# Properties of Cast Films from Pickle Fermentation Brine Protein

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A proteinaceous fibrous material formed during commercial fermentation of cucumbers was used for manufacturing films. The effects of pH and heat treatments on tensile strength (TS), puncture strength (PS), water vapor permeability (WVP), protein solubility in water, and color of pickle fermentation brine protein (PFBP) cast films were determined. Nine types of films at three different pH values (7.0, 9.0, and 11.0) and three different heat treatments (no heat, heated at 70 and 90 °C for 30 min) were prepared by casting 5% (w/w) PFBP aqueous solution, containing 2.25% (w/w) glycerol, onto polystyrene plates and overnight drying at room temperature. The TS of pH 7.0 films was higher than the TS of pH 9.0 and 11.0 films ($P < 0.05$). The highest PS was observed with pH 7.0 films prepared from PFBP solution heated at 70 °C ($P < 0.05$). Alkaline pH and temperature caused a decrease in both TS and PS of the films. Film thickness ranged from 58 to 74 $\mu$m. WVP values of films decreased with increasing pH and temperature. No heat/pH 7.0 films had the greatest WVP. Protein solubility of films in water increased with increasing pH and temperature.

**Keywords:** *Pickle protein exudate; protein film; tensile strength; water vapor permeability; protein solubility*

## 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).

Development of agriculturally derived alternatives to the petroleum-based packaging materials currently used by the food industry provides an opportunity to strengthen the agricultural economy and reduce importation of petroleum and petroleum products (Parris and Coffin, 1997). Increased consumer demands for both higher quality and longer shelf life foods in combination with environmental needs for reduction in the use of nondegradable disposable packaging materials have led to increased interest in edible film research (Chen, 1995). Edible films offer alternative packaging without serious environmental pollution because of their biodegradable nature. Edible films are not meant to totally replace synthetic packaging films. However, they do have the potential to reduce packaging and to limit moisture, aroma, and lipid migration between food components where synthetic packaging cannot function (Krochta and De Mulder-Johnston, 1997). An important aspect of these films is the renewable nature of the raw materials used for their production.

Edible coatings and films can prevent quality changes in foods by acting as barriers to control the transfer of moisture, oxygen, and carbon dioxide, lipid oxidation, and loss of volatile flavors and aromas. They can carry food ingredients and additives. Edible films and coatings used on pharmaceutical products, confections, fruits, vegetables, and some meat products are typically derived from lipids, proteins, carbohydrates, or composites of the three (Kester and Fennema, 1986; Brandenburg et al., 1993). Potential application and properties of protein-, lipid-, and polysaccharide-based edible films have been reviewed (Kester and Fennema, 1986; Guilbert, 1986; Krochta, 1992). The properties of protein-based edible films (Gennadios et al., 1994) and milk protein based films (McHugh and Krochta, 1994; Chen, 1995) have been comprehensively examined.

The composition and functional properties of pickle fermentation brine protein (PFBP) were reported previously (Hettiarachchy et al., 1998). The objectives of this research were to produce films from PFBP and to determine the effects of pH and heat treatments on tensile strength (TS), puncture strength (PS), water vapor permeability (WVP), protein solubility in water, and color of these films.

## MATERIALS AND METHODS

**Materials.** Fibrous material was collected from the surfaces of brine tanks using a perforated stainless steel spoon during commercial cucumber pickle fermentation and stored at 4 °C for further processing. Glycerin and NaOH were purchased from Fisher Scientific Co. (Pittsburgh, PA). $AgNO_3$ was obtained from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of analytical grade.

**Fermentation Brine Protein Processing.** Pickle fermentation brine protein was washed in running tap water [approximately 1:5 PFBP/water (v/v) ratio per washing]. After five tap water washings, deionized water was used until the washings were free of NaCl, which was determined using $AgNO_3$ solution in the presence of $K_2CrO_4$ as an indicator (AACC, 1983), and the protein was freeze-dried for 4 days at −45 °C condenser temperature and <100 mTorr vacuum using

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**Table 1. Selected Physical Properties of Cast Films from a Pickle Fermentation Derived Proteinaceous Material[a]**

| sample | thickness ($\mu$m) | relative humidity[b] (%) | water vapor permeability (g·mm/kPa·h·m$^2$) | tensile strength (MPa) | puncture strength (N) | protein solubility in water (%) |
|---|---|---|---|---|---|---|
| Con-7 | 73 ± 1.6a | 66 ± 1.1a | 10.56 ± 1.53a | 5.08 ± 0.46a | 4.65 ± 0.13b | 6.8 ± 1.4a |
| 70-7 | 74 ± 2.5a | 72 ± 1.4b | 5.78 ± 0.93b | 4.57 ± 0.43ab | 5.29 ± 0.39a | 7.3 ± 0.5a |
| 90-7 | 72 ± 9.8a | 71 ± 1.6b | 6.29 ± 1.21b | 4.27 ± 0.88b | 4.36 ± 0.16bc | 8.2 ± 0.8a |
| Con-9 | 70 ± 2.6ab | 73 ± 0.9b | 5.31 ± 1.88b | 3.50 ± 0.28c | 4.69 ± 0.27b | 9.8 ± 2.5a |
| 70-9 | 70 ± 3.6ab | 75 ± 1.1b | 4.18 ± 0.79b | 3.36 ± 0.52c | 3.93 ± 0.48c | 16.6 ± 0.7b |
| 90-9 | 69 ± 3.7ab | 77 ± 1.5b | 3.72 ± 0.49b | 3.64 ± 0.30c | 4.06 ± 0.18c | 17.6 ± 1.4b |
| Con-11 | 73 ± 7.4a | 76 ± 1.8b | 4.21 ± 1.26b | 3.22 ± 0.41c | 4.67 ± 0.21b | 17.5 ± 1.2b |
| 70-11 | 61 ± 4.2bc | 72 ± 1.3b | 4.72 ± 0.55b | 3.48 ± 0.32c | 4.09 ± 0.41c | 29.9 ± 3.2c |
| 90-11 | 58 ± 5.5c | 76 ± 1.4b | 3.75 ± 0.89b | 3.88 ± 0.68c | 3.36 ± 0.37d | 74.9 ± 3.9d |

[a] Means of three replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly different ($P > 0.05$). Con-7, -9, -11: no heat, pH 7.0, 9.0, and 11.0 films. 70-7, -9, -11: prepared from PFBP solution heated at 70 °C for 30 min at pH 7.0, 9.0, and 11.0. 90-7, -9, -11: prepared from PFBP solution heated at 90 °C for 30 min at pH 7.0, 9.0, and 11.0. [b] Calculated relative humidity values.

a VirTis 10-no$_3$ model freeze-dryer (The VirTis Co., Gardiner, NY). After freeze-drying, protein, ash, lipid, total carbohydrate, and moisture contents of PFBP were 72.5 ± 0.82, 3.2 ± 0.02, 0.7 ± 0.08, 20.2 ± 2.14, and 3.0 ± 0.35%, respectively (Hettiarachchy et al., 1998).

**Protein Determination.** Protein solubility was determined according to the microKjeldahl method.

**Film Preparation.** Film-forming solutions were prepared by dispersing PFBP (5%, w/w) and glycerol (2.25%, w/w) in deionized water. The solution was mixed using a mechanical homogenizer (Virtishear Tempest, The VirTis Co.) at setting 6 for 30 min. The pH value of the solutions was adjusted to 7.0, 9.0, or 11.0 using either 0.1 or 1.0 N NaOH. Subsequently, the solutions were heated in a water bath at 70 or 90 °C for 30 min. Control samples were prepared without heat treatment. After filtering through cheesecloth, to remove foam and undissolved impurities, and degassing under vacuum for 20 min, 12 mL of solution was poured onto polystyrene plates (casing surface area = 75 cm$^2$). Cast solutions were allowed to dry at room temperature (~23 °C) overnight. Nine types of films at three different pH values (7.0, 9.0, and 11.0) and three different heat treatments (no heat, heated at 70 and 90 °C for 30 min) were prepared.

**Film Thickness.** Film thickness was measured using a micrometer with a sensitivity of 1.25 $\mu$m (Digitrix-Mark II, model 2804-10, Mitutoyo, Japan). Film strips were placed between the jaws of the micrometer and the gap was reduced until the first indication of contact. Mean thicknesses of films were determined from the average of measurements at five locations.

**WVP.** Circular film samples of ~12 cm diameter were placed over the open mouth of aluminum cups (area = 33 cm$^2$) and secured between a metal rim and rubber gasket. WVP was measured in a chamber at 22 °C, conditioned at 50% relative humidity (RH). Air velocity was ~163 m/min over the surface of the cups to remove the permeating water vapor. Distilled deionized water was placed in the cups with an air gap of 1.4 cm above the water surface. The cup assembly was weighed every 30 min for a minimum of 10 h. Calculations for WVP were performed as described by McHugh et al. (1993).

**Mechanical Properties.** Two mechanical tests, TA and PS, were performed on films using a texture analyzer (TA.XT2, Texture Technologies Corp., New York). Film samples were conditioned at ambient temperature and 50% RH for 48−52 h prior to textural analyses according to ASTM Standard Test Method D 882-91 (ASTM, 1991). TS measurements were performed by securing film strips (75 mm × 10 mm) in the grips of the texture analyzer. Initial grip separation was 50 mm, and cross-head speed was set at 2 mm/s in a tension mode. Fifty millimeter initial grip separation and 2 mm/s cross-head speed were chosen because, under our experimental conditions, these settings produced more accurate data. TS was computed as peak force divided by initial cross-sectional area of the specimens. PS of films was measured by mounting circular film samples of 16 mm diameter on a cylindrical glass cup (12 mm diameter and 30 mm length) and securing between a plastic rim and a rubber gasket. Using a 3 mm probe at 5 mm/s constant speed in a compression mode (TA.XT2), the films were punctured and the force at puncture was read from a computer monitor (in newtons) and expressed as PS.

**Protein Solubility.** Small pieces of films (25−30 mg) were placed in 5 mL of deionized water. Film samples were conditioned at ambient temperature and 50% RH for 48−52 h prior to the solubility test. The film suspension was incubated at room temperature (22−25 °C) for 24 h by shaking gently. Solubility was determined by measuring the protein content of the supernatant using the micro-Kjeldahl method (AOAC, 1990), and percent solubility was calculated as follows:

$$\text{solubility (\%)} = \text{protein in supernatant} \times 100/\text{total protein in sample} \quad (1)$$

**Color Values.** Color values of prepared films were measured with a Gardner Colorimeter (ColorGard System/05, Pacific Scientific, Silver Spring, MD). Film specimens were placed on the surface of a white standard plate (calibration plate white 1415), and Hunter $L$, $a$, and $b$ color values were measured. The ranges of the three color coordinates were $L$ (0, black, to 100, white), $a$ (−, greenness, to +, redness), and $b$ (−, blueness, to +, yellowness) (Francis and Clydesdale, 1975). Total color difference ($\Delta E$) was calculated as

$$\Delta E = [(L_{\text{film}} - L_{\text{standard}})^2 + (a_{\text{film}} - a_{\text{standard}})^2 + (b_{\text{film}} - b_{\text{standard}})^2]^{0.5} \quad (2)$$

Standard values refer to the white calibration plate ($L = 92.93$, $a = -0.83$, $b = -0.89$).

**Statistical Analysis.** Each replication included individual preparation of film from film-forming solutions. Three replications were performed in a completely randomized design. A minimum of three observations for each property was collected. Data were analyzed using the general linear model (GLM) 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 treatment means shown to be significantly different by GLM was performed using the least significant difference (LSD) procedure with significance defined at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Film Thickness.** Mean film thicknesses for various types of films are presented in Table 1. Film thickness ranged from 58 to 74 $\mu$m. Control films had a less even surface and less transparent appearance compared to other film samples. The thickness of control films was close to the upper limit due to the bumpy surface, although this did not create a problem for thickness measurement. Therefore, property evaluation on control films was performed with confidence. There were no significant differences ($P > 0.05$) among Con-7, Con-9,

Con-11, 70-7, 70-9, 90-7, and 90-9 film thicknesses. However, the film thickness of 58 $\mu$m for 90-11 was significantly ($P < 0.05$) thinner than that of the other films except for 70-11 film (61 $\mu$m). The high pH and temperature probably cleaved the carbohydrate portion of PFBP into smaller units and made it more soluble. In addition, high temperature denatured the protein molecule and enhanced protein−protein interaction, resulting in a transparent and thinner film with a more uniform structure.

**WVP.** WVP is an important functional film property that determines utility in food systems. Measured WVP values of films were compared along with calculated actual RH conditions at the underside of film during testing (Table 1). WVP values of films decreased with increasing pH and temperature, although there were no significant ($P > 0.05$) differences among WVP values of Con-9, Con-11, 70-7, 70-9, 70-11, 90-7, 90-9, and 90-11 films. Con-7 showed the highest WVP value, which was significantly ($P < 0.05$) different from that of other films. In addition to changes in the carbohydrate portion, heating the film-forming solution denatures proteins, thus allowing increased interaction and higher packing and reduced mobility of polymer chains, causing a decrease in WVP (Stuchell and Krochta, 1994). Therefore, the lower WVP of films from alkaline heated solutions was attributed to alkaline pH conditions and high temperature, resulting in a denser and smoother film structure. Similarly, Gennadios et al. (1993) reported that WVP of soy protein isolate films decreased with increasing pH of film-forming solutions. WVP values were higher for films cast from unheated solution than for those from solution heated at 85 °C (Stuchell and Krochta, 1994). However, McHugh et al. (1994) did not observe any significant differences among WVP values or whey protein films prepared from solutions treated at pH 7.0 or pH 9.0 and 75 or 100 °C.

**Mechanical Properties.** Mechanical properties of protein films provide an indication of expected film integrity under conditions of stress that would occur during processing, handling, and storage. TS values of film are given in Table 1. The highest TS was for Con-7 film (5.08 MPa). TS values of pH 7.0 films were significantly ($P < 0.05$) higher than those of pH 9.0 or 11.0 films. There were no significant ($P > 0.05$) differences between pH 9.0 and 11.0 films. Again, alkaline pH and high temperature caused a decrease in TS probably due to decomposition of PFBP. Gennadios et al. (1993) reported that under high alkaline conditions the TS of soy protein isolate films was significantly reduced. Wheat gluten film-forming solutions were heated at 55, 65, 75, 85, or 95 °C for 10 min, and it was found that TS increased with increasing temperature (Roy et al., 1995).

The highest PS was observed with pH 7.0 films prepared from PFBP solution heated at 70 °C ($P < 0.05$) (Table 1). The 90-11 film had the lowest PS value. In general, alkaline pH and temperature caused a decrease in PS.

**Protein Solubility.** Protein solubilities of films were determined to evaluate their integrity in an aqueous environment (Table 1). High solubility may be desired for some applications such as in water soluble coatings and films. Protein solubilities of films increased with increasing pH and temperature. The pH 11.0 film prepared from PFBP solution heated at 90 °C gave the highest protein solubility (74.9%) ($P < 0.05$), whereas the lowest solubility was observed with Con-7 films (6.8%). The 90-11 and 70-11 film pieces immersed in water were broken apart at the end of 24 h of incubation. The combination of high alkaline pH and temperature treatment probably caused a cleavage of the carbohydrate portion of PFBP (contains ~20% carbohydrate; Hettiarachchy et al., 1998) into smaller units (BeMiller and Whistler, 1996). Heat treatment denatures proteins, possibly releasing small peptides that remained bound in the unheated proteins (Stuchell and Krochta, 1994). In addition, to a smaller extent, degradation of proteins could be possible due to high alkaline pH and temperature. As a result of these, a highly soluble film structure was observed. Similarly, an increase in protein solubility was observed with soy protein isolate films heated at 85 °C (Stuchell and Krochta, 1994). However, Roy et al. (1995) reported that after heating wheat gluten film-forming solutions at 55, 65, 75, 85, or 95 °C for 10 min, the solubility of films in water decreased with increasing temperature. The highly soluble nature of 90-11 film in water might be useful for preparation of hot water soluble pouches.

**Table 2. Hunter Color Values (*L*, *a*, and *b*) and Total Color Differences ($\Delta E$) of Films[a]**

| sample | *L* | *a* | *b* | $\Delta E$ |
|---|---|---|---|---|
| Con-7 | 68.7 ± 0.27ef | −1.36 ± 0.06a | 31.0 ± 0.03b | 40.1 ± 0.19a |
| 70-7 | 67.8 ± 0.12fg | −1.92 ± 0.05b | 30.6 ± 0.25bc | 40.3 ± 0.14a |
| 90-7 | 67.6 ± 0.28g | −3.27 ± 0.08d | 29.4 ± 0.49ef | 39.6 ± 0.54a |
| Con-9 | 70.6 ± 0.68bc | −2.56 ± 0.09c | 30.6 ± 0.47bc | 38.6 ± 0.49b |
| 70-9 | 70.2 ± 0.32cd | −3.36 ± 0.19d | 30.1 ± 0.41cd | 38.6 ± 0.20b |
| 90-9 | 69.4 ± 0.38de | −3.99 ± 0.05e | 29.6 ± 0.18de | 38.6 ± 0.33b |
| Con-11 | 70.3 ± 0.59bcd | −3.12 ± 0.16d | 31.8 ± 0.38a | 39.8 ± 0.41a |
| 70-11 | 71.2 ± 1.15b | −4.32 ± 0.35f | 32.1 ± 0.23a | 39.6 ± 0.79a |
| 90-11 | 73.4 ± 0.59a | −5.28 ± 0.14g | 28.9 ± 0.71f | 35.9 ± 0.64c |

[a] Means of three replicates ± standard deviation. Any two means in the same column followed by the same letter are not significantly different ($P > 0.05$). Con-7, -9, -11: no heat, pH 7.0, 9.0, and 11.0 films. 70-7, -9, -11: prepared from PFBP solution heated at 70 °C for 30 min at pH 7.0, 9.0, and 11.0. 90-7, -9, -11: prepared from PFBP solution heated at 90 °C for 30 min at pH 7.0, 9.0, and 11.0.

**Film Color.** Color is an important property of protein films because it could affect consumer acceptance of such films in potential edible or nonedible packaging applications (Kunte et al., 1997). Hunter *L*, *a*, and *b* color values and total color differences for films are shown in Table 2. Heat-treated films were generally clearer and more uniform than control films. Control films had a bumpy surface appearance due to the solubility problem. Our previous study showed that the isoelectric point of PFBP was approximately pH 4.0, and its solubility at pH 6.0 was 2.4% (Hettiarachchy et al., 1998). Therefore, pH is a critical factor in film preparation from PFBP. The 90-11 film showed the highest *L* value (lighter) (73.4) ($P < 0.05$), whereas the 90-7 film gave the lowest *L* value (darker). Similar to *L* values, the yellowness values (+*b* values) of 90-7 (29.4), 90-9 (29.6), and 90-11 (28.9) films were lower than those of the other films. Alkaline pH and temperature caused formation of a greener color (−*a* values). High alkaline pH and temperature unexpectedly gave lighter and less yellow films, even though such conditions would be expected to be more favorable for nonenzymatic browning. This discrepancy was probably due to increased solubility of PFBP under the experimental conditions (alkaline pH and temperature) compared to pH 7.0 films because particle size and distribution significantly affect color measurement (Hutchings, 1994).

**Conclusions.** TS of films ranged from 3.22 to 5.08 MPa. The highest PS was observed with pH 7.0 films prepared from PFBP solution heated at 70 °C ($P < 0.05$). High alkaline pH and temperature caused a decrease in both TS and PS of the films. Thickness of films ranged from 58 to 74 $\mu$m. WVP values of films decreased with increasing pH and temperature. To produce film from PFBP, a pH value ranging from 7.0 to 9.0 and a heat treatment from 70 to 90 °C were needed. The soluble nature of PFBP films in water might be useful for preparation of hot water soluble pouches.

## ABBREVIATIONS USED

Con-7, -9, -11, no heat, pH 7.0, 9.0, and 11.0 films; PFBP, pickle fermentation brine protein; PS, puncture strength; RH, relative humidity; TS, tensile strength; WVP, water vapor permeability; 70-7, -9, -11, prepared from PFBP solution heated at 70 °C for 30 min at pH 7.0, 9.0, and 11.0; 90-7, -9, -11, prepared from PFBP solution heated at 90 °C for 30 min at pH 7.0, 9.0, and 11.0.

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