Composition and Functional Properties of an Unusual Proteinaceous Fibrous Material from Cucumber Fermentation

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The composition and functional properties of an unusual proteinaceous fibrous material that developed on the surface of cucumber fermentation brines were investigated. Protein, ash, oil, total carbohydrate, and moisture contents of the fermentation brine protein (FBP) were 72.5, 3.2, 0.7, 20.2, and 3.0%, respectively. SDS−PAGE revealed two major components with molecular masses >200 kDa. FBP was a good source of essential amino acids. Solubility of FBP in water was 8.0, 0.0, 2.4, 34.4, and 57.1% at pH 2.0, 4.0, 6.0, 8.0, and 10.0, respectively. The foaming capacity of FBP was higher than that of egg albumen at pH 8.0 (30.0 versus 5.5 mL) and pH 10.0 (35.5 versus 7.0 mL). The emulsifying activity of FBP was less than that of bovine serum albumin at all pH values except for pH 6.0. Differential scanning calorimetry analysis showed that FBP was stable up to 130 °C under experimental conditions.

Keywords: *Pickle protein exudate; composition; amino acids; foaming; emulsifying*

INTRODUCTION

An unusual fibrous material is occasionally observed on the surface of brine during cucumber pickle fermentation. This material first appears as small strands that eventually develop into long fibrous structures resembling a cotton mop. The origin and conditions for formation of the fibrous material are unknown. Preliminary observations indicated the material to be primarily protein. Phloem exudate from cucumbers is reported to have a high protein content and may be the source of the fibrous protein (Reed and Northcote, 1983; Alosi et al., 1988).

Physicochemical characteristics and interactions with other components in foods determine the value of a protein as a functional ingredient in food systems. These characteristics, collectively referred to as functional properties, are important in influencing the processing, preparation, and quality attributes of foods (Kinsella, 1981). Solubility, emulsifying, and foaming properties of proteins contribute to the quality attributes of various food products. Each functional property of proteins requires different protein characteristics. For example, efficient formation of foams requires flexible molecules with few secondary and tertiary structures, while intermolecular cohesiveness and elasticity are important to produce stable foams (Kinsella, 1981; Damodaran, 1990). Any native proteins with good functional properties will easily find use as ingredients in food systems since costly chemical, enzymatic, or physical modification processes are not required to attain good functional properties.

The use of food ingredients is based primarily on their functionalities. This study was undertaken to investigate the composition and functional properties of a proteinaceous fiber material that developed on the surface of cucumber fermentation brines.

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MATERIALS AND METHODS

Materials. Egg albumen (EA, grade II) and bovine serum albumin (BSA, fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO). Soy protein isolate (SPI) was prepared from soybeans (*Glycine max* var. Walters, 1995 crop) obtained from the Department of Agronomy, University of Arkansas. The fibrous material was collected from the surface of brine tanks during commercial cucumber pickle fermentation. The reagents were of analytical grade and purchased from Fisher Scientific (Pittsburgh, PA) and Sigma.

Fermentation Brine Protein (FBP) Processing. FBP obtained from commercial cucumber fermentation was washed in running tap water followed by deionized water until the washings were free of NaCl and freeze-dried.

Chemical Analysis. Protein, ash, moisture, and petroleum ether extractable material (percent oil) contents of FBP were determined according to procedures described by the AOAC (1990). Total carbohydrates was calculated using the following formula:

$$\% \text{ total carbohydrates} = 100 - (\% \text{ protein} + \% \text{ oil} + \% \text{ ash} + \% \text{ moisture}) \quad (1)$$

Amino Acid Analysis. For cysteine and methionine determination, protein samples were first oxidized with performic acid for 16 h in an ice bath and then neutralized with hydrogen bromide. Oxidized and unoxidized protein samples were hydrolyzed at 121 °C with 6 N HCl for 18 h (AOAC, 1990). After the hydrolysis, amino acids were separated by HPLC using an ion exchange column. Postcolumn modification was performed with ninhydrin for detection at 570 nm.

Hydrophobicity Determination. Surface hydrophobicity of FBP was determined according to the 1-anilino-8-naphthalenesulfonate (ANS) method (Hayakawa and Nakai, 1985). Protein samples having concentrations ranging from 0.0015 to 0.015% were prepared by serially diluting a stock solution having a concentration of 0.015% with 0.01 M phosphate buffer (pH 7.0). Twenty microliters of ANS (8 mM in 0.01 M phosphate buffer, pH 7.0) was added to 4.0 mL of protein solution. The fluorescence intensity of ANS−protein conjugates was measured with a Kontron model SF23/B spectrofluorometer (Zurich, Switzerland) using excitation and emission wavelengths of 390 and 470 nm, respectively. The slope of the fluorescence intensity versus percent protein concentra-

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tion was calculated by linear regression and was used as an index of protein hydrophobicity.

Nitrogen Solubility. The method of Bera and Mukherjee (1989) was used. Samples (100 mg) were dispersed in 10 mL of NaCl solutions (0.0, 0.1, 0.2, 0.5, and 1.0 N NaCl), and the pH of each dispersion was adjusted to values ranging from 2.0 to 10.0 using either 1 N NaOH or 1 N HCl. The sample dispersions were shaken for 30 min at room temperature and then centrifuged at 1000g for 20 min. Nitrogen content in the supernatant was determined according to the micro-Kjeldahl method (AOAC, 1990), and percent nitrogen solubility was calculated as follows:

$$\text{N solubility (\%)} = (\text{N in supernatant/N in sample}) \times 100 \tag{2}$$

Emulsifying Properties. Emulsifying properties were measured according to the method of Pearce and Kinsella (1978). Two milliliters of pure corn oil and 6 mL of 0.1% protein solution in 0.1 M phosphate buffer (pH 7.0) were homogenized in a mechanical homogenizer (Virtishear Tempest, The VirTis Co., Gardiner, NY) at setting 6 for 1 min. Portions of the emulsion (50 μL) were pipetted out at 0 and 10 min after preparation and mixed with 5 mL of 0.1% sodium dodecyl sulfate (SDS). Absorbance of these mixtures was measured at 500 nm (Varian series 634 double-beam spectrophotometer, Springvale, Australia). The absorbance measured immediately after emulsion formation was expressed as emulsifying activity, and the emulsion stability index was determined as

$$\text{emulsion stability index} = T_0(\Delta t/\Delta T) \tag{3}$$

where ΔT is the change in the turbidity, T, occurring over the time interval, Δt (10 min), and T_0 is the turbidity at 0 time.

Foaming Properties. The foaming capacities of FBP, SPI, and EA were determined by measuring the volume of foams immediately after the introduction of air (90 cm³/min) for 15 s into 5 mL of 0.1% protein solutions in 0.1 M phosphate buffer (pH 7.4) in a glass tube (2.4 × 30 cm). Foam stability was calculated from the equation

$$\text{foam stability} = V_0(\Delta t/\Delta V) \tag{4}$$

where ΔV is the change in the volume of foam, V, occurring during the time interval, Δt (30 min), and V_0 is the volume of foam at 0 time (Kato et al., 1983).

Thermal Properties. Differential scanning calorimetric (DSC) studies were performed with a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT). Protein sample (40–60 mg/mL) was suspended in 0.06 M phosphate buffer at pH 7.0. In all DSC runs, 45 μL of protein suspension was hermetically sealed in a stainless steel pan. Another pan containing 45 μL of buffer with no protein was used as reference. Samples were heated from 30 to 130 °C at a rate of 10 °C/min (Raeker and Johnson, 1995).

Electrophoresis. SDS–PAGE (under reducing conditions) was carried out on a slab gel (4% stacking and 12% separating gels) using an SDS-Tris–glycine discontinuous buffered system described by Laemmli (1970). Prior to electrophoresis, the sample proteins were heated at 95 °C for 4 min in the presence of 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.002% bromophenol blue. Electrophoresis was performed at a constant current (13 mA for 1 h and 18 mA for the next 3.5 h). The gel was stained by 0.1% Coomassie brilliant blue in 10/40/50 acetic acid/ethanol/water (v/v/v) and destained in the same solvent system without dye.

Statistical Analysis. Data were analyzed using the general linear models procedure of the SAS package (version 6.03, 1995) developed by the SAS Institute Inc. (Cary, NC) to determine differences between treatment means. Pairwise comparison of all means was performed using the least significant difference (LSD) procedure at the 5% level. Experi-
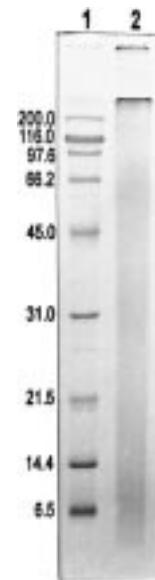
Figure 1. Electrophoretic patterns of FBP: (lane 1) molecular mass markers; (lane 2) FBP.

ments were replicated three times with three samples in a completely randomized design.

RESULTS AND DISCUSSION

Proximate Analysis. Protein, ash, oil, total carbohydrate, and moisture contents of FBP were 72.5 ± 0.82, 3.2 ± 0.02, 0.7 ± 0.08, 20.2 ± 2.14, and 3.0 ± 0.35%, respectively. SDS–PAGE under reducing condition revealed the presence of two major protein components with molecular masses >200 kDa and several minor components with approximate molecular masses of 7, 31, and 60 kDa (Figure 1). One of the major components did not enter the stacking gel, and the other component stayed at the top of the separating gel. The molecular weight distribution of FBP was much higher than those of the conventional proteins found in soy and milk. The heat stable nature of FBP could be attributed to its high molecular weight (see Thermal Properties).

Amino Acid Composition. Amino acid contents of FBP are shown in Table 1. The amino acid contents of casein and SPI, egg essential amino acid profile, and amino acids required by preschool children are also included in Table 1 for comparison. The essential amino acid profile of FBP surpassed the amino acid requirement of preschool children (FAO/WHO/UNU, 1985) except for Trp, which was not determined. FBP had higher Val, Leu, Ile, Thr, and Lys content than the egg essential amino acid profile. Compared to casein, FBP had higher Met + Cys, Thr, Ile, Leu, and Val content. Among the essential amino acids, Leu content of FBP was the highest. Among nonessential amino acids, Asp and Glu contents of FBP were found to be very high compared to egg essential amino acid profile (nonessential amino acids are also given for egg in Table 1). Asp, Ala, and Gly contents were higher than in SPI. In general, amino acid analysis results showed that FBP from pickle fermentation was rich in essential amino acid Lys, Met + Cys, Thr, Ile, Leu, Val, and Phe + Tyr.

Nitrogen Solubility. The solubility profiles of FBP at different pH values and various NaCl concentrations are shown in Figure 2. The solubility of FBP in water (0.0 N NaCl solution) was 8.0, 0.0, 2.4, 34.4, and 57.1% at pH 2.0, 4.0, 6.0, 8.0, and 10.0, respectively. Maxi-

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Table 1. Amino Acid (AA) Composition of FBP (Milligrams per Gram of Protein)

	FBP[a]	EEAA[b]	CAS[c]	SPI[d]	AARC[e]
essential AA					
Lys	71 ± 1.2	70	74	62	58
Met + Cys	36 ± 0.9	57	34	24	25
Thr	53 ± 1.8	47	38	32	34
Ile	61 ± 1.1	54	45	44	28
Leu	97 ± 2.2	86	90	77	66
Val	79 ± 1.9	66	56	41	35
Phe + Tyr	90 ± 1.4	93	93	90	63
Trp	ND[f]	17	12	14	11
nonessential AA					
Asp	129 ± 3.2	66		122	
Glu	130 ± 2.6	25		210	
His	12 ± 0.8	25	19	26	
Arg	55 ± 1.5	32		80	
Ser	48 ± 0.9	53		50	
Ala	64 ± 0.6	23		38	
Gly	51 ± 1.3	16		38	

[a] Values are means ± standard deviations. [b] Egg essential amino acid profile, from Pellett and Young (1980). [c] Amino acid content of casein, from Wu et al. (1994). [d] Amino acid composition of soy protein isolate, from Wang and Damodaran (1990). [e] Amino acid required by preschool children (2−5 years) suggested by FAO/WHO/UNU (1985). [f] Not determined.
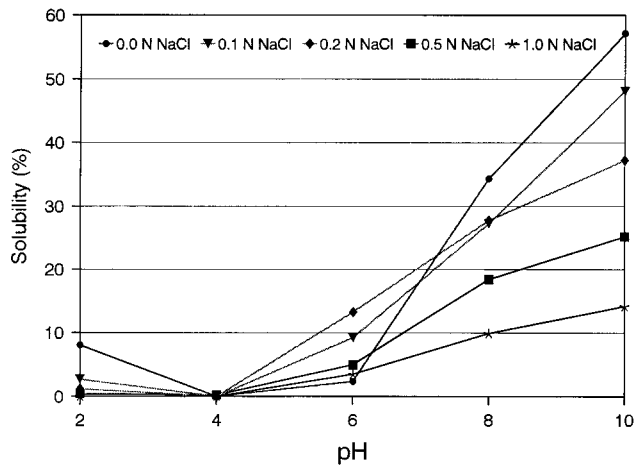
Figure 2. Solubility profiles of FBP at different pH values and NaCl concentrations.

mum solubility (57.1%) was observed in water (0.0 N NaCl solution) at pH 10.0 (P < 0.01). Minimum solubility (∼0%) was obtained at pH 4.0 at all NaCl concentrations (0.0, 0.1, 0.2, 0.5, and 1.0 N), indicating that the isoelectric point of FBP was close to this pH. Solubility increased with increasing pH from 4.0 to 10.0. At all pH values except pH 6.0 (2.4, 9.2, 13.2, 4.9, and 3.5% in 0.0, 0.1, 0.2, 0.5, and 1.0 N NaCl, respectively) solubility of FBP in water was significantly higher than those at all NaCl concentrations (P < 0.01). All NaCl concentrations caused a salting-out effect except for pH 6.0.

Emulsifying Properties. The emulsification properties of FBP, SPI, and BSA are presented in Figure 3. The emulsion activity of FBP at pH 2.0 was lower than that of BSA (0.667 versus 1.203) (P < 0.01) but similar to that of SPI (0.667 versus 0.806) (P > 0.05). At pH 4.0, FBP had insignificant emulsion activity, indicating that its activity strongly depended on solubility, since the solubility of FBP was almost 0% at this pH. The emulsion activity of FBP was lower than that of BSA at pH 6.0, 8.0, and 10.0. BSA has a superior emulsion stability index and is often used as the standard for comparing emulsification properties of proteins. The
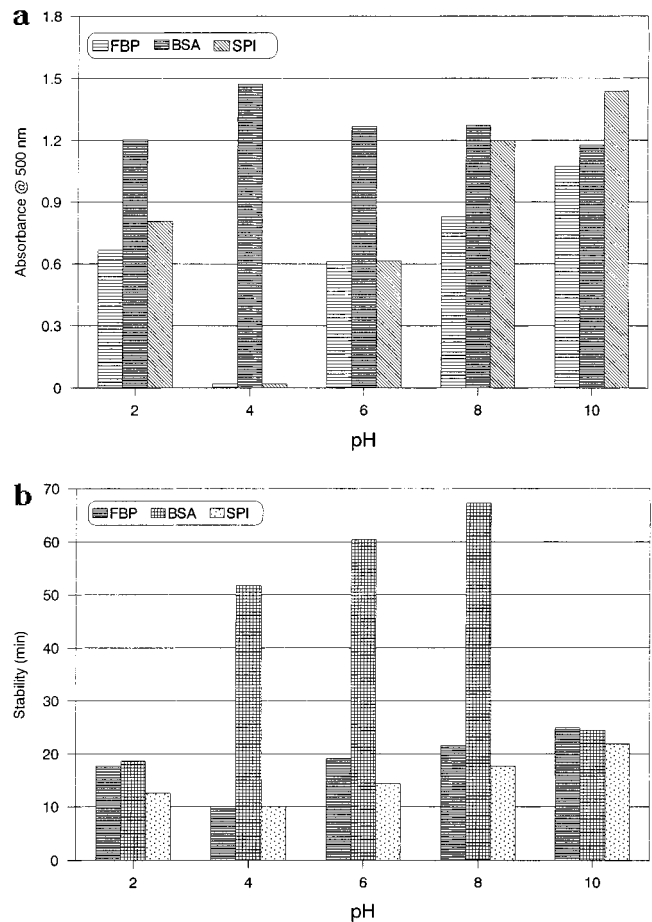

Figure 3. Emulsifying activity (a) and emulsion stability index (b) of FBP, BSA, and SPI.

emulsion stability index of FBP was similar to those of BSA and SPI at pH 2.0 and 10.0. However, BSA had a higher emulsion stability index than FBP and SPI at pH 4.0, 6.0, and 8.0.

Foaming Properties. The foaming properties of FBP, EA, and SPI were compared (Figure 4). The foaming capacity of FBP was higher than that of EA at pH 8.0 (30.0 versus 5.5 mL) and pH 10.0 (35.0L versus 7.0 mL) (P < 0.01) but similar to that of SPI (P > 0.05). At pH 2.0, 4.0, and 6.0, EA had higher foaming capacity than FBP.

The ability of a protein to form stable foams is critical in food applications (Kinsella and Whitehead, 1989). EA has a superior foaming stability and is used as the standard for comparing foaming properties of other proteins (Cheftel et al., 1985). The foaming stability of FBP was similar to that of EA at pH 8.0 (30 versus 32 min) and 10 (30 versus 30 min) (P > 0.05). However, FBP had lower foaming stability than EA at pH 2.0, 4.0, and 6.0 (P < 0.05), indicating that solubility was an important factor in foaming capacity and stability. Although the hydrophobicity value of FBP was high (19.2 for FBP and 7.1 for SPI), its low solubility seemed to mask the beneficial effect of hydrophobicity on the surface properties of FBP.

Thermal Properties. DSC thermograms of FBP and SPI (SPI was included in this experiment to make a reasonable comparison) are given in Figure 5. SPI produced two endothermic peaks at the peak temperatures of 82.8 °C for 7S globulin and 99.7 °C for 11S globulin. The peak temperature for 7S globulin was similar to 80.5 °C as reported by Hermansson (1978).
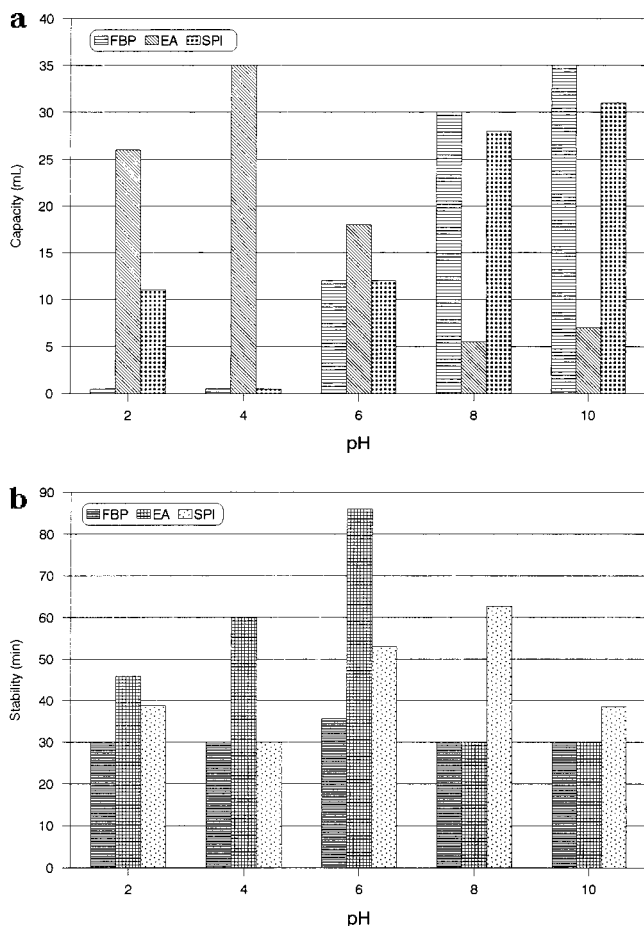
Figure 4. Foaming capacity (a) and stability (b) of FBP, EA, and SPI.
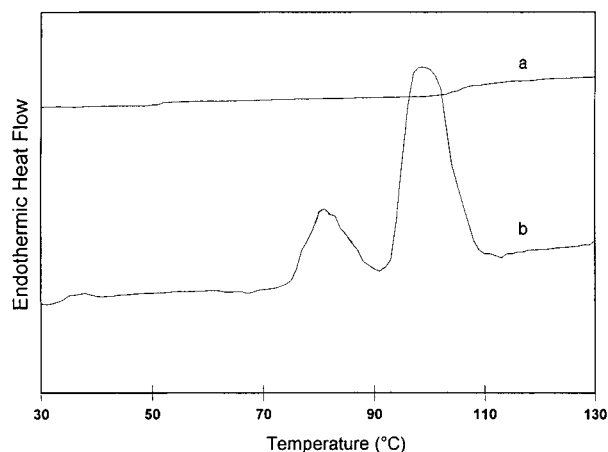
Figure 5. DSC thermograms of FBP (a) and SPI (b).

The value for 11S globulin was in agreement with 100 °C as reported by Hermansson (1978) and Sheard et al. (1986) but higher than 90 °C reported by Kim and Rhee (1989). However, FBP did not show any endothermic peak up to 130 °C. These findings demonstrated that FBP was more heat stable than SPI.

Since the FBP is heat stable, is a good source of essential amino acids, and has moderate emulsifying and foaming properties, it could find application in food products such as soup, meat, and bakery products. However, the source of the protein and the optimum condition required for its consistent development remain to be investigated.

ABBREVIATIONS USED

ANS, 1-anilino-8-naphthalenesulfonate; BSA, bovine serum albumin; DSC, differential scanning calorimetry; EA, egg albumen; FBP, pickle fermentation brine protein; SDS, sodium dodecyl sulfate; SPI, soy protein isolate.

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