

**Proteins extracted from whole milk
chloroform-methanol (2:1) and with
fifty percent aqueous ethanol**

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24 Eiweiß-Fraktionierung (Chloroform-Extraktion)

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Introduction

Cerbulis and Custer previously reported the isolation of a soluble casein fraction, named chloroform-methanol extractable proteins, CMEP, from freeze-dried casein with chloroform-methanol (2:1, v/v) and with 50 % ethanol (1). Those findings showed approximately 2 % by weight of pooled milk casein on a dry weight basis, with either chloroform-methanol or 50 % aqueous ethanol used as extractant. This was equivalent to 0,6 g of CMEP per liter of pooled whole milk. The CMEP fraction consisted of γ -casein, temperature-sensitive casein, and six or more minor components. Later studies (2) showed that these extractants did not remove all of the CMEP fraction from dry casein.

The work here reported indicates isolation of the CMEP fraction in considerably greater yield from pooled whole milk and from skim milk with chloroform-methanol (2:1) and 50 % aqueous ethanol.

Experimental procedure

Extraction with chloroform-methanol. Fresh pooled milk from cows (milked at 6 a. m.) was cooled immediately to 2 ° C on the farm. At the laboratory it was extracted at 8:30 that morning, as shown in Figure 1. Whole milk or skim milk (250 ml), into which 2.5 liters chloroform-methanol (2:1, v/v) was vigorously stirred for 3 hours, showed precipitated proteins in the mixture. The three-phase system formed consisted of an aqueous methanol layer on top, coagulated proteins, and a chloroform layer.

Filtered by gravity, the mixture yielded Filtrate I in two phases, an aqueous methanol layer and a chloroform layer. The coagulated proteins were mixed with 800 ml solvent, continuously stirred for 3 hours, and filtered (Filtrate II). Both filtrates were combined and evaporated *in vacuo* to dryness. The residue was taken up with 200 ml chloroform-methanol (2:1)

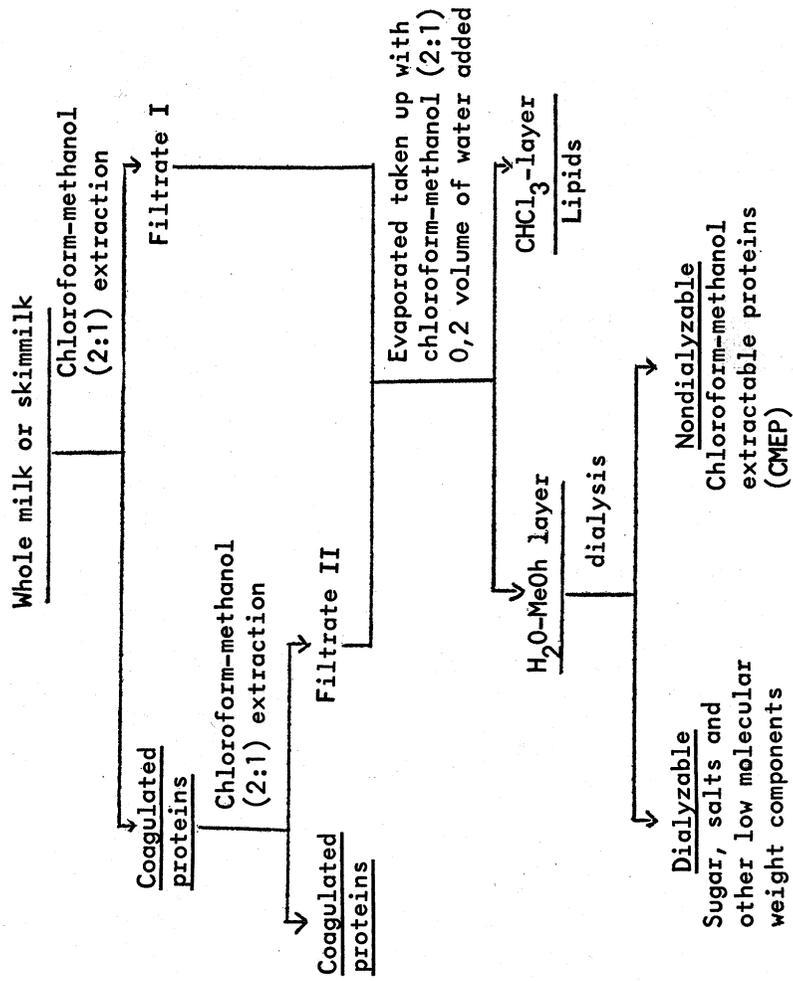


Figure 1. Extraction of whole milk or skimmilk.

and 40 ml water added, as described by FOLCH et al. (4), forming another three-phase system: aqueous methanol, interphase solids, and chloroform.

The chloroform layer (lipids) was separated and washed once with water, and the chloroform fraction was discarded. The washing was added to the aqueous methanol phase with interphase solids and extracted once again with chloroform, and the latter extract discarded. The aqueous methanol layer with interphase solids was dialyzed against distilled water for 4 days, with the water changed four times daily. The non-dialyzable fraction (CMEP) was freeze-dried and used for further studies.

Extraction with 50% aqueous ethanol. Pooled whole milk (250 ml) was mixed with 250 ml absolute ethanol and stirred for 2 hours, then filtered. The added ethanol precipitated milk proteins, which were extracted once more with 750 ml of 50% aqueous ethanol by stirring for 4 hours and then filtering. The extracts were combined, evaporated *in vacuo* to small volume, and dialyzed against distilled water as described above. The non-dialyzable fraction was freeze-dried. The residue contained a considerable amount of lipids, removed from the proteins by extracting the residue with diethyl ether.

All protein fractions were studied by polyacrylamide gel electrophoresis using Tris-EDTA-borate buffer at pH 8.6 (1).

Results and discussion

In six experiments, the yield of chloroform-methanol (2:1) extractable, non-dialyzable product was 2.0—2.5 g/liter in pooled milk, a yield considerably higher than that from acid precipitated and freeze-dried casein or casein micelles. The latter are approximately 2% by dry weight of pooled milk casein, or 0.6 g/liter of pooled whole milk (1, 3). This unexplained difference may be due to changes in the properties of the casein fraction brought on by acid precipitation or freeze-drying.

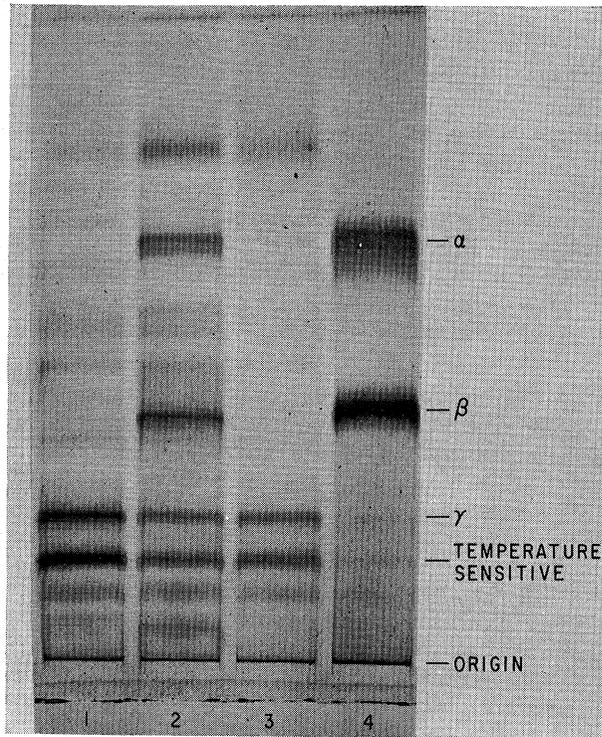


Figure 2. Polyacrylamide gel electrophoresis patterns of caseins: 1) and 3) Proteins obtained by chloroform-methanol. 2) Proteins obtained by ethanol. 4) Whole casein.

Homogenization and pasteurization changed the properties of the casein fraction considerably (3). The CMEP content was decreased from 2% of the total acid precipitated casein in fresh milk (1) to 0.52—0.55% in homogenized pasteurized milk (3).

The chloroform-methanol (2:1) method works equally well on skim milk as on homogenized pasteurized commercial milk.

Electrophoresis patterns showed that the chloroform-methanol extract contained up to 13 components (Figure 2). Besides previously reported components, that is, γ -casein, temperature-sensitive casein, and

other minor components, obtained from casein (1), two unidentified components migrated faster than the known casein components. The minor bands did not coincide with β -lactoglobulin or α -lactalbumin.

The 50 % aqueous ethanol extraction gave 5.2 g/liter extractable material, containing all the chloroform-methanol extract components plus some α -casein and β -casein. Therefore, the ethanol extraction method was not suitable for the preparation of minor proteins from milk.

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Zusammenfassung

CERBULIS, J.: **Aus Vollmilch mit Chloroform-Methanolgemisch (2 : 1) und mit 50%igem wässrigem Äthanolgemisch extrahierte Proteine.** „*Milchwissenschaft*“ **24**, (3) 140—142 (1969).

24 Eiweiß-Fraktionierung (Chloroform-Extraktion).

Mit einem Chloroform-Methanolgemisch (2 : 1) wurden etwa 2,2 g Protein aus 1 Liter Mischmilch extrahiert. Diese Fraktion enthält vorzugsweise γ -Casein und temperaturempfindliches Casein sowie 11 weitere Proteinfractionen in geringerer Menge. Mit der Chloroform-Methanolbehandlung lassen sich die Proteine aus Milch einfacher als aus Trockencasein isolieren. Da die Extraktion mit wässrigem Äthanol zu weniger reinen Konzentraten des γ -Caseins und der temperaturempfindlichen Caseinfraktion führt, ist sie für die Isolierung der in geringen Mengen vorkommenden Proteinfractionen nicht geeignet. Dok.-Ref.

CERBULIS, J.: **Proteins extracted from whole milk with chloroform-methanol (2 : 1) and with fifty percent aqueous ethanol.** „*Milchwissenschaft*“ 24. (3) 140—142 (1969).

24 Protein fractionation (Chloroform extraction).

Chloroform-methanol (2 : 1) extracted approximately 2.2 g of proteins from 1 liter of pooled milk. This fraction contained predominantly γ -casein and temperature-sensitive casein, with up to 11 other protein components in smaller amounts. The chloroform-methanol (2:1) treatment isolates these minor protein components more simply from milk than from dry casein. Since aqueous ethanol extraction produced less pure concentrate of γ -casein and temperature-sensitive casein fraction, this method is not suitable for isolating minor protein components of milk.

CERBULIS, J.: **L'extraction des protéines du lait entier au moyen d'un mélange de chloroforme et méthanol (2 : 1) et d'une solution aqueuse (50 %) d'éthanol.** „*Milchwissenschaft*“ 24. (3) 140—142 (1969).

24 Fractionnement de protéines (extraction au moyen de chloroforme).

CERBULIS, J.: **La extracción de proteínas de leche entera mediante una mezcla de cloroformo y metanol (2 : 1) y de una solución acuosa (50 %) de etanol.** „*Milchwissenschaft*“ 24. (3) 140—142 (1969).

24 Fraccionamiento de proteínas (extracción mediante cloroformo).