



Protein Determination

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Protein is one of the main constituents of milk along with fat, lactose and water. Proteins are comprised of individual amino acids linked together by peptide bonds. There are 20 amino acids that are common to most living organisms. The different protein molecules are folded into complex structures and perform a range of functions in liquid milk.

Proteins are an important component in the nutritional value of milk, with liquid milk typically containing around 3.5% protein. Based on relative solubility, dairy proteins are divided into casein (contained in colloidal micelles) and whey proteins. There are four different caseins (α_1 , α_2 , β and κ -caseins) and they comprise around 80% of total milk protein. There are four principal whey proteins (α -lactoalbumin, β -lactoglobulin, bovine serum albumin, immunoglobulins) that together comprise around 20% of total milk protein. Besides these main proteins there are numerous minor proteins. The determination of protein content of milk and milk products is an important process that underpins the international trade in dairy products.

The distinction between methods for determination of protein quality for nutrition purposes and chemically defined protein is important. In this fact sheet we refer only to the latter.

Methods for the determination of proteins in milk can be divided into three broad types:

1. Determination of total nitrogen
2. Direct protein determination
3. Indirect protein determination¹

Each of these methods has advantages and disadvantages; all are currently in routine use.

¹ Although methods based on total nitrogen determination are essentially also indirect methods, we have treated them as a separate type because of their wide spread acceptance and use in dairy testing laboratories.

Determination of total nitrogen

Nitrogen is an important component of protein, and each protein has a unique nitrogen content. For more than a century, food analysts have determined the protein content of foods by determining the total nitrogen content and then calculating the protein content by using a suitable “nitrogen conversion factor”. This factor is determined according to the amount of nitro-



gen in the protein amino acid sequence. The analysis method for determining total nitrogen content may involve chemically digesting or, alternatively, combusting the sample, and converting the resulting material to a form that allows the total nitrogen to be measured.

The total nitrogen value is converted to total crude protein using a nitrogen conversion factor of 6.38 for milk proteins and 6.25 for milk based infant foods. True protein nitrogen is obtained by subtracting from the total

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nitrogen content the nitrogen content of the filtrate of a sample treated with trichloroacetic acid. The result (true protein nitrogen) is multiplied with the conversion factor (6.38) and gives the true protein content.

The so-called Kjeldahl method and Dumas method, which are current international standards, respectively use the chemical digestion and combustion approaches. The advantage of these methods is that they have high reliability and accuracy. A disadvantage is that they require dedicated laboratory equipment and skilled staff which makes them expensive and time-consuming to carry out. Using these methods, around 95% of nitrogen in milk is found to be present as proteins, with the remainder as non-protein nitrogen sources such as urea.



Direct protein determination

Besides measuring the total nitrogen content, it is also possible to quantify specific protein components. A variety of methods are available for protein component determination.

- a. **Dye-binding assays** utilise dyes that specifically bind to proteins. By measuring the intensity of the dye colour, which depends on the protein concentration, the protein content is determined.
- b. **Chromatographic methods** involve separating the intact proteins based on their physical properties such as size, electrical charge or hydrophobic/hydrophilic properties and then measuring the relative amounts of each. There are several different types of chromatography methods available such as size-exclusion chromatography, ion-exchange chromatography, reverse phase chromatography and size exclusion chromatography. Once the proteins are separated, there are various ways in which they can be detected, e.g. by using ultraviolet, fluorescence or mass spectroscopy detectors. Calibration with appropriate commercially available protein standards allows quantitation.
- c. **Electrophoresis** is the separation of proteins by charge using an electric field. In order to make sure that basic and acidic proteins migrate in the same direction in the electric field, the proteins are heat-denatured in the presence of sodium dodecylsulfate (SDS). SDS binds to the hydrophobic parts of the proteins exposed upon denaturation, resulting in an almost equal relation between negative charge and molecular mass for basically every protein. In a polyacrylamide gel, every protein thus migrates during application of the electrical field a distance that is determined by its molecular mass. After separation, the individual protein bands are quantified by dye binding.
- d. **Immunology** relies on using the interaction between an antigen and its corresponding antibody. This methodology is very suitable for the quantification of minor proteins.

It is also possible to quantify individual amino acids chromatographically following thorough chemical digestion of the intact proteins. Using the ratios of selected marker amino acids that have been shown to be more common in casein or whey proteins, the whey and casein contents of milk and milk products can be estimated.

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To ensure accuracy for each of these methods suitable stable protein standards are important. These are commercially available and a robust quality assurance procedure is essential to ensure long term accuracy for any **protein** analytical methodology.

Indirect protein determination

Spectroscopic methods can be carried out rapidly at low costs in a production environment, but rely on calibration to a chemical or reference method to ensure consistency. Thanks to robust and well validated ISO/IDF standard methods as listed in the bibliography, it is possible to anchor them to validated reference assays on specific products. Near and mid infrared spectroscopy are in widespread use for both liquid and solid dairy products in production laboratories and independent testing laboratories alike.

Bibliography

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3. ISO 8968-4|IDF 020-4:2001 - Milk - Determination of nitrogen content - Part 4: Determination of non-protein-nitrogen content.
4. ISO 17997-1|IDF 029-1:2004 - Milk - Determination of the casein-nitrogen content - Part 1: Indirect method
5. IDF Standard 185: 2002(E), ISO 14891:2002(E). Milk and Milk Products. Determination of Nitrogen Content Routine Method by Combustion According to the Dumas Principle.



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