종자 단백질의 Foam fractionation에 미치는 pH의 영향

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Effect of pH on the Foam Fractionation of Seed Proteins

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INTRODUCTION

Mimosa, known as the sensitive plant or sleeping grass , is a member of the Mimoseae family. The botanists genus *Mimosa* is entirely different. Although it belongs in the same pea family, the LEGUMINOSAE, its name comes from the Greek *mimos*, a mimic. This genus consists of about 400 to 500 species of trees, shrubs, and herbaceous plants. These are mostly found in tropical- and subtropical America and in warm areas of Africa and Asia. The bark, leaves, seeds, and roots of Mimosa contain many chemicals. For example, components of *M. tenuiflora* (Willd.) can be used to treat skin wounds and burns. Mimosa bark tea, powder, ointment and products containing mimosa chemicals are commercialized in the form of shampoos, creams, capsules and soaps for many applications. Mimosa can cause a rapid increase in the number of cells of other organisms. Also, mimosa has an antibacterial effect. It also can neutralize the formation of free radicals. Mimosa contains a group of diverse bioflavonoids that form the major active substances. Other compounds found in the mimosa plant are tannins, triterpenoid saponins (mimonosides A, B, and C), three steroid saponins, alkaloids, lipids, phytosterols, glucosides, xylose, rhamnose, arabinose, lupeol and methoxy chalcones.

Foam fractionation is a separation technique that has been applied for the concentration of surface-active biological materials such as proteins, enzymes, polysaccharides, and surfactants. The potential application of this method lies in the early stages of a downstream process, where the separation and concentration of a dilute material from a large volume of crude material is required. In this study, we report on the use of foam fractionation to determine the protein separation ability of *M. pudica* L. seed extract solution over a wide pH range. The resulting enrichment ratio, mass recovery and optimal conditions are determined in this study.

MATERIALS AND METHODS

Sample Preparation

Mimosa seeds used in these experiments were gathered in Nashville, TN (in early 2002) from fallen mimosa seed pod, most of which had released their contents to the ground. The seeds were washed and dried, revealing a tough, intact and shiny coat. In a given run, about six grams of seed were combined with one liter of distilled water and ground using the liquefy setting on a 350-W blender (Blend Master 10, Hamilton Beach/Proctor-Silex, Washington, NC) for one minute.

Experimental Procedure

The foam fractionation apparatus consisted of a Pyrex glass cylinder of a 2.5 cm inside diameter and 36 cm length bed by an air line which had passed through a humidification chamber and a rotameter in series, as illustrated in Figure 1. The nominal ca. 500 ppm extract solution of mimosa seed protein was used initially for the foam fractionation experiments. 100 mL of feed solution was employed for each foam fractionation run. Each sample was initially adjusted to a desired pH value is the range of 3 to 10 by the addition of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. Humidified air was introduced to the porous ceramic disk sparger at the bottom of the foam column, creating bubbles in the feed solution, at a constant flow rate of 1.29 centimeters per second. The supplied air was pre-humidified by bubbling it through two water-containing Erlenmeyer flasks (the humidification chamber) in series to minimize both water loss from the feed solution and contamination from the influent air. Air bubbles were formed throughout the feed solution starting from the sparger at the column bottom and up to the top of the feed liquid. Produced foam, formed above the feed liquid phase (the major air-water interface), was collected in the beaker at the top part of the column (the each foamate collector) from a side port and collapsed using a magnetic stirrer. This experiment was run until no more foam was generated. The residual foam in the column was not recovered in the foamate. The foamate volume was measured using a pipette. Each sample of feed solution, foamate, and residue was, in turn, used to determine the respective total protein concentration, surface tension and pH.

The total protein concentrations of each sample were determined using the Bradford Coomassie Blue method (Bradford, 1976) by measuring the optimal absorbance with a spectrophotometer (Spectronic 20, Bausch & Lomb, USA) at 595 nm. The protein (here, BSA) calibration curve of C(mg/L) = 103.16A(OD595) was used to convert absorbance to concentration is 40 mg/L. This curve fit the underlying BSA data to on R2 value of 0.994.

The surface tension of each sample was measured using a Sigma70 surface tension apparatus (KSV Instruments Ltd., Finland) using the Wilhelmy plate technique. The first five trials for each sample were averaged to determine the reported surface tension value. The pH of each sample was measured using a laboratory pH meter (Model 5984-50, Cole-Parmer Instrument Co., USA).

RESULTS AND DISCUSSION

The pH of the feed solution, the initial protein feed concentration and the air superficial velocity in the bubble/foam column are the major variables studied in the mimosa batch foam fractionation process in order to optimize the recovery and separation of proteins. Other parameters, which can be considered in the performance of a foam fractionation process are the foam height, foam unit size, liquid pool height, liquid viscosity, surface tension of the feed solution and the feed temperature.

The pH of a typical mimosa seed protein prepared solution ranged from about 5.8 to 6.0. After filtering, a mimosa seed solution displayed a cloudy and white appearance without little significant solid precipitation, even after sitting overnight in a refrigerator. It is important to note that when NaOH was added to the mimosa seed protein solution for pH adjustment, it caused the solution to take on an increasingly greenish-yellow color. The addition of HCl did not result in any color change, but it did seem to increase the opacity of the solution. Either of these pH changes could result in a reaction in the solution such as that between a protein and a polysaccharide (such as galactomannan). Here, proteins seemed to coagulate and precipitate, especially at pH 4.0, as observed in the suspended particles in the foamate. Generally, when the pH of the protein solution is the same as its pI (the isoelectric point), the solution is cloudy and the protein precipitates as colloids or particles and becomes easier to separate (say, by foam fractionation) from the feed solution. There are no literature precedents for how this may occur for mimosa seed solutions.

Protein separation is clearly dependent on the relative surface activities of proteins involved in the initial feed solution. Surface activity is measured in terms of surface tension. A low surface tension indicates a high surface activity. Typically, the local minimum of a surface tension vs. pH profile gives the isoelectric point (the pH where the charge on the protein is zero) of a protein in solution. The pI (neutral point) can be achieved because proteins have both hydrophilic and hydrophobic parts. The hydrophobic part of the protein is likely to be concentrated at an air-liquid surface of a solution causing the surface tension of that protein solution to fall below that of water (72.6 mN/m). It thus follows that the concentration of hydrophobic proteins at an air-liquid interface of a solution is much higher than that in the feed solution. Building on this effect, a foam fractionation process can (by generating many air-liquid surfaces in a foam phase) separate and concentrate hydrophobic proteins from a feed solution to an aerated foam phase.

It can be concluded from this foam fractionation study of mimosa seed protein extract solution that both concentration and isolation of proteins is not reality achieved at high initial protein concentration. Foaming is readily reached and occurs in abundance. Local minimum surface tensions occurred for the feed solution at three pHs: 4, 7 and 9 when the feed protein concentration was 560 mg/L. The maximum foamate volume was observed at pH 4 and the minimum at pH 7. The shape of the foamate volume vs. pH curve seems to correspond more closely to the surface tension vs pH trajectory. This may indicate that either an isoelectric point or a neutrally charged point occurs around pH 4 and around pH 9, keeping in consideration that the standard deviation of these points may negate the local minima resulting from these measurements. The pH 4 and 9 depressed surface tension points

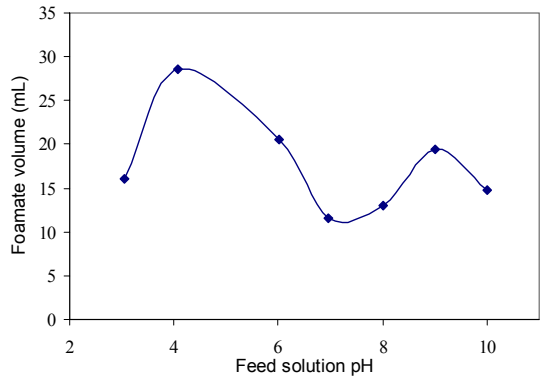

Figure. Total foamate volume generated in a mimosa seed protein batch experiment.

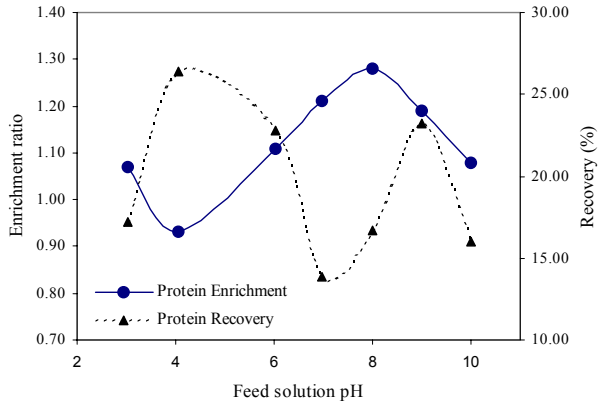

Figure. Protein enrichment ratio and protein recovery in the batch foam fractionation of mimosa seed protein extract solution.

correspond to local maximum protein recovery points. The foamate concentration is relatively high at pH 7. The protein recovery (vs pH) has the same shape as the recovered foam volume (vs pH) trajectory, implying that when more material carries over to the interstitial liquid spaces in the foam it leads to more protein recovery. The enrichment ratio ranged between 0.9 and 1.3. These low values confirm the fact that the starting protein concentration was relatively high (560 ppm). The shape of the enrichment ratio curve is opposite to that of the protein recovery curve. The highest objective function (optimal recovery) values were achieved at pH 9.

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