DETERMINATION OF THE PROTEIN CONTENT OF MILK BY A MODIFICATION OF THE STEAM DISTILLATION METHOD OF KOFRANYI[1]

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A method is described for the determination of the protein content of milk by steam distillation following addition of NaOH and $BaCl_2$. Excellent agreement was obtained between the protein values as determined by this method and the Official Macro-Kjeldahl procedure.

In recent years increased interest has been shown in procedures suitable for the routine determination of the solids-not-fat constituents of milk. A rapid and simple method for the determination of the protein content of large numbers of milk samples would be useful in obtaining more extensive information on the influence of environmental and genetic factors on the composition of milk. According to Politiek (*3*) the protein level in milk is heritable with heritability estimates ranging from 0.70 to 0.75. He also reported that fat and protein content were to a high degree inherited independently of each other. Some of the methods that may be used for the routine determination of the protein content of milk are (*a*) direct steam-distillation, (*b*) dye-binding with dyes such as Orange G or Buffalo Black and (*c*) formol titration.

In 1950 Kofranyi (*2*) reported on a direct steam distillation method for the determination of the protein content of milk. In this method a sample of milk made strongly alkaline with NaOH is submitted directly without previous digestion, to steam distillation in a Parnas-Wagner micro-nitrogen distillation apparatus. The experimental conditions were arranged such that a consistent amount (approx. 11%) of protein nitrogen was released. This nitrogen is mainly amide-nitrogen and a small amount is derived from alkaline hydrolysis of certain amino acids. The ammonia was received in 0.025 *N* H_2SO_4 and the excess standard acid was determined by titration with 0.025 *N* NaOH with a mixed indicator (methylene blue-methyl red). A factor then was used to convert the amount of standard acid into terms of total protein. This factor was established by comparing the results (ml. standard acid) obtained with the Kofranyi method with those (% protein) obtained on the same samples with the Kjeldahl procedure. Preliminary data on modified Kofranyi steam-distillation techniques applicable for large scale determinations of the total protein content of milk were reported independently by Vanderzant *et al.* (*5*) and Stone *et al.* (*4*). The present paper describes a modification of the Kofranyi technique which yielded the same precision as the Official Macro-Kjeldahl procedure.

EXPERIMENTAL METHODS

Milk samples were obtained from (*a*) individual Holstein and Jersey cows in the herd of the Dairy Science Department at Texas A and M College, and (*b*) mixed herd milks from individual producers in Brazos County. The samples represented two complete milkings from animals in good health. The samples were collected in half-pint glass bottles, placed in an ice chest, transported to the laboratory and were stored in a refrigerator at 40° to 45°F. The protein contents of these samples were determined within two to three days.

Procedure

The milk samples were placed in a waterbath and warmed to 70°F. Each sample then was mixed thoroughly to mix the cream and the serum portion of the milk. To 10 g. of milk delivered with a special pipette into a 250-ml. Soxhlet extraction flask were added 10 ml. of a 10 *N* NaOH solution and 10 ml. of a 10% $BaCl_2$ solution with automatic burettes. This mixture then was steam distilled for 9 minutes. The 9-minute distillation period was started as soon as the first drop of distillate turned the color of the indicator from purple to green. The released ammonia was received in a 250-ml. beaker containing 20 ml. of a 3% boric acid solution to which 5 drops of a mixed indicator were added. This indicator consisted of a mixture of 0.026% ethanolic methyl red and 0.013% ethanolic methylene blue. The amount of nitrogen released was determined by titration with 0.05 *N* HCL. A conversion factor then was used to convert milliliters of 0.05 *N* HCL into percent protein.

The apparatus (Figure 1) consisted of a steam generator (A), steam trap (B), 2 Kjeldahl connecting bulbs (D) and 2 Liebig condensers (E, length 300 mm., jacket 35 mm., tube 12 mm.). The steam gen-

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TABLE 1 — COMPARISON OF MILK PROTEIN VALUES AS DETERMINED BY THE MACRO-KJELDAHL AND STEAM-DISTILLATION METHODS

Breed	No. of samples	Kjeldahl protein mean	Steam-dist. protein mean	Conversion factor Range	Conversion factor Mean	Correlation coef.
Holstein	35	3.18	3.19	.382-.410	.395	.99
Jersey	35	3.92	3.93	.382-.406	.395	.98
Mixed Herd	20	3.18	3.19	.383-.410	.393	.97
Total	90	3.47	3.48	.382-.410	.394	.99

erator consisted of a 2-liter resin flask and was electrically heated. The heat was controlled by a variable autotransformer which was kept at a certain setting. Approximately one liter of distilled water was kept in the steam generator. The steam generator was also connected with a supply of distilled water for the purpose of adjusting the water level after each run. In this arrangement each steam generator supplied steam through one steam trap to two extraction flasks (C). Two distillation units were available so that four single determinations or two in duplicate could be run by one technician. Kjeldahl determinations were made according to the Official Macro Kjeldahl Method (*1*).

RESULTS AND DISCUSSIONS

In Figure 2 are presented data on the amount of ammonia (expressed as ml. 0.05 *N* HCL) distilled over at various times of steam-distillation. Other milk samples showed a similar pattern. After 9 minutes of steam distillation very little additional nitrogen was released.

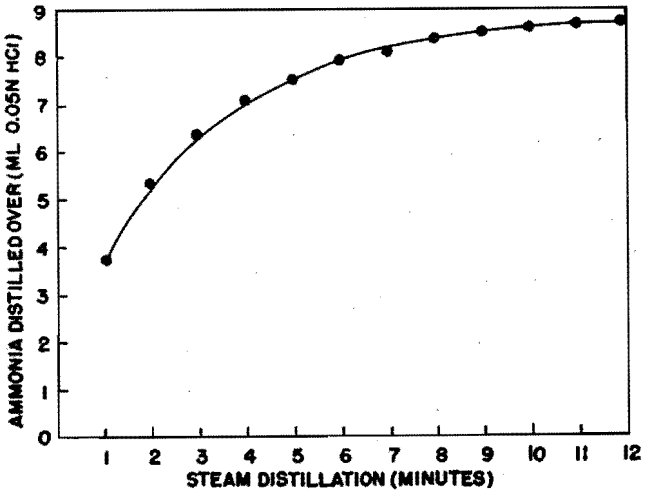

Figure 2. Amount of nitrogen released at various lengths of the steam-distillation period.

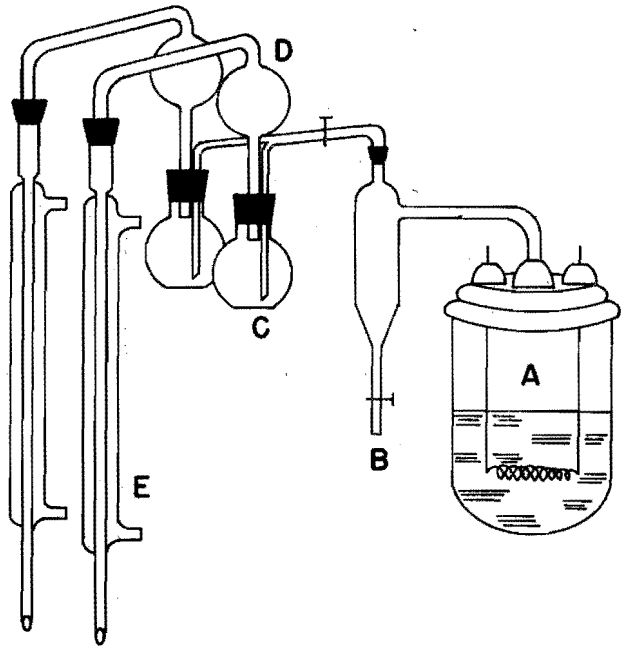

Figure 1. Steam distillation apparatus

The amount of Kjeldahl protein nitrogen and steam distilled nitrogen was determined in duplicate on a total of 90 samples of raw milk (Table 1). An analysis of the data indicates that the steam distillation method of determining protein in milk is as accurate as the macro-Kjeldahl method. The amount of steam distilled N expressed as percent of total protein N in the 90 samples ranged from 10.89 to 11.69 percent, with a mean value of 11.33 percent. The conversion factors were established by dividing the Kjeldahl protein values by the ml. of standard (0.05 *N*) acid used in titrating the steam-distilled N. The steam-distillation protein values were obtained by multiplying the ml. of standard acid used by 0.394.

A comparison of the steam distillation and Kjeldahl protein values of each of the 90 samples showed that these values differed by more than 0.1% (0.11% and 0.13%) in only two of these samples. In six samples the steam distillation protein values differed by 0.1% from the Kjeldahl protein values. In subsequent experiments, milk samples were stored at 40° to 45°F. for 7 days prior to testing with and without preservative. A commercial preparation of bichromate in

tablet form was used as preservative. Samples were withdrawn daily for protein determinations by the steam distillation method. Very little if any difference was found in the protein values of the samples stored for different periods (up to 7 days) at 40° to 45°F. with or without preservative. The data reported in the present study and those of Stone *et al.* (*4*) indicate that the steam distillation method can be used successfully to determine the protein content of milk. The average amount of steam distilled N expressed as percent of total protein N reported by Stone *et al.* (*4*) was 11.88 percent and slightly higher than the value reported in this study (11.33%). On the basis of the same standard acid (0.05 *N*) the conversion factors reported by Kofranyi (*2*), Stone *et al.* (*4*) and in this study would have been 0.382, 0.367, and 0.394 respectively. The small differences in these values can be explained by minor differences in the distillation procedure (equipment, distillation time etc.). However, they all reported excellent agreement between the protein values obtained with the two methods.

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