

R54-32

**BIURET METHOD FOR SOLUBLE WHEY PROTEINS IN  
NONFAT DRY MILK SOLIDS<sup>a,b</sup>**

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One of the most important of the factors which influence the baking quality of nonfat dry milk solids is adequate heat treatment of the skim milk before drying. In the manufacture of nonfat dry milk solids for beverage use, however, it is desirable to avoid excessive heat which induces changes in milk proteins associated with cooked flavor, rough tactual quality, and reduced solubility of milk powder. Accordingly, the military specifications for beverage quality nonfat dry milk solids have long required that this ingredient of recombined milk be manufactured under mild conditions of heating. This requirement creates a need for a laboratory test which will serve as an indication of the extent of heat treatment.

The undenatured protein content has been suggested as such an index, and Harland and Ashworth (9) developed a very useful turbidimetric method for the estimation of whey proteins. The results of collaborative tests by that method, however, were not always in close agreement. It was thought that a colorimetric method might lend itself toward greater reproducibility, and so the biuret reaction was used for replacing the turbidity measurement by colorimetry. The influence of non-protein constituents of the whey upon the reliability of the method was investigated. The major interference was caused by lactose which exhibited the properties of a polyhydric alcohol by reacting with the copper sulfate and sodium hydroxide of the biuret reagent to form a dark-blue complex. Its reducing action on the copper sulfate was an additional source of error. The method which was developed corrects for the interference by lactose without the necessity for a time-consuming lactose-removal operation. The method is simple but close attention to its details is necessary for best results. This paper is confined to a description of the method, the steps in its development, and its application to nonfat dry milk solids of known preheat treatment.

**MATERIALS AND METHODS**

Equipment. Constant temperature bath at  $40^{\circ} \pm 1^{\circ}\text{C}$ .

Centrifuge for operation at 2,000 to 2,400 r.p.m. (International Equipment Company<sup>c</sup> size No. 2, equipped with No. 831 and No. 845 angle heads, was used.)

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<sup>c</sup>The mention of this and other commercial products does not imply that they are endorsed or recommended by the Department of Defense over similar products not mentioned.

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Spectrophotometer. (Coleman Universal Model 14 with 13-mm. square cuvettes was used.)

Test tubes, 25 X 150 mm., fitted with No. 5 rubber stoppers.

Centrifuge tubes with constricted necks, 100-ml. capacity (Corning No. 8460).

Conical centrifuge tubes, 15-ml. capacity (Corning No. 8960 or 8080), fitted with No. 1 rubber stoppers.

Rubber caps for 15-ml. centrifuge tubes (International Equipment Company No. 580.)

Filter paper, Whatman No. 2, 11-cm. and 24-cm. diameter.

Reagents. Sodium chloride, granular, reagent grade.

Saturated sodium chloride solution, prepared by mechanically stirring for at least 30 minutes a mixture of 370 g. of reagent grade granular sodium chloride and 1,000 ml. of distilled water.

Lactose-sodium chloride solution, prepared fresh daily by dissolving 4.450 g. of reagent grade lactose monohydrate in saturated sodium chloride solution and diluting to 100 ml. with saturated sodium chloride.

Trichloroacetic acid solution (7.5% TCA), prepared fresh daily by dissolving 7.5 g. of reagent grade TCA in distilled water and diluting to 100 ml.

Sodium hydroxide solution (3%), prepared fresh daily by dissolving 7.5 g. of reagent grade sodium hydroxide pellets in 25 ml. of distilled water, cooling, and diluting to 250 ml.

Copper sulfate solution (5%), prepared fresh daily by dissolving 2.50 g. of reagent grade cupric sulfate pentahydrate in 35 ml. of distilled water and diluting to 50 ml.

Procedure. The procedure is presented schematically in Figure 1. The casein-free filtrate, hereafter referred to as "whey," is prepared according to the procedure of Harland and Ashworth (9). Weigh 8.0 g. of sodium chloride into a 25 X 150 mm. test tube and add 2.00 g. of nonfat dry milk solids. Pipette 20 ml. of distilled water into the tube; stopper the tube, shake it vigorously with a vertical motion for 40 seconds,

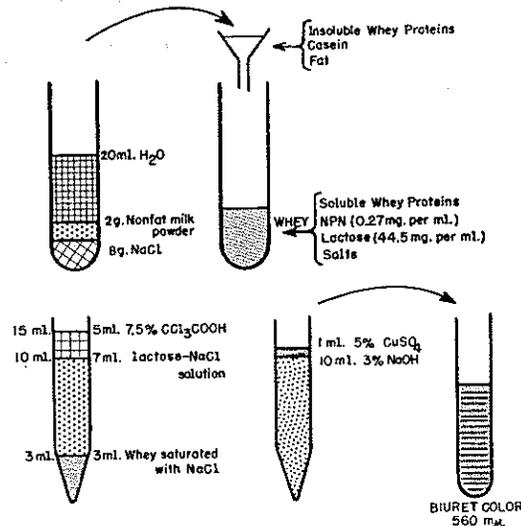


Figure 1. Diagram of the biuret method for whey proteins.

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and immerse it, without further agitation, in a 40°C. water bath for 30 minutes. Filter the mixture through an 11-cm. Whatman No. 2 paper into a test tube. The funnel should be supported by the receiving tube and covered with a watch glass. Stopper the receiving tube and swirl to mix contents. The filtrate can be held until the following day, if desired, but it should be held at room temperature.

Using a volumetric pipette, transfer a portion of the whey to a 15-ml. conical centrifuge tube. Three ml. of whey are sufficient in all cases. However, if the sample is known or suspected to have had high-heat treatment, it is preferable to use a 10-ml. aliquot of the whey. If less than 10 ml. are used, dilute the whey to 10 ml. with lactose-sodium chloride solution. Add 5 ml. of 7.5% trichloroacetic acid (TCA) solution and, without delay, stopper the tube and invert it gently 5 times in about 10 to 15 seconds total time. The motion should be gentle and at uniform rate with insufficient agitation to start flocculation. The rubber stopper should fit the tube tightly with no dead space around the rim and leave an enclosed volume of at least 15.5 ml. In some cases it is necessary to shorten a No. 1 rubber stopper by cutting off approximately 5 mm. at the smaller end. Graduations on the tube can be used for measuring the lactose-sodium chloride and TCA solutions, but it is better to measure these with pipettes. Twelve to 14 tubes can be run conveniently in a group. Twenty minutes after adding the TCA to the last tube of a group, remove the stoppers, and centrifuge the tubes for 10 minutes at a speed of 2,000 to 2,200 r.p.m. It is not necessary to balance each pair of tubes at time of use if all the tubes for a day's run are weighed in advance and selected within a tolerance of 0.5 to 0.6 g. of each other. The centrifugation should be done under conditions which prevent evaporation from the tubes. This can be accomplished by closing the bottom opening of the centrifuge guard bowl and wetting the inside of the bowl with water, or by closing each tube with a rubber cap.

Decant the supernatant solution. It is generally perfectly clear. Occasionally, it may have a slight turbidity, but protein flakes should be entirely absent. Invert the tube on a paper towel or filter paper and allow it to drain for 20 minutes. Wipe off the last remaining drop on the rim of the tube with a cleansing tissue and return the tube to an upright position.

Add 10 ml. of 3% sodium hydroxide solution, measured with a volumetric pipette, and stopper the tube with the same stopper used previously. Shake the tube until the precipitate dissolves completely. When the tube is mounted horizontally in a Kahn shaking machine parallel to the direction of travel, approximately 12 to 15 minutes are required. Set the tube in a rack until the foam disappears, generally 30 minutes, or 15 minutes if unstoppered. The tubes may be allowed to stand for a longer period if desired.

Add 1 ml. of 5% copper sulfate solution, measured from a 10-ml. burette, and without delay, stopper the tube with the same stopper used previously and shake it vigorously 10 times with a vertical motion. The shaking procedure should be uniform for all tests, and in the case of a series of tubes each copper sulfate addition and shaking should be completed before proceeding to the next tube. Run a blank determination consisting of 10 ml. of 3% sodium hydroxide and 1 ml. of 5% copper sulfate at the same time. Note the time of addition of copper sulfate to the first tube and make successive additions at 30-second intervals. Remove the stoppers and, 10 minutes after the last mixing, centrifuge the tubes for 10 minutes at a speed of 2,000 r.p.m. in order to settle the precipitate of copper hydroxide. Prevent evaporation from the tubes as in the previous centrifugation.

Decant the solutions into cuvettes and measure the intensity of the colors with reference to the blank at 560  $m\mu$ . Make the first reading 40 minutes after the first addition of copper sulfate and continue with succeeding tubes at 30-second intervals. Convert the absorbency readings to milligrams of protein nitrogen by reference to a standard curve, and finally to mg. of whey protein nitrogen per g. of nonfat dry milk

solids by multiplying by the factor  $\frac{11.75}{\text{ml. of whey tested}}$

Preparation of standard curve. Select a sample of nonfat dry milk solids of low-heat treatment or high whey protein content. Combine 60 g. of sodium chloride with 15.0 g. of the nonfat dry milk solids and 150 ml. of distilled water in a 500-ml. Erlenmeyer flask, close the flask with a rubber stopper, and shake it vigorously for 40 seconds.

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Prepare 3 such flasks and immerse them in a 40°C. water bath for 30 minutes. Filter each mixture through a fluted 24-cm. Whatman No. 2 paper into an Erlenmeyer flask. Cover the funnel with a watch glass and close the mouth of the receiving flask around the funnel stem with paper to minimize evaporation. Combine the 3 filtrates in a single flask, stopper the flask, mix the contents and store at room temperature. The yield of whey is 300 to 350 ml.

For nitrogen determination, pipette aliquots of the whey into 100-ml. centrifuge tubes. The aliquots should be from 25 to 50 ml. in volume, and from 2 to 6 determinations should be made. Dilute each aliquot of whey to 50 ml. with saturated sodium chloride solution and add 30 ml. of 7.5% TCA solution. The sodium chloride and TCA solutions may be measured carefully in 50-ml. graduated cylinders. Stopper the tubes with rubber stoppers and invert them gently 5 times in about 10 to 15 seconds total time. Each tube should be stoppered and inverted immediately after the TCA addition and before proceeding to the next tube. After 20 minutes, centrifuge the tubes at 2,400 r.p.m. Decant the supernatant solution, being careful not to lose any of the precipitate. Add 25 ml. of a wash solution composed of 1 volume of 7.5% TCA plus 2 volumes of saturated sodium chloride solution. Mix the solution and precipitate by swirling the tube gently and by breaking up lumps with a glass rod. Add 60 ml. more of the wash solution, which is used also for washing the glass rod, remove the rod, stopper the tube, and invert it several times. After 20 minutes, centrifuge and decant as before. Subject the precipitate to a second washing of the same kind.

Dissolve the precipitate in 60 ml. of 3% sodium hydroxide solution and transfer quantitatively with washing to an 800-ml. Kjeldahl flask. (Note: If facilities for centrifuging 100-ml. tubes are not available, 10-ml. aliquots of whey can be treated in 15-ml. tubes and the contents of 3 or 4 such tubes combined in a single Kjeldahl flask at this point.) Determine nitrogen by the Kjeldahl procedure and correct for blank determinations on the reagents in accordance with the A.O.A.C. procedures (1). For the digestion use 35 ml. of concentrated sulfuric acid, 10 to 12 g. of anhydrous sodium sulfate, 0.7 g. of mercuric oxide, and glass beads. Digest the mixture for 30 minutes after it is colorless. Express the results as milligrams of nitrogen per ml. of whey.

Prepare 1:10 whey by diluting exactly 5 ml. of whey to 50 ml. with lactose-sodium chloride solution. Prepare 1:1 whey, similarly, by diluting 25 ml. to 50 ml. with lactose-sodium chloride solution. With volumetric pipettes, transfer 0, 1, and 3 ml. of 1:10 whey and 1, 2, 3, 4, 5, 6, 8, and 10 ml. of 1:1 whey to 15-ml. centrifuge tubes. Dilute each aliquot to 10 ml. with lactose-sodium chloride solution, and continue with the procedure described previously. Prepare a standard curve relating absorbency to milligrams of nitrogen.

#### DEVELOPMENT OF METHOD

Size of sample. Almost identical results were obtained when the method was applied to the whey separated from 1-, 5-, 10-, and 20-g. samples of nonfat dry milk solids. Consequently, it is permissible to make a bulk separation of whey in order to obtain sufficient material for calibration purposes and to use a smaller sample for the test.

Total volume of whey. In computing the mg. of whey protein nitrogen per g. of nonfat dry milk solids in the last step of the analytical procedure, the factor 11.75 represents the volume of whey in ml. obtained from one g. of sample. This value was obtained in two ways. The first was a double-dilution procedure in which lactose was determined in 2 whey solutions of different concentration and different volume by a modification of Cajori's (2) iodometric procedure for the determination of aldoses. The second was a gravimetric method in which the total volume of whey was determined by establishing the relationship between volume of whey and weight of water contained in it.

The factor 12.0 used by Harland and Ashworth was apparently determined by the method which they described in an earlier paper (3). In that method, the volume occupied by the precipitated protein was ignored on the basis of Rowland's (11) work on the volume occupied by casein and fat separated from whole milk. However, Rowland's factor of 0.995 to correct for the volume of the precipitated casein and fat was arrived at by analysis of filtrates from milk diluted 10-fold. The factor is not applicable to undiluted milk in which case it would be reduced from 0.995 to 0.95. Using the value 1.313 for the density of casein, as reported by Chick and Martin (3) and cited later by Cohn and Edsall (4), the volume correction for 1.0 g. of nonfat dry milk solids

is 0.28 ml. The Harland and Ashworth factor reduced by this amount agrees closely with the factor used in this work.

Composition and stability of whey. The major constituents of the whey are listed in Figure 1. The figure for non-protein nitrogen was obtained by subtracting the protein nitrogen from the total nitrogen. These were determined on the whey from a typical low-heat powder by the Kjeldahl method, using TCA for the protein separation as described under "Preparation of standard curve." The value of 0.27 mg. per ml. agrees closely with the amounts reported by others (8, 9, 11, 12). The figure shown for lactose, 44.5 mg. per ml., represents lactose monohydrate. It was determined by the iodometric procedure referred to previously (2) and is nearly constant for all samples.

Kjeldahl nitrogen values on a sample of whey they determined 8 days apart differed by less than 1%. Variations in biuret determinations 9 days apart were less than 2%. A white precipitate separates from the whey upon prolonged standing. This separation can be minimized by holding the whey at room temperature rather than under refrigeration. A Kjeldahl nitrogen determination on the insoluble material which had separated from a sample of whey after several weeks' standing amounted to 0.04 mg. of nitrogen per g. of the original milk powder.

Volume of whey for analysis. Three ml. of whey from a low-heat sample of nonfat dry milk solids or 10 ml. from a high-heat sample provide an amount of protein which can be measured within the most accurate range of the Coleman spectrophotometer or Evelyn colorimeter. The final result by the biuret method was found to be independent of the volume of whey tested. This was not always the case with the Harland and Ashworth procedure, in which the degree of turbidity, measured in absorbency units, bore a linear relationship with volume of whey tested in some cases, but not in others. This variability with concentration cast some doubt on the reliability of the turbidimetric results.

Lactose-sodium chloride solution. The salt concentration influences the recovery of protein. Dilution of the whey with water rather than with saturated sodium chloride solution reduces the intensity of the final biuret color. The concentration of lactose in the lactose-sodium chloride solution is the same as the concentration of lactose in the whey. Regardless of the amount of whey taken for analysis, after it is diluted to 10 ml. the solution contains 445 mg. of lactose monohydrate. The significance of this is discussed under the heading "Compensating the error due to lactose."

Trichloroacetic acid solution (TCA). The final concentration of TCA is 2.5%. This concentration gave the greatest recovery of protein, and, according to Hiller and Van Slyke (10), is optimum for the separation of protein from non-protein nitrogenous material. As the final concentration was varied from 0 to 20%, a second, but smaller, maximum recovery point occurred at about 13%. Above 13%, the protein separated as an oil from which a solid precipitate could be recovered by shaking with water.

Precipitation of whey protein. In some instances the head space above the 15-ml. mark in the conical centrifuge tubes was so small that it was necessary to shorten the No. 1 rubber stoppers by cutting off approximately 5 mm. from the smaller end. There are a number of advantages in using the full capacity of the tube. Greater accuracy was provided in measuring the largest possible volumes of reagents. Also, dilution of the whey insured greater purity of the protein precipitate. If the tubes contained less than 15 ml. and were unstoppered during centrifugation, drying on the sides above the liquid allowed lactose and other non-protein material to be retained, as well as some protein which may be lost, in the subsequent decantation.

Recovery was best when only gentle mixing with TCA was employed, and the use of tubes with only 0.5 to 1 ml. of air space insured the required gentle mixing. When the tubes were shaken or when the concentration of TCA was high, protein was frequently lost during decantation. Immersing the tube in a boiling water bath for 10 minutes coagulated the precipitate to a non-coherent mass which did not stick to the glass, even after centrifuging.

When the time for coagulation of the protein was varied from 0 to 30 minutes the maximum variation in final color density was 2.5%. The period for optimum recovery was 20 minutes.

Re-solution of whey protein. Approximately 15 minutes on the Kahn shaking machine with 3% sodium hydroxide solution was satisfactory for re-solution of the protein precipitate. There were no apparent advantages in using higher concentrations of

sodium hydroxide or shaking times longer than necessary to effect solution of the protein. Although the concentration of sodium hydroxide affects the intensity of the final color, the concentration selected is not important if it is kept uniform throughout the calibration, tests, and blank determinations.

Development of biuret color. The concentrations and volumes of sodium hydroxide and copper sulfate solutions correspond closely with those used by Fine (5). Gornall, Bardawill, and David (6) recommended a biuret reagent containing copper sulfate, sodium potassium tartrate, and sodium hydroxide in a single solution. This "one piece" reagent was tried and discarded because the final solutions were always turbid.

The copper sulfate solution was prepared fresh daily because a precipitate forms in the solution after several days. Somewhat higher results were obtained with a 3-day-old solution.

One ml. of 5% copper sulfate solution appeared to be adequate for color development. Increased amounts of copper sulfate reduced the color intensity, but variations of 10% in the volume added changed the color density less than 1%.

When the period of contact with copper sulfate from the point of addition until the start of centrifugation was varied from 8 to 16 minutes, the variations in color intensity were not more than 2%.

Upon addition of copper sulfate there is an immediate precipitation of cupric hydroxide. Thorough mixing is necessary for complete color development, apparently to effect contact between the remaining copper sulfate and the protein adsorbed by the precipitate. The method of mixing at this point is important inasmuch as it affects the density of the final color. Consistent results between operators were obtained with the procedure described and variations from day to day with one operator did not exceed 2.5%. With more gentle mixing, ranging from 5 inversions of the tube at slow speed to 5 gentle rockings of the tube, results were as much as 14% lower. With more vigorous mixing, from 30 seconds of vertical shaking to 16 minutes on the Kahn shaking machine, results varied by less than 3%, and in no case did this amount to more than 0.12 mg. of nitrogen per g. of powder. Centrifugation of the precipitate should be done under conditions tending to prevent concentration of the solution by evaporation.

Stability of the biuret color. The biuret color fades, but in the period from 30 to 90 minutes after addition of the copper sulfate the color did not fade more than 2.4% for a low-heat sample nor more than 4% for a high-heat sample. The maximum fading encountered during any 10-minute interval between 30 and 90 minutes represented 0.04 mg. of nitrogen per g. of powder.

Effects of non-protein components of the whey. When the whey proteins were precipitated from solution, and the supernatant liquid decanted, portions of the other constituents remained, either adsorbed by the protein or adhering to the walls of the tube. Lactose added to the final color by reacting with the copper sulfate and sodium hydroxide to form a dark-blue complex. The reducing action of the lactose was probably responsible for the subsequent fading of the color discussed in the previous section. Of the non-protein components of the whey, only lactose interfered with the reaction. This is demonstrated by the data in Table 1. The whey proteins were precipitated from a solution made up of 2 volumes of saturated sodium chloride and one volume of 7.5%

TABLE 1  
Effect of non-protein components of whey on the biuret reaction

Material reacting with biuret reagent	Biuret color. Absorbency, 560 m $\mu$			
(1) Whey protein (impurities removed by washing precipitate with CCl <sub>3</sub> COOH-NaCl solution).....	0.400	0.402	0.411	Av. 0.404
(2) Whey protein (not washed).....	0.475	0.484	0.486	Av. 0.482
(3) Lactose associated with (2) (determined by analysis) <sup>4</sup> .....	0.073	0.078	0.080	Av. 0.077
(4) Whey protein, corrected for lactose, (2)-(3).....	0.402	0.406	0.406	Av. 0.405

<sup>4</sup>5.27, 5.78, and 5.91 mg. lactose monohydrate, respectively; average 5.65 mg.

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TCA, as in the regular procedure with the exception that the sodium chloride solution used for diluting the whey to 10 ml. contained no lactose. A wash solution of sodium chloride and TCA made up in this proportion was found to be effective in removing impurities from the precipitate without loss of protein. The biuret reaction was applied to the protein precipitated from 3-ml. portions of whey. The impurities were removed from 3 of the precipitates by re-suspending them in 14 ml. of the wash solution and separating again by centrifuging. The remaining 3 precipitates were not purified, and the lactose contained in them was determined by analysis. The amount of color due to lactose was then estimated from a calibration curve for the biuret reaction with lactose. When this figure was subtracted from the color density produced by the crude precipitate, the corrected measurement agreed within 1% of the value obtained with the purified precipitate. This was taken as evidence that lactose is the only non-protein component of the whey which contributes to the biuret color.

In order to demonstrate the magnitude of the errors due to non-protein materials in the whey, the data of Table 1 are presented again in Table 2. The figure 1.69 mg.

TABLE 2  
Magnitude of errors introduced by lactose and non-protein nitrogenous materials

Whey component	Amount in 3 ml. whey	Amount in CCl <sub>3</sub> COOH precipitate	Biuret color. Absorbency, 560 m $\mu$	Nitrogen equivalent of biuret color
	mg.	mg.		mg.
Protein.....	10.8	10.8	0.405	1.69
Lactose.....	133.5	5.65	0.077	0.34
NPN.....	0.81	0.05	.....	.....

$$\text{Lactose error} = \frac{0.34}{1.69} \times 100 = 20\%$$

$$\text{NPN error} = \frac{0.05}{1.69} \times 100 = 3\%$$

corresponds to absorbency 0.405 on a calibration curve for the biuret reaction with whey protein in which each protein precipitate was purified with a TCA-sodium chloride wash solution. The figure 0.34 mg. corresponds to absorbency 0.077 on the same curve, and represents the nitrogen equivalent, in terms of biuret color, of 5.65 mg. of lactose monohydrate. The figure 10.8 mg. was obtained by multiplying 1.69 mg. by the factor 6.38. These data indicate that the error due to lactose may be as much as 20%. The figure 0.05 mg. for NPN was obtained by difference between Kjeldahl nitrogen determinations on crude and purified protein precipitates. Although this amount is 3% of the total nitrogen, the error in measuring the biuret color would be smaller because the NPN consists of some fractions which give no biuret reaction, and of other fractions which, if they do react, form products which do not have absorption maxima at 560 m $\mu$  (7). The protein-washing step described under "Preparation of standard curve" is intended to eliminate the 3% error in Kjeldahl nitrogen determinations.

The possibility of reducing or eliminating the error due to lactose by measuring the color at some other wave length was investigated. Figure 2 demonstrates that in going from 560 to 455 m $\mu$ , the color due to lactose is reduced by four-fifths, but the color due to protein is reduced also by two-thirds. A 20% error at 560 m $\mu$  would still be an 11% error at the shorter wave length.

Dialysis was tried for removing lactose from the whey. This time-consuming step made the procedure less practicable and led to variable results. The error due to lactose was eliminated by removing the lactose from the precipitated protein with the wash solution described previously and the method gave satisfactory results. However, the extra time required for the purification step made it difficult to complete a large number of determinations in a single day. In the shorter procedure described in this paper, instead of removing lactose, more is added.

Compensating the error due to lactose. The data in Table 3 demonstrate that lactose would produce a variable error if no correction were made for it. Each point on the standard curve would then be made under the condition of a constant lactose-nitrogen

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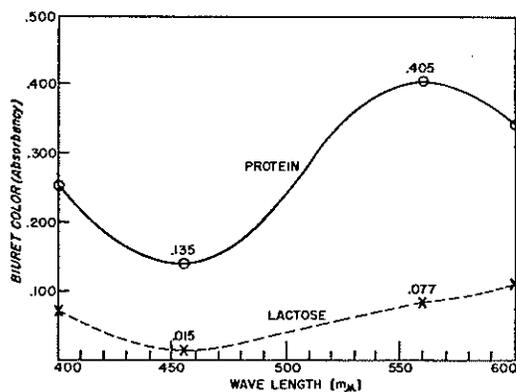


Figure 2. Biuret reaction absorption spectra.

ratio in the whey. If the nonfat dry milk solids chosen for the standard curve contained 5.88 mg. of whey protein nitrogen per g., determined by Kjeldahl, this fixed ratio would be 89. In the examples of sample tests in Table 3, however, the lactose-nitrogen ratio varies from 67 to 445.

Table 4 shows the effect of having 44.5 mg. of lactose monohydrate dissolved in each ml. of saturated sodium chloride solution used for diluting the whey. Each point on the standard curve and each test is then made in the presence of 445 mg. of lactose monohydrate. By this means, the error due to lactose is compensated for and the procedure is appreciably shortened.

**Standard curve.** The calibration curve for the method was prepared for the Coleman Universal Spectrophotometer, Model 14, with 13 mm. square cuvettes, at 560 mμ. The

TABLE 3  
Variability of error due to lactose

Whey protein nitrogen	Standard curve			
	Whey	Saturated NaCl solution	Protein nitrogen	Lactose
mg./g.	ml.	ml.	mg.	mg.
5.88	0	10	0	0
	1	9	0.5	44.5
	2	8	1.0	89
	3	7	1.5	133.5
	4	6	2.0	178
	7	3	3.5	311.5
	10	0	5.0	445
	Sample tests			
Low heat				
7.83	3	7	2.0	133.5
3.92	3	7	1.0	133.5
High heat				
1.96	3	7	0.5	133.5
1.18	10	0	1.0	445

TABLE 4  
Standardization of error due to lactose

Whey protein nitrogen	Standard curve			
	Whey	Saturated NaCl containing 44.5 mg. lactose per ml.	Protein nitrogen	Lactose
mg./g.	ml.	ml.	mg.	mg.
5.88	0	10	0	445
	1	9	0.5	445
	2	8	1.0	445
	3	7	1.5	445
	4	6	2.0	445
	7	3	3.5	445
	10	0	5.0	445
	Sample tests			
Low heat				
7.83	3	7	2.0	445
3.92	3	7	1.0	445
High heat				
1.96	3	7	0.5	445
1.18	10	0	1.0	445

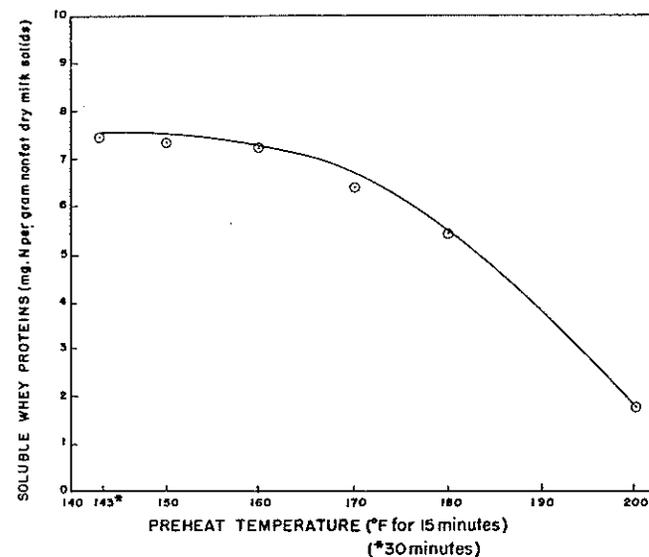


Figure 3. Effect of preheat treatment on soluble whey protein content of nonfat dry milk solids, determined by the biuret method.

regression line, obtained by the method of least squares, is  $N = 3.327 A - 0.090$ , in which  $N$  is the number of mg. of protein nitrogen in the final solution, and  $A$  is the absorbency of the biuret color. The standard error of estimate is 0.018 mg. of nitrogen. For the procedure in which the lactose is removed from the precipitated protein with a wash solution, the equation is  $N = 4.125 A + 0.018$ , and the standard error of estimate is 0.016 mg.

#### APPLICATION OF THE METHOD TO NONFAT DRY MILK SOLIDS

As little as 0.06 mg. of protein nitrogen can be determined. The effect of preheat treatment on the soluble whey protein content of nonfat dry milk solids is illustrated in Figure 3. In applying the method to 24 commercially-prepared milk powders, samples designated as having high-preheat treatment were found to contain less than 2.0 mg. of whey protein nitrogen per g., whereas low-heat samples generally contained from 5.0 to 8.0 mg. of whey protein nitrogen per g. Table 5 summarizes the results

TABLE 5  
Analysis of commercial samples of nonfat dry milk solids

Commercial designation of samples	Whey protein nitrogen mg. per g.
Low-preheat treatment*	
Average of 12 samples.....	6.62
Range of 12 samples.....	5.39-7.98
3 samples.....	1.66, 3.02, 3.80
High-preheat treatment <sup>f</sup>	
Average of 9 samples.....	1.13
Range of 9 samples.....	0.36-1.64

\*From 165°F. for 30 seconds to 170°F. for 10 to 20 seconds.  
<sup>f</sup>From 175°F. for 30 minutes to 265°F. for 40 seconds.

of these tests which indicate that determinations of soluble whey protein nitrogen provide a fairly reliable differentiation among milk powders of different preheat treatment.

#### SUMMARY

A biuret method for the determination of soluble whey proteins in nonfat dry milk solids is described. The composition and properties of the whey, the interference by non-protein components, and the analytical details which influence the reliability of the method are discussed. Errors due to the reaction of lactose are standardized and compensated for by a calibration curve. Commercially-prepared nonfat dry milk solids having high-preheat treatment were found to contain less than 2.0 mg. of whey protein nitrogen per g. whereas low-heat samples generally contained from 5.0 to 8.0 mg. of whey protein nitrogen per g.

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