Lipid and Chloroform-Methanol-Extractable Protein Content of Casein Micelles and Other Milk Fractions

J. Cerbulis

Micelles from unhomogenized milk contained 0.11 to 0.63% lipids but micelles from homogenized milk contained 5.13% lipids. The unsedimented supernatant casein, obtained by acid precipitation, from fresh whole milk contained 12.40% lipids and from homogenized milk 31.21% lipids. The acid whey from the ultracentrifuge experiments from both fresh milk and homogenized milk contained considerably more lipids, particularly phospholipids, than whey obtained from uncentrifuged skim milk. None of the phospholipids were free. The micellar casein fraction obtained by ultracentrifugation was approximately 93% of the total casein in fresh cow's milk, 77% in commercial homogenized pasteurized cow's milk, and 81% in goat's milk.

The casein in milk is present in two distinct states: in the micellar form, which can be removed by ultracentrifugation, and in smaller polymeric units (soluble casein), which does not centrifuge out with the micelles (von Hippel and Waugh, 1955).

Previous studies (Cerbulis, 1967; Cerbulis and Custer, 1967; Cerbulis and Zittle, 1965) determined the lipid content and composition of lipids of acid-precipitated casein and other milk fractions. It was desirable to investigate the lipids of casein micelles and other fractions of milk in relation to the ultracentrifugation of skim milk.

Lipids were determined in casein micelles, in supernatant casein, and in whey obtained in these experiments. Ultracentrifugation was performed on natural skim milk (without added $CaCl_2$) in order to understand better the constitution of the casein micelles and the supernatant casein (nonmicellar casein). Then the supernatant casein was obtained by precipitation at pH 4.6. The soluble casein fraction (Cerbulis and Custer, 1967) was obtained from the micelles and from the acid-precipitated supernatant casein with the chloroform-methanol (2 to 1) solvent system. This soluble casein fraction (Cerbulis and Custer, 1967) is here called chloroform-methanol-extractable proteins (CMEP). This CMEP fraction contained γ-casein, temperature-sensitive casein, and six or more other casein components. This CMEP fraction (Cerbulis and Custer, 1967) could not be identical in composition with the soluble casein described by von Hippel and Waugh (1955), since that casein fraction contained α-casein and β-casein. These studies were performed with samples of fresh cow and goat milk and with commercial homogenized, pasteurized cow milk. The nature of the lipid fraction was determined by thin-layer chromatography (TLC). These data were compared with data obtained in previous studies. The protein fractions were studied by polyacrylamide gel electrophoresis.

Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa. 19118

EXPERIMENTAL

Milk. Fresh pooled cow's milk was cooled immediately to 2° C. on the farm, then transported to the laboratory and separated. Commercial homogenized, pasteurized, vitamin D–enriched milk was obtained from a local distributor. (The milk was homogenized at 79° C., applying 2500-p.s.i. pressure, then pasteurized at 62° C. for 30 minutes).

Preparation of Milk Fractions. The complete procedure is shown in Figure 1. The separation of the whole milk was performed at 33° C. by a laboratory separator (De Laval Model 100). The cream (cream I) was freeze-dried and saved. The skim milk was centrifuged in a Beckman Model L4 ultracentrifuge equipped with a No. 30 rotor for 60 minutes at 25° C. and 30,000 r.p.m. Three layers were observed in the centrifuge tubes: top layer (cream II), compact and relatively very small from skim milk samples but large from commercial homogenized milk; supernatant; and a gel-like micelle pellet at the bottom.

The cream II layer was removed carefully with a spatula and wiped away from the supernatant completely to avoid the contamination of supernatant with cream particles. The cream layer of skim milk samples was negligible and was not analyzed but the commercial homogenized milk sample was analyzed. The supernatant fraction was removed by decantation. Then the walls of the tubes and the surface of the pellet were wiped with absorbent tissue to remove the fatty layer and the sediment was removed with a spatula. Cream II and micelle pellets were freeze-dried without additional treatment (Cerbulis and Ard, 1967).

The supernatant fraction was adjusted to pH 4.6 with 1*N* HCl and filtered. The precipitate, called supernatant casein, was freeze-dried without additional treatment. The whey fraction was dialyzed for 3 days and then the nondialyzable fraction was freeze-dried.

The whole casein was precipitated from skim milk, from commercial homogenized, pasteurized whole milk, and from fresh whole milk by adjusting to pH 4.6 with HCl and the precipitate was recovered by filtration. The whey fraction was dialyzed, then freeze-dried. The cream

I and whole casein fractions were freeze-dried without additional treatment.

Lipid Extraction and Separation. The lipids were extracted with petroleum ether and with chloroform-methanol (2 to 1, v./v.) as described previously (Cerbulis, 1967). The extraction scheme is shown in Figure 2. All lipid extracts were analyzed by TLC (Cerbulis, 1967). The developing solvents were petroleum ether–diethyl ether–acetic acid, 90:10:1 (v./v.) for neutral lipids and chloroform–methanol–water–acetic acid, 65:25:4:2 (v./v.) for phospholipids. The lipid fractions were separated by the silicic acid method described by Rouser *et al.* (1967). The recovery of lipids was 96 to 102.5%. The CMEP fraction was detected as described previously (Cerbulis and Custer, 1967).

Polyacrylamide Gel Electrophoresis was described previously (Cerbulis and Custer, 1967).

RESULTS AND DISCUSSION

Micelle Content in Skim Milk. The micelle content of various fresh milks and of commercial milk is shown in Table I. Nonmicelle casein (supernatant casein) comprised 6.7% of the total casein in the fresh Holstein milk and 22% in the commercial homogenized pasteurized milk. Goat milk contained 19% of nonmicelle casein.

Liquid Content in Various Casein Fractions. Table II shows the lipid distribution in freeze-dried milk fractions. Micelles from fresh unhomogenized milk of cow and goat contained 0.11 to 0.63% lipids, and micelles from homogenized pasteurized milk contained 5.13% lipids. The unsedimented supernatant casein (obtained by acid precipitation) from fresh cow and goat skim milk contained 12.4 and 7.37% lipids, respectively; that from commercial homogenized cow milk contained 31.21% lipids, but acid-precipitated whole casein from commercial homogenized

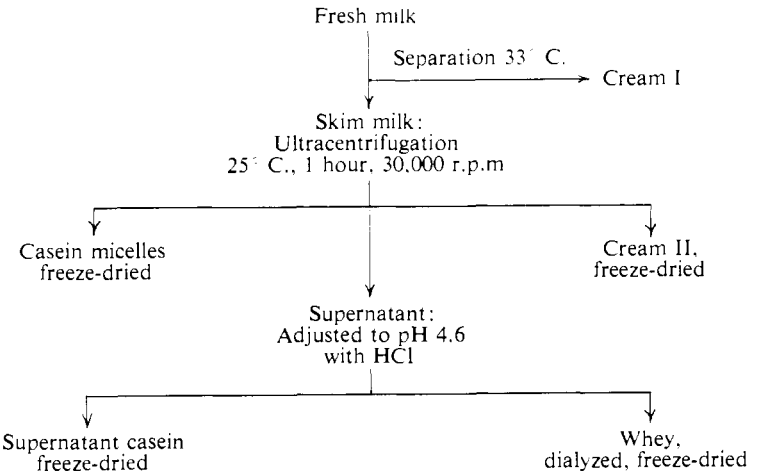

Figure 1. Fractionation of Milk

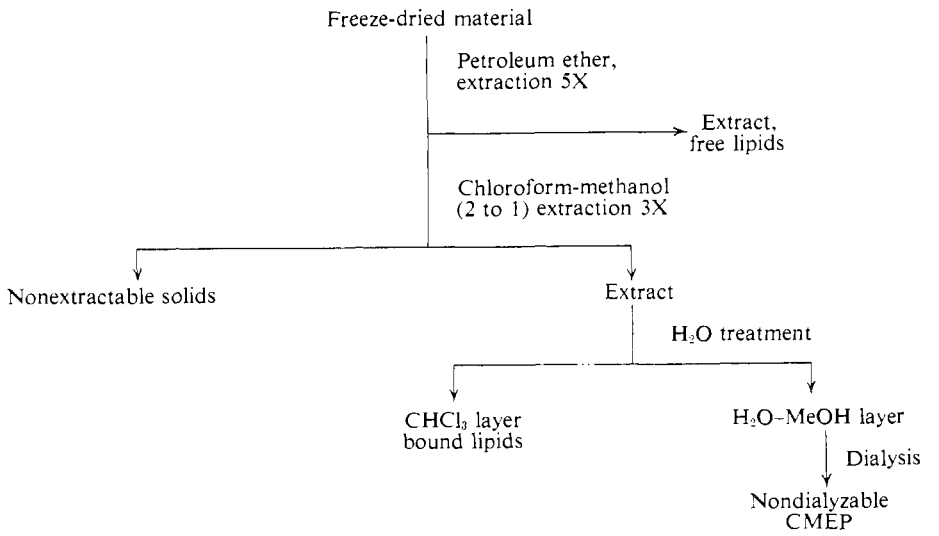

Figure 2. Extraction of Lipids

milk contained 32.67% lipids. Acid-precipitated whole casein from fresh cow milk contained only 1.52% lipids (Cerbulis, 1967). Stevenson and Bacharach (1937) had reported 1 to 2.5% lipids in acid-precipitated casein.

These data suggest that the high lipid content of casein aggregates resulting from homogenization limits their participation in the formation of casein micelles, or the aggregates are not sedimented by the procedure used because of the lower density resulting from the associated lipid.

Chloroform-Methanol-Extractable Proteins (CMEP). The CMEP (Figure 2) content of micelles was 1.60 to 2.25% from fresh milk of Guernsey, Jersey, and Holstein breeds (milk from Brown Swiss cows contained 0.9%) (Table II). This range of values is in agreement with previous findings (Cerbulis and Custer, 1967) on acid-precipitated whole casein in which 1.9 to 2.0% of CMEP was found. The CMEP content of supernatant casein and of micelles from homogenized milk was only 0.10 to 0.52%. Also acid-precipitated whole casein prepared from homogenized milk contained only 0.55% CMEP. These data suggest that the CMEP was associated mostly with the micelles in fresh milk but became nonextractable in homogenized pasteurized milk.

Influence of Homogenization. Homogenization and pasteurization changed the properties of each casein fraction considerably. The CMEP content was decreased from 2% of the total casein in fresh milk (Cerbulis and Custer, 1967) to 0.52 to 0.55% in homogenized pasteurized milk. The lipid content was increased from 1.52% in fresh milk casein (Cerbulis, 1967) to 32.67% in homogenized milk casein.

Bound lipid content of homogenized milk was 25.3%. Bound lipid content of fresh milk varied from 3.3 to 27.9% (Table III).

The homogenization and pasteurization of milk probably redistributed the milk lipids, produced new lipid-protein complexes, and released new extractable phospholipids in milk. Greenbank and Pallansch (1961) have noted that the migration of phospholipids from the fat globule surface to the aqueous phase occurs to some extent whenever milk undergoes mechanical agitation. Fox *et al.* (1960) and Jackson and Brunner (1960) observed the formation of fat-protein complexes on homogenization.

Lipid Content of Whey Fractions. Whey fractions from micelle preparation experiments and from homogenized milk, from which casein was precipitated at the isoelectric point, contained a considerably larger amount of phospholipids than the whey fraction of whole milk from which casein was precipitated at the isoelectric point (Table II).

In milk, particularly in homogenized milk, lipid fractions are present which are not removed by the separator but are partially removed by ultracentrifugation, producing cream II fraction and leaving a considerable amount of lipids in the supernatant (supernatant casein and whey fractions).

Table I. Distribution of Micelles, Supernatant Casein, and Whey Proteins

(Grams per liter of milk)

Milk	Micelles	Supernatant Casein	Whey[a]
Jersey	32.2	...	...
Guernsey	31.9	...	...
Brown Swiss	29.4	...	...
Holstein	27.4	...	...
Holstein	27.8	2.0	3.1
Goat	26.7	6.3	5.2
Commercial homogenized pasteurized cow's milk	25.6	7.2	3.1

[a] Dialyzed.

Table II. Lipid and CMEP Content of Milk Fractions

(Grams per 100 grams of freeze-dried solids)

Milk Fraction	Total Lipids	Neutral Lipids		P-Lipids, Bound Only	CMEP
		Free	Bound		
Guernsey					
Micelles	0.46	0.03	0.29	0.14	1.60
Jersey					
Micelles	0.11	0.01	0.07	0.03	1.90
Brown Swiss					
Micelles	0.27	0.04	0.10	0.13	0.98
Holstein					
Micelles	0.63	0.19	0.16	0.28	2.25
Supernatant casein	12.40	10.10	0.92	1.38	0.10
Whey[a]	2.59	0.39	1.04	1.16	0.06
Goat					
Micelles	0.25	0.06	0.09	0.10	0.60
Supernatant casein	7.37	5.80	0.89	0.68	0.70
Whey[a]	2.90	0.70	0.77	1.43	0.06
Homogenized pasteurized cow's milk					
Micelles	5.13	1.07	3.57	0.49	0.52
Supernatant casein	31.21	28.20	1.70	1.31	0.38
Whey[a]	2.18	0.16	1.06	0.96	0.40
Cream II[b]	80.45	79.53	0.41	0.51	0.02
Whole milk[a]	50.15	37.50	12.20	0.45	0.013
Cream I[b]	71.21	70.00	0.88	0.33	0.05
Whole casein	32.67	30.70	1.66	0.31	0.55
Whole whey[a]	2.88	0.28	0.39	2.21	2.05
Holstein[c]					
Cream I[b]	79.40	79.10	0.03	0.27	...
Skim milk	1.80	1.15	0.36	0.29	...
Whole casein	1.52	0.82	0.55	0.15	2.00
Whey[a]	0.83	0.12	0.53	0.18	0.05
Separator slime	76.00	72.90	0.84	2.26	...

[a] Nondialyzable fraction. [b] Total nonlipid solids. [c] Cerbulis (1967).

Table III. Free and Bound Lipids in Freeze-Dried Whole Milk

(Total lipids = 100%)

	Free	Bound
Fresh whole milk, sample 1	83.5	16.5
Sample 1, pasteurized	91.1	8.9
Fresh whole milk, sample 2	91.2	8.8
Fresh whole milk, sample 3	72.1	27.9
Homogenized pasteurized milk	74.7	25.3
Fresh whole milk, sample 4	96.7	3.3

Components of Lipid Fractions. All lipid fractions prepared in these experiments were analyzed by TLC. The free lipid fractions from all preparations, including homogenized milk experiments, contained neutral lipids only (triglycerides, diglycerides, monoglycerides, cholesterol, pigments, hydrocarbons, and free fatty acids). As an example, the composition of the free lipids of Holstein micelles determined by preparative TLC (Cerbulis, 1967) is as follows: cholesterol esters, hydrocarbons, and pigments, 18.4; triglycerides, 57.1; di- and monoglycerides, 5.3; free fatty acids, 9.5; and cholesterol, 9.7%. A characteristic phenomenon was the high hydrocarbon-pigment-cholesterol ester content in the free lipid fractions of micelles and whey. This was observed also in the previous experiments (Cerbulis, 1967). Stevenson and Bacharach (1937) had reported that the lipids extracted from acid-precipitated casein contained neutral fats only.

Phospholipids were found in the bound lipid fraction only. Even homogenization did not release petroleum ether–extractable phospholipids in milk. By using two-dimensional TLC (Rouser *et al.*, 1967), lecithin, cephalin, sphingomyelin, and four unknown minor components were found in the phospholipid fraction of all preparations from fresh milk. Preparations from homogenized milk contained several more uncharacterized phospholipids, which accounted for the increase of the phospholipid fraction in homogenized milk. These unknown substances of homogenized milk probably were released and redistributed during the homogenization of milk. However, these new substances were not observed before homogenization from milk fractions obtained by extraction with chloroform-methanol (2 to 1).

Polyacrylamide Gel Electrophoresis of Casein Fractions. Electrophoresis showed that the composition of micelle fractions and supernatant casein fractions from samples of fresh milk and of homogenized milk were identical. Both α-casein and β-casein were present. However, differences were observed in the CMEP fractions (Cerbulis and Custer, 1967) in milk from different breeds. CMEP fractions from the micelles of milk of Holstein, Jersey, and Brown Swiss cows showed identical composition of two major bands (γ-casein and temperature-sensitive casein) and several minor bands, identical to those previously obtained (Cerbulis and Custer, 1967). The sample from Guernsey showed somewhat different composition: No distinct bands but only a streak was present. The CMEP fraction from the micelles of homogenized milk also showed only a streak.

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