

Detection of Adulteration of Buttermilk Powder by Gel Electrophoresis

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ABSTRACT

Six commercial samples sold as buttermilk powder were compared with an authentic sample by SDS-PAGE, using either large gels (13.5 × 18 × .3 cm) with a manual system or precast miniature gels (4.3 × 5.0 × .045 cm) with an automated system. After being stained with Coomassie blue R, protein bands were quantitated by densitometry. Two unique fat globule membrane proteins, in addition to the caseins and whey proteins, were clearly visible in authentic buttermilk powders, but densities of fat globule membranes were reduced or absent in commercial nonfat dry milk and adulterated powders. The extent of adulteration was estimated by comparison of the relative percentage of fat globule membrane proteins in a suspect sample with their percentage in an authentic sample. The limit of detection for significant fat globule membrane protein bands was 12 μg of a 229-μg sample applied to a large gel and 420 ng of a 5-μg sample on a miniature gel. Although large gels facilitated visual observation of results, several days were required to cast a gel, separate the proteins, stain, and destain. In contrast, SDS-PAGE on miniature gels with the automated system required less than 3 h and offered a rapid screening procedure for detection of adulterated buttermilk powders.

(Key words: buttermilk powder, fat globule membrane proteins, nonfat dry milk, whey)

Abbreviation key: FGM = fat globule membrane.

INTRODUCTION

Dry buttermilk is a close, but not exact, substitute for nonfat dry milk in many applications. Buttermilk is the liquid remaining after cream has been churned into butter and should not be mistaken for the cultured lowfat product. When dried, buttermilk has a distinctive flavor and more milk fat (about 5%) than nonfat dry milk (about 1%) (5). Ice cream manufacturers have traditionally used large quantities of buttermilk solids because of the higher percentage of milk fat (3). Buttermilk is a very desirable ingredient in many baked goods and mixes, but its flavor limits its use in other applications.

During peak milk production, commercial outputs of nonfat dry milk, butter, and buttermilk powder reach high levels. Nonfat dry milk and butter can be sold to the USDA's Commodity Credit Corporation, but surplus dry buttermilk must be cleared through commercial sources. As a result, prices of buttermilk solids must be low enough to encourage use by customers who might prefer to use nonfat dry milk. Relative prices depend on two seasonal forces: the use of nonfat dry milk for cheese and cultured dairy product manufacture when fresh milk is scarce and its use in ice cream during spring and summer months.

Because prices of dry buttermilk are sensitive to nonfat dry milk prices and the two products are compositionally very close (5), food processors are concerned about substitution of nonfat dry milk for buttermilk solids when prices of buttermilk are high. In this paper, we describe an approach to detection of adulteration of buttermilk powder by gel electrophoresis and quantitation by densitometry.

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MATERIALS AND METHODS

Materials

Authentic and suspect commercial buttermilk samples were provided by Herb Struss, Laboratory Services Division (Minnesota Department of Agriculture, St. Paul). Fred Pepper, Western Commodities Support Laboratory (Agricultural Marketing Service, Chicago, IL) provided commercial samples of nonfat dry milk. Total milk proteins were from New Zealand Milk Products (Petaluma, CA).² Dialyzed whey proteins and sodium caseinate were prepared as previously described (1). Whey protein concentrate was a product of Land O'Lakes (Minneapolis, MN). An adaptation of the method of Herald and Brunner (4) was used to prepare fat globule membrane (FGM) proteins by churning (2). Acrylamide was purchased from Bio-Rad (Richmond, CA); *N,N'*-methylene bisacrylamide and thiourea were products of Eastman Kodak (Rochester, NY); 30% H₂O₂ was from Fisher (Pittsburgh, PA). All other reagents were the reagent grade or better.

Electrophoresis on large gels was performed with a vertical slab gel apparatus (E-C Apparatus Corp., St. Petersburg, FL) and a power supply (model 21; A. H. Thomas, Philadelphia, PA). The slab gel was maintained at 14°C by an external refrigerated bath. The Phast-System[®] automatic refrigerated instrument (15°C) and 20% homogeneous precast Phast-Gels[®] (Pharmacia Biotech, Piscataway, NJ) were used for the miniature gels.

Electrophoresis

Buttermilk powders, nonfat dry milk, total milk proteins, sodium caseinate, whey proteins, and FGM proteins were examined with SDS-PAGE. Both the large gels that were cast in place (13.5 × 18 × .3 cm) and the precast miniature gels (4.3 × 5.0 × .045 cm) included a stacking gel. The procedure for large gels was that described by Basch et al. (1). Acrylamide concentrations were 4% for the stacking gel and 15% for the separating gels; both were 2.67% crosslinked with bisacrylamide. Sample

concentration was 61 mg/ml; 15 μ l were applied to each lane of the large gel.

The SDS-PAGE with the PhastSystem[®] was based on Pharmacia's SDS-PAGE separation technique (file number 110). Samples were solubilized at a final concentration of 63 mg/ml in protein solvent [10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA, 2.5% SDS (wt/vol), and 5% 2-mercaptoethanol (vol/vol)] and heated at 100°C for 5 min. After cooling, bromophenol blue was added to a final concentration of .01% (wt/vol), and the samples were centrifuged at 8800 × *g* for 15 min to remove insoluble material; 1 μ l of sample was deposited onto each lane. Electrophoresis on PhastGel[®] 20% homogeneous gels was continued until 135 Vh accumulated. Protein bands were stained manually for 15 min at ambient temperature in .1% (wt/vol) Coomassie brilliant blue R dissolved in 30:10:60 (vol/vol/vol) methanol:acetic acid:water. The gels were destained in methanol:acetic acid:water (30:10:60) until the backgrounds were colorless, transferred to 10:5:85 glycerol:acetic acid:water (vol/vol/vol) for at least 16 h, and dried overnight in a forced-air oven at 55°C.

Gel Densitometry

Band densities on both types of gels were quantitated; the gels were scanned at 600 nm in a Bio-Rad (model 620) video densitometer interfaced to a computer, and scans were analyzed (1-D Analyst II, version 3.10 software; Bio-Rad). For each sample, the total band area of two unique FGM proteins in the samples ($A_I + B_{If}$) was calculated as a percentage of the sum of major band areas: FGM proteins (A_I and B_{If}), caseins (α_{s1} - and β -caseins), and whey proteins (β -lactoglobulin and α -lactalbumin). Of all protein bands observed, these six are the most accurately and reproducibly quantitated by densitometry and provide a basis for detecting adulteration by whey powders as well as by nonfat dry milk. The extent of adulteration was determined by comparing the percentage of $A_I + B_{If}$ in unknown samples to that in an authentic sample.

RESULTS AND DISCUSSION

Large Gels

Results of SDS-PAGE on large gels of the seven dry powder samples from the Minnesota

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

Department of Agriculture are shown in Figure 1. The identity of all samples was unknown except for MN1, authentic buttermilk powder. Of the proteins characteristic of FGM (2), two marker proteins, A_I and B_{If} , were clearly visible in the MN1 sample in lane 1. B_{If} appeared as a doublet because of differences in degree of glycosylation. Both A_I and B_{If} were always present in buttermilk powder. Sample MN1 also contained bands for all the caseins and the major whey proteins. Lanes 2 and 7 also clearly contained FGM proteins, and the sample in lane 3 may have contained a trace.

Figure 2 shows the electrophoretic patterns (lanes 3 and 4) for two samples (MN6 and MN7, Figure 1), along with FGM. The FGM bands in lane 4 were clearly visible, but these bands were absent in lane 3.

Densitometer data for Figure 1 are listed in Table 1. Sample MN1, the authentic buttermilk control, showed strong FGM and casein bands; the other samples were compared with this control. Sample MN2 contained buttermilk, as shown by the presence of FGM proteins, but the increased percentages of β -lactoglobulin and α -lactalbumin suggested adulteration with whey proteins—perhaps as much as 50%. Sample MN3 contained low percentages of casein; this sample appeared to be mostly whey protein. Sample MN4 contained high

percentages of casein and whey protein; this sample was probably nonfat dry milk or total milk protein. Samples MN5 and MN6 contained mostly whey proteins with little casein. Sample MN7 appeared to be an authentic buttermilk sample because of the presence of FGM proteins and a strong concentration of casein. The densitometer data for this sample closely resembled the scan for sample 1, the authentic sample.

Limit of Detection. The limit of detection of FGM marker proteins in authentic buttermilk powder on the large gels was investigated. A stock solution, prepared by dissolving 9.8 mg of buttermilk powder in 160 μ l of protein solvent, was diluted 1:1, 1:3, 1:9, and 1:24 (vol/vol) with protein solvent; 15 μ l of each dilution were placed on each lane. The amount of sample applied to each lane in 15 μ l ranged from 916.9 μ g (no dilution) to 36.7 μ g (1:24 dilution). Although the FGM protein bands in the 1:9 dilution were clearly visible on the gel, the band for α -lactalbumin was too faint to be quantitated; the absence of this protein would lead to slightly higher percentages for the other proteins (A_I + B_{If} , α_{S1} -casein, β -casein, and β -lactoglobulin), thus making adulteration of buttermilk powder more difficult to confirm. In the 1:3 dilution, α -lactalbumin was readily observed; quantitation by densitometry indicated

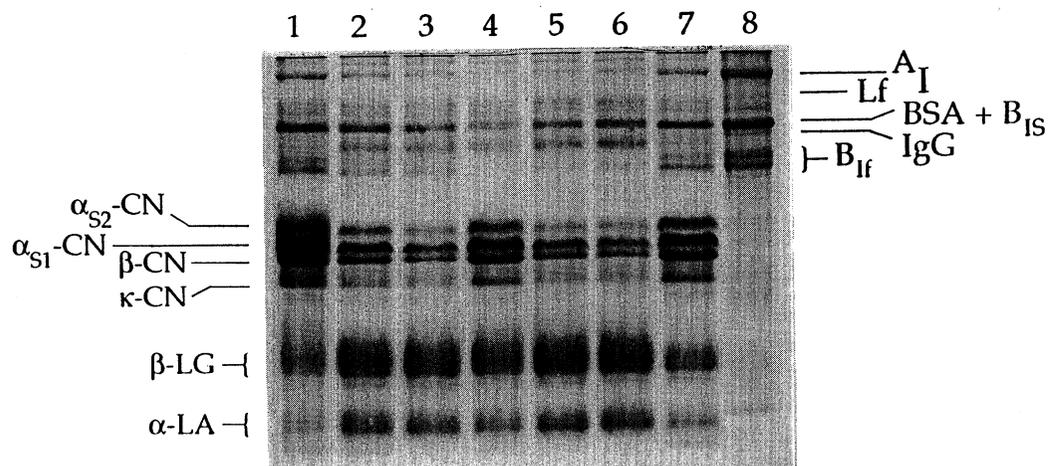


Figure 1. The SDS-PAGE of Minnesota (MN) samples on large gels. Lane 1, MN1 (authentic buttermilk powder); lane 2, MN2; lane 3, MN3; lane 4, MN4; lane 5, MN5; lane 6, MN6; lane 7, MN7; and lane 8, fat globule membrane (FGM) proteins. A_I , B_{IS} , and B_{If} = FGM proteins.

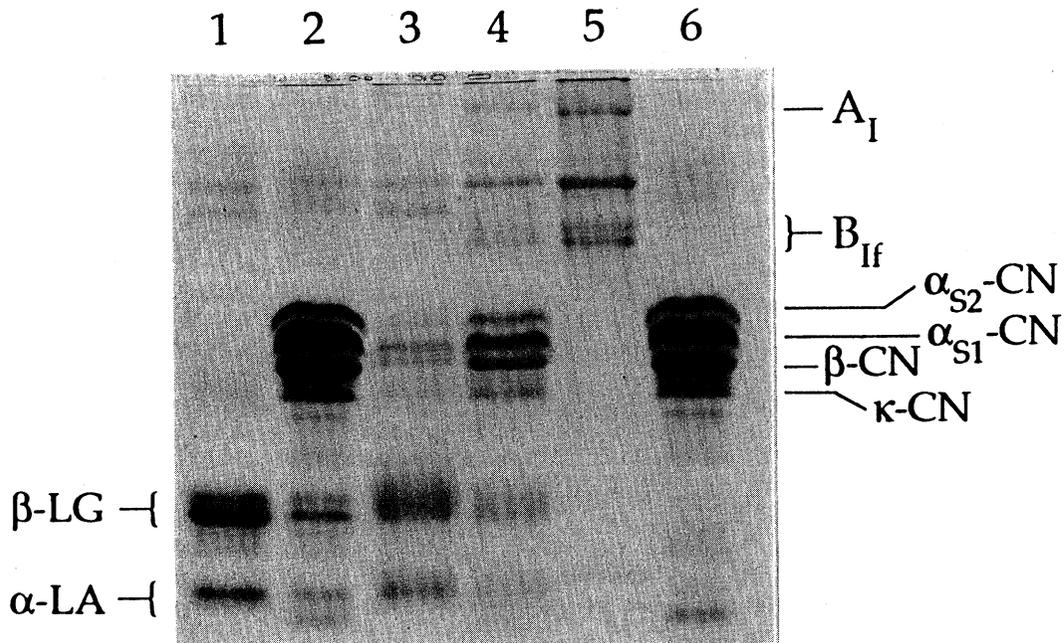


Figure 2. The SDS-PAGE of milk proteins, fat globule membrane marker (FGM) proteins, and two Minnesota (MN) samples on a large gel. Lane 1, dialyzed whey (pH 4.6); lane 2, total milk proteins (New Zealand); lane 3, MN6; lane 4, MN7; lane 5, FGM proteins; and lane 6, sodium caseinate. A_I and B_{If} = FGM marker proteins.

that band A_I represented $3.25 \mu\text{g}$ and band B_{If} $8.7 \mu\text{g}$ (1.4 and 3.8%, respectively, of the $229\text{-}\mu\text{g}$ sample). The latter calculations were based on all bands observed for the 1:3 dilution on the gel, not only the major bands used for determining extent of adulteration.

Unknowns. Five blends of buttermilk containing varying amounts of nonfat dry milk

were prepared and compared with the authentic sample on a large gel (not shown). The composition of each sample was unknown to the laboratory staff. These samples were A, 100% buttermilk powder; B, 100% nonfat dry milk powder; C, 50% A and 50% B; D, 25% A and 75% B; and E, 75% A and 25% B. Densitometry of the gel (Table 2) showed that sample B

TABLE 1. Densitometric analysis of Minnesota samples by SDS-PAGE on large gels.

Sample	$A_I + B_{If}^1$	$\alpha_{s1}\text{-CN}$	$\beta\text{-CN}$	$\beta\text{-LG}$	$\alpha\text{-LA}$
MN1 ²	10.00	36.38	31.29	16.99	5.33
MN2 ³	5.60	17.05	10.94	42.88	23.52
MN3 ³	4.54	12.36	7.24	54.64	21.21
MN4 ³	2.59	25.61	27.54	32.97	11.29
MN5 ³	1.92	13.90	11.85	50.94	21.39
MN6 ³	1.49	10.98	9.01	55.71	22.81
MN7 ³	10.59	32.83	29.60	20.78	6.20

¹ $A_I + B_{If}$ = Fat globule membrane marker proteins.

²Authentic buttermilk powder.

³Identity of samples unknown.

TABLE 2. Densitometric analysis of blends of buttermilk and nonfat dry milk by SDS-PAGE on large gels.

Unknown	A _I + B _{If} ¹	α _{s1} -CN	β-CN	β-LG	α-LA	Adult. ²
(% of total band area)						
A ³	6.45	31.24	32.71	17.85	11.76	0
B ⁴	0	35.28	35.68	18.04	11.00	100.0
C ⁵	3.91	32.98	34.82	16.62	11.67	39.4
D ⁶	1.36	32.44	35.76	18.56	11.88	78.9
E ⁷	5.50	34.87	32.08	17.12	10.43	14.7

¹A_I + B_{If} = Fat globule membrane marker proteins.

²Percentage of adulteration compared with sample A, authentic buttermilk powder.

³A: 100% authentic buttermilk powder.

⁴B: 100% nonfat dry milk.

⁵C: 50% buttermilk powder plus 50% nonfat dry milk.

⁶D: 25% buttermilk powder plus 75% nonfat dry milk.

⁷E: 75% buttermilk powder plus 25% nonfat dry milk.

was not buttermilk powder. Percentages of FGM proteins were significant in samples A and E, indicating that they were buttermilk powders; E was judged to be slightly adulterated because of the lower percentage of A_I + B_{If} (5.5%), although the extent of adulteration was underestimated. The remaining samples (C and D) appeared to be heavily adulterated (3.91 and 1.36%, respectively). The extent of adulteration was also underestimated for sample C and slightly overestimated for sample D.

Miniature Gels

The success of SDS-PAGE on the large gels prompted adaptation of the procedure to automated gel electrophoresis with the Phast-System[®]; use of precast miniature gels and a short automated run time would provide a far more useful procedure for routine screening and detection of adulteration.

Figure 3 shows SDS-PAGE of a miniature gel with the seven samples from the Minnesota Department of Agriculture that are shown in Figure 1. The FGM bands (A_I and B_{If}), caseins, and whey proteins are all clearly visible in the sample of authentic buttermilk (lane 1). Analysis of the bands in this gel showed that the contents of each sample probably were the same as those found for the large gel (Figure 1). The densitometer data for the miniature gel are shown in Table 3. The SDS-PAGE analyses of authentic buttermilk powder were repeated five times on miniature gels to

obtain the following average values for the significant protein bands: A_I + B_{If}, 9.6% ± 2.4; α_{s1}-casein, 46.8% ± 4.1; β-casein, 28.9% ± 3.2; β-lactoglobulin, 11.5% ± 3.3; and α-lactalbumin, 3.1% ± 1.1.

Limit of Detection. The limit of detection for the FGM protein bands on miniature gels was determined in the same way as for the large gels except that the initial concentration of authentic buttermilk powder in the stock solution was 25 mg/ml. Dilutions were made with protein solvent (1:1, 1:2, 1:3, 1:4, and 1:5 vol/vol); the amount of sample applied to each lane of a PhastGel in 1 μl ranged from 25 μg (no dilution) to 4.2 μg (1:5 dilution). The band for α-lactalbumin was too faint to be detected in dilutions greater than 1:4. Quantitation by densitometry indicated that in the 1:4 dilution band A_I represented 125 ng and band B_{If}, 295 ng (1.65 and 5.9%, respectively, of the 5-μg sample). These percentages are somewhat greater than those of the large gels; the relative length of the separating portion of a PhastGel[®] gel was shorter than for a large gel, fewer minor bands were observed, and the total band area was therefore smaller.

Unknowns. The blended samples described were compared with authentic buttermilk powder in the same way as for the large gel (Figure 4). The results, shown in Table 4, were similar to those for the large gel (Table 2), but the amount of adulteration estimated for sample D was somewhat low (i.e., the percentage of

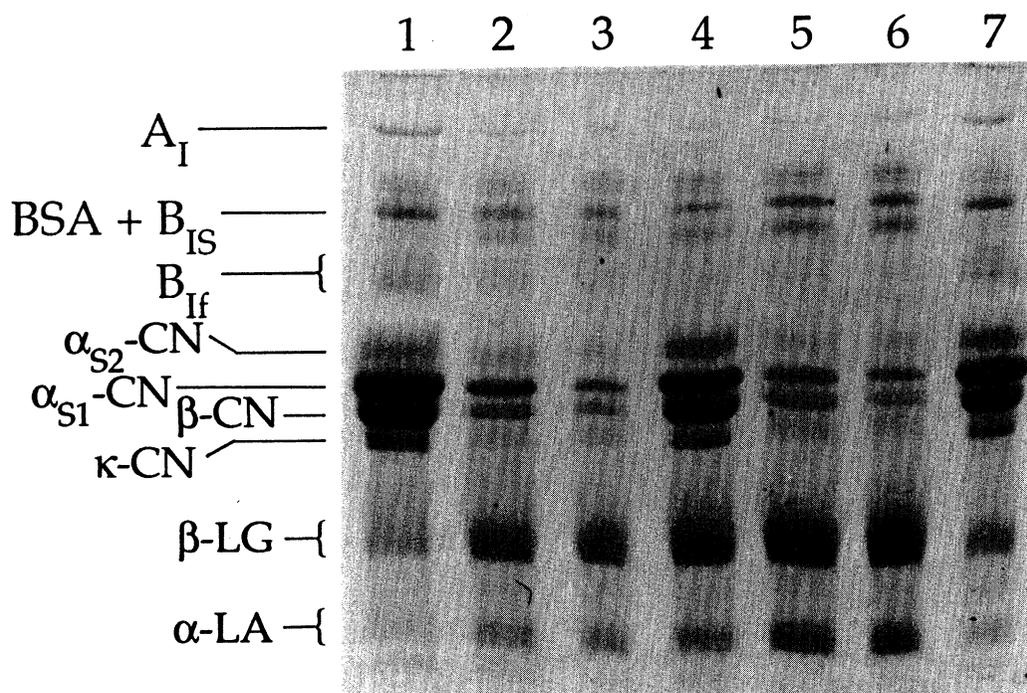


Figure 3. The SDS-PAGE of Minnesota (MN) samples on miniature gels. Lane 1, MN1 (authentic buttermilk powder); lane 2, MN2; lane 3, MN3; lane 4, MN4; lane 5, MN5; lane 6, MN6; and lane 7, MN7. A_I , B_{IS} , and B_{If} = FGM proteins.

FGM bands was too high). Overall, the miniature gels provided greater accuracy in determining the amount of adulteration.

Interference. To determine whether traces of milk fat present in nonfat dry milk can be observed on the miniature gels, and thus inter-

fere in detection of adulteration of buttermilk powder by nonfat dry milk, seven samples of nonfat dry milk powder from producers throughout the US were examined on both miniature gels and large gels (results not shown). No FGM bands could be detected in

TABLE 3. Densitometric analysis of Minnesota (MN) samples by SDS-PAGE on miniature gels.

Sample	$A_I + B_{If}^1$	α_{S1} -CN	β -CN	β -LG	α -LA
(% of total band area)					
MN1 ²	9.92	31.71	30.97	14.39	3.09
MN2 ³	5.79	13.97	9.09	48.71	22.44
MN3 ³	3.21	8.30	4.93	60.30	23.25
MN4 ³	0	22.60	25.65	36.16	15.58
MN5 ³	.87	6.47	9.50	60.85	22.30
MN6 ³	2.48	4.15	5.94	64.46	22.96
MN7 ³	4.84	27.02	31.59	25.89	10.64

¹ $A_I + B_{If}$ = Fat globule membrane marker proteins.

²Authentic buttermilk powder.

³Identity of samples unknown.

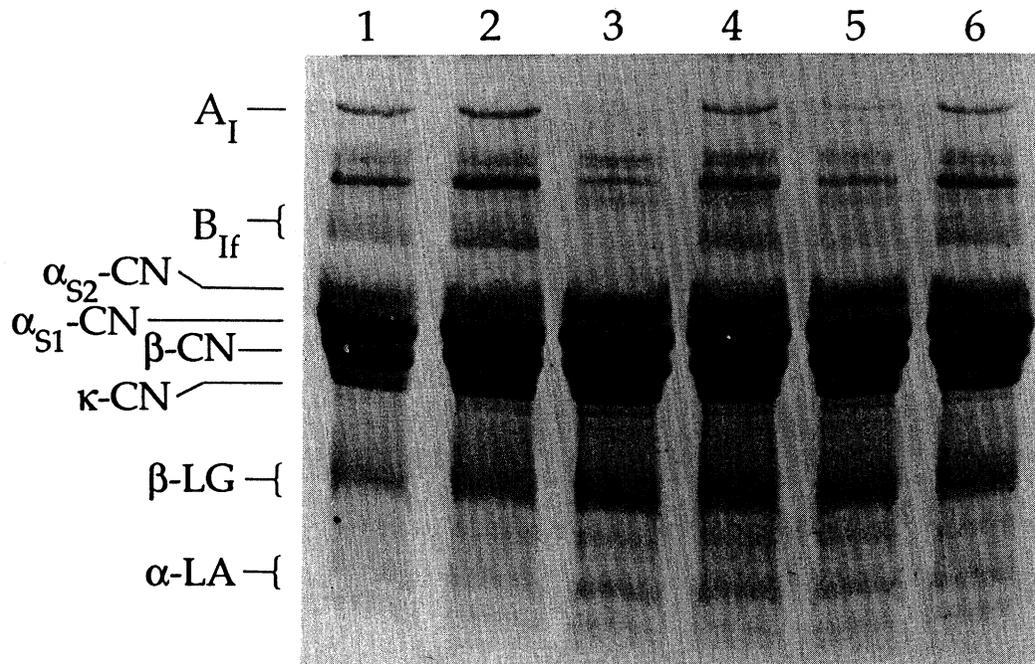


Figure 4. The SDS-PAGE of unknown samples on 20% homogeneous miniature gels. Lane 1, MN1 (authentic buttermilk powder); lane 2, A (100% buttermilk powder); lane 3, B (100% nonfat dry milk); lane 4, C (50% buttermilk powder plus 50% nonfat dry milk); lane 5, D (25% buttermilk powder plus 75% nonfat dry milk); and lane 6, E (75% buttermilk powder plus 25% nonfat dry milk). A_I and B_{If} = fat globule membrane marker proteins.

any of the samples on either type of gel. The FGM proteins have distinct mobilities, and the only bands migrating more slowly than the casein bands were those of high molecular weight whey proteins—lactoferrin, BSA, and

IgG—and B_{Is} , an FGM protein that migrates with BSA. The mobilities of these proteins are greater than that of FGM protein A_I and less than that of B_{If} and therefore would not affect detectability.

TABLE 4. Densitometric analysis of blends of buttermilk and nonfat dry milk by SDS-PAGE on miniature gels.

Unknown	$A_I + B_{If}$ ¹	α_{s1} -CN	β -CN	β -LG	α -LA	Adult. ²
						(%)
(% of total band area)						
A ³	9.08	42.26	29.33	12.63	3.69	0
B ⁴	0	43.83	36.08	16.22	3.87	100.0
C ⁵	4.48	49.25	32.34	11.44	2.49	50.7
D ⁶	4.08	42.86	41.33	9.69	2.04	55.1
E ⁷	6.77	44.79	35.42	11.46	1.56	25.4

¹ $A_I + B_{If}$ = Fat globule membrane marker proteins (averages of five replicates).

²Percentage of adulteration compared with sample A, authentic buttermilk powder.

³A: 100% authentic buttermilk powder.

⁴B: 100% nonfat dry milk.

⁵C: 50% buttermilk powder plus 50% nonfat dry milk.

⁶D: 25% buttermilk powder plus 75% nonfat dry milk.

⁷E: 75% buttermilk powder plus 25% nonfat dry milk.

A similar test of possible interference by FGM bands in whey powder was also conducted. Samples of whey protein concentrate were examined on both large and miniature gels. Neither of the marker proteins, A_I or B_{IF}, was observed.

CONCLUSIONS

Gel electrophoresis provides an ideal method for detecting adulteration of buttermilk powder because the unique FGM protein bands, A_I and B_{IF}, are marker proteins present only in buttermilk. Moreover, these A_I and B_{IF} markers have unique mobilities. No other milk proteins can be mistaken for the FGM bands. The automated system offers three distinct advantages for any screening procedure that depends on gel electrophoresis: availability of precast gels, minimum sample requirements, and minimum time required for running, staining, and destaining. These features permit many samples to be examined in a very short time. Our investigation of this system indicated that adulteration of buttermilk can be detected reproducibly using SDS-PAGE on precast miniature gels with accuracy greater

than that provided by SDS-PAGE on large gels.

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REFERENCES

- 1 Basch, J. J., F. W. Douglas, Jr., L. G. Procino, V. H. Holsinger, and H. M. Farrell, Jr. 1985. Quantitation of caseins and whey proteins of processed milks and whey protein concentrates. Application of gel electrophoresis and comparison with Harland-Ashworth procedure. *J. Dairy Sci.* 68:23.
- 2 Basch, J. J., R. Greenberg, and H. M. Farrell, Jr. 1985. Identification of the milk fat globule proteins. II. Isolation of major proteins from electrophoretic gels and comparison of their amino acid compositions. *Biochim. Biophys. Acta* 830:127.
- 3 Commodity Economics Division. 1991. Page 7 in *Dairy Situation and Outlook Yearbook*. DS-143, August. Econ. Res. Serv., USDA, Washington, DC.
- 4 Herald, C. T., and J. R. Brunner. 1957. The fat-globule membrane of normal cow's milk. I. The isolation and characteristics of two membrane-protein fractions. *J. Dairy Sci.* 40:948.
- 5 Posati, L. P., and M. L. Orr. 1976. Composition of Foods. Dairy and Egg Products: Raw, Processed, Prepared. *Agric. Handbook No. 8-1*, Agric. Res. Serv., USDA, Washington, DC.