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Elemental Analysis: N/Protein and sulfur determination in brewery industry by combustion method

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Goal

To assess the performance of the elemental analyzer for the N/Protein determination in alternative to the Kjeldahl method, and the sulfur determination.

Introduction

The brewing industry plays a key role in the food and beverages market worldwide. Beer is, in fact, one of the oldest and most widely alcoholic drinks consumed in the world, and the third most popular drink overall after water and tea. Beer is brewed from cereal grains, most commonly from malted barley, though wheat, maize (corn), and rice are also used. During the brewing process, the fermentation of the starch sugars in the wort produces ethanol and carbonation in the resulting beer. Most modern beer is brewed with hops, which add bitterness and other flavors and act as a natural preservative and stabilizing agent. Other flavoring agents such as gruit, herbs, or fruits may be included or used instead of hops. Sulfites are widely used as additives in beverages to prevent spoilage by oxidation and bacterial growth during production and storage. In particular, sulfur dioxide is considered as the most important factor in preserving the shelf life of beer, because inhibits beer oxidation. Off-flavors in beer are frequently due to the presence of sulfur compounds.

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In brewery industry, several tests are performed for quality control and R&D purposes. Two of these tests are the determination of the amount of protein, through the determination of the nitrogen content, and the sulfur content, fundamental in achieving high quality and securing the safety of the raw materials (barley, malt), intermediate (wort) and final products (beer). The protein content is important to ensure the survival, growth and productivity of the yeast used to convert the sugar into ethanol and carbon dioxide while the nitrogen content in beer is important to evaluate the stability and texture of head foams.

For the protein determination, the alternative to the Kjeldahl method, based on Dumas technology (combustion method) has been greatly improved to make faster, safer and more reliable. Combustion Dumas method has been approved and adopted by the ASBC (American Society of Brewing Chemists) for nitrogen determination in barley and total nitrogen in wort and beer by combustion method.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), based on the dynamic flash combustion of the sample, can be the alternative to the Kjeldahl method for nitrogen and protein determination. The Flash*Smart* EA copes with a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput.

Figure 1. Thermo Scientific™ Flash*Smart*™ Elemental Analyzer.

Considering the need for cost efficiencies and the likely increase in helium gas cost, for the possible shortage, argon can be used as alternative to helium in the Flash*Smart* EA for N/Protein determination. With the Flash*Smart* EA the sulfur determination by combustion method can be performed by using the same system with a specific single combustion-reduction reactor for all type of matrices analysis, both solid and liquid samples.

This note presents N/Protein and sulfur data of several barley, malt, wort and beer samples in a large range of concentrations to show the repeatability, accuracy and precision.

Methods

The Elemental Analyzer operates according to the dynamic flash combustion of the sample. Barley and malt samples are weighed in tin containers and introduced into the combustion reactor via the Thermo Scientific™ MAS Plus Autosampler with oxygen. Wort and Beer samples are injected by a syringe through the Thermo Scientific™ AS 1310 Liquid Autosampler or manually into the combustion reactor with oxygen.

For N/Protein determination, after combustion, the produced gases are carried by a helium flow to a layer of copper, then swept through CO_2 and H_2O traps, a GC column and finally detected by a Thermal Conductivity Detector (TCD) (Figure 2). For sulfur determination, after combustion the resulted gases are carried by a helium flow to a layer filled with copper, then swept through a GC column which provides the separation of the combustion gases, and finally, detected by a Thermal Conductivity Detector (TCD) or Flame Photometric Detector (FPD) if the sulfur content is in trace level (Figure 3).

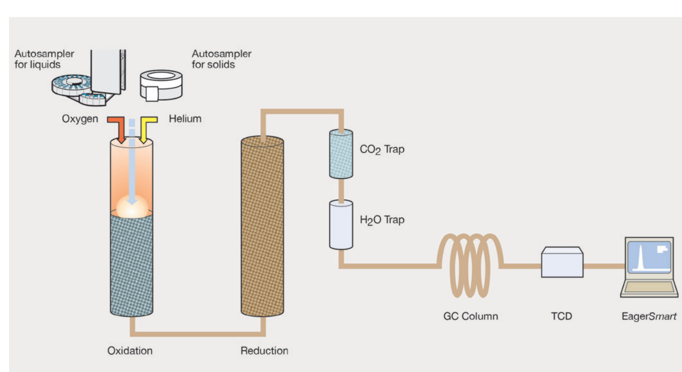

Figure 2. N Brew configuration.

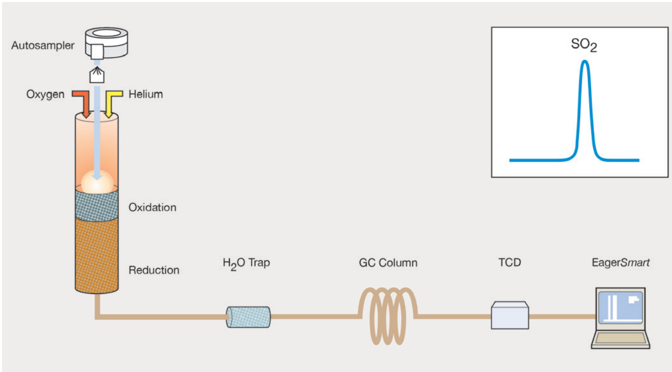

Figure 3. Sulfur configuration.

A complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis. The Eager*Smart* Data Handling Software controls all analytical parameters of the instrument including the oxygen flow and the timing of oxygen injection. It calculates automatically the amount of oxygen, relative to the sample matrix and sample weight, through the dedicated Thermo Scientific™ OxyTune Function ensuring the complete combustion of the sample. The dedicated software converts automatically the nitrogen % in protein content using a specific protein factor.

The standard configuration for N/Protein determination is based on a double reactors system: first reactor for combustion and catalytic oxidation of the combustion gases, the second is used to reduce nitrous oxides as N_2. The superior performance of the Flash*Smart* EA offers advantages such as the possibility to install two analytical circuits which are used alternatively (for example N/Protein using a single reactor in the left furnace and sulfur by TCD Detector in the right furnace, or N/Protein using a single reactor in the left furnace and sulfur by FPD Detector in the right furnace) in a single analyzer. With two autosamplers, the switching time from one analytical circuit to the second can be reduced. With the Thermo Scientific™ Multivalve Control Module the need for tools or manual intervention is removed and ensures very low helium consumption by switching from helium to nitrogen or argon gas, when the elemental analyzer is in Stand-By Mode. The cost of analysis is significantly reduced.

Results

For N/Protein determination, several brewery samples in a large range of nitrogen concentration were analyzed to demonstrate the performance of the Flash*Smart* Elemental Analyzer using helium and argon as carrier gas. The protein factor 6.25 was used to calculate the protein content.

For malt analysis, the calibration was performed with aspartic acid standard (10.52 N%) using K factor as calibration method. The calibration was evaluated by the analysis of aspartic acid and Thermo Scientific Pasta Reference Material as unknown before and after the samples. The data obtained fall within the technical specification of the system for aspartic acid (theoretical 10.52 N%, accepted range 10.42 – 10.62 N%). Malt samples were homogenized by a ball mill. Using helium carrier gas, sample weight was 250 – 300 mg while using argon carrier gas, the sample weight was 130 – 140 mg. Table 1 shows the N/Protein data obtained of three malt samples using helium and argon carrier gas, the data are comparable and the repeatability is more than acceptable. Samples were analyzed five times.

Table 1. N/Protein data using helium and argon carrier gas of malt samples.

Malt samples	N/Protein using helium carrier gas				N/Protein argon carrier carrier gas			
	N%	Av. N% Std.Dev. RSD%	Protein %	Av. N% Std.Dev. RSD%	N%	Av. N% Std.Dev. RSD%	Protein %	Av. N% Std.Dev. RSD%
Malt 1	1.70 1.71 1.69 1.69 1.71	1.70 0.0100 0.59	10.61 10.68 10.59 10.57 10.67	10.62 0.0488 0.46	1.70 1.71 1.69 1.71 1.69	1.70 0.0100 0.59	10.62 10.70 10.57 10.69 10.53	10.62 0.0740 0.70
Malt 2	1.56 1.55 1.55 1.53 1.54	1.55 0.0114 0.74	9.73 9.69 9.70 9.57 9.65	9.67 0.0618 0.64	1.55 1.54 1.54 1.56 1.57	1.55 0.0130 0.84	9.71 9.60 9.65 9.74 9.83	9.71 0.0879 0.91
Malt 3	1.74 1.75 1.75 1.73 1.75	1.74 0.0089 0.51	10.89 10.91 10.94 10.82 10.95	10.90 0.0517 0.47	1.76 1.75 1.74 1.74 1.78	1.75 0.0167 0.95	11.03 10.95 10.87 10.89 11.14	10.98 0.1108 1.01

For beer analysis, the calibration was performed with urea water solution (1000 ppm N) using K factor as calibration method. The calibration was evaluated by the analysis of urea water solution (1000 and 480 ppm N) as unknown before and after the samples. Beer samples were shaken to eliminate the CO_2. Using helium carrier gas, 125 µl sample was injected by the AS 1310 Liquid Autosampler while using argon carrier gas, 250 µl sample was injected manually. Table 2 shows the nitrogen data obtained of four beer samples using helium and argon carrier gas. Data are comparable, and the repeatability is more than acceptable. Samples were analyzed 10 times.

Table 2. Nitrogen data of beer samples using helium and argon carrier gas.

Sample	Beer 1		Beer 2		Beer 3		Beer 4	
Carrier gas	Helium	Argon	Helium	Argon	Helium	Argon	Helium	Argon
ppm N	1048	1005	924	918	921	925	953	974
	1040	1026	932	934	906	908	961	953
	1051	1027	920	925	916	914	956	958
	1046	1044	936	933	917	925	952	964
	1050	1021	931	933	914	912	951	956
	1052	1032	919	924	906	917	944	969
	1045	1033	923	921	906	928	945	956
	1039	1036	919	926	912	934	958	974
	1047	1039	924	931	916	933	961	962
	1045	1023	920	938	907	918	953	968
Av. N%	1046	1029	925	928	912	921	953	963
Std Dev.	0.0004	0.0011	0.0006	0.0006	0.0006	0.0009	0.0006	0.008
RSD%	0.41	1.07	0.66	0.69	0.61	0.97	0.62	0.79

Table 3 shows nitrogen data of wort and beer samples using helium as carrier gas. Wort is rather viscous due to the sugar content, samples can be analyzed without any pre-treatment or diluted.

Table 3. Nitrogen data of wort and beer samples using helium carrier gas.

Sample	ppm N	RSD%	No. of runs
Wort A	620	0.65	2
Wort B	526	0.36	2
Wort C	553	0.11	2
Wort D	309	1.01	2
Wort E	1242	1.09	16
Beer A	867	0.92	20
Beer B	662	1.31	20
Beer C	634	0.83	20
Beer D	671	0.71	2
Beer E	705	0.63	2

Table 4 and 5 shows N/Protein comparison between Kjeldahl method and Flash*Smart* EA of barley, malt, wort and beer samples. Data are comparable demonstrating the performance of the combustion method.

Table 4. N/Protein data comparison of barley and malt samples.

Sample	Kjeldahl method		Flash*Smart* EA		
	N%	Protein %	N%	Protein %	RSD%
Barley 1	1.39	8.69	1.42	8.88	0.46
Barley 2	1.35	8.44	1.34	8.38	0.87
Barley 3	1.56	9.75	1.57	9.81	0.56
Barley 4	1.47	9.19	1.45	9.06	1.01
Malt 1	1.66	10.38	1.67	10.44	0.25
Matl 2	1.75	10.94	1.78	11.12	0.67
Malt 3	1.54	9.62	1.53	9.56	0.51
Malt 4	1.43	8.94	1.40	8.75	0.66

Table 5. Nitrogen data comparison of wort and beer samples.

Sample	Kjeldahl method	FlashSmart EA	
	ppm N	ppm N	RSD%
Wort 1	889 - 893	899	1.21
Wort 2	1140 - 1150	1170	0.87
Wort 3	1300 - 1310	1320	0.91
Wort 4	995 - 993	993	1.11
Wort 5	824 - 827	821	1.09
Beer 1	587 - 592	594	1.13
Beer 2	650 - 666	659	0.96
Beer 3	614 - 6019	618	1.01
Beer 4	628 - 630	630	0.89
Beer 5	640 - 645	637	0.91

Finally, sulfur determination in malt and beer were performed using helium as carrier gas. For malt analysis by TCD Detector, the calibration was performed with 2 – 3 mg BBOT (7.44 S%) as standard using K factor as calibration method, sample weight was 3 – 4 mg. For beer analysis by FPD Detector, the calibration was performed with 0.1 – 1.5 mg Thermo Scientific Soil Reference Material (230 ppm S) using Quadratic Fit as calibration method, beer sample were weighed at 1 – 2 mg. Table 6 shows the sulfur data of the malt samples while Table 7 shows the sulfur data of the beer samples.

Table 6. Sulfur determination of malt samples by TCD Detector.

Sample	Malt 1	Malt 2	Malt 3
S%	0.0568 0.0575 0.0561 0.0576 0.0565	0.0328 0.0334 0.0331 0.0332 0.0331	0.0203 0.0206 0.0205 0.0280 0.0209
Average S%	0.0569	0.0331	0.0206
Standard deviation	0.0006	0.0002	0.0002
RSD%	1.13	0.65	1.16

Table 7. Sulfur determination of beer samples by FPD Detector.

Sample	Beer 1	Beer 2	Beer 3	Beer 4
ppm S	99 101 101 99 99	71 71 69 71 70	74 74 75 75 75	68 69 67 69 68
Av. ppm S	100	70	75	68
Std. Dev.	0.0001	0.0001	0.0001	0.0001
RSD%	1.10	1.27	0.73	1.23

Conclusions

The Thermo Scientific Flash*Smart* Elemental Analyzer, based on the combustion method (Dumas), offers advantages over the Kjeldahl Method for the Nitrogen/Protein determination in terms of automation, ease of use and cost per sample.

The Flash*Smart* Elemental Analyzer, using argon as carrier gas enables to perform Nitrogen/Protein analysis in a large range of concentrations in many types of brewery samples without matrix effect. The N/Protein data obtained are comparable with those obtained using helium as carrier gas.

The RSD% obtained was less than 2% of the performance requirements of the Official Methods.

No memory effect was observed, indicating complete combustion and detection of the element independent of the sample matrix.

The application showed that the Dumas Method meets manufacturers and laboratories requirements, including compliance to official methods. The Dumas Combustion method has been approved and adopted by Official Organizations such as ASBC, AOAC, AACC, AOCS, IDF, IFFO and ISO. The ASBC (American Society of Brewing Chemists) has two Official Methods approving the combustion method for nitrogen determination in barley and total nitrogen in wort and beer. The Flash*Smart* EA allows also the sulfur determination with TCD and FPD Detectors. The data obtained shows a good repeatability.

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