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DEVELOPMENT OF MICROSTRUCTURE IN RAW, FRIED, AND FRIED AND COOKED PANEER
MADE FROM BUFFALO, COW, AND MIXED MILKS

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Abstract

Paneer was made from cow, buffalo, and mixed cow and buffalo milk by coagulation with citric acid at pH 5.5. All milk samples were heated to 90°C. Cow milk was coagulated at this temperature but buffalo and mixed milks were cooled to 70°C before coagulation. Differences in the composition and the treatments of the cow and buffalo milks were reflected in the composition and structure of the paneers. Electron microscopy revealed that raw paneer samples had a granular structure consisting of protein particles having a core-and-lining ultrastructure. Deep-frying in vegetable oil at 175°C for 4-5 min led to the compaction of the paneer structure and also the individual protein particles. Cooking of the fried paneers by boiling in salt water (1.5% NaCl) for 5 min resulted in partial restoration of the overall structure of the paneers and the ultrastructure of the protein particles. The restoration was most obvious in the paneer made from cow milk.

Introduction

Paneer is the curd obtained by acid coagulation of hot milk, subsequent drainage of whey, and washing and pressing of the curd [1]. The milk (cow, buffalo, or mixed milk) is first heated to 90°C, cooled to 70°C, and coagulated with citric acid. Citric acid is the acidulant of choice for large-scale manufacture but tartaric acid is often used by street vendors who prepare fresh paneer in small quantities for their customers. The curd is drained and washed in cold water to reduce the lactose content and then is pressed manually into patties. In India, raw paneer is very popular as an ingredient in vegetarian dishes and is also consumed after frying in peanut oil. Fried paneer may be further cooked by boiling in salt water. Paneer is also available in Canada, where it is made from cow milk.

Coagulation of cow milk at 90°C and pH 5.5 is known to lead to the formation of a core-and-lining ultrastructure of protein particles [6]. This structure is also found in Queso Blanco, the Latin-American White cheese [7], which is made by a procedure [9] similar to that used to produce paneer. One of the objectives of this study was to examine whether a similar structure develops in paneer.

Frying of paneer in oil introduces additional heat treatment to milk curd and dehydrates it to some extent. Additional structural changes may develop while fried paneer is cooked in salt water. The other objective, therefore, was to study changes in the microstructure of the fried and subsequently cooked paneer.

Materials and Methods

Preparation of paneer

Separate batches of pooled buffalo and cow milks were obtained from the herds maintained at the National Dairy Research Institute in Karnal. Cow milk had 4.0% fat and 12.8% total solids. Buffalo milk (7.7% fat and 18.2% total solids) was clarified and standardized to 6.0% fat and 16.5% total solids. Standardized buffalo milk and cow milk were mixed in equal proportions (1:1) to obtain mixed milk, which contained 5.0% fat and 14.5% total solids. Each batch (5 L) of milk was heated to 90°C without holding. It took 5 min for the milk to attain the temperature of 90°C. Cow

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milk was coagulated at 90°C by the addition of 1% (w/v) citric acid. Buffalo and mixed milks were first cooled from 90°C to 70°C (it took 2 min to cool the milk) and then coagulated within 1 min. The coagulated curd was placed in cheese cloth and transferred into a wooden hoop (16x7.5x5 cm). The curd was pressed with a pressure of 0.05 kg/cm² for 15 min and then chilled for 2-3 h by immersion in tap water at 8° to 10°C. The chilled curd (paneer) was drained for 15 min and analyzed.

The paneer was cut into cubes (3.0x2.0x1.5 cm) and deep-fried in hydrogenated vegetable oil at 175°C for 4-5 min. The fried paneer was boiled in a 1.5% sodium chloride (w/v) solution for 5 min to simulate the usual cooking procedure.

Characterization of paneer

The moisture content of the paneer was determined by drying samples (1 to 2 g) at 100±2°C to constant weight. The fat content of raw, fried, and fried-and-cooked paneer was determined gravimetrically by taking 1.0 to 1.5 g accurately weighed samples, digesting them in a hydrochloric acid-water (2:1, v/v) solution in a fat-extraction flask and by extracting the fat using diethyl ether and petroleum ether according to the method by Roese and Gottlieb as outlined in the Official Methods of Analysis [2].

Firmness of all paneer samples was measured using an Instron Model 4301 Universal Testing Machine equipped with a 100 N cell. A cylindrical paneer plug, 20 mm in diameter and 20 mm high, was placed on the platform and compressed to 80% of its initial height at crosshead speed of 50 mm/min. The results are expressed in mN/mm² [5].

Electron microscopy

Samples (approx. 1x1x10 mm) obtained at the National Dairy Research Institute in Karnal at

various stages of production were fixed in a 2.8% glutaraldehyde solution, sealed in vials, and mailed to the Food Research Centre in Ottawa for electron microscopy [1]. For scanning electron microscopy (SEM), the samples were dehydrated in a graded alcohol series, defatted in chloroform, returned to alcohol, rapidly frozen in Freon 12 cooled to its freezing point with liquid nitrogen, and freeze-fractured. The fragments were melted in absolute alcohol, critical-point dried from carbon dioxide, mounted on aluminum SEM stubs, coated with gold in a Technics Hummer II sputter coater, and examined in an AMR-1000A scanning electron microscope operated at 10 kV. Micrographs were taken on 100 ASA 35-mm film [8].

For transmission electron microscopy (TEM), the samples fixed in glutaraldehyde were trimmed into pieces approximately 0.5x0.5x0.5 mm, post-fixed for 2 h in a 2% OsO₄ solution in 0.05 M veronal-acetate buffer, pH 6.8 [8], dehydrated in alcohol, embedded in a Spurr's low-viscosity medium (J. B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada), and sectioned. Sections, approximately 90 nm thick, were stained with uranyl acetate and lead citrate solutions and examined in a Philips EM-300 electron microscope operated at 60 kV. Micrographs were taken on 35-mm film [8].

Results and Discussion

Chemical composition

Heating of the milk to 90°C prior to coagulation increases the yield of the curd because whey proteins are coprecipitated with casein in the form of a β -lactoglobulin-k-casein complex [12]. Cooling of buffalo and mixed milk to 70°C prior to coagulation is necessary as coagulation at 90°C produces excessively firm curd with a low yield.

Table 1. Characterization of paneer made from cow, buffalo, and mixed milks

CHARACTERISTICS	COW MILK PANEER			MIXED MILK PANEER			BUFFALO MILK PANEER		
	Raw	Fried	Fried + cooked	Raw	Fried	Fried + cooked	Raw	Fried	Fried + cooked
pH of milk	6.75	-	-	6.7	-	-	6.65	-	-
Total solids of milk (%)	12.8	-	-	14.5	-	-	16.5	-	-
Temp. of coagulation	90°C	-	-	70°C	-	-	70°C	-	-
pH of coagulation	5.50	-	-	5.40	-	-	5.4	-	-
Yield (%)	14.50	-	-	18.75	-	-	21.0	-	-
Moisture of paneer (%)	52.4	28.7	64.6	49.0	26.8	68.7	48.3	22.8	70.1
Moisture (%) relative to raw paneer	100.0	54.8	123.3	100.0	54.7	140.2	100.0	47.2	145.1
Total solids of paneer (%)	47.6	71.3	35.4	51.0	73.2	31.3	51.7	77.2	29.9
Fat content:									
(a) in paneer (%)	23.4	36.1	17.6	26.1	39.6	16.0	29.2	43.7	16.6
(b) rel. to total solids (%)	49.2	50.6	49.7	51.2	54.1	51.1	56.5	56.6	55.5
Protein content:									
(a) in paneer (%)	20.3	30.3	15.0	20.8	28.5	12.8	18.5	27.7	10.4
(b) rel. to total solids (%)	42.6	42.5	42.4	40.8	38.9	40.9	35.8	35.9	34.8
Firmness (mN/mm ²)	15.8	43.0	9.2	12.5	36.8	5.7	16.4	29.5	5.5

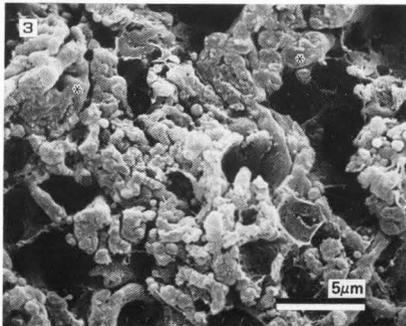
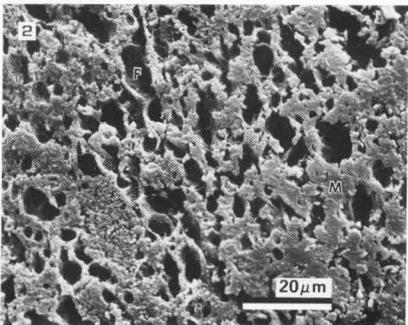
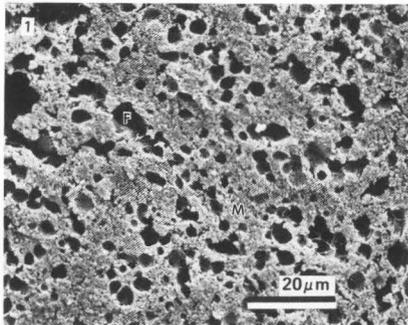
The differences in the composition of cow and buffalo milks and in the temperature of coagulation affected the composition and structure of the paneer (Table 1). Thus, the total solids content of the cow milk was 12.8%, whereas the buffalo milk contained 16.5% total solids, *i.e.*, 29% more. Because of the higher fat content of the buffalo milk, the yield (21.0%) of buffalo milk paneer was considerably higher than the yield (14.5%) of the cow milk paneer. The difference in the yield was almost 45% higher with the buffalo milk paneer, not taking its higher total solids content of 51.7% into consideration (compare with 47.6% total solids in the cow milk paneer). Of the total mass of the paneer, fat represented more than one half of it (56.5%) in the buffalo milk paneer and slightly less than one half of it (49.2%) in the cow milk paneer.

Frying of the paneers in vegetable oil substantially reduced their moisture contents. Relatively more water was lost from the cow milk paneer, where the moisture content decreased to 28.7% (*i.e.*, to 54.8% of the initial moisture present in the raw paneer) than in the buffalo milk paneer, where the moisture content decreased to 22.8% (*i.e.*, to 47% of the initial value). Contrary to what was expected, the fat content remained relatively stable during frying as well as during the subsequent cooking of the paneer; it was approximately 50% of the total solids content in the cow milk paneer and approximately 56% in the buffalo milk paneer.

Cooking of the fried paneer increased its moisture content, and consequently, the total solids contents dropped to 29.9% in the buffalo milk paneer and to 35.4% in the cow milk paneer. Frying and cooking had a considerable effect on the firmness of the paneer made from both kinds of milk. The firmness of the cow milk paneer almost tripled by frying but dropped to about 60% of the initial value in cooked paneer. The lower protein content and the higher fat content of the raw, fried, and fried-and-cooked buffalo milk paneer probably caused the products to be softer than the cow milk paneers. The paneer samples made from mixed milk had all their parameters between the paneers made from pure cow and from buffalo milks (Table 1).

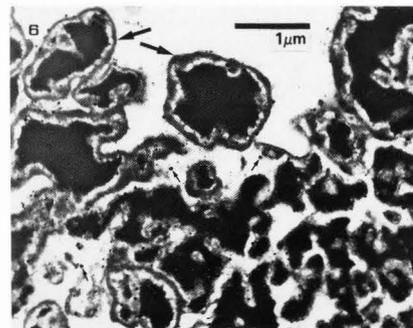
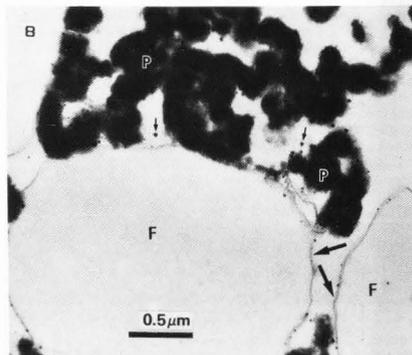
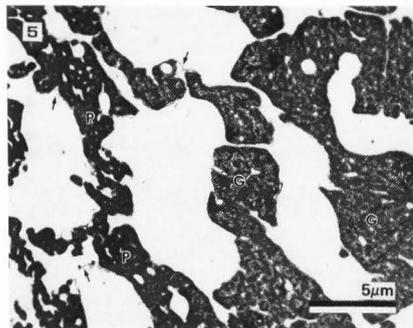
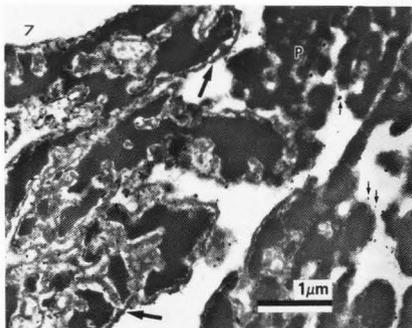
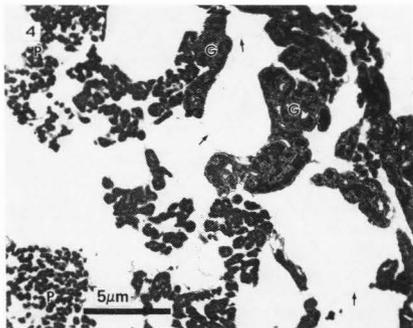
Microstructure

Milk in all three experimental variants was heated to 90°C, but the cow milk was coagulated with citric acid at a higher temperature (90°C) than the buffalo milk (70°C). However, the final pH values were similar in both paneers, *i.e.*, 5.4



Figs. 1 and 2. Structures of raw cow milk paneer (Fig. 1) and raw buffalo milk paneer (Fig. 2). Void spaces (F) in the protein matrix (M) indicate the location of fat prior to its removal during preparative steps for SEM.

Fig. 3. Detail of the protein particles in raw cow milk paneer. Large particles are marked with asterisks.



membranes (arrows). The core-and-lining structure is noticeable in the large protein particles.

Fig. 5. Thin section of raw buffalo milk paneer shows large protein particles having the core-and-lining structure (G) and clusters of individual compact protein particles (P). Remnants of fat globule membranes (arrows) are also noticeable.

Fig. 6. Detail of the core-and-lining structure (large arrows) in raw cow milk paneer. Minute black dots (small arrows) are contaminants.

Fig. 7. Detail of the core-and-lining structure (large arrows) in buffalo milk paneer. Some compacted protein particles (P) are free of the core-and-lining structure. Minute black dots (small arrows) are contaminants.

Fig. 8. Detail of fat globules (F) with compact protein particles (P) attached to the fat globule membranes (large arrows). Minute black dots (small arrows) are contaminants.

Fig. 4. Thin section of raw cow milk paneer shows large protein particles (G), small protein particles (P), and fat globule

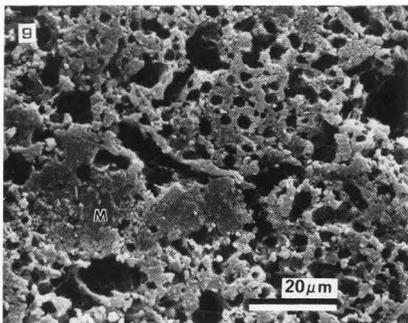
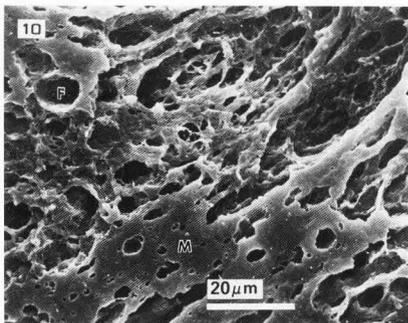


Fig. 9. SEM of cow milk paner which had been fried in oil shows a compacted protein matrix (M).

Fig. 10. Buffalo milk paner fried in oil consists of a severely compacted protein matrix (M). Void spaces (F) indicate the presence of fat particles in the paner prior to the preparation of the samples for SEM.

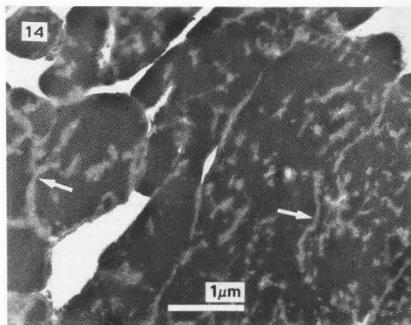
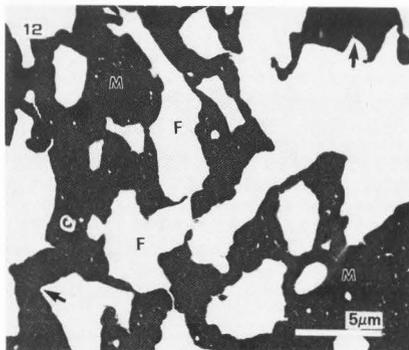
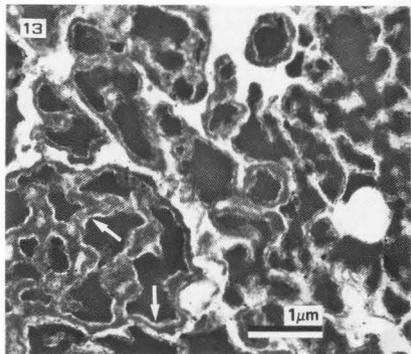
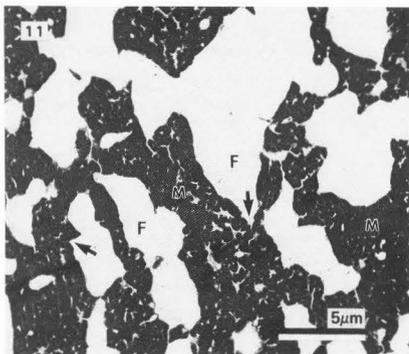


and 5.5, respectively. In the raw state, both paners consisted of aggregated protein particles. At a low magnification, SEM showed that the structures were apparently uniform and fat globules were evenly distributed in the protein network (Figs. 1 and 2). At a higher magnification, however, protein particles varying in dimensions (Fig. 3) were observed. TEM confirmed the existence of the granular structure in the paner and also revealed the internal ultrastructure of the protein particles (Figs. 4 to 8). In raw cow milk paner, the small protein particles were uniform in density (Figs. 4 and 8) and resembled those found in other milk products such as Cottage cheese [4]. In larger particles, the core-and-lining structure (Fig. 6), characteristic of curd obtained by the acidulation of hot milk to pH 5.5, was well developed. A similar core-and-lining structure was found in the buffalo milk paner (Figs. 5 and 7). Protein particles lacking this structure were also present and, in contrast to the cow milk paner, were more densely packed and were fused. Micrographs showing areas where different structures were in close proximity to each other have been used for illustration (Figs. 4 to 7). Intact fat globules with casein particles attached to the fat globule membranes (Fig. 8) were frequently seen in the raw paner. Minute black dots in Figs. 6 to 8 are probably contaminants consisting of a glutaraldehyde-osmium tetroxide complex which developed during the preparation of the samples for electron microscopy [10].

The heterogeneity in the structure of the paner may be explained by local differences in the pH value during the coagulation of the milk.

It was shown earlier [6] that pH of 5.5 is essential for the development of the core-and-lining structure. It is possible that a part of the milk may have been acidified below the critical pH value before uniform acidity was achieved in the curd by stirring. The casein particles in the areas of localized overacidulation thus did not develop the core-and-lining structure. The development of the core-and-lining ultrastructure in bovine protein particles was shown to depend on the temperature at which the milk is coagulated and the final pH value [6]. The ultrastructure develops fully only if the milk had been heated to 90°C, apparently as the result of the formation of a complex between κ -casein and β -lactoglobulin [12]. At a lower temperature, only a part of β -lactoglobulin present in the milk reacts with κ -casein; consequently, the lining around the casein particles does not develop fully. The structure of the mixed milk paner was more similar to the buffalo milk paner than to the cow milk paner probably because the same lower temperature of 70°C was used to coagulate the milk. In this study, the paner was made in accordance with the commercial operations and all three milks had been heated to 90°C. It is recognized, however, that a separate study is required to establish the relationship between the temperature at which the milk is coagulated and the texture of the resulting paner and to examine the role of the core-and-lining ultrastructure in this relationship.

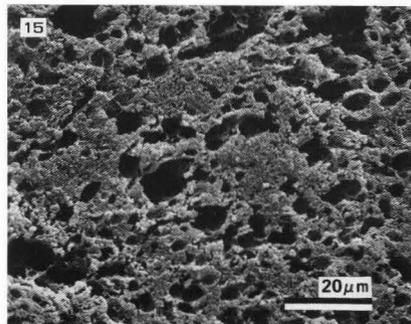
Frying in oil severely altered the structure of the paner. SEM shows that compaction suppressed the fine granularity of the protein matrix in the cow milk paner (Fig. 9). The granularity completely vanished in the buffalo milk paner (Fig. 10). The compaction is even more clearly evident in TEM micrographs (Figs. 11 and 12). The structure of the protein matrices resembles that in young Cheddar cheese [3]. In spite of a severe compaction of the core-and-lining structure observed at a low magnification, the existence of this structure has been confirmed by an examination at a higher magnification (Fig. 13) although it deviates from the original structure in the buffalo paner (Fig. 14). The compaction of the

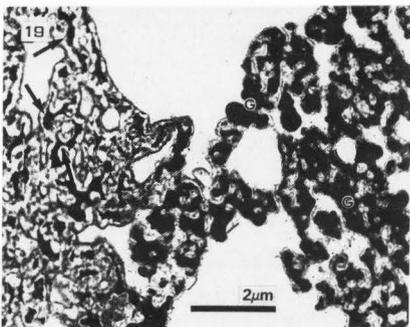
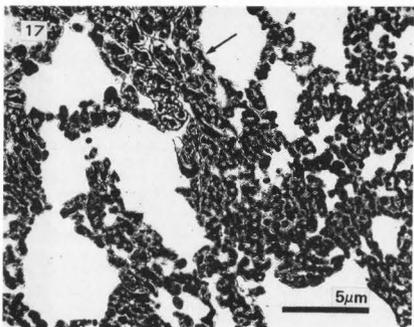
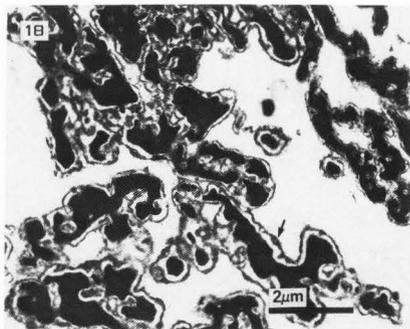
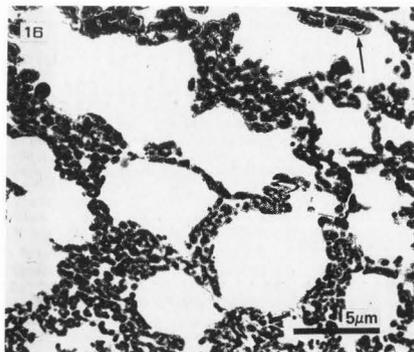


Figs. 11 and 12. Thin sections of cow milk paneer (Fig. 11) and buffalo milk paneer (Fig. 12) which had been fried in oil show compacted protein matrices (M) and areas with sharp and pointed (arrows) outlines presumed to contain fat (F).

Figs. 13 and 14. Details of the cow milk paneer (Fig. 13) and buffalo milk paneer (Fig. 14) which had been fried in oil show differences in the core-and-lining structures (arrows) in both paneers.

Fig. 15. SEM of cow milk paneer which had been fried and subsequently cooked in salt water. The granular structure appears to be restored (compare with Fig. 1).





Figs. 16 - 19. Thin sections of cow milk paneer (Figs. 16 and 18) and buffalo milk paneer (Figs. 17 and 19) which had been fried in oil and subsequently cooked in salt water. The restoration of the core-and-lining structure (small arrows) to a varying

extent is noticeable in all micrographs. Localized disintegration of the protein particle 'cores' (large arrows) and partial disintegration of the 'lining' (G) are noticeable in the buffalo milk paneer (Fig. 19).

paneer structure caused by frying also altered the shapes of the fat globule clusters. The fat particles acquired sharp and pointed outlines (Figs. 11 and 12) as compared to their near-globular shapes in the raw paneer. The fat globule membranes vanished or were broken and the fragments became convoluted.

Cooking in salt water restored both the granular structure of the fried paneer (Fig. 15) and the core-and-lining structure of the protein particles, particularly in the paneer made from cow milk (Fig. 16). Interestingly, swelling of the protein matrix during cooking considerably loosened even the initially more compact curd of

the buffalo milk paneer (Fig. 17). The core-and-lining structure of the protein particles was also restored in both paneer samples (Figs. 18 and 19). Disintegration of the 'cores' and abundant occurrence of the 'lining', as well as the opposite situation characterized by partial disintegration of the 'lining', were localized to small areas; in this respect, the structure of the cooked buffalo milk paneer appeared to be quite different from the structure of the cooked cow milk paneer.

The finding of a stable core-and-lining structure of the milk protein particles resistant to the effects of frying in vegetable oil is in

agreement with an earlier report that this structure, which also develops in Latin-American White cheese made from cow milk, was found almost intact in process cheese in which the White cheese was part of the natural cheese blend [3].

This study showed that the microstructure of paneer, irrespective of the milk used, is similar to other milk products obtained by coagulating hot milk with an acid to a pH in the vicinity of 5.5. Frying in oil expelled water and compacted the protein matrix but did not alter its fat content to any considerable extent. Cooking of the fried paneer in salt water increased the moisture content of the product beyond the initial level and restored the structure of the curd as well as the ultrastructure of the protein particles.

Acknowledgments

Skillful technical assistance provided by Mrs. Paula Allan-Wojtas and Mr. J. Emery is acknowledged. The authors thank Dr. H. W. Modler for useful suggestions. Electron Microscope Unit, Research Branch, Agriculture Canada in Ottawa provided facilities. Contribution 760 from the Food Research Centre.

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Discussion with Reviewers

R. Nath: Please comment on organoleptic preference of variously prepared paneer by the consumer.
Authors: Most paneer in Indian cuisine is fried and is used as an ingredient in curried vegetable dishes such as muttar paneer. Some customers may prefer to buy nonfried paneer and process it at home to their liking. Paneer may also be breaded and then deep-fried.

Reviewer I: Please explain how the buffalo milk was standardized.
Authors: Addition of skimmed buffalo milk was used to reduce the fat content in the whole buffalo milk. Fine adjustment was achieved using separated buffalo cream.

Reviewer I: What is clarification and what effect has it on milk composition or microstructure?
Authors: Clarification means the removal of corpuscular contaminants such as leucocytes from milk by centrifugation. It is not supposed to affect the composition and microstructure of the milk.

Reviewer I: The authors state that "...the fat content remained relatively stable during frying...". Do the authors know whether there was any interchange of the vegetable (frying) oil and the milk fat? This means that although the total content of fat was "relatively stable", its composition could have changed. It might be interesting to know what microstructures would allow such exchange to take place. One might wonder if a "fat exchange" experiment could be done in a way analogous to the common deuterium experiment done with proteins.

Authors: The exchange of vegetable oil and milk fat was not studied. In our opinion, fat globule membranes represent a barrier which would, to some extent, limit such an exchange. The porosity of the paneer protein matrix would certainly play the essential role. Chemical differences in the fatty acid composition between vegetable oil and milk fat as detected by gas chromatography (GC) could be used to study the fat and oil exchange.

R. Cartwright: The observation of the core-and-lining structure is important in understanding the behavior of these products. Do the authors agree that this phenomenon would be a good candidate for application of gold-labeling technology

currently being practised? Also are the authors aware of any attempts to study this phenomenon using gold-labeling technology?

Authors: The core-and-lining structure in milk products has received little attention although its incidence is common to product obtained by the acidulation of hot milk to final pH of around 5.5. The gold-labeling technique will probably help explain the development of the structure. The technique would require the preparation of antibodies against κ -casein, β -lactoglobulin, and their heat-induced complex, and interacting them with gold granules in order to stain thin sections of the core-and-lining structure.

R. Cartwright: In your paper you state that a pH of 5.5 is essential for development of the core-and-lining structure. An article recently published by Haque and Kinsella [13] acknowledges the interaction of heat treated β -lactoglobulin and κ -casein at a pH of 6.8. Is it possible that the core-and-lining structure develops during heat treatment, due to the interaction between β -lactoglobulin and κ -casein? Would it then be possible that the core-and-lining structure is already present before coagulation and is reduced as the pH is lowered to 4.6 indicating a dependence on colloidal calcium or net charge of the micelle for the existence of the lining seen in the micrographs?

Authors: High temperature (>70°C) is necessary for the core-and-lining structure to develop. The presence of β -lactoglobulin is also essential for

this development to take place. Recently, the milk salt system has been found to be involved in the formation of the structure [14]. Effects of the fixation of the acidulated milk at varying temperatures and the stability of the core-and-lining structure at varying pH would be interesting to study.

R. Cartwright: In the Harwalkar and Kaláb reference [6], it appears as if the micelles may have an individual lining structure. However, in some of the micrographs you present here, it appears as if a group of micelles may be surrounded by a single lining. How do you interpret the micrographs in this respect?

Authors: The appearance of the lining depends on the acid used. As the development of the core-and-lining structure in general is not yet fully understood, we don't know why in some cases the cores are considerably larger than in others.

Additional References

13. Haque Z, Kinsella JE. (1988). Interaction between heated κ -casein and β -lactoglobulin: Predominance of hydrophobic interactions in the initial stages of complex formation. *J. Dairy Res.* 55, 67-80.
14. Harwalkar VR, Kaláb M. (1988). The role of β -lactoglobulin in the development of the core-and-lining structure of casein particles in acid-heat-induced milk gels. *Food Microstruc.* 2 (in press).