Effect of Bubble Velocity and pH Step Changes on the Foam Fractionation of Sporamin†

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Foam fractionation can be employed to concentrate and separate sporamin (ipomoein) from a sweet potato protein−water extract. In this foam fractionation study, the bulk solution pH and the air superficial velocity, V_0, were the two primary control variables. It was determined that the protein separation ratio is strongly dependent on the bulk solution pH, with the highest sporamin recovery occurring at pH 3. Two types of experiments were conducted here. The first was to hold the air superficial velocity constant and step down the pH in small increments, with 10 mL of foamate collected at each pH level. The second was to hold the pH constant and step up the air superficial velocity, V_0, in small increments, again with 10 mL of foamate collected at each V_0 level. The lowest feasible air superficial velocity generally corresponds to the maximum protein recovery at a given pH. One strategy for separating β-amylase from sporamin in the sweet potato extract is to first foam the bulk solution at pH 5 to concentrate the sporamin, followed by foaming at pH 3 to collect the concentrated β-amylase.

Keywords: *Foam fractionation; protein separation; sweet potato protein; sporamin; ipomoein*

INTRODUCTION

Foam and bubble fractionation processes are promising methods for separating proteins in water solutions (Lemlich, 1972; Rodger, 1975). Besides the addition of air or other carrier gases such as carbon dioxide, no additional substances are needed, except possibly the addition of acid or base to achieve a desired pH (Montero et al., 1993). Thus, contamination by exogenous separating agents is minimized. These fractionation phenomena occur naturally in commercial fermentation processes, in protein foam head formation in glasses of beer, and in biological waste treatment processes. They also occur in breaking waves along ocean shorelines (Blanchard, 1972). These bubble-based separation and concentration processes are relatively inexpensive and easy to scale up (Prokop, 1993). Like other protein separation processes, they can inactivate enzymes under certain conditions. Such bubble inactivation, however, does not seem to apply to such proteins as the sweet potato storage protein, sporamin (ipomoein), which has no apparent enzymatic effect. This storage protein (also the major protein) supplies the nitrogen to the germinating sweet potato (Osuji and Cuero, 1992). Thus, if protein were desired, without regard for activity, removing denatured protein as a precipitate either directly or with the aid of a foaming process would be desirable.

Sweet potato processing normally recovers only the starch, leaving a foaming wastewater processing stream rich in sporamin, which comprises ∼80% of the total proteins (Maeshima et al., 1985). The concentration of such proteins in the process water stream is typically ∼1%. Foam fractionation, using air as a carrier, seems to be appropriate for concentrating sporamin since it foams readily in water (Ko et al., 1998). Thus, removal of sporamin by foam fractionation could provide a simple low-cost method for removing a waste product, which may have potential commercial application as a foaming agent. This paper differs from the paper by Ko et al. (1998) in that step changes were not explored in their paper. When invertase and α-amylase water solutions (Loha et al., 1997) are aerated, by contrast, foam is not easily formed. In our foam fractionation system, air bubbles are created using a porous ceramic distributor at the bottom of a column, about half-filled with a water solution containing sporamin. Foam is created at the surface of the solution (above the air−water interface). These foam cells become more concentrated in sporamin as the foam rises in the column, due to bulk water drainage from the foam cells. Concentrated sporamin is collected in the efflux foam stream, formed by collapsing foam cells effectively removing the air from the liquid water product. This work builds on the extensive studies of foam fractionation conducted over the past 50 years (Lemlich, 1972). Much of the past research dealt with synthetic water systems, containing a detergent (often a protein), to create a single-solute system. A characteristic found to be important in past separation studies (Charm, 1972) of natural systems (without added synthetic surfactants) is that the presence of a protein by itself can provide the agent to create a stable separating foaming process.

The total protein content of a typical commercial sweet potato is ∼0.5−2.5% on a fresh root basis (Osuji and Cuero, 1992). The major proteins in a sweet potato are sporamin and β-amylase, with sporamin comprising ∼60−80 wt % (Maeshima et al., 1985) and β-amylase ∼5 wt % (Kays, 1992) of the total soluble proteins. The

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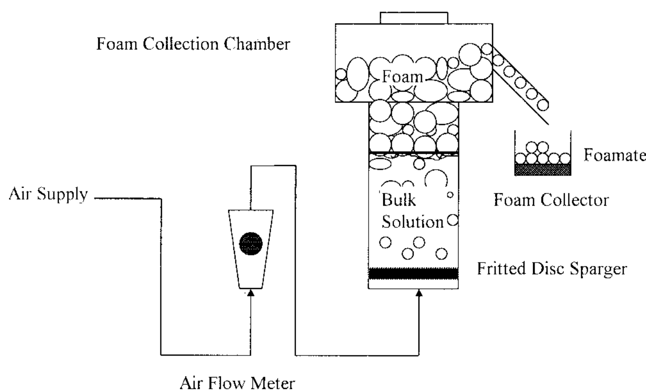

Figure 1. Schematic of the bubble/foam fractionation process.

insoluble proteins, in turn, comprise ~15 wt % of the total protein and can be filtered off along with the fiber. In this study, the wet fresh sweet potato root was comprised of 11.6% starch, 2.6% reducing sugar, 3.9% fiber, 80.1% water determined directly gravimetrically using evaporation, and 1.8% protein, by difference.

MATERIALS AND METHODS

Sample Preparation. Sweet potatoes (Beauregard cultivar, which is distributed by the Dixie Lamb Alabama Sweet Potato Co., Cullman County, AL) were purchased from a local grocery store. Each sweet potato was rinsed with water, and the wash water was evaporated at room temperature. The desired sample solution was prepared by first cutting the sweet potato into small pieces of ca. ~20 g. These pieces were combined with 100 mL of deionized water in a food blender (Blend Master 10, with a 350 W motor, made by Hamilton Beach/Proctor-Silex Inc., Washington, NC) and then chopped for 5 min. The extract was then filtered through Whatman No. 40 filter paper. The filter cake was washed several times until only residual fiber could be observed on the filter paper. No fiber was visible in the wash water in the 500 mL Pyrex flask. The final filtered solution was comprised primarily of starch, protein, sugar, carotene, and tannin. The starch was allowed to gravity settle to the bottom of the flask for 2 h and was then collected following bulk liquid decanting. Deionized water was then added to this (visually) starch-free bulk (filtrate) protein−sugar−tannin solution to bring the volume to 1 L. Next, the fiber on the filter paper and the settled starch were dried and weighed. The remaining protein-rich liquid extract (~450 mg/L protein at pH 6, as observed at the starting point in Figure 4) was stored in the refrigerator at 10 °C until used in the foam fractionation experiments (typically within 2 days). A small amount of filtrate solution, tannin and β-carotene, was removed in the foaming process as observed by clearing of the brown and orange colors, respectively.

Experimental Procedure. The experimental bubble/foam fractionation column used was a 1 L Nalgene tall graduated cylinder shown on the right side of Figure 1. The upper rim was modified by adding a cylindrical plastic cover with an added effluent collection tube to make a foam collection chamber (DeSouza et al., 1991). In addition, a porous ceramic (fritted) disk sparger (medium size porosity) was imbedded in the center of the bottom of the graduated cylinder, typically fitted (with silicon glue) flush to the inner cylinder wall. The initial protein-rich sweet potato extract solution was adjusted to a desired pH (between 2 and 10) by adding either 1 N hydrochloric acid or 1 N sodium hydroxide. The pH of the extract was around ~6 without addition of acid or base, and this liquid remained at room temperature (23 ± 2 °C) throughout each experiment. The initial volume of the solution used in a bubble/foam fractionation column in a batch experiment ranged from 400 to 800 mL. Air (flow rates ranging from 15 to 60 cm^3/s) was introduced under pressure

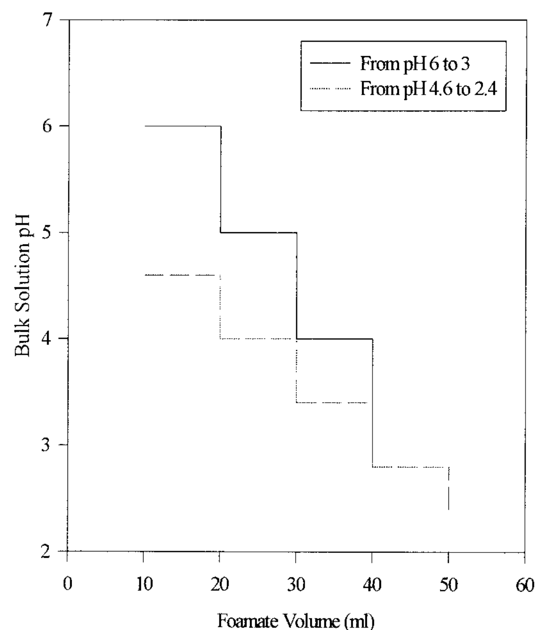

Figure 2. Typical pH−foamate volume step function profiles for pH decreases from 6 to 3 and from 4.6 to 2.4. A 10 mL foamate sampling was taken at each pH for constant air superficial velocity.

to the sparger to create the bubbles in the column. The presence of white foam, seen in the upper part of the column, indicated that the sweet potato contained surface active proteins. The white foam is rich in sporamin, as seen in the electropherogram in Figure 6. That foam passed to the foam collector through the effluent collection tube, where the foam was collapsed mechanically (here, using a glass rod stirrer). Foam was withdrawn at various times in a manner similar to a batch distillation process. The resulting volume of the liquid foamate "cut" was measured along with the pH and the total protein content. The pH and the total protein concentration were also determined in the residual solution in the column at the end of each run (total time for all of the step changes was ~20 min).

The effect of bulk solution pH, when the foamate volume sample was 10 mL, was studied in these experiments. Additional foam was generated by lowering the pH in increments (ΔpH) to impose an increasingly acidic driving force. Again, another foamate sample of 10 mL was collected, as shown in Figure 2. The pH step changes were continued in this fashion until foaming stopped. Subsequently, the effect of the air superficial velocity (as shown in Figure 3) was measured at various pH values.

The V_0 step changes, ΔV_0, were made from the lower to the upper air superficial velocities. In these experiments the bulk solution pH was held constant (unlike the experiment reported in Figure 4) to directly observe the effect of bulk solution pH. The step change of the air superficial velocities was conducted in a similar manner for the bulk solution pH cases as shown in Figure 3. About 10 mL of foamate was collected at each step. In both the foamate and the residual bulk solution, the total protein concentration was measured using the Coomassie Blue method (Bradford, 1976). A sweet potato total protein solution calibration curve ($A = 0.014C$) was generated, where A is the optical absorbance at 595 nm of a Bausch & Lomb Spectronic 20 spectrophotometer and C the total protein concentration in mg/L. C was determined by weighing the sample after drying in an oven. The measured sugar residue (in glucose equivalents) was subtracted from the mass of the dried sample to give the total protein value.

Electrophoresis. Gel electrophoresis was used to identify the molecular weights of the two primary proteins and their respective approximate relative concentrations within both the foamate and the bulk liquid of the foam fractionation process.

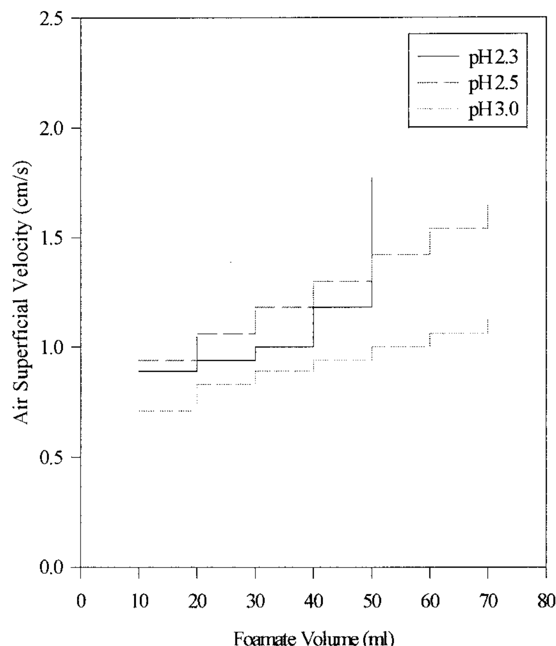

Figure 3. Air superficial velocity−foamate volume step function profile. A 10 mL foamate sampling was taken at each V_0 for constant bulk solution pH.

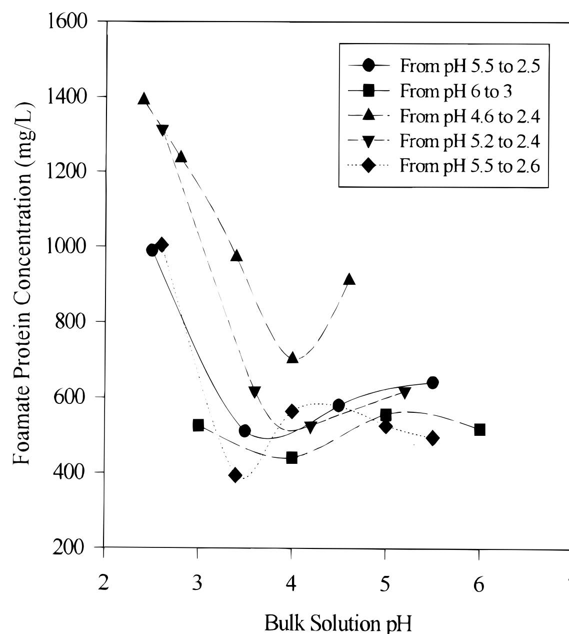

Figure 4. Effect of stepping down (in increments) the bulk solution pH on the total foamate protein concentration at the constant air superficial velocity of 1 cm/s.

Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was employed in this study following the Laemmli procedure (Laemmli, 1970). The proteins (40 μL sample size) passing through the 11% polyacrylamide gel were stained with Coomassie Brilliant Blue R-250 to mark the protein bands. Typically, this stain produced sharp bands when between 15 and 20 μg of protein was applied (Hames and Rickwood, 1990). In these experiments, bovine serum albumin (BSA; lot 41F-9300), ovalbumin (lot 55F8510), and carbonic anhydrase from bovine erythrocytes (lot 43F-8050), purchased from Sigma Chemical Co. of St. Louis, MO, were used as the protein molecular weight markers to calibrate the gel at the approximate molecular weights of 68 000, 43 000, and 29 000 kDa, respectively. α-Amylase (lot 113F-0516) from Sigma was used separately to calibrate the gel at 51 000 kDa.

The reducing sugar concentration was measured using the dinitrosalicylic acid reagent (DNS) method (Miller, 1959). The fiber mass was determined by drying the wet fiber at 100 °C in an oven for 10 h, so that the fiber concentration is this mass divided by the mass of the wet sweet potato sample. The water concentration was also determined gravimetrically using evaporation (for 10 h), based on the difference between the wet mass and the 100 °C oven-dried mass. The protein mass (~450 mg/L) was determined gravimetrically by difference in the combined water, sugar, and protein sample (see Experimental Procedures).

RESULTS AND DISCUSSION

The primary independent variables selected for these foam fractionation experiments, based on preliminary experiments and prior studies in the literature (Kays, 1992; Ko et al., 1998; Lemlich, 1972; Montero et al., 1993), were the bulk solution pH and the air superficial bubble velocity.

Effect of Bulk Solution pH. The pH of the sweet potato extract liquid strongly affected the foam recovery of proteins (Ko et al., 1998). In general, the lower the pH, the higher the protein concentration in the foamate. As shown in Figure 4, the protein concentration in the foamate rises rapidly as the pH drops from 4 to 2.5. At pH 2.5, the protein concentration in the foamate is ~2−3 times more than that in the pH 4 and 5.5 cases. Usually, at pH 3 or lower, both the foam cell and bubble cell sizes are small and uniform (~2 mm diameter) and very resistant to collapse. Bubble sizes were measured here with a "ruler" to get a qualitative measure of small or large. Smaller bubbles (~1−2 mm diameter) provide a larger effective surface area than the larger ones. Larger bubble surface areas can lead to larger amounts of protein adsorbed at the foam/solution interface. However, larger foam cells (larger bubbles) provide higher liquid drainage, which can lead to higher foamate protein concentrations. At pH 4 or above, foam cells are larger and nonuniform in size (the biggest diameters reach ~2 cm) and foam drainage is clearly observed in the wet foam when the air superficial velocity is low.

At the beginning of the run, when the bulk solution pH is <3 and the air superficial velocity is 1 cm/s (air flow rate 28 cm^3/s), the foam cells are very small, ~3 mm in diameter. Bubbles gradually increase in size as the foamate is removed, perhaps indicating that proteins with lower surface tension are removed first. For late times in the foam fractionation when foam generation stops, the foam cells may reach 3 cm in diameter. At this time, the experiment is terminated. Large-diameter foam cells occur later in the experiment, after 30 min, because the surface active proteins (which create and enhance the foam) are being depleted.

Decreases in pH increase the hydrophobicity of the remaining proteins, thus resulting in additional foam formation. At very low pH, the foamate became turbid, presumably caused by precipitation of denatured proteins and colloidal particles present in the foamate. The colloidal particles in the sweet potato extract usually settle out when the extract pH reaches pH 3.

Effect of Air Superficial Velocity. At superficial velocities <2 cm/s, the foam was stable but broke down at higher velocities. High velocities caused turbulence and backmixing and, hence, bubbles sheared in the foam fractionation column. With high air superficial velocities, bubbles move up rapidly to the top of the column and stable foams do not form. Stable foams are created

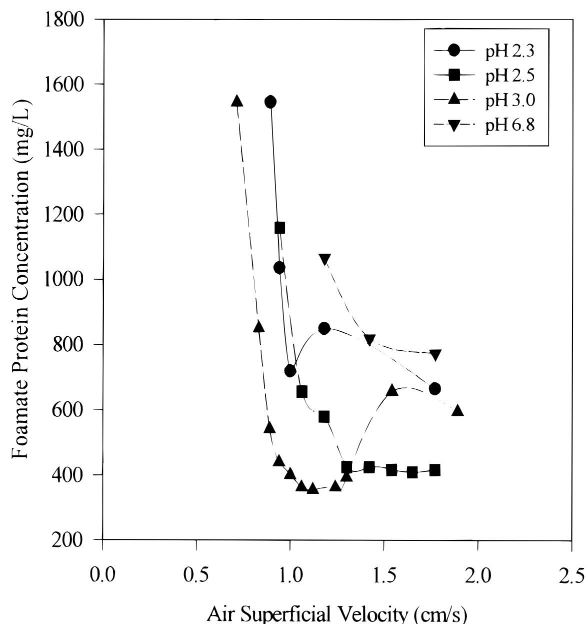

Figure 5. Effect of stepping up (in increments) the air superficial velocity on foamate protein concentration at constant pH values of 2.3, 2.5, 3.0, and 6.8.

only when the air superficial velocities are between 0.5 and 2 cm/s. At velocities <0.5 cm/s, however, the foam fractionation column is difficult to operate because the air flows unevenly through the porous sparger. Hence, the superficial air velocity of 0.5 cm/s is called the minimal feasible velocity. Conversely, the superficial air velocity of 2 cm/s is a feasible upper bound because above this V_0 unstable and discontinuous foams are formed.

Stable foams are desirable not only for easy control of semibatch or continuous foam fractionation operations, but shear (resulting from unstable foams) in the foam phase can substantially denature the separated proteins (Handa-Corrigan et al., 1989). One way to minimize rupturing of the foam is to recycle part of the effluent foamate phase. Protein recovery and foamate protein concentration were also affected by the interactive effects of the air superficial velocity and the bulk solution pH. For example, the maximum foamate protein concentration at pH 3 was ~1500 mg/L at 0.75 cm/s, but the maximum foamate protein concentration at pH 6.8 was ~1100 mg/L at a velocity of 1.1 cm/s. Proteins could no longer be separated at velocities >2 cm/s, because the bulk liquid became turbulent and high shear forces tended to break down the foam, leaving only the bulk phase.

The foamate total protein concentration increased when the air superficial velocity decreased and tended to level off when velocities were >1.2−1.5 cm/s (Figure 5). At velocities below 1 cm/s (large bubble residence times), protein concentrations in the foamate tended to increase very rapidly, presumably indicating that equilibrium conditions were approached and shear degradation was minimized. Although in these experiments low velocity led to the highest protein concentrations, the volume of foamate was low. Therefore, the protein mass recovery (foamate flow rate × foamate protein concentration) was not maximized at the lowest superficial air velocity but at a somewhat higher value.

Electrophoresis. SDS−PAGE of sweet potato proteins from the foam fractionation process is illustrated

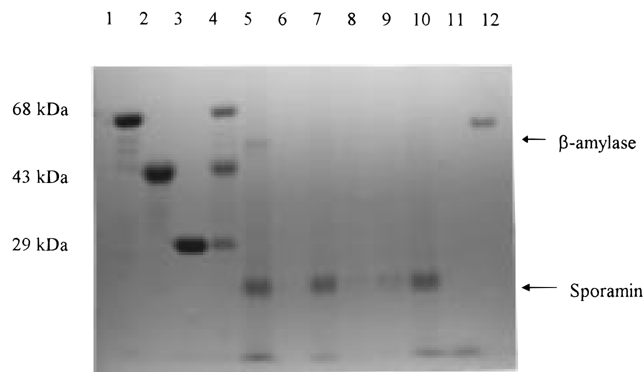

Figure 6. SDS−PAGE of marker and foam fractionated sweet potato proteins: (lane 1) BSA; (lane 2) ovalbumin; (lane 3) carbonic anhydrase; (lane 4) mixture of the proteins observed in lanes 1−3; (lane 5) foamate at pH 3 (first run at pH 5 and then at pH 3); (lane 6) bulk solution at pH 3 (first run at pH 5 and then at pH 3); (lane 7) foamate at pH 5; (lane 8) bulk solution at pH 5; (lane 9) sweet potato feedstock solution; (lane 10) foamate at pH 3; (lane 11) bulk solution at pH 3; (lane 12) α-amylase. Reprinted with permission from Ko et al. (1998). Copyright 1998 Humana Press.

in Figure 6. This figure was developed for this study and it had also been used to illustrate the paper of Ko et al. (1998). Lanes 1−3 and 12 contain the marker proteins. Lane 4 contains a mixture of the marker proteins found in lanes 1−3. Lanes 7 and 10 contain foamate proteins, and lanes 8 and 11 contain bulk solution proteins at pH 3 and 5, respectively. Lane 9 depicts the proteins found naturally in the sweet potato. Lanes 5 and 6 contain foamate and residual bulk solution proteins, respectively. For lanes 5 and 6, the sweet potato proteins were foam fractionated at pH 5 and then the residue was foam fractionated at pH 3 to obtain the foamate and the bulk solution for lanes 5 and 6, respectively. From previous studies (Maeshima et al., 1985; Osuji and Cuero, 1992), the molecular weight of sporamin was estimated at 25 000, 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 concentrations at both pH 3 and 5 are ~3−5 times greater than sporamin in the original feedstock solution (lane 9).

The second most abundant protein, β-amylase, has a molecular weight ~200 000 with four equal molecular subunits, each subunit being ~50 000 (Fasman, 1989). A subunit of this protein can be observed as a small sharp band at the 50 000 position in lane 5, close to the 51 000 band of α-amylase seen in lane 12. It is difficult to observe the β-amylase subunit band in lanes 7 (pH 5) and 10 (pH 3) because the sporamin is much more abundant than β-amylase and has a stronger surface activity than β-amylase. Sporamin has a much higher surface activity than β-amylase because when a 90% sporamin−10% amylase solution is added to water, it foams when aerated, and amylase by itself does not foam. When the sweet potato extract is foam fractionated at pH 5, the concentrated sporamin in the foamate comes out of the bulk solution before the β-amylase. The β-amylase can be recovered at concentrations higher than that in the feedstock solution when the sporamin is depleted first in the bulk solution, as shown in lane 5. Thus, one way to selectively deplete the sporamin is to first foam the bulk solution at pH 5, concentrating sporamin in the foamate. Then, at pH 3 the β-amylase

can be recovered along with residual sporamin by foaming the bulk solution. There is, however, less foam present at pH 5 than at pH 3. After much of the sporamin has been removed, the β-amylase attaches more readily to the adsorptive sites on the air bubbles, not having to compete for those sites as much as sporamin. Generally, α-amylase does not foam (DeSouza et al., 1991) when air bubbles are introduced into a foam fractionation column containing only this protein. Presumably this is the case for β-amylase, as well. It has been shown in these experiments that β-amylase is concentrated only in the foamate in conjunction with a foaming protein such as sporamin and is not likely to foam on its own. In the natural sweet potato system both proteins are present together and no foaming surfactant needs to be added to recover β-amylase in the foamate.

Conclusions. Sweet potato storage protein (sporamin) recovery in the foam fractionation of a sweet potato protein−water extract increases as the bulk solution pH is reduced. At pH <3, the foam cell is small in size and uniformly spherical in shape. Since a small foam cell has a large adsorption surface area and a long residence time in the foam fractionation column, it would be expected to lead to a high amount of protein recovery in the foamate through enhanced adsorption and longer water drainage times during the foam fractionation process. In fact, this did occur. The protein recovery in the foamate is enhanced further by reducing the air superficial velocity. At pH 3 and 5, the concentration of the proteins recovered in the foamate is ∼3−4 times that of the original bulk protein concentration and ∼90% of the sporamin (25 000) is recovered. The β-amylase subunit (50 000) can also be recovered in the foamate at a concentration higher than the bulk solution by first foaming the bulk solution at pH 5 (to remove much of the sporamin) and then foaming the remaining bulk solution at pH 3 to recover the β-amylase along with some of the residual sporamin. Foam fractionation of a sweet potato−water extract seems to offer a promising low-cost first step in recovering sporamin and β-amylase from sweet potatoes.

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