ABSTRACT

Nonprotein nitrogen in pooled sweet (Cheddar and Dagano) and acid (cottage) wheys was estimated by currently proposed dialysis and chemical precipitation methods. Kjeldahl nitrogen analyses of (a) membrane retentates after water dialysis, and (b) 12% trichloroacetic acid plus 0.2% phosphotungstic acid filtrates indicated that nonprotein nitrogen values vary significantly with the method of sample preparation. Membrane porosity influence nonprotein nitrogen values for molecular weight cut-offs of 3500, 6000 to 8000 and 12,000 to 14,000. Dialyzable nitrogen values with all membranes were lower than 12% trichloroacetic acid soluble nitrogen for both wheys and higher than the 12% trichloroacetic acid + 0.2% phosphotungstic acid soluble nitrogen for all but the 3500 molecular weight cut-off membrane. The dialyzable nonprotein nitrogen fraction was heterogeneous but more than 80% was less than 3500 in molecular weight.

INTRODUCTION

Current emphasis by industry on the utilization of cheese whey and cheese whey protein in applications of food products has created renewed interest and concern about methods used to quantify protein and nonprotein nitrogen. Large but variable amounts of nonprotein nitrogen (NPN) are in cheese whey and must, therefore, be taken into account in calculating the protein content (3, 6, 8). Additionally, NPN content may influence the final properties and stability of whey subjected to membrane processing and/or spray drying or other processing treatments. However, controversy exists over the most suitable method of estimating NPN in whey systems (3). The classical 12% trichloroacetic acid (TCA) soluble nitrogen method does not appear suitable for estimation of NPN in whey systems because of the differential solubility of macropeptides (2, 10). However, deKoning et al. (4), who studied detection of adulteration of milk powder with whey powder, found that rennet whey macropeptides soluble in 12% TCA could be precipitated by addition of phosphotungstic acid (PTA).

Prolonged dialysis against water currently is suggested as an appropriate indirect method to estimate the NPN content of whey systems (3). However, the type and probable porosity of dialysis membranes have not been reported. Furthermore, dialysis is a laborious and time-consuming process. Therefore, an attempt was made in our investigation of commercial sweet and acid wheys: a) to determine the effect of membrane porosity on the dialyzability of NPN compounds, b) to compare the dialysis and chemical precipitation methods of NPN estimation, and c) partially to characterize the NPN fraction.

MATERIALS AND METHODS

One sample each of pooled acid (five lots of cottage) and sweet (six lots of Cheddar and two lots of Dagano) whey were obtained from commercial sources. The whey were dialyzed in four commercial cellulose membranes, three (Spectrapor, National Scientific Co., Cleveland, OH) having reported molecular weight (mol wt) cut-offs of 3500, 6000 to 8000, and 12,000 to 14,000, and one (Union Carbide Corp., Chicago, IL) of an unknown porosity. Whey samples (in duplicate) of 20 to 25 ml were pipetted and secured tightly in the cellulose membranes. Exhaustive dialysis was done against three changes of deionized distilled water for 72 h at <5 C. Dialyzable nitrogen (N) values were estimated by taking the difference of average Kjeldahl analyses (1) on original wheys (in duplicate) and on membrane retentates (single...
Determinations on duplicate samples). Included in the reagent blank N determinations were the dialysis tubing since the whey N determinations were run on samples (known volume) still in the dialysis tubing. The contribution of the dialysis tubing to the blank titration was negligible.

Twelve percent TCA soluble N determinations (12) and those for N soluble in 12% TCA plus .2% PTA (by weight) (4) were made in duplicate on filtrates obtained from all whey samples. Nitrogen values were determined by mixing 50 ml whey and 50 ml of a 24% TCA solution for TCA soluble N and 50 ml whey and 50 ml of a 24% TCA and .4% PTA solution for TCA + PTA soluble N and allowing the mixtures to stand for 10 min before filtering through Whatman 42 filter paper. A correction factor of 1.015 was employed for N values determined on filtrates to account for precipitation losses.

RESULTS AND DISCUSSION

Differences in NPN between commercial samples as estimated by dialysis and chemical precipitation methods are in Table 1. Increasing dialyzable N values were obtained with increasing membrane porosity for both types of whey with relatively higher values for acid whey than sweet whey. These results point out that there is a need to know and report membrane porosity to obtain reproducible results if dialysis is to be used as a method of NPN estimation. One might use the dialyzable N values for membranes of known porosity to estimate the porosity of the membrane of unknown molecular weight cut-off, which for the membrane in Table 1 would be about 8000 to 12,000. Calculation of the range of molecular weight for the NPN fraction, based on dialyzable N values for the highest porosity membrane as 100%, yielded estimates that >80% of the total dialyzable nitrogenous components are <3500 mol wt.

Comparison of dialyzable N and soluble N from chemical precipitation methods shows that the classical 12% TCA chemical method gave highest values followed by the membrane of highest porosity. Values were lowest for the membrane of minimum porosity but were close to those by the modified chemical method employing the combination of 12% TCA and .2% PTA.

The material released by rennin action on κ-casein and soluble in 12% TCA is essentially a large peptide in the mol wt range of 6000 to 8000 (10) with variable carbohydrate content. From its amino acid composition, the minimum molecular weight of the peptide portion of the glycomacropeptide has been close to 6000 (5, 11). Using a membrane of large porosity has the risk of dialyzing out the macropeptides as well as the low molecular weight proteose-peptone components 5 (mol wt ~ 14,300) and 8 (mol wt ~ 4100 and 9900) (7). Therefore, a mem-

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of various methods of estimating soluble nitrogen in cottage and sweet cheese wheys.</th>
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<tbody>
<tr>
<td><strong>Cottage wheya</strong></td>
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<tr>
<td>mg/100 ml</td>
</tr>
<tr>
<td><strong>Nitrogen</strong></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>12% TCA soluble</td>
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<tr>
<td>12% TCA + .2% PTA soluble</td>
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<tr>
<td><strong>Dialyzable</strong></td>
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<td>12,000 to 14,000 mol wt cut-off</td>
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<td>6000 to 8000 mol wt cut-off</td>
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<tr>
<td>3500 mol wt cut-off</td>
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<tr>
<td>Unknown mol wt cut-off</td>
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</table>

a Average of duplicate determinations on pooled cottage or sweet whey sample.

b A factor of 1.015 was used to convert determinations on filtrates to an original whey basis assuming a precipitation loss of .7% protein (sp gr 1.25) and .5% fat (sp gr .9) and a sp gr for whey of 1.010. Bound water of protein was assumed to be .5 ml/g.
brane porosity of 3500 might be expected to reflect more approximately the "true" NPN content of whey and be the best estimate of "true" protein.

porosity membrane and the TCA plus PTA chemical precipitation method may suggest application of the modified precipitation method for determination of the "true" NPN and protein content of whey systems rather than the classical 12% TCA method.

CONCLUSIONS

The results of whey NPN determination indicate that: a) the values of NPN vary significantly with the method of sample preparation, b) knowledge of membrane porosity is necessary for obtaining reproducible results if dialysis is employed to estimate NPN and that low porosity membranes may yield the most meaningful results, c) the combination of 12% TCA with .2% PTA may be the better chemical precipitation method because of the closer similarity of values to low porosity dialysis membrane results, and d) the NPN fraction is heterogeneous but predominantly less than 3500 mol wt.

REFERENCES