

Research Article

Utilization of Chicken By-Products to Form Collagen Films

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Chicken collagen casings could be an alternate source of collagen casings that are manufactured for sausages. The overall objective of this project was to extract chicken collagen from by-products of the broiler processing industries and to explore the possibility of making films. Chicken skin was washed, ground, and pretreated to remove the noncollagenous compounds. Collagen was extracted using acetic acid and pepsin. Solubilized collagen was salted-out and centrifuged at 20,000 ×g at 4°C for one hour. The precipitates were dissolved in 0.5 M acetic acid and dialyzed against 0.1 M acetic acid and distilled water before freeze-drying. Molecular weight, collagen solubility at different pH values, and NaCl concentrations were determined. TA-XT2 texture analyzer was used to characterize mechanical properties of collagen films. The highest collagen solubility was obtained at pH 2 and 2% NaCl. Hand-homogenized, nonfiltered, and conditioned samples had the highest hardness (3,262 g) and the least brittleness (30.5 mm). These results demonstrate that chicken collagen extracted from chicken by-products has the ability to form films and could be considered for making casings or be used in various other industries.

1. Introduction

Natural casings may have high bacterial loads, need to be preserved, and are more labor-intensive to handle than reconstituted casings [1]. Edible collagen films can be formed into collagen casings. Collagen casings play an important role in manufacturing process of sausages. Collagen casings can inhibit bacterial attachment as well as enhance the hygienic properties of sausages [2].

The chicken processing industry is an important income generator in Delmarva Peninsula. Annual broiler production is approximately 8.9 million pounds in 2009 [3]. Waste generated by the chicken processing plants can be minimized by utilizing chicken by-products. Collagen extraction from chicken by-products minimizes the waste production of chicken industry.

Lucca and Ferraz da Silva Torres [4] studied the conditions of hot dog processing in Brazil and found that the hygienic conditions for food preparations were extremely poor. Consumers were at high risk due to unsuitable hygienic

practices during food preparation and the lack of basic knowledge regarding safe food handling that causes public health problems. Although all hot dogs are fully cooked, they should always be reheated before eating. The effect of reheating on the viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters following refrigerated or frozen storage was studied by Porto et al. [5]. It was suggested that frankfurters which may become contaminated with low levels of *L. monocytogenes* prior to packaging and after unpackaging should be adequately reheated prior to consumption. Thus, for added precaution, persons at risk may choose to avoid eating sausages type foods unless they are reheated to 140°C [6]. So to speak, incident of *L. monocytogenes* can be minimized if sausages have edible casings.

Collagen casings are important in manufacturing sausages to maintain its specific shape. The earliest forms of natural collagen casings are casings from bovine and porcine intestines. Casings made from extruded, reconstituted collagen fibers are popular compared to natural casings

because fabricated collagen casings offer the advantages of uniformity, strength, flexibility, and greater consistency in processing environments compared to natural casings.

Chicken collagen was mixed with acetic acid, glutaraldehyde, carboxymethyl cellulose (CMC), sorbitol, sodium chloride (NaCl), hydrochloric acid (HCl), and glycerol to form collagen films. Acetic acid and carboxymethyl cellulose were used to improve the strength. Plasticizing agent glycerol/sorbitol and cross-linking agent glutaraldehyde were used to improve the pliability of the film and also to overcome film brittleness caused by extensive intermolecular forces [1]. It was found that if the concentration of glutaraldehyde exceeds 0.2%, the maximal viscosity of collagen gel solutions reaches a plateau [7]. Drying, conditioning, and neutralizing provide additional cross-linking of the collagen casings made up of bovine collagen, Gennadios [8]. Fernandes et al. [9] used acrylic containers to dry collagen films made up of porcine in a vacuum atmosphere.

The ability to form chicken collagen films from the extracted collagen samples from chicken by-products would indicate the possibility of making collagen casings which are important in the production of a variety of value-added or processed meat products. Collagen extracted from one-step acid and pepsin procedure was selected to make collagen films because this method gave higher yield and had less processing time [10].

2. Material and Methods

2.1. Raw Material Preparation and Pretreatment. Material preparation was adopted from Kittiphattanabawon et al. [11]. Broiler chicken (Perdue Farms, Salisbury, MD) skin was cleaned with tap water and skin samples were cut into small pieces ($0.5 \times 0.5 \text{ cm}^2$) before storing at -20°C . Removal of noncollagenous proteins, fat, and inorganic matter was conducted at 4°C with continuous stirring. Noncollagenous protein was removed by constantly mixing the samples of skins with 0.1N NaOH at a sample/alkali solution ratio of 1:6 (w/v). The mixture was stirred for 6 hours. Every 2 hours, the alkali solution was changed. Then the sample was washed with distilled water, until the water was clear and colorless. The deproteinized skin samples were defatted with 10% butyl alcohol with a solid/solvent ratio of 1:6 (w/v). The mixture was stirred for 24 hours. Every 6 hours, the solvent was changed. Then the defatted samples were washed with distilled water three times. Inorganic compounds were removed by soaking the defatted sample in a 0.1N HCl solution with a solid/solvent ratio of 1:6 (w/v). The mixture was stirred for 24 hours. Then, the sample was filtered through double layer cheesecloth and washed three times with distilled water [12].

2.2. Collagen Extraction and Precipitation. 0.5 M acetic acid containing pepsin (20 kU/g, Sigma-Aldrich Corp., St. Louis, MO) was used to soak defatted samples for 48 hours at 4°C . The solid/solvent ratio was 1:6 (w/v). The mixture was filtered with a double layer of cheesecloth and the filtrates were collected for precipitation [13]. The collagen extractions were precipitated according to the method described by

Kittiphattanabawon et al. [11]. NaCl was used for the precipitation until final concentration reaches 2.6 M in the presence of 0.05 M tris(hydroxymethyl)aminomethane (pH 7.0). The precipitates were collected by centrifuging at $20,000 \times g$ for 60 min at 4°C and the pellets were dissolved in 0.5 M acetic acid. Then the pellets were dialyzed against 0.1 M acetic acid and distilled water.

2.3. Collagen Solubility. The method described by Montero et al. [14] was used to determine solubility of collagen with slight modifications. To obtain a stock solution of 8 mg/mL, collagen was dissolved in 0.5 M acetic acid and the mixture was stirred at 4°C until the collagen completely solubilized.

2.3.1. Effect of pH on Collagen Solubility. Six aliquots of the collagen stock solution (10 mL) were placed in 50 mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and the pH adjusted to 2, 4, 6, 8, 10, and 12 using 6 N NaOH or 6 N HCl. Samples were centrifuged at $20,000 \times g$ and 4°C for 30 min. Supernatants were subjected to protein measurement. The Bradford assay was used to measure the absorbance at 595 nm wavelength [15]. Bovine serum albumin was used as the standard and calculations were directly proportional to the standard curve.

2.3.2. Effect of NaCl on Collagen Solubility. Collagen solution (5 mL) was mixed with 5 mL of NaCl at various concentrations (0%, 2%, 4%, 6%, 8%, 10%, and 12% w/v). The mixtures were stirred for 30 min at 4°C and centrifuged for 30 min at $20,000 \times g$ at 4°C . Supernatants were collected separately and protein content was measured using the Bradford assay following the same procedure described in Section 2.3.1.

2.4. Collagen Film Formation. Film-forming solutions were prepared by mixing 8 g of collagen samples with glycerol, sorbitol, carboxymethyl cellulose (CMC), and glutaraldehyde. The mixture was poured on polystyrene weighing dish to cast films. Then they were air-dried at room temperature for 48 hours. The dry films which had a water activity of 0.8 were carefully recovered from the weighing dishes to conduct conditioning procedures. Conditioning was conducted with 0.1 M HCl and 0.1 NaOH several times with slow agitation for 15 min. During conditioning collagen films were washed with distilled water between each treatment with 0.1 M HCl and 0.1 NaOH.

2.5. Texture Analysis. TA-XT2 texture analyzer was used to analyze the texture of collagen films using the flat blade probe (Table 3). The hardness and brittleness of the films which were formed in different ways were measured. The hardness is the peak force (g) of the compression required for the sample's breakage and the brittleness is the distance from zero force to peak force. Higher peaks indicated higher hardness and samples which have longer distance indicated less brittleness of the film.

3. Results and Discussion

3.1. Collage Extraction Using One-Step Acetic Acid and Pepsin Extraction Procedure. Jongjareonrak et al. [16] reported that

TABLE 1: Collagen solubility in different pH values.

| Sample ID | Protein content (mg/mL) ^a |
|-----------|--------------------------------------|
| pH 2 | 0.16 ± 0.51 |
| pH 4 | 0.05 ± 0.08 |
| pH 6 | 0.05 ± 0.07 |
| pH 8 | 0.04 ± 0.04 |
| pH 10 | 0.08 ± 0.07 |

^aMean of two observations.

the pepsin digested procedures gave a complete digestion for chicken skin and also there was no intact skin left with pepsin after 48 hours digestion. Nalinanon et al. [13] suggested that use of acetic acid makes skin more porous, therefore, enhancing pepsin digestion. Further, they suggested that more molecular cross-links between collagen and other components can be broken by the additional digestion with pepsin. After comparing yields of five different extraction procedures with 0.5 M acetic acid, 0.5 M citric acid, alkali extraction, one-step acetic acid and pepsin extraction and two-step acidic acid and pepsin extraction [10], one-step acetic acid and pepsin extraction was performed to extract collagen to form collagen films because of the high yield of collagen (38.7%).

3.2. Collagen Solubility

3.2.1. Chicken Collagen Solubility at Different pH Values.

Pepsin extracted collagen was dissolved in 0.5 M acetic acid to obtain a final concentration of 8 mg/mL and the pH of the solution was adjusted using 6 N NaOH or 6 N HCl. The Bradford assay was used to measure the absorbance at a wavelength of 595 nm. Bovine serum albumin was used as the standard and calculations were directly proportional to the standard curve. The highest solubility of 0.16 mg/mL occurred at pH 2 and the lowest solubility of 0.05 mg/mL occurred at pH 8 (Table 1). Collagen solubility in pH 10 was 0.08 mg/mL. This solubility behavior is typical for proteins, which tend to carry a positive charge at low pH and a negative charge at high pH. Molecules with the same charge repel each other and increase solubility. At an intermediate pH, proteins reach their isoelectric point.

At the isoelectric point net charge of a protein molecule is zero and the repulsion forces are minimized. This leads to the aggregation of protein molecules and decreased solubility [14].

Jongjareonrak et al. [16] found that acid and pepsin extracted collagens from bigeye snapper skin exhibited the lowest solubility between pH values of 6 and 7. Kittiphatanabawon et al. [11] observed the lowest solubility of acid extracted collagen from bigeye snapper skin was at pH 7. The result of this study shows that the lowest chicken collagen solubility was at pH 8. However, the pH values tested in this study were pH 6 and pH 8 and did not include pH 7 so that a direct comparison for the solubility at this pH is not possible.

3.2.2. Chicken Collagen Solubility at Different Concentration of NaCl.

Five milliliters of the collagen solution (8 mg/mL) was

TABLE 2: Collagen solubility in different NaCl concentrations.

| Sample ID | Protein content (mg/mL) ^a |
|-----------|--------------------------------------|
| NaCl 2% | 0.11 ± 0.1 |
| NaCl 4% | 0.02 ± 0.02 |
| NaCl 6% | 0.01 ± 0.04 |
| NaCl 8% | Not detected |
| NaCl 10% | Not detected |
| NaCl 12% | Not detected |

^aMean of two observations.

TABLE 3: Collagen film parameters for texture analyzer.

| Filter ID number | Maximum force (g) | Distance (mm) |
|------------------|-------------------|---------------|
| Film 1 | 3262 | 30.5 |
| Film 2 | 16037 | 5.8 |
| Film 3 | 500 | 26.3 |
| Film 4 | 4102 | 26.7 |
| Film 5 | 740 | 14.8 |

mixed with 5 mL of NaCl solution of different concentrations (2, 4, 6, 8, 10, and 12%). The highest solubility (0.11 mg/mL) was obtained at 2% NaCl (Table 2). Solubility at 4 and 6% NaCl was 0.02 and 0.01 mg/mL, respectively. No solubility was detected for the protein at NaCl concentrations of 8, 9, and 10%. The Bradford test may not be sensitive enough to measure low values of proteins because the sensitivity of Bradford assay is 0.01 mg/g of protein [17]. This result is in agreement with the report by Nalinanon et al. [13] who found that the solubility of collagens decreased gradually with increasing NaCl concentration. Vojdani [18] explained that a higher concentration of NaCl might result in decreased protein solubility via a salting-out effect.

Collagen solubility in different NaCl concentration (Table 2) decreased by increasing hydrophobic interaction and aggregation, and also NaCl competes with the protein for water.

In addition, ions from NaCl could also neutralize charges on proteins reducing electrostatic interaction and repulsion; thereby it was favoring the hydrophobic aggregation as well.

3.3. Film Formation with Collagen. The ability of forming films from the extracted collagen samples would indicate the possibility of making collagen casings which are important in the production of a variety of value-added or processed meat products. Collagen extracted from one-step acid and one-step pepsin was selected to make collagen films because this method gave higher yields (38.7%) and the samples were easier to handle than those from the two-step acid and one-step pepsin method during collagen extraction procedures [10].

Glycerol and sorbitol were added to the film-forming solutions as plasticizers. This reduced the brittleness caused by extensive intermolecular forces. Gennadios et al. [1] showed that collagen films have limited resistance to water vapor transmission and require a significant amount of plasticizers (sorbitol and glycerin) to impart flexibility.

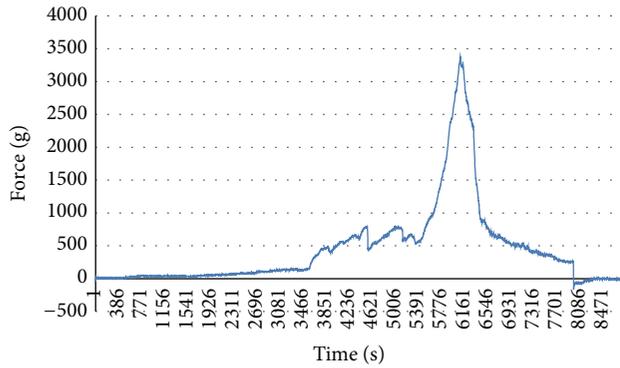


FIGURE 1: Film 1: hand-homogenized, nonfiltered, and conditioned film (8 g).

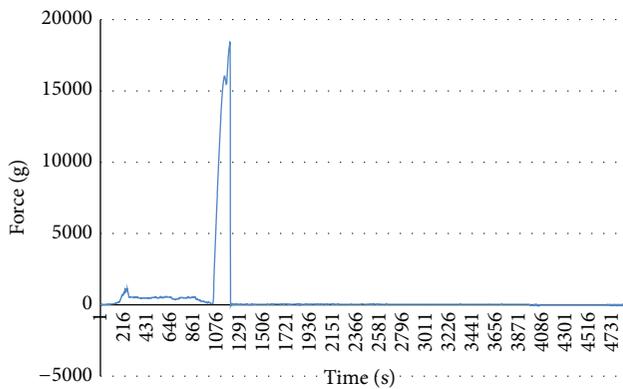


FIGURE 2: Film 2: hand-homogenized, nonfiltered, and conditioned thin film (8 g).

3.4. Comparison of Collagen Films. Figures 1, 2, 3, 4, and 5 show the texture analysis results for the films which had different processing procedures. Film 1 was hand-homogenized, nonfiltered, and conditioned and used 8 g of slurry and Film 2 was hand-homogenized, nonfiltered, and conditioned and it was a thinner film using less than 8 g of slurry. Film 3 was formed from a hand-homogenized, filtered, and nonconditioned sample using 8 g of slurry to make a film. Film 4 was formed from hand-homogenized filtered slurry (8 g) and was conditioned. Film 5 was from a machine homogenized sample and used 8 g of slurry. Machine homogenized samples did not form a film that could go through in the conditioning steps. Film 2 had the highest peak of 16037 g (160 N) and the shortest distance of 5.8 mm. It was the hardest film and also the most brittle film. Films 1 and 4 were much harder and less brittle than Films 3 and 5 which were the softest and least brittle.

The film obtained from the collagen slurry mixed with the Ultra-Turrax T25 high speed homogenizer maintained a soft texture and was hard to remove from the weighing dish. However, films obtained from hand-mixed samples were very easy to remove from the weighing dishes when they were properly dried.

Proper appearance could be obtained when the hand-mixed samples were filtered through a double layer of

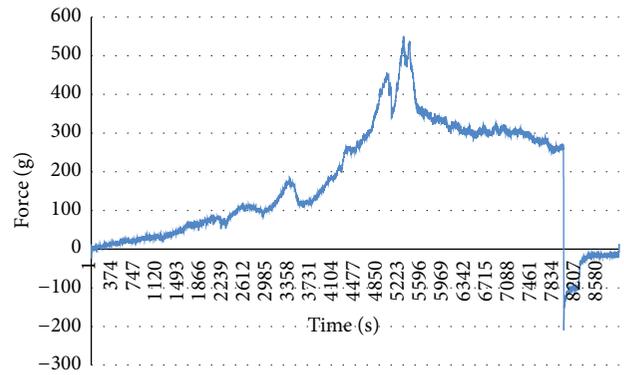


FIGURE 3: Film 3: hand-homogenized, filtered, and nonconditioned film (8 g).

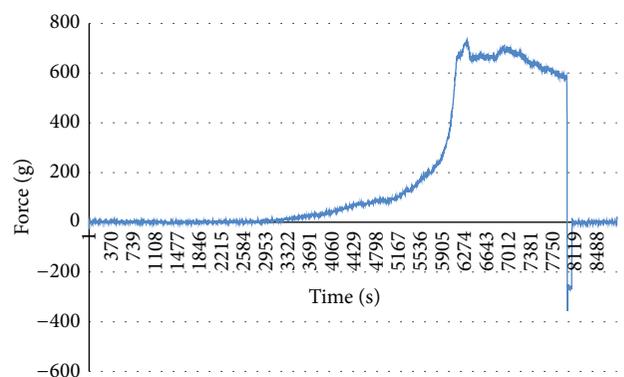


FIGURE 4: Film 4: hand-homogenized, filtered, and conditioned film (8 g).

cheesecloth. However, those films were more brittle than nonfiltered collagen films.

Yellow coloration of the collagen film could be reduced by dipping collagen film in a HCl bath during the conditioning steps. Dipping collagen film in a NaCl bath can increase cross-links according to Gennadios et al. [1]. Also drying, conditioning, and neutralizing provided additional cross-linking of the collagen film as reported by Hood et al. [19]. After being washed with distilled water, films were dried under an air flow at room temperature until the water activity reached a level of 0.8. Then the collagen films were stored at room temperature.

4. Conclusion

Chicken skin extracted with one-step acid and one-step pepsin was selected as the source for the experimentation to manufacture collagen film because of its high yield (38.7%) after conducting pretreatment procedures to remove fat and minerals.

The extracted colorless and odorless chicken collagen was used to find the solubility with different acid concentrations and salt concentrations. Collagen solubility was affected by pH level and NaCl concentration. The highest solubility

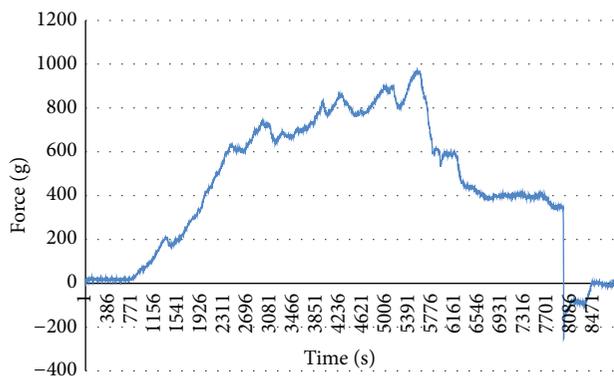


FIGURE 5: Film 5: machine homogenized film (8 g).

(0.16 mg/g) of collagen was obtained at pH 2 and lowest solubility was obtained at pH 8 (0.04). Highest solubility (0.11 mg/mL) of collagen with NaCl was at 2% and solubility decreased with increasing NaCl concentration. Soluble protein was not detected at 8% to 12% NaCl concentration.

Collagen was mixed with acetic acid, glutaraldehyde, CMC, sorbitol, and glycerol. High levels of NaCl shrink the collagen fibers in order to form a stronger film during the conditioning step. In addition, HCl improved the color. Texture analysis was conducted using five different methods of making collagen films to test their properties for hardness and brittleness. Hand-homogenized, nonfiltered/filtered, and conditioned (8 g) film gave comparatively higher values for the hardness (3262 g/4102 g) and less brittleness (30.46 mm/26.7 mm). The results of this study indicate that opportunities exist for further development of collagen film by making casings from chicken collagen as an alternate source of collagen. Collagen extracted from the processing of different by-products could be used as an alternate source of collagen for other industries as well.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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