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Formation and Stability of Foam Made with Various Potato Protein Preparations

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In the present study, foam-forming and -stabilizing properties of potato proteins were studied using whipping and sparging tests. The soluble potato proteins are mainly composed of patatin and protease inhibitors. The whipping tests showed that less foam was formed from untreated patatin than from the protease inhibitors, but patatin foam was much more stable. The foam-forming properties of patatin could be strongly improved by partial unfolding of the protein. Whipping tests, at both low (0.5 mg/mL) and high (10 mg/mL) protein concentration, also indicated that foams made with an ethanol-precipitated protein isolate were more stable than those made with β-casein and β-lactoglobulin. More generally, it can be concluded that when proteins are used as a foaming agent, a high concentration is required, because the protein available is inefficiently used. Also, there are several variables that may all, in different ways, affect both foam formation (amount of foam, bubbles size distribution) and foam stability. These variables include the type and concentration of protein, solvent conditions (pH, *I*), and the method used to make the foam.

KEYWORDS: Potato; *Solanum tuberosum*; proteins; patatin; protease inhibitors; pH; heat; ethanol; ionic strength; foam formation; foam stability; whipping; sparging

INTRODUCTION

Potato fruit juice (PFJ) is a byproduct from industrial starch manufacture and contains approximately 1.5% (w/v) soluble protein. The soluble potato proteins can be tentatively classified into three classes: patatin (41 kDa), protease inhibitors (5−25 kDa), and others (mostly high molecular weight) (*1*). Potato protein has a relatively high nutritional quality (*2*, *3*), and it thereby has good potential for utilization in foods. However, recovering the protein by heat coagulation from PFJ results in a complete loss of most of its functional properties. Several efforts have, therefore, been made to recover potato proteins from PFJ that have retained their functional properties.

The ability to form and stabilize foams is considered an important functional property of food proteins, which is exploited in several food products. The foam properties of undenatured potato proteins have only been studied to a limited extent (*4*−*7*) and were observed to be inferior to the properties of whey proteins (*5*). However, the foaming properties of potato protein obtained by ultrafiltration, CMC complexation, and anion-exchange chromatography were shown to be very good in model systems (*6*, *7*), as well as in a number of food systems (*8*), being at least comparable to those of casein and egg albumin. The purpose of the present study was to examine foam forming and foam stabilizing properties of various potato protein preparations and to compare these properties to those of β-casein and β-lactoglobulin, as reference proteins.

When studying foam properties, a distinction should be made between formation and stability, although the two processes cannot be studied separately (*9*). For the making of a foam, air, liquid, energy, and a surfactant, in this study protein, are needed. The surfactant serves two purposes, of which the most important is its ability to form interfacial tension gradients to stabilize the newly formed bubbles against immediate coalescence. Surfactants also lower the interfacial tension (γ), thus making the break-up of bubbles, opposed by the Laplace pressure ($p_L = 2\gamma/R$), easier (*9*). Proteins differ from small-molecule surfactants in that much higher surface loads (Γ) are needed to significantly reduce γ, as needed to create γ gradients (*10*). Also, the conformation of proteins needs to change to effectively

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reduce γ. Proteins vary markedly in the rate at which these conformational changes take place (*11*).

In this study, two different methods were used to make foam: (1) sparging and (2) beating or whipping. During sparging, bubbles are released from a grit due to buoyancy forces. This method is more suited than whipping to produce foams from structurally rigid proteins, because the proteins have more time to adsorb and unfold at the interface. During whipping, large bubbles are introduced in the solution, which are subsequently broken up into smaller ones (*9*). During whipping, the beaters cause velocity fluctuations that not only facilitate bubble break-up but also, according to Bernoulli's law, cause pressure fluctuations: $\Delta p = \rho(\Delta v)^2/2$, in which Δp is the pressure fluctuation, ρ the density of the liquid, and v the velocity of the whisk (*12*). These pressure fluctuations cause the bubbles in the solution to pulsate and their interfacial area (A) to change with time via $\Delta \ln A/\mathrm{d}t = \rho v^3/3Lp$, in which L is the distance between the pins on the whisk (*12*). The change in area leads to a change in surface tension via $\Delta\gamma = \rho\eta_{SD}v^3/3Lp$, in which η_{SD} is the surface dilational viscosity ($=\Delta\gamma/(\Delta \ln A/\mathrm{d}t)$) (*13*). This implies that, above a certain whipping speed, $\Delta\gamma$ will become too large, and the film between two encountering bubbles will break, allowing them to coalesce, according to Prins (*13*). This has been shown experimentally for low-molecular-weight surfactants (*12*). When proteins are used, whipping time can also be expected to show an optimum. Increasing the whipping time will initially result in an increase in foam volume since more and smaller bubbles can be formed. However, when whipping times become too long, the continuous surface expansion and protein desorption, due to bubble breakage, may result in extensive protein unfolding and subsequent aggregation, leading to a decrease in the effective molar protein concentration and a decrease in foam volume.

Once a foam is formed, various instabilities may occur. Coalescence, i.e., the rupture of the film between two bubbles, can occur in foams when spreading particles (e.g., fat) are present or when hydrophobic particles with a diameter larger than the film thickness are present (*13*). Drainage is the flow of liquid out of the foam due to gravity. The rate of drainage is, among other factors, affected by the bubble size, the viscosity of the liquid, and the amount of liquid in the foam. Compared to that in foams from small-molecule surfactants, drainage in protein foams is greatly retarded, as the adsorbed proteins can form fully stagnant surfaces (*14*). Ostwald ripening, the most important instability in most foams, is fast because of the high solubility of air in water. Due to a higher Laplace pressure, the solubility of air in the liquid phase is higher around smaller bubbles than around larger bubbles, resulting in the growth of larger bubbles at the cost of small ones. This process can be retarded or stopped if the surfactant stays adsorbed at the interface of the shrinking bubble, which occurs with proteins, because then the surface tension will decrease when the surface area is reduced. The relation between the surface tension and change in surface area is given by $E_{SD} = \mathrm{d}\gamma/\mathrm{d} \ln A$, in which E_{SD} is the surface dilational modulus. It has been shown that Ostwald ripening in foam will completely stop if E_{SD} becomes equal to or larger than $\gamma/2$ (*15*). This theory, however, does not consider relaxation processes, which may cause Ostwald ripening to proceed further.

Studying these aspects is by no means easy. Proteins differ greatly in their behavior upon adsorption onto an air−water surface. The "protein" studied is generally a mixture of species. Foam formation is governed by several variables, of which surfactant type and concentration, and whipping time and intensity, may be most important. Once a foam has been made, it is subject to a number of instabilities. These instabilities depend on a number of variables, especially bubble size and properties of the bubble surfactant layer, which depend, in turn, on the method of formation. Moreover, the relations are quite different for the various instabilities. Unfortunately, no good methods are available to determine bubble size distribution, rate of Ostwald ripening, and coalescence rate. Nevertheless, it is essential to distinguish among the types of instability in order for the work to lead to further understanding. In the present case, where the bubbles are not very small, careful visual observation allows estimates to be made of bubble size and of the occurrence of Ostwald ripening and coalescence; drainage and (change in) amount of foam can be measured. Altogether, it was considered worthwhile to make a study of the aspects mentioned for some groups of reasonably well characterized industrial proteins. We realize that the conclusions are, to some extent, tentative.

MATERIALS AND METHODS

Preparation of Protein Preparations. *1. Potato Fruit Juice (PFJ).* Potatoes of the cultivar *Elkana* were used, and PFJ was made according to method described in ref *16*. The resulting clear yellowish filtrate, which has a pH of 5.7−6.0, is known to be similar to industrial PFJ (AVEBE B.A., Foxhol, The Netherlands) and is further denoted as PFJ.

2. Potato Protein Isolate (PPI). Potato protein isolate was prepared by slowly adding 95% (v/v) ethanol (−20 °C) to stirred PFJ (4 °C) to a final concentration of 15 or 20% (v/v) and adjusting the apparent pH of the clear mixture to 5.0 by addition of 0.5 M H_2SO_4. After 1 h at 4 °C, the suspension was centrifuged (30 min, 19000*g*, 4 °C), and the precipitate was washed twice with a 0.1 M ammonium acetate buffer (pH 5) containing 15 or 20% (v/v) ethanol. Subsequently, the precipitate was suspended in water, and the suspension was adjusted to pH 7 using 0.1 M NaOH and then freeze-dried and stored at −20 °C. The preparations obtained were denoted PPI (15% EtOH) and PPI (20% EtOH).

PPI contains most of the proteins present in PFJ, but these are known to be unfolded to some small extent due to their precipitation in the presence of ethanol (*17*). PPI was prepared in two different variants, PPI (15% EtOH) and PPI (20% EtOH), which differ mainly in the lower protein solubility of the latter. The indication of simply "PPI" in this study signifies PPI (15% EtOH).

3. Ammonium Sulfate Precipitate (ASP). Ammonium sulfate precipitate was prepared from PFJ according to the method described in ref *18*. ASP was prepared to simulate total undenatured potato protein.

4. Patatin. Patatin was purified according to the method described in ref *18* and stored at −20 °C. Ethanol-precipitated patatin (PAT-5E) was prepared by slowly adding 95% (v/v) ethanol (−20 °C) to a stirred patatin solution (5 mg/mL, 4 °C) to a final concentration of 20% (v/v) ethanol. The apparent pH of the clear mixture was adjusted to 5.0 by addition of small volumes of 0.5 M H_2SO_4. After 1 h at 4 °C, the suspension was centrifuged (30 min, 19000*g*, 4 °C), and the precipitate was suspended in water, adjusted to pH 7 using 0.1 M NaOH, extensively dialyzed against 9 mM sodium phosphate buffer (pH 7), and then stored at −20 °C.

Patatin is the major protein in PFJ (*19*), with a molecular mass of 40.5 kDa (*1*), and is estimated to make up 38% of potato protein in PFJ. PAT-5E is patatin that has a partly unfolded tertiary structure, as evidenced by its precipitation at pH 5 in the presence of ethanol (*17*).

5. Protease Inhibitor Pool (PIP). Protease inhibitor pool protein was prepared by gel filtration of PFJ according to the method described in ref *18* and stored at −20 °C.

Ethanol-precipitated PIP (PIP-5E) was prepared by slowly adding 95% (v/v) ethanol (−20 °C) to a stirred PIP solution (6 mg/mL, 4 °C) in 9 mM sodium phosphate buffer (pH 7) to a final concentration of 20% (v/v). The apparent pH of the clear mixture was adjusted to 5.0 using 0.5 M H_2SO_4. After 1 h at 4 °C, the suspension was centrifuged (30 min, 19000*g*, 4 °C), and the precipitate was suspended in distilled

water and adjusted to pH 7 using 0.1 M NaOH. Next, the solution was extensively dialyzed against 9 mM sodium phosphate buffer (pH 7) and then stored at −20 °C.

PIP contains mainly protease inhibitors of molecular mass between 15 and 25 kDa (*18*) and is estimated to contain 45% of the protein present in PFJ (*20*). PIP-5E has approximately the same protein composition as PIP but has been precipitated in the presence of ethanol, which is expected to have caused small irreversible changes in the conformation of the proteins (*17*).

6. Other Proteins. β-Lactoglobulin was obtained as described previously (*21*). β-Casein (90% pure on dry weight) was purchased from Eurial (Nantes, France). β-Casein and β-lactoglobulin were used as reference proteins, because their foam properties have been extensively studied (*22*) and have been repeatedly tested using the applied whipping test (*23*, *24*).

Protein Content. The protein content of the protein solutions used was estimated using the method of Bradford (*25*), with bovine serum albumin (Sigma A-7511, lot no. 92H93131) as a standard.

Foam Properties. *Whipping Method.* Protein solutions of β-lactoglobulin, β-casein, PPI, ASP, PIP, PIP-5E, PAT-5E, and patatin were dispersed to a protein concentration of 0.5 mg/mL in a 9 mM sodium phosphate buffer containing 35 mM NaCl (pH 7, $I = 50$ mM), unless stated otherwise. Formation and stability of foam were estimated by the whipping method described by Caessens and co-workers (*23*). Generally, a volume of 100 mL of protein solution in a graduated glass cylinder was stirred at a speed of 2500 rpm for 70 s. The foam volume was monitored for 60 min (the first measurement 2 min after stirring started), and the foam quality (bubble size, coalescence, and Ostwald ripening) was evaluated visually. All measurements were performed at least in duplicate. The average standard deviation for the initial foam volume was 5 mL for the standard whipping test. For the measurements at various stirring speeds and stirring times, the average standard deviation was 11 mL.

Variation of the stirring speed was tested on 0.5 mg/mL protein solutions using speeds from 2000 to 5000 rpm, with a stirring time of 70 s. Variation of the stirring time (10−360 s) was applied at 2500 rpm. Initial foam volumes were measured 50 s after the stirring was stopped. Foam measurements as a function of protein concentration were performed with solutions containing 0.1, 0.2, 0.35, 0.5, and 1.0 mg of protein per milliliter.

The effect of pH on the foam properties of PPI, ASP, PIP, and patatin was investigated by dispersing these proteins to a concentration of 0.5 mg/mL in 9 mM sodium phosphate (pH 7), 24 mM sodium acetate (pH 5), or 17 mM sodium phosphate (pH 3), all adjusted to an ionic strength of 50 mM by addition of NaCl to a concentration of 35 mM. The effect of pH unfolding was tested for PPI, ASP, and patatin by dissolving these proteins in 9 mM sodium phosphate buffer (pH 3). After 30 min, the pH was adjusted to 7 by addition of 0.1 M NaOH, and NaCl was added to a final concentration of 35 mM. Next, 9 mM sodium phosphate buffer (pH 7) containing 35 mM NaCl (pH 7, $I = 50$ mM) was added to make the final protein concentration 0.5 mg/mL. This treatment will be further indicated as pH 3 → pH 7.

The effect of heat treatment on the foam properties of PPI, ASP, PIP, and patatin was investigated by dispersing these proteins to a final concentration of 2.5 mg/mL in 9 mM sodium phosphate buffer (pH 7, $I = 15$ mM) and subsequently heating these dispersions in a thermostated water bath at 80 ± 1 °C for 10 min. After heating, the solutions were immediately cooled in ice. Next, NaCl was added to a final concentration of 35 mM, and the solutions were filtered over a 0.2-μm filter (Schleicher & Schuell, Dassel, Germany). The protein concentration of the heated solutions was estimated, and the final concentration was adjusted to 0.5 mg/mL using 9 mM sodium phosphate buffer containing 35 mM NaCl (pH 7, $I = 50$ mM).

The effects of ionic strength on foam properties were investigated by dispersing PPI, ASP, PIP, and patatin to a final concentration of 0.5 mg/mL in 9 mM sodium phosphate buffer (pH 7) that contained no NaCl ($I = 15$ mM), 35 mM NaCl ($I = 50$ mM), or 185 mM NaCl ($I = 200$ mM).

Ledoux Apparatus. The formation and stability of foam were also studied using a "whipped-cream tester" (Ledoux Machine Factory, Dodewaard, The Netherlands), which was designed by NIZO (*26*) and will be further denoted as the Ledoux apparatus. The apparatus is schematically shown in **Figure 1**. Into the beaker of the instrument was poured 200 mL (3.0 cm) of protein solution, and the two whisks were used to stir at a speed of 360 rpm (80%) for 70 or 360 s. After whipping, the whisks were kept in place. The foam height was monitored for 60 min (the first measurement was 50 s after stirring was stopped), and the foam quality (bubble sizes, coalescence, and Ostwald ripening) was evaluated visually. All measurements were performed at least in duplicate. The average standard deviation for the measurements performed on the Ledoux apparatus was 0.4 cm. For foam prepared using the Ledoux apparatus, 10 mg/mL solutions of β-lactoglobulin, β-casein, PPI, and ASP in 9 mM sodium phosphate buffer containing 35 mM NaCl (pH 7, $I = 50$ mM) were used.

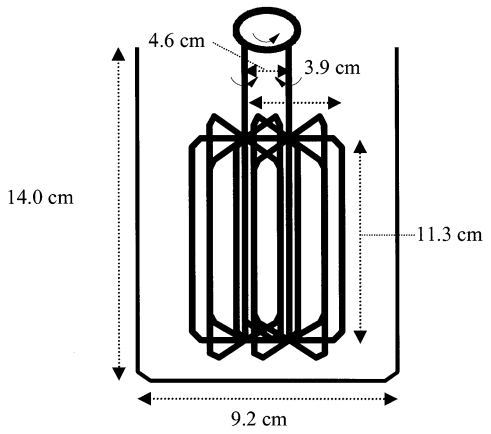

Figure 1. Schematic representation of the Ledoux apparatus

Sparging Method. Foams were also made using a sparging method. The formation and stability of foams were estimated by introducing air at a constant flow rate for 40 s through a G-2 filter (pore-size, 40−90 μm) in a glass column (3.2 × 20 cm) with 40 mL of protein solution. The foam height was monitored for 60 min (the first measurement immediately after stopping the air flow), and the foam quality (bubble sizes, coalescence, etc.) was judged visually. All measurements were performed at least in duplicate. The average standard deviation for the sparging measurements was 1.9 mm. Protein dispersions (pH 7, $I = 50$ mM) with a protein concentration of 0.5 mg/mL, prepared from β-lactoglobulin, β-casein, PPI, ASP, PIP, and patatin, were used.

RESULTS

Effects of Whipping Speed and Whipping Time Using the Whipping Method. The effect of whipping speed on the foam volume formed from the various protein preparations is shown in **Figure 2**. For most preparations, foam volume increased gradually with whipping speed between 2000 and 3000 rpm. Above 3000 rpm, foam volume generally increased much more, the foam became much firmer, and the bubble size became visibly smaller. Speeds ≥3500 rpm resulted in protein aggregation, as the serum became turbid. Only in the case of β-casein did the foam remain liquid-like at higher whipping speeds, and in this case no aggregation was observed. For β-casein, β-lactoglobulin, and PIP, no optimal speed was found, while for ASP, PPI (15% EtOH), PAT-5E, and PIP-5E, foam formation seemed to be maximal at 4000 rpm. Foam formation of PPI (20% EtOH) was maximal at 3000 rpm. Using the standard whipping time of 70 s for patatin, almost no foam remained at any whipping speed. When the whipping time was reduced to 30 s, substantially more foam remained, resulting in an optimum speed of about 3000 rpm.

The influence of whipping time on foam formation at 2500 rpm is shown in **Figure 3**. The curves in **Figure 3** show two different shapes. The shape of the curves, obtained for both PPI variants and patatin, is characterized by a strong relative increase

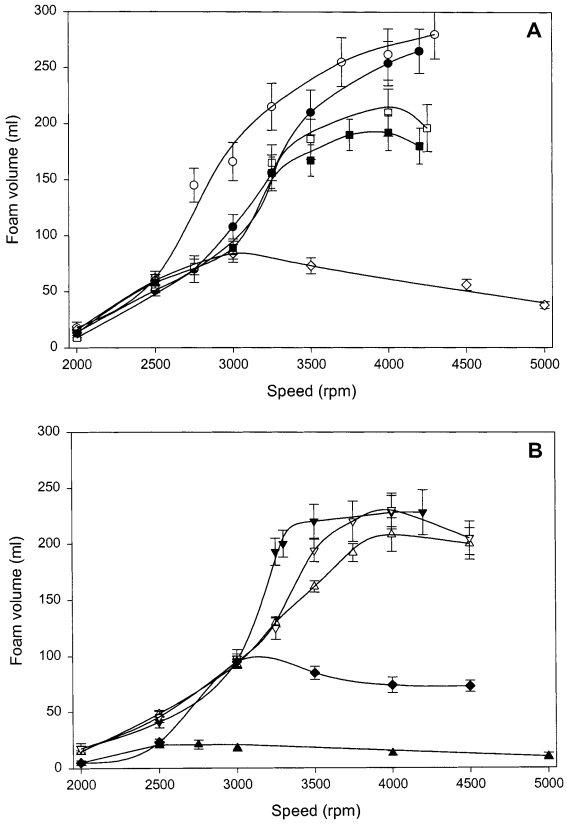

Figure 2. Effect of whipping speed on foam formation at pH 7 and I = 50 mM using a whipping time of 70 s, unless otherwise stated, with 0.5 mg/mL solutions of (A) β-lactoglobulin, ●; β-casein, ○; ASP, □; PPI (20% ethanol), ◇; and PPI (15% ethanol), ■; and (B) patatin (30 s), ◆; patatin (70 s), ▲; PIP, ▼; PAT-5E, △; and PIP-5E, ▽.

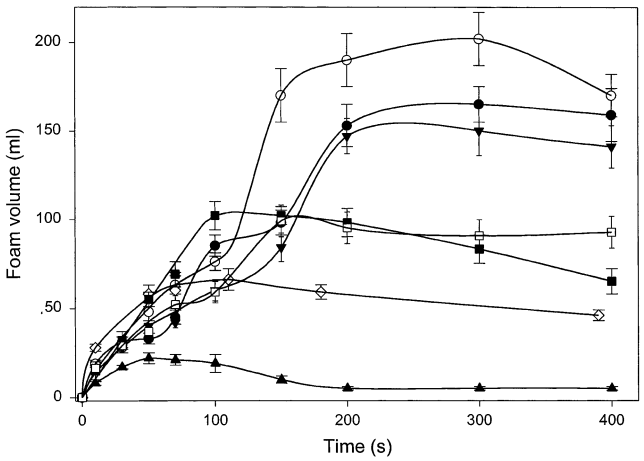

Figure 3. Effect of whipping time on foam formation at pH 7 and I = 50 mM using a whipping speed of 2500 rpm with 0.5 mg/mL solutions of PPI (20% ethanol), ◇; PPI (15% ethanol), ■; ASP, □; β-lactoglobulin, ●; β-casein, ○; patatin, ▲; and PIP, ▼.

in foam formation with whipping time until a maximum is reached at 60−100 s. Longer whipping times resulted in a gradual decrease in foam formation. Another curve shape is characterized by an initial strong increase in foam formation with whipping time but with a longer optimum whipping time of 150−300 s; this concerned β-casein, β-lactoglobulin, ASP, and PIP. During the whipping of these proteins, distinct transitions at 80 and 130 s were also observed, in that whitening of the liquid and a strong increase in viscosity occurred; the resulting foams had visibly smaller bubbles and a greater

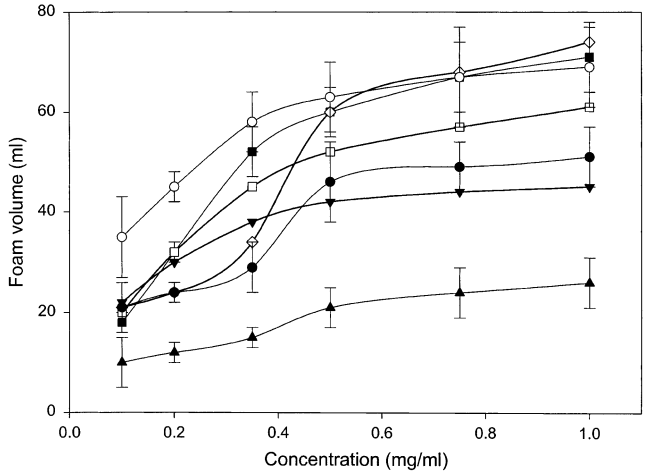

Figure 4. Effect of protein concentration on foam formation at pH 7 and I = 50 mM using a whipping speed of 2500 rpm and a whipping time of 70 s with solutions of PPI (20% ethanol), ◇; PPI (15% ethanol), ■; ASP, □; patatin, ▲; PIP, ▼; β-lactoglobulin, ●; and β-casein, ○.

firmness. In contrast to high whipping speeds, long whipping times usually did not result in visible protein aggregation. Only β-casein (at whipping times >100 s) formed precipitates on the whisk.

Higher whipping speeds or longer whipping times had similar effects; both resulted in increased foam formation and foam firmness and decreased bubble size, up to a certain level.

Effects of Protein Concentration. In **Figure 4**, the influence of protein concentration on foam formation can be seen. All of the protein preparations showed an increase in foam formation with increasing protein concentration. Two curve shapes could be distinguished. An S-shaped curve was found for PPI (20% EtOH), patatin, and β-lactoglobulin, with a remarkable increase in foam formation between 0.35 and 0.5 mg/mL. The curve shape found for ASP, PPI (15% EtOH), PIP, and β-casein featured a strong increase in foam formation with concentration up to 0.35 mg/mL, followed by a more gradual increase above this concentration. For all protein preparations, a visible decrease in bubble size was observed when the protein concentration was increased above 0.35 mg/mL. Therefore, if the surface area of the foam bubbles rather than the foam volumes were depicted, a much stronger increase would have been observed at higher concentrations. Indications that protein was not efficiently used during foam formation were obtained when the average bubble size was estimated by microscopy (results not shown). The average bubble size (d_{32}) of PPI at 0.5 mg/mL was estimated to be 150 μm. If a surface load of 3 mg/m^2 is assumed, then the amount adsorbed (in 60 mL of foam) is merely about 10% of the protein available.

Foam Properties at Various pH Using the Whipping Method. In **Figure 5A**, foam volume as a function of time is presented for foam made with various protein preparations (pH 7, I = 50 mM) using the whipping method. Curves of foam volumes as a function of time in **Figure 5** are examples of the curves generally obtained. Further results obtained using the whipping method are summarized in **Table 1**.

At pH 7, foam formation was highest for β-casein and PPI. Significantly less foam was formed with ASP, PIP-5E, and PAT-5E. Foam formation was lowest for patatin. Remarkably, ethanol precipitation of patatin and, to a lesser extent, PIP resulted in a marked increase in foam formation compared to that observed with untreated patatin and PIP. Patatin, PAT-5E, and PIP-5E formed the smallest bubbles, while the largest bubbles were

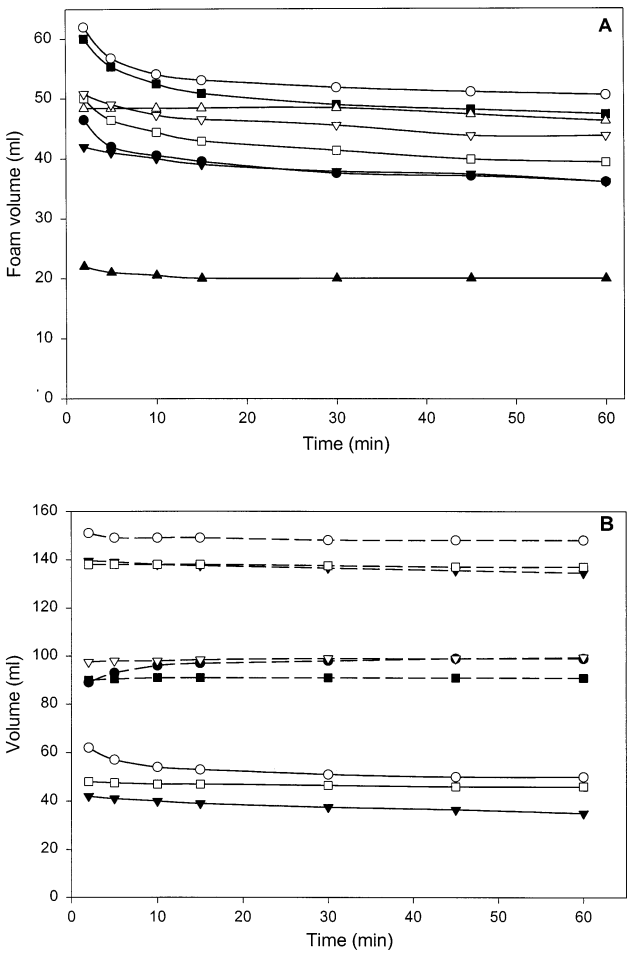

Figure 5. (A) Foam volume as a function of time for foam formed using the whipping method (2500 rpm, 70 s) at pH 7 and $I = 50$ mM with 0.5 mg/mL solutions of β-lactoglobulin, ●; β-casein, ○; ASP, □; PPI, ■; patatin, ▲; PIP, ▼; PAT-5E, △; and PIP-5E, ▽. (B) Foam volume (solid line) and upper and lower foam boundaries (dashed lines) of foam formed using the whipping method (2500 rpm, 70 s) at pH 7 and $I = 50$ mM with 0.5 mg/mL solutions of β-casein, ○(volume), ○(upper), ●(lower); PIP, ▼(volume), ▼(upper), ▽(lower); and PAT-5E, □(volume), □(upper), ■(lower).

observed in foam whipped from ASP, PPI, and β-lactoglobulin. In **Figure 5B**, examples are shown of foam volume and the upper and lower foam boundaries as a function of time for foam made with β-casein, PIP, and PAT-5E. The change in the lower foam boundary gives an indication of the amount of liquid drained from the foam, while the upper foam boundary indicates foam volume decrease due to other instabilities. Data on drainage are also presented in **Table 1**, together with the initial volume fraction of air in the foam. From **Figure 5B** it can be seen that the decrease in foam volume with time in foam made with β-casein is mainly due to drainage, while in foam made with PIP other instabilities prevail. Foams whipped from patatin and PAT-5E showed the slowest drainage, while drainage of foams formed from the other preparations increased in the order PIP and PIP-5E < ASP < β-lactoglobulin < PPI and β-casein (**Table 1**). Foam formed from PAT-5E was also the most stable against Ostwald ripening, while foams prepared from PIP, PIP-5E, and β-casein were the least stable against Ostwald ripening. Coalescence was observed only in the case of PIP and PIP-5E (**Table 1**).

Since the isoelectric pH of patatin is about 5, the effect of pH on the foam properties of various potato protein preparations was tested at pH 5, when protein solubility permitted. Because patatin is known to unfold irreversibly at pH $\leq$ 4.5 (*27*), the influence of unfolding was also tested by adjusting the pH of patatin containing preparations to 3 and subsequently back to pH 7 (pH 3 → 7).

In **Table 1**, the influence of pH on formation and stability of foams formed by various protein preparations can be seen. PPI formed less foam at pH 3 than at pH 7, but the foam at pH 3 drained more slowly and was more stable against Ostwald ripening, resulting in a volume decrease of 12% rather than 22% in 60 min. When PPI was dispersed at pH 3 and subsequently brought to pH 7, foam formation increased compared to that at pH 3. The foam drained faster than at pH 3, but it was more stable against Ostwald ripening and drained slower than that formed from PPI at pH 7, that had not previously been at pH 3.

ASP formed similar volumes of foam at pH 5 and pH 7. The foam formed at pH 5 drained faster than foam prepared at pH 7 and showed a higher relative decrease in foam volume for 60 min: 27% at pH 5 as compared to 22% at pH 7. At pH 3, ASP formed a bit more foam than at pH 7 and pH 5, with significantly smaller bubbles, but it showed somewhat faster drainage. If the pH had been adjusted from 3 to 7, the foam formed showed properties similar to those of the foam formed at pH 3 but seemed to be more stable against Ostwald ripening (**Table 1**).

PIP formed more foam at pH 5 than at pH 7 and pH 3, with smaller bubbles and a higher stabilty against Ostwald ripening. Also, the coalescence observed at pH 3 and pH 7 was not observed at pH 5. The foam properties of PIP at pH 3 were similar to those at pH 7, except for a somewhat enhanced stability against Ostwald ripening at pH 3. The relative decrease of foam volume after 60 min was 14% at pH 7, while it was 18% at pH 5 and pH 3 (**Table 1**).

The largest changes in foam formation with pH were observed with patatin. At pH 3, 2.5 times more foam was whipped from patatin than at pH 7. The resulting foam, however, drained faster and was less stable against Ostwald ripening than at pH 7. When the pH of patatin was first adjusted to pH 3 and then to pH 7 (pH 3 → 7), almost 3 times more foam was formed than at pH 7, and the foam was more stable against Ostwald ripening than at pH 3, although it drained faster. Foam whipped from patatin at its isoelectric pH (pH 5) was similar in amount but more stable against drainage than that from patatin (pH 3 → 7). The relative decrease in foam volume after 60 min was only 9% at pH 7 and 12% at pH 5, while it was about 24% for pH 3 and pH 3 → pH 7 (**Table 1**).

Effects of Ionic Strength. The effects of ionic strength on the formation and stability of foam whipped from PPI, ASP, PIP, and patatin at pH 7 are also shown in **Table 1**. In the case of ASP and PIP, a decrease in ionic strength resulted in a slight decrease in foam formation. Decreasing the ionic strength from 50 to 15 mM resulted, for all potato protein foams, in an increase in bubble size and a decreasing stability against Ostwald ripening (**Table 1**).

Increasing the ionic strength from 50 to 200 mM resulted, for PIP and patatin, in an increase in foam formation but also an increase in bubble size. Foam whipped from PIP at high ionic strength did not show coalescence. In the case of ASP and PIP, increasing the ionic strength resulted in the formation of foams that showed faster drainage (**Table 1**).

Effects of Heat Treatment. The effects of heating (80 °C, 10 min) solutions of PPI, ASP, PIP, and patatin at pH 7 on the foam properties are shown in **Table 1**. Heating of PPI did not result in increased foam formation. The resulting foam, however, contained smaller bubbles and was substantially more stable

Table 1. Summary of Results Obtained Using the Whipping Method (2500 rpm, 70 s) with Solutions Containing 0.5 mg of Protein per Milliliter

				foam volume (mL)[a]					occurrence of	
fraction	pH	I (mM)	ΔT[b]	2 min	60 min	φ (air)[c]	bubble size[d]	drainage (%)[e]	coalescence[f]	Ostwald ripening[g]
β-casein	7	50		62	50	0.67	++	49	–	++
β-lactoglobulin	7	50		47	36	0.66	+++	38	–	+++
PPI	7	15		58	45	0.70	++++	52	–	++++
	7	50		60	47	0.68	+++	52	–	++
	7	200		64	46	0.66	+++	46	–	++
	7	50	yes	61	48	0.68	++	51	–	+
	3	50		41	36	0.67	++	30	–	+
	3→7	50		56	45	0.66	++	37	–	+
ASP	7	15		44	41	0.70	++++	23	–	+++
	7	50		50	39	0.66	+++	29	–	++
	7	200		50	33	0.66	+++	41	–	++
	7	50	yes	37	33	0.71	+++	9	+	+
	5	50		51	37	0.63	+++	48	–	+++
	3	50		56	43	0.64	++	45	–	++
	3→7	50		58	44	0.66	++	46	–	+
PIP	7	15		33	30	0.79	++++	14	+	++++
	7	50		42	36	0.73	++	18	+	+++
	7	200		49	36	0.65	+++	41	–	+++
	7	50	yes	53	41	0.67	+++	40	+	+++
	5	50		60	49	0.65	+	19	–	++
	3	50		39	32	0.72	++	18	+	+++
PIP-5E	7	50		51	44	0.66	+	17	+	++++
patatin	7	15		22	20	0.72	++	0	–	+++
	7	50		22	20	0.65	+	0	–	++
	7	200		31	29	0.61	++	0	–	++
	7	50	yes	32	30	0.80	+	0	–	–
	5	50		58	51	0.67	++	5	–	++
	3	50		55	42	0.68	++	23	–	+++
	3→7	50		60	46	0.67	++	45	–	++
PAT-5E	7	50		48	46	0.61	+	5	–	–

[a] Average standard deviation, 5 mL. [b] Heat treatment (80°C, 10 min). [c] Volume fraction. [d] More plus signs indicates larger bubbles. [e] Percent drained of liquid initially present in foam. [f] +, Coalescence observed; –, coalescence not observed. [g] –, Slow Ostwald ripining; ++++, fast Ostwald ripening.

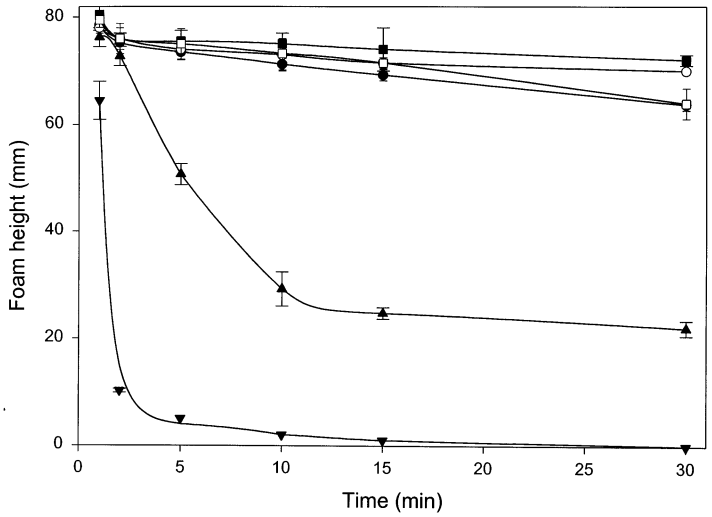

Figure 6. Foam height as a function of time for foam prepared using the sparging method at pH 7 and I = 50 mM with 0.5 mg/mL solutions of β-lactoglobulin, ●; β-casein, ○; ASP, □; PPI, ■; patatin, ▲; and PIP, ▼.

against Ostwald ripening. ASP formed less foam if it had been heated. The foam formed showed slower drainage but was less stable against coalescence. The foam volume had decreased by 22% for unheated ASP and by 11% for heated ASP after 60 min. PIP formed more foam after heat treatment, but the resulting foam drained faster, which may have resulted in the observed decrease in stability against coalescence. The relative volume decrease observed was 14% for unheated PIP and 22% for heated PIP. Heating of patatin resulted in the formation of more foam, which was very stable against Ostwald ripening (**Table 1**).

Foam Properties Using the Sparging Method. Sparging was used as an alternative to whipping because it is known that some proteins have a low foamability when whipped, while they do form foam when sparging is used. In **Figure 6**, foam height as a function of time is presented for foam formed from various protein preparations (pH 7, I = 50 mM) by sparging. It can be seen in **Figure 6** that the amount of foam formed by sparging was very similar for all protein preparations, quite unlike the results obtained with whipping. Even patatin, which showed a low foamability when whipped (**Figure 5**), produced a foam height comparable to that of the other proteins. Only PIP formed

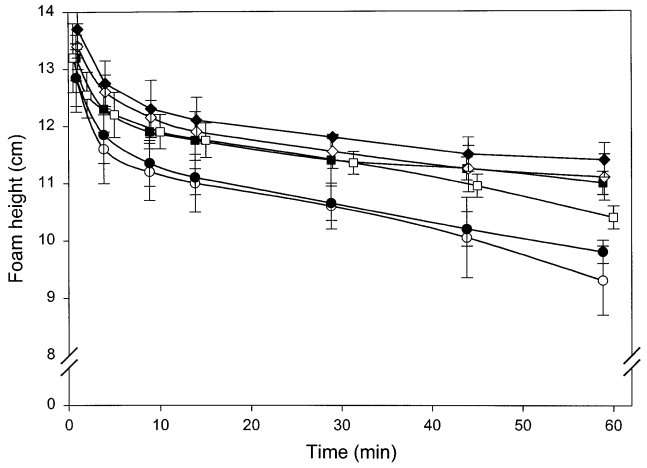

Figure 7. Foam height as a function of time for foam prepared with the Ledoux apparatus using whipping times of 70 and 360 s at pH 7 and I = 50 mM with 10 mg/mL solutions of β-casein (70 s), ○; β-lactoglobulin (70 s), ●; PPI (70 s), ■; PPI (360 s), ◆; ASP (70 s), □; and ASP (360 s), ◇. Initial liquid height: 3.0 cm.

somewhat less foam than the other preparations. Bubble sizes in sparged foams were much larger than in whipped foams and increased in the order β-casein and PPI < β-lactoglobulin < ASP ≪ patatin ≪ PIP. Foam formed by PPI and β-casein also drained the least, while foam stabilized by patatin and PIP drained the most and fastest. It is possible that the fast and extensive drainage resulted in the poor stability against coalescence and Ostwald ripening observed in foam stabilized by patatin and PIP.

Foam Properties Using the Ledoux Apparatus. With the whipping method, low protein concentrations were used to emphasize the differences in foam properties between the protein preparations. To test the foam properties of some potato protein preparations at conditions more similar to food manufacture, we used the Ledoux apparatus at a protein concentration of 10 mg/mL. **Figure 7** shows the foam height as a function of time for foam whipped from solutions of ASP, PPI, β-casein, and β-lactoglobulin. As can be seen in **Figure 7**, no significant differences in initial foam formation were observed. Using a whipping time of 70 s, foam prepared from ASP and PPI was visibly more stable against Ostwald ripening than foam prepared from β-casein and β-lactoglobulin. Also, foam made with ASP and PPI under these conditions showed less drainage than foam prepared from β-casein. Increasing the whipping time for ASP and PPI from 70 to 360 s caused a decrease in bubble size. At both whipping times, a smaller bubble size was observed for foam made with PPI than for that made with ASP.

DISCUSSION

Foam Properties of Patatin. The foam properties of patatin at pH 7 (**Figure 5**) resemble those of a structurally rigid protein like, e.g., lysozyme (*28*, *29*), being characterized by the formation of only a small volume of foam consisting of small bubbles. Such a structurally rigid protein would presumably unfold slowly at the interface (*11*), which would also agree with the results shown in **Figure 2**. At a whipping time of 30 s, the optimum whipping speed was observed to be 3000 rpm, which is low compared to those for the other protein preparations. At higher whipping speeds, patatin is presumably not able to unfold fast enough to produce a surface pressure sufficient to stabilize the newly formed bubbles against immediate coalescence by forming γ-gradients (*11*). Longer whipping times may result in increased foam formation due to the adsorption of surface-denatured proteins, as is the case when whipping ovalbumin (*30*, *31*). The rate of unfolding of patatin at the interface must be very slow, or its rate of refolding, when desorbed from the interface, must be fast, since longer whipping times do not result in increased foam formation. Foam formation from patatin by sparging, which is characterized by low surface expansion rates as compared to whipping, is similar to that of other protein preparations (**Figure 6**). But even then, the unfolding of patatin is apparently too slow to stabilize the foam against extensive drainage, Ostwald ripening, and coalescence (**Figure 6**). In contrast, Ralet and Guéguen (*7*) found that very stable foams could be formed by sparging air through a 1 mg/mL patatin solution. The higher protein solution in the latter case, 1 mg/ml instead of 0.5 mg/mL, may have contributed to the observed stability.

When whipped at its isoelectric pH (pH 5), patatin forms much more foam, which has been observed for various proteins (*32*, *33*). However, in the case of patatin the tertiary structure is already affected at pH 5 (*18*) which may have affected its foaming properties. Ralet and Guéguen observed a decrease in both foam formation and stability against drainage when sparging a patatin solution at pH 5 as compared to pH 7 (*7*). As can be seen from **Figures 2** and **5** and **Table 1**, PAT-5E, which differs from patatin in having a partially unfolded tertiary structure, forms much more foam than patatin when both are whipped at the same conditions. Apparently, as emphasized by Damodaran and co-workers (*34*, *35*), the flexibility of the tertiary structure is important in foam formation. The increase in foam formation is not accompanied by a decrease in foam stability, since drainage rate, presumably due to a smaller bubble size, and Ostwald ripening are lower than those in foams produced from β-casein and β-lactoglobulin (**Table 1**). Patatin is unfolded more extensively and irreversibly by adjusting the pH to 3 (*27*), and foam formation at pH 7 then increases even more, though it also results in faster drainage (**Table 1**). In contrast to these observations, Ralet and Guéguen observed a decrease in foam formation at pH 4, at which patatin is already unfolded, and also an increase in drainage rate compared to the properties at pH 7 (*7*). Irreversible unfolding of patatin by heating at 80 °C resulted in a much smaller increase in foam formation, although the amount of residual structure in the protein after heat-induced unfolding was shown to be similar to that after pH-induced unfolding (*27*). This difference in foam formation may be caused by a decrease of the effective molar protein concentration due to a higher degree of aggregation after heating, which has been well established for emulsion formation (*36*).

The effect of various pretreatments on the foaming properties of patatin indeed shows that these properties are very sensitive to the structural stability of the protein and to the degree of unfolding of the tertiary (*37*) and secondary structure (*38*) of the protein. Unfolding may enhance both foam formation and foam stability, but complete loss of protein structure may result in extensive aggregation and a decrease in solubility, and thus be detrimental to its foaming properties (*34*, *35*, *39*).

Foam Properties of the PIP. When whipped at pH 7, PIP forms more foam than patatin. The protease inhibitors in PIP generally have an even higher structural stability than patatin (*18*). However, their pI's are known to cover a wide range (pH 5.1→ 9) (*20*), which has been observed to increase foam formation when mixtures of proteins differing substantially in pI were compared to pure proteins (*33*). The stability of foam formed from PIP at pH 7 did, however, show a low stability against Ostwald ripening and especially against coalescence

(**Table 1**). The same instabilities became even more apparent when foam was produced from PIP by sparging. The formation of very unstable foams by sparging of potato protease inhibitor solutions has also been observed by Ralet and Guéguen (*7*). In contrast, at pH 5, no coalescence was observed. Since PIP was prepared by gel filtration chromatography, the presence of coalescence-inducing impurities in this preparation is very unlikely. The authors have, until now, no explanation for the coalescence in PIP-stabilized foams.

Foam Properties of PPI. PPI (15% EtOH) formed much more foam than PPI (20% EtOH) at whipping speeds >3000 rpm (**Figure 2**). This difference can be explained by the observation that dispersions of PPI (20% EtOH) contain substantial amounts of large aggregates, as observed by gel filtration chromatography (results not shown), thereby lowering the effective molar protein concentration. At low whipping speeds (<3000 rpm), this reduction of the effective protein concentration is not noticed, presumably because new surface is not formed extremely fast and the large aggregates may even contribute to the stabilization of the newly formed bubbles against coalescence. At high whipping speeds, however, the decrease in effective molar concentration presumably prevails, since the rate at which new interface is created is higher, and foam formation with PPI (20% EtOH) decreases. In the remainder of this section, only the properties of PPI (15% EtOH) will be discussed.

PPI at pH 7 formed more foam than PAT-5E, PIP-5E, and ASP. The stability of the foam formed from PPI at pH 7 is similar to that of PIP-5E and ASP (**Table 1**). When the foam properties of un-denatured potato proteins in ASP are compared to those of ethanol-precipitated proteins in PPI (**Table 1**), it can be seen that foam formation with PPI is higher. Foam prepared from ASP generally drains more slowly than that prepared from PPI, but the relative decrease in foam volume after 60 min is similar.

Foam Properties of (Partially) Unfolded Proteins. Various degrees of unfolding of the tertiary and secondary structure of potato proteins by ethanol, pH variations, or heat treatment generally resulted in a substantial increase in the stability against Ostwald ripening (**Table 1**). The driving force for Ostwald ripening is the Laplace pressure difference over the curved surface of a bubble: $\Delta p = 2\gamma/R$ (*40*). If the proteins do not desorb when the bubble shrinks, then γ will continue to decrease and thereby reduce the driving force for Ostwald ripening (*40*). Restricted unfolding of proteins prior to foaming has been shown not only to increase foam formation, but also to increase intermolecular interactions between proteins in the interface in several cases (*30*, *33*, *35*, *38*). The increase of these interactions will presumably reduce the probability that the proteins desorb when the interface is compressed, and thereby stabilize the bubbles against Ostwald ripening (*41*). Lowering of the ionic strength was shown to reduce the stability against Ostwald ripening (**Table 1**) (*42*), which can possibly be explained by an increase in electrostatic repulsion distance in the interface, thereby reducing the strength of attractive interactions between the proteins in the interface. Increasing the ionic strength, on the other hand, results, in some cases, in an increase in the amount of foam formed and, more generally, in an increased drainage rate (**Table 1**). When an increase in drainage rate was observed with increasing ionic strength, the initial volume fraction of liquid in the foam was also found to be higher. Ralet and Guéguen (*7*) did not find a general effect of ionic strength on drainage rate. In general, the drainage rate could not be related to the initial volume fraction of air in the foam. A strong correlation ($R^2 = 0.828$) was, however, found between the total volume of liquid in the foam and the rate of drainage (results not shown), excluding the foams made with patatin.

In conclusion, it was shown that less foam could be formed from untreated patatin than from the protease inhibitors, but patatin foam was much more stable. The foam-forming properties of patatin could be strongly improved by partial unfolding of the protein. Whipping tests, at a concentration of 0.05% (w/v), also indicate that foams made with PAT-5E and the industrially more relevant PPI are more stable than those made with β-casein and β-lactoglobulin (**Table 1**), also at industrially more relevant protein concentrations (1% (w/v)). Partsia and Kiosseoglou also found that whipping 0.25−1.0% (w/w) potato protein solutions resulted in foams with a higher foam expansion and a higher stability against drainage than foams made with the same concentration of freeze-dried egg white (*6*).

More generally, it can be concluded that when proteins are used as a foaming agent, a high concentration is required, because the protein available is inefficiently used. Various proteins differ markedly, however, in the concentration that is needed to make a certain amount of foam. Also, there are several variables that may all, in different ways, affect both foam formation (amount of foam, bubbles size distribution) and foam stability against various instability processes. These variables include the type and concentration of protein, solvent conditions (pH, *I*), and the method used to make the foam. A simple conclusion, therefore, cannot be drawn.

ABBREVIATIONS USED

A, interfacial area (m^2); ASP, ammonium sulfate precipitate; E_{SD}, surface dilational modulus $\equiv d\gamma/d \ln A$; *I*, ionic strength; *L*, distance between pins on beater (m); p_L, Laplace pressure $= 2\gamma/R$ (Pa); PAT-5E, patatin resolubilized at pH 7 after precipitation at pH 5 in the presence of 20% (v/v) ethanol; PFJ, potato fruit juice; PIP, protease inhibitor pool; PIP-5E, PIP after precipitation at pH 5 in the presence of 20% (v/v) ethanol; PPI or PPI (15% EtOH), potato protein isolate prepared by precipitation at pH 5 in the presence of 15% (v/v) ethanol; PPI (20% EtOH), potato protein isolate prepared by precipitation at pH 5 in the presence of 20% (v/v) ethanol; *R*, bubble radius (m); υ, whipping speed (m/s); γ, interfacial tension (N/m); η_{SD}, surface dilational viscosity ($=d\gamma/(d \ln A/dt)$); ρ, density of liquid phase (kg/m^3).

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