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Membrane Filtration of Food Suspensions

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Factors affecting the membrane filtration of food suspensions were studied for 58 foods and 13 membrane filters. Lot number within a brand, pore size (0.45 or 0.8 μm), and time elapsed before filtration had little effect on filterability. Brand of membrane filter, flow direction, pressure differential, age (microbiological quality) of the food, duration of the blending process, temperature, and concentration of food in the suspension had significant and often predictable effects. Preparation of suspensions by Stomacher (relative to rotary blender) addition of surfactant (particularly at elevated temperature) and prior incubation with proteases sometimes had dramatic effects of filterability. In contrast to popular opinion, foods can be membrane filtered in quantities pertinent to the maximums used in conventional plating procedures. Removal of growth inhibitors and food debris is possible by using membrane filters. Lowering of the limits of detection of microorganisms by concentration on membrane filters can be considered feasible for many foods. The data are particularly relevant to the use of hydrophobic grid-membrane filters (which are capable of enumerating up to 9×10^4 organisms per filter) in instrumented methods of food microbiological analysis.

Sharpe and co-workers (22, 23, 25-27) showed that a novel growth vehicle—the hydrophobic grid-membrane filter (HGMF)—has great attractions in the automation of enumerative microbiology because of its potential ability to simplify the engineering requirements for diluting and counting and to eliminate the likelihood of false low counts at high levels of contamination. Its attractions also extend to manual counting procedures, because its use could often eliminate the need to make dilutions and the loss of data through misjudging contamination levels.

Demonstration of the ability of HGMF to provide linear recoveries at levels as high as 9×10^4 colony-forming units per filter (26) and maintain a linear performance with suspensions of several common foods (23) reinforced hopes that the unique properties of the HGMF might be exploited in the instrumentation of general food microbiology. However, thorough knowledge of the filterability of food suspensions through membrane filter (MF) material and the factors affecting it was seen to be an important requirement before further developments in HGMF-based instrumentation could be considered. A literature search revealed an apparent apathy towards MF techniques by food microbiologists; little data on the membrane filtration of foods, at levels equivalent to those used in, for example, plate count analyses, appear to exist.

The most relevant published uses of MF are restricted to easily filterable beverages such as wines, beers, and sugar liquids (10-12, 15, 19, 20) and to a few dairy products such as milk, butter, and ice cream (1-4, 6, 8, 9, 16-18). The membrane filtration of wines, beers, and sugar liquids for microbiological analysis would appear to present little problem. An interesting method of improving the filterability of egg albumin for *Salmonella* determinations by pectic enzyme hydrolysis followed by clarification with celite was described by Kirkham and Hartman (14). Apart from this, the published uses of MF in food microbiology appear to be limited to filtration of swabbed or shaken extracts (7) and a pressure method of transferring surface organisms to the MF (28). The American Public Health Association *Compendium* (29) recommends microbiological analysis by filtration for foods which "can be dissolved and passed through a bacteriological membrane filter. . .," without, however, offering any examples of suitable subjects.

There has been more enthusiasm from the dairy industry, but the problems and solutions have not been well defined. Some workers report difficulty filtering milk, for example (8, 17). The problem was avoided in one case by centrifuging and resuspending the milk (4). Improvement in filterability resulting from dilution of the specimen has been claimed (9, 18). A rather uncon-

trolled method, whereby a milk sample to be filtered was added to warm (45 to 50°C) surfactant solution already in the filter, was described by Fifield and co-workers (6) and others (2, 3). In another case, ice cream was made filterable by liquefaction, settling, and addition of surfactant (16).

Apart from these examples, the microbiological applications of MF appear to be limited to the analysis of waters (which needs no further comment) and the sterilization of fluids. The absence of publications pertinent to foods was not surprising because (i) the numerical operating range of the ubiquitous 47-mm MF is so small (e.g., lower and upper counting limits of 20 and 80 colonies, respectively) that many disks would be required per analysis to accommodate the range of counts found in most foods, and (ii) most microbiologists in our experience tend to dismiss food suspensions as being generally unfilterable.

The HGMF has a numerical operating range far greater than any conventional MF (or even petri dish) so that point (i) above does not now apply. The potential applicability of MF methods to food microbiology thus rests on the validity of point (ii). There seemed to be so much unverified opinion about the factors affecting membrane filterability that we decided to obtain a definitive data base for any future work on either HGMF or MF.

MATERIALS AND METHODS

Filtration apparatus. The filtration apparatus is shown in Fig. 1. Positive pressure was used above the MF rather than the normal underside vacuum, to allow more precise control of pressure differential. No significant alteration in flow rate was expected, except, perhaps, where boiling or drastic outgassing of the filtrate may occur under excessive vacuum. MF support was provided by a flattened stainless steel mesh A (Michigan Wire Cloth, Ambac Industries, Garden City, Mich.; 40 by 40; 0.010-inch [ca. 0.025-cm] wire). The extent to which this may have interfered with flow rates was not calculable, but is believed to be negligible. Cylinder B contained the sample, and defined the filtration area (1.905-cm diameter, 2.850 cm^2). The apparatus was capped and sealed with screw clamp (C). Pressure, applied through a valve D via a two-stage reducer from a nitrogen cylinder and monitored by an open mercury manometer, varied by less than 1% during a filtration. The filtrate outlet height was arranged to be level with disk A; a small, additional time-variable pressure resulting from the head of sample in B was ignored. Valve E allowed filtrate to be piped to a Sartorius 3716MP electronic balance (Canadian Laboratory Supplies Ltd.) fitted with a draft-excluding cover and a suction line for removal of filtrates. Analog output from the balance was recorded on tracing paper by a Servogor recorder (Brinkmann 2541, Brinkmann Instruments, Canada Ltd.) running at 60 mm/min.

MF. In all cases, the manufacturer's orientation of MF material was preserved. The following MF were purchased as 25-mm disks or were punched from larger format material already on hand: Amicon microporous filter, 0.45 μm (Amicon Canada Ltd.), lot number BB 0050F SUBA; Gelman Metricel, 0.45 μm, 6N, lot number 81913, and GA-6, lot number 81859; Gelman Metricel 0.8 μm, 4N, lot number 81264, and GA-4, lot number 81681 (Gelman Instrument Co.); Millipore 0.45 μm HAWP, lot numbers 22819, 14247-19, 109876, 106515, and 26945-19, 0.45 μm HABP, lot number 09242-3; 0.8 μm AAWP, lot number C7M21886A (all Millipore Ltd., Mississauga, Ont., Canada) and Millipore 0.45 μm HABP, lot number unknown (BDH Chemicals Ltd.); Oxoid Nuflow 0.45 μm, lot number 3722, and 0.8 μm, lot number 3680 (Medex Chemicals

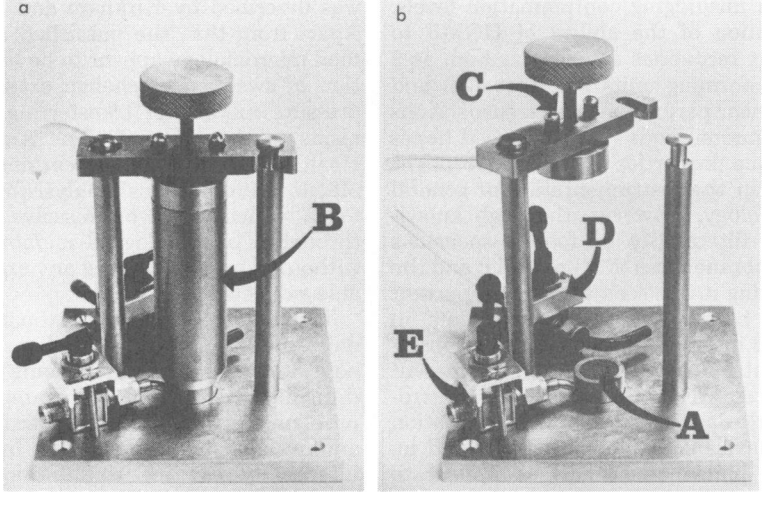


FIG. 1. *The filtration apparatus with (a) and without (b) cylinder B in place. For explanation of components, see text.*

Ltd.); Sartorius 0.45 μm SM11306, lot number 061233726, and 0.45 μm SM11106, lot number 060964702; Sartorius 0.8 μm SM11304, lot number 300814572, and SM11104, lot number 401836572 (BDH Chemicals Ltd.). Unless otherwise stated, all data refer to filtration through Millipore HAWP material, lot number 106515.

Foods. The following foods were purchased locally and stored at ambient, 4 or −20°C as appropriate: bacon; beef chunks; lean (15.6% fat), medium (25.0% fat), and regular (30.1% fat) ground beefs; frozen chicken; frankfurters (Maple Leaf); ground pork (31.2% fat); pork sausages (Maple Leaf); canned clams (Clover Leaf); cod fish sticks (High Liner); ocean perch; cooked shrimp (Brilliant); turbot; apple sauce (Stokely); baked beans (Heinz); frozen brussels sprouts and frozen carrots (Arctic Gardens); pickled gherkins (Bick); frozen green beans (Loblaw); lima beans (York); canned mushrooms (Clover Leaf); potato (Dollar Chips, McCain); frozen strawberries (York); basil, ground cinammon, and ground nutmeg (all McCormick); walnuts (McNair); whole and skim milk (Clark); skim milk powder (Carnation); table cream (Borden); sour cream (Sealtest); vanilla ice cream (Loblaw); yoghurt—Set Style and Swiss Spun (both Delisle); butter (Loblaw); cheddar cheese (Black Diamond); mozarella, Philadelphia, and Velveeta cheeses (all Kraft); apple turnovers (Pepperidge Farm); breakfast cereal (Quaker's Captain Crunch); Egg Beaters (Fleischmann); vanilla instant pudding (Jello); pizza (Gusto); puff pastry (Gainsborough); ravioli with meat (Nelia); spaghetti (Primo); turkey pot pie (Loblaw); frozen waffles (Loblaw); chicken and rice instant soup mix (Loney); vegetable beef soup (Aylmer); tomato soup (Campbell); mushroom soup (Clark); apple juice (Martin); and orange juice (Loblaw). For age studies, frozen or refrigerated subsamples were removed to the bench (room temperature 22 ± 2°C) at appropriate intervals before the experiment.

Filtration and recording. The operational details should be self-evident, and only the following points are noted. For each new filter disk, a preliminary run was made with distilled/deionized water to clear any bubbles from the line. With the unit pressurized (valve D) and the recorder zeroed, with the chart in motion, valve E was opened to pass filtrate when the chart was at the desired (x-coordinate) position. In this way, several corresponding curves could be drawn from the same starting point to aid visualization. Incompletely filtered samples were aspirated out before removing B. The data are displayed in terms of weight of food filtered out per square centimeter of MF (rather than volume of suspension) unless otherwise indicated.

Preparation of food suspensions. Decimally diluted suspensions in distilled/deionized water were prepared from 10- to 40-g specimens according to the general homogeneity of the food. Suspensions were prepared in Mason jars on an Osterizer blender (Sunbeam Corp. of Canada Ltd.) at 25,000 rpm, or in a Stomacher 400 (Canadian Laboratory Supplies Ltd., or Dynatech Corp.). Centimally diluted suspensions were prepared separately by blending 4 g of food in 396 ml of diluent. For experiments on time elapsed before filtration, suspensions were transferred to beakers and either stirred continuously (magnetic stirrer) at room temperature or allowed to settle and stirred immediately before use. Except where otherwise stated, blending times were 60 s for the Osterizer and 30 s for the Stomacher; suspensions were used within 15 min of preparation.

Pressure differential studies. Headspace pressures of 10, 20, 30, 60, and 95 kPa (101.4 kPa = 760 mm of Hg) were employed. The highest figure is equivalent to the pressure drop achieved by a good water pump. Investigation of higher pressures was deemed unnecessary. Unless otherwise stated, data refer to a pressure differential of 95 kPa.

Surfactant studies. In preliminary studies, foods were blended in Triton X-100 and Tween 80 solutions, up to 10% (wt/vol). Most of the data reported here are for 1% solutions of these surfactants. For stomaching, air was removed from the bag by sliding it down into the stomaching compartment; this avoided the frothing which is inevitable with the rotary blender.

Protease studies. Trypsin (Nutritional Biochemical Corp., Cleveland, Ohio) at a final concentration of 100 μg/ml in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.8, was stomached with food (10%) and incubated for up to 70 min at 37°C before filtration. Similar suspensions with Pronase (Calbiochem, Los Angeles, Calif.) at the same concentration in 0.02 M sodium acetate buffer, pH 6.0, were also incubated at 37°C for up to 70 min.

Temperature dependency studies. The filtration unit and suspensions were immersed in a water bath and equilibrated. The unit was raised from the bath to add MF, water, or suspension, and returned immediately. Where not stated, data refer to ambient temperature (22 ± 2°C).

RESULTS

General considerations regarding filterability. The filterability of a suspension of food in water, under a constant pressure differential, may be interpreted in several ways. Filtration rate (the slopes of the graphs) defined as either the volume of filtrate issuing from the filter or the weight of food brought into intimate contact with the filter (the cake) per second, is relatively meaningless, changing as it does while filtration progresses. At the instant of commencing filtration, every food suspension had a high filtration rate (Q_0) indistinguishable from that of water. Thereafter, the rate decreased as a function of food, preparation conditions, temperature, etc. In some instances filters became quite impermeable when a certain amount of food had been filtered, whereas in others a more or less steady state was reached. A cake filtration model (13), which assumes that flow rate decreases in inverse proportion to the amount of food accumulated on the filter, leads to the following flow rate equation:

$$t = a_1 v + a_2 v^2 \quad (1)$$

(v = volume, t = time). This relation, which

would have allowed comparisons to be made easily from tables of the constants a_1 and a_2, was quickly shown to be inadequate at describing the shapes of many of the graphs. Some curves are better described by a complete blocking mechanism (13) in which particles plug pores completely, i.e., by the following relation:

$$v = Q_0(1 - e^{-kt}) \quad (2)$$

Brief examination of the data by using the following general model:

$$t = a_0 + a_1v + a_2v^2 + a_3v^3 + \ldots \quad (3)$$

led to sets of figures from which the practicality was only discernible by recalculating the curves. Other forms of data, for example, tables of quantity filtered during various time intervals, became cumbersome when attempting to compare the effects of different variables. It was decided, therefore, that the curves themselves conveyed information in the most practical and compact manner.

Intrinsic variability of filtration rates. Figure 2 exemplifies the many graphs obtained by filtering stock food suspensions through different manufacturer lot numbers and different blendings of the same food specimen through several MF from one lot number. Successive filterings of stock suspensions through MF from a given lot number yielded almost perfectly superimposable graphs, and the effect is not illustrated in Fig. 2. However, successive blendings of the same food specimen yielded minor variations which could either be due to inhomogeneity of the specimen or blending inconsistencies. Similarly, there were differences between lot numbers from the same manufacturer; Millipore lot number 22819, for example, consistently filtering slower than lot number 106515.

Effect of filter brand and pore size. Figures 3 and 4 illustrate that for ground beef, green beans, turbot, and milk, at least, permeability differences between the MF brands in a given pore size were as great as or greater than those between pore sizes in a given brand. In fact, differences in flow rate between 0.45- and 0.8-μm pore sizes of a brand were generally barely noticeable, and for stomached ground beef the Oxoid Nuflow 0.8-μm MF actually filtered less

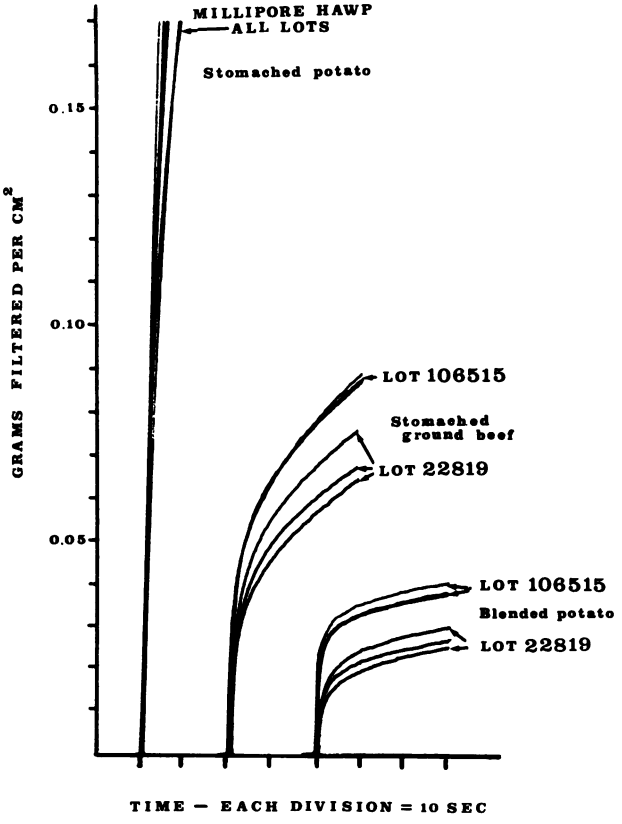


FIG. 2. *Variation of membrane filtration rate with lot number in Millipore MF. The three curves for each lot (center and right hand groups) are different blendings of the same sample.*

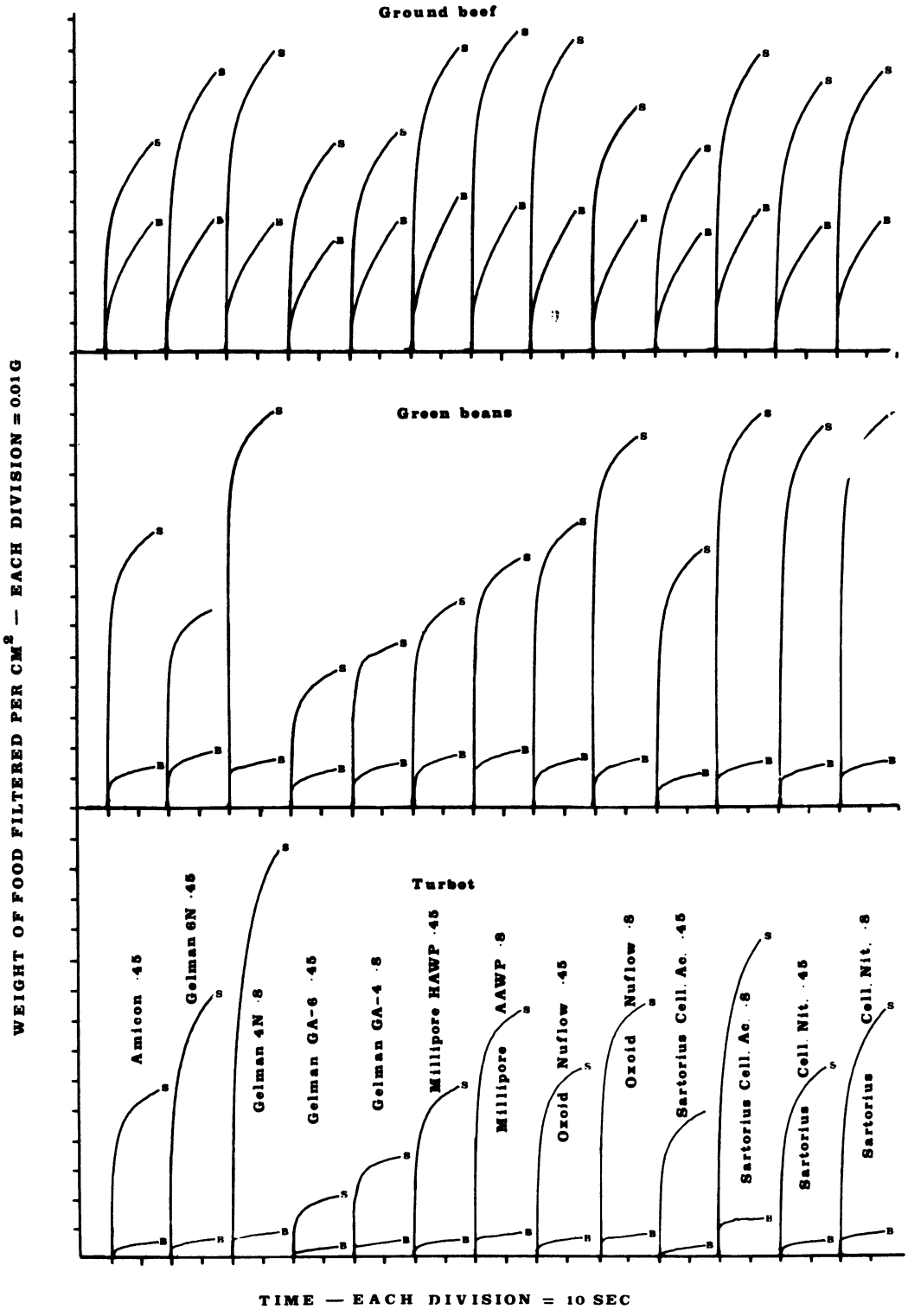


FIG. 3. *Variation of membrane filtration rate with MF brand and pore size, for stomached (S) and rotary-blended (B) ground beef, green beans, and turbot.*

rapidly than the 0.45-μm pore size. The differences between filters for these foods do not correspond to the relative flow rates determined for water, shown in Table 1.

Effect of flow direction through MF. Figure 4 shows that flow direction had a very pronounced effect for all the 0.45-μm filters, except the Sartorius cellulose nitrate with milk. All filters except both the Sartorius (cellulose acetate and nitrate) were more permeable when used in the orientation supplied (i.e., with the suspension to be filtered in contact with the side uppermost, as packed). These findings should be compared with the following manufacturer's instructions regarding orientation: Amicon, "Insert either side up"; Gelman, "Facing up towards material being filtered"; Millipore, no instructions; Oxoid, "Filter through top as packed"; Sartorius, no instructions. The cause of the variability is undoubtedly complex; the large difference observed for Millipore filters with milk does not follow the small differences for stomached ground beef, green beans, and turbot, for example.

Effect of pressure differential. The filtration rate of water (and presumably of food suspensions also, at the instant of commencing filtration) varied with the type of MF, but was always proportional to the pressure differential across the MF. The coefficient was, for example, 0.009 g/cm^2 per s/kPa for the 0.45-μm-pore-size Sartorius cellulose acetate MF (Table 1). With foods, however, although increasing pressure differential always resulted in faster filtration, the effect rapidly became nonlinear; after 10 or 60 s, for example, the quantity filtered at 95 kPa was always less than 9.5 times that at 10 kPa (Fig. 5). The effect of pressure differential became less important as filtration progressed, but in no instance was the quantity filtered at a low pressure observed to overtake that from a higher pressure.

TABLE 1. *Flow rates and specific flow rates of water through MF at 20°C*

Filter	Pore size (μm)	Flow at 95 kPa (g/cm^2 per s)	Specific flow rate (g/cm^2 per s/kPa)
Amicon	0.45	1.18	0.0124
Gelman GA-6	0.45	1.10	0.0115
Gelman GA-4	0.8	1.90	0.0201
Gelman 6N	0.45	1.14	0.0120
Gelman 4N	0.8	2.00	0.0211
Millipore HAWP (lot no. 106515)	0.45	0.83	0.0087
Millipore AAWP	0.8	2.22	0.0234
Oxoid Nuflow	0.45	1.00	0.0105
Oxoid Nuflow	0.8	1.60	0.0168
Sartorius SM11306	0.45	0.85	0.0090
Sartorius SM11304	0.8	2.00	0.0211
Sartorius SM11106	0.45	1.07	0.0112
Sartorius SM11104	0.8	2.00	0.0211

Effect of time elapsed before filtration. No significant changes were observed with either stomached or blended ground beef and potato, when suspensions were either stirred or allowed to stand for up to 45 min before filtration. Graphs were virtually superimposable and are not shown.

Effect of suspension preparation procedure. Except in the case of vanilla ice cream, for which the blended specimen filtered more rapidly than the stomached one, and Egg Beaters, for which there was no noticeable difference, all food suspensions prepared by Stomacher filtered more rapidly than those prepared by rotary blender (see Fig. 6, 7, and 9). The differences were dramatic for relatively intact foods (e.g., carrots and beef chunks) but decreased in importance for those foods which were already disrupted or dispersed by the manufacturing process (e.g., ground beef, soups). As would be expected, also, from the continued breakdown of tissues into smaller particles, increased blending time in either instrument resulted in decreased filterability (Fig. 7).

Effect of food concentration. Although the quantity of filtrate obviously increases with decreasing concentration of food in the suspension, the total weight of food brought into contact with the MF may change either way (Fig. 8). Thus, chicken became less, whole milk slightly more, and skim milk much more filterable.

Effect of age of the food. The filterability of both stomached and blended ground beef, green beans, and turbot decreased with increasing age up to 48 h (Fig. 9). This trend might be expected from the decreasing tissue integrity caused by autolysis and microbial growth and the increased microparticulate content of the suspensions which would result. In contrast, the filterability of (stomached) whole milk increased dramatically by 48 h—a consequence of the removal of particulate material during clotting, which was not effectively resuspended by the homogenization.

Effect of surfactant at 20°C. At this temperature, the effect of adding 1% Tween 80 or Triton X-100 was very variable. The filtration rate for stomached ocean perch increased moderately with Triton X-100 and greatly with Tween 80 added (Fig. 10). Whole milk and blended green beans filtered slightly faster with surfactant added, whereas stomached ground beef and blended potato filtered less rapidly

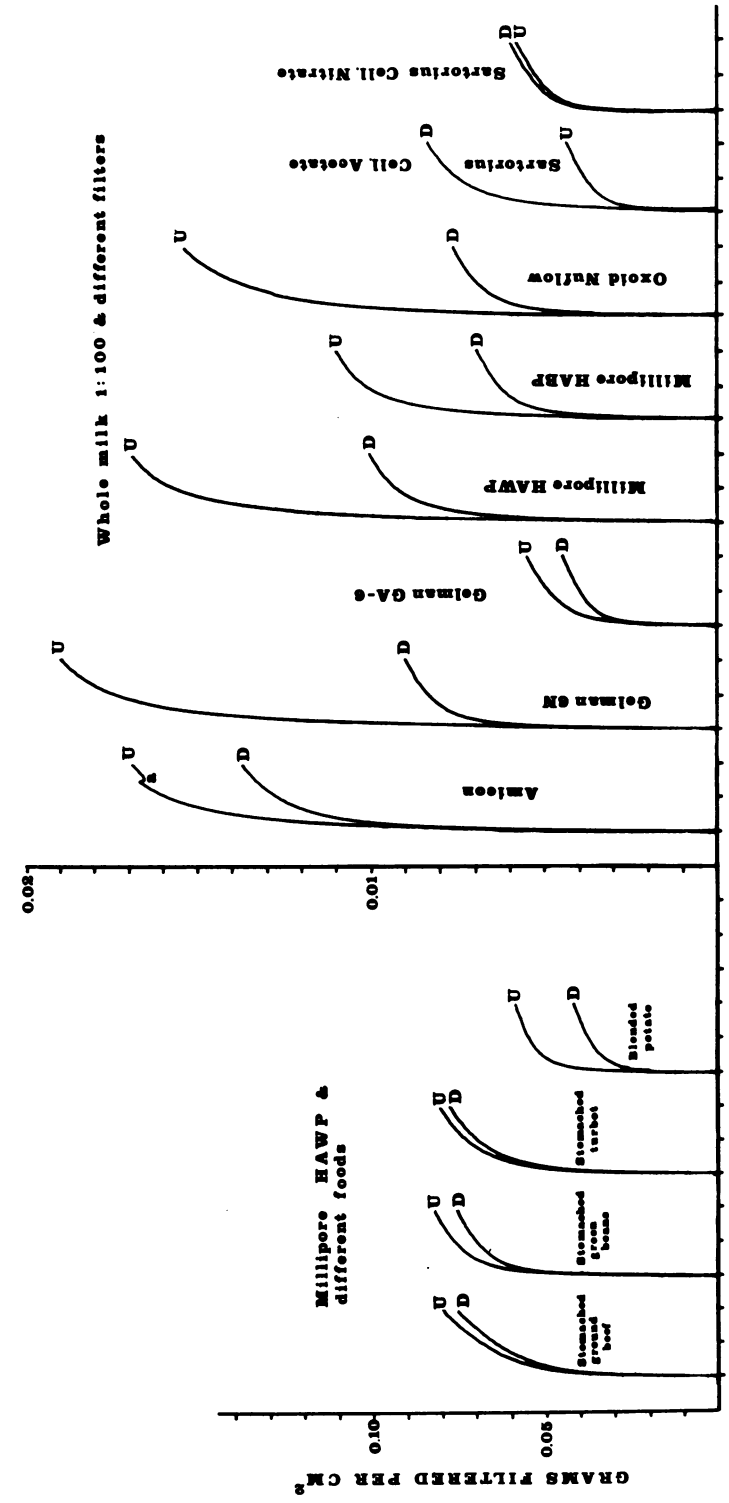


FIG. 4. *Variation of membrane filtration rate with direction of flow through 0.45-μm MF. U, Upper side of MF in box (as packed) placed uppermost in apparatus (i.e., in contact with liquid to be filtered); D, flow direction reversed; a, inflexion caused by bubble in delivery tube.*

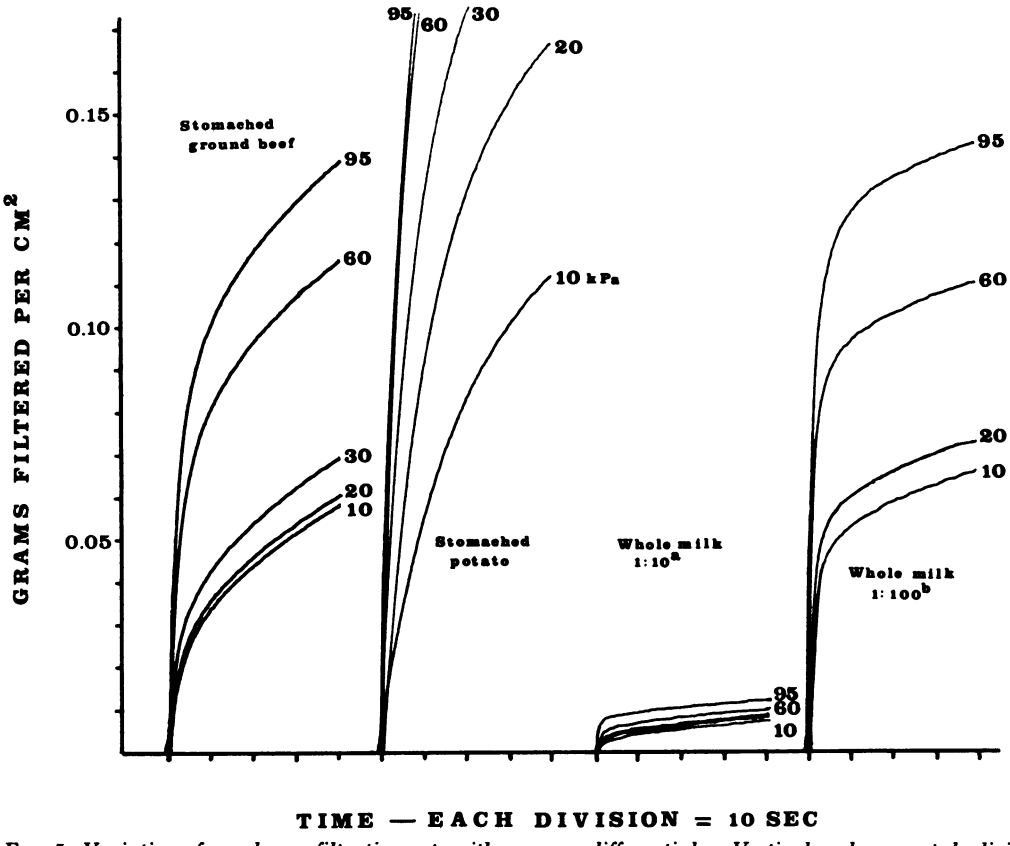


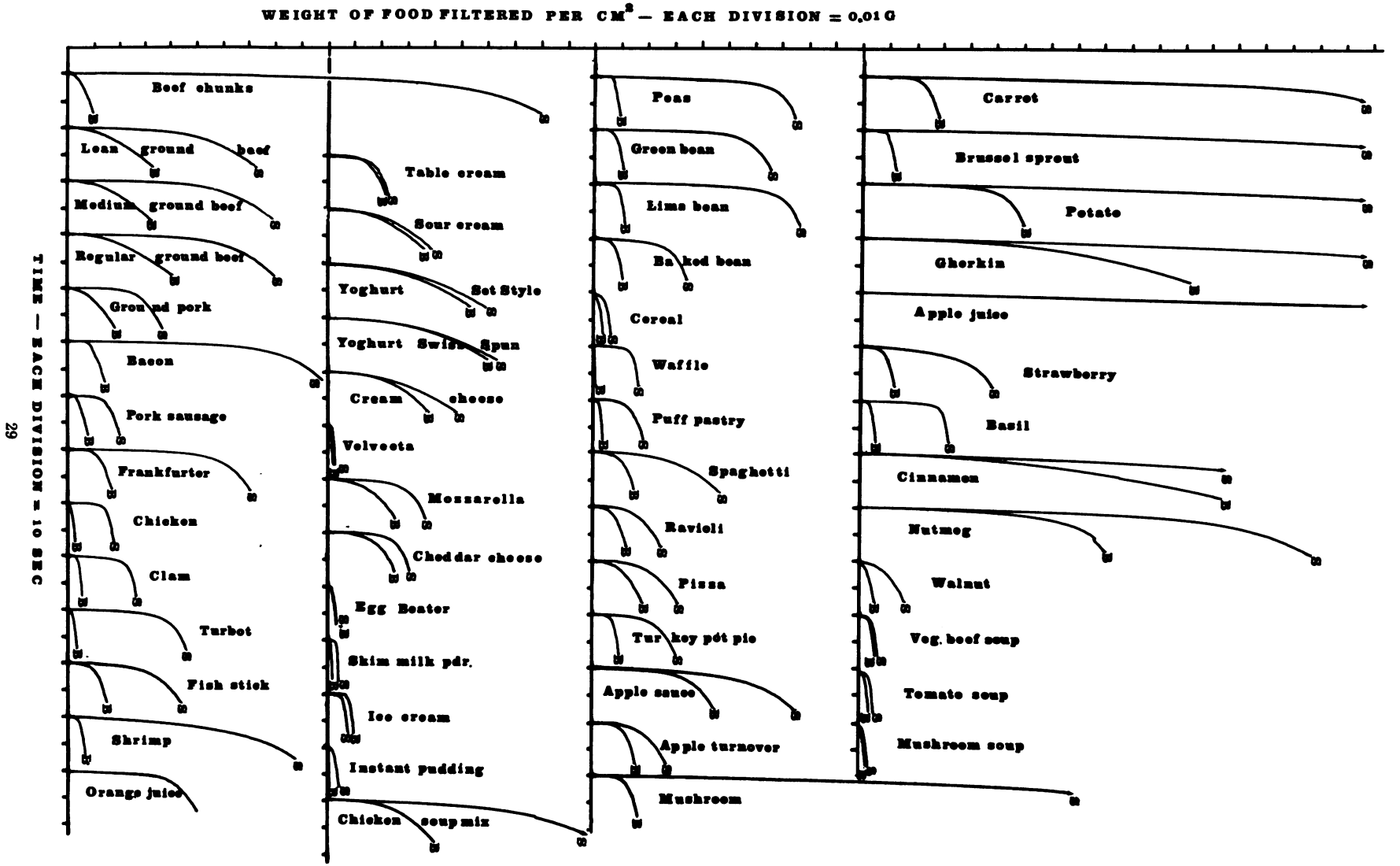
FIG. 5. *Variation of membrane filtration rate with pressure differential. a, Vertical scale correct; b, divide vertical scale by 10.*

(Fig. 10). Vegetable beef soup and Velveeta (Fig. 11) were not noticeably affected by addition of Triton X-100. Its addition to stomached butter (Fig. 11) decreased the filtration rate dramatically, and higher concentrations (e.g., 2, 5, and 10%; data not shown) decreased the rate still further.

Effect of temperature without surfactant. Over the range 10 to 40°C and without added surfactant, increasing temperature produced an increased filtration rate. For blended potato, vegetable beef soup, Velveeta, and stomached whole milk (Fig. 11), the increase was modest. In the first three, the increase is believed to be mainly due to the decreasing viscosity of water. (Thus, plotting the quantity of blended potato filtered after 15 or 30 s against the reciprocal of the viscosity of water at the different temperatures yielded an almost straight line; graph not shown). Whole milk filtered faster at 40°C than would be expected from viscosity decrease alone, indicating that some conformational change may be important. Stomached butter, which filtered very rapidly at 20 to 40°C, was dramatically slower at 10°C—presumably an effect of the cohesivity of fat in the Stomacher bag.

Combined effects of surfactant and temperature. The effect of these two factors together was complex. All foods filtered more rapidly at the higher temperatures, with or without Triton X-100. Blended potato filtered less rapidly at all temperatures with the surfactant present (Fig. 11), and vegetable beef soup was not greatly affected (Fig. 11), whereas the poor filterability of Velveeta improved very slightly at 40°C with the surfactant present (Fig. 11). However, concentrations of Triton X-100 up to 5% at 40°C did not improve the filterability of Velveeta further (Fig. 12). Filtration of stomached butter at 20 to 40°C was dramatically inhibited by Triton X-100 (Fig. 11); in this case, the surfactant presumably increased the amount of fat

FIG. 6. *Membrane filtration rates for 10% suspensions of 54 common foods. Except for apple and orange juices, graphs show both stomached (S) and rotary-blended (B) preparations. For data on whole and skim milk, butter, and ocean perch, see Fig. 5 and 8 to 11.*



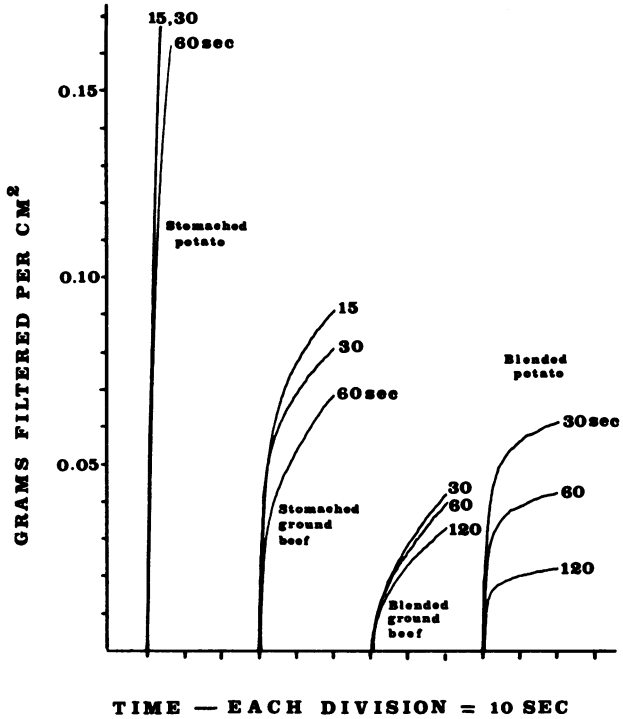


FIG. 7. *Variation of membrane filtration rate with suspension preparation time.*

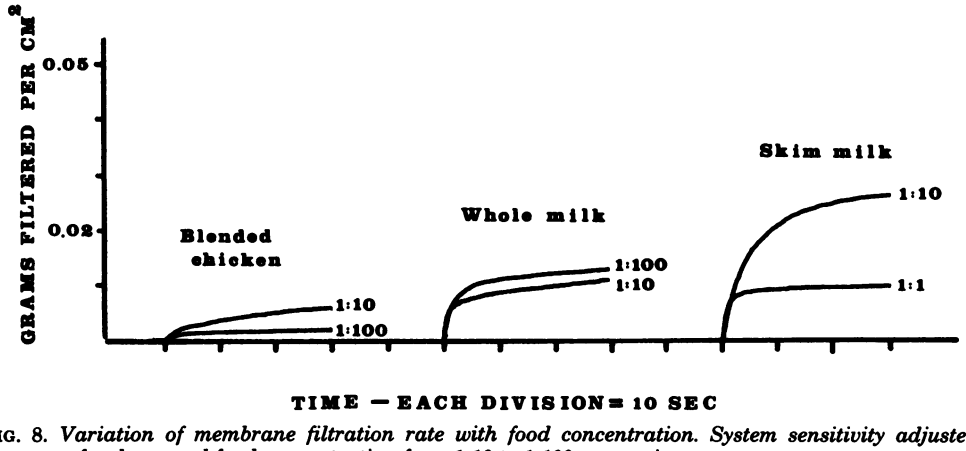


FIG. 8. *Variation of membrane filtration rate with food concentration. System sensitivity adjusted to compensate for decreased food concentration from 1:10 to 1:100 suspensions.*

dispersed as globules by the Stomacher. Whole milk, on the other hand, although being relatively unaffected at 10 and 20°C, filtered somewhat better at 30°C and dramatically better at 35 and 40°C (Fig. 11); in this case, fat globules were presumably removed by solubilization in the surfactant.

Effect of protease. The dramatic improvement in filterability of Velveeta after 70 min of incubation with Pronase contrasts markedly with the insignificant improvement caused by up to 5% Triton X-100 (Fig. 12). Trypsin produced a smaller improvement during this time; the effect of both proteases increased greatly between 60 and 70 min, evidence that the distribution of particle sizes passes through a critical range. Similar improvements were noted for skim milk powder (Fig. 12). In this case, however, the relative effect of the two proteases was reversed.

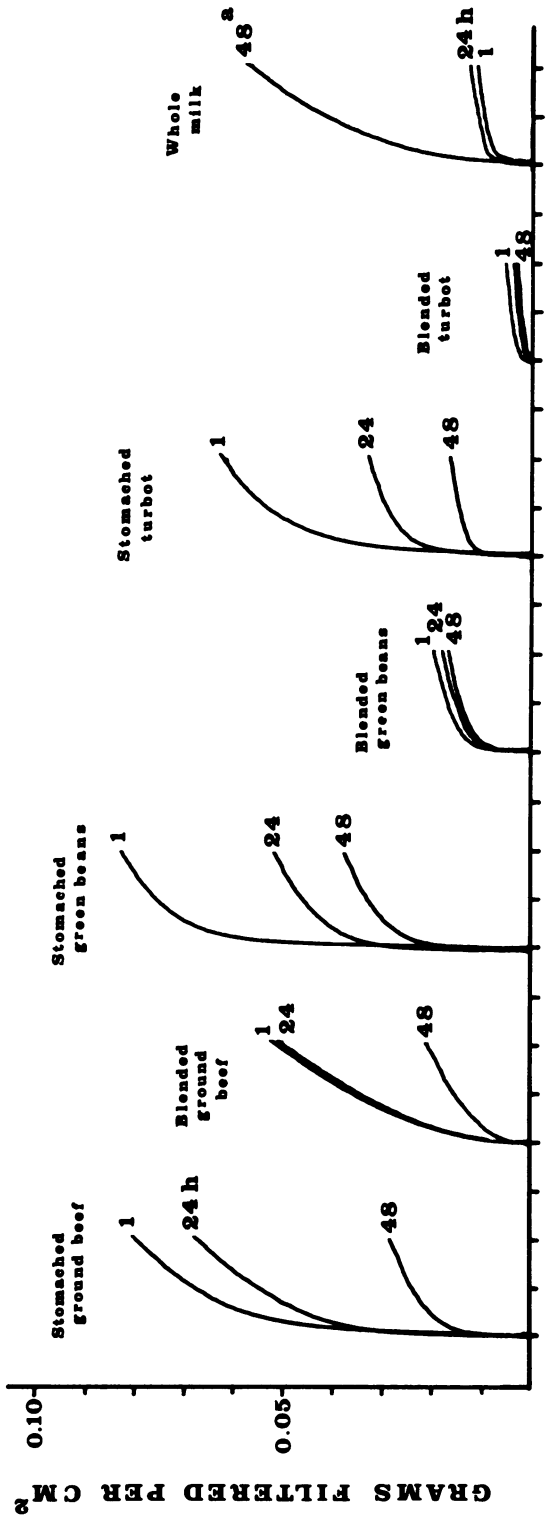


FIG. 9. *Variation of membrane filtration rate with age of food (1, 24, and 48 h). a, Stomached before filtration.*

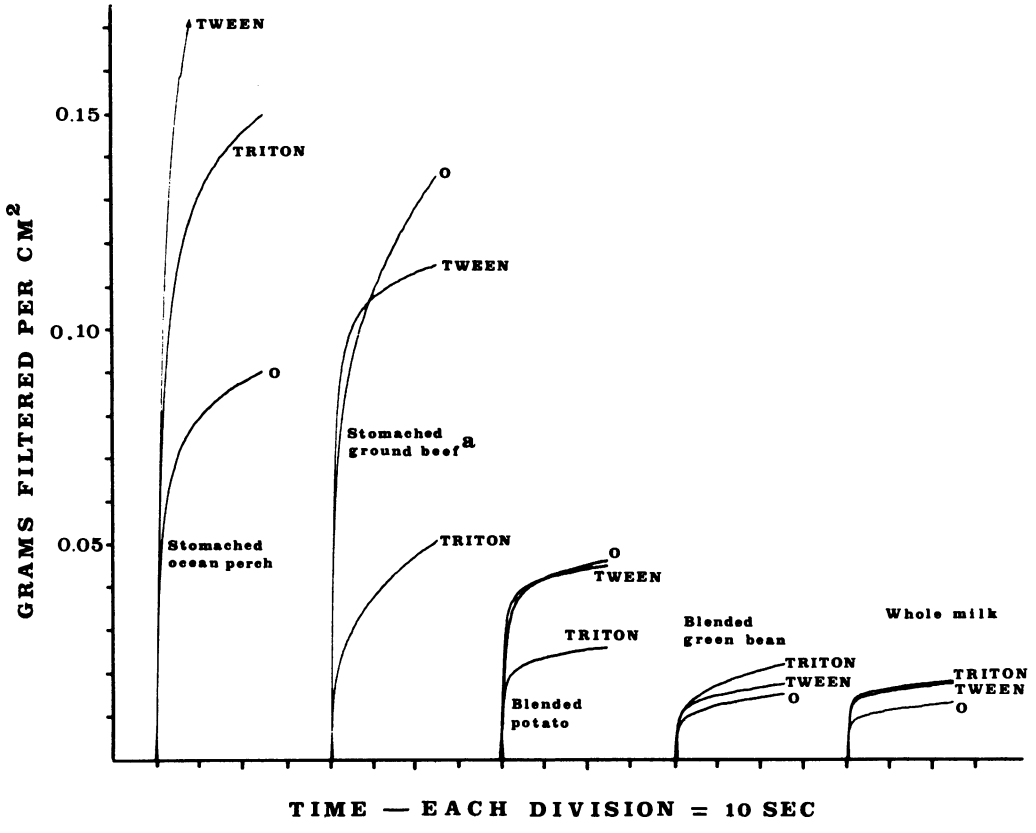


FIG. 10. *Variation of membrane filtration rate with addition of 1% surfactant at 20°C. a, Different regular ground beef specimen to those in Fig. 2 to 9.*

DISCUSSION

The microbiological attractions of MF are generally seen to be in situations exploiting their ability to concentrate organisms from a relatively dilute and easily filterable specimen, i.e., lower the limit of detection of the standard plating procedure (29) or allow removal of microbial growth inhibitors (2, 23). There are certainly instances where these properties would be valuable in food microbiology, for example, in the determination of *Salmonella* or yeasts in certain foods. Many of the foods examined in this study will be seen to be filterable at levels which would allow concentration of organisms 10-fold or more, compared with conventional plating procedures. The finding of Sharpe and co-workers (23) that the relative affinities of common food bacteria and food particles for MF are different enough to allow food debris to also be selectively removed from the filter indicates that the MF method has potential for the rapid detection (or even enumeration) of, say, *Salmonella*. For example, it may be possible to produce fluorescent antibody-stained colonies directly, having sufficient signal-to-noise ratio for electronic detection.

The membrane filterability of a food suspension is determined by its microparticulate content. These particles cause a steadily increasing resistance to flow through their adsorption along the MF pores or their accumulation as a filter cake. Alternatively, they may completely block the pores. At the beginning of this study we expected that starch or protein particles would be likely to cause the first (standard) type of blocking and fat or oil globules would cause the second (complete blocking). However, the data indicate that either effect may occur without distinction. Visible particles do not block MF and are preferable because their presence implies a lower concentration of smaller particles. The observed effects of suspension preparation by Stomacher and rotary blender are consistent with those expected from the different mechanical and physical actions of the two blending

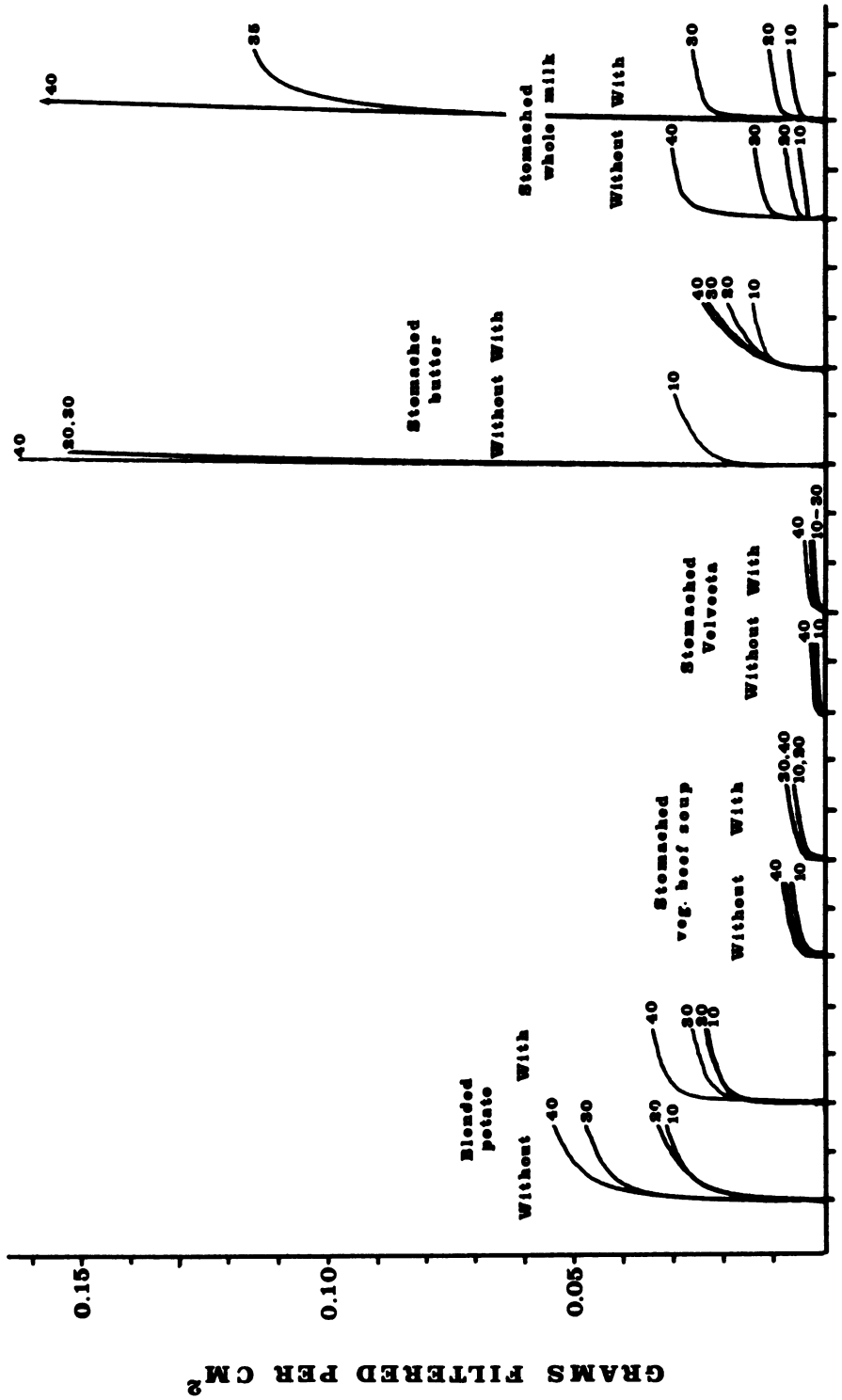


FIG. 11. *Variation of membrane filtration rate with temperature, with and without Triton X-100 (1%) added.*

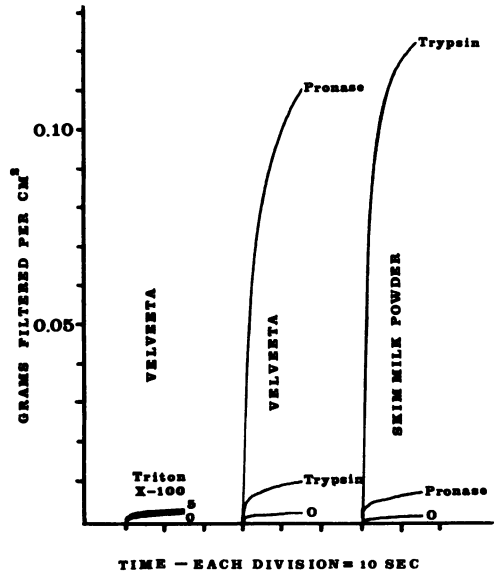


FIG. 12. *Relative effects of surfactant (0 to 5% Triton X-100) and protease treatments (70 min) on the membrane filtration rate of Velveeta (left and center). Also relative effects of trypsin and Pronase treatments (70 min) on filterability of skim milk powder (right).*

methods. The Stomacher is always observed to be less destructive to tissues than rotary blenders (5, 21, 24, 31). Use of the Stomacher and the minimum possible stomaching time for adequate dispersal of organisms are thus to be preferred for any preparation of samples for membrane filtration.

Lot to lot consistency and even choice of 0.45- versus 0.8-μm pore size from a given manufacturer appear to be minor considerations in membrane filtration. However, the chemical or physical composition of MF from each manufacturer may be relevant in the reliability of filtration. Similarly, the best direction of flow through the MF should be determined. Direction of filtration is usually quoted with maximum recovery (particularly of fecal coliform organisms) in mind (30) but its importance to flow rate is obviously significant. Except for the Sartorius filters examined here, the best direction for filtration seemed to coincide with the best direction (implied) for microbial recovery. However, it should be borne in mind that when the organisms are filtered in the presence of appreciable quantities of food debris any effect on recovery ascribed to surface roughness, etc., may be considerably perturbed (most probably being eliminated). In the absence of further data, filtering in the direction of maximum flow rate is to be preferred.

In the ordinary laboratory filtration set-up, pressure differential is largely uncontrollable, varying according to the pump, filtration rate, aeration of the sample, etc. There is no evidence to indicate the desirability of filtering at anything other than the maximum pressure differential obtainable with any given apparatus. Warm suspensions are preferable to cold ones, simply on account of the reduced viscosity of water.

The effect of diluting the sample is most interesting. Reports of improved filtration rates on diluting dairy products (9, 18) are supported by our data. Changes in the degree of dispersal, dissolution and conformation of tissue components, etc., are obviously important. It is quite possible that changes in ionic strength, pH, etc., might also be significant, and these should be investigated if difficulties in filtering are experienced.

It is apparent that combinations of the use of elevated temperatures, suitable surfactants, and incubation with proteases (and possibly amylases) are capable of producing dramatic increases in the filterability of foods, according to whether fat, protein, or starch particles are the principle cause of filter pore blocking. Thus, if filtration difficulties are experienced and if the organisms are relatively tolerant, an array of procedural modifications is available to provide the desired level of filterability. It can be safely concluded that most foods are (or can be made) filterable at levels relevant to most microbiological analytical requirements.

From the point of view of the straightforward trading of many conventional manual agar plating methods (for example, the standard plate, coliform, and *Staphylococcus aureus* counts) for instrumented methods based on HGMF, concentration of the food is unnecessary. In these cases, it is more important to know whether MF or HGMF of convenient size will filter at least the maximum weight of food encountered in a normal plating procedure within a reasonable time and particularly what factors limit the procedure. For example, if a procedure is to be carried out unattended, it is important that the probability of incomplete filtration be very low.

The filtration area provided by a typical 6.0-cm square HGMF of 10,000 grid cells, with hydrophobic barriers 0.013 cm wide, is 14.4 cm^2. Because for most plate counts the greatest quantity of food taken is 1.0 ml of a 1:10 homogenate (i.e., 0.1 g) it is required that the filtration curve reliably pass 0.007 g/cm^2 within the time allowed for the food to be considered filterable at a level pertinent to the conventional analysis. It will be seen from the graphs that, without the use of

procedural modifications to facilitate filtration, only Velveeta, Egg Beaters, and skim milk powder, out of 58 food specimens, failed to reach this level within 15 s, and five others (vanilla instant pudding, Captain Crunch cereal, and vegetable beef, mushroom, and tomato soups) were in a range where the reliability of filtration might be doubted. By modifying the preparation procedure, even the rather intractable Velveeta could be made easily filterable at this level. We conclude that the attractions of instrumented methods of food microbiological analysis based on HGMF need not be limited by the filterability of food suspensions and that reduction of the limit of detection of microorganisms from any food by concentration using MF is distinctly feasible.

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LITERATURE CITED

1. **Barber, F. W., C. P. Burke, and H. Fram.** 1954. The Millipore filter technique in the dairy industry. J. Milk Food Technol. **17**:109–112.
2. **Busta, F. F., and M. L. Speck.** 1965. Enumeration of *Bacillus stearothermophilus* by use of membrane filter techniques to eliminate inhibitors present in milk. Appl. Microbiol. **13**:1043–1044.
3. **Claydon, T. J.** 1975. A membrane-filter technique to test for the significance of sublethally injured bacteria in retail pasteurized milk. J. Milk Food Technol. **38**:87–88.
4. **Ehrlich, R.** 1953. Membrane filter method for determination of coliforms in pasteurized and certified milk. J. Milk Food Technol. **16**:6–8.
5. **Emswiler, B. S., C. J. Pierson, and A. W. Kotula.** 1977. Stomaching *vs* blending. Food Technol. (Chicago) **31**:40–42.
6. **Fifield, C. W., J. E. Hoff, and B. E. Proctor.** 1957. The Millipore filter for enumerating coliform organisms in milk. J. Dairy Sci. **40**:588–589.
7. **Frazier, W. C., and D. F. Gneiser.** 1968. Short-time membrane filter method for estimation of numbers of bacteria. J. Milk Food Technol. **31**:177–179.
8. **Goff, J. H., T. J. Claydon, and J. J. Iandolo.** 1972. Revival and subsequent isolation of heat-injured bacteria by a membrane filter technique. Appl. Microbiol. **23**:857–862.
9. **Graves, D. C., and I. A. Schipper.** 1966. Membrane filtration technique for isolating organisms from raw milk of normal udders. Appl. Microbiol. **14**:535–538.
10. **Halden, H. E.** 1957. Methods for count of microorganisms in liquid sugar. J. Am. Soc. Sugar Beet Technol. **9**: 393–399.
11. **Halden, H. E., D. D. Leethem, and F. G. Eis.** 1958. Developments in methods for microbiological control in liquid sugar. J. Am. Soc. Sugar Beet Technol. **10**: 138–141.
12. **Halden, H. E., D. D. Leethem, and F. G. Eis.** 1960. Determination of microorganisms in sugar products by the Millipore method. J. Am. Soc. Sugar Beet. Technol. **11**:137–142.
13. **Hermans, P. H., and H. L. Bredée.** 1936. Principles of the mathematical treatment of constant pressure filtration. J. Soc. Chem. Ind. London Trans. Commun. **55**: 1–4.
14. **Kirkham, W. K., and P. A. Hartman.** 1962. Membrane filter method for the detection and enumeration of *Salmonella* in egg albumen. Poult. Sci. **41**:1082–1088.
15. **Moroz, R.** 1957. Determination of yeast in sugar liquids using membrane filters. Int. Sugar J. **59**:70–71.
16. **Nutting, L. A., P. C. Lomot, and F. W. Barber.** 1959. Estimation of coliform bacteria in ice cream by use of the membrane filter. Appl. Microbiol. **7**:196–199.
17. **Reusse, U.** 1959. Quantitative determination of *Brucella* in cow's milk by means of membrane filters. Bull. W.H.O. **21**:782–784.
18. **Rodriguez, R. B., and W. G. Walter.** 1957. Use of the membrane filter for the microbiological testing of dairy products. Sanitarian (Los Angeles) **19**:196–197 & 218.
19. **Roth, K.** 1970. Methodik einer mikrobiologischen Kontrolle bei der Faswasche. Brauwelt **110**:567–573.
20. **Scheuermann, E. A.** 1970. Betriebskontrollen und Prozessfiltrationen: Membranfilter in der Nahrungsmittelindustrie. Ernahrungswirtschaft **10**:840–844.
21. **Schiemann, D. A.** 1977. Evaluation of the Stomacher for preparation of food homogenates. J. Food Protect. **40**: 445–448.
22. **Sharpe, A. N.** 1978. Some theoretical aspects of microbiological analysis pertinent to mechanization, p. 19–40. *In* A. N. Sharpe and D. S. Clark (ed.), Mechanizing microbiology. Charles C Thomas, Publisher, Springfield, Ill.
23. **Sharpe, A. N., M. P. Diotte, I. Dudas, and G. L. Michaud.** 1978. Automated food microbiology: potential for the hydrophobic grid-membrane filter. Appl. Environ. Microbiol. **36**:76–80.
24. **Sharpe, A. N., and A. K. Jackson.** 1972. Stomaching: a new concept in bacteriological sample preparation. Appl. Microbiol. **24**:175–178.
25. **Sharpe, A. N., and G. L. Michaud.** 1974. Hydrophobic grid-membrane filters: new approach to microbiological enumeration. Appl. Microbiol. **28**:223–225.
26. **Sharpe, A. N., and G. L. Michaud.** 1975. Enumeration of high numbers of bacteria using hydrophobic grid-membrane filters. Appl. Microbiol. **30**:519–524.
27. **Sharpe, A. N., and G. L. Michaud.** 1978. Enumeration of bacteria using hydrophobic grid-membrane filters, p. 140–153. Chapter 10 *In* A. N. Sharpe and D. S. Clark (ed.), Mechanizing Microbiology. Charles C Thomas, Publisher, Springfield, Ill.
28. **Silliker, J. H., H. P. Andrews, and J. F. Murphy.** 1957. A new non-destructive method for the bacteriological sampling of meats. Food Technol. (Chicago) **11**: 317–318.
29. **M. L. Speck (ed.).** 1976. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
30. **Tobin, R. S., and B. J. Dutka.** 1977. Comparison of the surface structure, metal binding, and fecal coliform recoveries of nine membrane filters. Appl. Environ. Microbiol. **34**:69–79.
31. **Tuttlebee, J. W.** 1975. The Stomacher—its use for homogenization in food microbiology. J. Food Technol. **10**:113–122.