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Membrane Filtration of Dairy Products for Microbiological Analysis

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Incubation with protease or Tween 80 or both dramatically improved the membrane filterability of liquid milks, powdered skim milk, and frozen dairy products without reducing the viability of five common species of bacteria. The technique can permit isolation and enumeration of microorganisms from test samples of these foods as large as 5 g. Flow direction through the filter was an important factor in filterability of dairy products.

According to a recent report (4), 93% of the 1.3×10^6 microbiological analyses of food carried out in Canada each year involve dairy products. The figures are probably similar in the United States and many other countries. Any improvement in the methodology of these analyses could lead to significant savings of time and money. In the 1950's the use of membrane filters (MF) for dairy products was reported (3, 8, 10) and actively advocated by a leading manufacturer (Millipore Corp., *Recommended Procedure for Bacteriological Analysis of Milk*, 1959). However, a 1958 report of the American Public Health Association (1) stated that "many unforeseen filtration problems soon became apparent" and ended by not recommending the MF method for milk samples. The filterability problem has remained to the present day, such that the 1978 edition of *Standard Methods for the Examination of Dairy Products* (7) contains only one use for MF—that of the somatic cell count in raw milk. Since Sharpe and co-workers (11-16) have recently shown the attractiveness in food microbiology of MF, and particularly hydrophobic grid MF techniques, a solution to the problem of dairy product filterability was sought. Use of a 4% protease-2.2% Triton X-100 incubation to improve membrane filtration of raw milk for somatic cell counts was recently described by Cousins et al. (5), but these conditions may be too severe for viable cell counts. We investigated the modification of these conditions to maintain complete bacterial viability while improving the filterability of dairy products.

In the course of the investigation, it became apparent that flow direction through the filter is an important factor in the filterability of dairy products. For example, a decimal dilution of liquid skim milk filtered 50 times faster through one side of an MF than through the other side. A study was made of this phenomenon with several MF brands and different types of the same brand.

MATERIALS AND METHODS

Materials. Chemicals were purchased from the following suppliers: bovine pancreas trypsin, lot number 118C-8050, and *Streptomyces grisues* protease, lot number 117C-0042 (Sigma Chemical Co., St. Louis, Mo.); Tween 80 (polyoxyethylene sorbitan monooleate, J. T. Baker Chemical Co.); and bacterial culture media (Difco Laboratories, Detroit, Mich.). The following membrane filters (47-mm diameter, 0.45-μm pore size) were used: Gelman Tuffryn, lot number 82146 (Gelman Instrument Co., Montreal, Canada); Millipore HAWP, lot numbers 08M72478 and C8N78271; Millipore HAWG, lot number C8N7935; and Millipore HABP, lot number C7N26281 (Millipore Ltd., Mississauga, Ontario, Canada); Oxoid Nuflow, lot number 3911 (Medox Chemicals Ltd., Ottawa, Canada). Unless otherwise indicated, data refer to Millipore HAWP, lot number 08M72478.

Foods were purchased locally and stored at ambient, 4 or −20°C as appropriate: homogenized, 2%, and skim milk (Clark Dairy), powdered skim milk (Magic Instant), vanilla deluxe ice cream (Laura Secord), vanilla economy ice cream (Domino), and soft-serve ice milk (Dairy Queen). All foods were filtered as decimal dilutions in sterile peptone water prepared by mixing 30 s in a Colworth Stomacher 400 or Stomacher 80 (Canadian Laboratory Supplies Ltd.).

Filtration apparatus and filtration. The filtration apparatus and its method of operation were as previously described (15); the filtration cylinder was larger, exposing a filtration area of 13.07 cm^2. Analog output from the electronic balance was recorded directly onto paper. Unless otherwise indicated, the filtrations were performed at ambient temperature by using a pressure differential of 95 kPa. The filter side providing the fast flow rate was established with each freshly opened box by using liquid skim milk (1:10) as the probe; except where otherwise indicated, this side was uppermost during filtration. Results are shown as weight of food filtered per square centimeter of MF.

Proteolytic digestion. A stock solution of 10%

(wt/vol) trypsin or *S. griseus* protease in 1.0 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.6, was prepared daily and stored on ice. A mixture of 1 part food plus 8 parts diluent was warmed to 35°C, 1 part stock enzyme solution was added to it, and the mixture was re-incubated (final concentration, 1% protease in 0.1 M Tris-HCl, pH 7.6). At appropriate intervals 50-ml portions were removed, and their filterability was determined. In other studies, the concentration of the stock solution was appropriately adjusted; the incubation time was 20 min.

Surfactant treatment. A stock solution of 50% (wt/wt) aqueous solution of Tween 80 was prepared by heating and stirring. This solution, stored at ambient temperatures, kept for an indefinite period; for use, it was heated to 85°C. Decimal dilutions of foods were prepared with Tween 80 at a final concentration of 1%. The mixtures and apparatus were equilibrated to the indicated temperature in a water bath before the filtration; unless otherwise stated, the temperature was 40°C.

Toxicity. Cells from an 18-h culture in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) were inoculated into 50 ml of a decimal dilution of powdered skim milk to give a final plate count between 50 and 250 cells. Samples (10 ml) were removed to each of three small Stomacher bags and treated as follows: (i) 1% Tween 80, warmed to 40°C; (ii) 1% *S. griseus* protease in 0.1 M Tris-hydrochloride, pH 7.6, incubated at 35°C for 20 min; and (iii) a combination of i and ii incubated 20 min at 35°C. The remaining inoculated food provided the control. Duplicate pour or spread plates were prepared from each sample and incubated at 35°C as follows: *Escherichia coli* biotype 1 (violet red bile agar, 24 h), *Staphylococcus aureus* MF31, S6, FRI47, FRI161 (Baird-Parker agar, 48 h), *Streptococcus faecalis* and *S. faecium* (KF streptococcus agar, 48 h) and *Salmonella typhimurium* K1-2B (bismuth sulfite agar, 24 h). The protease solution, inoculated onto Trypticase soy agar and the four selective media, showed no growth after 48 h at 35°C.

RESULTS

Filter orientation. The membrane filterability of food was affected by the orientation of the MF towards the filtrant. Figure 1 shows that the filtration rate of a decimal dilution of liquid skim milk was 3 to 10 times faster through one side of the MF than through the other in the first 30 s of filtration; this increased to 50 after 2 min. However, the filter orientation varied between brands, with Gelman Tuffryn having the fast side and Millipore HAWP and Oxoid Nuflow having the slow side uppermost. A study of two brands and four types of MF (Fig. 2) suggested that orientation was generally consistent from box to box within the same lot number, although, occasionally, this was not so (Fig. 3). In our experience, there has always been a within-box uniformity of this property.

Filtration rate of liquid milks. Preliminary

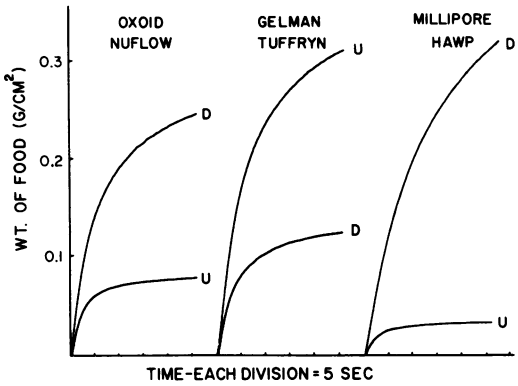

FIG. 1. *Dependence of filtration rate of 10% liquid skim milk on flow direction through three brands of MF. U, Upper side of MF as packed toward filtrant; D, flow direction reversed.*

experiments were conducted to determine optimum filtration conditions. Figure 4 shows that the filterability of homogenized and 2% milks was increased 20 to 25 times by treatment with 1% Tween 80 at 40°C. The filtration rate of skim milk at 40°C was three times greater than at 20°C; the addition of Tween 80 made no further difference.

Filtration rate of powdered skim milk. Untreated powdered skim milk is almost unfilterable. Since digestion for 70 min with 0.01% trypsin dramatically improves its filterability (15), higher enzyme concentrations were studied in an attempt to reduce digestion time. Surprisingly, for a given digestion time, increasing the trypsin concentration 100-fold did not change the filtration rate (Fig. 5). The first 10 min of the trypsin digestion resulted in a four- to sixfold increase in filtration rate; increasing the time to 60 min did not substantially increase this rate. In contrast to trypsin, the filterability of powdered skim milk during digestion with 0.1% *S. griseus* protease increased linearly with time. After 20 min of incubation with 1% protease, powdered skim milk filtered at a rate of 0.42 g/cm^2 in 30 s. This rate was virtually unchanged by a further 10-min digestion, so the 20-min incubation was chosen as our final condition.

Filtration of frozen dairy products. The filterability of deluxe ice cream (Fig. 6) improved fivefold by increasing the temperature from 10 to 40°C. The addition of 1% Tween 80 had little effect at 10 and 20°C but improved filterability a further fourfold at 40°C. The filterability of economy ice cream improved only slightly after proteolytic digestion with either trypsin or *S. griseus* protease, even when the concentration of the latter was raised to 4%.

A combined treatment of surfactant and pro-

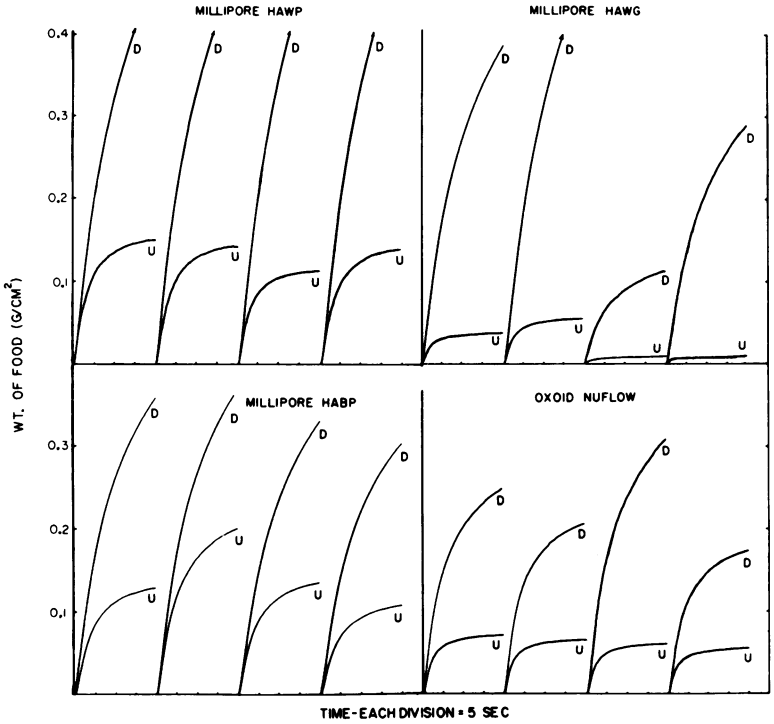

FIG. 2. *Dependence of filtration rate of 10% liquid skim milk on flow direction: comparison of MF brand, type, and box-to-box variation. Each curve in a pair are MF from the same box; different curve pairs are for different boxes. U, D, as for Fig. 1.*

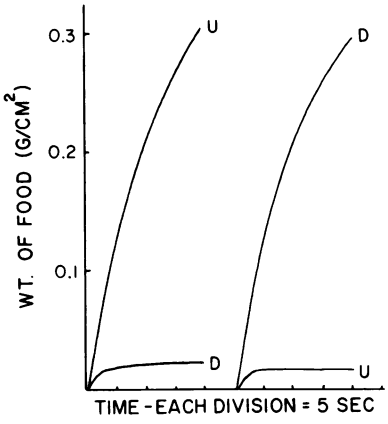

FIG. 3. *Dependence of filtration rate of 10% liquid skim milk on flow direction: comparison of two boxes from the same MF shelf pack. U, D, as for Fig. 1.*

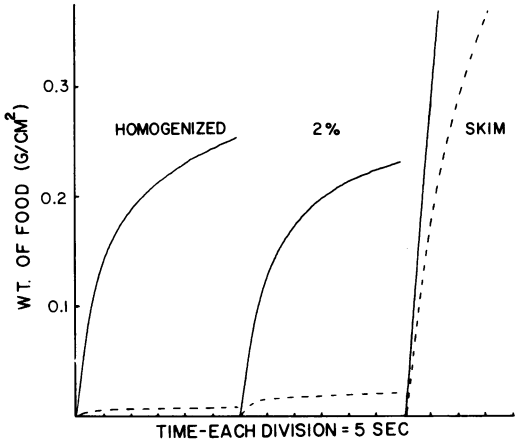

FIG. 4. *Membrane filtration rates of 10% liquid milks under the recommended conditions (see text). Broken lines show filtration rates at 20°C without Tween 80.*

teolysis considerably improved the filterability of all dairy products tested (Fig. 7). Whereas Tween 80 alone has little effect on the filterability of ice milk, it tripled the filterability of deluxe and economy ice creams. The additional step of proteolytic digestion increased the throughput of ice milk fivefold and that of economy and deluxe ice creams by about one-third.

Toxicity. By using the recommended conditions (see Discussion), a study was made of the effect of *S. griseus* protease and Tween 80 on bacterial viability. The data in Table 1 show that eight strains of commonly encountered food microorganisms were unaffected by this treatment.

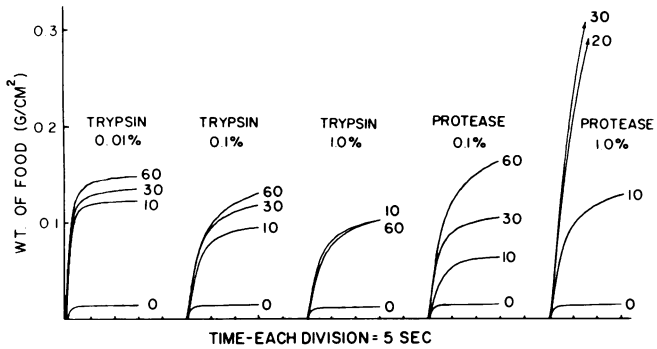

FIG. 5. *Effect of proteolytic digestion on membrane filtration rate of 10% powdered skim milk. The number after each curve states time of hydrolysis with enzyme. See text for details.*

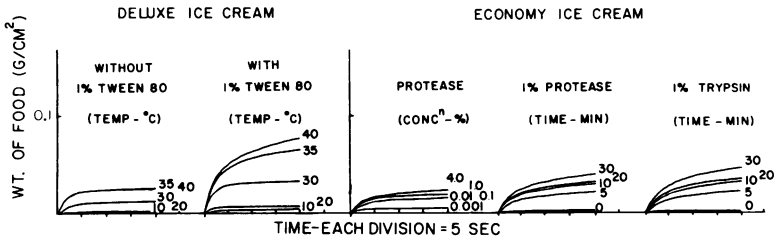

FIG. 6. *Factors affecting membrane filterability of 10% ice creams: effect of temperature, with and without Tween 80, on deluxe ice cream; effect of proteolytic enzyme concentration and incubation time on economy ice cream.*

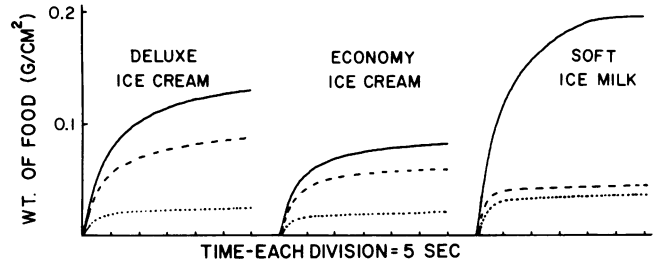

FIG. 7. *Membrane filtration rates of 10% frozen dairy products after treatment with 1% Tween 80, 1% S. griseus protease in 0.1 M Tris-hydrochloride, pH 7.6, and incubation for 20 min at, and filtration at, 35°C. Dotted lines show filtration rate at 20°C with no additions; broken lines show that at 40°C with 1% Tween 80.*

DISCUSSION

Advantages of improved limit of detection, removal of inhibitors, elimination of false positives, greater numerical operating range, reduction of interference from other organisms and shorter analytical time possessed by MF (and particularly hydrophobic grid MF) have been discussed previously (16). For the dairy industry to be able to take advantage of these techniques, dairy products must be made easily filterable. On the basis of the results reported here, the following procedures are recommended: (i) liquid skim milk: dilute 1:10 and filter at 40°C; (ii) homogenized and 2% milks: dilute 1:10, make 1% with respect to Tween 80, and filter at 40°C; (iii) powdered skim milk: dilute 1:9, add 1 part of 10% *S. griseus* protease in 1.0 M Tris-hydrochloride (pH 7.6), incubate 20 min at 35°C, and filter; (iv) frozen dairy products: dilute 1:9, add 1 part of 10% *S. griseus* protease in 1.0 M Tris-hydrochloride (pH 7.6), make 1% with respect to Tween 80, incubate for 20 min at, and filter at, 35°C. By using these methods, the weights of dairy products filterable through a 47-mm MF with an exposed area of 13.07 cm^2 in times up to 2 min were determined and are shown in Table 2. When translating these results to hydrophobic grid MF, it should be remembered that the hydrophobic grid occludes a significant propor-

TABLE 1. *Effect of 1% Tween 80 or 1% S. griseus protease treatments or both on viable bacterial counts*[a]

Organism	% Recovery[b] compared with untreated controls		
	1% Tween 80	1% Protease	1% Tween 80 plus 1% protease
E. coli biotype 1	108	89	94
S. aureus MF31	119	92	99
S. aureus S6	86	106	81
S. aureus FRI47	101	108	94
S. aureus FRI161	109	102	95
S. faecalis	111	106	106
S. faecium	103	106	98
S. typhimurium K1-2B	92	87	92
$\bar{x}$	103.6	99.5	94.9
s	10.6	8.7	7.1

[a] See text for temperatures and contact times.
[b] Minimum plate count 81, maximum 252. All results means of duplicate platings.

TABLE 2. *Quantities of dairy products filterable through 47-mm-diameter MF as decimal dilutions*

Food	Prefiltration treatment[a]	Quantity of food filtered (g)	Time (min)
Liquid skim milk	None	5.0	1.5
Homogenized milk	A	3.5	2.0
2% Milk	A	5.0	1.0
Powdered skim milk	B	5.0	0.5
Economy ice cream	C	3.5	2.0
Deluxe ice cream	C	3.5	2.0
Soft-serve ice milk	C	3.5	1.0

[a] A, 1% Tween 80, filter at 40°C; B, 1% *S. griseus* protease in, 0.1 M Tris-hydrochloride (pH 7.6), incubate for 20 min at 35°C; C, A plus B, incubate for 20 min at, and filter at, 35°C.

tion of the total area. For example, only 52% of the exposed area of the 1984 grid-cell hydrophobic grid MF (16) is available for filtration.

Cousins et al. (5) used unbuffered 4% *Aspergillus oryzae* protease to improve the filterability of raw milk. In a preliminary study we showed that powdered skim milk digested for 20 min with buffered 1% trypsin (final pH 7.5) filtered six times more rapidly than after an unbuffered digestion (pH 6.2). The filterability of undigested powdered skim milk was unchanged by pH adjustment alone. We therefore conclude that a substantial improvement in filterability is gained by buffering the proteolytic digestion mixture to the pH optimum of the enzyme.

It is apparent that the effectiveness of surfactant or protease treatment or both is consistent with the composition of the food. For example, warm surfactant treatment improves the filterability of liquid milks containing bufferfat but has no effect on liquid skim milk. Powdered skim milk which contains 35.5% protein and 1% fat is made filterable by a proteolytic digestion and needs no additional treatment. Economy and deluxe ice creams with milk fat contents of 10 to 18% (2) show their greatest increase in filterability from the surfactant alone. Soft-serve ice milk, on the other hand, with a fat content of 5% and a milk solids non-fat content of 12%, shows very little change from the detergent treatment but a marked increase when coupled with a proteolytic digestion.

Whereas Fifield and other workers (6) used 0.1% Triton X-100 as a filtering aid, Reusse (9) reported that there was a 30% loss of *Brucella abortus* after 1-min contact with 0.5% Triton X-100 and a 41% loss after 2 min. In a preliminary study we observed that *E. coli* was unaffected by a 5-min contact at 40°C with 1% Tween 80, whereas under the same conditions Triton X-100 resulted in a 71% loss of viability. We conclude that the use of Triton X-100 is not acceptable for viable counts. With Tween 80 and *S. griseus* protease in the recommended procedures, there was no loss of viability among the eight strains of food-borne microorganisms tested.

The question of the best direction of flow through the MF does not usually arise with water or aqueous solutions. However, Tobin and Dutka (17) reported differences between the two sides in bacterial recovery and flow rates when filtering tap water and showed that the orientation of this effect varied between brands. With many foods, the effect is measurable but not of practical significance (15). However, our results with liquid skim milk (Fig. 1, 2, and 3) show that assurances of proper filter orientation could be critical to the feasibility of MF procedures for microbial analysis of dairy products.

ACKNOWLEDGMENTS

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