Reprinted from *American Laboratory* October 2008

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Protein Testing Enters the 21st Century: Innovative Protein Analyzer Not Affected by Melamine

Global resourcing and production have increased the demand for food manufacturers to ensure the safety and integrity of their products. Though a weakening U.S. dollar and rising costs originally forced U.S. food manufacturers to consider less expensive imported protein sources, they have had to reevaluate that strategy because of the vast opportunity for adulterated shipments and lack of accurate and timely testing procedures. Customs inspectors and food manufacturers receive a multitude of large shipments every day of wheat gluten, rice protein, soy meal, and other protein-rich ingredients. Laboratories are already backlogged, and having to wait days for test results creates a bottleneck that costs suppliers and end users time and money. More importantly, test methods that do not measure protein directly are inaccurate and can inadvertently allow tainted or substandard ingredients into the food supply, jeopardizing consumers' health as well as their trust in the manufacturers of the products they use.

The need for better protein testing methods was pushed into the spotlight recently when thousands of Chinese children under the age of 2 fell ill with a growing number of reported deaths caused by kidney stones resulting from the presence of melamine in dairy products. Melamine is an organic compound used in plastics, fertilizer, and flame-resistant materials that causes renal failure in humans as well as animals. Melamine, which is rich in nitrogen, had been added to diluted milk to boost the nitrogen content and cheat the standard protein tests, which actually measure the total amount of nitrogen. The price of milk and other similar products is dictated by their protein content, with higher levels commanding elevated prices. Hence, without an effective means of testing for protein, unscrupulous suppliers have a strong incentive to adulterate these commodities.

Standard protein tests

Everyone wants to measure protein routinely and accurately, but until recently, these two desires were mutually exclusive. The most accurate protein measurement techniques typically involve chromatography followed by mass spectrometry and can be prohibitively expensive, since they also require trained chemists and long analysis times, further adding to the expense.

The standard Kjeldahl and Dumas protein tests used in the food industry measure the total amount of nitrogen in a sample and then calculate the protein based on the nitrogen levels. Protein contains nitrogen, and though there can be naturally occurring nonprotein sources of nitrogen in a food product, it is not found in the other two major components, fats and carbohydrates. Thus, for many years, chemists have used these tests for total nitrogen to determine protein content. However, neither test can distinguish the nitrogen in protein from nonprotein nitrogen, a significant blind spot in the global marketplace.

In addition, these tests are not fast enough for process control. They also require either hazardous chemicals, analytical chemistry skills, or both. Kjeldahl involves heating sulfuric acid and a copper catalyst to high temperatures for hours at a time, requires extensive laboratory safety procedures, and produces hazardous waste. Dumas is a combustion method, and all of the products given off must be dealt with within the instrument. Combustion systems require frequent routine maintenance and cannot easily handle liquids or larger sample volumes.

Another option is infrared, which is an indirect technique that requires frequent calibration. Infrared systems are used as a secondary method, since they are easily affected by sample matrices and other components.

Table 1 Standard Kjeldahl protein test vs SPRINT

Sample ID	Kjeldahl	SPRINT
Nonfat dry milk powder	35.33	35.58
NIST infant formula	11.17	11.14
Milk, chocolate	3.27	3.27
Milk	3.27	3.27
Sour cream, regular	2.85	2.83
Sour cream, light	5.71	5.7
Ice cream, vanilla	3.11	3.13
Ice cream, chocolate	3.46	3.41
Plain yogurt	3.46	3.46
Cheese, natural cheddar	23.57	23.64
Cheese, processed	10.53	10.59
Soy milk	3.07	3.075
Soy milk, chocolate	2.08	2.12
High-fiber beverage	9.91	9.92
Egg with butter	10.52	10.57
Whole egg	10.77	10.74
Frankfurters, turkey/pork	9.13	9.2
Frankfurters, turkey	11.5	11.6
Bologna, chicken/pork	11.33	11.39
Beer malt	7.94	7.84
Pet food, canned, moist	10.29	10.30

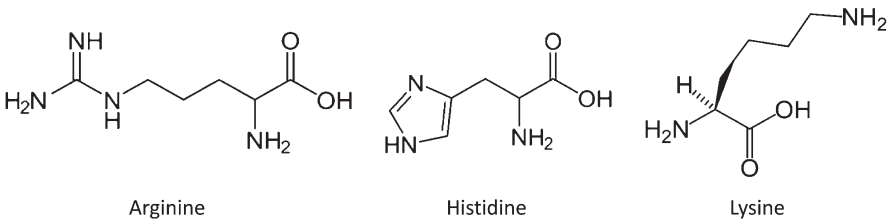

Figure 1 *Amino acids arginine, histidine, and lysine.*

A better way

A rapid, accurate, direct method has long been needed for protein determination: one that would be easy enough for anyone to use, but provide the accuracy needed to ensure product integrity and give food processors the information they need for formulation and regulatory compliance. The SPRINT™ rapid protein analyzer (**CEM Corp.**, Matthews, NC) offers such an alternative—it is fast, accurate, and easy to use; can operate on the production floor or in the laboratory; and utilizes methods that are approved by the AOAC and AACC. The analyzer's accuracy is equal to or better than Kjeldahl, and yields results in just 2–3 min for most sample types (see *Table 1*). This accuracy is the result of a technique known as protein tagging, which has been in use in the bioscience field for years. It is a direct measurement of the protein a sample contains that ignores nonprotein nitrogen.

Protein tagging

Proteins are made up of chains of peptides, which are in turn made up of amino acids. There are 20 different amino acids commonly found in foods, and the order in which they are linked determines the protein's function. Of these 20 amino acids, arginine, histidine, and lysine have a high pK_a and therefore readily react with acidic groups found on reagents, such as azo dyes (see *Figure 1*).

A multitude of proteins abound in food. For instance, α-casein, one of the five types of caseins found in cow's milk, has 201 amino acids with 15 arginine, four histidine, and seven lysine amino acids. Including the N-terminus of the amino acid chain, there are 27 basic sites on this protein. In other words, 13.4% of the amino acids that make up an α-casein are basic.

The SPRINT uses iTAG™, a proprietary solution that has an acidic group that readily attaches to the basic groups found on these amino acids. It also has an associated structure featuring an extensive aromatic character, and readily absorbs light at 483 nm. The combination of these two structures creates an agent that stoichiometrically binds to the basic amino acids and is easily detected using ordinary absorption spectroscopy.

Essentially, a known amount of iTAG is added to a weighed sample and allowed to bind to the protein. The solution only binds to the basic amino acids in the protein, ignoring nonprotein sources of nitrogen. The iTAG is then filtered and measured by the built-in detector and results are displayed, all of which takes place in 2–3 min, from start to finish, for most foods.

Results not affected by adulterants

Though it is possible to inflate the apparent protein content of a substance by adding an agent that is rich in nitrogen, such as melamine, the iTAG solution is target selective and will only attach to the basic amino acids found in protein, arginine, histidine, or lysine, none of which is found in melamine or similar adulterants.

Reducing the sensitivity of routine analysis techniques to nonprotein nitrogen improves the situation for many stakeholders in the food and food ingredient industry. Pricing of raw materials is more accurate since the errors caused by nonprotein nitrogen are removed. This greatly reduces the incentive to include adulterants used to inflate the apparent protein content and, thankfully, improve the safety of the food supply.

Sample results

The Kjeldahl and Dumas methods are currently widely accepted, and companies have their formulations and processes based on the results of these tests. They measure total nitrogen and convert that number to a theoretical (crude) protein content. The results are susceptible to operator technique and, worst of all, any source of nitrogen is counted as protein. SPRINT takes this into account and may be set for crude protein or true protein. It is a simple matter of loading a different program. The test time is no different. Either way, SPRINT analysis is not susceptible to nonprotein sources of nitrogen.

Fast approach to protein analysis

SPRINT offers food manufacturers, testing laboratories, and governmental agencies a faster, more accurate way to measure protein in any type of food or ingredient. Anyone can operate the easy-to-use system. Food samples are gathered using standard methods. Approximately 1 g is transferred into a disposable cup and weighed on an analytical balance. The cup is placed in the analyzer along with a disposable filter. The system dispenses iTAG solution into the cup and homogenizes the sample. The proteins bind with the solution, forming an insoluble complex. A portion of the solution is drawn through the disposable filter into the colorimeter and analyzed. The results are displayed on the instrument's screen. All parts that touch the sample are either self-cleaning or disposable.

SPRINT is a green method technology and iTAG is completely nontoxic, providing an environmentally friendly, cost-effective solution to the need for faster, more accurate protein determinations. The analyzer is an important development in protein testing and is poised to become the method of choice for the next century.

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