

## EFFECT OF HOMOGENIZATION ON CASEIN MICELLES AND MOZZARELLA CHEESE PROPERTIES

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### ABSTRACT

Homogenization of cheese milk results in weakened cheese body and texture. To study these effects on a molecular level, casein micelles were examined after homogenization for changes in size distribution and possible protein interactions. Photon correlation spectroscopy showed a shift to smaller micelles after homogenization and a reduction of up to 10% in average micelle size after homogenization in milk dialysate at 87.6 MPa with a French press. A  $\kappa$ -casein- $\beta$ -lactoglobulin interaction was observed by gel electrophoresis when micelles were homogenized in the presence of the whey protein. Because homogenization can be a part of cheese milk preparation, the effect of shear pressure on proteolysis and texture of Mozzarella cheese was investigated. Cheese milk homogenized at 17.2 MPa produced both lowfat and full fat cheeses with reduced meltability and increased hardness. Proteolysis of  $\alpha_{s1}$ -casein was 85% less than in control (non-homogenized) lowfat cheese and about half of that in control full fat cheese. Results suggest that altered size distribution and protein interactions affect curd formation and structure during initial cheesemaking steps in such a way as to retard texture development by proteolysis during ripening.

### INTRODUCTION

The effect of shear on milk proteins has been of interest for many years, ever since it was demonstrated that homogenization of skimmed milk followed by processing into nonfat dry milk resulted in a functional product that would whip into a stable foam when reconstituted (Tamsma, et al., 1968). Nonfat dry milk powders made from skimmed milks that had not been homogenized during the processing sequence would not whip.

Dairy products like cheese can be thought of as composite or filled gels of milk proteins that form a three dimensional network that entraps the dispersed phase containing the fat droplets, water and air. Fat globules in cheese behave as a pliable filler which interacts with the protein (casein) matrix to reinforce or weaken the gel structure depending on the degree of homogenization (Armbruster, et al., 1995; Desai and Nolting, 1995). When cheese milk is homogenized, there is a reduction in curd firmness and heat stability and altered (weakened) body and texture in such ripened cheeses as Cheddar and Tilsit, suggesting that homogenization has effects beyond disruption and size reduction of the milkfat (Peters, 1964). On the other hand, homogenization may reduce fat leakage, oiling off at elevated temperatures and increase lipolysis in the case of blue

cheese (Peters, 1964.) Tunick (1994) demonstrated that homogenization of cheese milk virtually eliminated free oil formation in heated Mozzarella cheese.

Concerns about saturated fat and cholesterol in the diet have stimulated U.S. consumer interest in lowfat and nonfat cheeses. The manufacture of such cheeses that have good flavor, body and texture is technologically challenging. We have been studying the rheological properties of reduced fat Mozzarella cheese and decided to investigate the effects of homogenization on the textural qualities, since removal of milkfat impacts the salt/water ratio, an important determinant in controlled texture development (Lawrence, et al., 1987). To do this, we decided it was necessary to examine homogenization effects at the molecular level, that is, on the size distribution of the casein micelles and on protein-protein interactions in the absence of milkfat.

## **MATERIALS AND METHODS**

### **Fundamental Studies**

Milk: Fresh raw whole milk was obtained from the mixed dairy herd at Delaware Valley College, Doylestown, PA. Milk was skimmed by warming to 37°C and separated in an open DeLaval laboratory cream separator, followed by chilling and storage at 4 C.

Preparation of Casein Micelles: Casein micelles were separated by ultracentrifugation at 100,000 x g for 1 hr at 37°C from fresh raw skimmed milk, and resuspended in milk dialysate by continuous gentle stirring for two days at 4 C. Milk dialysate was made by suspending membrane tubing with a molecular weight cutoff of 6000-8000 Da filled with distilled water in raw skimmed milk for 3 to 5 days at 4°C.

Homogenization: Ten mL of resuspended micelles were homogenized at 21°C in an SLM/Aminco 20K French pressure cell at 14.7 or 87.6 MPA on an SLM/Aminco Laboratory Press; nonhomogenized micelles served as controls. (A French pressure cell consists of a steel barrel and plunger, with exit valves that permit the release of the liquid contents under the selected pressure. It was designed for the rupture of microbial cells and spores.) Exit temperatures ranged from 25 to 35°C. The sample was collected and analyzed immediately. Skimmed milk,  $\beta$ -lactoglobulin + casein micelles (0.231 g/g casein) and  $\alpha$ -lactalbumin + casein micelles (0.117 g/g casein) were homogenized in the same manner.

Photon Correlation Spectroscopy (PCS): PCS was performed with a Malvern PCS 4700c system equipped with a SpectraPhysics Argon laser with a wavelength of 488 nm. The light scattering angle was 90 degrees. The casein suspension was diluted 1:100 with milk dialysate previously filtered through a 1  $\mu$ m Nucleopore polycarbonate membrane filter to remove precipitated calcium phosphate. PCS data were collected with the Malvern Automeasure™ program that computed size distribution, Z-average mean and average micelle size.

**Transmission Electron Microscopy (TEM):** Less than 1 min after homogenization, samples were chemically cross-linked by addition of glutaraldehyde to a concentration of 1%, incubated at 25°C for 1 hr and stored at 4 C. Drops of sample were adsorbed to Formvar-carbon coated specimen grids for 1 min, washed with 20 drops of 0.1 M Tris buffer at pH 7.4 and negatively stained with 2% uranyl acetate solution. Photographic images of micelles were recorded at an instrumental magnification of 20,500 in a Zeiss 10B transmission electron microscope. Perimeters of individual micelles were traced and digitized. Digital images were processed and analyzed to measure and plot the distribution of circular diameters for populations of micelles using a Dapple Systems image analyzer running Imageplus™ software.

**Gel Permeation Chromatography (GPC):** After homogenization, 5-mL samples were analyzed by GPC on a 1.5 x 120 cm column of Sephacryl S-1000 (Pharmacia LKB) fitted with a flow adaptor (BioRad). After sample application, the column was eluted with 0.2M sodium phosphate buffer, pH 6.8, at ambient temperature (ca. 22 C) with a flow rate of 40 mL/hr. The elution was monitored at 280 nm; peak fractions were pooled and lyophilized for electrophoretic analysis. Only the control (0 MPa) and the high pressure homogenized sample (87.6 MPa) were compared.

**Gel Electrophoresis:** Native electrophoresis in the presence of urea was conducted on lyophilized pools from gel permeation separations with the PhastSystem™ (Pharmacia) using 8 - 25% gradient gels. Samples were dissolved in 6.6M urea-0.112M Tris-0.112M acetate, pH 6.4 (Van Hekken and Thompson, 1992). Concentrations were based on relative peak heights in the chromatograms to assure detection. After addition of 2-mercaptoethanol, sample solutions were boiled for 5 min. Protein zones were stained for 10 min at ambient temperature in Coomassie Brilliant Blue R dissolved in 30:10:60 methanol:acetic acid:water. The gels were destained until the background was clear and restained with silver stain to show the bands more clearly. Total milk protein isolate (New Zealand Milk Products, Inc.) was used as a standard.

## **Cheesemaking**

**Cheese Preparation:** Lowfat Mozzarella cheese was prepared as described by Tunick, et al. (1993). Cheese was prepared from 22.7 Kg of milk and one batch was prepared on a given day. Milk was standardized with cream or skimmed milk to 1% milkfat before pasteurization at 63°C for 30 min. Milk for homogenized milk cheeses was homogenized double stage at pressures totalling 10.3 or 17.2 MPa at 63 C. The designation "0 MPa" is used for milk that was not homogenized. The general make procedure flow diagram is shown in Figure 1.

Cook temperature was examined as part of the study. Cheeses were cooked at either 45.9°C or 32.4 C. Cheese milk at 32.4°C was inoculated with 125 mL of CR7 starter culture (50% *Streptococcus thermophilus* and 50% *Lactobacillus bulgaricus*; Marschall Laboratories Division, Rhone-Poulenc, Inc.). After the pH decreased 0.1 unit, 4.4 g of single strength calf rennet (chiefly, chymosin) (Chr. Hansen's Lab., Inc.) were added. The curd was held for 35 min, cut and held for another 15 min. High temperature cheeses

were heated over 45 min to 45.9°C and held for 50 min. Low temperature cheeses were stirred at 32.4°C for 10 min and held for 90 min. The whey (pH 6.3-6.4) was drained; the curd was rinsed and cut into slabs. After the pH dropped to 5.2-5.3, the slabs were covered and iced overnight. The curd was hand-stretched the next day by kneading for 7 min in 70-80°C water. Samples were pressed into 8-oz polyethylene cups, cooled, removed from the cups and brined for 2 hr in 23% sodium chloride solution, dried and stored in vacuum sealed pouches for up to 6 wks at 4 C.

**Gel Electrophoresis:** Cheese samples were prepared for electrophoresis by extracting after 1, 3, and 6 wks of storage with a pH 8.0 solution of 0.166 M Tris, 1mM EDTA, 2.9% SDS and 1.7mM dithiothreitol, as described by Tunick et al.(1995). Extracts were lyophilized and stored at -20°C until used. SDS-PAGE of extracts was performed with the PhastSystem, using 20% homogeneous gels; gels were stained with 0.1% Coomassie Blue R250, destained and dried. Gels were scanned with a BioRad model 620 Video Densitometer interfaced with a PC and ID Analyst II™ (Version 3.1) software and peak areas integrated.

**Rheology:** Texture profile analysis (TPA) (hardness and springiness measurements) was performed with an Instron Universal Testing Machine as previously described (Tunick, et al., 1993) at 1 and 6 wks of storage. Cheeses were tempered at 23 - 26°C for 1 hr and a slab was cut from the sample's interior by cutting with a piano wire mounted in a frame. Four to six cylinders (14mm diameter x 14mm high) were cut from each slab with a cork borer. Storage and loss moduli were measured at 1 and 6 wks of storage with a Rheometrics Model RDA-700 Dynamic Analyzer at 23-26°C at a frequency of 100 rad/s at 0.8% strain. Three discs (25.4 mm diameter x 4-5 mm thick) were cut from the slab of cheese and glued to pairs of parallel aluminum plates with cyanoacrylate bonding agent for the analyses.

**Other Analyses:** Moisture was determined by a forced draft oven method of the AOAC (1990). Meltability was measured at 1 and 6 wks of storage by the Schreiber test. A disc of cheese (18 mm diameter x 5 mm thick) was measured on a graph of concentric circles after heating 5 min at 232°C (Kosikowski, 1982). A meltability of 1 means no change, 2 means a spread of 5 mm, 3 means an expansion of 10 mm, etc.

Statistical evaluations were done with the General Linear Models Procedures of SAS (1989). Compositional data, rheological and electrophoretic responses were analyzed by factorial ANOVA to examine effects of interactions of fat, temperature, and time. An interaction was considered significant at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

Data in Figure 2 show the effect of homogenization on the Z-average mean size of casein micelles. These data were obtained by photon correlation spectroscopy (PCS), which measures the amount of laser light scattered by particles suspended in solution. Values of the Z-average mean are close to those obtained by other methods such as

electron microscopy, if the size distribution range is small; however, if the range is large, the larger particles skew the value, resulting in a larger mean than can be measured by other techniques. The Z-averages shown here, about 180 nanometers at 87.6 MPa, are 60 to 80 nanometers larger than those we obtained by electron microscopy; this can be attributed to the large size distribution range (50 to 600 nanometers) of casein micelles. The results shown here indicate that at very high homogenization pressure, a significant reduction of 8 to 10% in the Z-average micelle size occurred. There was no significant difference between the control and the sample homogenized at 14.7 MPa, although the trend was for smaller micelles.

Pictured in Figure 3 are transmission electron photomicrographs of casein micelles before (Picture A) and after (Picture B) homogenization at 87.6 MPa. Careful examination of the photomicrographs shows little apparent difference but some micelles appear to be distorted and there also appears to be a greater number of smaller micelles.

The histogram in Figure 4 shows that homogenization of the casein micelles did result in a slight decrease in micelle size. An increase in the number of micelles smaller than 100 nanometers in diameter was observed and the two histograms are significantly different from one another at  $p < 0.05$ . Average micelle size was smaller for TEM than for PCS, falling in the 118-120 nanometer size class. The number of small micelles may actually be larger than reported because the imaging software used was unable to differentiate among individual micelles clustered together.

Analysis of homogenized (87.6 MPa) samples of casein micelles by gel permeation chromatography on a Sephacryl S-1000 column had some unexpected results. The chromatogram shown in Figure 5 indicates that homogenization at high pressure (87.6 MPa) did affect micelle size. The elution pattern for the unhomogenized control is similar to that reported by Creamer (1984). The chromatogram of the homogenized sample, instead of showing a decrease, showed a shift to the left, indicating a larger particle. This is in direct contrast to results from TEM and PCS. We suggest that during the homogenization step, some micelles are disrupted but reaggregate into larger particles in the presence of the phosphate buffer. We probably erred in not using milk dialysate or Jenness-Koops buffer (Jenness and Koops, 1962) as the eluting medium.

The chromatograms in Figure 6 show the effect of homogenization on the elution pattern of raw skimmed milk. In this case, the pattern shifts to the right, suggesting smaller particles, although the general shape of the major peaks of the pattern remains the same. The small leading peak that disappeared upon homogenization may contain somatic cells, membrane fragments or large protein aggregates broken up by the shearing force used (87.6 MPa).

Still unanswered was the question of whether high pressure homogenization generated sufficient mechanical energy to induce protein-protein interactions. Calculations showed that there was insufficient energy to disrupt disulfide linkages, but distortion of protein secondary and tertiary structures could occur as a result of homogenization. Therefore, the casein micelles were homogenized in the presence of the

whey proteins  $\beta$ -lactoglobulin or  $\alpha$ -lactalbumin. The chromatogram shown on Figure 7 demonstrates the effect of high pressure homogenization on the elution pattern of casein micelles homogenized in the presence of  $\beta$ -lactoglobulin. In this case, the second peak is larger than for casein micelles alone. This apparent increase in size may be due to the amount of  $\beta$ -lactoglobulin added (0.231g/g casein), which was slightly more than that normally found in milk. We believe that the size increase suggested by the chromatogram might be due to the binding of  $\beta$ -lactoglobulin to  $\kappa$ -casein as is known to occur as a result of heat treatment (Parris, et al., 1990). Although every effort was made to minimize temperature rise during homogenization, at 87.6 MPa, "micro hot spots" could have occurred during passage through the valve of the French press that resulted in complex formation.  $\beta$ -lactoglobulin is also known to denature as a result of exposure to high hyperbaric pressure (Hayashi, et al., 1987). We believe that homogenization could distort and denature the protein in such a way as to expose the buried reactive sulfhydryl group in  $\beta$ -lactoglobulin.

We turned to native urea gel electrophoresis to determine if homogenization of casein in the presence of whey proteins had produced a protein-protein interaction. The gel patterns shown in Figure 8 present preliminary evidence for an induced interaction between  $\beta$ -lactoglobulin and  $\kappa$ -casein. Lane 1 is the unhomogenized mixture of casein micelles and  $\beta$ -lactoglobulin; lane 2 is the same sample homogenized at 14.7 MPa; lane 3 is the sample homogenized at 87.6 MPa. It is clear that the middle band is disappearing; this band corresponds to that shown in lane 6,  $\beta$ -lactoglobulin alone. Lane 4 is casein alone and lane 5 is a sample of total milk protein obtained from New Zealand Milk Products, Inc. As might be expected, application of the same procedures to casein micelles homogenized in the presence of  $\alpha$ -lactalbumin showed no evidence of interaction with the casein micelles (data not shown).

We were also interested in the effects of homogenization on the proteolysis and texture of lowfat Mozzarella cheeses prepared from milks homogenized at 17.2 MPa. Homogenization has previously been used to improve the yield of Mozzarella but no studies of the effects of homogenization on textural qualities of lowfat Mozzarella prepared from cows' milk had been reported. Table 1 shows the effects of homogenization on hardness and springiness of Mozzarella cheeses. In all cases, hardness depended on cook temperature; the high cook temperature cheeses contained slightly less moisture in nonfat substance, suggesting less protein hydration and a firmer casein matrix. Hardness was also dependent on homogenization pressure; hardness increased in both homogenized samples, regardless of cook temperature. Homogenization is known to induce milkfat-protein complex formation (Fox, et al., 1960) and also disrupts the fat globule membrane, replacing it, at least in part, with casein submicelles spread out over the fat droplet surface (Keenan, et al., 1988). It has been proposed that this change increases the interfacial tension of the fat globules, making them more rigid; therefore the cheese is harder (Luyten, 1988). Springiness is related to the elasticity of the cheese and homogenization reduced springiness regardless of cook temperature.

Refrigerated storage for 6 wks had the greatest effect on hardness with the greatest decline being seen in the homogenized sample cooked at low temperature.

An important functional property of Mozzarella cheese is its melting quality. Data in Table 2 demonstrate the effect of homogenization on meltability and on  $G'$ , the storage modulus associated with elasticity, that was measured by dynamic oscillatory shear, with a mechanical spectrometer. Homogenization is known to decrease meltability, as adsorption of casein submicelles onto the lipid droplets apparently prevents the melted fat from spreading (Keenan, et al., 1988). Meltability was not significantly decreased by homogenization as shown by these data; however, homogenized samples generally did not melt as well as controls. Meltability improved in all cases during 6 wks of storage under refrigerated conditions.  $G'$  decreased with storage time. With the exception of the lowfat high temperature cook cheeses,  $G'$  was at least 7 dyn/cm<sup>2</sup> when meltability was poor (0.9 or 1.1);  $G'$  was below 7 when meltability was 1.2 or more. Higher values of the storage modulus show a greater ability to store energy while maintaining structural integrity, so samples with high values of the storage modulus would not be expected to melt well.

Proteolysis of the  $\alpha_{s1}$ -casein took place during the 6 wks of refrigerated storage and was primarily responsible for many of the effects noted. Initial cleavage of the  $\alpha_{s1}$ -casein by rennet results in the formation of  $\alpha_{s1}$ -casein, the large peptide remaining after removal of residues 1 to 23. Data in Table 3 show the percentage of  $\alpha_{s1}$ -casein remaining and the amount of  $\alpha_{s1}$ -I-casein formed during 6 wks of refrigerated storage. In all samples cooked at 45.9 C, reduced levels of proteolysis occurred. The moisture in nonfat substance is higher in the low temperature cheeses; scanning electron photomicrographs indicated a 50% greater survival of starter culture bacteria in these cheeses (data not shown). The low cook temperature and high moisture also promoted the survival of chymosin and plasmin activity. A totally unexpected finding was that homogenization at normal pressure (17.2 MPa) apparently retards proteolysis. As can be seen from the data of Table 3, homogenization retarded formation of  $\alpha_{s1}$ -I-casein, even in the low temperature cook cheese, over the 6-wk storage period.

Finally, we compared the effect of homogenization of the cheese milk in the presence or absence of fat on the rheological and proteolytic characteristics of a lowfat high moisture Mozzarella cheese, produced at the low cook temperature of 32.4°C. These milks were homogenized at only 10.3 MPa. In one case, milk was homogenized with 1% fat; in the other, the milk was skimmed, the skim portion homogenized, and recombined with the unhomogenized cream. As listed in Table 4, initial hardness of the homogenized skimmed milk cheese was the same as that of the control cheese whereas the homogenized milk cheese was slightly but not significantly lower; after 6 wks, there was no significant difference in hardness as a result of homogenization. Results for springiness were also unaffected by homogenization, indicating that these properties are influenced by fat content and not fat globule size; the proteolytic breakdown of the casein matrix during storage led to the observed decreases in hardness and springiness with

time. Although values of the storage modulus ( $G'$ ) decreased with time, homogenization had no significant effect. The loss tangents ( $G''/G'$ ) were 5 to 10% higher after 6 wks of storage than in the 1-wk-old samples (data not shown); this increase with refrigerated storage represents the contribution of the viscous modulus, which has been attributed by others to proteolysis (Diefes, et al., 1993).

As shown in Table 5, the breakdown of  $\alpha_{s1}$ -casein to  $\alpha_{s1}$ -I-casein during the 6-wk storage period was observed in the three cheeses. No significant correlations with homogenization at 10.3 MPa were found, although the homogenized skim milk cheeses appeared to undergo less proteolysis of  $\alpha_{s1}$ -casein. Evidently, higher homogenization pressure is required before a significant effect on proteolysis is observed.

Additional investigations have been conducted into this effect which have not yet been published. We have concluded from these studies that homogenization at 17.2 MPa of cheese milks, even for cheeses where the curd is cooked at a low temperature, induces changes that retard the formation of  $\alpha_{s1}$ -I-casein. These changes could be alterations in protein conformation that obscure cleavage sites or alter the local environments of these sites, effectively reducing the enzymatic activity of the rennet. Since preliminary studies suggested that homogenization induced covalent linkages between  $\beta$ -lactoglobulin and  $\kappa$ -casein and similar effects have been observed on heating, the presence of crosslinked  $\beta$ -lactoglobulin and  $\kappa$ -casein fragments might have a retarding effect on proteolysis.

As a result of our studies, we recommend that homogenization of cheese milk for lowfat Mozzarella manufacture be limited to no more pressure than required to recombine ingredients, unless retardation of proteolysis is desired, to extend shelf life, for example. Future studies should aid in clarifying the relationship between homogenization and proteolysis.

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Table 1. Effect of homogenization on moisture-in-nonfat-substance (MNFS), hardness and springiness of Mozzarella cheese as measured by texture profile analysis during storage at 4 C.

SAMPLE	MNFS (%)	HARDNESS (N)		SPRINGINESS (mm)	
		1 wk	6 wk	1 wk	6 wk
0 MPa (CONTROL) (n = 16)					
LOWFAT: 45.9C	59.0	127	119	9.38	8.33
LOWFAT: 32.4 C	60.4	96	90	8.78	7.62
17.2 MPa (n = 8)					
LOWFAT: 45.9 C	60.5	193	194	7.62	7.57
LOWFAT: 32.4 C	63.7	119	74	7.81	7.34

From Tunick, et al., 1993.

Table 2. Effect of homogenization on meltability (Schreiber test) and storage modulus (G', dynamic oscillatory shear) of Mozzarella cheese during storage at 4 C.

SAMPLE	MELTABILITY		G' (N/cm <sup>2</sup> )	
	1 wk	6 wk	1 wk	6 wk
0 MPa (CONTROL) (n = 12)				
LOWFAT: 45.9 C	0.9	1.6	8.65	6.19
LOWFAT: 32.4 C	1.3	1.5	6.28	6.23
17.2 MPa (n = 6)				
LOWFAT: 45.9 C	0.9	1.4	6.31	8.88
LOWFAT: 32.4 C	1.1	1.3	7.00	4.66

From Tunick, et al., 1993.

Table 3. Effect of homogenization on proteolysis of Mozzarella cheese during storage at 4 C.

SAMPLE	$\alpha_{s1}$ -CASEIN (%)		$\alpha_{s1}$ -I-CASEIN (%)	
	1 wk	6 wk	3 wk	6 wk
	0 MPa (n = 4)			
LOWFAT: 45.9 C	46.8	46.6	4.4	6.0
LOWFAT: 32.4 C	45.2	24.0	8.9	22.4
	17.2 MPa (n = 2)			
LOWFAT: 45.9 C	43.8	42.3	6.1	10.0
LOWFAT: 32.4 C	57.3	43.7	5.7	4.2

Data obtained from densitometry measurements of SDS-PAGE on 20% homogeneous gels. Percentages based on total amounts of  $\alpha$ - and  $\beta$ -caseins.

From Tunick, et al., 1993.

Table 4. Effect of milk treatment on values of hardness, springiness and storage modulus (G') of Mozzarella cheeses during storage at 4 C. (n = 3). (Homogenization 10.3 MPa). (Cook temperature 32.4 C).

SAMPLE	HARDNESS (N)		SPRINGINESS (mm)		G' (N/cm <sup>2</sup> )	
	1 wk	6 wk	1 wk	6 wk	1 wk	6 wk
	NONHOMOGENIZED					
LOWFAT:	105	83	8.59	7.66	6.40	6.85
	HOMOGENIZED					
LOWFAT:	95	70	8.85	7.15	7.18	4.64
	HOMOGENIZED SKIMMILK					
LOWFAT:	109	77	8.55	7.10	7.65	6.50

From Tunick, et al., 1995.

Table 5. Effect of milk treatment on values of  $\alpha_{s1}$ -casein and  $\alpha_{s1}$ -I-casein of Mozzarella cheeses during storage at 4 C. (n = 3). (Homogenization 10.3 MPa). (Cook temperature 32.4 C).

SAMPLE	$\alpha_{s1}$ -CASEIN (%)		$\alpha_{s1}$ -I-CASEIN (%)	
	1 wk	6 wk	1 wk	6 wk
	NONHOMOGENIZED			
LOWFAT:	44.0	28.0	3.2	19.5
	HOMOGENIZED			
LOWFAT:	43.8	27.9	1.2	22.5
	HOMOGENIZED SKIMMILK			
LOWFAT:	42.4	33.7	0	16.2

PERCENTAGES BASED ON TOTAL OF  $\alpha$ - AND  $\beta$ -CASEINS.

From Tunick, et al., 1995.

Figure 1. Make procedure for Mozzarella cheeses

	MILK Standardize Pasteurize Homogenize at 63C Cool
ADD STARTER	33 C
ADD RENNET (SETTING)	pH > 0.1; Hold 35 min.
CUTTING	Rest 15 min; Stir 15 min.
COOK	Low Temperature: Hold 45-60 min, 33 C. High Temperature: Raise temperature over 60 min to 45.9 C.
DRAIN (DIPPING)	Low Temperature: Drain 1/2 whey, Hold 30-45 min, drain remainder of whey. High Temperature: Drain 1/2 whey, Hold 45 min at 45.9 C, drain remainder of whey.
RINSE	Low temperature: Cover with 33 C water, drain; rinse with 33 C water, drain. High temperature: Rinse with 40 C water, drain; cover with 40 C water, Hold 30 min, drain.
CHEDDAR	Form slabs; Turn until pH = 5.2-5.3.
ICE CURD	16 hr.
HAND STRETCH	Stretch 7 min in 70 - 80 C water.
PACK	8-oz Cups; Cool 30 min.
BRINE	2 hr; Saturated NaCl (23%); Drain.
STORE	Vacuum Pack; Store at 4 C.

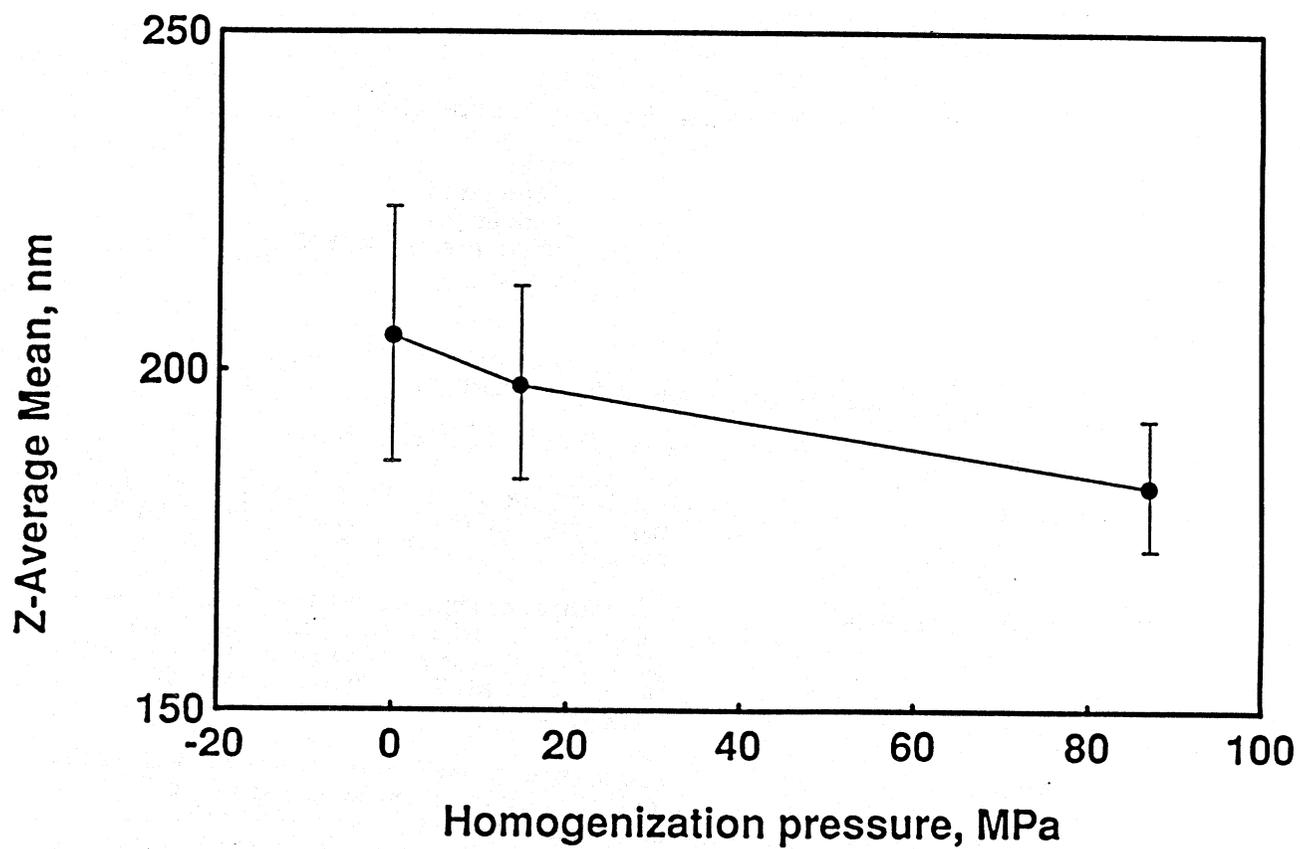


Figure 2. Effect of homogenization on the Z-average mean of casein micelles for 0, 14.6 and 87.6 MPa homogenization pressures measured at 90 degrees by photon correlation spectroscopy.

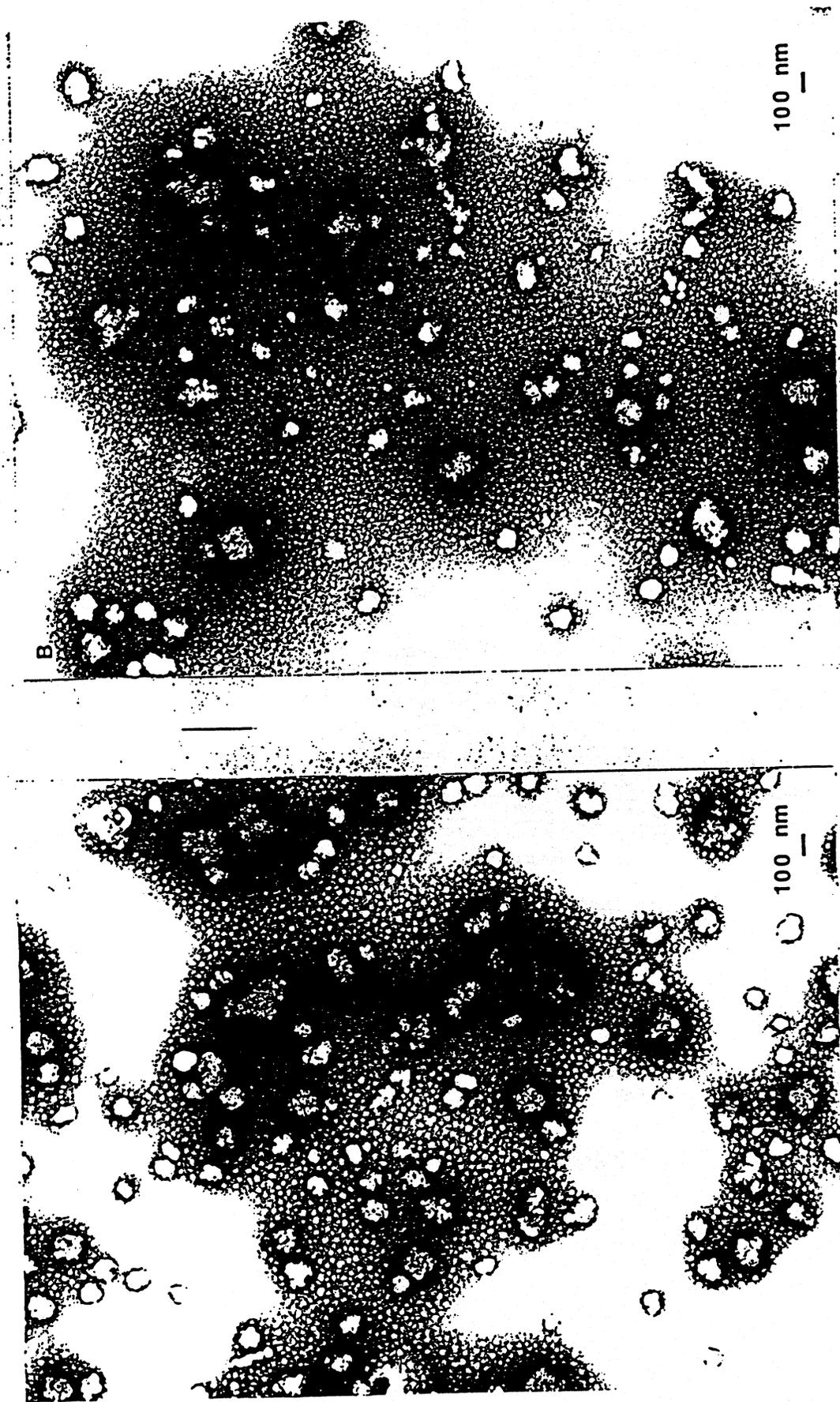


Figure 3. Typical photomicrographs of negatively stained (2% uranyl acetate solution) suspensions of casein micelles, adsorbed to Formvar/carbon coated grids, used to determine size distribution. Magnification is 55,600X. A. Control (Unhomogenized). B. Homogenized 87.6 MPA.

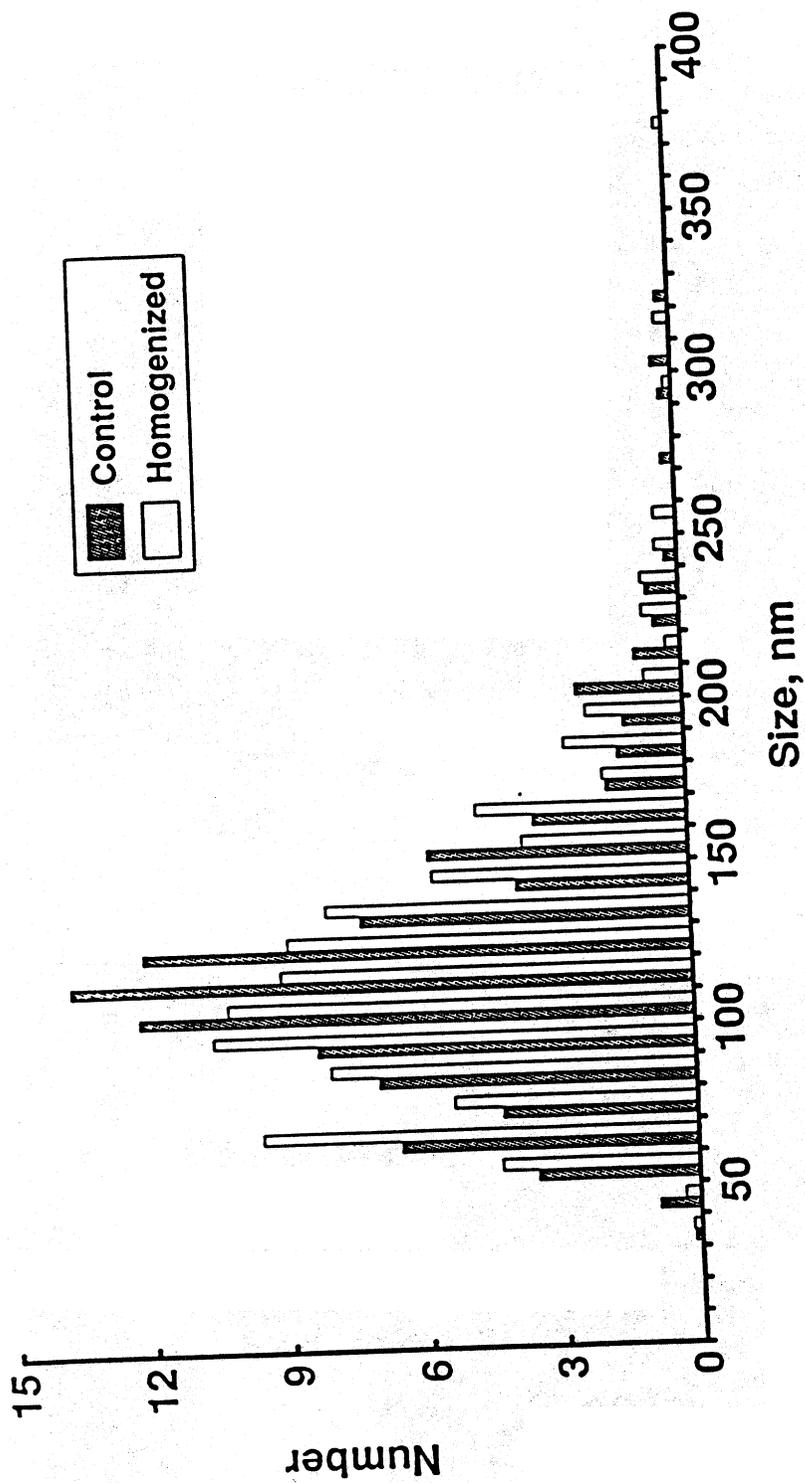


Figure 4. Effect of homogenization at 87.6 MPa on size distribution of casein micelles as measured by transmission electron microscopy.

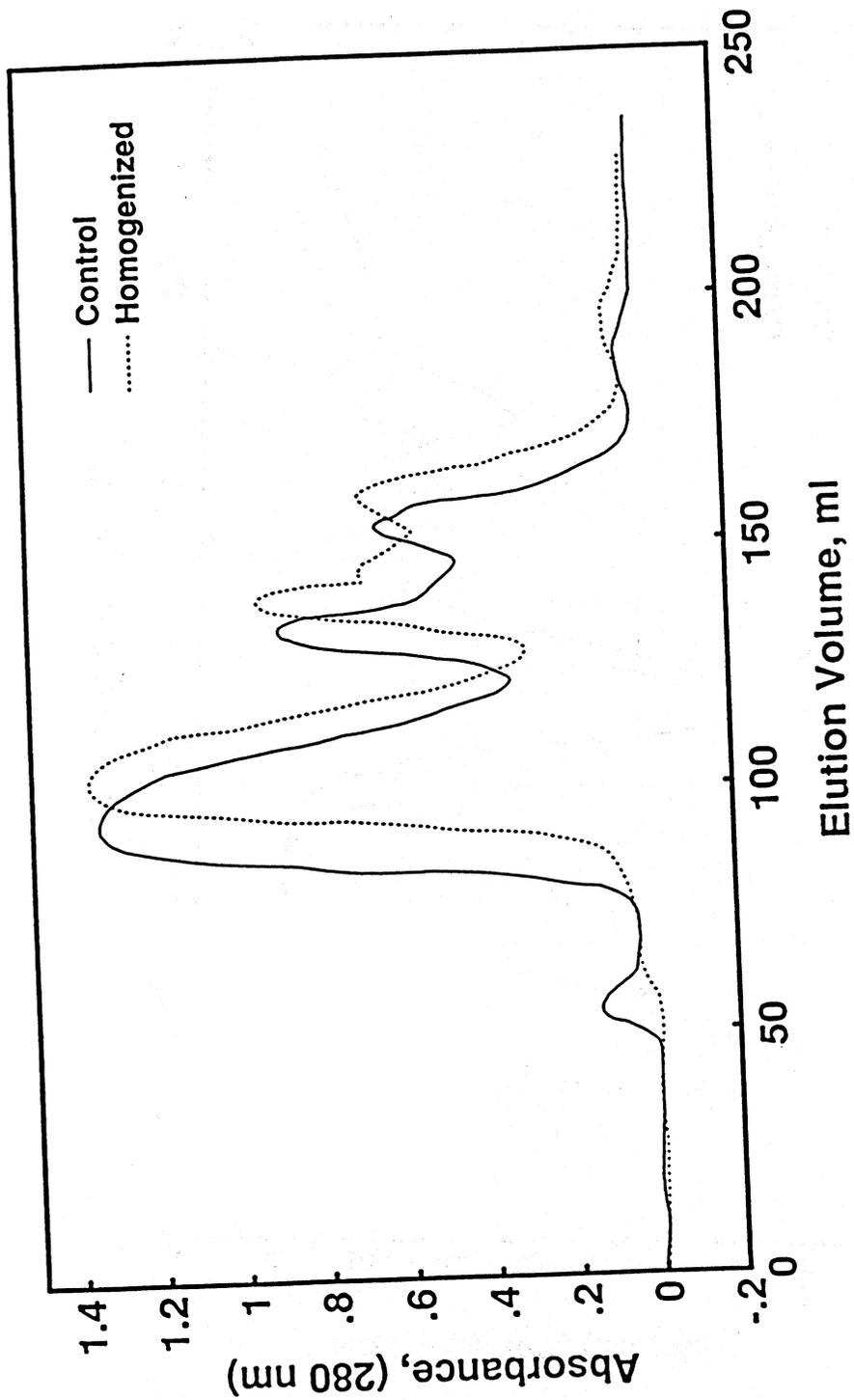


Figure 5. Gel permeation of casein micelles on a Sephacryl S-1000 column at 40 mL/hr of 0.02 M NaPO<sub>4</sub>, pH 6.8 buffer before and after homogenization at 87.6 MPa.

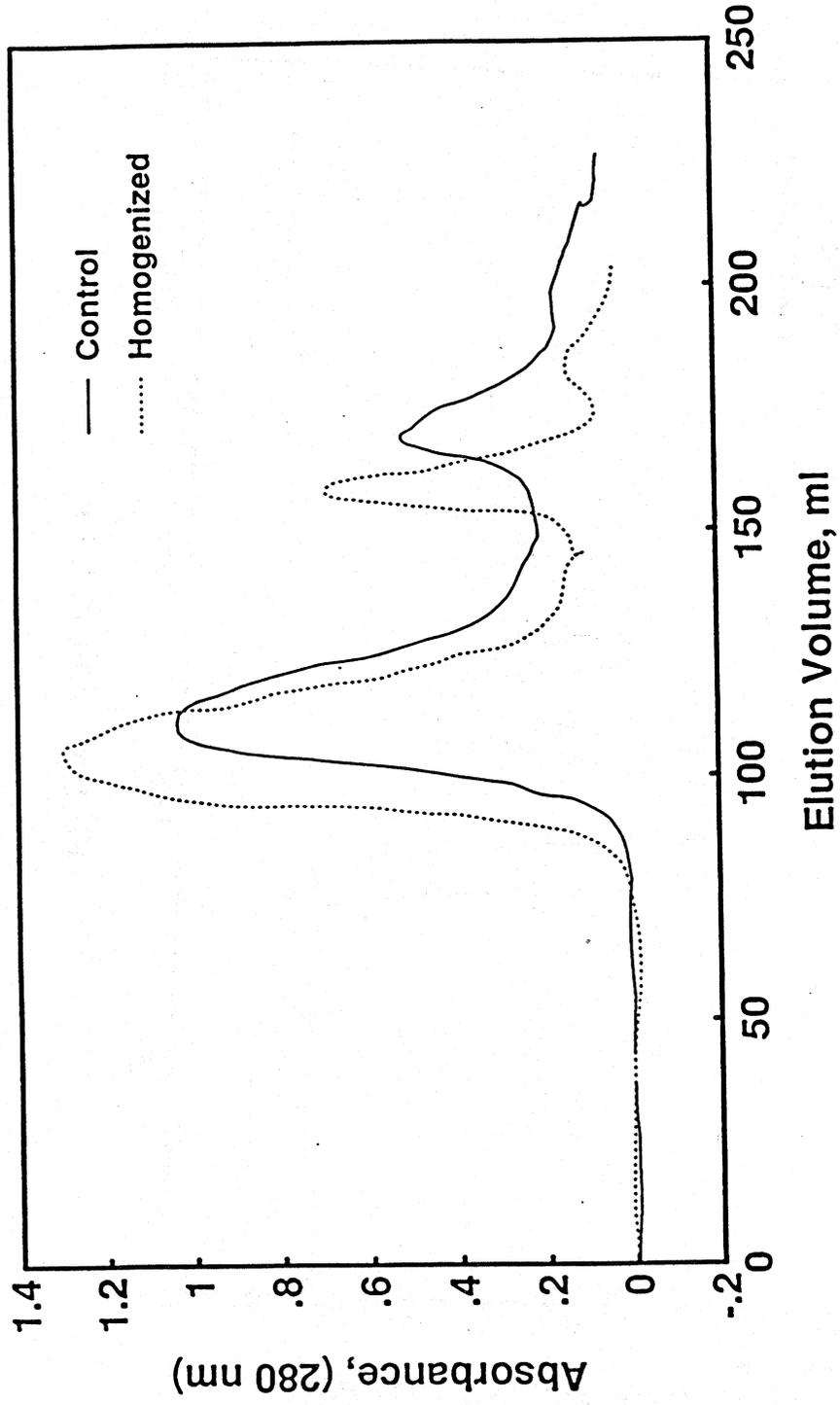


Figure 6. Gel permeation of skim milk on a Sephacryl S-1000 column at 40 mL/h of 0.02 M NaPO<sub>4</sub>, pH 6.8 buffer before and after homogenization at 87.6 MPa.

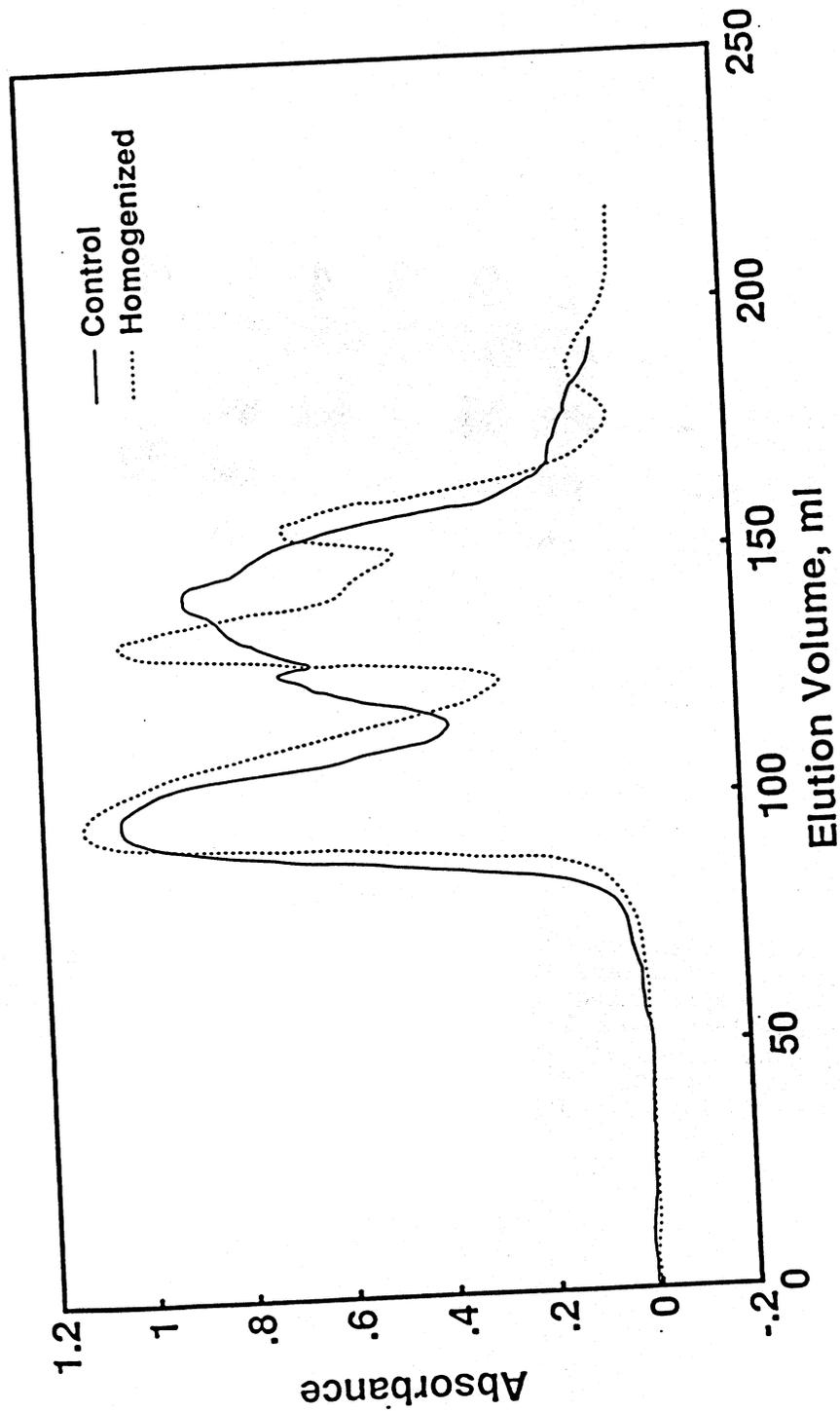


Figure 7. Gel permeation of casein micelles and  $\beta$ -lactoglobulin before and after homogenization at 87.6 MPa on a Sephacryl 1000 column at 40 mL/h of 0.02 M  $\text{NaPO}_4$ , pH 6.8 buffer.

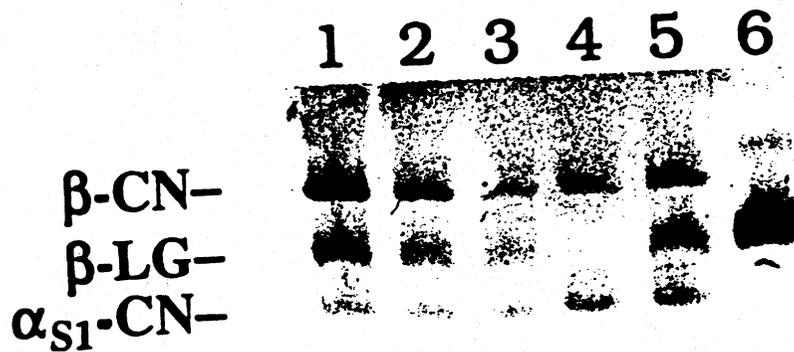


Figure 8. Electropherogram; Pharmacia PhastSystem, 8 to 25% gradient. Gels were stained with Coomassie blue, destained and restained with silver stain. Lane 1: Casein +  $\beta$ -lactoglobulin, unhomogenized; Lane 2: Casein +  $\beta$ -lactoglobulin, homogenized 14.7 MPa; Lane 3: Casein +  $\beta$ -lactoglobulin, homogenized 87.6 MPa; Lane 4: Whole Casein; Lane 5: Total milk protein; Lane 6:  $\beta$ -lactoglobulin.