Effects of pH modification in proteins from fish (Whitemouth croaker) and their application in food packaging films

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ARTICLE INFO

Article history:

Received 4 June 2017

Received in revised form

13 August 2017

Accepted 14 August 2017

Available online 18 August 2017

Keywords:

Proteins from fish (Whitemouth croaker)

Packaging films

Protein gelation

Secondary structure

ABSTRACT

The commercial use of biodegradable proteins films is still limited due to their poor mechanical and barrier performance. However, processing of proteins to modify their structure may have a significant impact on films properties. Thus, the effect of thermal or pH-induced denaturation on the gelation behavior of Whitemouth Croaker proteins was investigated in the development of films for packaging. Denaturation at pH 2 and pH 10 yielded the toughest protein gels. Gelation through pH modification is related to conformational changes in the structure of fish proteins, especially in secondary structure. Rheology tests demonstrated that a stronger gel was formed at pH 2, which might be due to the positively charged proteins. This behavior is related to the lower mesh size of the gel as demonstrated by small-angle X-ray scattering experiments. Gels prepared at both pH 2 and pH 10 had the ability to form cohesive films; however, no significant differences were observed between these treatments for water vapor permeability and mechanical properties. The modification of protein structure through gelation produced more soluble and less permeable films.

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1. Introduction

Hydrocolloids including polysaccharides and proteins are known for their propensity to form viscous dispersions and/or gels when dispersed in water. In food systems, they have the ability to modify the rheology, including the viscosity and the texture (Saha & Bhattacharya, 2010). The wide array of functional properties of hydrocolloids includes thickening, gelling, emulsifying and stabilization (Glicksman, 1982). Proteins in general have been frequently used because of their relative abundance, nutritional qualities and functional properties including their film-forming ability (Jiang, Xiong, Newman, & Rentfrow, 2012; Ramos et al., 2013). In particular, fish proteins have the ability to form cohesive and continuous matrices during the evaporative film-forming process due to the presence of myosin. It is the most abundant protein of myofibrils (60–70% of muscle protein), and it is responsible for their functional properties, such as gelation (Bourtoom, Chinnann, Jantawat, & Sanguandeekul, 2006; Xiong, 1997). However, the commercial use of materials from sustainable sources for food packaging is still

limited due to their poor mechanical and barrier performance that could compromise food quality and safety; therefore, efforts are required for the improvement of these materials.

Gelation is one of the most important processes for proteins in food formulations, materials development and biomedical applications. The use of protein gels for these purposes is attractive due to their inherent biocompatibility, biodegradability and renewable nature (Chen, Remondetto, & Subirade, 2006; Thawornchinsombut & Park, 2007; Wang et al., 2015). Different gelation methods have been documented in the literature, including heating (Comfort & Howell, 2002), chemical action (Katsuta, Hatakeyama, & Hiiraki, 1997) and enzymatic treatments (Eissa, Bisram, & Khan, 2004; Tarhan, Spotti, Schaffer, Corvalan, & Capanella, 2016). The process of thermal gelation includes initial unfolding upon heating and subsequent aggregation of protein molecules. The heating is usually carried out near the protein isoelectric point or at high ionic strength (Thawornchinsombut & Park, 2007; Wang et al., 2015). Depending on the processing method used to induce gelation, gels with different network structures may have different functional properties. These structures are induced by modulating protein conformation and interactions through the treatment conditions such as pH, temperature, protein and salt concentration (Yang,

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Wang, Vasanthan, & Chen, 2014).

The aggregation and gelation behavior of proteins from whey (Li & Zhong, 2016; Zhang, Arrighi, Campbell, Lonchamp, & Euston, 2016a; Zhang, Arrighi, Campbell, Lonchamp, Euston, & 2016b), soy (Chen, Zhao, Chassenieux, & Nicolai, 2016), quinoa (Ruiz, Xiao, Boekel, Minor, & Stieger, 2016), canola (Yang, Wang, Vasanthan, & Chen, 2014), oat (Yang, Wang, & Chen, 2017) and chicken (Zhao et al., 2016) have been widely studied. Whey and soy proteins were widely investigated to produce beverages, infant foods, tofu, shoyu, baked goods and other products (Maubois & Lorient, 2016; Singh, Kumar, Sabapathy, & Bawa, 2008; Zhou, Liu, & Tang, 2017) because of their high content of essential amino acids, good digestibility, and health benefits (Jose, Pouvreau, & Martin, 2016; Li & Zhong, 2016; Yang, Wang, Vasanthan, & Chen, 2014). These proteins have also been studied because of their capacity to entrap important molecules within gel networks (Chen et al., 2006). However, studies on gelation of proteins derived from fish are limited, despite the existence of an abundant waste product from fisheries worldwide (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013).

The modification of muscle proteins structure from Withe-mouth croacker (*Micropogonias furnieri*), a low cost fish species available in Atlantic Ocean from Northern Venezuela to the Gulf of St. Mathias (Argentina), has not yet been investigated. Herein, the effect of thermal and pH-induced denaturation on the gelation behavior of these proteins was investigated, and films were cast in order to establish the effects of protein modification on their properties.

2. Material and methods

2.1. Raw material

Whitemouth croaker (*Micropogonias furnieri*), obtained from fish processing industries in Rio Grande, Brazil, was utilized as the raw material. The fish was washed with chlorinated water, beheaded, eviscerated, and filleted. The muscle was crushed, placed in plastic containers and stored frozen at -18°C .

2.2. Preparation of fish protein isolate

A pH-shifting process adapted from Nolsøe and Undeland (2009) was used to separate muscle proteins. In this process, alkaline solubilization and isoelectric precipitation were performed. Samples were homogenized with distilled water (ratio 5:1, v/w). The protein separation was performed in a closed reactor, under stirring and controlled temperature. Alkaline solubilization was carried out at pH 12 for 20 min at 4°C . After solubilization, the substrate was centrifuged at $7500\times g$ for 15 min. During centrifugation, the samples were separated into three phases: the upper phase (neutral lipids) and the bottom phase (insoluble proteins) were discarded, and the middle phase (soluble proteins) was retained. The middle phase was titrated to pH 5.5 until precipitation near the isoelectric point and centrifuged at $7500\times g$ for 15 min. The precipitate was stored at -18°C and lyophilized afterwards. The separation process generated protein with 96% of purity according to the Association of Official Analytical Chemists (AOAC) method (2000).

2.3. Modification of fish proteins

The samples of fish proteins for all experiments were prepared at a concentration of 10% (w/v) protein in distilled water. Protein solutions (4 mL) were titrated to different pH using 1M NaOH or 1M HCl. All the experiments were performed at two temperatures (60 and 80°C) for 45 min. Initial tests were performed in order to

evaluate samples under the specified conditions. Proteins treated at different pH were able to form gels (data not shown). Modified proteins with the best characteristics (pH 2 and pH 10 at 80°C) were studied in more details, as described below.

2.4. Rheology measurements

Rheology was performed using a stress-controlled Anton-Paar MCR-301 rheometer outfitted with an environmental enclosure and Peltier heater. Measurements were performed in Direct Strain Oscillation mode (DSO) at 1% strain, within the linear regime. Samples were loaded into a 25 mm diameter parallel-plate geometry at a typical gap distance of 1 mm, sealed with mineral oil to prevent evaporation and allowed to equilibrate for 1 h. Frequency sweeps were acquired from 0.001 to 100 rad/s at 25°C . For temperature sweeps, the temperature ramps were performed at $1^{\circ}\text{C}/\text{min}$. Temperature ramps were performed from 5°C to 70°C , 5°C to 80°C , and 5°C to 90°C . At the end of each ramp, the sample was held at constant temperature for 5 min.

2.5. Raman spectroscopy

Room temperature Raman spectra were recorded in a LabRAM HR Evolution spectrometer equipped with a 532 nm laser (Laser Quantum Torus 532, power 50–750 mW). The Raman spectra of the samples were acquired in the range $600\text{--}3500\text{ cm}^{-1}$ (acquisition time: 60 s; accumulations: 60; RTD time: 40; grating: 600 gr/mm; ND filter: 1%; hole: 400). Nuclear magnetic resonance glass tubes were used as support samples for the Raman analysis.

2.6. Small-angle X-ray scattering experiments (SAXS)

SAXS measurements were performed at beamlines 12-ID-B and 12-ID-C at the Advanced Photon Source (APS) and Argonne National Lab (ANL), respectively. Samples were scooped into 1 mm thick anodized aluminum washers with a 5 mm bore, and sealed between two pieces of Kapton[®] tape. Sample acquisition time was 0.1 s at room temperature. 1D reductions were performed at the beamline, accounting for sample transmission. Data was then fit to a modified correlation length model, originally developed by Hammouda, Ho, and Kline (2004) through a nonlinear, least-squares fit. The scattering intensity is modeled by the following equation:

$$I(q) = \frac{A}{q^n} + \frac{C}{1 + (q\xi)^m} + B$$

where $I(q)$ is the scattering intensity, q is the scattering vector, and B is scattering from background. The Porod exponent, n , characterizes the fractal structure of the gel, and the Lorentzian exponent, m , characterizes the polymer–solvent interactions, describing the system's thermodynamics. The exponent, p , provides a minor correction that improves fitting at higher q values, especially for scattering curves of low temperature and concentration, where a plateau in the intensity is not present. The Lorentzian screening length, ξ , is the correlation length for polymer chains. In the case of gel networks, it gives an indication of the gel mesh size (Hammouda et al., 2004; Horkay & Hammouda, 2008).

2.7. Film preparation

After protein modification at pH 2 and pH 10, films of 5% protein were prepared. The pH of the mixture was adjusted to 11.0 in order to solubilize proteins. The sample was heated to 80°C and held for 1 h. After cooling, it was cast in Petri dishes and left to dry at 40°C .

Then, the films were placed in desiccators (50% relative humidity - RH) for 24 h, and the evaluations were performed. Unmodified protein was used to prepare control films.

2.8. Film evaluation

Film thickness, tensile strength, elongation, color, opacity, solubility and water vapor permeability were evaluated.

2.8.1. Thickness and mechanical properties

Film thickness (mm) was determined using a digital micrometer (IP65, Insize, Brazil) with precision of 0.001 mm.

Mechanical properties were determined using a Texture Analyzer (TA.XTplus, Stable Micro Systems, UK) according to ASTM Standard Method D882-02 (ASTM, 2002). The films were cut into 25 by 85 mm strips and the initial grip separation and cross-head speed were set at 50 mm and 1 mm/s, respectively. Tensile strength (MPa) was calculated by dividing the maximum force by the initial cross-sectional area of the film. Elongation (%) was calculated by dividing film elongation at break by the initial gauge length of the specimen.

2.8.2. Water vapor permeability and solubility

Water vapor permeability (WVP) was determined gravimetrically according to ASTM Standard Method E96-00 (ASTM, 2000) with modifications. The films were sealed on permeation cells containing anhydrous calcium chloride (0% RH). The cells were placed in desiccators with a saturated sodium chloride solution (75% RH), and they were weighed at 24 h intervals over a period of 7 days.

Solubility tests were performed similar to Gontard, Duchez, Cuq, and Guilbert (1994) based in the percentage of dry matter solubilized in water after 24 h.

2.8.3. Films color and opacity

Film color and opacity was measured using a CR-400 Minolta Chroma Meter (Minolta, CR-400, Japan), and the CIELab color space was used. The color of the films was expressed as total difference in color (ΔE^*) obtained using illuminant D65. The total difference in color (ΔE^*) was calculated as follows:

$$\Delta E^* = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$

where L^* , a^* and b^* are the values of the standard color parameters ($L^* = 97.39$, $a^* = 0.14$, $b^* = 1.94$) and L , a and b are the values of the sample color parameters.

2.8.4. Statistical analysis

One-way analysis of variance (ANOVA) was carried out. Differences between means were compared using Tukey's test. The significance level was set at 0.05.

3. Results and discussion

In the present study data obtained with modification of protein structure through pH change (pH 2 and pH 10) are shown.

3.1. Effect of pH modification in rheological properties of the gels

The effect of pH modification of proteins on the rheological properties of the resulting gels was investigated. These properties are directly related to the interactions between protein molecules. Such interactions are usually through hydrogen bonds, hydrophobic interactions, electrostatic interactions and disulfide linkages.

Rheological properties strongly affect the intended use of the gel and the materials of interest for further development using gels (Wang et al., 2015). Frequency sweeps (Fig. 1) demonstrate that the storage modulus (G') for pH 2 and pH 10 was higher than loss modulus (G''), indicating that elastic modulus predominated in the systems.

Both protein gels behaved as elastic solids, and the larger difference between G' and G'' for the gel prepared at pH 2 indicates its more elastic behavior compared to the gel prepared at pH 10. In the present study, G'' did not become larger than G' over the frequency range studied. This suggests that the material does not flow; therefore, the microstructure of the materials does not collapse and the mechanical energy given to the material is retained. The gel at pH 10 showed stronger frequency dependence, especially at lower frequencies, for G' and G'' . According to Nunes, Raymundo, and Sousa (2006), such behavior characterizes weaker gel shear strength, manifest in the pH 10 gel in the present study.

There are a large variety of applications for protein gels, including thickening and gelling aqueous dispersions, stabilizing foams, emulsions and dispersions, suspended particulate materials, inhibitors of syneresis, and agents to increase water retention (Sutherland, 2007). In general, a rigid structure of the gel is required for these applications; therefore, the gel formed at pH 2 in the present study showed more promising properties for food formulations applications and for materials development.

The proteins treated at pH 2 maintained their mechanical properties even after repeated heating cycles. Despite the influence of temperature on the gels modulus, the superposition of temperature sweeps suggests that stiffness of the gels is fully reversible on cooling. G' values were higher than G'' for the entire temperature range (Fig. 2), which is likely due the ionization and subsequent partial unfolding of fish protein induced by the acid/alkaline environment that led to the exposure of buried active sites, promoting protein-protein interactions prior to thermal denaturation (Léger & Arntfield, 1993). However, the sample behavior is dependent on the temperature, as shown by decreasing G' values when the temperature is increased.

Two different regions are clearly distinguished in terms of the evolution of G' in Fig. 2. Minimum values are observed at the end of the heating step (70, 80 and 90 °C) due to a balance between the increase in mobility. Then, during the cooling cycle, the increase in G' reflects the strengthening of the network. This is attributed to the formation of enhanced interactions between protein molecules, primarily hydrogen bonds (Molina, Defaye, & Ledward, 2002). The highest G' values were observed in the cycle 5–80 °C, indicating 80 °C as the best gelation temperature. Increasing temperature in the third cycle (to 90 °C) resulted in a decrease in G' due to breaking of the network as a result of protein denaturation through conformational and hydrophobic changes, as stated by Weegels, de Groot, Verhoek, and Hamer (1994). A similar trend was observed by Chen et al. (2016) for the thermal aggregation and gelation of soy proteins. The G' values at the end of the cooling step were comparable to amaranth protein (up to 3800 Pa) (Shevkani, Singh, Rana, & Kaur, 2014), pea protein (2000 Pa) (O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004) and quinoa protein (3300 and 5000 Pa) (Ruiz et al., 2016) previously reported for similar heating profiles.

The gel prepared at pH 2 was able to return to its initial loss and storage modulus after heating, indicating its suitability for application in products subjected to heat treatment such as processed meat and bakery products, as well as products stored at refrigerated temperatures, including dairy products. This behavior indicates that the modified fish protein is a promising food constituent to act as an emulsifying, texturizing or thickening agent. Moreover, the

Fig. 1. Frequency sweeps of 10% fish protein gel at pH 2 and pH 10 at 1% strain.

conformational changes in protein structure due to denaturation led to gel formation with different rheological properties, which are interesting for materials development. Commercial applications of sustainable materials are still limited due to their low mechanical and barrier performance, and structurally modified proteins may improve the properties of these materials.

3.2. Structural modification of fish protein

The Raman spectra of the original protein and gels at pH 2 and 10 (Fig. 3) present a broad band in the amide I region, between 1700 and 1600 cm^{-1} , which is due to the contribution of the C=O stretching of the peptide carbonyl groups, coupled with the in-plane N-H bending and C-N stretching vibrations. The so-called amide I band corresponds to the sum of coupled modes of the polypeptide backbone.

Deconvolution of this band gives quantitative information on the secondary structure (Fig. 4a, b and 4c). The untreated fish protein has 40% α -helix (1648–1652 cm^{-1}), 35% β -turns (1673–1682 cm^{-1}) and 25% low frequency β -sheets (1626–1640 cm^{-1}). Different secondary structure is observed for the gels at pH = 2 and pH = 10; at both pHs samples have mainly β -sheets (71 and 69% for pH = 2 and pH = 10, respectively) and a lower amount of α -helix

(29 and 23%) than in the native protein. The gel at pH = 10 also exhibits 8% β -turns. Therefore, the two different treatments performed on the fish protein changed the secondary structure resulting in decreased α -helix content and an increase in the low frequency β -sheets.

3.3. Microstructure of the gels

Scattering results showed no qualitative change for the modifications at pH 2 and pH 10, showing predominantly amorphous structure (Fig. 5). The data was fit with the correlation length model, originally developed by Hammouda et al. (2004). At pH 2, the effect of intermolecular correlations became more pronounced, as indicated by the appearance of a broad peak and considerable increase in the low q scattering intensity. The same behavior was observed by Ruiz et al. (2016) in the study of quinoa protein isolate gels. According to these authors, lower pH could have formed a more agglomerated network, and this trend seems to be responsible for the higher G' values. Moreover, moving the pH away from pI makes the protein more positively or negatively charged, which impacts conformational changes in the structure due to protein-protein and protein-water interactions.

Fig. 2. Temperature sweeps of 10% fish protein gel at pH 2 at 1% strain and 1 rad/s.

Fig. 3. Raman spectra of 10% fish protein gels prepared at pH 2 and pH 10.

Fig. 4. Raman spectra, amide I peak (1600–1700 cm^{-1}) deconvolution of a) fish protein and gels prepared at b) pH 2 and c) pH 10.

According to Hammouda (2010), a Porod exponent between 2 and 3, as observed in the present study (Table 1), is characteristic of mass fractal, such as branched systems (gels) or network structures. The denaturation of fish protein structure without splitting of disulfide bonds at pH 2 impacted the aggregation of proteins, which was confirmed by the fractal structures demonstrated by the Porod number. However, the denatured structures with splitting of disulfide bonds at pH 10 allowed them to develop a gel with a larger mesh size (ξ) (4.30 nm). Besides that, below the pI (pH 5.5 for fish protein), the protein is positively charged, and the protein-protein interactions could be predominant in relation to protein-water

Table 1

Modified Correlation Length Model fits for Porod Exponent, Correlation Length and Lorentzian exponent.

Sample	Porod	ξ (nm)	M
pH 2	2.20 ± 0.05	2.48 ± 0.08	4.35 ± 0.30
pH 10	2.41 ± 0.08	4.30 ± 0.17	3.22 ± 0.13

interactions. This might be a reason for the lower mesh size for the gel prepared at pH 2.

According to Stading, Langton, and Hermansson (1992), at pH away from the isoelectric point and high ionic strength, fine stranded gels are formed. Thus, the gel prepared at pH 10 had lower ionic strength and weaker gel network was formed probably due to the larger mesh sizes. The smaller gel mesh size of the pH 2 sample is in agreement with stronger gel networks observed by rheology.

The M value characterizes the polymer-solvent interactions, describing the system's thermodynamics (Hammouda et al., 2004; Horkay & Hammouda, 2008). According to Abdelhedi et al. (2016), higher pH gels are essentially maintained by hydrophobic and hydrogen bonds, while at acidic pH, the disulfide binding and noncovalent interactions play a crucial role in gel structure formation. Thus, a large number of hydrogen bonds could suggest better interaction with water (solvent) and might be related to the

Fig. 5. SAXS plots of 10% fish protein gels at pH 2 and pH 10.

Table 2
Properties of films prepared with modified fish proteins and without modification.

Properties	Film		
	Without Modification	pH 2	pH 10
Thickness (mm)	0.115 ^a ± 0.006	0.114 ^a ± 0.001	0.107 ^a ± 0.003
Solubility in Water (%)	38.8 ± 0.4	Totally soluble	Totally soluble
Water Vapor Permeability (g.mm/dia.m ² .kPa)	12.55 ^a ± 0.48	8.64 ^b ± 0.37	7.56 ^b ± 0.36
Tensile Strength (MPa)	5.55 ^a ± 0.24	4.19 ^b ± 0.09	4.15 ^b ± 0.17
Elongation (%)	60.1 ^a ± 1.8	28.4 ^b ± 1.4	29.4 ^b ± 0.8
Total difference in color (ΔE*)	10.56 ^c ± 0.31	12.65 ^a ± 0.12	11.62 ^b ± 0.16
Opacity (%)	10.2 ^a ± 0.2	11.1 ^b ± 0.1	10.2 ^a ± 0.1

Mean values of triplicate determinations ± standard error. Means in same line with different superscript are significantly different ($p < 0.05$).

lower M value for the fish protein modified with pH 10. At alkaline conditions, the inter- and intra-chain disulfide bonds which stabilize the secondary and tertiary structures could split, strongly affecting the properties of the gels. In contrast, at heated, acidic or neutral conditions they remain stable (Schwenke, Drescher, Zirwer, & Raab, 1988; Yang, Wang, Vasanthan, & Chen, 2014).

3.4. Fish protein modification for packaging films development

The aggregation and gelation process after heat and pH-induced gelation showed significant impact on the properties of films to be used as plastic packaging materials (Table 2). Most of the properties were not influenced by the different pH modifications. The films produced with modified proteins were completely soluble in water. For foods which have water addition for consumption, the complete solubilization of the film is interesting. For edible sachets for salts and sugars and products that are heated prior to consumption, high solubility is also advantageous (Bourtoom & Chinnan, 2008). Thus, the modifications showed good trends in the development of materials for these applications. It is worth noting that these soluble materials have the ability to protect packaged products from environmental effects, even if they had lower mechanical properties compared to the non-modified proteins. According to Hewage and Vithanarachchi (2009), extreme pH and thermal treatments induce protein solubilization due to the unfolding of the protein chains, supporting film formation after water evaporation. The solubility increases due to uncoiling of the protein, which resulted from changes to more negative charge of the groups in the alkaline system and more positive charge in the acidic system. Therefore, the coils repelled each other moving the pH further away from the pI of fish proteins (Hewage & Vithanarachchi, 2009). As a consequence, it bonded with water, and solubility of film protein is increased.

The WVP was significantly lower ($p < 0.05$) for the films prepared using modified proteins. Partial unfolding of the protein resulted in increased availability of reactive hydrophobic side groups of amino acid residues in polypeptides (Jiang, Chen, & Xiong, 2009). As a consequence, the WVP decreased in comparison to the non-modified protein film. In the present study, it was observed that water solubility was not related to WVP. According to Hanani, O'Mahony, Roos, Oliveira, and Kerry (2014), who found similar trend for gelatin films, WVP is related to physical properties i.e., the microstructure and the presence of any pores, cracks or fissures, whereas water solubility is related to the molecular properties of the macromolecules of the film.

In terms of mechanical properties, both tensile strength and elongation were superior for the film without protein modification. Mechanical properties of the films are important in the context of material handling, and both control and pH modified films were easily handled. Despite the decrease in mechanical

properties, the pH modification still produced films with sufficient performance for food packaging materials development. Results of the present study were superior to soy protein films (Jiang et al., 2012) prepared with acid and alkaline pH, mainly in comparison to elongation values which were lower than 1%. The elongation results of modified protein films were comparable to methylcellulose-based packaging films (around 20%) (Tharanathan, 2003). Lower mechanical properties might be due to the acid and alkaline treatments, which form protein aggregates from dissociated protein subunits, as also reported by Jiang, Chen, & Xiong (2010).

The aggregation and gelation of proteins influenced the color and opacity due to the modified structure of the proteins. In general, high transparency and low ΔE^* difference are requisites in films for food applications. However, when transparency must be limited in products with high fat content, darker and opaque films are indicated.

4. Conclusion

The modification of fish proteins through pH change was promising as a method to form stiff gels. Rheological properties showed that gelation induced by lowering the pH to 2 formed a stronger gel, as observed by the higher storage modulus. Temperature sweeps demonstrated the ability of the gel to keep its structure after multiple heating-cooling cycles. The gelation process through pH modification may be related to conformational changes in the structure of the protein, especially in secondary structures which increased with protein denaturation. The gel formed at pH 2 had a lower mesh size than gels formed at pH 10, consistent with its more elastic behavior that is due to the positively charged protein below the pI affecting protein-protein and protein-water interactions.

Cohesive films were formed using modified proteins structures through pH adjustments and small differences were observed between pH 2 and pH 10. Despite the inferior mechanical properties, the modifications of proteins structure through gelation produced more soluble and less permeable films, which are advantageous characteristics for some food packaging applications. These films may be used to design a new generation of sustainable sourced materials; however, further studies are required to assess their ability to keep food quality and safety, including thermal stability and the influence of humidity on mechanical and barrier properties. Besides the development of films for food packaging materials, the modification of proteins to form gels might be used for food formulations improvement.

Acknowledgments

The authors acknowledge the financial support by the Coordination for the Improvement of Higher Education Personnel and

National Counsel of Technological and Scientific Development of Brazil.

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