Recent Methods in Total Protein Quantification of Milk

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Abstract

Quantitative analysis of total protein in milk is usually associated with conventional, time-consuming methods. Although there are rapid UV-vis measurements, they have interferences from different molecules which effect the accuracy of the tests. By using the latest developments in nanotechnology, there are fast and accurate methods with great potential use in industry. Surface-enhanced Raman spectroscopy (SERS) enables the development of sensitive total protein assay with a small sample volume. In addition, chemometric methods and imaging techniques have been utilized with this field. Therefore, this review aims to summarize latest developments in the total protein quantification of milk.

Keywords: Milk, Protein, Quantification, Raman, SERS, Digital imaging, Diffuse reflectance

Abbreviations: BCA: Bicinchinonic Acid; PMA: Phosphomolybdic Acid; PLS: Partial Least Squares; LEDS: Light-Emitting Diodes

Introduction

Total protein quantification is important in many areas, from clinical research to basic science. Among them, food quality control is the critical issue and milk needs to be monitored routinely at every stage of the production. Milk composition depends on many factors and milk contains % 3.0 - 3.5 total protein varying with the breed, feeding and climate conditions. There are conventional methods for the quantification of the total protein in dairy products, such as Kjeldahl [1] and spectroscopic methods. All these existing methods have drawbacks and time-consuming steps of analysis. There is a demand for fast, accurate and easy-to-operate techniques for the protein quantification of raw milk and the dairy products. Kjeldahl method starts with the digestion of samples with sulfuric acid which total nitrogen converts to ammonium sulfate. This solution is neutralized under alkali conditions and then distilled into a boric acid. The final step is the titration of borate anions with hydrochloric acid. It is clear that Kjeldhal method is the most reliable method so far, however there are three different steps which are both time and chemical consuming. On the other hand, most preferred methods are Biuret, Lowry and Bicinchinonic acid (BCA) [2-4] which are required UV-vis spectroscopy for the measurement, but there is the risk of false quantitation due to the reduction capability of glucose or any other reducing molecules in the sample. Vibrational spectroscopy has mostly used for qualitative analysis; however, Raman spectroscopy has preferred in quantitative analysis nowadays. Different attempts have been pointed out on the total protein quantification of milk protein with spectroscopic techniques,

however each of them has their own drawbacks. Although there are many sensitive and rapid techniques for peptides and specific proteins with Raman spectroscopy, there is still a demand for total protein evaluation of milk and dairy products. Raman spectroscopy based total protein assays for milk will be discussed in this short review since it is sensitive and easy-to-operate.

Within this scope, we proposed a technique for the total protein quantification of milk with surface-enhanced Raman spectroscopy [5]. This technique has the advantage of direct analysis of milk with only ten-fold dilution with water. Rod-shaped nanoparticles were synthesized and the reaction took place between OPA and protein in the presence of these nanoparticles. The measurement time of the developed assay was 10 seconds and the total assay time was less than 5 minutes when the nanoparticles were ready-to-use. In addition, real milk samples were analyzed with high accuracy. The high-affinity character of OPA to proteins and the remarkable sensitivity feature of SERS have presented an advantageous method compared to other spectroscopic methods5. The comparison of the existing techniques is given in Table 1 and the developed OPA-SERS assay is featured with selective and sensitive total protein quantification of milk. Likewise, the method we have proposed, Huang et al. developed phosphomolybdic acid (PMA) mediated SERS technique for the detection of total protein in milk [6]. A good linear correlation was found between casein and SERS intensity of PMA. With the limit of detection of 1.5μg/mL, this simple and rapid method was also able to quantify the total protein in milk. The results were in correlation with the

Lowry and the Kjeldahl methods. It was important to note that the proposed method has no interference with melamine at the range of 5 mM [6].

Table 1: Comparison of existing total protein quantification methods.

Assay	Principal	Technique	Sensitivity (mg/mL)	Interference
Kjeldahl [6]	Amount of nitrogen (N)	Titration	-	Other N sources
Biuret [3]	Copper chelating	UV-VIS Spectroscopy	1.0 – 10	Reducing sugars, Tris buffer, ammonia
Lowry [4]	Copper chelating	UV-VIS Spectroscopy	0.5 – 1.5	Free thiol groups, buffer salts
BCA	Copper chelating	UV-VIS Spectroscopy	0.5 – 1.5	EDTA
OPA	Protein-Ligand Recognition	Fluorescence Spectroscopy	0.01 – 0.5	-

In general, Raman spectra of liquid milk has poor signal to noise ratio which has forced to develop aforementioned methods requiring a label or a reaction. To obtain relevant information from the Raman spectra, chemometric methods were utilized in this field. Mazurek et al. quantified macronutrients in cow milk using FT-Raman spectroscopy [7]. The Raman spectra of liquid milk samples were analyzed with PLS calibration model and with RSEPtest errors of 3.4-5.6% relative to the reference method. This study was also an effective tool for simultaneous quantification of fat, protein, carbohydrates and dry mater in milk. Besides Raman spectroscopy, there are different approaches for the total protein quantification of milk. Kucheryavskiy [8] developed a new algorithm for obtaining information from digital images for the quantification of total protein and fat in cow milk. The conventional digital imaging has never performed for the milk analysis before this study. Histograms of intensity, Angle Measure Technique and first-order statistics were chosen for image features, then partial least squares (PLS) regression was calculated. However, the obtained results were worse than the Vis/SW-NIR spectroscopic method, this study had the technical simplicity and the potential use in the field [8].

Bogomolov [9] developed a technique for the diffuse reflectance spectroscopic analysis of total protein and fat in milk, using the visible and near infrared spectroscopic measurements with eight fiber probes. One of the fibers was used as an illumination and the rest of the fibers detected the diffusely reflected light. Using one of the channels as an internal reference improved the data quality and model optimizations improved the calibration accuracy. The method was tested with industrial milk samples with different fat and total protein content successfully. The proposed method had two important sensor suggestions comparing the full-spectrum analysis. By performing illumination with nine light-emitting diodes (LEDS), fat could be analyzed, whereas for the total protein quantification seven LEDs were required. The developed technique had the potential use in-line process analysis in the dairy industry [9]. The capability of latest methods for accurate quantification of total protein in milk with different techniques, ranging from Raman spectroscopy to digital image analysis were proposed. Although there are still problems, the field is open to new opportunities and developments in nanotechnology have gained tremendous attention for fast and sensitive analysis with small amount of sample.

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