

Quantitation of Caseins and Whey Proteins of Processed Milks and Whey Protein Concentrates, Application of Gel Electrophoresis, and Comparison with Harland-Ashworth Procedure

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ABSTRACT

Alternate methods for quantitation of caseins and whey proteins in milk products were investigated. The Harland-Ashworth and Leighton procedures, which are used for routine determinations of soluble whey proteins in milk, could not be adapted satisfactorily to quantitation of whey protein in blends of nonfat dry milk solids and whey protein concentrates because of problems of precipitation techniques. Gel electrophoresis in sodium dodecyl sulfate does not require fractionation prior to analysis and works well for nonfat dry milk solids, whey protein concentrates, and blends of these products, as well as total milk protein concentrates. Use of thiourea and hydrogen peroxide as gel catalysts improves band resolution and allows for easy handling and better quantitation. This method, which is an adaptation of the Laemmli procedure, may be of use for detecting adulteration of nonfat dry milk solids or even fluid milk with whey protein concentrates and may find other applications; 10 major milk proteins can be visualized and quantitated on one gel electrophoretogram.

INTRODUCTION

Nonfat dry milk and whey protein concentrates are used in the food industry for manufacture of baked goods, dairy products, and processed foods. Not only do caseins and whey proteins differ from each other in their nutritional values and functional properties, but

their ratios in processed dairy products are prescribed by federal regulations (7). The large differential in price between nonfat dry milk (NFDM) and whey protein concentrate (WPC) makes processed dairy products a prime target for adulteration by altered ratios of NFDM to WPC. Therefore, in products that contain both NFDM and WPC, methods should be developed to quantitate the ratio of casein to whey protein.

Harland and Ashworth (10) developed a turbidimetric method for routine estimation of nondenatured whey proteins in heat-treated milk and NFDM solids. Leighton (14) modified the Harland-Ashworth (HA) method to improve turbidity development and introduced whey protein index (WPI) for skim milk powder. The WPI is defined as milligrams of whey protein nitrogen soluble in saturated NaCl solution per gram of milk powder. These procedures are satisfactory for routine analyses of soluble whey protein in skim milk powders and possibly could be extended to analysis of other products for which the ratio of casein to whey protein is required.

We studied use of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) to quantitate the ratio of casein to whey protein in dairy products. Results by the electrophoretic method are compared with those from the HA and Leighton (LE) methods. In addition, a Coomassie blue dye binding method was used to test for problematic turbidity development in the HA method.

MATERIALS AND METHODS

Sources of Caseins and Whey Proteins

Fresh warm raw milk was obtained from Holstein cows. Within an hour after milking, milk was skimmed by centrifugation at 5,000 × g for 30 min at 5°C. Raw skim milk was acidified

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to pH 4.6 at 20°C, and the casein precipitate was separated from the whey by filtration. The filtrate, containing the whey proteins, was dialyzed against distilled water for several days, rechecked for pH change, and then lyophilized. The casein fraction was washed with distilled water three times, maintaining the pH of the water at 4.6 by addition of dilute HCl. After washing, the caseins were suspended in water, neutralized with NaOH, and lyophilized as sodium caseinate.

The NFDM, total milk protein concentrates (TPC), and WPC were obtained from commercial sources. Low and high-heat NFDM standards were obtained from the American Dry Milk Institute (ADMI), Rosemont, IL.

Purified caseins were obtained as described: crude α_{s1} -, β -, and α_{s2} -caseins were prepared by urea fractionation (1) followed by column chromatography on DEAE cellulose in urea (19); κ -casein was prepared by the method of McKenzie and Wake (15).

Purified whey proteins, β -lactoglobulin and α -lactalbumin, were prepared by the method of Aschaffenburg and Drewry (2). Bovine serum albumin, bovine immunoglobulin G Cohn fraction II, and all other standard proteins were purchased from Sigma Chemical Company. Lactoferrin and γ_1 - and γ_3 -caseins were gifts of M. L. Groves of this laboratory.

Protein Analysis

Protein was determined by the Coomassie blue method of Bradford (4) as modified for milk proteins by Douglas et al. (8). For total protein assay, sodium caseinate in Tris-citrate-urea buffer pH 9.0 was used as a standard; for soluble whey protein the standard was lyophilized whole whey protein in saturated NaCl solution containing 50 mM phosphate, pH 6.7.

Whey Protein Index

The WPI of NFDM powder was estimated by the method of Harland-Ashworth (10, 12) or by the LE method (14).

²Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

³Warning: produces cancer in animals; causes irritation.

Kjeldahl Nitrogen

Total nitrogen of sodium chloride filtrates from the HA assay was determined according to the official AOAC method (3) and calculated on a dry powder basis. The nonprotein nitrogen (NPN) of each sample was determined as follows: 10-g subsamples reconstituted to 10% total solids (wt/wt) were treated with 50-ml quantities of 15% (wt/wt) trichloroacetic acid (12% final concentration) and filtered. Nitrogen content of 10-ml samples of the filtrate was determined as described (3). The WPN was calculated by subtracting NPN from total nitrogen.

Polyacrylamide Gel Electrophoresis

Proteins derived from skim milk, NFDM, TPC, and WPC were examined by polyacrylamide gel electrophoresis (PAGE) by the discontinuous system described by Laemmli (13), which we modified for slab gel systems. An E-C Vertical Slab Gel Apparatus,² yielding gels of 3 mm thickness, was used with a pH 8.3 electrode buffer (3.03 g/liter Tris-base, 14.4 g/liter glycine, and .3 g/liter sodium dodecyl sulfate). The stacking and separating gels were prepared to be 4 and 15% acrylamide, respectively, 2.67% crosslinked with N,N'-bis-methylene acrylamide, and polymerized with 2% (wt/wt total acrylamide) thiourea³ and 1.3% (vol/wt total acrylamide) hydrogen peroxide (30% solution) as catalysts. The final buffer concentrations in the stacking and separating gels were .375 M Tris-HCl (pH 6.8) and 1.0 M Tris-HCl (pH 8.8), respectively. Protein samples (2 mg sodium caseinate, 1 mg dialyzed whey protein, or 5 mg NFDM or TPC or WPC) were solubilized in 50 μ l of protein solvent system [.166 M Tris and 1 mM Na₂ ethylenediaminetetraacetate (EDTA), pH 8.0], 50 μ l of 7% SDS, and 20 μ l of 2-mercaptoethanol, and heated at 100°C for 5 min. After cooling, 20 μ l of bromophenol blue dye solution (.1%) and 20 μ l of glycerol were added to the samples. The gels were run for 13 h at 80 mA and maintained at 14°C. After the run, they were stained for protein with .03% Coomassie blue (CB) in 10% trichloroacetic acid/10% methanol/7% acetic acid (total volume 330 ml) for 24 h. They were destained with a similar volume of 10% methanol/7% acetic acid in the presence of an activated charcoal destaining

cartridge, after 24 h the cartridge was removed, and destaining continued for another 24 h.

Gel Scanning

Quantitative analyses of electrophoretic separations were by scanning the gels at 550 nm in a Gelman Model 18 automatic computing densitometer; each lane was scanned twice and readings were averaged. The WPI for SDS-PAGE experiments was calculated by the adjusted percent whey protein multiplied by the total protein content, grams per gram of powder as determined by the CB method, and converted to milligrams N by a factor of 15.5% N.

RESULTS AND DISCUSSION

Determination of Soluble Whey Proteins

The HA procedure (10, 12) represents a well-accepted method for determining the undenatured (soluble) whey protein content of NFDM solids and has been used for classification of NFDM heat treatments for many years. Leighton (14) modified the method by the use of a buffered saturated sodium chloride solution to keep the pH of the filtrate-salt solutions between 2.7 and 3.1, regardless of the type of NFDM powder under test. This method improved sensitivity and reproducibility of the test. We wished to determine if these methods could be extended to samples that contain blends of NFDM solids and whey protein concentrate or other milk-derived protein products.

Twenty-four commercial samples of NFDM (high-heat treated) were analyzed in duplicate by both the HA and LE procedures. In each case, turbidity determinations on the filtrates were compared to total Kjeldahl nitrogen (KN), whey protein nitrogen (WPN), and whey protein estimated by the CB dye binding method. Means for these determinations are in Table 1. All 24 samples were high-heat products based on the HA standard curve. Each method of analysis appeared to give its own range.

Data from the 24 NFDM samples were analyzed statistically by correlation coefficients and comparisons among responses measured by the HA method. The turbidity response of HA method is significantly correlated with CB, KN, and WPN responses (Table 2). All direct measurements of protein in the filtrates (CB,

KN, and WPN) correlate closely with each other with higher coefficients. Table 3 indicates that correlations among turbidity by the LE method and CB, KN, and WPN are larger than .6. Comparison of both tables shows that the LE turbidity response appears to give high correlations with the methods that directly measure protein than does the HA response. When this premise was tested by comparison of correlation coefficients (18), LE turbidity correlated with CB and WPN responses significantly more than did HA turbidity ($P < .05$ for .92 vs. .59, and for .71 vs. .26).

Samples of WPC were run by both the HA and LE methods. As expected, measures were too high for the standard curve because of the excessive whey protein in WPC. Thus, the procedure was altered slightly in two ways: 1) .25 g of WPC samples was weighed instead of 2.0 g; or 2) weight of the sample remained at 2.0 g, but the amount of filtrate transferred into the cuvette for turbidity measurement was reduced to one-tenth. Both modifications also were tested by the CB dye binding method to analyze for whey proteins.

Results for the various methods of analysis are in Table 4. All of the methods appear applicable to WPC; however, WPI range from

TABLE 1. Whey protein indices and variance associated with analyses of nonfat dry milk (NFDM) samples.¹

Method ²	WPI ³		Coefficient of variation ⁴
	\bar{X}	SD	
HA-Turbidity	1.16	.43	37
HA-CB	1.88	.53	28
HA-WPN	.93	.24	25
LE-Turbidity	1.00	.35	35
LE-CB	1.82	.40	22
LE-WPN	.90	.29	32

¹ n = 24.

² HA = Turbidity development by the standard Harland-Ashworth method (10); CB = whey protein content of filtrate by Coomassie blue dye binding method (4); LE = turbidity development by modification of Leighton (14). WPN = Kjeldahl nitrogen adjusted for nonprotein nitrogen.

³ WPI = Whey protein index: milligrams soluble whey protein N per gram NFDM.

⁴ $100 (\sigma / \bar{x})$.

TABLE 2. Correlation coefficients¹ for various methods of quantitating soluble whey proteins of 24 nonfat dry-milk samples analyzed by the Harland-Ashworth procedure.

	HA ²	CB	KN	WPN
HA	1.00
CB	.59	1.00
KN	.41	.76	1.00	...
WPN	.26	.68	.88	1.00

¹Correlation coefficients were all significantly nonzero with $P < .001$.

²HA = Turbidity development by the standard Harland-Ashworth procedure (10); CB = whey protein content of filtrate by Coomassie blue dye binding method (4); KN = total Kjeldahl nitrogen of filtrate; WPN = Kjeldahl nitrogen adjusted for nonprotein nitrogen.

50 to 80% of their actual total protein contents. Thus, for 90 to 95% whey protein in WPC, these methods give qualitative but not quantitative results. The LE method is more consistent with a smaller coefficient of variation, but all of the correlation coefficients among methods were less than .25 and were not significantly different from 0. We encountered difficulties in the filtration step with both the HA and LE procedures with all samples that contained WPC. After precipitation with saturated sodium chloride, both

TABLE 3. Correlation coefficients¹ for various methods of quantitating soluble whey proteins of 24 nonfat dry milk samples analyzed by the Leighton procedure.

	LE	CB	KN	WPN
LE	1.00
CB	.92	1.00
KN	.64	.67	1.00	...
WPN	.71	.70	.84	1.00

¹Correlation coefficients were all significantly nonzero with $P < .001$.

²LE = Turbidity development by modification of Leighton (14); CB = whey protein content of filtrate by Coomassie blue dye binding method (4); KN = total Kjeldahl nitrogen of filtrate; WPN = Kjeldahl nitrogen adjusted for nonprotein nitrogen.

procedures specify sample filtration through Schleicher & Schuell No. 602 filter paper. With NFDM samples, filtration was rapid, and filtrates were water clear after the first few drops had passed through the filter. However, with samples containing WPC, filtration was slow indicating much denatured whey protein. The filtrates were frequently cloudy and could be clarified only by repeated filtration or by centrifugation at 10,000 rpm. Therefore, incomplete filtration before the samples were used for turbidity development could give irregular results and account for some of the variation of samples containing WPC.

During the heating process, the major whey protein, β -lactoglobulin, is denatured and then may interact with other whey proteins or with κ -casein to form a complex that precipitates with the caseins, thus reducing the quantity of protein in the whey fraction (8). In formulated products that may contain added whey proteins, these effects may be even more problematic, because the degree of interaction is dependent upon concentration of whey protein (17). These results show that methods that depend on solubility of whey protein for subsequent measurement are not reliable when the whey protein content is high and when the product contains considerable denatured protein. Therefore, SDS-PAGE was attempted to determine

TABLE 4. Whey protein indices and variance associated with analyses of whey protein concentrates.¹

Method ²	WPI ³		Coefficient of variation ⁴
	\bar{X}	SD	
HA-Turbidity	43.3	9.5	22
HA-CB	32.6	7.8	24
LE-Turbidity	46.0	2.4	5
LE-CB	50.0	5.9	12

¹n = 24.

²HA = Turbidity development by the standard Harland-Ashworth method (10); CB = whey protein content of filtrate by Coomassie blue dye binding method (4); LE = turbidity development by modification of Leighton (14).

³WPI = Whey protein index: milligrams soluble whey protein N per gram nonfat dry milk.

⁴100 (σ / \bar{x}).

quantitatively caseins and whey proteins without fractionation of samples.

Qualitative Identification of Milk Proteins

The SDS-PAGE was on sodium caseinate and individual purified caseins (Figure 1). Molecular weights of major caseins are in the range 19,000 to 25,000 (20); however, in this system they appear to migrate in the region near or above carbonic anhydrase (M_r 30,000). Thus, the caseins exhibit abnormal behavior in the Laemmli gel. The apparent molecular weights agree with the work of Green and Pastewka (9), who reported that the caseins behaved abnormally in SDS-PAGE. Reynolds and Tanford (16) found that a wide variety of proteins bind nearly identical amounts of SDS. The authors recommended the maximum binding ratio of 1.4 g SDS/g protein. However, Cheeseman and Jeffcoat (6) found that binding of SDS to individual caseins ranged from .9 g SDS/g for para- κ -casein to 3.4 g SDS/g for β -casein. This may lead to anomalous results that may be due

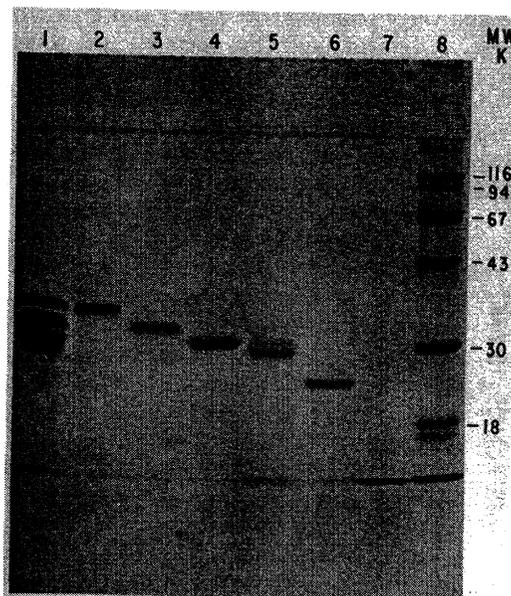


Figure 1. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sodium caseinate and individual purified caseins. 1, sodium caseinate; 2, α_{S2} -casein; 3, α_{S1} -casein; 4, β -casein; 5, κ -casein; 6, γ_1 -casein; 7, γ_3 -casein; 8, standard purified proteins (see text).

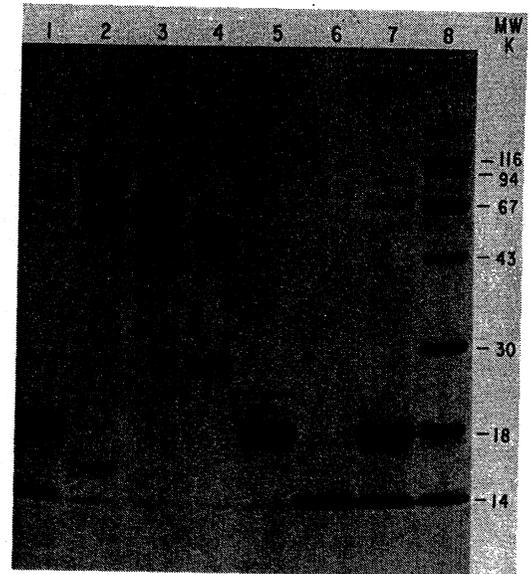


Figure 2. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis of dialyzed whey (pH 4.6) and individual purified whey proteins. 1, dialyzed whey (pH 4.6); 2, lactoferrin; 3, bovine serum albumin; 4, immunoglobulin G, Cohn fraction II; 5, β -lactoglobulin; 6, α -lactalbumin; 7, dialyzed whey (pH 4.6); 8, standard purified proteins (see text).

to competing equilibria as the caseins either bind SDS or interact with other casein molecules. To identify positively the casein band, electrophoretic patterns of individual purified caseins prepared in our laboratory are compared with sodium caseinate as illustrated in Figure 1. Electrophoretic separation of a standard protein mixture consisting of myosin (M_r 200,000), β -galactosidase (116,000), phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), β -lactoglobulin (18,400), and α -lactalbumin (14,300) is shown in slot 8 of Figure 1 for comparison.

Figure 2 shows protein patterns in the SDS-PAGE system for whey proteins. The dialyzed whey protein (in slots 1 and 7) has three faint bands in the upper part of the gel and two heavily stained bands in the lower part. The purified protein bands in slots 2 to 6 represent lactoferrin (86,000), bovine serum albumin (67,000), immunoglobulin, heavy chain (~55,000), β -lactoglobulin (18,400),

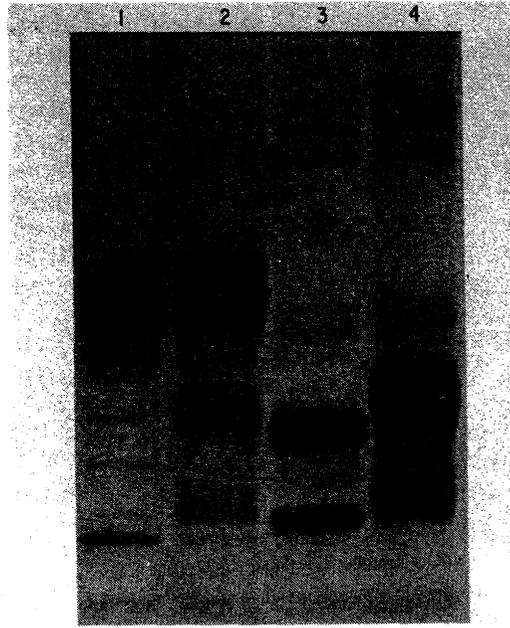


Figure 3. Comparison of laboratory prepared casein and whey fractions (without heat treatment) and processed dairy products (with heat treatment). 1, sodium caseinate (not heated); 2, nonfat dry milk (heated); 3, dialyzed whey (not heated); 4, whey protein concentrate (heated).

and α -lactalbumin (14,300). The standard protein mixture is in slot 8 for comparison. Thus, the major whey proteins, which appear in slots 1 and 7 are in order of descending molecular weight: lactoferrin, bovine serum albumin, immunoglobulin heavy chain, β -lactoglobulin, and α -lactalbumin. β -Lactoglobulin appears as a doublet in this system. The major genetic variants (A, B, and C) of β -lactoglobulin were tested with identical results. The reasons for this behavior are unclear but may relate to buffer fronts in this region.

The system was applied to NFDM and WPC. Figure 3 illustrates electrophoretic patterns for sodium caseinate, NFDM powder, dialyzed whey, and spray-dried WPC. Casein patterns are similar, but casein bands in NFDM powder are broader and fuzzier than those in sodium caseinate. Likewise, β -lactoglobulin and α -lactalbumin bands are broader in WPC than

those in dialyzed whey. This property may be from heat treatment of NFDM solids and WPC. Sodium caseinate and dialyzed whey were prepared without heat treatment. Commercial TPC gave bands with properties intermediate between the laboratory and the NFDM samples.

Quantitative Determination of Milk Proteins

Figures 4 and 5 show typical densitometric scans from SDS-PAGE of NFDM powder and WPC. Initially ammonium persulfate and 3-dimethylaminopropionitrile were used as cocatalysts in the gels. These reagents produced distortions that affected the gel scans. The use of thiourea and hydrogen peroxide as described in Materials and Methods overcame these problems. In the gel scan of NFDM (Figure 4), the numbered protein components are as follows: 1) lactoferrin, 2) bovine serum albumin, 3) immunoglobulins, 4) α_{S2} -casein, 5) α_{S1} -casein, 6 and 7) β -casein and κ -casein, 8) γ -casein, 9) β -lactoglobulin, and 10) α -lactalbumin. Protein components of WPC (Figure 5) are numbered in the same way. The integrator in the densitometer measures the area under the curve. The percent of casein (components 4 to 8) and whey (components 1 to 3 and 9 to 10) were computed and the percent of whey protein was determined from these percents. Figure 6 illustrates the linearity of densitometer response to mixtures of sodium caseinate and dialyzed whey proteins. From this plot the equation is $y = .667x + 3.80$.

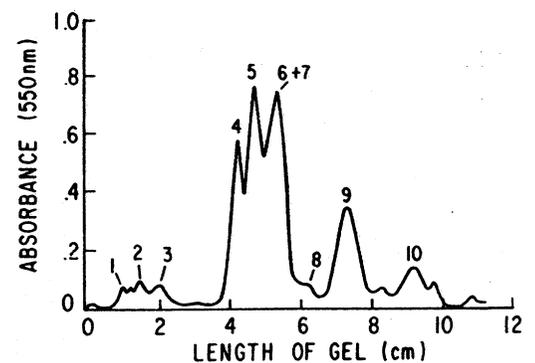


Figure 4. Gel scan of the protein pattern of typical electrophoretic gel of nonfat dry milk: 1) lactoferrin, 2) bovine serum albumin, 3) immunoglobulins, 4) α_{S2} -casein, 5) α_{S1} -casein, 6, 7) β & κ -casein, 8) γ -casein, 9) β -lactoglobulin, 10) α -lactalbumin.

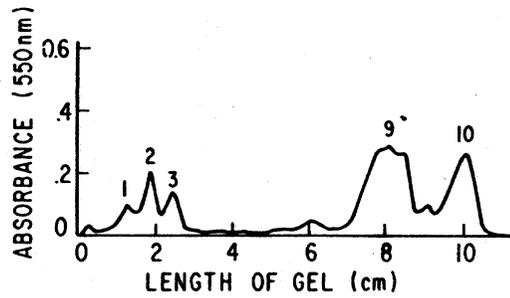


Figure 5. Gel scan of the typical electrophoretic pattern of whey protein concentrate: 1) lactoferrin, 2) bovine serum albumin, 3) immunoglobulins, 4) α_{S2} -casein, 5) α_{S1} -casein, 6, 7) β & κ -casein, 8) γ -casein, 9) β -lactoglobulin, 10) α -lactalbumin.

Variations of staining from gel to gel were encountered. This problem was overcome by a low-heat NFDM included as an internal standard on the calibration gels and on each unknown gel. The raw densitometer reading for percent whey in the low-heat NFDM internal standard was compared with the reading from calibration gels. This difference was added to or subtracted from the raw percent whey in the unknown samples. After this correction, percent whey protein was found from the calibration curve.

The same 24 commercial samples of NFDM were electrophoresed, and their percents whey protein calculated. The whey protein content of these samples varied considerably with a

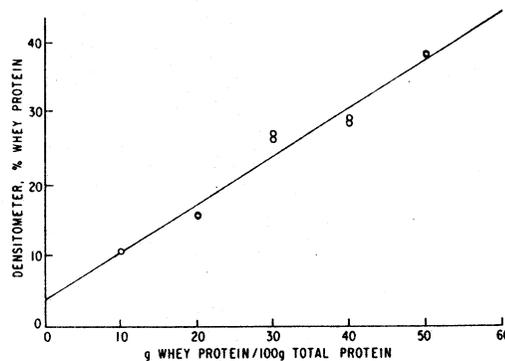


Figure 6. Standard relationship of the weight percent whey in gels as determined by the densitometer to known weight percent whey of the samples.

mean of 15.8% whey protein and a standard deviation of 4.8. The standard deviation appears large; however, the coefficient of variation 30 is within the range for WPI by the fractionation methods (Table 1). (One NFDM sample analyzed on three gels over 3 mo had an average percent whey protein of 17.4, standard deviation 1.5, and a coefficient of variation 9.0). Biological variation of milks across breeds and regions does occur. Cerbulis and Farrell (5) reported the analysis of fluid milks from commercial dairy herds in southeast Pennsylvania with mean for six breeds $18.1 \pm .9\%$ whey protein. Jenness (11) concluded that fluid milk varies considerably in gross composition, particularly in protein content. In Manitoba the mean for six breeds was $17.3 \pm .9\%$, which is lower than that in Pennsylvania. In six areas of California the average mean of bulked milks was $18.4 \pm .4\%$ whey protein (11). Our 15.8% mean is in relative agreement with these other means, but our standard deviation of 4.8% is larger than standard deviations for fluid milks. There seem to be three sources for the variation: method of analysis, biological variability of milk, and nature of the samples (fluid vs. dried milks). As mentioned, dry milks have less defined electrophoretic bands, perhaps leading to some of the variance.

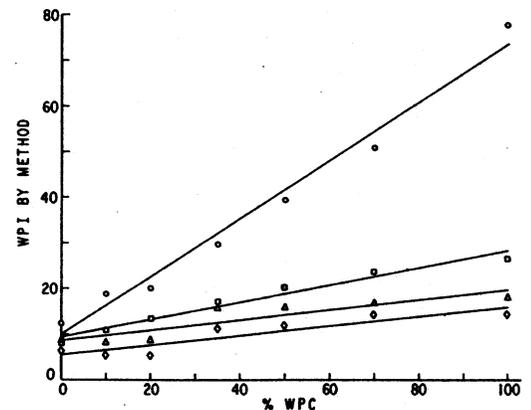


Figure 7. Plot of whey protein index (WPI) determined by various methods against known mixtures of nonfat dry milk and whey protein concentrate (WPC). \diamond , Harland-Ashworth-turbidity; Δ , Coomassie blue of filtrate; \square , WPI from true whey protein by Kjeldahl nitrogen; and \circ , sodium dodecyl sulfate-polyacrylamide gel electrophoresis; here WPI is calculated from the adjusted percent whey protein as given in Materials and Methods.

TABLE 5. Analyses of blends of whey protein concentrate (WPC) and nonfat dry milk.

Unknown sample	Known % WPC	% WPC determined by gel ¹
A	17.0	18.5
B	13.0	15.8
C	26.0	27.9

¹ Calculated from standard curve of Figure 7.

Comparison Methods

As a test of the SDS-PAGE method, five blends of NFDM solids and WPC were prepared and analyzed along with the original materials. These samples were fractionated by the standard Harland-Ashworth method, and the filtrates were analyzed by turbidity development (HA), CB dye binding, and Kjeldahl determination of true whey protein nitrogen. The WPI by HA fractionation for these known blends were compared with those calculated by the SDS-PAGE method as in Figure 7. A straight line was calculated by the method of least squares for each set of points. Figure 7 shows that three lines, HA, CB, and WPI give a linear response but have small slopes (.1 to .2), which indicate that although these methods measure an increase of soluble WPN proportional to the amount of added WPC, they fail to measure accurately the total concentration of whey proteins as more WPC was blended with NFDM solids. Cause of failure is filtration of whey, which contained denatured proteins as the result of heat treatment. As more WPC is added, more denatured whey protein precipitates on the filters. Analyses of protein retained on the filters bore out this conclusion. The line for SDS gel measurements has a larger slope (.639), which indicates that the densitometric measurement of the whey proteins as they were increased in the blends is most responsive and yields the most accuracy.

Mixtures of WPC and NFDM of unknown composition were prepared. Percent WPC was determined by the SDS-PAGE method by the curve in Figure 7. Table 5 shows agreement between calculated and actual percents with sample B at 13% WPC giving the largest error. With precipitation methods, A and B crossed over and gave results not valid statistically,

whereas C was determined accurately only by KN and WPN determination of filtrates. For these figures, variability of the NFDM samples tested (Table 1) and their associated coefficients of variation, it appears as though 15% adulteration of NFDM with WPC would be a conservative lower limit of detection for the gel method and 30% for the salt precipitation methods with KN.

Quantitative SDS-PAGE, thus, appears to be an effective method for obtaining the ratio of casein to whey protein in dairy products and a useful procedure for detecting adulteration of NFDM solids or even fluid milk with WPC. The method can measure as low as approximately 15% adulteration of NFDM solids with WPC and by extrapolation 2% adulteration of fluid milk with WPC.

Obtaining Percent Whey Protein

General outline for obtaining percent whey protein by the SDS-PAGE densitometry procedure is:

1) A standard curve (Figure 6) is constructed by running a gel containing varying ratios of purified caseinate and whey proteins. Bands 1 to 3 and 9 to 10 are whey proteins and 4 to 8 are caseins (Figure 4); the relative areas are computed by densitometry and their ratios obtained. Included on this gel is an ADMI low-heat NFDM standard whose percent whey protein is computed.

2) Unknown samples are run on gels along with the same ADMI standard. The raw percent whey protein of the ADMI standard is compared with that obtained on the gel in step 1. The difference is added to or subtracted from the raw percent whey protein of the unknowns. By the curve in Figure 6, the adjusted percent whey protein is then obtained for the unknown. The WPI may be calculated as described in Materials and Methods.

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