

## Microporous Ultrafiltration of Skim Milk

J.H. WOYCHIK, P. COOKE, and D. LU

### ABSTRACT

Membranes with porosities of 100 and 200 nm were used to obtain a 4:1 milk volume reduction. Average micelle diameters determined from electron micrographs were 46 nm (permeate) and 52 nm (permeate) for the 100-nm-pore fractions and 46 and 55 nm for the 200-nm-pore fractions. The calculated average micellar volumes of the retentate fractions were about twice those of the corresponding permeate fractions. Casein-whey ratios were 0.7-0.9 in the permeates and 5.0-7.7 in the retentates. Higher  $\alpha_{s2}$ - and lower  $\beta$ -casein contents were found in the permeate micelles than in the retentates.

Key Words: milk, ultrafiltration, microporous, micelle, permeate

### INTRODUCTION

CASEIN in milk is in the form of stable colloidal micelles having a size range from 30 nm in diameter to greater than 600 nm (Donnelly et al., 1984). Micelle fractionation according to size has been achieved by permeation chromatography using controlled-pore glass columns (McGann et al., 1979; Ekstrand et al., 1981; Donnelly et al., 1984) and by ultracentrifugation (Davies and Law, 1983) for the study of composition and physical properties of various sized micelles. Casein composition has been reported to vary with micelle size (Rose and Colvin, 1966; McGann et al., 1980) in agreement with reports that a higher  $\kappa$ -casein content accompanies a decrease in micellar size. However, considerable variation was reported (McGann et al., 1980) for the content of the remaining caseins in micelles of different size ranges. Donnelly et al. (1984) reported that  $\kappa$ -casein content increased with decreasing micellar size, while  $\alpha_s$ - and  $\beta$ -casein decreased which was the opposite of their previous report (McGann et al., 1980). Davies and Law (1983) reported that the  $\alpha_{s1}$ -casein content did not change with micelle size. Yoshikawa et al. (1982) reported increasing  $\beta$ -casein content as micelle size decreased in fractions obtained by controlled-pore glass chromatography. These inconsistencies may be related to variations in fractionation methods, separation temperatures, and to methods of casein analysis.

Membrane technologies such as reverse osmosis and ultrafiltration are commonly used by the dairy industry for the concentration of whey and the concentration of milk prior to cheesemaking. The advent of microporous membranes with porosities in the range of 100 nm to 400 nm raises the possibility of using such membranes to effect micellar fractionations on an industrial scale. Our study was undertaken to evaluate the potential of microporous ultrafiltration to produce permeate and retentate fractions with different mean micellar sizes, possibly varying casein composition and altered casein/whey protein ratios.

### MATERIALS & METHODS

#### Materials

Raw pooled milk was obtained from a local herd and skimmed and pasteurized (30 min at 145°F) in the pilot plant.

#### Methods

**Composition.** Moisture was determined as the weight loss after heating a sample of about 2 g in a porcelain crucible for 75 min in an oven at 130°C. Ash was determined by combustion of dried samples overnight in a muffle furnace at 500°C. Lactose was determined by the spectrophotometric procedure of Miller and Burton (1959). Fat was determined by extracting 1-g samples 3X with chloroform:methanol (2:1 v/v) for 1 hr on a shaker. Supernatants were collected after centrifugation at 10,000 rpm for 30 min. Supernatants were combined and filtered through Whatman #1 filter paper. The combined supernatants were taken to dryness by roto-evaporation and the residue dissolved in methylene chloride. The methylene chloride was evaporated in a weighed beaker under a stream of nitrogen.

**Microfiltration.** Skim milk was warmed to room temperature and allowed to equilibrate for 2 hr prior to microfiltration. Filtration was done using a Minitan Ultrafiltration System (Millipore) with 8 membrane plates having a total surface area of 480 cm<sup>2</sup> at 800 mL/min in a recirculating retentate mode until the initial milk volume was concentrated 4:1. Membranes (polyvinylidene difluoride) with 100 and 200 nm porosities were used to prepare permeate and retentate fractions. Permeates were concentrated 10:1 using a 10,000 MW cut-off membrane prior to lyophilization. Concentrates were lyophilized directly.

**Electron microscopy.** Micelles were sedimented using a Beckman Model L8-70 ultracentrifuge at 25,000 rpm for 30 min using the Ti-50 rotor at 25°C. The sedimented micelles were resuspended in ultrafiltrate at a concentration of 5% and chemically fixed by the addition of glutaraldehyde to 1% (v/v). Aliquots (10  $\mu$ L) of the liquid suspension were encased in 2% agarose gels, treated with 1% osmium tetroxide in 0.1M sodium cacodylate buffer (pH 7.2), dehydrated in a graded series of ethanol solutions, and embedded in an epoxy resin mixture. Thin sections (80 nm) were stained with solutions of 2% uranyl acetate and lead citrate. Photographic images of micelle profiles were recorded using a transmission electron microscope at an instrumental magnification of 20,500 X, measured from an average line spacing (463.5 nm) of a carbon replica grating. The distributions of circular diameters of micelle profiles in samples of photographic negatives were measured and tabulated from binary images using a digital image analyzer (Dapple Systems, Sunnyvale, CA). Micelle diameters of 25 to 200 nm were counted in 20 bins with a size increment of 8.75 nm/per bin. Data were compared as number average micelle diameters with standard deviation calculated from 5-8 ran-

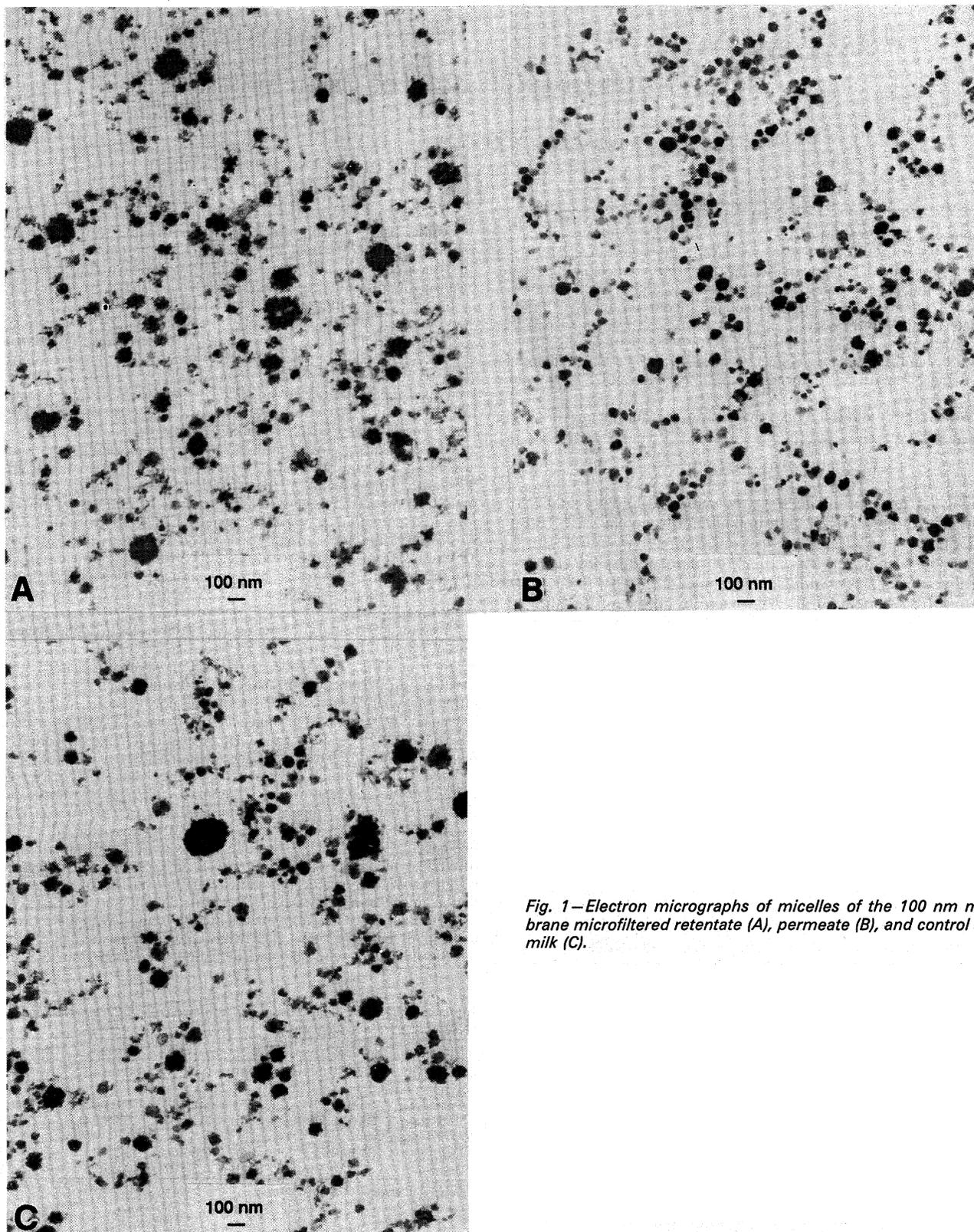
Table 1—Compositional analysis (%) of dried microfiltered fractions and comparison with original milk

Product	Protein	Fat	H <sub>2</sub> O	Ash	Lactose
Skim milk <sup>a</sup>	35.0 (1.0)	0.9 (.03)	6.7 (.10)	8.5 (.13)	51.6 (.58)
100-nm membrane					
Permeate	35.0 (0.7)	0.4 (.06)	5.2 (.14)	5.8 (.07)	54.0 (.71)
Retentate	39.3 (0.3)	1.1 (.04)	3.9 (.07)	3.9 (.14)	49.0 (.71)
200-nm membrane					
Permeate	31.5 (0.7)	0.5 (.04)	6.5 (.00)	6.2 (.14)	55.0 (.71)
Retentate	38.0 (0.7)	0.9 (.01)	4.6 (.07)	7.6 (.28)	49.0 (.71)

<sup>a</sup> Lyophilized sample of original skim milk.

Table 2—Casein-whey protein distribution in microfiltered fractions compared to original skim milk<sup>a</sup>

Fraction	Casein (%)	Whey (%)	Casein/Whey
Skim milk	84.9 (0.5)	15.1 (0.5)	5.62
100-nm permeate	43.1 (1.9)	56.9 (1.6)	0.75
Retentate	82.7 (1.3)	17.3 (1.3)	5.06
200-nm permeate	47.6 (2.0)	52.4 (1.9)	0.90
Retentate	88.5 (0.6)	11.5 (0.6)	7.70



*Fig. 1—Electron micrographs of micelles of the 100 nm membrane microfiltered retentate (A), permeate (B), and control skim milk (C).*

domly selected micrographs. Relative volume fraction was calculated according to McGann et al. (1980).

**Electrophoretic analysis.** The various fractions and controls were examined by sodium dodecyl sulfate electrophoresis in the presence of 2-mercaptoethanol using the "PhastSystem"™ (Pharmacia, Piscataway, NJ) with homogeneous 20% acrylamide gels. Densitometry of the electrophoretic patterns was done with a Biorad Model 620 video densitometer to determine the casein-whey protein ratios and casein component composition in the permeate and retentate fractions.

## RESULTS & DISCUSSION

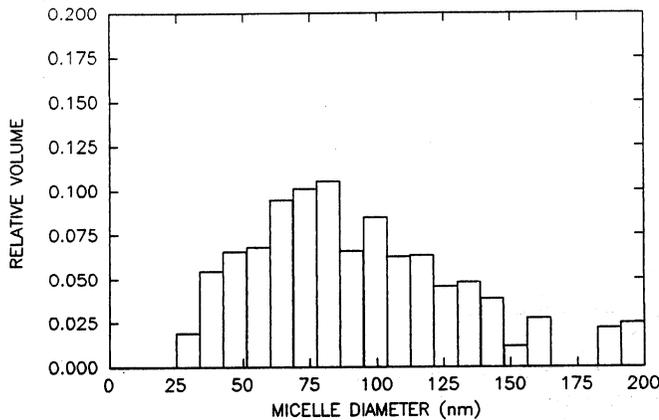
### Microfiltration of skim milk

Microfiltration of skim milk with either the 100 nm or 200 nm membranes proceeded normally while operating in a recirculating retentate mode until a 4:1 concentration of the original milk volume was achieved. Significant changes in both flux rate and operating backpressure did not occur until the

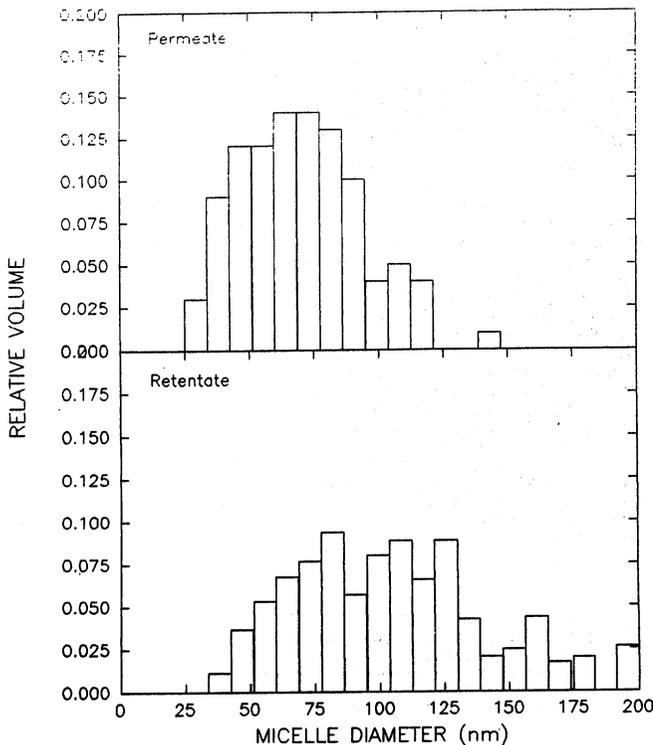
**Table 3—Average micelle size and volume in microfiltered fractions and comparison with skim milk<sup>a</sup>**

	Diameter (nm)	Volume (nm <sup>3</sup> × 10 <sup>3</sup> )	No. particles
Skim milk	50.2 (1.1) A	152	1035
100-nm permeate	45.7 (3.4) B	90	1248
Retentate	52.1 (0.7) C	173	868
200-nm permeate	46.4 (3.0) B	110	1028
Retentate	55.2 (4.2) C	191	932

<sup>a</sup> Values with different letters are significantly different by the Independent Student T-Test at the 0.05 level.



**Fig. 2—Relative volume fraction of skim milk micelles.**



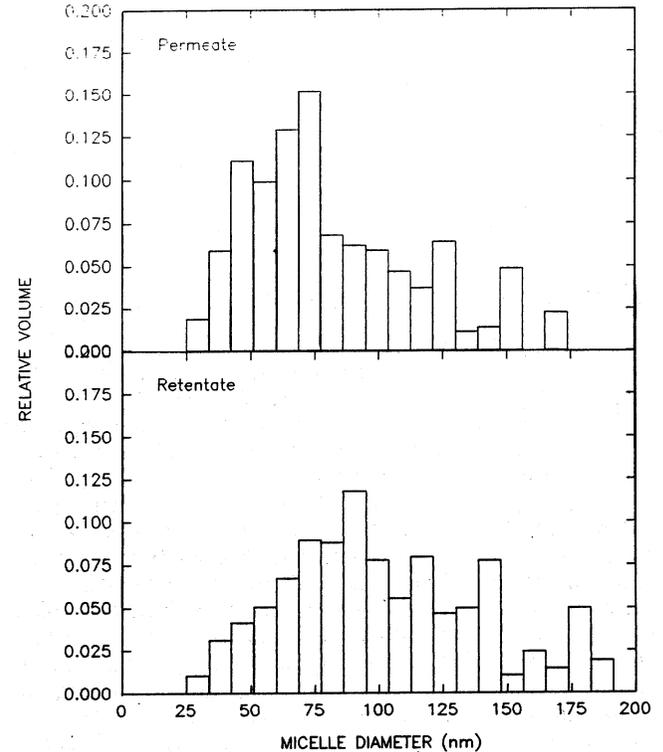
**Fig. 3—Relative volume fraction of permeate and retentate micelles fractionated using a 100 nm membrane.**

level of concentration reached 6:1. Two L of milk could be concentrated 4:1 in about 3 hr using either the 100 or 200 nm membranes (480 cm<sup>2</sup> surface area) using a tangential flow rate of 800 mL/min. The compositional analysis of the dried microfiltered fractions is presented in Table 1 and compared with lyophilized original milk. Protein compositions of the fractions were 32 to 39%; fat content was significantly higher in retentate fractions while moisture, ash and lactose content were

**Table 4—Micellar casein component distribution (%) in microfiltered fractions<sup>a</sup>**

Fraction	$\alpha_{s-2}$	$\alpha_{s-1}$	$\beta$	$\kappa$
100-nm permeate	13.3 (0.9)A	40.7 (1.6)A	34.2 (0.8)C	11.0 (0.8)A
Retentate	8.8 (0.8)C	39.2 (1.0)A	40.2 (0.6)A	11.0 (0.3)A
200-nm permeate	11.8 (0.5)B	39.2 (0.2)A	37.7 (0.3)B	11.1 (0.4)A
Retentate	8.9 (0.6)C	39.0 (0.6)A	39.3 (0.1)A	11.2 (0.5)A

<sup>a</sup> Means calculated from 3-4 analyses. Values with different letters are significantly different by the Independent Student T-Test at 0.05 level.



**Fig. 4—Relative volume fraction of permeate and retentate micelles fractionated using a 200 nm membrane.**

essentially comparable. The casein and whey protein compositions of the permeate and retentate fractions (Table 2) indicated permeate casein concentrations about half those of the retentates. Casein/whey ratios were 0.75-0.90 in the 100 and 200 nm permeates, respectively, and 5.0 and 7.7 in the retentates. This is in comparison to a ratio of 5.6 in original skim milk.

### Micelle size

Electron micrographs of micelles of control skim milk and of permeate and retentate fractions were obtained using the 100 nm membrane (Fig. 1). Evidently, from the micrographs the permeate fraction consisted primarily of smaller micelles (< 100 nm) (Fig. 1, B) while in the retentate (Fig. 1, A) micelles with diameters greater than 100 nm were readily evident. Many smaller micelles were still present in the retentate fractions. Extensive diafiltration would probably be required to further reduce the population of smaller micelles. This would be especially true if significant micellar dissociation (McGann et al., 1980) occurred during or following the microfiltration process.

The average micelle diameters and volumes shown in Table 3 indicate differences in values for the fractions compared to those of skim milk micelles. The average micellar volumes for the permeate fractions were about half those of the retentates.

A comparison of the relative volume of control skim milk micelles (Fig. 2) with that of micelles comprising the permeate and retentate fractions (Fig. 3 and 4) reflects the expected volume differences between permeate and retentate fractions. A more effective size separation was obtained with the 100 nm membrane (Fig. 3) with relatively small numbers of micelles larger than 100 nm present in contrast to permeate obtained using the 200 nm membrane (Fig. 4). Both retentate fractions still contained large numbers of micelles with diameters less than 100 nm indicating that microfiltration would need to be extended past the 4:1 concentration level, together with possible use of diafiltration, to effect a greater removal of smaller micelles from the retentate fractions. However, the possibility of rearrangements in the size of micelles in retentates or permeates should also be considered (McGann et al., 1980) since the skim milk system is in dynamic equilibrium, dependent upon a variety of environmental factors.

#### Micellar casein composition

Results of the densitometric analysis of the micellar casein component composition of the permeate and retentate fractions are given in Table 4. The  $\alpha_{s1}$ - and  $\kappa$ -casein contents were comparable in all of the fractions, however, the  $\alpha_{s2}$ -casein content was significantly higher and the  $\beta$ -casein significantly lower in both permeates. The lower  $\beta$ -casein content in the permeate fractions (smaller micelles) was in agreement with the report of Donnelly et al. (1984). However, the magnitude of this difference between large and small micelles was much greater in our study. Since the permeate fractions only represent an enrichment of smaller micelles, comparison of our

compositional data with those for narrowly defined micelle size fractions of Donnelly et al. (1984) is necessarily limited.

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