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Batch Foam Recovery of Sporamin from Sweet Potato

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ABSTRACT

The major sweet potato root protein, sporamin (which comprises about 80–90% of the total protein mass in the sweet potato) easily foams in a bubble/foam-fractionation column using air as the carrier gas. Control of that foam fractionation process is readily achieved by adjusting two variables: bulk solution pH and gas superficial velocity. Varying these parameters has an important role in the recovery of sporamin in the foam. Changes in the pH of the bulk solution can control the partitioning of sporamin in the foam phase from that in the bulk phase. A change in pH will also affect the amount of foam generated. The pH varied between 2.0 and 10.0 and the air superficial velocities (V_0) ranged between 1.5 and 4.3 cm/s. It was observed in these ranges that, as the pH increased, the total foamate volume decreased, but the foamate protein (mainly sporamin) concentration increased. On the other hand, the total foamate volume increased significantly as the air superficial velocity increased, but the foamate concentration decreased slightly. The minimum residual protein concentration occurred at pH 3.0 and V_0 = 1.5 cm/s. On the other hand, the maximum protein mass recovery occurred at pH 3.0 and at V_0 = 4.3 cm/s.

Index Entries: Batch foam fractionation; bioseparation; protein separation; sweet potato; sweet potato proteins.

INTRODUCTION

Plants usually store their food in seeds and roots for subsequent germination and budding. The storage-root plants, such as cassava, taro, and sweet potato, have been used as important food-starch sources for many centuries. Sweet potatoes are also nutritionally important for their beta-carotene, vitamin C, fiber, potassium, and protein content *(1,2)*. The sweet

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potato protein level generally varies according to the way it was cultivated (3), as well as the environmental growing conditions, genetic factors (4,5), and storage conditions (6). The protein level typically ranges between 1.3 and 10% (on a dry basis) (2). The water content is generally 80% by weight. The protein distribution in a sweet potato is spread quite evenly throughout the stored root (7). Sporamin (ipomoein), a globulin protein, is a major protein comprising about 80–90% of the total protein content in the sweet potato (1,8). One interesting property of sporamin is that it can act as a strong surfactant, creating foam in water when air bubbles are introduced to a sweet potato extract solution. Since sporamin foams easily and naturally by itself, a batch foam fractionation process may be a promising technique for collecting, concentrating, and separating it from the other proteins. The other proteins are mostly α and β amylase, which comprise about 5% of the total soluble proteins (4).

A batch foam-fractionation process generally uses air or an inert gas as a carrier gas. The gas is introduced to the process through a nozzle or a sparger at the bottom of the foam-fractionation column. The gas then creates bubbles in the liquid (mainly water) solution. If one of the proteins in the solution foams, a foam layer can be developed above the bulk-liquid phase. Because of its high surface activity, sporamin can create a high protein concentration at the air–liquid interface above the bulk liquid and below the foam layer (9). Typically, sporamin from the bulk solution moves by diffusion to the air–liquid bubble interface and attaches to a rising bubble. Once bubbles reach the liquid surface, they are transformed into a foam. In the foam phase, foam cells move upward to the top of the column, and then to the foam collector as foamate (collapsed foam) (10). From experience and reported work in the literature (10–12), the foamate protein concentration for this foam-fractionation process is controlled by the initial solution pH, the initial bulk-protein concentration, the air superficial velocity (11), and the processing time (τ) taken to generate the foam, referred to here as the batch-processing time. The initial bulk solution pH plays an important role in controlling the ionic charge balance in the sweet potato extract solution. When the net ionic charge in the protein solution is equal to zero (called the isoelectric point [pI]), the protein coagulates and becomes easier to separate from the bulk solution. The pI condition is generally a desired setting for separating proteins from a water solution and is expected to play the same role for sporamin in a sweet potato extract solution.

MATERIALS AND METHODS

Sample Preparation

Sweet potatoes (marketed by Dixie Lamb Alabama Sweet Potato Company, AL) were purchased from a local grocery store. This stored sweet potato root was cleaned with water and air-dried at room temperature. A large section was removed from the sweet potato perpendicular

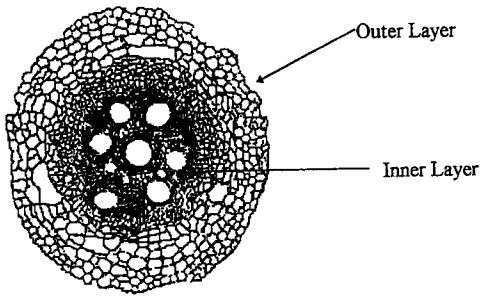

Fig. 1. Anatomy of the cross-section of sweet potato storage root.

to the longitudinal axis (approx 10 cm long) and used for the experimental samples. Both of the tapered ends of the sweet potato stored root were discarded. The typical anatomy of the cross-section of the sweet potato root is shown in Fig. 1. For the purpose of this study, the slice was dissected into two zones: the outer layer and the inner layer. Typically, the outer layer is comprised of the epidermis, cortex, and lacuna, and the inner layer is comprised of the parenchyma, phloem, protoxylem, primary cambium, secondary xylem, metaxylem, and endodermis *(13)*. The diameter, outer layer, and inner layer thickness of a sweet potato were determined to be approx 4–6 cm, 4–5 mm, and 4–6 cm, respectively. Outer layer, inner layer, and combined layer samples were analyzed for protein *(14)*, starch, fiber, reducing sugar *(15)*, moisture content, and others (tannins, beta-carotene, and so on).

The combined layer samples were used for the batch foam-fractionation experiments. A large center section of the sweet potato (about 5 cm in diameter, 1 cm thick, and ca. 20 g) was used as the experimental sample, and chopped into small pieces. These pieces were prepared for the foam fractionation run by combining them with 200 mL of deionized water in a 350-W motor food blender (Blend Master 10, Hamilton Beach/Proctor-Silex, Washington, NC). The blender chopped up these pieces for 4 min at the "liquefy" speed setting. The produced liquefied sweet potato extract solution was then filtered through Whatman (Clifton, NJ) No. 4 filter paper. The filter cake was washed several times, until only residual fiber remained on the filter paper. The filtered solution was then decanted and the starch was removed from the solution in a centrifuge (Marathon 21K, Fisher Scientific, Pittsburgh, PA) at a speed of 1073*g* for 4 min. The supernatant was collected and the starch was discarded. Water was added to the starch-free supernatant to increase the volume of the solution to 1 L. The protein-rich solution was stored in the refrigerator at 10°C, until used for the foam-fractionation experiment (typically within 2 d).

Experimental Procedure

The batch foam-fractionation apparatus was comprised of a glass cylinder 2.5 cm in diameter and 36-cm long, as shown in Fig. 2. The top port

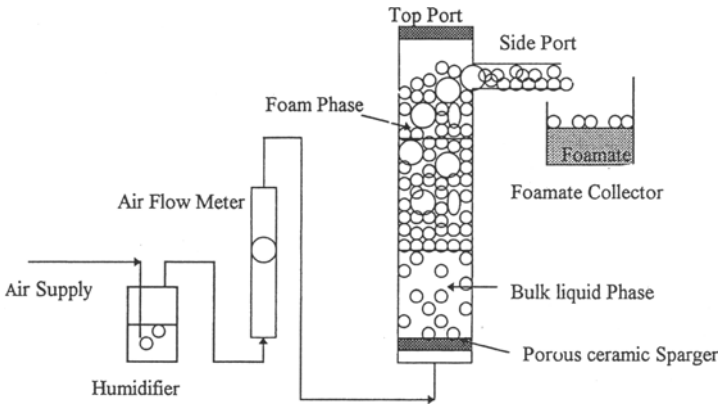

Fig. 2. Schematic of the batch foam-fractionation process.

was used for loading the sample, and closed with a rubber stopper during foam fractionation. The opened side port allowed the foam to flow into the foam collector. A porous ceramic disk sparger was inserted into the bottom of the foam-fractionation column, and connected to the air supply copper tube by aluminum flanges. The air-flow rate was measured using an air-flow meter (rotameter type). The air supply was prehumidified by bubbling it through water to minimize both column water loss and protein contamination from the influent air. The incoming air was therefore held close to constant relative humidity, since its temperature was 23 ± 2°C. No attempt was made to determine the effect of influent air humidity on foam formation in these experiments.

Sweet potato protein-extract solution was adjusted initially to the desired pH (between 2.0 and 10.0) by adding hydrochloric acid or sodium hydroxide. This initial volume of extract solution used for batch experiment in the foam-fractionation column was 100 mL. The air (superficial velocity ranged between 1.5 and 4.3 cm/s) was introduced to the porous sparger at the bottom of the column. The bubbles entered the bulk-protein mixture, rose up to the liquid surface, and then generated foam above the liquid surface. Foams were continuously carried up to the top of the column in the open space above the liquid, eventually passing through the side port to the foam collector. Foam collapsed on its own (~10 min) and created liquid high-protein concentrate (foamate). All of the experiments were carried out at room temperature (23 ± 2°C). Typically, the bubble diameter is a function of the liquid surface tension, viscosity, and density, and these in turn are functions of temperature. Keeping the temperature fixed in these experiments helped maintain the bubble at a constant diameter. No attempt was made to vary the temperature in these experiments. The batch foam-fractionation experiments were terminated when foam flow to the foam collector stopped (batch processing time, τ, ca. 2–10 min).

Both the foamate and the residual volumes and also pHs were measured. The total protein concentrations in both the foamate and the residual liquid were determined at this processing time (τ), using the Bradford Coomassie blue method *(14)*. The optical absorbance, measured spectrophotometrically, was converted to total protein concentration in mg/L, using a total protein–optical absorbance calibration curve for sweet potato proteins, originally developed by determining the foamate protein mass gravimetrically.

Electrophoresis

Gel electrophoresis was used to identify the mol wt of the two primary proteins (sporamin and β-amylase), and their respective approximate relative concentrations within both the foamate and the bulk liquid of the foam-fractionation process. α-amylase (ca. 20% of the total amylase concentration) did not show up on the gel. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed in this study, following the Laemmli procedure *(16)*. The proteins passing through the 11% polyacrylamide gel were stained with Coomassie brilliant blue R-250 to mark the protein bands. Typically, use of this stain leads to easily seen sharp bands on the gel when the protein mass in the analyzed solution ranges between 15 and 20 μg *(17)*. In these experiments, bovine serum albumin (Lot No. 41F-9300), ovalbumin (Lot No. 55F8510), and carbonic anhydrase (Lot No. 43F-8050), purchased from Sigma (St. Louis, MO), were used as the protein mol wt markers to calibrate the gel at the approximate mol wt of 68 kDa, 45 kDa, and 29 kDa, respectively.

RESULTS AND DISCUSSION

The outer, inner, and combined layers of the sweet potato used for the foam-fractionation experiments were evaluated for starch, fiber, reducing sugar *(15)*, protein *(14)*, and moisture content, as shown in Table 1. The colors of the sweet potato extract solution of the outer, combined, and inner layers were observed to be dark brown, brownish orange and bright orange, respectively. The dark brown color of the outer layer sweet potato extract solution may be caused by its high tannin content in the root epidermis. The outer layer of the root is comprised of fiber, tannin, and lignin, in order to reinforce and protect the root from environmental damage. The fiber from the outer layer was observed to be coarse and thick, and the fiber from the inner layer was much finer. The inner layer is used by the plant for storing food in the form of starch. In particular, the starch is stored in the parenchyma cells *(13)* within the inner layer. It is seen in Table 1 that the outer layer is high in fiber, but low in starch. The protein content of the outer layer is slightly higher than that in the inner layer. This observation seems reasonable, because the outer layer is the place

Table 1
Percentage of Compositions of Outer, Inner, and Combined Layers of Sweet Potato on a Fresh Root Basis

Compositions	Outer layer % wt	Inner layer % wt	Combined layer % wt
Starch	4.32	5.98	5.60
Fiber	6.37	6.03	6.13
Reducing sugar	4.86	8.23	6.36
Proteins	0.53	0.45	0.46
Moisture	80.05	79.03	79.43
Others	3.87	0.28	2.02

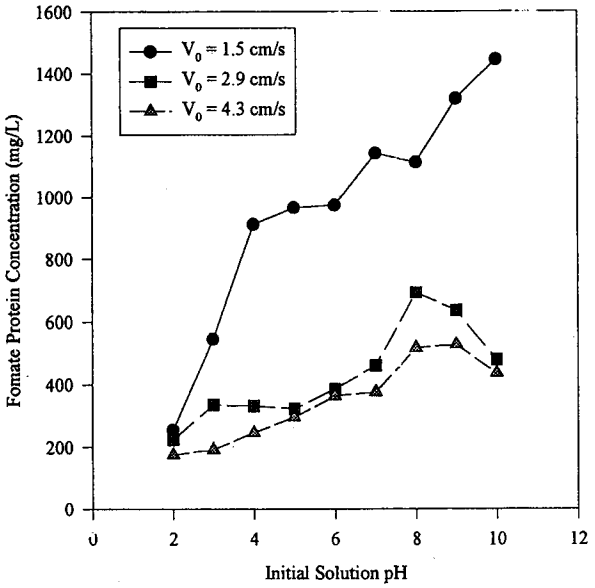

Fig. 3. Foamate protein concentration for V_0 1.5, 2.9, and 4.3 cm/s, and $2 \leq \tau \leq 10$ min in a batch foam fractionation.

where the sweet potato is germinated. From these results, the total protein content (~80% of which is sporamin, the storage protein) is not much different between outer and inner layer. If the percentage of sporamin content remains a constant in both layers, then any cutting of the sweet potato root would be representative and suitable for a foam fractionation.

The air superficial velocity and the initial solution pH seem to be important independent variables in the foam-fractionation process. For example, as the air superficial velocity increases, both the foamate concentration and the foamate volume decreases. It can be seen in Fig. 3 that the foamate protein concentration increases as the initial bulk-solution pH

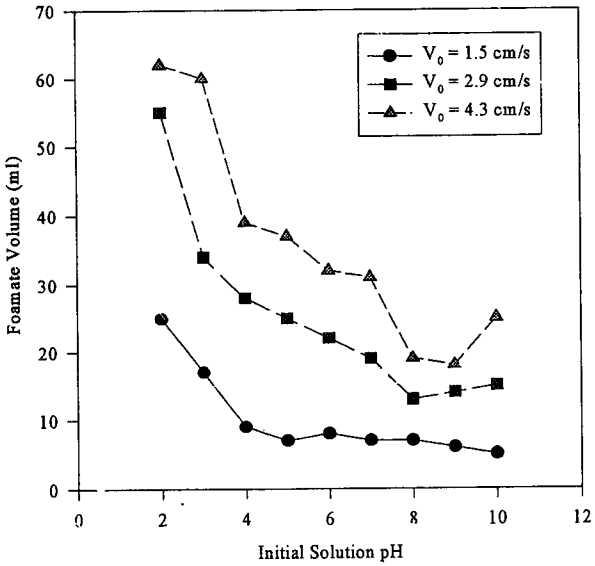

Fig. 4. Foamate volume for V_0 = 1.5, 2.9, and 4.3 cm/s, and $2 \leq \tau \leq 10$ min a batch foam fractionation.

increases. In the acidic range, it was observed that proteins seem to coagulate and precipitate (denature), as evidenced by suspended particles in the foamate. In the basic range, the soluble foamate protein concentration rises, while the concentration of suspended particles (insoluble protein) in the foamate decreases. When the air superficial velocity increases from 2.9 to 4.3 cm/s, the foamate protein concentrations increase slightly, particularly at the high pHs; the foamate volume increases considerably over the entire pH range. Air bubbles at the air superficial velocities of 2.9 and 4.3 cm/s rise very fast and are turbulent in the liquid-bulk phase. Both the bubble diameters and the foam cell diameters for these velocities are approximately constant in the range of 2–4 mm. These diameters typically increase near the end of a run when the amount of generated foam (protein surfactant) decreases. At the air superficial velocity of 1.5 cm/s, both the bubble and foam cell sizes are constant, at about 2 mm in diameter, and foam cells move slowly upward in the column. Liquid drainage from the foam can be seen clearly. The high liquid drainage from the foam phase typically enhances the foamate protein concentration, particularly at an air superficial velocity of 1.5 cm/s curve, as shown in Fig. 3. Because the bulk liquid can be entrained in the foam cellular structure at high air superficial velocities, the foamate volume increases as the air superficial velocity increases, as shown in Fig. 4. On the other hand, an increase in the initial solution pH lowers the foamate volume. This decrease of the foamate volume with an increase in pH may be caused by the changes in resulting ionic charges.

This change, in turn, reflects the underlying changes in sweet potato protein-water structure, which causes the proteins to be less surface-active, and, hence, create less foamate volume.

After the experiment is terminated (when foam ceases to flow to the foam collector), the residual bulk-liquid volume, bulk-protein concentration, and bulk-solution pH are determined. It was found that the changes in bulk solution and foamate pHs were approximately: $\Delta pH_{Foamate} = 0.5$ (acidic range) and -0.5 (basic range), where $\Delta pH_{Foamate} = pH_{Foamate} - pH_{Initial}$ and also $\Delta pH_{Residue} = -0.1$ (acidic range) and 0.1 (basic range). "Initial" pH here refers to the initial bulk pH. The residual protein concentration is lowest for the case in which the initial solution pH was 3.0, as shown in Fig. 5. The effect of a change in superficial velocity is generally less significant than the effect of a change in pH. The relative response to changes in V_0 depends on pH, and it is seen in Figs. 4–6 that the response to this effect is not obvious. Figs. 5 and 6 represent the tradeoff between protein recovery and protein concentration. Typically, before air is introduced to the foam-fractionation column, the initial solution is cloudy. After the foam-fractionation experiment has been completed, the residual bulk liquid is cleared of the colloidal matter and looks like normal deionized water. Generally, when the pH of the protein in solution is at its p*I*, the solution is cloudy and protein precipitates as colloids or particles. The sweet potato-water solution is most cloudy at pH 3.0, indicating that the sweet potato-protein solution pI is close to this value. Because sporamin is the major protein in the sweet potato storage root, the precipitation phenomena seems to imply that the pI of sporamin is around 3. As the initial solution pHs depart from 3, the residual protein concentrations rise, and the total protein recovery decrease, the proteins become more soluble in the bulk-liquid phase and less precipitated in the foam phase.

Typically, for pHs above 3.0, an increase in the initial solution pH enhances the foamate protein concentration, but reduces the generation of foamate volume (Fig. 4). The total protein recovery (the percentage of recovered protein mass in the foamate, relative to the initial protein mass in the sweet potato extract solution) is an important variable for determining a local maximum in the different protein-separation cases. Figure 6 shows that the total protein recovery reaches a maximum at pH 3.0 (which corresponds to the lowest residual protein concentration seen in Fig. 5), and then decreases for the initial solution pHs away from 3.0. It is also observed in Fig. 6 that an increase in air superficial velocity in the studied V_0 range tends to enhance the total protein recovery. The total protein recovery (mostly sporamin) ranged between 77 and 87% over the studied range at pH 3.0.

Another important variable to consider is the batch processing time (τ) (as shown in Fig. 7 which is to be minimized. The global optimum is resolved when the pH and V_0 variables are different for these possibly conflicting objectives through a cost analysis. Here, the optimum is clearly at pH 3.0, close to the pI.

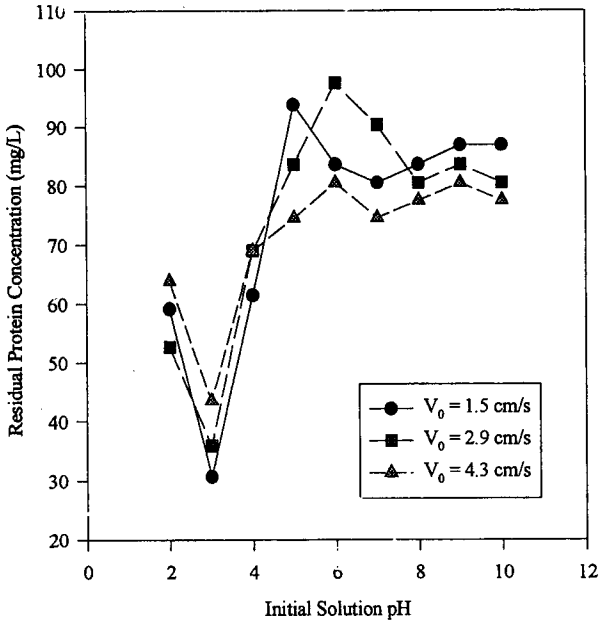

Fig. 5. Residual protein concentration for air superficial velocity 1.5, 2.9, and 4.3 cm/s in a batch foam fractionation.

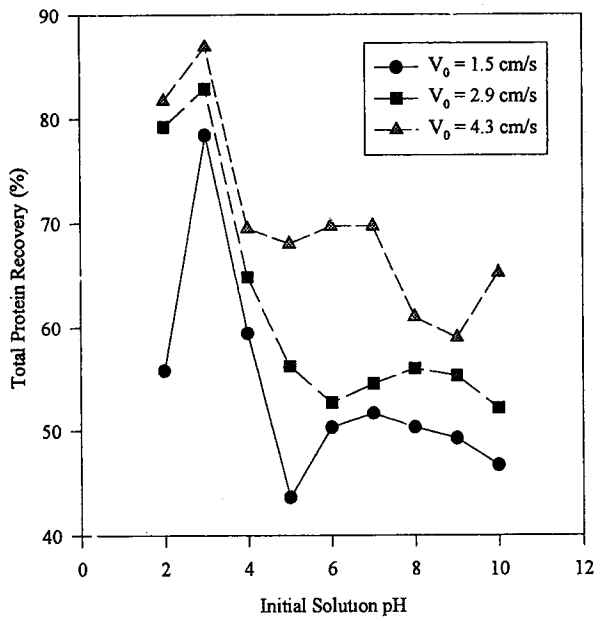

Fig. 6. Total protein recovery for air superficial velocities 1.5, 2.9, and 4.3 cm/s in a batch foam fractionation.

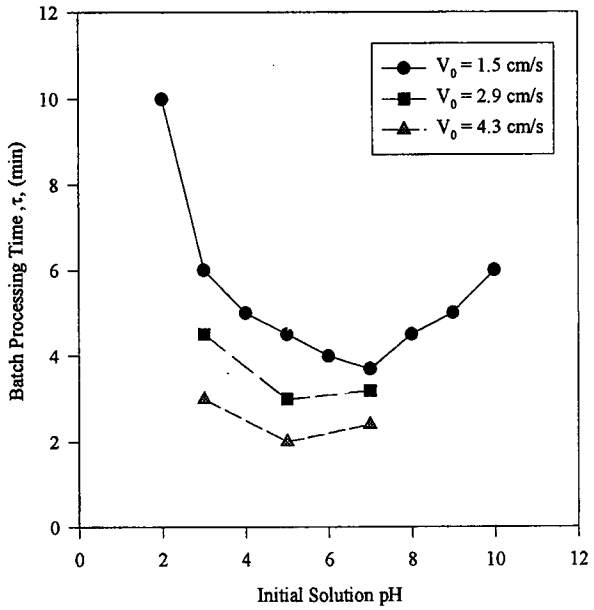

Fig. 7. Sweet potato batch processing time (τ) as a function of pH and V_0.

SDS-PAGE of foam fractionation of sweet potato proteins is illustrated in Fig. 8. Lanes 1–4 contain the three marker proteins: bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), and lane 12 contains α-Amylase (51 kDa). Lanes 7 and 10 contain foamate proteins, and lanes 8 and 11 contain bulk-solution proteins at pH 3.0 and 5.0, respectively. Lane 9 represents the sweet potato feed stock proteins. Lanes 5 and 6 contain foamate and bulk-solution proteins, respectively. For lanes 5 and 6, the sweet potato proteins were foam-fractionated at pH 5.0, and then the residue was foam-fractionated again at pH 3.0 to obtain the foamate and the bulk solution for lane 5 and 6, respectively. From previous studies *(3,8)*, the mol wt of sporamin was estimated at 25 kDa, which corresponds to the major strong band in lanes 5, 7, 9, and 10. As shown in lanes 5, 7, and 10, it is clear that foam fractionation can be used to concentrate sporamin from the sweet potato extract. Sporamin concentration at both pH 3.0 and 5.0 is 3–5× greater than sporamin in the original feed stock solution.

The second abundant protein, β-amylase, has a mol wt around 201 kDa, with four equal molecular subunits, each subunit being about 50 kDa *(18)*. This protein can be observed as a small sharp band in lane 5. It is difficult to observe the band of β-amylase in lanes 7 (pH 5.0) and 10 (pH 3.0), because the sporamin is much more abundant than β-amylase, and has a stronger surface activity than β-amylase. When the sweet potato extract is foam-fractionated, the foamate containing sporamin comes out before the β-amylase. The β-amylase can be recovered at higher concentrations than that in the feedstock solution when the sporamin is depleted

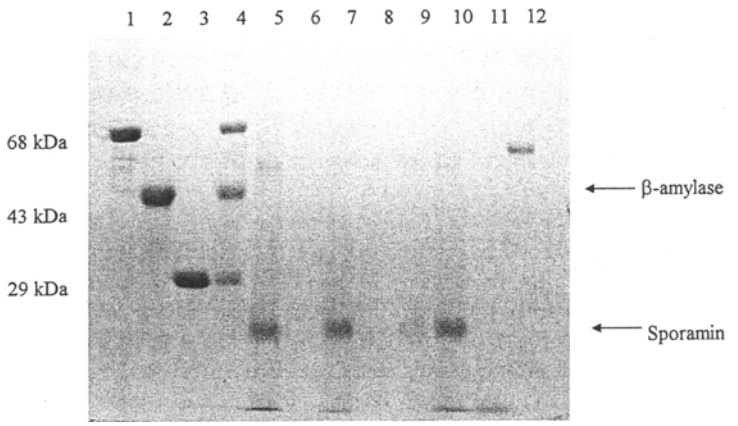

Fig. 8. SDS-PAGE of marker and foam fractionated sweet potato proteins: Lane 1, bovine serum albumin; lane 2, ovalbumin; lane 3, carbonic anhydrase; lane 4, the mixture of lane 1, 2, and 3; Lane 5, foamate at pH 3.0 (run at pH 5.0 and the pH 3.0); lane 6, bulk solution at pH 3.0 (run at pH 5.0, and then pH 3.0); lane 7 foamate at pH 5.0; lane 8, bulk solution at pH 5.0; lane 9, sweet potato feed stock solution; lane 10, foamate at pH 3.0; lane 11, bulk solution at pH 3.0; and lane 12, α-Amylase.

first in the bulk solution, as shown in lane 5. One way to selectively deplete the sporamin is to first foam the bulk solution at pH 5.0, concentrating sporamin into the foamate. Then, at pH 3.0 the β-amylase can be recovered, along with residual sporamin, by foaming the bulk solution. Sporamin appears to be more surface-active than β-amylase; thus, sporamin is more easily separated by foaming. After much of the sporamin has been removed, the β-amylase attaches more readily to the adsorptive sites on the air bubbles. Generally, amylase does not foam when the air bubbles are introduced into a foam-fractionation column containing only this protein. Thus, it has been shown in this experiment that β-amylase is concentrated only in the foamate, in conjunction with a foaming protein such as sporamin. In the natural sweet potato system, both proteins are present together, and no foaming surfactant is required to recover β-amylase in the foamate.

CONCLUSIONS

Sweet potato storage protein (primarily sporamin) recovery in batch foam fractionation from a sweet potato-water extract is maximized at pH 3.0, decreasing as the initial solution pH increases. In addition, an increase in the air superficial velocity enhances the total protein recovery, which is highest (about 87%) at an air superficial velocity of 4.3 cm/s. However, the β-amylase subunit (50 kDa) can also be recovered in the foamate, at a higher concentration than the bulk solution, by first foaming the bulk solution at pH 5.0 (to remove much of the sporamin), and then foaming at pH

3.0 to recover the β-amylase, along with the residual sporamin. Foam fractionation of a sweet potato-water extract solution seems to offer a promising low-cost first step in recovering sporamin and β-amylase from sweet potato.

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