J. Dairy Sci. 98:1–17
http://dx.doi.org/10.3168/jds.2015-9580

Modification of the Kjeldahl noncasein nitrogen method to include bovine milk concentrates and milks from other species[1]

Karen L. Wojciechowski and David M. Barbano[2]
Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, 289 Stocking Hall, Ithaca, NY 14853

ABSTRACT

The objective of our research was to modify the current indirect casein method for bovine milk to enable it to be applied to bovine milk, bovine milk concentrates, and milks of other species that contain a protein concentration up to 9% (wt/wt). Our work used a series of bovine milk concentrates from about 3 to 9% protein with the same casein as a percentage of true protein to determine the amount of buffer required and pH of the noncasein nitrogen (NCN) filtrate to achieve consistent estimates of casein and casein as percent of true protein. As the concentration of protein in milk increased (either in bovine milk concentrates or in milks of other species), the amount of buffer needed for the NCN sample preparation method to achieve a filtrate pH of 4.6 increased. In the first part of the study using a series of bovine milk concentrates, it was demonstrated that the method gave more consistent predictions of casein as a percentage of true protein when the final NCN filtrate pH was between 4.5 and 4.6 at 38°C. When the amount of buffer added to the sample was not sufficient (i.e., the filtrate pH was too high), the filtrates were not clear. A polynomial equation was developed for prediction of the amount of acetic acid or sodium acetate buffer required to achieve pH 4.5 to 4.6 for milk protein concentrations from 3 to 9% protein using bovine milk and milk concentrates. When the equation developed using cow milk was applied to goat, sheep, and water buffalo milks, it correctly predicted the volume of reagents needed to achieve a final NCN filtrate pH of 4.6 at 38°C. We also verified as part of this work that the ability to measure NPN content of milk was not influenced by protein content of milk in the range from 3 to 9% protein. The results of this study will be used as the basis for proposed changes in the official methods for measurement of the casein content of milk to expand the scope of the method so it can be used to achieve accurate results for milk concentrates and milks of other species.

Key words: casein, Kjeldahl, noncasein nitrogen, nonprotein nitrogen

INTRODUCTION

In 1938, Samuel J. Rowland published a paper (Rowland, 1938a) describing the analytical procedures for fractionation, measurement, and classification of N-containing compounds in milk. The fractions that he defined were total nitrogen (**TN**), noncasein nitrogen (**NCN**), NPN, proteose-peptone, and globulin N. The methods described by Rowland (1938a,b) were used with good results for the determination of the N distribution in a large number of samples of normal and abnormal bovine milk. The TN, NCN, and NPN are the main N fractions for the determination of N distribution in bovine milk used commonly today. The NCN method uses 2 solutions (acetic acid and sodium acetate) which, when combined, form an acetate buffer. However, Rowland (1938a,b) did not combine the 2 solutions and then add them to milk. The results of Rowland (1938a,b) indicated that it was important to lower the pH of the milk and water mixture beyond 4.6 with the addition of acetic acid, and then after a short incubation period at 38°C the acetate solution was added to bring the pH back up to approximately 4.6. The 2-step addition allows the pH to go below the isoelectric point with acetic acid addition, which provides ruggedness to the method to accommodate milks of other species with slightly lower isoelectric points of CN than bovine milk. Rowland (1938a,b) analyzed only bovine milks and used a fixed volume of the buffer solution with a 10-mL milk sample. This is reflected in the current International Dairy Federation (IDF, 2004, method 29–1) and AOAC International methods (AOAC International, 2010, methods numbers 991.20, 991.21, and 998.05) for indirect measurement of casein N content of milk. Need exists in the global dairy industry for a modified NCN method that works bovine milks, milks of other species (e.g., sheep, goat, and

Received March 16, 2015.
Accepted June 3, 2015.
[1]Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors by Cornell University or the Northeast Dairy Foods Research Center.
[2]Corresponding author: dmb37@cornell.edu

water buffalo), and bovine milk concentrates. Recent work with milk protein concentrates has demonstrated that more buffering capacity of the reagents is needed (Zhang and Metzger, 2011). It is likely that milks with higher protein content will require a different amount of buffer to achieve the optimum pH for preparation of an NCN filtrate that is free of CN. The objective of our research was to modify the current indirect CN method for bovine milk to enable it to be applied to bovine milk, bovine milk concentrates, and milks of other species that contain a range of protein concentration up to 9%.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The study was conducted in 2 phases. In phase I, 4 bovine milk protein concentrations (from about 3 to 9%) in combination with 6 different buffer concentrations at constant buffer volume addition in the NCN method were evaluated to determine the effect of buffer concentration on filtrate pH, clarity, and NCN content with milks with a wide range of protein content. All percentages reported in the current paper are weight/weight unless otherwise stated. This was replicated 3 times. In phase I, we encountered difficulties keeping the highest concentration of sodium acetate in solution during refrigerated storage, which prevented us from exploring higher buffer concentrations to achieve the target pH of the NCN filtrate. Therefore, we used a different strategy in phase II of our work. We increased the volume of addition of the low-concentration buffer that is specified in the original NCN method to progressively increase the buffering capacity used for precipitation of the CN for higher-protein samples instead of increasing buffer concentration. In phase II, 3 different volumes of buffer addition were used, one that equaled the buffering capacity of the highest buffer concentration used in phase I, and 2 volumes that exceeded the highest buffer concentration used in phase I. In phase II, the performance of the NCN method was evaluated for bovine milk and milk concentrates from about 3 to 9% protein, sheep, water buffalo, and goat milks using 3 different volumes of buffer addition.

To determine if milk protein and buffer concentration had an effect on estimated NCN content, casein as a percentage of TP (**CN%TP**), and NCN filtrate pH, an ANOVA using the Proc GLM procedures of SAS (SAS version 8.02, SAS Institute Inc., Cary, NC) was conducted using a split-plot model. Milk protein concentration and replicate were category variables in the whole plot, whereas buffer concentration was used as a continuous variable with linear, quadratic, and cubic terms for buffer concentration and their interaction with whole plot terms as the subplot. The milk by replicate interaction was used as the error term to test the significance of terms in the whole plot whereas the model error term was used to test for significance of all the other terms in the model. Distortion of the ANOVA by multicollinearity in the model was minimized by mean centering the buffer concentration using a mathematical transformation (Glantz and Slinker, 2001). Buffer concentration was transformed as follows: buffer concentration = buffer concentration – [(highest buffer concentration – lowest buffer concentration)/2]. This mathematical transformation made the data set orthogonal with respect to buffer concentration.

Current Status of the NCN Method for CN Determination

The official methods for measurement of NCN in both IDF and AOAC are based on the principles of the Rowland (1938 a,b) method for fractionation of the N-containing compounds of milk and the collaborative study of the NCN method by Lynch et al. (1998). The current method uses a 10-mL milk test portion at 38 ± 1°C that is diluted with 75 mL of water at 38 ± 1°C. To the diluted milk, 1 ± 0.02 mL of 10% acetic acid was added to lower the pH below 4.6; then, the mixture was swirled and held for 10 min at 38 ± 1°C. After 10 min, 1 ± 0.02 mL of 1 *N* sodium acetate solution is added to bring the pH back up to about 4.6. Lynch et al. (1998) discussed the use of a correction factor in the calculation of the casein result to take into account the volume occupied in the milk by the CN and fat removed from the NCN filtrate. In the present study, we measured the fat content of the milks by ether extraction (AOAC International, 2010; method 989.05) and calculated the applicable correction factor for each sample.

It was assumed that buffer capacity was sufficient with these two 1-mL additions for bovine milks of all protein levels; the principle goes back to the original work by Rowland (1938a,b). Prior to the collaborative study conducted by Lynch et al. (1998), ruggedness testing was not done to determine the range of protein over which the buffer concentration specified in the method was adequate because the NCN method was a long-standing official method and the primary purpose of the collaborative study was to develop the new direct CN method that would produce results that would agree with the more traditional indirect method of CN determination on bovine milk. Recent interest in the application of the direct and indirect CN determination methods (Lynch et al., 1998) to milks from other species and bovine milk concentrates that contain higher protein content has raised a question about the