternative to the classical Kjeldahl method, based on the Dumas (combustion) method, has been developed and approved by different associations.

The Thermo Scientific™ FlashSmart™ Elemental Analyzer (Figure 1) requires no sample digestion or toxic chemicals, while providing important advantages in terms of time, automation and quantitative determination of nitrogen in a large range of concentrations.



Figure 1. Thermo Scientific FlashSmart N/Protein Analyzer.

Method

The Elemental Analyzer operates according to the dynamic flash combustion of the sample. Samples are weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen. After combustion, the produced gases are carried by a helium flow to a second reactor filled with copper, then swept through CO₂ and H₂O traps, a GC column and finally detected by a thermal conductivity detector (Figure 2).

A complete report is generated by the Thermo Scientific™ EagerSmart™ Data Handling Software and displayed at the end of the analysis. From the nitrogen data obtained, the dedicated software allows the automatic calculation of the protein content.

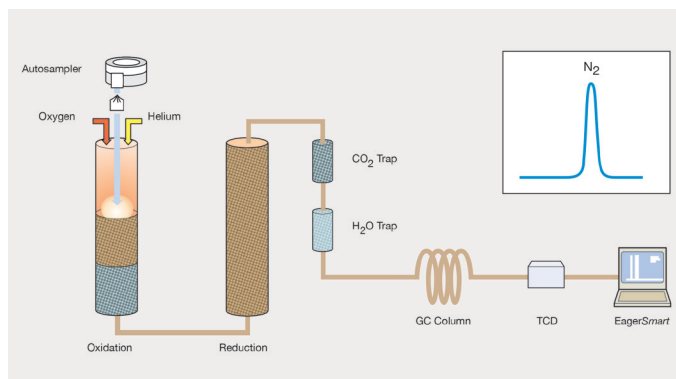


Figure 2. FlashSmart N/Protein configuration.

Analytical Conditions

Left Furnace Temperature	950 °C
Right Furnace Temperature	840 °C
Oven Temperature	50 °C
Carrier Flow	140 mL/min
Reference Flow	100 mL/min
Standard	Aspartic acid (10.52 N%)

Note: The oxygen amount necessary for the complete combustion of samples is calculated automatically by the OxyTune function present in the Thermo Scientific EagerSmart Data Handling Software.

Results

Dairy samples were selected representing different type and nitrogen content. The data obtained demonstrates no-matrix effect in the determination of nitrogen, indicating complete combustion for all types of samples. The calibration was performed with 50–100 mg of aspartic acid (10.52 N%); and K factor was used as the calibration method. The protein factor used to calculate the protein content through the nitrogen value was 6.38.

Table 1 shows the N/Protein data obtained from a UHT milk reference material (ultra high temperature processing, at about 135 °C) from Ceca Lait (Centre D'Etude et de Controle des Analyses en Industrie Laitière), the certified value was 0.5298 N%, an average of the results from five laboratories using the Kjeldahl method. Table 2 shows the N/Protein data obtained in different liquid milk samples, raw and thermally treated according to different procedures, analyzed in duplicate and compared to the Kjeldahl data. Liquid milk samples were weighed in tin containers adsorbed on the inert material Chromosorb. Table 3 shows the N/Protein data obtained of different milk powder samples analyzed five times and the compared to the Kjeldahl data. Samples were weighed at 200–250 mg.

Table 1. N/Protein data of UTH milk reference material.

Weight (mg)	N%	Protein %
101.4	0.5312	3.3891
103.9	0.5286	3.3722
100.0	0.5321	3.3948
105.5	0.5339	3.4065
101.3	0.5306	3.3854
102.4	0.5251	3.3504
102.4	0.5335	3.4035
101.0	0.5264	3.3584
101.0	0.5288	3.3735
99.7	0.5276	3.3659
Average	0.5298	3.3800
RSD%	0.5604	0.5605

Table 2. N/Protein data of liquid milk samples in comparison to Kjeldahl method.

Milk sample	FlashSmart EA		Kjeldahl		Milk sample	FlashSmart EA		Kjeldahl	
	N%	Prot. %	N%	Prot. %		N%	Prot. %	N%	Prot. %
Raw 1	0.498	3.18	0.498	3.18	Raw 3	0.433	2.76	0.429	2.74
	0.497	3.17	0.498	3.18		0.434	2.77	0.428	2.73
Raw 2	0.574	3.66	0.571	3.64	Raw 4	0.422	2.69	0.415	2.65
	0.577	3.68	0.567	3.62		0.414	2.64	0.412	2.63
Pasteurized 1	0.500	3.19	0.497	3.17	UHT 1	0.531	3.39	0.520	3.32
	0.495	3.16	0.495	3.16		0.527	3.36	0.528	3.37
Pasteurized 2	0.495	3.16	0.494	3.15	UHT 2	0.522	3.33	0.525	3.35
	0.497	3.17	0.492	3.14		0.528	3.37	0.523	3.34
Pasteurized 3	0.469	2.99	0.465	2.97	UHT 3	0.500	3.19	0.500	3.19
	0.467	2.98	0.464	2.96		0.498	3.18	0.500	3.19

Table 3. N/Protein data of milk powder samples in comparison to Kjeldahl method.

Milk powder sample	FlashSmart EA		Kjeldahl		Milk powder sample	FlashSmart EA		Kjeldahl	
	N%	Prot. %	N%	Prot. %		N%	Prot. %	N%	Prot. %
A	4.91	31.30	4.91	31.31	C	4.19	26.73	4.18	26.64
	4.89	31.19	4.93	31.45		4.21	26.87	4.19	26.73
	4.93	31.45	4.88	31.13		4.19	26.73	4.19	26.71
	4.94	31.50	4.92	31.39		4.19	26.73	4.22	26.92
	4.93	31.45	4.91	31.29		4.22	26.92	4.19	26.73
Average	4.92	31.38	4.91	31.32	Average	4.20	26.80	4.19	26.75
RSD%	0.41	0.41	0.38	0.39	RSD%	0.34	0.34	0.36	0.39
B	4.43	28.24	4.41	28.14	D	5.46	34.80	5.46	34.82
	4.42	28.20	4.42	28.20		5.46	34.80	5.46	34.82
	4.38	27.96	4.38	27.94		5.48	34.96	5.44	34.70
	4.44	28.33	4.41	28.10		5.47	34.90	5.43	34.66
	4.43	28.24	4.43	28.24		5.44	34.69	5.44	34.69
Average	4.43	28.19	4.41	28.12	Average	5.46	34.83	5.45	34.74
RSD%	0.53	0.49	0.42	0.41	RSD%	0.27	0.30	0.25	0.22

Table 4 and 5 show the N/Protein data obtained from cheese samples with different protein, lipid and water content, analyzed several times to evaluate the repeatability; the weight of sample was 200–500 mg.

Table 4. N/Protein data of cheese samples.

Emmental			Parmesan			Seasoned Provolone		
Weight (mg)	N%	Prot. %	Weight (mg)	N%	Prot. %	Weight (mg)	N%	Prot. %
315.0	3.33	20.76	408.7	5.19	33.11	270.2	4.33	27.66
365.9	3.41	21.26	351.3	5.16	32.94	294.2	4.39	28.03
244.1	3.40	21.25	285.9	5.17	33.00	255.0	4.36	27.84
260.3	3.36	20.97	284.7	5.18	33.05	236.8	4.29	27.39
224.9	3.36	20.96	394.2	5.26	33.57	292.9	4.35	27.75
201.2	3.40	21.22	344.5	5.27	33.60	268.9	4.36	27.85
269.2	3.35	20.92	374.9	5.23	33.36	237.6	4.36	27.81
308.2	3.35	20.94	435.0	5.26	33.56	213.7	4.38	27.96
310.5	3.39	21.16	303.9	5.17	32.98	250.1	4.32	27.59
334.5	3.41	21.31	322.0	5.23	33.37	279.9	4.36	27.81
Average	3.38	21.07	Average	5.21	33.25	Average	4.35	27.77
RSD%	0.88	0.88	RSD%	0.80	0.80	RSD%	0.67	0.67

Table 5. N/Protein data of spread cheese samples.

Singles			Spread cheese		
Weight (mg)	N%	Prot. %	Weight (mg)	N%	Prot. %
370.5	2.24	14.31	216.6	3.40	21.22
512.9	2.28	14.52	304.3	3.34	20.88
489.7	2.28	14.55	245.4	3.33	20.77
388.2	2.28	14.53	278.9	3.34	20.87
447.7	2.28	14.52	383.3	3.40	21.20
Average	2.27	14.49	Average	3.36	20.99
RSD%	0.78	0.68	RSD%	0.99	0.99
Caprino (goats milk cheese)			Mascarpone*		
Weight (mg)	N%	Prot. %	Weight (mg)	N%	Prot. %
385.0	2.28	14.56	250.0	0.64	4.09
404.0	2.30	14.66	262.9	0.63	4.01
Average	2.29	14.61	Average	0.63	4.05
RSD%	0.48	0.48	RSD%	1.51	1.51

* Mascarpone: Italian cheese made from cream coagulated by the addition of certain acid (lemon juice, vinegar, etc).

Table 6 shows the N/Protein data obtained of yogurt sample weighing 200–300 mg adsorbed on Chromosorb. Table 7 shows a comparison between FlashSmart EA and Kjeldahl protein data of different dairy products.

Table 6. N/Protein data of yoghurt sample.

Weight (mg)	N%	Prot. %
271.9	0.520	3.326
288.8	0.519	3.314
277.6	0.522	3.329
268.4	0.518	3.306
273.0	0.515	3.286
277.5	0.508	3.238
276.5	0.510	3.252
280.7	0.512	3.265
273.8	0.512	3.265
285.0	0.510	3.256
Average	0.515	3.284
RSD%	0.948	1.006

Table 7. Protein comparison data between FlashSmart EA and Kjeldahl method.

Sample	FlashSmart EA protein %	Kjeldahl protein %
UHT milk	3.38	3.37
Milk powder	4.32	4.30
Yoghurt	0.51	0.50
Parmesan cheese	33.25	33.26
Provolone cheese	27.77	27.75
Mascarpone cheese	4.05	4.07
Caprino cheese	14.61	14.60

The FlashSmart EA can analyze the fractions of milk and dairy products by liquid injection using the Thermo Scientific™ AS 1310 Liquid Autosampler. The volume injected was 80–125 ul. As a standard glycine water solution was prepared, and K factor was the calibration method. The formula used for the calculation of the fractions is:

$$N_{\text{Total}} = N_{\text{Caseine}} + N_{\text{Serum}} + N_{\text{NPN}}$$

Determination of Non Proteic Nitrogen (NPN): The acidification of the sample with trichloroacetic acid (60% conc.) produces the complete precipitation of both the serum and the caseinic fractions. After centrifugation, the analysis of the liquid fraction permits the determination of the contribution of the non proteic fraction.

Determination of Non Caseine Nitrogen (NCN): The acidification of another aliquote of the same sample produces the complete precipitation of the caseine fraction, after centrifugation, the determination of Nitrogen permits the measurement of the sum of the NPN and the Serum protein contributions.

Finally, the concentration of Nitrogen in the Serum fraction is calculated by subtraction of the two results:

$$N_{\text{Serum}} = (N_{\text{NPN}} + N_{\text{Serum}}) - N_{\text{NPN}}$$

Table 8 shows the nitrogen data in soluble protein of dairy products while Table 9 shows the nitrogen data of different cheese and the related fractions.

Table 8. Nitrogen data in soluble protein of dairy products.

Sample	N%	RSD%	Sample	N%	RSD%
Soluble protein from cheese 1	0.0132 0.0140 0.0139	3.182	Soluble protein from cheese 2	0.0121 0.0124 0.0120	1.711
Soluble whey protein 1	0.1512 0.1519 0.1516	0.232	Soluble whey protein 3	0.1416 0.1441 0.1437	0.938
Soluble whey protein 2	0.1610 0.1609 0.1600	0.343	Soluble whey protein 4	0.1498 0.1497 0.1483	0.562

Table 9. Nitrogen data of different cheese and the related fractions.

Sample	Total N		NCN		NPN	
	%	RSD%	%	RSD%	%	RSD%
Crescenza	2.43 2.47 2.42 2.43 2.46	0.889	0.407 0.411 0.400	0.347	0.132 0.128 0.136	2.985
Ricotta	1.39 1.36 1.38 1.37 1.37	0.826	0.212 0.216 0.212	1.089	0.0799 0.0764 0.0721	5.141
Mascarpone	0.614 0.635 0.627 0.611 0.600	2.267	0.138 0.130 0.136	3.039	0.0431 0.0486 0.0486	6.925

Conclusions

The Thermo Scientific Flash*Smart* Elemental Analyzer is a valuable solution for nitrogen/protein determination for dairy products analysis, thanks to excellent reproducibility and no memory effects observed when changing the type of dairy sample. This indicates the complete and accurate detection of the nitrogen present.

As a complete automatic system, the Flash*Smart* EA is able to analyze nitrogen in a wide range from low to high content, solid and liquid samples without the use of sample digestion or the toxic chemicals applied in traditional methods.

The Dumas Combustion method has been approved and adopted by Official Organizations as ASBC, AOAC, AACC, AOCS, ISO and IDF (International Dairy Federation) Standard.

Find out more at thermofisher.com/OEA

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