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Identification of protein fractions in chhana whey and its powders

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Identification of the various chhana whey protein fractions was carried out after separation with polyacrylamide gel electrophoresis. The compositions of the protein fractions of chhana whey powders produced after concentrating by ultrafiltration and reverse osmosis were also studied, and compared to commercial cheese whey powders. The composition of the chhana whey protein fractions was quite different from the protein fractions of commercial cheese whey powders. Most of the whey proteins in the chhana whey powders were more highly denatured than those of commercial cheese whey powders.

INTRODUCTION

Chhana is an Indian dairy product made from cows' milk by acidifying with whey or food grade acids and heating to about 80°C. It is the basis of several Indian sweets, and is rapidly spreading outside India (e.g. the chhana whey used in this study was produced in a factory in London). It is estimated that, in India, there is sufficient chhana whey to produce 100 000 tonne of powder annually, and this in itself justifies the study of this product. An earlier paper has reported that chhana whey contained less protein than Cheddar cheese, acid casein and cottage cheese wheys and the protein composition was different (Jindal *et al.*, 1993). It is well documented that the functional properties of protein products depend on the protein source, its composition, method of production and certain environmental factors (Kinsella, 1984). Since the method of production and protein composition of chhana whey is different from that of cheese whey, it was very important to identify the various chhana whey protein fractions, which will be helpful in understanding the proper utilisation of chhana whey products. There has been no reported study on the identification of chhana whey protein fractions or protein composition of its products.

During this study an attempt has been made to identify the various chhana whey protein fractions on the basis of measured molecular weight values, assisted by references from the literature and standard proteins run with the samples, using polyacrylamide gel electrophoresis without sodium dodecylsulphate (PAGE), and with sodium dodecylsulphate (SDS–PAGE). The protein compositions of the various chhana whey powders produced by ultrafiltration (UF) and reverse osmosis (RO) were compared with commercial cheese whey powders.

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MATERIALS AND METHODS

Materials

UF chhana whey retentate and chhana whey powders produced as described previously (Jindal & Grandison, 1992) were used for this study. The chhana whey was obtained from Bombay Halwa Limited (Southall, Middlesex, UK), and was derived from cows' milk (Channel Island breeds). Commercial cheese whey powder samples were obtained from Carbery Milk Products Limited, Ballineen, County Cork, Ireland.

Electrophoresis

Protein analysis was carried out on different chhana and cheese whey powders by PAGE and SDS–PAGE, by the discontinuous system (Laemmli, 1970). A Bio-Rad Mini Protein II dual slab cell (Bio-Rad Laboratories, Richmond, USA) with spacers of 0·75 mm thickness was used.

The protein standards and all reagents (analytical grade) were obtained from Sigma Chemical Company, London, UK. Solutions for electrophoresis were prepared according to Hames (1981). To study all the fractions present in the chhana whey, larger amounts of protein were loaded when using UF retentate because some of the fractions were not visible otherwise. For the preparation of samples from powders, samples were dispersed in distilled water to a concen-

Fig. 1a. SDS–PAGE pattern of chhana whey and chhana whey protein concentrates. 1. Separated chhana whey; 2, ×4 chhana whey; 3, ×27 chhana whey; 4, molecular weight standards (SDS).

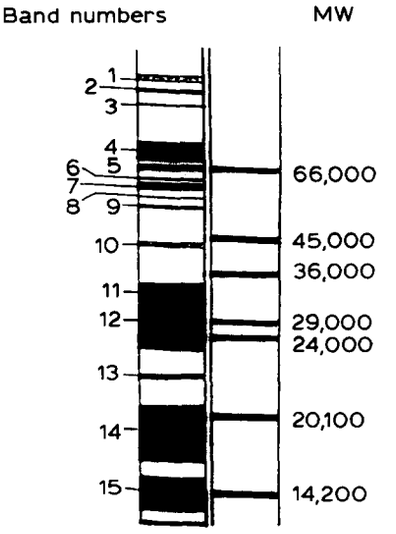

Fig. 1b. Illustration of protein bands (slot 3 and 4).

tration of 1% protein and allowed to stand overnight before further dilution. A calculated amount of 20 μg protein was loaded for each powder sample. The stacking and separating gels contained 4% and 12·5% acrylamide respectively.

RESULTS AND DISCUSSION

Determination of the protein molecular weight (MW)

A calibration curve (log molecular weight vs relative mobility) was drawn from the molecular weight standards using SDS–PAGE (Figs 1 and 2). It was difficult to characterise all the protein bands but attempts have been made to identify them on the basis of the measured molecular weight values, assisted by references from literature and standard proteins run with the samples. Molecular weights (MW) of the chhana whey proteins estimated from Fig. 1 are presented in Table 1 and described as below:

Table 1. Estimated Molecular weights of chhana whey proteins from Fig. 1

Band no. from top to bottom	Relative mobility	Molecular weight
1	0·005	
2	0·027	
3	0·066	
4	0·176	66 800
5	0·214	61 700
6	0·242	58 200
7	0·264	55 000
8	0·286	52 500
9	0·308	49 800
10	0·368	43 700
11	0·484	34 900
12	0·571	27 900
13	0·670	22 400
14	0·791	17 600
15	0·912	13 500

1. For bands 1, 2, and 3 MW was not estimated because standard proteins up to only 66 000 MW were run. Obviously these are high MW proteins. For band 4, a MW of 66 800 was estimated. Other authors have observed high MW proteins in whey or whey protein concentrates. Lee *et al.* (1975) reported bands corresponding to MW 110 000; 94 000 and 84 000 using SDS–PAGE, and suggested that these may be casein complexes. Parnell-Clunies *et al.* (1988) observed components in the MW range 67 000–78 000 which they suggested were disulphide-linked polymeric forms of κ-casein. Talbot & Waugh (1970) noted several polymeric forms of κ-casein with MW up to 200 000. It is possible that the high MW proteins observed in the present study correspond to some of these reported proteins, although it is unlikely that the disulphide bonds were present under the conditions used for SDS-PAGE.

2. Band 5 is equivalent to the standard serum albumin band.

3. Lee *et al.* (1975) observed (but did not identify) bands equivalent to MW 53 000 and 58 000 in whey protein concentrates (> 4-fold concentration); the bands

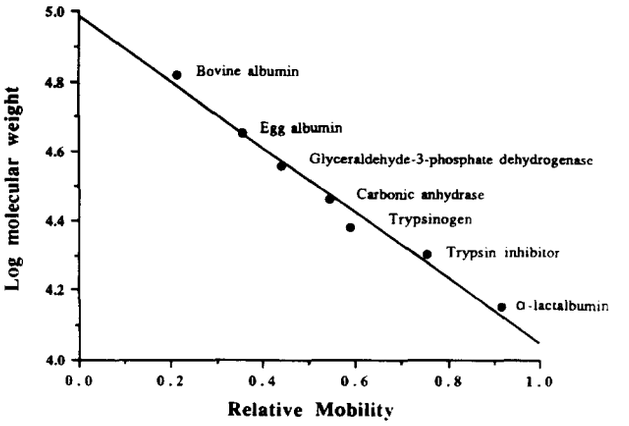

Fig. 2. Calibration curve for protein molecular weight using 12·5% SDS–PAGE.

were not observed in separations of whole whey. These proteins were either present in low levels in whole whey, or formed during ultrafiltration. Similar bands with estimated molecular weight values of 58 200, 55 000, 52 500 and 49 800 were present in UF-concentrated chhana whey during the present study (bands 6, 7, 8 and 9) which were either absent or very faint in original chhana whey.

4. The caseins tend to give anomalous molecular weight values when subjected to SDS–PAGE; values of 34 000, 24 000 and 26 000 respectively have been reported for α-casein, β-casein and κ-casein (Mullin & Wolfe, 1974). The actual MW values determined from the amino acid sequence) are in the region of 24 000, 24 000 and 19 000 for a α_{s1}-casein, β-casein and κ-casein respectively (Whitney *et al.*, 1976; Eigel *et al.*, 1984). In the present study band 11 with MW 34 900, corresponds to α_s-casein and a broad bands 12, MW 27 900 corresponds to β-casein and κ-casein standards.

5. Another broad band (band 14) with an estimated MW of 17 600 is β-lg which runs as a single band on the SDS–PAGE system.

6. Band 15 with estimated MW of 13 500 corresponds to the α-lactalbumin standard.

7. The components of the proteose–peptone fraction cannot be identified by this method but are probably present in chhana whey. Reported molecular weight values of components 3, 5, 8F and 8S are: 40 800, 14 300, 4100 and 9900 (Whitney *et al.*, 1976; Mulvihill & Donovan, 1987). Component 5 (MW 14 300) is probably masked by α-lactalbumin while the MW of components 8F and 8S (4100 and 9900) are too low for identification; but it is likely that the band running faster than α-lactalbumin contained these components. Component 3 separates into four components (MW 18–32 000) during PAGE after treatment with SDS and mercaptoethanol (Kester & Brunner, 1982). Whitney *et al.* (1976) have reported that PP3 is possibly a heterogeneous component with MW of 40 800. It is likely these were masked by β-lactoglobulin and casein bands, although bands 10 (MW 43 700) and 13 (MW 22 400) may represent these components.

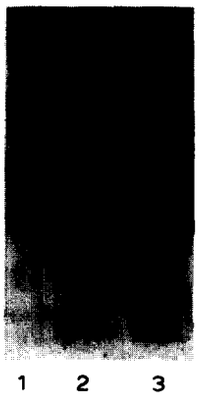

Fig. 3a. PAGE pattern of chhana whey and chhana whey protein concentrates. 1, Whey protein standards; 2, ×4 chhana whey; 3, ×27 chhana whey.

Identification of the chhana whey protein fractions on the PAGE gel

To study all the fractions (native protein) present in chhana whey, larger amounts of protein were loaded using UF retentate as before. The various protein bands are illustrated in Fig. 3.

In the case of separated chhana whey all the bands were faint compared to the UF concentrated chhana whey. Some of the bands were present in UF concentrated chhana whey but not visible in separated chhana whey. This was probably because the concentrations of these proteins in the whey were too low to detect with coomassie blue. It seems unlikely that these were formed during the UF process. There were two bands (8,9) corresponding to β-lactoglobulin A and B, and one band (7) corresponding to α-lactalbumin. There were two prominent bands (4,5) and one minor band (6) between α-lactalbumin and BSA. Band 5 corresponded to α_s-casein standard. No BSA band could be identified on the PAGE gel of chhana whey. A band (3) corresponding to β-casein was present, and another band (2) close to the second band of BSA which cannot be identified. There was a slow running band (1) corresponding to κ-casein and immunoglobulin. With UF concentrated chhana whey there were four (10, 11, 12, 13) bands running faster than β-lactoglobulin.

Comparison of the electrophoretic separation pattern of protein fractions with studies on milk by Andrews (1983) and Andrews & Alichanidis (1983) revealed that chhana whey may contain PP3 (band 2) PP5 (bands 4, 5, 6), PP8S and PP8F (bands 10, 11, 12, 13) proteose–peptone components. Comparison with the studies of Li-Chan (1983) and Donovan & Mulvihill (1987) also suggested that band 2 was a proteose–peptone component. Donovan & Mulvihill (1987) reported that proteose–peptone was the most heat-resistant protein.

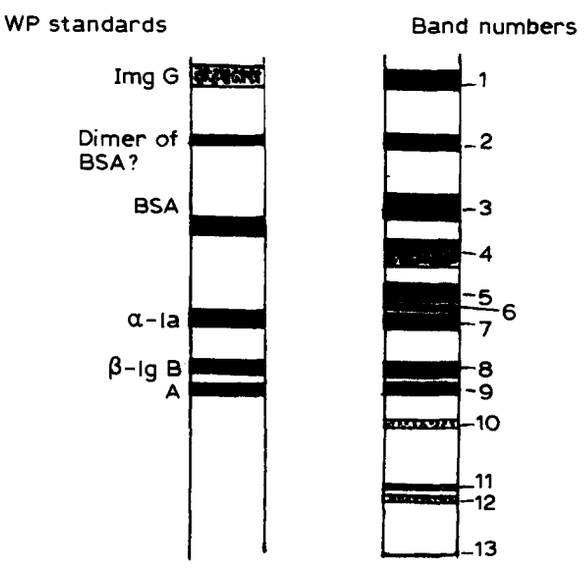

Fig 3b. Illustration of protein bands (slot 1 and 3).

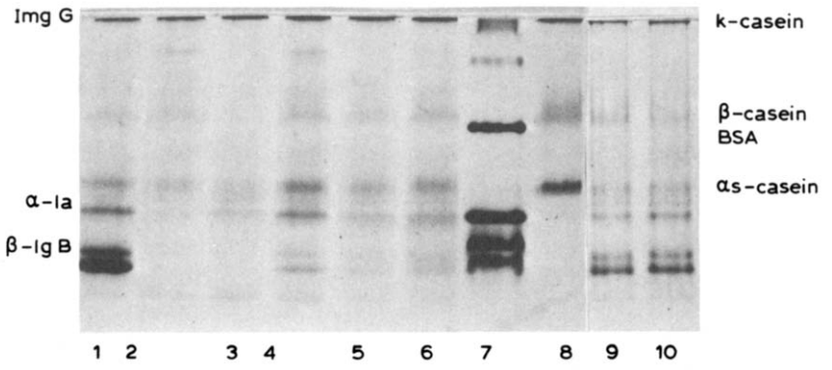

Fig 4. PAGE pattern of chhana powders. Powder 1 (35%); 2 powder 2 (22%); 3 powder 3 (40%); 4, powder 4 (58%); 5, powder 5 (27%); 6, powder 6 (40%); 7, Whey protein standards; 8, casein standards; 9, RO powder (2%), 10, RO powder (2%).

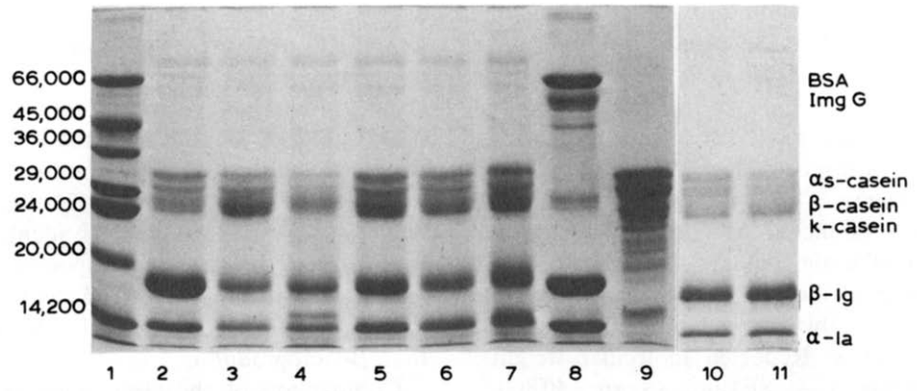

Fig. 5. SDS–PAGE pattern of chhana powders. 1, Molecular weight standards; 2, powder 1 (35%); 3, powder 2 (22%); 4, powder 3 (40%); 5, powder 4 (58%); 6, powder 5 (27%); 7, powder 6 (40%), 8, whey protein standards; 9, casein standards; 10, RO powder (2%), 11, RO powder (2%).

Protein composition of powers

The electrophoretic separation of protein fractions of chhana whey powders on PAGE (Fig. 4) and SDS–PAGE (Fig. 5) gels are presented. For comparison, the protein composition of commercial cheese whey powders was also investigated (Figs 6 and 7).

Compositions of the protein fractions of chhana whey powders differ considerably from commercial cheese whey proteins. Most of the whey proteins in the chhana whey powders were denatured compared to the commercial cheese whey powders. This is due to the high heat-treatment of milk during the chhana production. Chhana whey powders had much more prominent casein bands than the commercial cheese whey powders. Chhana whey powders contain a higher proportion of casein than cheese whey powders, as much more whey protein is incorporated in chhana than in cheese. The BSA band was absent in all the powder samples of chhana whey on the

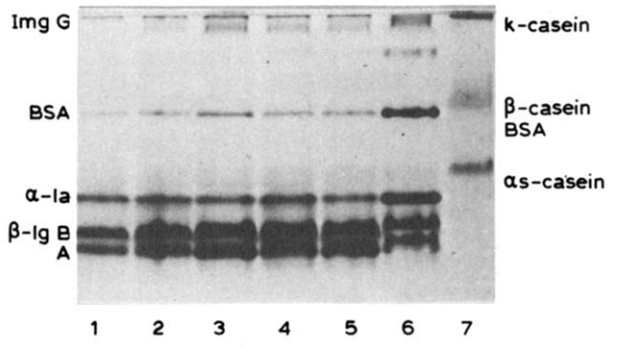

Fig. 6. PAGE of commercial cheese whey powders. 1, Powder 1 (12·5%); 2, powder 2 (35%); 3 powder 3 (55%); 4, powder 4 (75%); 5, powder 5 (75% high gel); 6, whey protein standards; 7, casein standards.

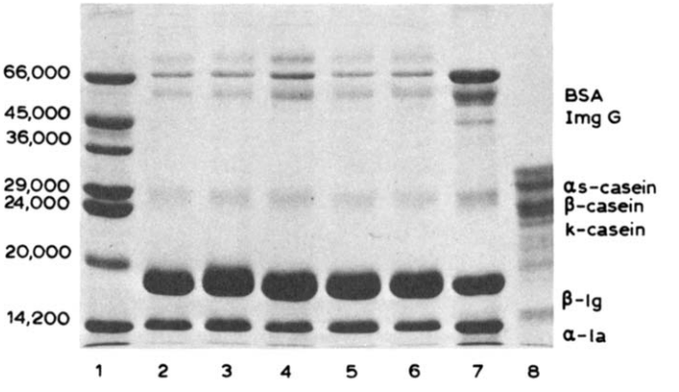

Fig. 7. SDS–PAGE of commercial cheese whey powders. 1, Molecular weight standards; 2, powder 1 (12·5%); 3, powder 2 (35%); 4, powder 3 (55%); 5, powder 4 (75%); 6, powder 5 (75% HG); 7, whey protein standards; 8, casein standards.

PAGE gel but a very faint band appeared on the SDS–PAGE gel, whereas it was present on both PAGE and SDS–PAGE gels of commercial cheese whey powders. There is no information in the literature on the protein composition of chhana whey products.

CONCLUSION

Chhana whey contains prominent casein bands and proteose–peptone components along with the whey proteins. The protein composition of chhana whey powders was quite different from that of commercial cheese whey powders.

ACKNOWLEDGEMENT

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