

Physical and Chemical Analyses of Film Formed at Air-Water Interface on Reconstitution of Whole Milk Powders

B. E. Leach,¹ N. H. Rainey, and M. J. Pallansch

Dairy Products Laboratory, Eastern Utilization Research and Development Division
USDA, Washington, D. C.

Abstract

When whole milk powder is added to water a major portion of it dissolves but some material remains insoluble and forms a film at the air-water interface making further powder wetting impossible without agitation. Studies were undertaken to determine the physical characteristics and composition of this film. Film balance studies showed the material to be multimolecular in depth, compressible but non-elastic. Small amounts of the interfacial product have been collected and analyzed for total lipid, cholesterol, fatty acids, nitrogen, phosphorus, hexose, hexosamine and neuraminic acid. Gas-liquid chromatography showed that the film contained a relatively high concentration of C₁₈ saturated fatty acids. Gel electrophoresis showed that the film contained a number of sugar-protein complexes. Increasing the temperature of the water on which the film was spread or heating the powder before solution reduced the film strength. The surface film formed by milk powders containing liquid fat was so weak that the powder had self-dispersing properties.

The rapid self-dispersion of any particulate solid into water demands speedy penetration of the air-water interface. Commercially, self-dispersing nonfat milk powders have been successfully made by agglomeration processes known as instantizing (5).

These processes do not produce self-dispersing whole milk powder granules. Instantized whole milk particles, like other whole milk powders when poured on to the water surface, seem incapable of penetrating the interface. This defect has been ascribed to a lack of wettability of the powder particles (3).

During the course of a study of factors influencing the dispersibility of whole milk powders we noted that, in general, all whole milk powder granules by all spray- and vacuum-drying techniques which minimized the temperature rise

in milk during dehydration, wet rapidly when brought in contact with water. However, on passing into the water the particles left a residue in the interface which could coalesce with other similar material to form a surface film. This film prevented the wetting of any additional powder poured on to the surface.

Our paper describes some of the physical and chemical properties of the film formed on the surface of water during the reconstitution of whole milk powder and suggests methods to overcome its formation.

Material and Methods

All powders used in this study were made from fresh milk which had received minimum heat treatment, consistent with pasteurization requirements, prior to concentrating and drying.

The vacuum-foam-dried whole milk powders were prepared by using a procedure described by Sinnamon et al. (10). This procedure was also used to dry a concentrate in which the normal fat phase was replaced by a fraction of butteroil melting at 20 C. The butteroil fraction was obtained by filtering butteroil after it had stood approximately 24 hr at 20 C.

Spray-dried foam powders were made as described by Hanrahan et al. (6) and a conventional spray-dried powder was made using standard industrial techniques in conjunction with a 9-ft Swenson dryer.

Samples of surface film, for chemical analysis, were obtained using a 25-cm. I.D. desiccator filled to the rim with distilled water and equipped with a magnetic stirring device. After sprinkling 75 mg of powder on to the water surface, the film so formed was washed free of occluded milk constituents by slowly stirring the water under the film for 5 min. The washed film was collected on the desiccator rim by moving a stainless steel barrier across the surface. The collected material was lyophilized and stored at -20 C before use.

Film pressure-area relationships were studied, using a CENCO hydrophyl balance. Temperature of the water, on which the films were studied, was maintained at the desired level by use of heating tape cemented to the bottom of the water tank.

¹Baptist Memorial Hospital, Department of Pathology, Memphis, Tennessee.

Gas-Liquid chromatography was carried out, using a Micro Tek Model 2000-R chromatograph equipped with columns and dual-flame detectors.

Canalco Model 12 disc electrophoresis apparatus was used for electrophoretic analysis in acrylamide gels, employing the method of Davis (4).

Published procedures determined total lipid (9), cholesterol (1), phosphorus (2), nitrogen (8), sialic acid (12), total hexoses (13), and hexoseamines (14). Methyl esters of the fatty acids in the triglyceride fraction of the film were made, using the method of James (7).

Results

The passage of 1 g of whole milk solids through an air-water interface was found to form a film having a dry weight of approximately 0.0025 g. The chemical composition of the film is presented in Table 1. From this it can be seen that the film contains a higher level of fat than is found in the milk powder from which it was formed.

TABLE 1

Analytical data obtained for a dried whole milk powder and its air-water interfacial film

	Milk powder	Air-water interfacial film	
		————— (%) —————	
Total lipid (Mojonnier)	24.5	68.7	
Cholesterol	0.10	0.15	
Phosphorus	0.38	0.25	
Total Nitrogen	4.60	4.40	
Sialic acid	0.22	0.12	
Total hexoses ^a	39.30	3.38	
Hexoseamine	0.30	0.20	

^a Includes the products of lactose hydrolysis.

Gas-liquid chromatography of the methyl esters of the fatty acids derived from milk fat and the fat in the film showed the materials to be similar in composition except for the C₁₈ saturated fatty acid fractions. Figure 1 shows a typical chromatogram obtained on analysis of the film lipid fraction. The area under the C₁₈ saturated fatty acid peak is 37% larger than that obtained by analyzing milk fat under similar conditions.

Gel-electrophoresis of the proteins in the film extractable in pH 8.4 tris-glycine buffer showed the presence of very limited amounts of soluble material that stained with bromphenol blue. Analysis of 2-ml samples of solution obtained by extracting 1.6 mg of film material with .4 ml of buffer showed two faint, slow-moving bands in the gel and one slightly stronger band

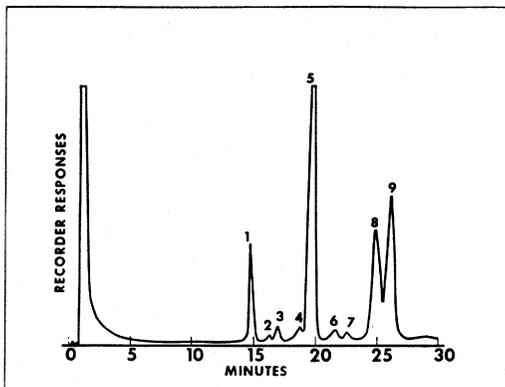


FIG. 1. Chromatogram of methyl esters obtained from fat isolated from surface film. Peak 8 is the unsaturated C₁₈ acid ester; Peak 9 the saturated C₁₈ acid ester. Resolution achieved on 4-ft column packed with SE30 on Chromsorb support. Temperature programmed from 80 C, start at the rate of 7.5 degrees, rise/minute for the first 2 min and 2.0 degrees rise/minute for the next 18 min.

having the mobility of β -lactoglobulin. By using a densitometer to compare the intensity of light absorption by the bands obtained during the analysis of the film with those of the band produced by analysis of a solution of β -lactoglobulin of known concentration, it was estimated that the film material contained .1% undenatured β -lactoglobulin or less. However, the high molecular weight fraction of the film was found to be characterized by the presence of relatively high levels of sugar-protein interaction products. One zone, in particular, gave a strong response to the anthrone sugar reagent, as shown in Figure 2.

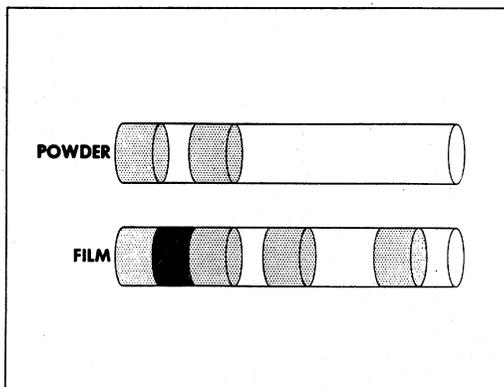


FIG. 2. Schematic diagram of disc electrophoresis pattern of soluble sugar-protein complexes in milk powder and the surface film. Shading indicates relative intensity developed by treatment of plug with anthrone reagent. Electrophoresis carried out in pH 8.4 tris-glycine buffer. Bands migrated from left to right.

From these analyses it is apparent that the film arises from a fractionation of the milk constituents effected by the passage of the particle through the interface.

Some information pertaining to the relative strength and dimensions of the surface film was obtained by use of a film balance. Figure 3 shows that the film formed by dusting 0.2 g of

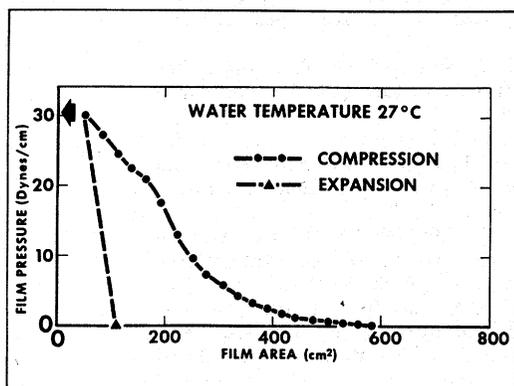


FIG. 3. Effect of lateral pressure change on the area of the surface film formed by dissolving milk powder in water. Solid arrow indicates film collapse point.

milk powder on to the surface of 27 C water can be compressed into an area of 50 cm² by exerting a lateral pressure of 30 dynes/centimeter. Application of higher pressures results in film collapse. Release of pressure on the compressed film was not accompanied by any appreciable area increase. Reduction of the collapse-point pressure, indicating a reduction in film strength, could be achieved by increasing the temperature of the water on which the film was formed, as shown in Figure 4. This tempera-

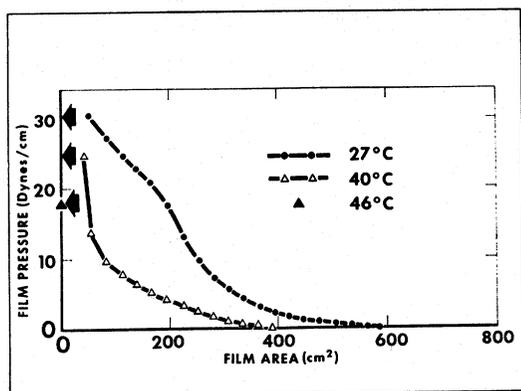


FIG. 4. Effect of water temperature on strength of surface film formed by dissolving milk powder particles.

ture effect is of limited use, since it was found that a film collapse point, sufficiently low to permit penetration of the interface, could not be obtained at temperatures below those causing reduction in milk protein solubility.

More effective reduction of collapse-point pressures could be observed when the powder was heated before spreading on to the water surface, or if the milk powder used to form the film was so constituted as to contain only the low-melting fraction of milk fat, as demonstrated in Figure 5.

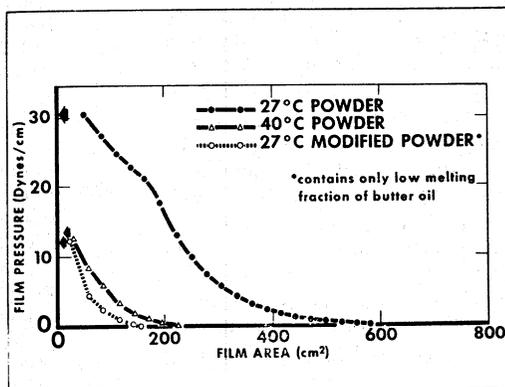


FIG. 5. Effect of heating milk powder and changing the composition of its fat phase on the strength of surface films formed by dissolving milk powder particles.

The surface film formed on dispersion of powders containing only low-melting-point fats was so weak, in fact, that it could be broken by the weight of any added milk powder. Therefore, this product was self-dispersing.

It was also observed that vapors of volatile fat solvents completely destroyed the surface film formed by dispersing whole milk powders on water. Experimental milk powders were, therefore, made by equilibrating material against the vapors of carbon tetrachloride and ether. These powders sank through the interface rapidly without film formation and could, in an impractical sense, be considered instantized.

Discussion

The collapse pressures of films formed on the surface when whole milk powder is dissolved in 27 C water are considerably higher than those reported in the literature for monomolecular films of protein or fatty acids spread on aqueous substrates. These collapse pressures range from 15 to 25 dynes/centimeter. The observed strength of the films formed by dissolving whole milk powders is not unexpected, since simple

calculations based on our data indicate that the film is not monomolecular and may be considered to be approximately four molecules thick.

Being unable to observe any contact angle between a droplet of water and the upper surface of the spread film, we speculate that the film may consist of a layer of two of triglyceride molecules sandwiched between two proteinaceous monolayers.

The film, therefore, can be considered as a protein-carbohydrate-lipid interaction product whose integrity, surprisingly, depends upon the physical state of the lipid phase. Any method which can be used to liquefy the lipid phase will result in film weakening to the point where self-dispersion of the milk powder becomes possible. This may indicate that self-dispersing whole milk powders suitable for room-temperature storage will be made only from milk whose constituents have been modified by fractionation or by use of additives. Even though fractionation of butteroil by partial crystallization followed by filtering seems impractical, it has been found that by crystallizing out of an acetone solution 75 to 80% of the butteroil can be recovered in a form melting at 18 C (11).

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