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V. V. Mistry

H. N. Hassan

D. J. Robison

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EFFECT OF LACTOSE AND PROTEIN ON THE MICROSTRUCTURE OF DRIED MILK

V.V. Mistry^{*}, H.N. Hassan¹, and D.J. Robison²

Minnesota-South Dakota Dairy Foods Research Center, Dairy Science Department, and 2 Animal Disease Research and Diagnostic Laboratory

South Dakota State University, Brookings, SD 57007.

¹Present address: Department of Dairy Science, Faculty of Agriculture, Tanta University, Kafr-Elsheikh, Egypt.

Abstract

Skim milk of approximately 3% total protein was ultrafiltered to 16.2% protein and 4.1% lactose, and then diafiltered to reduce lactose to 2.1% and 0.9%. Total protein content was maintained at approximately 17%. A portion of the skim milk was condensed in a rising film evaporator to 14.7% protein and 15.7 % lactose . All concentrates were spray dried at 120 to 125 °C inlet air temperature and 75 to 80 °C outlet temperature using a rotary atomizer in a pilot plant spray dryer. Moisture content of the powders were 4.7 to 6.3% and lactose content ranged from 3.1% in diafiltered milk powder to 51.4% in the condensed milk powder. Scanning electron microscopy showed that powder particles with the highest lactose content had a wrinkled surface and dents. Powder particles with lower lactose, including ultrafiltered and diafiltered milk powders, possessed dents but had no wrinkles and were smooth. When the lactose content of the diafiltered skim milk containing 0.9 % lactose was raised to 15.6% by addition of lactose powder and then the product was spray dried, the powder particles showed a wrinkled surface. Intermediate lactose products, such as whey protein concentrate (37.1% lactose) possessed particles with a wrinkly surface as well. The wrinkles were not as pronounced as in powders with high lactose. Permeate powder containing 82.8% lactose had smooth particles with some wrinkles. Results suggest that lactose and protein influence the surface structure of milk powder particles.

Key Words: Spray drying, Dried milk, Microstructure, Lactose, Protein, Particle surface, Wrinkles, Scanning electron microscopy, Dents

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Introduction

Microstructural characteristics of dried milks are dependent on a number of factors including method of drying, drying conditions, and composition of concentrates (5) . In most dried milk products, lactose is the predominant component (13). Dried skim milk, for example, contains approximately 50% lactose, and dried whole milk 35%, whereas dried whey may contain as much as 70% lactose (8, 13, 17). Lactose is present in these products as a continuous phase and, consequently, plays a significant role in microstructural characteristics. Lactose may exist in dried milk products in different forms which significantly affect the physicochemical and structural properties of the products. For example, if lactose is in the glassy amorphous state, it will absorb moisture from the atmosphere readily which will cause caking and clumping of the powder. Powder particle aggregates in this case are very large (13). Additionally, storage in a humid environment affects lactose crystallization, which in turn influences microstructural characteristics (11, 18). These effects are particularly pronounced in whey powders which contain 70% or more lactose. Under ordinary conditions, most of the lactose is in the glassy state but by including a pre-crystallization step prior to drying, 85 to 100% of the lactose can be converted to the crystalline α -monohydrate form. Powder containing this form of lactose will not cake (17) .

Certain milk products, e.g., caseinates and a new high milk protein powder, however, contain less than I% lactose (14, 16). The major component in these products is milk protein. Microstructural characteristics of these powders are, therefore, dependent almost entirely on components other than lactose. In previous studies, the manufacture and properties of a new high milk protein powder were reported (14, 15). In the manufacture of this powder, membrane technology was used to concentrate proteins and remove lactose prior to spray drying. The resulting powder contained approximately 85% total protein (which included both casein and whey proteins) and less than 1% lactose. It was observed during these studies that the high milk protein powder particles were characteristically smooth; unlike nonfat

dry milk but they did have dents.

The objective of this study was, therefore, to examine the effect of changes in composition of skim milk, especially that of lactose and protein, on the microstructural characteristics of dried products obtained by spray drying under identical conditions.

Materials and Methods

Preparation of powders

Approximately 360 kg of pasteurized skim milk was obtained from a commercial dairy and divided into two portions. One portion (110 kg) was condensed in a single stage rising film evaporator (Blaw-Knox, Mora, MN) at 43 °C and under 84 kPa vacuum. Concentration continued until a 5:1 concentration was obtained. The concentrate was cooled and maintained at 4 °C until drying commenced.

The second portion of skim milk (250 kg) was ultrafiltered at 38 °C to approximately 6:1 volume concentration ratio (VCR) in an Abcor UF model 1/1 sanitary pilot plant unit equipped with a 5.6 m² spiral wound membrane (Wilmington, MA). Forty kg concentrate (retentate) and 210 kg permeate were produced. Approximately 7 kg rctentate was removed for drying. To the remainder, 58 kg water at 32 °C was added to commence the first diafiltration (DF1). This water-diluted retentate was ultrafiltered to remove the added water (58 kg). Seven kg retentate was removed for drying. For the second diafiltration (DF2), 141 kg water at 32 $^{\circ}$ C was added to the retentate remaining from DF1 and ultrafiltered again to remove the added water. This was repeated for a third diafiltration (DF3). Retentate from the final diafiltration (DF3) was collected for drying and analysis. Permeate from the initial ultrafiltration and each diafiltration was discarded. To a portion of the retentate of DF3 (5.5 kg) , 1.4 kg lactose monohydrate (Curtis Matheson Scientific, Eden Prairie, MN) was added at approximately $30 °C$ so that powder produced from this mixture would contain approximately 50% lactose.

In a separate experiment, approximately 145 kg raw whole milk at 38 °C was ultrafiltered as above. Retentale was discarded and approximately 73 kg permeate was condensed in an evaporator as above to a 5: I concentration.

The above five concentrates were spray-dried in 11 to 16 kg batches in a Niro Atomizer pilot plant spray drier, model ASO 412/E (Columbia, MD). The spray drier was equipped with a rotary atomizer, propane-fired heater, two exits for the powder, and the capacity of removing 16 to 18 kg water/hour. Inlet air temperature was 120 to 125 °C, and the feed rate was adjusted to attain an outlet air temperature of 75 to 80 $^{\circ}$ C. A constant atomizer speed was maintained. Concentrate temperature was 38 °C. Powder obtained from the main exit of the drying chamber was sifted with a USA standard testing Sieve Number 18 (Tyler equivalent 16 mesh, Fisher Scientific Co., Minneapolis, MN) to remove any burnt

particles prior to analysis. Samples were fixed immediately for scanning electron microscopy (SEM). Details of sample preparation and fixing are provided below.

A commercial whey protein concentrate dried at 200 °C outlet air temperature using a rotary atomizer and a commercial sodium caseinate whose drying conditions were not known were also evaluated.

Analyses

Composition. Total protein in skim milk, condensed skim milk, UF, DF1, DF3 and all powders was determined by the macro-Kjeldahl method and ash using a muffle furnace at 550 °C (1). Total solids in skim milk and all concentrates was determined by oven drying, and fat in all products was measured by the Mojonnier method (2). Moisture in powders was determined with an Ohaus Model MB200 moisture balance (Florham Park, NJ) using standard methods (6) and lactose was calculated by difference.

Scanning electron microscopy. All powders were prepared for SEM according to published methods (10, 11). A SEM aluminum stub was sonicated in acetone for 5 minutes, allowed to air dry, and painted with a silver-based paint. A double sticky tape was attached to the stub and a thin layer of powder was applied on the exposed sticky surface of the tape. The powders were sputter-coated with approximately 20 nm gold:palladium for 3 minutes at 10 mA in an atmosphere of argon using a Hummer VI Technics sputter coater (Electron Microscopy Systems Jnc. , Munich, Germany). The sputtercoated powders were stored in a desiccator at room temperature (23 to 25 °C). Coated samples were examined in an International Scientific Instruments Super IliA SEM operated at 15 kV. Photomicrographs were taken on a Type SS Polaroid SO ASA film (Polaroid Corp., Cambridge, Mass).

Results and Discussion

Composition of milk and concentrates

Milk composition was varied by a series of processing steps. Pasteurized skim milk containing 3.1% protein was used as the base material for all products except permeate, for which whole milk was used (Table 1). The skim milk was condensed to approximately $5:1$ concentration giving 14.7% protein, 15 .7% lactose, and 34.4% solids. Since thermal evaporation of milk removes only water. all components were concentrated in an equal proportion (8).

Ultrafiltration and diafiltration were used to lower lactose concentration by various levels while maintaining protein content at approximately the same level as in condensed milk (Table 1). Ultrafiltration is a selective concentration/separation process in which lowprocess in which lowmolecular weight components are removed from milk, along with water, as permeate (7). If further removal of these low-molecular weight components such as lactose is desired, an additional step called diafiltration can be performed. In diafiltration, retentate obtained by ultra-

Microstructure of dried milk

Table 2. Composition of spray-dried condensed skim milk and permeate, ultrafiltered/diafiltered skim milks, whey protein concentrate, and caseinate.

Product	Moisture $(\%)$	Protein $(\%)$	Fat $(\%)$	Ash $(\%)$	Lactose $(\%)$	
Nonfat dry milk	4.7	35.6	1.1	7.2	51.4	
Whey protein concentrate	8.3	46.7	4.5	3.4	37.1	
Ultrafiltered skim milk (UF)	5.2	66.0	2.0	6.9	19.9	
Diafiltered skim milk (DF1)	6.3	75.8	2.1	6.8	9.0	
Diafiltered skim milk (DF3)	6.4	81.5	2.3	6.8	3.0	
Diafiltered skim milk (DF3) + Lactose	5.1	37.9	1.3	3.5	52.2	
Sodium caseinate	4.3	88.0	1.1	6.5	0.1	
Permeate	2.9	4.2	0.2	9.8	82.8	

filtration is diluted with water and then ultrafiltered again. This can be repeated several times until most of the low-molecular weight components are removed (7). Hence, ultrafiltration of skim milk to 5:1 produced a retentate (UF) that contained 16.2% protein and 4.1% lactose (Table 1). To further lower the lactose content, ultrafiltered skim milk was batch-diafiltered with water three times, producing retentates with lactose concentrations of 2.1 (DF1) and 0.9% (DF3) with corresponding protein contents being 15.9 and 17.5%, respectively.

To investigate the effect of mineral loss during ultrafiltration /diafiltration on microstructure, lactose was added back to DF3, resulting in 15.6% lactose and 14.3 % protein in the mixture (Table 1). The major difference between this mixture and condensed skim milk was the ash content (1.4 and 3.5%, respectively).

Permeate of ultrafiltration contained 4.9% lactose and traces of fat and protein. When condensed by thermal evaporation to approximately 5: I, a concentrate containing 26.2% lactose was produced (Table l). The total protein content in this concentrate was 1.2% (Table 1), which is likely to be non-protein nitrogen (7). Commercial liquid whey protein concentrate was not available for analysis.

Composition of dried products

Dried products ranged in lactose content from 0.1% in sodium caseinate to 82.8% in permeate, and total protein content ranged from 4.2 to 88% in permeate and sodium caseinate, respectively (Table 2). Nonfat dry milk contained 51.4% lactose. Ultrafiltration of skim milk lowered the lactose content of powder to 19.9, and diafiltration (DF3) lowered it further to a final concentration of 3.1%. There was an increase in protein content with the decrease in lactose with diafiltration. Composition of powder produced from diafiltered skim milk with added lactose was similar to that of nonfat dry milk in all respects except ash content. The ash content of the nonfat dry milk was almost twice as much as that of the diafiltered skim milk.

Microstructure of dried products

Microstructure of dried milk products depends on a number of factors including composition, drying conditions such as air temperature and type and speed of

Figure 1. SEM micrographs of nonfat dry milk (51.4% lactose) prepared by spray drying thermal evaporated skim milk. Particles were characterized by deep dents and wrinkles (marked by solid black arrows). a) At low magnification. Some particles were cracked by the electron beam (open arrow). Panicle marked T is an unusual tubular particle in this product, white arrow shows wrinkles around deep depressions. b) At high magnification.

Figure 2. SEM micrographs of dried whey protein concentrate (37.1% lactose) prepared by spray drying ultrafiltered whey. Particles had deep dents and the surface was slightly wrinkled (marked by arrows). a) At low magnification. The electron beam cracked some of the particles (open arrow); b) at high magnification.

Figure 3. SEM micrographs of dried ultrafiltered skim milk, UF, (19.9% lactose) prepared by spray drying ultrafiltered skim milk. Deep dents were evident but the particle surface was smooth (shown by arrows). a) At low magnification; b) at high magnification.

Figure 4. SEM micrographs of dried diafiltered skim milk, DF1, (9.0% lactose) prepared by spray drying diafiltered skim milk. These particles also were characterized by deep dents and a smooth surface (shown by arrows). a) At low magnification; b) at high magnification.

Microstructure of dried milk

Figure 5. SEM micrographs of dried diafiltered skim milk, DF3, (3.1% lactose) prepared by spray drying diafiltered
skim milk. As with UF, and DF1, these particles had deep dents and were smooth (shown by arrows). a) At low magnification; **b**) at high magnification.

atomization (5). Therefore, identical drying conditions were maintained in this study for all products except the two commercial products.

The microstructure of dried products as seen with SEM is shown in Figures I to 8. Powder particles of all products, regardless of composition of the product, were characterized by dents. Powder particle size was obtained by measuring particle diameter and using the scale on each micrograph to determine actual size. Particle size was generally the same for all products with the exception of permeate which had smaller particles. Particle size for permeate ranged from 3 to 17 μ m (Figure 8), whereas for the other products it ranged from approximately 6 to 30 μ m, with majority of the particles being in the 13 to 30 μ m range (Figures 1 to 7).

Distinct differences were observed in the surface structure of the products depending on composition. Particle surface of nonfat dry milk was characterized by deep wrinkles (Pigures Ia and lb), which is a common characteristic of spray-dried skim milk $(3, 9)$. Particles of unusual shapes were also observed in this product, e.g., tubular shaped particles (marked with letter T, Figure 1a). Whey protein concentrate, which had a lower lactose content (37.1%) than nonfat dry milk, had a considerably different surface. The surface was not as wrinkled as that of nonfat dry milk, but was wavy (Figures 2a and 2b). Additionally, nonfat dry milk particles also had wrinkles around deep depressions (white arrow, Figure Ia). These were absent in the lower lactose particles (Figures 2 to *5,* and 7).

As stated earlier, the three ultrafiltered/diafiltered skim milk powders had low lactose contents. Particles of these products were free of wrinkles and were smooth (Figures 3, 4, and 5). It had been observed earlier that particles of the high protein products were hollow (15). In this study, it was possible to create a wrinkly surface on particles of diafiltered skim milk by adding lactose to the concentrate prior to drying (Figures 6a and 6b). This product also had some unique particles, e.g., a large central particle to which smaller particles were attached (Figure 6a). The smaller particles appeared to be attached to the larger particles by the folds of the wrinkles on the latter (open arrow, Figure 6a). Commercial sodium caseinate (0.1% lactose) had a surface structure almost identical to that of the ultrafiltered/ diafiltered skim milks (Figure 7). Caseinate particles with dents and smooth surface have been reported by others as well $(4, 12, 15)$. On the other hand, particles of spray dried permeate, which contained the most lactose of all products (82.8%) , were generally smooth but wrinkles were occasionally observed (Figures 8a and Sb). Spray-dried permeate samples were difficult to analyze under the SEM. Particles of this product were very easily cracked by the electron beam of the SEM (open arrow, Figure 8b). This effect was possibly due to the very high lactose content of these particles which made them brittle. A similar phenomenon but with lesser severity was also observed in the intermediate lactose products such as nonfat dry milk (Figure 1a) and whey protein concentrate (Figure 2a) but never in the high protein products. The proteins in the high protein products probably made the particles more flexible than those high in lactose.

The above observations show that as the lactose content of skim milk was reduced, and as the protein content increased, wrinkles from particle surface gradually disappeared. At 19.9% lactose and lower, no wrinkles were evident in the powder particles. Wrinkles could be created in powder particles by adding lactose to diafiltered concentrates, suggesting that lactose plays a role in surface structure properties. Burna and Henstra (3, 4) stated that wrinkles on nonfat dry milk powder particle surface are caused by uneven shrinkage of casein during drying, and that lactose had no contribution. They also found that spray-dried particles of a lactose solution were free of wrinkles. In the present study, it was further observed that high protein, low-lactose dried products such as sodium caseinate (88 % protein) and diafiltered skim milk (81.5% protein) had an identical, smooth surface structure. Additionally, whey protein concentrate with an intermediate lactose content (37 .1 %) had fewer wrinkles than nonfat dry milk. 1t is interesting to note that while the total protein concentration of caseinate and diafiltered skim milk (DF3) was quantitatively similar (Table 2), the proteins in the two products were qualitatively different. Protein in caseinate consisted mainly of casein, whereas protein in DF3 consisted of both casein and whey proteins. Protein in whey protein concentrate was mainly all whey protein. These observations indicate that milk proteins, regardless of type (casein or whey protein or combination), may not have a direct influence on surface structure. Structure similar to that of milk protein powders (Figure 6) has also been observed with soy proteins (19). Lactose may not have a direct influence on surface structure as suggested by the surface of permeate powder. Interaction between protein and lactose at a lactose content of more than 33% coupled with uneven shrinking during drying may be responsible for surface wrinkles.

J:

Conclusions

Lactose and protein content of dried milk considerably affected surface structure of powder particles. Powder particles containing casein and/or whey proteins were characterized by deep dents regardless of lactose content. The surface of skim milk powder particles containing 51.4% lactose and 35.6% protein was extremely wrinkled. Whey protein concentrate with a slightly lower lactose content (37.1%) and higher protein content $(46.7%)$ had fewer wrinkles on the surface. Particles of dried ultrafiltered/diafiltered skim milk had a smooth surface with no wrinkles. These powders contained 3.1 to 19 .9 % lactose and 81.5 to 66% protein. Particles of a commercial caseinate containing less than 1% lactose and 88% protein (mainly casein) were also smooth. When lactose was added to diafiltered skim milk and dried, the resulting powder particles became wrinkly.

Microstructure of dried milk

Figure 6. SEM micrographs of dried diafiltered skim milk, DF3 + lactose, $(52.2\%$ lactose) prepared by spray drying diafiltered skim milk with added lactose. Particles had deep dents but wrinkles, as in nonfat dry milk, reappeared (shown by solid arrows). a) At low magnification. The open arrow shows how the smaller particles are attached to a larger central particle by the folds of wrinkles on the large particle. b) At high magnification.

Figure 7. SEM micrograph of commercial caseinate (0. I% lactose). Particles are characterized by deep dents and smooth surface (shown by solid arrow). The open arrow indicates a fairly large particle sitting in a depression in another particle.

Figure 8. SEM micrographs of permeate powder (82.8% lactose). Particles were spherical with small dents and smooth surface (shown by solid arrows). a) At low magnification; b) at high magnification. The electron beam cracked some particles (open arrow).

Dried permeate containing 83% lactose and 4% protein had smooth particles suggesting that lactose and milk proteins together in milk powder produce wrinkles on powder particles, thereby increasing surface area of the particles. High protein, low lactose powders and permeate powders (high lactose, low protein) had particles with smooth surface. Wrinkles were present only on particles of powders containing both lactose and protein. It would be interesting to evaluate the effect of surface morphology on functionality of protein powders. It has been shown earlier that high milk protein powders with smooth surface have good solubility (15).

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Discussion with Reviewers

D.J. McMahon: Why was water used for diafiltration? This will also change the salt balance in the resultant retentate so that when comparisons arc made they will be confounded. The effect of changing the lactose content can therefore not be separated from the effect of changing the calcium phosphate content.

Authors: To concentrate proteins and remove lactose at the same time, water would have to be used for diafiltration. Water will gradually dilute out the lactose and remove it through the membrane until none is left in the concentrate. It is true that during diafiltration salts will also be removed along with lactose. For this reason powder was prepared from a diafiltered, lactose-free concentrate to which pure lactose was added back (DF3 + lactose, Tables 1 and 2). Wrinkles similar to those in nonfat dry milk were observed in the absence of minerals. This would suggest that minerals do not play a major role in surface morphology.

D.J. McMahon: What was the ratio of α -lactose to Blactose in your samples? If these were different to that obtained in commercial drying of milk what is the application of this work to the dairy industry?

Authors: We did not measure the ratio of α -lactose to ß-lactose in the powders but in trials with different thermal histories (storage versus no storage of concentrates prior to drying, higher drying temperatures) we did not observe any differences in surface morphology of the particles. If lactose in dried milk products exists in the crystalline state, the lactose crystals will be clearly visible as large particles of irregular shape in the electron micrographs. No such particles were observed in any of the products evaluated. Hence, it may be surmised that under commercial drying conditions results similar to those reported in this paper would be obtained.

D.N. Holcomb: What is the purpose of painting the aluminum stub with silver based paint before application of the double sticky tape? The practice of painting the edge of the tape after it is applied to the aluminum stub assures that there is a conductive path to ground in case the sputter coating does not coat the edges of the tape. Maybe the tape was pressed into the silver paint before it had dried, thus coating the edges of the tape?

Authors: When the double sticky tape was attached to the painted aluminum stub, it was ensured that the edges of the tape stuck well and remained attached to the stub. This way the conductive path to ground was assured and no problems were encountered when the samples were sputter-coated and viewed.

D.J. McMahon: You imply that formation of wrinkles increases the surface area. Is not this also a function of particle size? And if the permeate powders are smaller wouldn't their surface area (on the basis of mass) therefore be larger?

Authors: It is true that surface area is a function of particle size but in a given product if lactose determines the presence or absence of wrinkles without a change in particle size, then wrinkles will be responsible for changes in surface area. Particle size for all products except permeate powder was the same.

D.J. McMahon: What explanation do you have for the lack of wrinkles on commercial caseinate powder? Does this imply that having the caseins in skim milk present as micelles changes the particle drying process compared to a product containing acid precipitated casein?

Authors: Smooth surface of commercial caseinates have been observed before by others (references 3 and 4). The smoothness has generally been attributed to implosion of caseinate particles and uneven shrinkage. In studies with soya protein isolates and concentrates (reference 19), it was observed that sodium proteinate isolates of soya had a different appearance than the isoelectric type. The former were smooth and partially collapsed whereas the latter were more clumped together. Differences between the two soy products were attributed to differences in drying conditions rather than composition. A similar explanation may apply to micellar versus acid precipitated casein.

V.R. Harwalkar: How would changing the heating conditions during spray drying affect the surface structure (smooth versus wrinkly) of the particles? Were the spray drying conditions for soy protein powders the same as in your experiments? Did the soy protein

powders contain any lactose or sugar?

Authors: Heating conditions per se during spray drying will not affect surface structure. It was observed in preliminary studies (micrographs not shown) that nonfat dry milk powder produced at 200 °C inlet temperature had wrinkled particles as well. Likewise, particles of high protein powders produced at higher drying temperatures were smooth as well. The drying conditions for preparing the soy powders have not been cited. Soy protein isolates contain more than 92% protein and have no lactose or other sugars. They do have 0.1 to 0.2% crude fibre [Meyer EW. (1970). Soy protein isolates for food. In: Proteins as human food, Lawrie RA (ed.), AVI Pub. Co. Westport, Conn. p. 346.

V.R. Harwalkar: Would substituting lactose by other sugars have similar effects on the surface structure?

Authors: We do not know what effect other sugars will have on the high milk protein powders, but with spray dried whey protein blends containing sugars other than, or in addition to, lactose (maltodextrins or hydrolyzed lactose) we observed that while particles do have some wrinkles, a large number of irregular shaped crystals of varying size were also present. These could be sugar crystals. Effect of sugars on surface structure would depend in part on type of sugar.

D.J. McMahon: It may be the form of lactose that influences surface structure as well as quantity. It would have been useful to fracture some of these particles to compare their interior structure. Were the permeate powders hollow or did they contain numerous vacuoles? Authors: In an earlier publication (15), it was reported that the high milk protein powder particles were hollow. We do not know if permeate powder particles were hollow as well. Because of their high lactose content, permeate particles cracked and melted very quickly under the electron beam and were therefore difficult to analyze under the SEM.

V.R. Harwalkar: Does the wrinkly surface affect the sorption or solubility or dispersibility of the dried powders?

Authors: It is not known if the wrinkly surface will itself directly affect solubility, sorption or dispersibility. Studies reported in another paper (reference 15) showed that the solubility of high milk protein powders with smooth particles was as good as that of nonfat dry milk especially at high temperatures (> 45 °C). The poor solubility at lower temperatures may have been due to higher protein content. In the same study, the protein powders also showed good foaming ability.

Reviewer IV: Figures 4, 5, and 7 are unnecessary as they are identical to Figure 3 and do not add new information that couldn't be expressed in a couple of sentences in the text!

Authors: We feel that these figures are useful. While the structure indicated by these Figures is similar to that of some of the other Figures, products represented in the Figures in question arc considerably different from the other products. For example, Figure 4 represents a
product with 9% lactose, Figure 5 with 3% lactose and
Figure 7 represents a commercial caseinate product with entirely different method of manufacture and composi· tion. We feel it would be of interest to the readers to visually see the immense structural similarity between products of differing composition.

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