pH and Heat Treatment Effects on Foaming of Whey Protein Isolate

L.G. PHILLIPS, W. SCHULMAN and J.E. KINSELLA

ABSTRACT

The overrun obtained by whipping whey protein isolate (WPI) was significantly (p<0.05) affected by changing pH. Heating WPI at pH 4.0 reduced rate and amount of overrun. The highest overrun values for unheated WPI were observed at pH 5.0 and 7.0 after heating at 55°C for 10 min. The maximum foaming stability for unheated WPI was obtained at pH 5.0. Heat treatment had little effect on stability at pH 4.0 or 7.0 but at pH 5.0, 80°C for 10 min improved stability by 65%. Based on surface pressure data, the rate of adsorption of β-lactoglobulin interfacial films and the work of compression correlated with overrun, maximum overrun, overrun development and foam stability.

INTRODUCTION

THE UTILIZATION of whey proteins in food products is limited principally because of the lack of uniform composition and variable functional properties (Liao and Mangno, 1987; Schmidt et al., 1984; Kinsella, 1984; Phillips et al., 1989b). A better understanding of the relationship between physiochemical and functional properties of whey proteins is needed to standardize production protocols and extend their use in foods (Morr, 1985; Kinsella, 1984; Schmidt et al., 1984).

Foaming is an important functional property required for the use of proteins in certain products. The interfacial behavior of proteins reflects physical interactions that are influenced by the composition and conformation of the protein in solution and at the air-water interface (Graham and Phillips, 1976, Kinsella and Phillips, 1989; Phillips et al., 1989a). Studies of the foaming behavior of food proteins is complicated by interactions of mixtures rather than individual proteins and by molecular changes brought about by processing treatments (Stainsby, 1986). Limited systematic studies have been conducted on foaming using well defined mixtures of pure proteins to elucidate behavior at the interface (German and Phillips, 1989; Stainsby, 1986). An understanding of protein interactions during foam formation is important to optimize their preparation and use in processing. Liao and Mangno (1987) showed the foaming properties of whey protein concentrates significantly correlated with β-lactoglobulin content and with the extent of whey protein denaturation. Both pH and temperature treatments can cause changes in the conformation and structure of whey proteins especially β-lactoglobulin (McKenzie, 1971; Kella and Kinsella, 1988). The objectives of our study were to determine the effects of pH and prior heat treatment on foaming properties i.e. overrun and foam stability) of whey protein isolate.

MATERIALS & METHODS

Proteins

The studies were conducted with whey protein isolate (WPI) obtained by ion exchange chromatography (Mitchelstown Isolates, County Cork, Ireland). The protein content was determined by macro-Kjeldahl (Bradstreet, 1965). Ash and moisture were determined according to AOAC (1980).

Turbidity

Before whipping, the aggregation of whey protein isolate after pH adjustment was determined by transmission at 500 nm using a Spectronic 700 spectrometer (Bausch and Lomb, Rochester, NY). The values were expressed as Tb = (1-T500) × 100. A value of 100 corresponded to a completely turbid solution (0 transmittance).

Foaming properties

The effects of pH and heat treatment on the foaming properties of WPI were assessed using the method of Phillips et al. (1987). Overrun was measured (3 replicates) using 75 mL whey protein isolate (5.0% w/w) solubilized for 30 min with adjustment to the appropriate pH (4.0, 5.0 or 7.0) using either 0.1N HCl or 0.1N NaOH. The solutions were held for 10 min at 25, 55 or 80°C, cooled to 25°C in a water bath (10 min) then whipped for 5-min intervals for a total of 20 min using a Sunbeam Mixmaster mixer (Sunbeam Corporation, Oak Brook, IL.). The amount of air incorporated was measured and recorded as percent overrun.

Foam stability was measured (3 replicates) after whipping for 15 min as described by Phillips et al. (1987). The weight of liquid separating from the foam was continuously recorded using a Sartis balance (model 1212MP Brinkman Instruments Co., Westbury, NY) connected to an Apple IIe computer using an interface board (IMI State College, PA). The time required for half the original weight of the foam to drain as liquid was reported as 50% drainage, an index of instability. The drained liquid was collected in a tared container on the balance pan.

Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method of Laemmli (1970) was used to estimate molecular weight of the proteins. A Hoefer SE200 miniature slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) was used. Bovine serum albumin, β-lactoglobulin, α-lactalbumin, lactoferrin and immunoglobulin G (Sigma, St Louis, MO) were used as molecular weight standards. The gels were scanned by an E-C densitometer (EC Co., St. Petersburg, FL) interfaced with an integrator (Hewlett-Packard, Corvallis, OR) to determine relative concentration of each protein.

Surface pressure

The effects of pH and heat treatment on the surface pressure of WPI (5.0% conc) were measured (3 replicates) using a Fisher tensiometer (Fisher Scientific, Springfield, NJ) with a du Nouy ring. Surface tension of the distilled water used for protein solubilization was measured. The surface tension was measured after hydration (30 min) and after heat treatment and cooling of the various protein solutions (50 min after start of hydration). Changes in temperature and instrument accuracy were accounted for using the surface tension of water at 25°C (72.14 mN/M) and the following equations:

Correction factor = 1/[1 - (72.14 - measured surface tension of water)]

Corrected surface tension = measured value × Correction factor

Surface Pressure (mN/M) = 72.14 × Corrected surface tension
Table 1—Proximate composition of WPI and the results from SDS-PAGE and densitometry

<table>
<thead>
<tr>
<th>Composition</th>
<th>g/100g powder</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>90.3</td>
<td>95%</td>
</tr>
<tr>
<td>Ash</td>
<td>3.0</td>
<td>3.2%</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.0</td>
<td>5.5%</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bovine serum albumin</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>92.0</td>
<td>92%</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>5.0</td>
<td>5.5%</td>
</tr>
<tr>
<td>Other</td>
<td>0.5</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Table 2—Effect of pH on surface pressure (σ) of WPI (5% conc)

<table>
<thead>
<tr>
<th>pH of Solution</th>
<th>σm/N</th>
<th>σc/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>23.7</td>
<td>24.7</td>
</tr>
<tr>
<td>5</td>
<td>23.5</td>
<td>26.7</td>
</tr>
<tr>
<td>7</td>
<td>23.6</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Statistics

Data were analyzed using the statistical analysis package, release 82.3, computer program with the general linear models option (SAS, 1985). The mean square error term from the analysis of variance was used to calculate 95% confidence intervals. Overrun development was determined from the slope measured by linear regression of the individual overrun curves. The Pearson correlation coefficient (R) was reported for each, as a measure of linearity.

RESULTS & DISCUSSION

WPI composition

The proximate composition and the SDS-PAGE demonstrated that this WPI contained 95% protein (dry basis) of which 92% was β-lactoglobulin (Table 1). The effects of pH on the turbidity of whey protein isolate were measured as an indicator of protein association. The values for turbidity were 64.9, 100.0 and 61.5 at pH 4.5 and 7 respectively. The WPI was predominantly β-lactoglobulin thus the increased turbidity at pH 5.00 reflects an increase in protein/protein association as the pH approaches the pl of β-lactoglobulin (pI 5.2) (McKenzie, 1971; Kella and Kinsella, 1988). The surface pressure of WPI changed very little with pH but the values were similar to those reported by Waniska and Kinsella, (1985) for β-lactoglobulin (Table 2). Heat treatment had no significant effect.

The overrun of the WPI solution was significantly (p<0.05) altered by changing pH (Fig. 1). The highest maximum overrun was obtained at pH 5.0. Overrun development at pH 5.0 increased (R = 0.995) with whiptime and reached a maximum of 1241% after 20 min whipping (Fig. 1 and Table 3). The rate of overrun development was also pH dependent with the highest value for overrun development (32.14) occurring at pH 5.0 and the lowest value (6.88) at pH 4.0 (Table 3). Overrun increased with increasing whiptime for each of the overrun plots (Fig. 1, Table 3). This is in contrast to egg white which has a maximum overrun at whip times less than 20 min (Phillips et al., 1987). This indicates a difference in the type of interactions. The basic protein lysozyme (pI 10.7) at the native pH of egg white (pH 7-8) is positively charged. Thus egg white electrostatic interactions between lysozyme and the negatively charged proteins lead to aggregation and reduced foam formation after whipping 15 min (Phillips et al., 1987). The strong electrostatic attractions are not present in whey which is predominantly protein with an isoelectric point below pH 7.0 (Phillips et al., 1989). In addition, β-Lg being a stable protein requires longer whipping time to effect protein unfolding and stable film formation (Kinsella and Phillips, 1989).

Heating whey protein isolate at pH 4.0 reduced overrun and rate of overrun development. After heating at 80°C, the rate of overrun development was reduced by 63% after heating at 80°C (25°C) to -1.20 (80°C) and the overrun was reduced after 20 min whipping by 34% (Table 3 and Fig. 2). The largest reductions in overrun with heat treatment occurred at pH 5.0 (Fig. 2 and Table 3). The overrun of WPI (pH 5.0, 20 min whipping) was reduced by 63% after heating at 80°C. The rate of overrun development dropped from 32.14 at 25°C to 2.78 at 80°C (Table 3).

Heating WPI (pH 7.0) at 55°C increased the overrun of WPI by 22% (Fig. 2). Overrun development was also higher after heating at 55°C than at 25°C or 80°C (23.68, 16.42 and 17.92 respectively) (Table 3). Thus, to enhance foaming of WPI a milder heat treatment and/or adjusting pH to less than 5.0 prior to heat treatment such that protein/protein interactions are minimized is recommended.

Foam stability

The highest foam stability values (i.e. 50% drainage = 60.8 min) for unheated WPI were obtained at pH 5.0 (Fig. 3), which were also conditions for maximum overrun (Fig. 1). Thus at pH 5.0 foams held more air and were more stable than at pH
Fig. 2—Effects of prior heat treatment on the overrun of whey protein isolate (5% concentration) at pH 4.0 (A), 5.0 (B) and 7.0 (C) (with 95% confidence intervals). The WPI solutions were heated (55 or 80°C) at the specified pH for 10 min then cooled to 25°C before overrun was measured.

Fig. 3—Effects of heat treatment and pH on the foam stability of WPI (5% concentration). The WPI solutions were heated (55 or 80°C) at pH 4.0, 5.0 or 7.0 for 10 min then cooled to 25°C before foam stability (time to 50% drainage, min) was measured.

Table 4—Correlations of relationships between interfacial adsorption of β-lactoglobulin and foaming characteristics of whey protein isolate

<table>
<thead>
<tr>
<th>Foam parameter</th>
<th>Work of compression</th>
<th>Adsorption</th>
<th>Rearrangement</th>
<th>Average area cleared</th>
<th>Correlation coefficient (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foam stability (15 min)</td>
<td>1.000</td>
<td>0.856</td>
<td>0.636</td>
<td>0.579</td>
<td>0.971</td>
</tr>
<tr>
<td>Overrun (maximum)</td>
<td>0.971</td>
<td>0.975</td>
<td>0.388</td>
<td>0.807</td>
<td>0.922</td>
</tr>
<tr>
<td>Overrun/Overrun (min)</td>
<td>0.990</td>
<td>0.922</td>
<td>0.514</td>
<td>0.692</td>
<td>0.982</td>
</tr>
</tbody>
</table>

The high overrun values for WPI were observed at pH 5.0, with no heat treatment and at pH 7.00 after heating at 55°C. Haggett (1976) demonstrated the overrun of cheese WPC was improved after using a similar heat treatment. These results are consistent with those observed by Cooney (1974) who reported maxima for whey protein foam volume at pH 5 and pH 7-8. The maximum at the higher pH was inhibited by adding disulfide blocking agents suggesting disulfide interchange was involved in film formation or stabilization at the higher pH (Cooney, 1974).

The improved overrun for WPI (pH 7.00) after heating at 55°C may reflect partial unfolding of β-lactoglobulin and a resultant increase in protein/protein interaction. The unfolding of β-lactoglobulin and exposure of free thiol (Kella and Kinsella, 1988) may have facilitated disulfide bond formation. Shimizu et al. (1985) reported that β-lactoglobulin was more flexible at pH 7.0 and more resistant to unfolding at pH 3.0. Heat treatment at 80°C reduced the overrun for all pH levels tested. Several investigators reported heat treatments ≥65°C impaired whipping ability (Cooney, 1974; Richert, 1979). Kato et al. (1983) observed less foaming but optimal foam stability for β-lactoglobulin following similar heat treatment. This could result from increased protein aggregation thereby reducing available protein for new film formation, but increasing film thickness and strength once the foam was formed.

The low overrun values for WPI at pH 4.0 may reflect the rigidity of the β-lactoglobulin molecule. At a pH below the isoelectric point, β-lactoglobulin is more compact (Waniska and Kinsella, 1985; Kella and Kinsella, 1988). This would allow more protein to reside at the interface. At pH 4.0, β-lactoglobulin is stable and less likely to unfold resulting in reduced foam. Shimizu et al. (1985) also reported a lower emulsifying activity for β-lactoglobulin at lower pH levels owing to reduced flexibility of β-lactoglobulin.

Waniska and Kinsella (1985) estimated the rate of adsorption and rearrangement, the area cleared per molecule during adsorption and rearrangement and the work required to clear an area at the interface ie, work of compression for β-lactoglobulin. The similarities between surface pressure data (Table 2) for WPI which we found and those obtained by Waniska and Kinsella, (1985) for the interfacial properties of β-lactog-


Ms received 8/21/89; revised 9/15/89; accepted 2/13/90.

This work was supported by the National Dairy Board.

REFERENCES


Ms received 8/21/89; revised 9/15/89; accepted 2/13/90.

This work was supported by the National Dairy Board.

ULTRASONIC HEAT TRANSFER... From page 1115

τ shear stress

Subscripts

g generalized

1 initial

1 - 9 numerical subscripts for π

∞ fluid medium

REFERENCES


Ms received 6/30/89; revised 11/20/89; accepted 11/25/89.

Salaries and research support provided by State and Federal Funds, appropriated to the Ohio Agricultural Research & Development Center, The Ohio State Univ. Journal Article No. 347-93.

Volume 55, No. 4, 1990—JOURNAL OF FOOD SCIENCE—1119