Selective Precipitation and Removal of Lipids from Cheese Whey Using Chitosan

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A simple method to remove fat from cheese whey was studied. Addition of 0.01–0.02% chitosan to Cheddar cheese whey at pH 4.5 resulted in formation of a chitosan–fat globule membrane complex. The complex flocculated and precipitated when incubated at ambient temperature for about 10–30 min. Centrifugation of the treated whey resulted in a clear supernatant that contained almost all the whey proteins but with a lipid content of less than 0.26 g/100 g of protein. No detectable amount of residual chitosan was present in the whey protein isolate. Preconcentration of whey to about 4-fold (v/v) only slightly increased the minimum concentration of chitosan needed to flocculate fat globule membranes. On the basis of these results, a simple industrial method to remove fat from cheese whey is proposed. Preliminary economic analysis indicated that the additional cost involved in the manufacture of fat-free whey protein isolate was about $0.50/kg of whey protein. Chitosan has been approved as a food additive in certain developed countries. Several toxicological studies have shown it to be nontoxic. Therefore, the use of chitosan as a processing aid to remove lipids from cheese whey should be safe.

Keywords: *Lipid removal; cheese whey; chitosan; flocculation; precipitation*

INTRODUCTION

One of the major factors that limit the use of whey protein concentrates (WPC) in processed foods is their high lipid content. Most of the whey lipid is derived from milk fat globule membrane fragments (MFGM), which remain dispersed in a stable colloidal form. The turbidity of untreated whey is mainly due to light scattering by these micron-sized fragments. During ultrafiltration, they foul the membrane and thus greatly reduce the efficiency. In addition, because of retention of MFGM in the retentate, WPC prepared by ultrafiltration usually contains about 5–15% fat depending upon the protein content of WPC (Morr *et al.*, 1973). Oxidation of lipids during processing and storage leads to development of off-flavors (DeBoer *et al.*, 1977). Pretreatment of whey to reduce the fat content has been shown to improve flavor stability (DeBoer *et al.*, 1977). The presence of lipids in WPC and whey protein isolates (WPI) also greatly impairs the foaming properties of whey proteins (Morr, 1986; Burgess and Kelly, 1979) and possibly the stability of WPC-based emulsions (Halling, 1981).

The colloidal stability of MFGM fragments in whey is due to their high negative charge contributed by the phosphate groups of membrane phospholipids. It follows then that neutralization of the negative charge by using a polycationic polymer should induce flocculation of MFGM fragments. Chitosan is a natural polyglucosamine polymer derived from chitin, an *N*-acetylglucosamine polymer found in crustacean shell (Knorr, 1984). The pK of the glucosamine residues is about 6.3 (Muzzarelli, 1985), and hence chitosan assumes a polycationic character at acidic pH. In the present study, we show that MFGM fragments in cheese whey can be selectively precipitated by chitosan under appropriate conditions.

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MATERIALS AND METHODS

Clarified sweet Cheddar cheese whey (pH 6.2) was obtained fresh from a local cheese factory and used immediately upon arrival. Chitosan (made from crab shell) was from Sigma Chemical Co. (St. Louis, MO). Commercial chitosan made from shrimp shell (81.3% deacetylation) was obtained from Protan Inc. (Raymond, WA). All other chemicals were of analytical grade.

Precipitation of Lipids. Precipitation of whey lipids by chitosan was as follows. To cheese whey at 25 °C was added an aliquot of chitosan solution (1% solution in 10% acetic acid). The pH was adjusted to 4.5 by adding 1 M HCl. The mixture was incubated for 10 min and centrifuged at 1116*g* for 5 min in a laboratory clinical centrifuge. The turbidity of the supernatant was measured at 500 nm. A control, containing no added chitosan, was performed under identical conditions. The differences in the turbidity between the supernatants of the control and the chitosan-treated whey represented the efficiency of removal of MFGM fragments from the whey.

Analytical Methods. The protein content and profile of whey before and after chitosan treatment were determined by HPLC using a Bio-Gel SEC 30-XL (300 × 7.8 mm) gel permeation column (Bio-Rad, Richmond, CA). The untreated whey was filtered through a 0.2 μm syringe filter to remove MFGM fragments. Filtrate (20 μL) was injected into the column, and the elution profile was recorded at 254 nm using 20 mM phosphate buffer (pH 6.8) as the eluent at a flow rate of 0.5 mL/min. This was compared with the elution profile of chitosan-treated whey. The loss of protein was determined from differences in the elution profiles and in the area under each protein peak of the profile.

SDS–PAGE using 5–20% linear gradient slab gels was performed as described by Laemmli (1970). The molecular weight markers phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 000), α-lactalbumin (14.2 000), and low molecular weight marker kit containing myoglobin peptide fragments were from Sigma. Samples for SDS–PAGE were prepared by heating whey with 2-fold concentrated sample buffer solution containing 2% SDS in boiling water for 5 min.

To determine the lipid content, the untreated whey and treated whey were dialyzed exhaustively against water and

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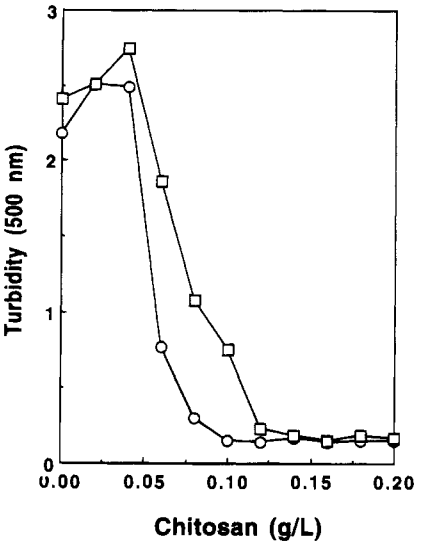

Figure 1. Effect of chitosan concentration on precipitation of milk fat globule membrane fragments in Cheddar cheese whey at 25 °C and pH 4.5. Experimental details are given in the text. (○) Crab shell chitosan; (□) shrimp shell chitosan.

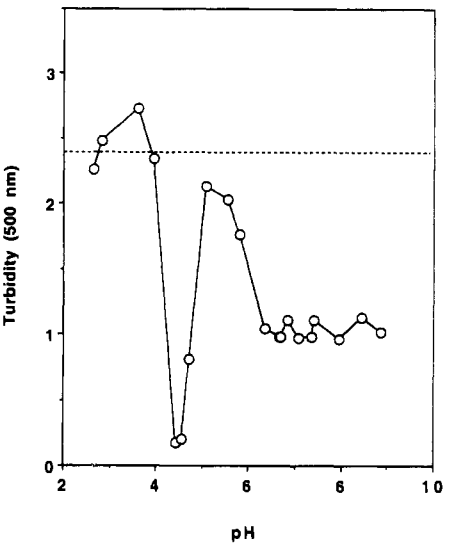

Figure 2. Effect of pH on precipitation of milk fat globule membrane fragments of Cheddar cheese whey by 0.024% chitosan using the conditions described in the text. The dotted line represents the turbidity of untreated whey.

then freeze-dried. The lipid contents were determined according to the Mojonnier method (Newlander and Atherton, 1977).

Protein content was determined according to the micro-Kjeldahl method. Chitosan was determined according to the method of Muzzarelli (1977). The residual amount of chitosan in the supernatant of treated whey was expressed as the percentage of total chitosan added to the whey.

Ultrafiltration. Ultrafiltration of sweet whey was performed using a bench-top unit (Tri-Clover Inc., Kenosha, WI) fitted with a U4-E500 (10 000 cutoff) spiral wound membrane (Desalination Inc., Escondido, CA).

RESULTS

Preliminary experiments indicated that the optimum pH for formation of an insoluble complex between chitosan and MFGM fragments in cheese whey was about 4.5. To determine the minimum chitosan concentration needed to cause maximum precipitation of MFGM fragments, the turbidity of the supernatant as a function of chitosan concentration at pH 4.5 was studied. The turbidity decreased with increase in chitosan concentration (Figure 1). At about 0.012%, both crab shell and shrimp shell chitosans removed more than 95% of the turbidity from cheese whey. The results suggest that addition of about 0.01–0.016% chitosan to a typical cheese whey at pH 4.5 should result in almost total removal of MFGM fragments.

Figure 2 shows the effect of solution pH on the efficiency of precipitation of MFGM fragments by 0.024% chitosan. In this case, an excess amount of chitosan (0.024% instead of 0.01%) was used to make sure that all MFGM fragments were complexed. Increase in pH from 3 to 3.7 caused an increase in turbidity of the supernatant, indicating that interaction between chitosan and MFGM fragments resulted in formation of a colloidal complex that did not sediment during centrifugation. The increase in turbidity might be because of the larger particle size of the chitosan–MFGM complex compared to the size of the MFGM fragments alone. When the pH was increased from 4.0 to 4.5, the turbidity of the supernatant dropped to a very low value and the supernatant was visually crystal clear. At pH 5.0 the turbidity of the supernatant increased and then progressively decreased at higher pH. However, the turbidity was minimal at pH 4.5, indicating that this was the optimum for flocculation and precipitation of the chitosan–MFGM complex. The results indicate that the chitosan–MFGM complex underwent a transition in a narrow pH range near 4.5. Below pH 4.0 and above pH 5.0 it possessed a significant amount of net positive or negative charge, which decreased the tendency of the complex to flocculate. At pH 4.4–4.6 the complex might have zero net charge, facilitating flocculation and precipitation via hydrophobic and possibly hydrogen bonding interactions.

To determine whether loss of whey proteins occurred during precipitation of MFGM with chitosan at pH 4.5, the HPLC profile of the supernatant was analyzed and compared with that of 0.2 μm filtered untreated whey. The profiles were identical in terms of number of peaks and peak area, except for a peak at the void volume of the column in the case of the untreated sample (data not shown). This high molecular weight species might be the result of the presence of smaller than 0.2 μm MFGM fragments in the filtrate. The results suggested that no soluble whey proteins were lost during precipitation of MFGM fragments by chitosan. This was further confirmed by SDS–PAGE of untreated and chitosan-treated whey (Figure 3). Both treated and untreated whey samples contained the same number of protein bands, and the intensities of these bands, especially the β-lactoglobulin, α-lactalbumin, serum albumin, and IgG bands, were similar, indicating that no soluble whey protein was lost during precipitation of MFGM fragments with chitosan. Determination of total nitrogen content of untreated whey and the supernatant of chitosan-treated whey indicated that about 5.8% protein (based on a 6.38 conversion factor) was lost after the chitosan treatment. This protein might be the lipoproteins associated with MFGM fragments.

The efficiency of precipitation of MFGM fragments by chitosan at 25 and 45 °C is shown in Figure 4. Below 0.018% chitosan, the turbidity of the supernatant of the sample treated at 45 °C was higher than that of the control as well as that of the sample treated at 25 °C. However, above this chitosan concentration the turbidities of the supernatants were very low and were practically independent of the temperature. This indicates that more efficient precipitation/flocculation of the

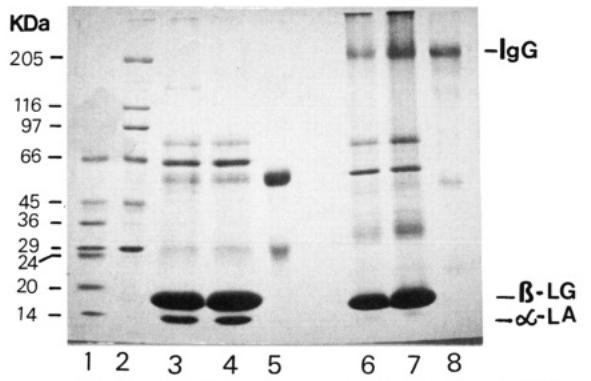

Figure 3. SDS–PAGE patterns of untreated and chitosan-treated Cheddar cheese whey samples. Twenty microliter samples were loaded onto the gel. Samples 1–5 contained 5% β-mercaptoethanol, whereas samples 6–8 did not. Samples 1 and 2 were molecular weight markers. Samples 3 and 6 were untreated and samples 4 and 7 were chitosan-treated (0.024%) whey supernatants. Samples 5 and 8 were IgG.

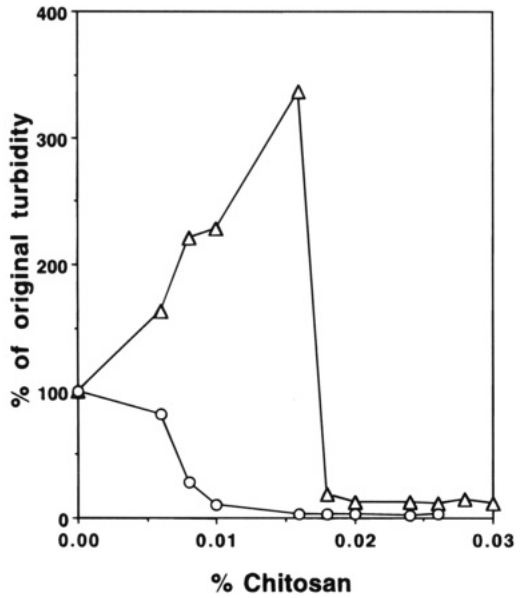

Figure 4. Effect of temperature on precipitation of milk fat globule membrane fragments by chitosan, with other experimental details as described in the text. (○) 25 °C; (△) 45 °C.

chitosan–MFGM complex was achieved at ambient (or lower) temperatures than at higher temperatures.

The stability of the insoluble chitosan–MFGM complex formed upon addition of 0.024% chitosan to whey at pH 4.5 was dependent on the ionic strength of the solution (Figure 5). Again, in this case, an excess amount of chitosan was used to make sure that all MFGM fragments were effectively complexed. The turbidity of the supernatant progressively increased with the addition of an increasing amount of NaCl. This might be attributed to ion binding and dispersion of the chitosan–MFGM precipitate into a stable colloidal form by the salt. As there was no increase in turbidity when NaCl was added to the untreated whey (Figure 5), the increase in turbidity of the chitosan-treated whey must have been due to formation of a chitosan–MFGM complex. At 0.15 M NaCl, adjustment of pH in the range of 3.5–5.0 did not decrease the turbidity (results not shown). This further confirmed that the increase in turbidity at higher ionic strength was not due to a shift in the pK values of the ionic groups involved in the interaction but was due to salt-induced dispersion of the chitosan–MFGM complexes.

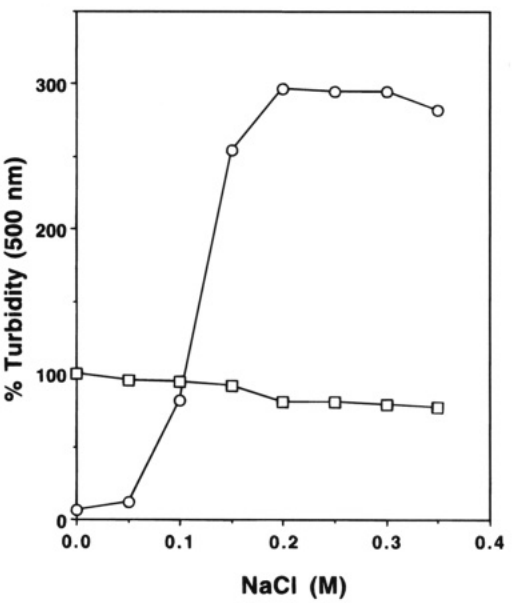

Figure 5. Effect of NaCl concentration on precipitation of milk fat globule membrane fragments by 0.024% chitosan at pH 4.5. (○) Treated whey; (□) untreated whey.

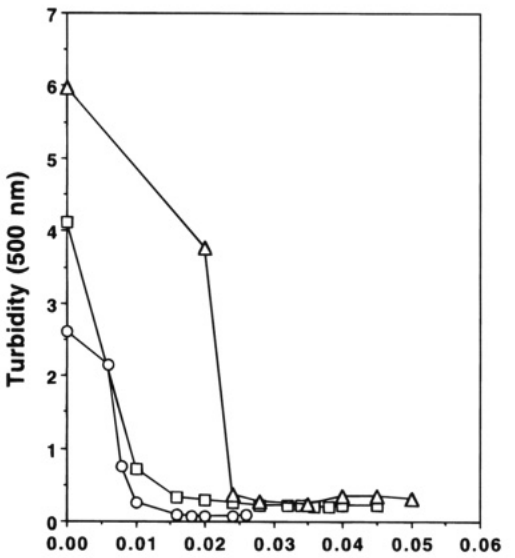

Figure 6. Effect of preconcentration of cheddar cheese whey on the efficiency of removal of milk fat globule membrane fragments by chitosan. Concentration factor of whey: (○) 1; (□) 2; (△) 4.

The effect of preconcentration of whey by ultrafiltration on the efficiency of precipitation of MFGM fragments by chitosan is shown in Figure 6. The minimum chitosan concentration required to precipitate MFGM fragments slightly increased with concentration of whey. For instance, the chitosan concentration required to give minimal turbidity was about 0.010–0.016% for unconcentrated and 2-fold concentrated whey, whereas it was about 0.025% for the 4-fold concentrated whey. For unconcentrated and 2-fold concentrated whey, the supernatants obtained after treatment with 0.016% chitosan at pH 4.5 were very clear (Figure 7), indicating efficient removal of MFGM fragments. In the case of 4-fold concentrated whey, the supernatant obtained after treatment with 0.024% chitosan was slightly turbid, but more than 90% of the original turbidity was removed.

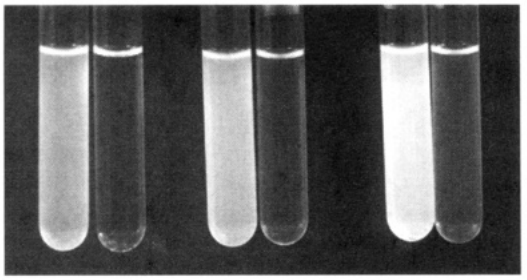

Figure 7. Photographs of untreated and chitosan-treated ultrafiltered whey samples. The solutions were centrifuged at 1116*g* for 5 min.

The dialyzed untreated whey and chitosan-treated whey supernatant (free of lactose and ash) contained about 3.25 ± 0.07% and less than 0.26 ± 0.02% fat in dry matter, respectively. Because the lipid content of the treated whey powder was so low, exact determination was difficult, and it is likely that the actual lipid content was lower than 0.26%. Nonetheless, the results indicated that treatment of whey with chitosan removed more than 92% of the lipids originally present.

The residual amount of chitosan in the supernatant is dependent on the initial amount of chitosan added to the whey. Since the optimum concentration of chitosan needed to cause more than 95% reduction in turbidity was about 0.01% (Figure 1), this condition was used to determine the residual amount of chitosan remaining in the supernatant. Under these conditions, no detectable amount of chitosan was present in the supernatant of the treated whey, indicating that all added chitosan has been removed in the form of chitosan–lipid complex.

DISCUSSION

Several approaches to reduce the fat content of cheese whey prior to ultrafiltration have been examined in the past (Pratt and Tinkler, 1952; Attebery, 1971). Attebery (1971) reported that addition of 75 mM Ca^{2+} to cheese whey at pH above 6.0 and at 60 °C resulted in formation of a precipitate which contained almost all of the original fat in the whey. Variations of this original approach have been reported (Fauquant *et al.*, 1985; Pierre *et al.*, 1992; Maubois *et al.*, 1987). Maubois *et al.* (1987) reported that addition of 1.2 g/kg calcium to whey at 2 °C, followed by rapid heating to 50 °C and holding for 8 min, caused precipitation of phospholipoproteins. Although this method decreases the lipid content, the extent of protein loss (11%), high ash content, and high energy cost involved in cooling and rapid heating of whey have limited its commercial exploitation

The mechanism of precipitation of MFGM by chitosan apparently involved electrostatic interaction at pH 4.5. Chitosan is a polyglucosamine polymer. Since the p*K* of the amino groups of glucosamine residues is about 6.3, chitosan is extremely positively charged at pH 4.5. This facilitated electrostatic interaction between chitosan and the negatively charged MFGM fragments. Since the major whey proteins, such as α-lactalbumin, β-lactoglobulin, lysozyme, and bovine serum albumin, are either positively charged or electrically neutral at pH 4.5, they did not interact with the positively charged chitosan. Furthermore, since these whey proteins are highly soluble at their isoelectric pH, they remained in solution at pH 4.5 and thus enabled selective precipitation of the chitosan–MFGM complex. Whereas the natural ionic strength of cheese whey was not detrimental to electrostatic complex formation, at higher ionic strength the complex was unstable and formation of a flocculum was inhibited. This suggests that whey concentrated by reverse osmosis may not be suitable for this method, unless it is diafiltered to reduce the ionic strength to below or equal to the level of natural whey. Previously, Bough and Landes (1976) reported that addition of 2.15% chitosan to cheese whey at pH 6.0 resulted in a coagulum that contained about 73% protein, 6% lactose, 9.5% ash, 7% moisture, and 0.15% fat in dry matter. Since whey proteins are negatively charged at pH 6.0, it is not surprising that electrostatic interaction of these proteins with chitosan resulted in a coagulum rich in protein and low in lipid content. However, it was reported that only about 2.27 g/L solids were recovered, indicating that the majority of protein was still in the effluent.

The method described here offers an industrially feasible process to remove lipids from cheese whey. It should be economic. The bulk price of practical grade chitosan with 70–85% deacetylation is in the range $10–15/kg. Assuming that the protein content of whey is 0.6%, and assuming the use of 0.01% chitosan for precipitation of whey lipid, additional material cost would be about $0.36–0.55/kg of lipid-free whey protein. Since the quality and functional properties of the lipid-free whey protein product would be superior to those of current WPC products, the additional cost appears to be justifiable. It could be further reduced by extracting lipids from the chitosan–MFGM complex with organic solvents, so that the chitosan could be recycled. The recovered lipids, mainly phospholipids (lecithin), could be used as a value-added product in food, cosmetic, and pharmaceutical products.

In addition, since the treated whey was free of suspended particles, the efficiency of ultrafiltration should be better than with untreated whey (Daufin *et al.*, 1993). Possibly 80–90% water removal could be achieved without a major reduction in the flux rate. Since no salt, other than those formed during pH adjustment, is added, the ash content of the resulting WPC or WPI should be low. The process appears to be suitable for all types of whey, including cottage cheese whey, which is already at about pH 4.5.

Several toxicological studies have indicated that only concentrations above 18 g of chitosan/kg of body weight showed oral toxicity in mice (Arai *et al.*, 1968; Knorr, 1984). The residual amount of chitosan in the treated whey was negligible. Since chitosan is practically insoluble at pH 7.0, any residual amount that might be present in the supernatant can be removed by adjusting the pH of the supernatant to 7.0 followed by filtration to remove the precipitated chitosan. Chitosan is currently approved as a food additive in Japan and Norway. In the United States, chitosan is approved as a feed additive. Human feeding studies in Japan have shown that when 3–6 g of chitosan/day was given in the diet of healthy males, it showed a beneficial effect of decreasing total serum cholestrol; no toxic effects were found (Maezaki *et al.*, 1993). In humans, chitosan functions mainly as a dietary fiber.

The process could be adapted to existing whey processing plants, without major additional capital investment. The only additional equipment needed would be a centrifuge and/or a filter to remove the chitosan–MFGM precipitate. Microfiltration using 2–3 μm pore membranes also may be used to remove the chitosan–MFGM precipitate. A process flow chart depicting the

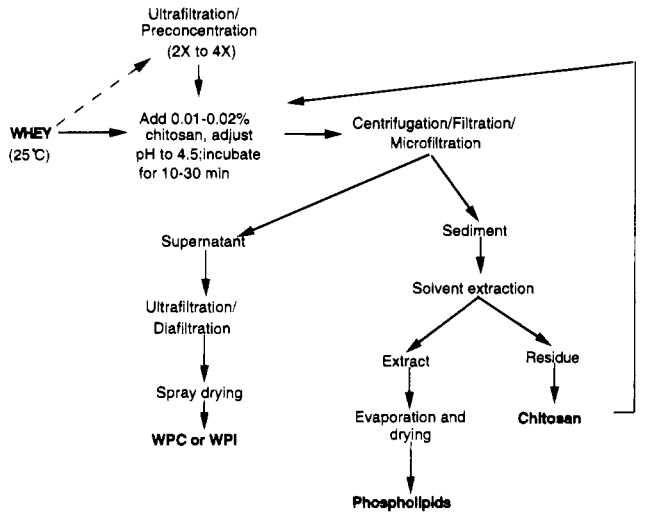

Figure 8. Process flow chart for removal of milk fat globule membrane fragments and lipids and production of fat-free whey protein concentrate (WPC)/isolate (WPI) from cheese whey.

various unit operations involved in the process is schematically shown in Figure 8.

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