Effect of Whey Composition on Ultrafiltration Performance

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The ultrafiltration (UF) of acid, cheese, and Shrikhand wheys is strongly affected by membrane fouling. UF membrane performance is significantly improved by a clarification procedure consisting of raising the pH to 7.5 and filtering out the precipitate. This improvement due to clarification can be attributed not only to the reduction in Ca salts but also to the reduced concentration of high molecular weight proteins such as immunoglobulin G, which have isoelectric points near the clarification pH. UF data with previously well-characterized membranes showed that the flux/protein rejection characteristics of the clarified wheys are similar to those for filtration of single-component (BSA) solutions.

Keywords: *Clarification; pretreatment; whey; ultrafiltration; immunoglobulins*

INTRODUCTION

The disposal of whey, a major effluent of the dairy industry, is a major problem worldwide. Ultrafiltration (UF) of acid whey (Hayes et al., 1974; Lee and Merson, 1976; Cheryan and Kuo, 1984; Patocka and Jelen, 1987) and sweet cheese whey (Taddei et al., 1988; Labbé et al., 1990) is commercially practiced; however, loss of membrane productivity is a perennial problem.

Membrane fouling during the processing of acid whey (Lee and Merson, 1976; Patocka and Jelen, 1987) or sweet cheese whey (Taddei et al., 1988; Labbé et al., 1990) is attributed mainly to the precipitation of Ca salts, especially calcium phosphates. Pretreatment of whey to remove Ca salts has been shown to increase membrane productivity. For example, flux improvements have been observed after removal of Ca by chelation with EDTA for acid whey (Lee and Merson, 1976; Patocka and Jelen, 1987), by addition of citrate to acid whey (Patocka and Jelen, 1987) or sweet cheese whey (Taddei et al., 1988), or by replacement of Ca with Na for acid whey (Patocka and Jelen, 1987).

It is also known that feed pH affects membrane fluxes during whey filtration. Lee and Merson (1976) found increased fluxes after acidification of acid whey. Nilsson (1988) has observed higher flux and lower flux loss when whey protein solutions were filtered at high pH. Mohr et al. (1989) reported that acid whey shows lower flux than cheese whey.

The present study is aimed at understanding the reasons for improved UF performance after a simple clarification procedure (Lee and Merson, 1976) consisting of raising the pH to 7.5 and filtering the precipitate. As mentioned above, previous studies have emphasized the reduction of Ca salts due to this procedure, whereas in this paper we also consider the role of various types of whey proteins. As a point of reference, we compare the ultrafiltration characteristics of unclarified and clarified wheys with those of a single standard protein [bovine serum albumin (BSA)] through two different previously characterized membranes.

Thus, we compare the UF performance of wheys from three different sources: acid (AW), cheese (CW), and Shrikhand (SW) with poly(acrylonitrile) (PAN) and poly(acrylonitrile-*co*-acrylamide) membranes. The copolymer contained 30 mol % acrylamide and is referred to as PAN-2 to be consistent with our earlier work (Musale and Kulkarni, 1996). We compare the UF of unclarified (without pretreatment) and clarified (adjusting the whey pH to 7.5 and then filtering) wheys with respect to permeate flux, protein rejection, and flux recovery as a function of pH.

EXPERIMENTAL PROCEDURES

Whey Preparation and Composition. All three wheys were prepared from buffalo milk of standard composition (6% fat, 14.8% total solids, 3.2% proteins) according to the procedures outlined below.

Acid Whey (AW). The pH of the milk kept at 30 °C was reduced to 4.5 using 2 N HCl. The milk was maintained at this pH for 1 h and then warmed at 40 °C for 10 min to settle the precipitated casein. The whey was filtered through muslin cloth and then centrifuged at 4000 rpm for 30 min.

Cheese Whey (CW). Milk, kept at 30 °C, was stirred for 20 min with 0.015 w/v % $CaCl_2$ and 5 v/v % curds as the starting culture, followed by addition of 20 ppm rennet. After thorough mixing, the mixture was kept at 30 °C for 45 min; the cheese was cut and further kept at 45 °C for 15 min. The whey was separated from cheese by filtering through muslin cloth and then by centrifugation at 4000 rpm for 30 min.

Shrikhand Whey (SW). Curds were added to the milk and kept at 40 °C for 6 h. The whey was obtained by filtering the formed curds through muslin cloth and centrifuged at 4000 rpm for 30 min.

Clarified Wheys. In a second set of experiments, all three wheys were clarified prior to UF by raising the

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Table 1. Experimentally Measured Composition (Weight Percent) and Properties of Different Unclarified and Clarified Whey Samples Used

	acid whey		cheese whey		Shrikhand whey	
constituent	unclarified	clarified	unclarified	clarified	unclarified	clarified
pH	4.2	7.5	5.9	7.5	4.8	7.5
specific conductivity (mS cm^{-1})	5.0	7.2	3.0	4.9	3.5	7.2
fat[a]	0.1	0.1	0.2	0.1	<0.1	<0.1
protein[b]	0.74	0.64	0.74	0.70	0.81	0.74
lactose[c]	5.65		4.77		5.05	
calcium[d] (ppm)	1770	232	650	367	1080	255
ash[e]	0.8	0.5	0.6	0.7	0.5	0.8
intrinsic viscosity[f] (dL/g)	0.022	0.024	0.024	0.026	0.023	0.024

[a] Determined by Gerber test (Pearson, 1970). [b] Determined by the method of Lowry et al. (1951). [c] Determined by the DNSA method of Robyt and Whelan (1972). [d] Determined by atomic absorption spectroscopy. [e] Determined by gravimetric analysis. [f] Measured in aqueous media using Ostwald viscometer at 28 °C.

Table 2. Membrane Characteristics

	acrylamide content[a]	contact angle[b]	surface free energy[c] (erg/cm^2)			pure water flux[d]
polymer	(mol %)	(deg)	γ_s^p	γ_s^d	γ_s	(lm^{-2} h^{-1})
PAN	0	73	8	31	39	200
PAN-2	30	56	26	13	39	200

[a] Determined by ^1H NMR (80 MHz) in DMSO-d_6. [b] Measured on polymer films by sessile drop method using goniometer. [c] Calculated as given by Musale and Kulkarni (1996). [d] At 200 kPa.

pH to 7.5. The wheys were allowed to stand at this pH for 10 min at 27−28 °C. The precipitated solids were then filtered out with Whatman paper (No. 1). Such pretreated wheys are designated clarified wheys.

The compositions of each of the unclarified and clarified wheys studied i.e. AW, CW and SW are listed in Table 1. The compositions reported are the average of two replicate measurements. The values for unclarified acid and cheese whey compare well with those reported by other investigators [e.g., Kosikowski (1979)].

Electrophoresis (SDS−PAGE) of both unclarified (natural pH) and clarified whey (pH 7.5) samples was carried out using 10% polyacrylamide gel, and the separated proteins were silver stained according to the procedure of Gross et al. (1987). The calcium content was determined by atomic absorption spectroscopy (Chemito, model 201, India). The protein concentrations in the feed and permeate samples were determined according to Lowry's method (Lowry et al., 1951). All other standard analytical methods are mentioned in Table 1.

UF Studies. The polymer and membrane preparation methods have been described before (Musale and Kulkarni, 1996). PAN was obtained from Indian Petrochemicals Ltd., and the copolymer (PAN-2) was based on acrylonitrile and acrylamide in the mole ratio 70:30.

Both PAN and PAN-2 membranes have similar pore size distributions (mode pore diameter of 20 nm) and similar pure water fluxes (Table 2). Zeta potential measurements (Musale and Kulkarni, 1996) indicate that PAN is more negatively charged than PAN-2 in the pH range studied. Contact angle measurements show that PAN-2 membrane is more hydrophilic, has a higher polar component of surface energy, and has a lower dispersion force component in comparison to PAN (Table 2). The data in Table 2 are the averages of 2 replicate measurements for contact angle and surface free energy and of 10 replicate measurements for pure water flux.

Each whey was ultrafiltered in a 13.4 cm^2 area stirred cell (Amicon) at 600 rpm, in the pH range 4.5−6.5 (for unclarified whey UF) and 4.5−7.5 (for clarified whey UF) at 200 kPa. In the case of unclarified whey UF, the pH was adjusted with 2 N NaOH or 2 N HCl, whereas in the case of clarified whey UF, the pH was reduced by 2 N HCl. The above pH range includes values both above and below the isoelectric points (IEPs) of the major whey proteins. Flux recoveries were determined after 5× volume concentration runs with unclarified wheys by measurement of the initial water flux before use and the final water flux after the used membrane had been washed for 10 min with distilled water. This water wash is intended to remove deposited material corresponding to "reversible" fouling and thereby measure the flux loss due to factors such as membrane adsorption.

RESULTS AND DISCUSSION

The differences in composition between the various whey types (AW, CW, and SW) as well as the effects of clarification on whey composition are discussed first. The characteristics of unclarified whey UF with two types of membranes (PAN, hydrophobic; PAN-2, hydrophilic) are then analyzed and compared with the corresponding UF data with clarified wheys.

Whey Composition. The compositions of all three wheys are shown in Table 1. Gel electrophoresis (SDS−PAGE) results (Figure 1) show that all three wheys contain approximately similar proteins: α-lactalbumin (α-LA) (MW ∼ 14000), β-lactoglobulin (β-LB) (MW ∼ 18000), BSA (MW ∼ 68000), and immunoglobulins (Igs) (MW ∼ 160000). The major whey proteins, namely α-LA, β-LB, and BSA, have an IEP at pH ∼5, whereas immunoglobulin G (IgG) (the major variant among all immunoglobulins which together constitute 12% of the total whey protein) has an IEP at pH 7 (Hanemaaijer et al., 1989).

Though the protein compositions in the three wheys appear to be similar, there are significant differences in pH as well as other components (Table 1). The acidity decreases in the order AW > SW > CW. The calcium content decreases in similar order AW > SW > CW. AW also contains slightly more lactose than CW and SW. An important difference in SW compared to the other wheys is that ∼7.5% of the lactose has been fermented to lactic acid (determined as titratable acidity).

Although it is well-known that fat content can affect UF fluxes markedly, the fat levels were constant at a low level (∼<0.1−0.2%) in the centrifuged whey samples studied here. Hence, no effect of fat content was observed in this study.

Whey Clarification: Effect on Whey Composition. It is reported that permeate fluxes can be

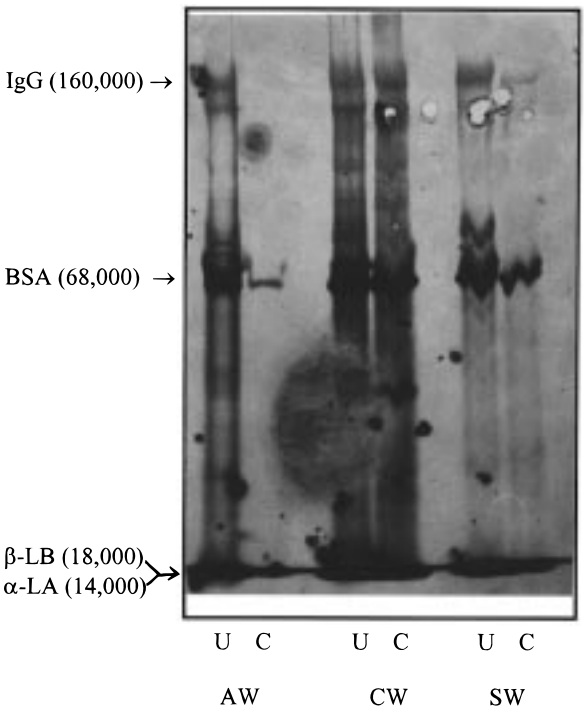

Figure 1. Electrophoresis (SDS−PAGE) staining patterns for unclarified (natural pH) and clarified (pH 7.5) wheys, 10% gel, silver stained: U, unclarified; C, clarified.

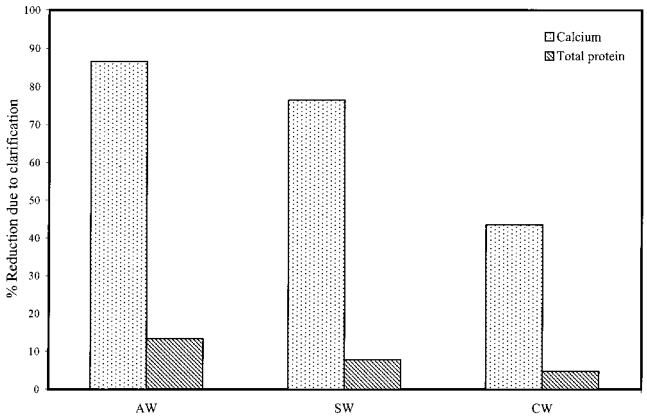

Figure 2. Effect of whey clarification on Ca and protein concentration for all three wheys.

increased by raising the pH of whey and filtering the precipitated $CaPO_4$ (Lee and Merson, 1976). In the present study this feed pretreatment was investigated from a dual viewpoint of examining this strategy for flux increase as well as identifying the factors controlling the UF flux.

It is apparent that the main differences between the clarified and original whey samples (other than pH) lie in the calcium and protein contents. The percent reductions in these two solutes after clarification are shown in Figure 2. Clarification reduces both the Ca content and the total protein content in the order AW > SW > CW. The reduction in protein content appears to be mainly due to the removal of the minor component IgG, which has an IEP at pH 7 and is thus most likely to precipitate at the clarification pH. This is supported by the gel electrophoresis results shown in Figure 1; the band intensity corresponding to IgG is almost completely invisible in clarified AW, reduced significantly in SW, and little affected in the case of CW. The BSA

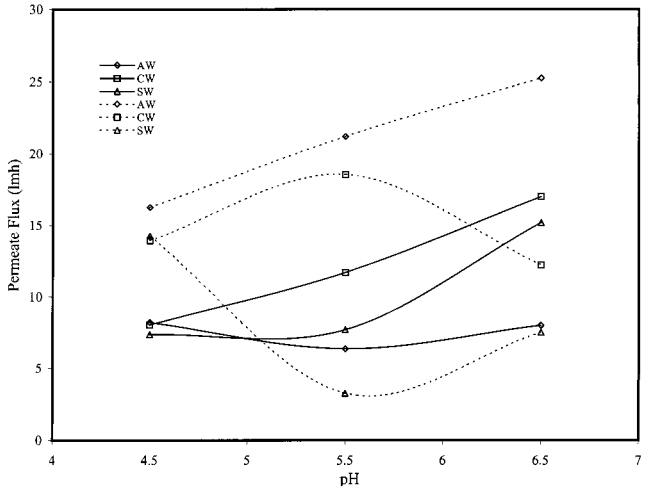

Figure 3. Effect of pH on permeate flux for UF of unclarified wheys for PAN and PAN-2 membranes, 200 kPa, 600 rpm: solid lines, PAN; dotted lines, PAN-2.

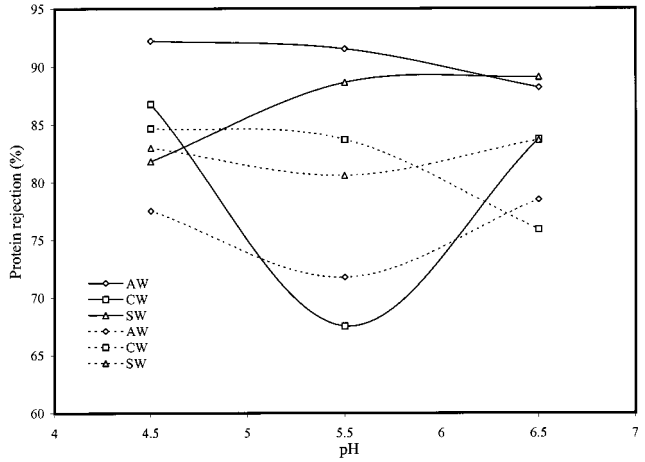

Figure 4. Effect of pH on total protein rejection for UF of unclarified wheys for PAN and PAN-2 membranes, 200 kPa, 600 rpm: solid lines, PAN, dotted lines, PAN-2.

band intensity in AW also appears reduced; however, this reduction is not seen in the other whey samples.

The intrinsic viscosity is slightly increased in all three wheys after clarification (Table 1); this may be attributed to the difference in mineral content.

Unclarified Whey UF Characteristics. The flux and rejection data for unclarified whey UF are shown at a constant VCF of 1.67 as a function of pH for PAN and PAN-2 in Figures 3 and 4

The flux decline with the three natural wheys was modeled by estimating the concentration polarization caused by protein rejection. The mass transfer coefficient, k, and the protein concentration at the membrane surface, C_m were calculated from eq 1 by the

$$J_v = k \ln \frac{C_m - C_p}{C_r - C_p} \quad (1)$$

nonlinear regression method used previously (Musale and Kulkarni, 1996). The values of k and C_m calculated by this method are shown in Table 3.

Although the hydrophilic copolymer membrane had consistently higher fluxes than PAN during our previous studies on standard BSA UF (Musale and Kulkarni, 1996, 1997), the whey data do not show this difference

Table 3. Mass Transfer Coefficient, k, and Protein Conentration at the Membrane Surface, C_m, for UF of Various Wheys

	acid whey		cheese whey		Shrikhand whey	
pH	$k \times 10^4$ (cm s^{-1})	C_m (mg/mL)	$k \times 10^4$ (cm s^{-1})	C_m (mg/mL)	$k \times 10^4$ (cm s^{-1})	C_m (mg/mL)
			PAN			
4.5	1.14	95	0.57	575	3.41	17
5.5	0.41	1119	0.92	192	1.25	29
6.5	0.49	1490	1.68	342	1.38	25
			PAN-2			
4.5	*a*	*a*	1.66	13	0.88	131
5.5	1.90	102	3.31	21	0.44	2383
6.5	4.06	68	*a*	*a*	1.86	114

[a] Data could not be fitted with satisfactory mean square errors; hence, the calculated parameters are not reliable.

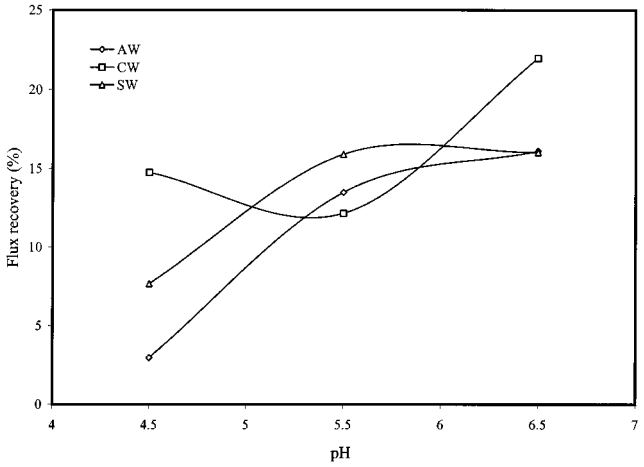

Figure 5. Effect of pH on flux recovery for UF of unclarified wheys for PAN membrane, 200 kPa, 600 rpm.

clearly (Figure 3; Table 3). The BSA ultrafiltration measurements were done at lower protein concentrations (0.1−0.2 g/dL) than those present in the wheys (0.6−0.8 g/dL). The higher protein content may overwhelm the increase in fouling resistance expected due to the improvement in PAN-2 membrane surface characteristics. Also, there is no clear trend among the various natural wheys, despite the differences in Ca content.

Figure 4 shows that in the case of PAN-2, the overall protein transmissions are more than that in PAN due mainly to its hydrophilicity, lower negative charge, and lower dispersive surface energy. Increased transmission in the hydrophilic PAN-2 membrane compared to the hydrophobic PAN membrane is consistent with our previous data on BSA (Musale and Kulkarni, 1996) and Hb UF (Musale and Kulkarni, 1997) and can be explained similarly.

The protein rejections for UF of unclarified wheys in the membranes do not show a clear trend with varying pH. It had been seen in earlier studies (Musale and Kulkarni, 1996, 1997) that BSA rejection decreased with increasing pH in these membranes; however, this trend was nullified when another protein (Hb, higher IEP) was also present. As was also the case for UF flux, the protein rejections do not show any consistent trend among the three wheys.

The flux recovery increases with pH for all three types of unclarified wheys for both membranes (Figures 5 and 6), similar to the data obtained with BSA UF (Musale and Kulkarni, 1996). The flux recovery increases with

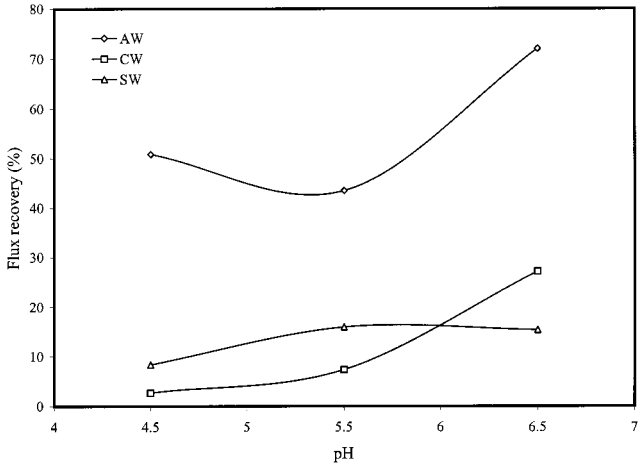

Figure 6. Effect of pH on flux recovery for UF of unclarified wheys for PAN-2 membrane, 200 kPa, 600 rpm.

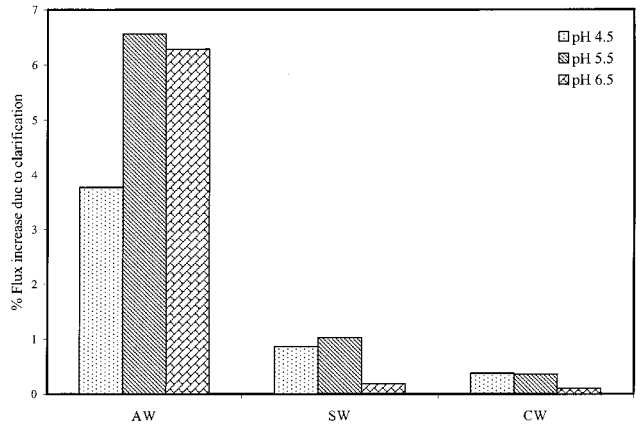

Figure 7. Effect of whey clarification on percent flux increase in PAN membrane.

pH due to decreased electrostatic attraction between the major whey proteins and membranes up to pH 5 and increase in electrostatic repulsion above pH 5. Because of its hydrophilic nature, PAN-2 has slightly higher flux recoveries than PAN, as seen previously for BSA UF (Musale and Kulkarni, 1996). As was also the case for comparison of fluxes, this advantage in the case of PAN-2 is not consistent.

Clarified Whey UF Characteristics. It has been proposed (Lee and Merson, 1976) that the reduction of Ca salts as a result of clarification is responsible for improved ultrafiltration performance. Ca salts are known to precipitate within the membrane pores; this gives rise to both reduced flux and increased protein rejections (Patocka and Jelen, 1987; Taddei et al., 1988; Hanemaiijer et al., 1989). The percent increase in permeate flux after clarification is shown in Figures 7 and 8 for PAN and PAN-2 and can be compared with the corresponding decrease in protein and Ca content shown in Figure 2. The flux increase in clarified whey UF compared to unclarified whey UF is consistent with the reduction in both Ca and protein content.

As shown in Figures 1 and 2, although there is a substantial reduction in Ca content, clarification also reduces the higher MW immunoglobulins, leaving in solution mainly the smaller proteins with IEP values close to pH 5. We propose that the improved ultrafiltration performance with the clarified wheys could also arise from the reduction in the high IEP proteins. Attractive electrostatic interactions between major pro-

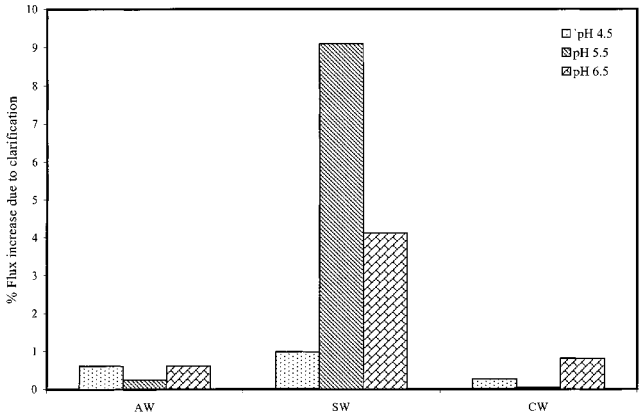

Figure 8. Effect of whey clarification on percent flux increase in PAN-2 membrane.

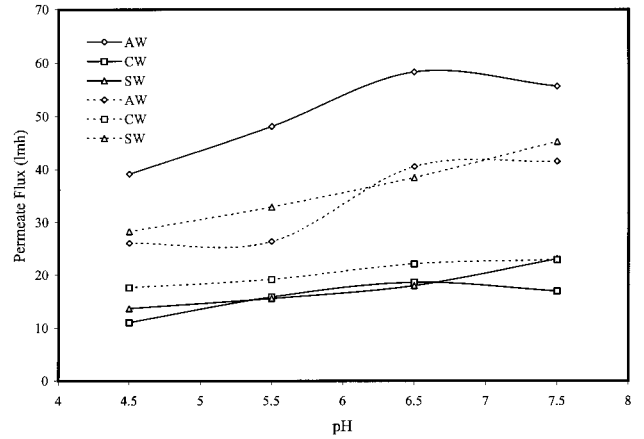

Figure 9. Effect of pH on permeate flux for UF of clarified wheys for PAN and PAN-2 membranes, 200 kPa, 600 rpm: solid lines, PAN; dotted lines, PAN-2.

teins and IgG between pH 5 and 7 would be diminished, and only repulsive interactions between major proteins would dominate after whey clarification. In other words, because the remaining proteins could then be treated as a single group, the UF characteristics (trends for flux/rejection with feed pH) for the clarified whey should more closely resemble those of standard BSA ultrafiltration. This difference in the protein−protein electrostatic interactions could also explain the different trends in flux and rejection with pH in unclarified and clarified whey UF.

The flux and rejections obtained with each clarified whey with PAN and PAN-2 membranes are shown in Figures 9 and 10. The data were obtained at the same experimental conditions as that for the natural wheys.

UF data with standard proteins (BSA or Hb) consistently showed that flux increased with increasing feed pH (Musale and Kulkarni, 1996, 1997). This trend was based on the increasingly repulsive interactions between proteins and the negatively charged membranes (Musale and Kulkarni, 1996). However, in the case of the unclarified wheys, the flux did not respond consistently to changes in feed pH (Figure 3). This expected trend is seen only in the case of clarified wheys (Figure 9). As hypothesized, the clarified whey behaves similarly to the single-protein solutions (BSA or Hb). This may be due to the reduction in attractive interactions of IgG with other whey proteins at pH values between 5 and 7 after clarification.

Similarly, in the case of pure BSA ultrafiltration (Musale and Kulkarni, 1996), the protein rejection

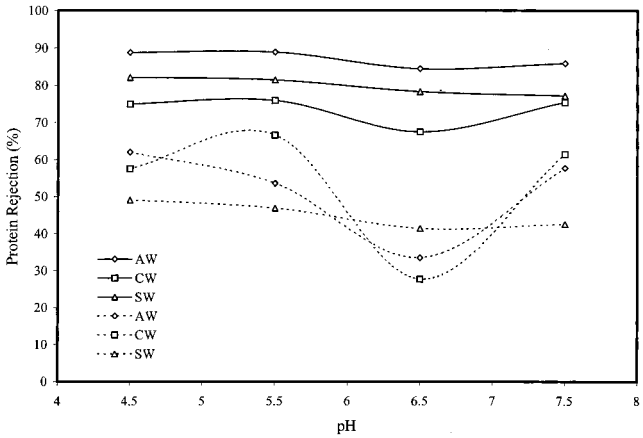

Figure 10. Effect of pH on total protein rejection for UF of clarified wheys for PAN and PAN-2 membranes, 200 kPa, 600 rpm: solid lines, PAN; dotted lines, PAN-2.

decreases at pH values above the protein IEP. Again, this trend was not seen with the unclarified wheys (Figure 4); however, for the clarified wheys, the observed rejections (Figure 10) do decrease above the IEP of major whey proteins, up to pH 6.5, similar to BSA or Hb rejection data (Musale and Kulkarni, 1997). As expected, this effect is more pronounced for the more hydrophilic PAN-2 membrane.

The rejections with the clarified wheys are less than those with the unclarified wheys. Gel electrophoresis of the permeate samples shows only the presence of the lower molecular weight proteins (α-lactalbumin and β-lactoglobulin) in the permeates. It is well-known that the presence of high molecular weight proteins can increase the rejection of the smaller proteins (Higuchi et al., 1991); hence, this effect is also consistent with IgG removal during clarification. Clarified AW shows more transmission than clarified CW and SW, which can be attributed to the highest reduction in IgG for this case.

Conclusions. The improvement in UF performance due to clarification can be attributed not only to the reduction in Ca salts but also to the decreased concentrations of high molecular weight proteins such as IgG, which have higher isoelectric points than the other whey proteins. Thus, in clarified wheys, protein−protein electrostatic interactions are mainly repulsive (the remaining proteins have similar IEPs) and the flux dependence on pH is similar to that of pure protein (BSA) UF (Musale and Kulkarni, 1996). The protein rejections in the clarified whey case also tend to decrease with increasing pH, similar to BSA UF (Musale and Kulkarni, 1996). These trends are not consistent in the case of unclarified whey UF. The reduced protein rejection in the clarified whey can also be explained by the reduction in the higher MW immunoglobulins.

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