Effect of different filtration membranes on composition of sweet and acid whey protein

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ABSTRACT

Whey (acid and sweet) is an important byproduct of dairy industry and rich in various nutritious components like lactose, whey protein, lactalbumin and lactoglobulin etc. Whey proteins in the form of concentrates are mainly used in the food industry due to their wide range of physical, chemical and functional properties like viscosity, water holding capacity, solubility, emulsification, gelation capacity and foaming. There is need to develop an efficient and affordable method of whey separation with minimum losses and membrane separation plays an important role in this regard over conventional methods. In this study three types of membranes like dialysis membrane, egg separation and fractional membranes are used and efficient results in terms of composition and yield were found by ultrafiltration after HPLC, Electrophoresis of retentate.

Key words: membrane filtration, whey protein, electrophoresis, HPLC

INTRODUCTION

Cheese, a very well-known dairy product was invented in 6,000 BC (Anonymous, 2005). Cheese is the product that is enriched in vitamins, minerals and protein that are necessary for good health. About 10.1 billion pounds of cheese was produced in USA (USDA, 2010). Whey is the greenish yellow by-product of cheese industry. Cheese industry produces about 115 million tons of whey annually and 47% of it is directly disposed in drain, which causes serious pollution problems having high biological oxygen demand (BOD) of 32,000 ppm or greater (Kosikowski and Mistry, 1997). The usage of the whey and its products in the food industry was started back in 1970 as it is a rich source of α-lactalbumin, β-lactoglobulin, proteosepeptones, lactose and minerals (Walzem et al., 2002).

Whey proteins are mainly used in the food industry due to their wide range of physical, chemical and functional properties like viscosity, water holding capacity, solubility, emulsification, gelation and foaming. β-Lactoglobulin is usually used as a stabilizer in food emulsions because of its surface-active properties. The antioxidant and gelation properties of Bovine serum albumin (BSA) makes it applicable in many therapeutic and food preparations. Immunoglobulin can improve immunological properties of infant formula. It has been reported that Lectoferrin has anti-inflammatory, immunostimulatry and antimicrobial properties. It plays a vital role in growth, iron metabolism and act as iron transporter. Recent studies showed that lactoferrin suppress tumor growth (Alm’ecija et al., 2007).

Liquid whey cannot be used directly in the food industry that’s why it is further processed into whey protein concentrates (WPC) and isolates (WPI) with a low lactose content (Walzem et al., 2002). Dried whey ingredients are used in a number of the food products like bakery products, sherbets, candies and cheese (Kosikowski and Mistry, 1997). Whey proteins separation and fractionations has led to the synthesis of good quality whey protein supplements manufactured as main products of cheese industry (Marshall, 2004). Successful and economical separation of whey protein is the major current issue. In their separation care should be taken to preserve the native and biological activity of proteins. Membrane is any material which can be used as an interface between two liquids, acting as a barrier to the flow of ionic and molecular species present in the liquid. Membranes have become an integral part of the processing because of the separation technique and selective transport as compare to other unit operations like evaporation in food processing (Marshall, 2004). Membrane separation techniques are energy efficient process that has been mostly used in separating fine particles in biotechnological, chemical, and food processing industries. The objective of the research is to determine the effect of dialysis membrane, egg membrane and ultrafiltration on whey protein composition.
MATERIALS AND METHODS

Procurement of raw material

Raw material, milk was purchased from the dairy farm University of Agriculture, Faisalabad. Milk (10L) standardized @ 3% fat content and heated to 65°C for 30 minutes followed by cooling to 6°C. Later on it was being divided into two equal parts for the production of Cheddar and acid cheese separately to get sweet and acid whey.

Production of Sweet whey from Cheddar Cheese

To get sweet whey of Cheddar cheese, milk was heated to 65°C/30min (pasteurized) and then cooled down to 31°C. Starter culture @ 2% was added in whey for acidification to reduce the pH to 6.5. For coagulation rennet 0.05% and 0.1% and calcium chloride 0.02% was added to facilitate coagulation. After the addition of rennet and calcium chloride whey was leave for curd formation without disturbing for 30-45 minutes at 31°C. The coagulum was checked after every 10-15 min with a stem of thermometer which was plunge below the surface layer and lifted the coagulum causing it to break in a cleavage line. A clear cleavage with green whey at the break of cleft indicated that the curd is ready to be cut. Then the curd was cut with knife to have small cubes (1-1.5”) both horizontally and vertically. Following cutting, curd was scalded at 39-40°C and stirred for 45-50 min. scalding enhances synersis and whey expulsion while stirring contributes uniform heat distribution. After scalding when the pH reduces to 6.2, the whey was drained from curd and the cheddaring was carried out till the pH reduces to 5.4. The whey obtained after whey removal was used as sweet whey for further processing and analysis.

Production of Acid whey from Acid Cheese

Buffalo milk is an ideal raw material for manufacture of good quality cottage cheese. The standardized whey was heated to 90°C/10min. The temperature of milk was dropped down to 70°C and was coagulated at this temperature using 10 percent citric acid solution. The temperature of citric acid solution is also maintained at 70°C. Citric acid solution was added with continuous stirring till clear whey separate out. After complete coagulation, the stirring was stopped and the coagulated mass (curd) is allowed to settle down for about 5 minutes. The whey is then drained through stainless steel strainer. The temperature of the content was not allowed to drop below 63°C until this stage. The whey is collected, and this whey was also being subjected to compositional and physic chemical analysis.

Chemical analysis of whey

pH

The pH of the whey samples was determined by electronic digital pH meter (Inolab WTW series pH-720) according to method given in AOAC (2003).

Acidity

Acidity of whey sample was determined by titration method given in AOAC (2003).

Moisture content

The moisture content of whey was determined according to AOAC (2003). 5 ml sample was taken in dried and weighted china dish. Then the sample was placed in oven at 100°C for 3 hours and cooled in desiccator and weighted.

Fat content

Fat determination of whey samples was carried out by the following method as described by (Marshall, 1993).

Nitrogenous and Non protein nitrogen fractions

Protein fractions like total protein and non-protein nitrogen (NPN) were determined using the Kjeldahl method using following formula (IDF, 2006).

\[
\text{Total Protein} \times \% = \frac{\text{Volume of K2504 used (ml)} \times 250 \times 0.0014 \times 100}{\text{Volume of Sample} \times \text{Sample used for Distillation}}
\]

Total Protein (%) = Nitrogen% x 6.3

The nitrogen (%) was converted to NPN contents by using the conversion factor 3.60

\[
\text{NPN} = \text{N} \times 3.60
\]

Lactose content

Lactose content of the whey sample was determined by the method described in AOAC (2003).

The lactose percent was calculated as followed
Total soluble solids
Total solids were determined according to the method described in AOAC (2003). A sample (5g) was taken in clean and dried and tarred china dish. It was heated for 15 min in water bath and was kept in hot air oven for 3-4 hours at 100°C and then cooled in a desiccator and weighed quickly. The total solids were calculated as under:

\[
\text{Total Solids (\%)} = \frac{\text{Residue After Drying}}{\text{Weight of Sample (g)}} \times 100
\]

Ash content
Ash was estimated by the method as given in AOAC (2003). Ash percentage in the whey samples was calculated as:

\[
\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of Sample (g)}} \times 100
\]

Filtration of whey
Whey sample was filtered by dialysis tube, egg membrane and ultra-filtration membrane as shown in Figure (1).

Physiochemical analysis of retentate
Nitrogenous fractions
Protein fractions like total protein, non-protein nitrogen (NPN) were determined using the Kjeldahl method (IDF, 2006).

Protein characterization
Separation of casein and whey proteins
Whey was filtered with different filtration membranes like ultrafiltration, dialysis tube and egg membrane at normal condition. The sample then froze at 80°C. Whey for electrophoresis was collected and dialysed at 4°C against several changes of distilled water. Dialysis tubing with a molecular weight cut off of 12-14000 Daltons was used. The dialysis was done for 24 hours and then whey samples were stored at -20°C to be used for electrophoresis (Bouaouina et al., 2006).

Electrophoretic profile of whey proteins of acid and sweet whey retentate on SDS-PAGE
Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed on Bio-Rad Mini Protean 3 System (Bio-Rad Laboratories, Hercules, CA, USA) according to the method of Laemmli (1970).

HPLC Analysis of whey proteins
10mg dried sample dissolved in 0.5ml urea solution and 2ml TFA solution. The sample was run on HPLC following the conditions as mentioned by Verdini et al. (2004). Approximately 20mg of the lyophilized whey powder was dissolved in 0.01 M imidazole (pH 7), 0.01 M dithioerytrol and 6.6M urea. The solution was mixed and filtered through a disposable 0.2um filter (Al-tech Associates, Inc., Deerfield, IL, USA) before 100ul was injected into the HPLC system. An HPLC system with a gradient programmer model 2360 (Isco, Inc., Lincoln, NE, USA), a V4 variable wavelength absorbency detector and a SyncChrpak RPP (250 x 4.6 mm) C18, 300,A column (SynChrom, Inc., Lafayette, IN, USA) at 30°C were used for chromatographic separations. Detection was at done at 220nm wavelength.

Statistical analysis
The collected data was statistically analyzed by analysis of variance techniques (ANOVA) using completely randomized design (CRD) (Steel et al., 1997).

RESULTS AND DISCUSSION
Chemical Composition of whey
Table: 1 holds the mean values of chemical characteristics of sweet and acid whey, it comprises of pH, acidity, moisture, fat, ash, total nitrogen, non-protein nitrogen (NPN), lactose and total soluble solids. It indicated that acid whey showed low pH and high acidity then sweet whey. The reason behind difference in pH and acidity of the whey is due to number of factors including the lactation stage, diseases, colostrum etc. (Fox et al., 2000). The results are in accordance to Jensen (1995) and Fox et al. (2000) who reported that whey has pH in 4.5-6.0 range at 25°C and acidity in the range of 0.14% to 0.21% respectively. Moisture content of both whey didn’t vary much from each other, however acid whey show higher value then sweet whey. In case of fat significant difference was observed with higher fat contents in sweet whey then acid whey. The fat value are in accordance with the finding of Fox and McSweeney (2003).
Table 1. Composition of whey as affected by membranes

<table>
<thead>
<tr>
<th>Whey type</th>
<th>pH</th>
<th>Acidity</th>
<th>Moisture</th>
<th>Fat</th>
<th>Ash</th>
<th>TN</th>
<th>NPN</th>
<th>Lactose</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid whey</td>
<td>4.52±0.07b</td>
<td>0.60±0.11a</td>
<td>93.08±3.62</td>
<td>0.05±0.02b</td>
<td>0.33±0.04b</td>
<td>0.90±0.05b</td>
<td>0.26±0.017</td>
<td>4.62±0.03b</td>
<td>6.91±0.43</td>
</tr>
<tr>
<td>Sweet whey</td>
<td>6.29±0.08a</td>
<td>0.53±0.12b</td>
<td>91.81±2.51</td>
<td>0.30±0.12a</td>
<td>0.51±0.08a</td>
<td>1.34±0.09a</td>
<td>0.26±0.027</td>
<td>4.97±0.02a</td>
<td>8.19±0.52</td>
</tr>
</tbody>
</table>

Data are means±SD. Means sharing similar letter are not differed significantly (p>0.05)

TSS = Total Soluble Solids; TN= Total Nitrogen

Table 2. Means for total nitrogen of retentate obtained after filtration through different membranes

<table>
<thead>
<tr>
<th>Technique</th>
<th>Total Nitrogen</th>
<th>Means</th>
<th>NPN</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid</td>
<td>Sweet</td>
<td>Acid</td>
<td>Sweet</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>47.91±1.45</td>
<td>51.94±0.94</td>
<td>49.92±2.84 a</td>
<td>2.39±0.07</td>
</tr>
<tr>
<td>Dialysis tube</td>
<td>44.44±1.30</td>
<td>47.41±0.66</td>
<td>45.92±2.10 b</td>
<td>2.21±0.06</td>
</tr>
<tr>
<td>Egg membrane</td>
<td>44.67±1.50</td>
<td>47.78±0.59</td>
<td>46.22±2.20 b</td>
<td>2.23±0.07</td>
</tr>
<tr>
<td>Mean for type</td>
<td>45.67±1.94 b</td>
<td>49.04±2.51 a</td>
<td>2.27±0.09 b</td>
<td>2.45±0.12 a</td>
</tr>
</tbody>
</table>

Data are means±SD. Means sharing similar letter are not differed significantly (p>0.05)

Figure 2. HPLC chromatogram of retentate of acid and sweet whey proteins
Physicochemical parameters

Nitrogenous fractions (Total Nitrogen and Non-Protein Nitrogen NPN)

Two major milk proteins are defined by acid precipitation as casein that precipitates at an isoelectric pH of 4.6 and whey proteins. The whey proteins are further subdivided into mammary synthesized protein. The former exists in many kinds with slight variation in their genetic makeup (sequence of amino acids) while later include the blood proteins (Creamer, 1996). Table: 2 shows the mean comparison of total nitrogen and Non-protein nitrogen of both types of whey and the technique used for filtration. With respect to the technique used highest mean value of total nitrogen and NPN was observed for the ultrafiltration in the retentate which is very high significantly more than the mean for egg membrane and dialysis tube. On the other hand mean for the sweet whey of total nitrogen and NPN are much more than mean for acid whey. Total nitrogen found in whey protein is not coming from the protein alone. There are other non-protein sources contributing towards the nitrogen content. Non-protein substances accounts for 5% of the nitrogen in whey and include amino acids, uric acid, ammonia and urea.

Electrophoretic profile of whey proteins of acid and sweet whey retentate on SDS-PAGE

Electrophoretic pattern of whey from different species reveal great difference among protein profile of these species. Pre-stained protein ladder of Sigma-
Aldrich ranging from 11-170 kDa was used for purpose of comparing the molecular weights of whey proteins of these species. In cow whey major three bands were noticed. In ultrafiltration retentate band having molecular weight of 14kDa was of α-LAC. The other main band was of β-Lg. It was at 17kDa. The concentration and peak height of β-Lg was more in comparison of α-LAC. Other major band was of BSA at 70kDa. The concentration and peak height of BSA was also less than β-Lg. The other band which was at 26, 61kDa were also observed. The band at 26 kDa might be dimer of α-LAC. The band of highest molecular weight was observed at 222kDa. It has less concentration, peak height than α-LAC and β-Lg and could be Ig. In dialysis retentate observed bands were four. The lowest molecular band was at 14kDa and was of α-LAC. The other band was at 27kDa and could be β-Lg because it had electrophoretic mobility similar to bovine β-Lg. The concentration of β-Lg was more in buffalo whey whey. The third band was observed at 84kDa and could be of buffalo serum albumin because it had electrophoretic mobility similar to bovine serum albumin. The last observed band was noted at 108kDa and it could be of Ig. In egg membrane α-LAC, β-Lg and BSA could be observed. Several faint bands could be dimer of α-LAC (28kDa) and β-Lg dimer and octamers. SDS-PAGE was performed to observe the difference in protein pattern of Holstein cow’s whey and Japanese-Saanen goat’s whey (Tomotake et al., 2006). They observed that α-LAC and β-Lg were the major bands in both kinds of whey and content of whey proteins were almost same in the two kinds of whey. The values of β-Lg and α-LAC were 15.0 ± 0.8% and 4.7 ± 0.6% for the cow whey.

**Quantification of protein through HPLC**

The whey proteins fractions had a retention time of 24 min and thus a trustworthy acknowledgement of different chromatographic peaks of serum albumin, α-lactalbmin and β-lactoglobulin proteins in whey of both acid and sweet whey samples were analyzed. The peaks were present at different molecular weights with serum albumin at 66,395 Dalton, α-lactalbmin for 14,234 Dalton and β-lactoglobulin for 18,264 Dalton for peak 1, 2 and 3 respectively. There were difference between cow and buffalo for β-lactoglobulin. These results were in close conformity with the results of Vohra et al., 2006 but with a slight difference of β-lactoglobulin that was observed in Asian buffalos. There arose certain small peaks beside the major peaks that might be due to glycosylation were found in both buffalo and cow whey samples but there were more glycosylation in cow whey as compared to that of buffalo. These findings were also in conformity to the findings of Slagen and Visser (1999).

**REFERENCES**


