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Protein and Melamine Testing in Food

The addition of the chemical melamine to Chinese dairy products has caused more than 10 infant deaths worldwide and over 50,000 children in China to be hospitalized. Melamine in itself is not harmful, but when levels are high enough, it can cause crystals to form in the liver, leading to renal failure. This scandal comes on the heels of last year's discovery of the addition of melamine to wheat gluten in pet food imported from China, which led to thousands of animal deaths (see ***IBO*** 11/15/07**)**. Melamine is added to food products to fake a higher protein level in a sample that has been diluted.

Currently, testing for protein content in food is done using methods that do not directly test protein, and instead obtain protein readings by measuring nitrogen levels, which the addition of melamine will increase. In food testing, protein analysis is used for determining properties of proteins, such as chemical composition, type and concentration, which are important in quality control and labeling. This article will primarily investigate the current state of direct protein detection methods, their advantages and disadvantages, and their possible future. In the absence of a standard direct protein test, melamine testing is the solution to the current crisis

The Kjeldahl and Dumas methods are the standard methods for protein testing of foods. Both methods rely on the measurement of nitrogen, which is released in the Kjeldahl method after an acid, such as sulfuric acid, digests the sample. Using the Dumas method, which is a faster variation of the Kjeldahl method, nitrogen is measured after a sample of a known mass is combusted. These methods are easy to run, recognized by regulatory agencies, low cost and reproducible. "Currently, Kjeldahl and Dumas are the classical methods important in modern food protein analysis and are the approved methods by international organizations," stated Dr. Anthony Fontana, technical director of Chemistry at the independent food testing company Silliker. "Kjeldahl is applied as a reference method for the evaluation of alternative protein determination techniques," he explained to ***IBO***.

The downsides of the Kjeldahl method include the use of hazardous substances and that it is relatively slow. For Dumas, the protein contained in the actual sample may be misrepresented because a small sample size is required for the reaction. But, currently, the most pertinent drawback is that these methods cannot detect some forms of adulteration since they do not directly measure protein. "If a regulatory agency, such as the FDA, determines that an alternative analytical method is superior to Kjeldahl or Dumas, then they may require this technique. If or how soon this may occur is anyone's guess," commented Dr. Fontana.

Two direct methods for protein testing in food that do not rely on testing nitrogen levels are UV-Visible and infrared spectroscopy (IR). UV-Vis can determine low concentrations of proteins in a sample, and thus is mainly used for pure protein analysis in laboratories rather than for quality control. With UV-Vis, certain chemical groups, such as peptide bonds, aromatic side-groups and aggregate proteins, can be targeted for protein determination, allowing for a clear verification of results. The run-

ning time of the whole process is relatively short, but sample preparation can be time consuming and labor intensive due to the necessity of obtaining a transparent sample. In addition, amino acid sequences differ in some proteins, which occasionally leads to a misinterpretation of absorbance readings.

In contrast, IR is good for quality control purposes. Because it requires less sample preparation and is nondestructive, it is used for on-line analysis. It is widely used for protein analysis of wheat, grain and dairy products. However, equipment calibration is a necessary but sometimes time-consuming step in IR analysis of food samples. Another drawback is the high cost of instruments. As Dr. Fontana explained, IR also has "accuracy, reproducibility, interference and quantification issues. These spectrophotometric methods . . . have responses that vary with the amino acid composition of the proteins, have possible interferences from nonprotein compounds, like carbohydrates, salts and lipids, that have to be taken into account, [and] may have instability of the reagents and may have a time dependency of the response based on reagent mixing, color development and so on." He added, "for a specific matrix these spectrometric techniques may be applied, but as a general method for direct protein detection in a wide variety of food matrices, they have limitations."

A recently introduced technique for direct protein analysis in food is CEM's SPRINT Rapid Protein Analyzer, which began shipping in the first quarter of this year. The SPRINT uses protein-tagging technology to determine protein levels in two minutes. According to CEM, it is less expensive, safer and easier to use than Kjeldahl and can be used in labs and on the production floor because it does not require the use of an acid. According to John Urh, CEM's SPRINT product manager, the company developed the product based on customer requests. As he told ***IBO***, "our tagging technology allows us to bind to proteins directly and measure that binding, and that gives us the protein measurements. We settled on this technology two years ago. We said we'd go ahead and build a product on this technology, one that would be automated and easy to use."

With the SPRINT, the company wanted to circumvent the weaknesses of indirect protein detection methods, such as the requirement of supplemental adulterant testing. "There are still other sources of nitrogen such as urea, so just testing for melamine will help reduce the incentive to add melamine to a product, but there are still ways to fool the tests by adding different sources of nitrogen to the material," said Mr. Urh. "To me, it seems that a test that measures protein directly will always win out in a situation when people are adding adulterants to a material."

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The company also shied away from the use of spectroscopy techniques such as IR in the development of the SPRINT because, as Mr. Urh told ***IBO***, "[it] measures the vibrations of the different molecular bonds. The problem with this is that it measures the similar types of bonds that are not in protein." He continued, "When you're looking at a signal for protein in IR, you're looking at a signal that includes a lot of other components. And there's a lot of mathematics and a deconvolution of data that has to be performed to get that protein number, so it is not a direct measurement."

According to Mr. Urh, the only barrier to adoption of CEM's technology is that it is new to the food industry, and that it will take time for CEM's customer base to learn about it. The SPRINT conforms to the Association of Analytical Communities' (AOAC) standards for dairy analysis and the American Association of Cereal Chemists' standards for grain analysis. Studies for it conformity to AOAC's meat standards are underway.

However, direct melamine testing is the current the way in which the melamine crisis is being handled. According to the World Health Organization (WHO), more current methods for melamine testing are LC-MS/MS and LC-MS. LC-MS/MS, with a melamine detection limit of 50 pg/mL, is both reliable and covers a wide range of samples. The downside is that the process is the most expensive and complicated of these techniques and, including sample preparation, analysis can take up to two days. LC-MS is similar to LC-MS/MS in detection limit and sample range, but its sensitivity is such that sample interference is possible. GC-MS, which was extensively used during the pet food scandal, is more sensitive than LC-MS with a sensitivity of 0.01 mg/kg. It differs from LC-MS/MS and LC-MS in that it requires the melamine to be reacted into trimethylsilyl derivatives before analysis. This step is labor intensive and sensitive enough to add a large margin of error if mishandled.

The nature of these tests has led China to seek faster and easier testing methods for melamine. At the start of October, China's Ministry of Science and Technology released an appeal to members of the public to develop a way to detect melamine in only 30 minutes, according to Xinhuanet.com. That same month, the WHO announced that it was developing a test kit that would rapidly determine melamine levels, according to *China Daily*. ELISA test kits have also been quite effective, according to the WHO, in quickly detecting melamine

in milk, wheat gluten and pet food samples, but until recently they have not been capable of detecting melamine at the limits set by governments. The most stringent limit is 0.05 ppm, set by Taiwan at the end of September. However, Hong Kong's limit for pregnant or breast-feeding women and children under three years of age is 1 ppm, while for all everyone else, it is 2.5 ppm, which is the limit in the US and the EU. Romer Labs' AgraQuant Melamine ELISA can meet government requirements as it detects melamine at 0.50–25 mg/kg for milk powder and 0.10–0.50 mg/kg for milk, yogurt and yogurt drinks.

Nevertheless, such adulterant-specific solutions are unlikely to be the answer. "There are other nonprotein nitrogen-containing adulterants that would interfere with the conversion of nitrogen into protein and not be detected by melamine analysis," explained Dr. Fontana. "Accurate conversion of nitrogen into protein only occurs if the nitrogen content of the protein fraction is known and other nonprotein nitrogen-containing compounds are accounted for. Separation of nonprotein nitrogen from true protein can be accounted for by the addition of protein precipitation testing."