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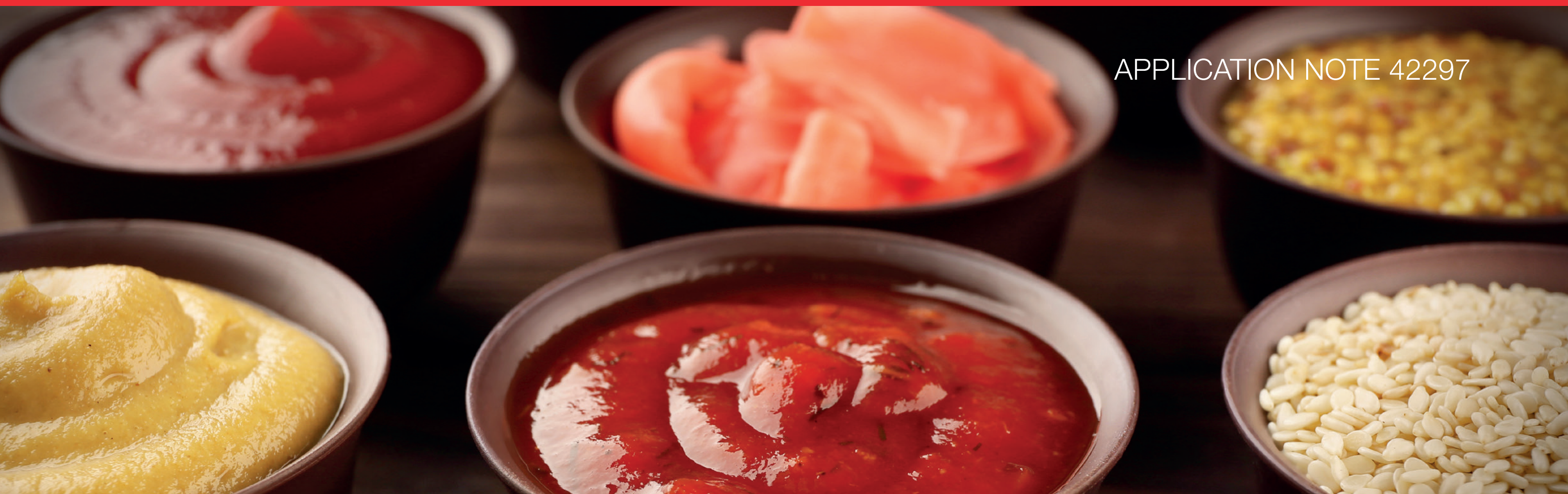

Elemental Analysis: Nitrogen/Protein and sulfur determination in soy and fish Asian sauces

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Goal

Demonstrate the performance of the Thermo Scientific Flash*Smart* Elemental Analyzer for food quality and labeling purposes for Asian sauces.

Introduction

Asian sauces are used in food preparation to enhance flavor and are an important part of Asian cuisine. Soy sauce, a traditional fermented product, is widely used. Its main ingredients are salt and protein hydrolisates (amino acids and peptides), using steamed defatted soybean flakes and baked wheat grains as the main starting materials. Its quality varies greatly with the raw materials used and the method of manufacture.

The Total Nitrogen (TN) and Amino-type Nitrogen (AN) contents are generally used as the quality indices for soy sauce products. According to the national standard in Taiwan, the first-grade soy sauce products should contain more than 1.4 TN% and more than 0.56 AN%. In Japan, the Japanese Agricultural Standard (JAS) specifies three grades of soy sauce: special, upper and standard.

Other traditional sauces, such as fish sauce, are used as condiments and sometimes as substitutes for soy-bean ones.

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Many fermented fish products are prepared in different parts of the world and the method of processing depends upon various factors such as availability of raw materials, consumers' preference and the climatic conditions of the region. In addition, fish sauce is a product that can be made cheaply from various fish raw materials, which are not normally used for food. Also, it is a brown liquid seasoning commonly used in most parts of Southeast Asia. It is called by different names in different countries and it contains a mixture of amino acids and other degradation products.

Sulfur is also an essential component of living matter. Sulfur deficiency has a negative influence in the quality of proteins as it is essential for the synthesis of amino acids such as cysteine, cystine, methionine and the synthesis of vitamins. The importance of sulfur testing in foods and feeds has grown in recent years and many of the classical methods are now no longer suitable for routine analysis.

Globalization of the food market has led to the need for accurate control of products for protecting commercial value, safeguarding consumer health and maintaining manufacturer's reputations. Official regulations establish the protein content and labelling requirements which enable consumers to make price and quality comparisons based on protein declarations. The use of a simple and automated technique allowing fast analysis with excellent reproducibility and which can avoid the risk of handling toxic chemicals is required. Regarding protein content, the alternative to the classical Kjeldahl method, is the Dumas (combustion) method which is approved by official associations (AOAC, AACC, AOCS, ASBC, IDF, IFFO and ISO).

For quality control purposes, the organic elements in food need to be determined. For the determination of carbon, hydrogen, nitrogen and sulfur the combustion method is used. Oxygen determination is obtained by pyrolysis.

The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (Figure 1), is based on dynamic combustion (using a modified Dumas method). It provides rapid and automated nitrogen and sulfur determination without the use of hazardous chemicals and offers advantages in precision over traditional methods. The Flash*Smart* Elemental Analyzer allows runs at low and high levels without matrix effects.

Trace sulfur content can be accurately determined. Sample protein content is calculated automatically using a conversion factor in the Thermo Scientific™ Eager*Smart*™ Data Handling Software.

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

Methods

The Elemental Analyzer operates using 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.

For N/Protein determination, the standard configuration is based on a double reactor system: the first reactor is used for combustion and catalytic oxidation of the combustion gases, the second is used to reduce nitrous oxides as N_2. 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). As an alternative to helium, argon can be used as the carrier gas.

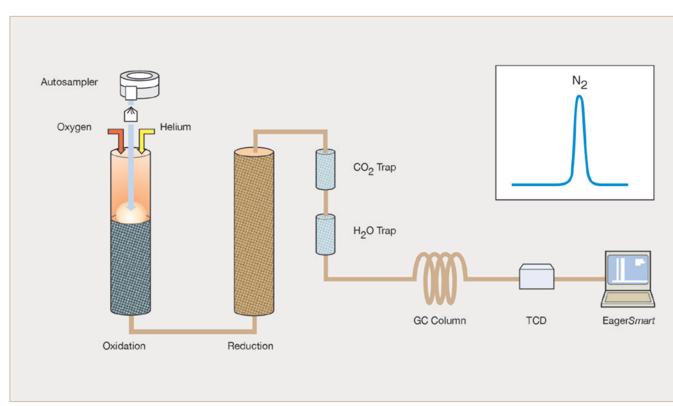

Figure 2. N/Protein configuration.

The Flash*Smart* EA is equipped with two totally independent furnaces allowing the installation of two different analytical circuits which are used alternately and completely automatically through the Thermo Scientific™ MultiValve Control™ (MVC) Module. Each analytical circuit can use its own autosampler. N/Protein can also be determined using a single combustion/reduction reactor installed on the left furnace and sulfur determination on the right furnace (Figure 3).

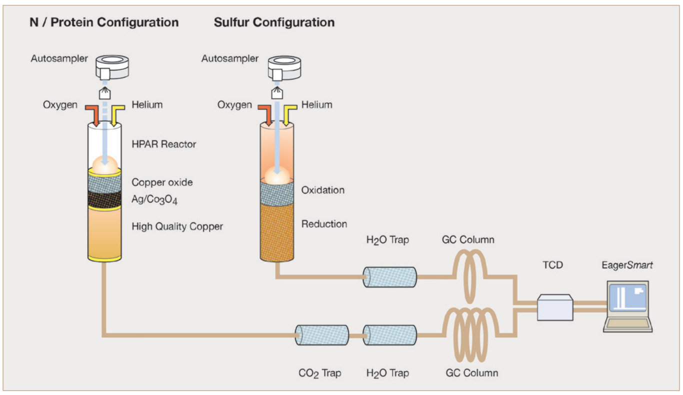

Figure 3. N/Protein and Sulfur configuration.

For sulfur determination, after combustion the produced 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) (Figure 3).

A complete report is automatically generated by the Thermo Scientific™ Eager*Smart*™ Data Handling Software and displayed at the end of the analysis. The software automatically converts the nitrogen % into protein content using a specific protein factor.

Results

Different soy and fish Asian sauces were analyzed to show the performance of the Flash*Smart* EA. Samples were analyzed without homogenization or treatment. The protein factor used to calculate the protein content was 6.25.

For N/Protein determination, the analyses were performed by the double reactor system and by the single combustion/reduction reactor to evaluate reproducibility. Helium and argon carrier gases were used in the double reactor system. The values obtained were then compared. The calibration of the Flash*Smart* EA was performed with aspartic acid using K factor as the calibration method. Samples were weighed in tin containers adsorbed on Chromosorb. The sample weight was 120 – 180 mg using helium carrier gas in the double reactor system, 120 – 150 mg using argon carrier gas in the double reactor system and 120 – 145 mg using the single combustion/reduction reactor. The sauces were analyzed five times to show the repeatability of the data. No relevant difference was observed. Table 1 shows the N/Protein data of the Asian sauces.

Table 1. Reproducibility of N/Protein data of Asian sauces.

Sample name	Double Reactor System								Single Comb./Red. Reactor			
	Helium carrier gas				Argon carrier gas				Helium carrier gas			
	N%	RSD%	Prot.%	RSD%	N%	RSD%	Prot.%	RSD%	N%	RSD%	Prot.%	RSD%
Soy sauce 1	0.91 0.91 0.90 0.91 0.91	0.49	5.69 5.66 5.66 5.66 5.69	0.29	0.91 0.91 0.91 0.90 0.90	0.60	5.66 5.67 5.69 5.64 5.65	0.34	0.91 0.90 0.89 0.91 0.91	0.99	5.68 5.65 5.59 5.69 5.68	0.72
Soy sauce 2	0.48 0.48 0.47 0.48 0.47	1.15	2.99 2.99 2.93 3.03 2.92	1.55	0.46 0.47 0.46 0.46 0.46	0.97	2.89 2.92 2.85 2.90 2.91	0.93	0.47 0.48 0.46 0.47 0.47	1.50	2.96 2.98 2.90 2.94 2.95	1.01
Soy sauce 3	2.23 2.21 2.21 2.22 2.22	0.38	13.92 13.83 13.80 13.90 13.85	0.36	2.20 2.20 2.22 2.20 2.21	0.41	13.74 13.77 13.89 13.73 13.84	0.50	2.20 2.21 2.20 2.20 2.21	0.25	13.72 13.83 13.76 13.75 13.81	0.33
Fish sauce 1	1.40 1.40 1.39 1.42 1.40	0.73	8.74 8.74 8.69 8.86 8.74	0.72	1.38 1.38 1.38 1.38 1.41	0.81	8.63 8.60 8.62 8.59 8.78	0.90	1.140 1.40 1.38 1.39 1.40	0.64	8.77 8.77 8.61 8.70 8.77	0.81
Fish sauce 2	0.85 0.84 0.85 0.85 0.85	0.45	5.31 5.25 5.29 5.30 5.29	0.43	0.83 0.82 0.82 0.82 0.83	0.66	5.18 5.12 5.10 5.11 5.19	0.81	0.83 0.84 0.84 0.84 0.84	0.49	5.19 5.23 5.23 5.24 5.26	0.49

For sulfur determination, helium only was used as the carrier gas. Samples were weighed directly into the tin container. The calibration was performed with BBOT (2,5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene) using K factor as the calibration method. The sample was weighed at 3 – 4 mg. The sauces were analyzed three times. Table 2 shows the sulfur data obtained.

Table 2. Sulfur data of Asian sauces.

Sample name	S%	Std. Dev.	RSD%
Soy sauce 1	0.0437 0.0425 0.0427	0.0006	1.50
Soy sauce 2	0.0358 0.0350 0.0353	0.0004	1.14
Soy sauce 3	0.0358 0.0350 0.0353	0.0003	0.57
Fish sauce 1	0.308 0.307 0.306	0.0010	0.33
Fish sauce 2	0.308 0.307 0.306	0.0046	1.66

Conclusion

The Flash*Smart* EA, based on the combustion method (Dumas), performed N/Protein and sulfur determination for the analysis of food and fermented products. The method provides excellent reproducibility, with no memory effects when analyzing another type of sample. This indicates the complete and accurate detection of the nitrogen present.

The N/Protein data obtained using helium and argon as carrier gas are comparable: this confirms argon as an alternative carrier gas to helium. The nitrogen data obtained using double and single reactor are comparable. The Flash*Smart* EA allows working with double channel configurations, switching automatically from N/Protein on the left furnace to sulfur on the right furnace. The switch is performed by the MVC Module, which enables an increase in productivity and reduction in helium consumption.

With the Flash*Smart* EA, nitrogen and sulfur can be analyzed from low to high content without matrix effects and without the use of sample digestion or toxic chemicals. The combustion Dumas method has been approved and adopted by Official Organizations (among others ASBC, AOAC, AACC, AOCS, ISO).

Acknowledgments

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