# MS: The Whey to Purity

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## The world's largest food company discovers the purifying analytical powers of mass spec.

As dietary requirements become more stringent and consumers more health-conscious, industry has become innovative in finding ways to introduce healthier products into the market. One such effort by researchers is the improvement of the quality of milk by analyzing the most abundant whey proteins, α-lactalbumin (α-Lac) and β-lactoglobulin (β-Lg) (Figure 1), to detect chemical modifications induced by processing conditions. Researchers have discovered that the most widely used thermal procedures used in industrial processes (i.e., pasteurization and sterilization) induce structural modifications that detrimentally affect milk quality in powder and liquid form.

Pasteurization is used to retard spoilage in many food products. The process is, of course, named after Louis Pasteur, who discovered that organisms such as coliforms, pseudomonads, streptococci, and staphylococci could be inactivated and rendered harmless in wine by heating at temperatures below the boiling point. The process has become one of the most widely practiced food safety techniques in the world, used to protect products such as fruit juices, beer, wine, ice cream, and milk. However, not surprisingly, the heating process that makes food safe can initiate changes in taste, odor, and even nutritional character. Recently, researchers have been working on a means of assessing the quality of milk by analyzing whey proteins in raw and pasteurized products.

The nature and the extent of covalent modifications in pasteurized milk whey were observed by researchers by integrating classical biochemical procedures with advanced mass spectrometric methodologies. According to Jörg Hau of the Nestlé Research Center (*www.nestle.co.uk*), "characterization of the influence of industrial processing on protein modifications and classification of whey-protein-based ingredients or milk powders from different suppliers" are the focus for developing an MS method to assess whey content (*1*).

### Challenges

Although it is known that the nutritional and functional properties of whey are linked to the folded state of proteins, the detailed relationship between structure and function is far from understood (*2, 3*). The origin of the milk, varied processing methods, as well as protein content and its relative amount in the whey can influence the physical properties of the protein mixture. These physicochemical changes that occur during the production process can affect the organoleptic or sensory quality, nutritional value, and solubility of the final food product. This is especially true in the case of beverages, where the clarity of the finished product is important. The food industry has observed batch-to-batch variability in protein content and bioavailability from what seemed to be "nominally identical products from different suppliers" affecting "foam formation and foam stability", according to Hau's team. The resulting protein destruction could yield powders with low protein content yields versus a desired protein yield of 25–89.9%, according to the U.S. Department of Agriculture's 21 *CFR* Part 184.1979c specification for whey protein concentrate (*4*).

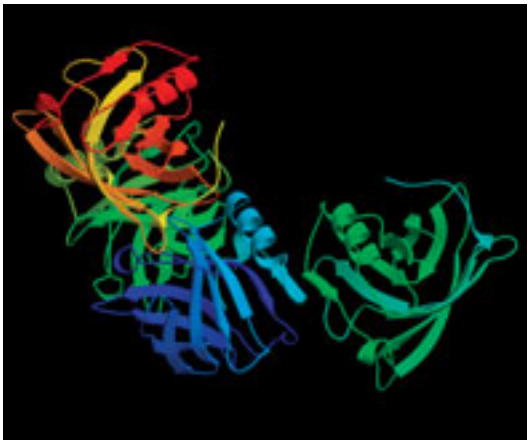

**FIGURE 1: β-Lactoglobulin molecule**

IMAGE: PROTEIN DATABANK

### Whey Mechanics

Several analytical methodologies have been developed to detect the physicochemical changes in whey protein. The most widely accepted methods seek to determine the amount of furosine by HPLC and/or GC methodologies following acid hydrolysis of lactosylated proteins (*5, 6*). An enzyme-linked immunosorbent assay has been shown "to be effective in detecting and quantitating lactosylated milk proteins." However, none of these approaches provide information on further possible modifications of milk proteins other than lactosylation (*7*). Nor can they identify lactosylation sites, and the question remains as to whether specific amino acid residues have been preferentially modified.

Mass accuracy determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis exhibits "considerable variation" and cannot "separate the two β-Lg variants or the glycosylated form of α-Lac from the β-Lg," according to research conducted by Nicki Kinghorn and colleagues at the New Zealand Dairy Research Institute (*www.fonterra.com/default.jsp)* (*8*). Capillary electrophoresis (CE) has also been used to determine α-Lac in milk powders by researchers Jose Gutierrez and Lisa Jakobovits of the U.S. Customs Service Research Laboratory. CE provides fast separation and high resolution, but the extraction process is crucial in determining α-Lac, and because of denaturing that occurs in processing, levels of α-Lac were lower than that of milk, which suggests "degeneration due to processing of the liquid milk."

Recently, new technologies using MS for the structural analysis of milk proteins have been developed to provide a correlation between the observed variability and structural modifications induced by industrial processes. Researchers have been able to determine that protein modifications

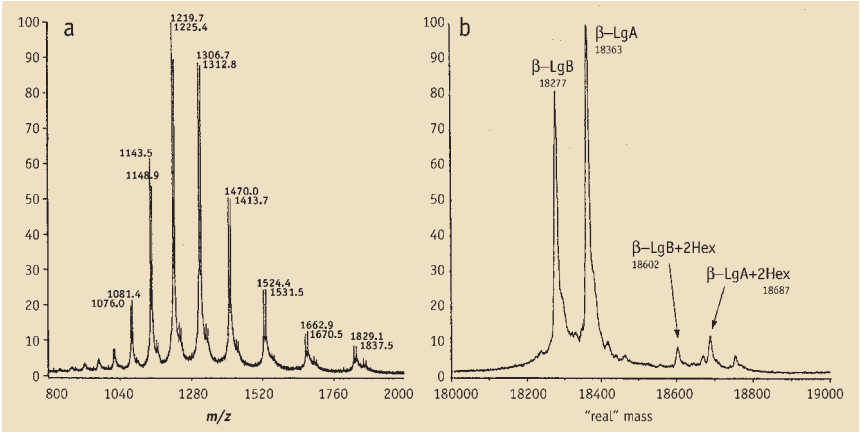


**FIGURE 2: Mass spectra of averaged, -lactoglobulin peaks (a) raw data and (b) deconvulated spectrum. Proteins are "almost pure" and exhibit "minor gylcosylation". (Reprinted with permission from Hau, J. et al. *J. Chromatogr. A* 2001, *926* (1), 105–112. Copyright 2001 Elsevier.)**

are essentially caused by the nonenzymatic glycation of amino groups by lactose (Maillard reaction) and from fat oxidation. In 2000 (*7*), a research team led by Rosa Siciliano at the Istituto di Scienze dell'Alimentazione del CNR (*www.isa.av.cnr.it*) concluded that "a detailed structural investigation of the modification sites, carried out by the mass mapping strategy, revealed the occurrence of preferably lactosylated sites in both α-Lac and β-Lg."

MS has also become an important technique in investigating the glycosylation and oxidation that occur in commercially manufactured whey protein. The combination of electrospray ionization (ESI) MS with HPLC allows the characterization and localization of lactosylation sites and the determination of protein glycosylation.

## The Technology

The Nestlé Research Center team has recently begun characterizing whey protein to differentiate the protein composition from various vendors. To gain more insight into batch variability, Nestlé researchers used a method that coupled LC with ESI MS. The method was tested using several samples with a mobile phase containing trifluoroacetic acid (TFA), but initial results indicated that the TFA could lessen the sensitivity depending on the electrospray interface. Subsequently, the TFA was replaced with 5% formic acid, which showed improved chromatographic separation and sensitivity as confirmed by MS.

Describing the ESI system, Hau says, "While conventional quadrupole mass analyzers allow the acquisition of raw spectra with approximately unit resolution, a time-of-flight instrument as employed in this study usually provides a working resolution of 5000 or higher." Compared with other techniques for mass determination, this has proven superior, says Hau.

The mass spectra of the whey samples allowed detection of the primary proteins α-Lac, β-Lg A, and β-Lg B in the sample mixture. The deconvoluted spectrum of the averaged β-Lg peaks shows signals of β-Lg B at 18,277 *m/z* and β-Lg A at 18,363 *m/z* with a small amount of glycosylation (Figure 2). Hau's team observed that as with the primary proteins, most of the other proteins in the samples "had undergone a variety of modifications" in the process, including glycosylation and oxidation. These modifications induced by process-related events in minor amounts or below the limit of detection are crucial to the functionality of the whey in the final product.

The LC-ESI-MS combination offers a very reliable, rapid, and accurate method of analysis of whey protein mixtures. Hau adds, "Protein modifications induced by processing can be identified and used to evaluate the impact of different processing technologies on whey protein." Although MS alone can separate charged atoms or molecules according to their *m/z* ratio and provide structural information, it "cannot determine the exact nature of a structural modification in a protein."

**MS is providing an improved mechanism for detecting structural modifications.**

## Future Development

To further investigate the relationship between proteins and the severity of the imposed manufacturing processes, Nestlé researchers have collaborated with scientists from Proteome Systems Ltd. *(www.proteomesystems.com)* "to address questions related to the biological significance of the glycosylation of dietary proteins," according to Jean-Richard Nesser, Head of Nestlé's Bioscience Department (*9*). Their efforts are focused on establishing a "glycoprotein research program to analyze the sugars attached to proteins in milk." The researchers hope that this offers technologies that the food industry can use as a guideline in monitoring milk production.

MS is providing an improved and more accurate mechanism for detecting structural modifications of whey proteins induced by industrial treatments. Through improved ESI-MS methodology, the procedure has enhanced the analyses of milk powders relative to previous methods. And the ability to characterize and identify whey proteins using this technique could provide a powerful tool to the dairy and food industry.

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