Effect of Heat Curing of Film-Forming Solution on the Properties of Soy Protein Films

Abstract

This study aimed to investigate the impact of heat treatment on the properties of soy protein isolate film-forming solutions. The solutions, consisting of soy protein isolate, glycerol, and phosphate buffer solution (pH 7.4), were subjected to heat treatment at 65, 75, and 85°C for 2, 4, or 6 h. The resulting films were then analyzed for tensile strength, elongation at break, film solubility, color parameters, and transparency. The results indicated that increasing the heat-curing temperature increased tensile strength, film transparency, and yellowish color in the films, as well as decreased water solubility and elongation at break. Notably, the film cured at 85°C for 2 h exhibited the highest tensile strength, film solubility, and elongation at break compared to both the control and other heat-cured films. However, the film heated at 75°C showed a better elongation at break.Overall, the findings suggest that heat curing of film-forming solutions at temperatures between 75 and 85°C has the potential to improve the properties of soy protein isolate films.

Keywords: Biodegradable film, Film-forming solution, Heat Curing, Soy protein film, Tensile strength,

1. INTRODUCTION

The primary purpose of packaging is to preserve and prolong the shelf life of products while ensuring their quality. However, the extensive global production of petroleum-based plastics, which exceeds 280 million tonnes annually with an average growth rate of approximately 5%, poses significant environmental challenges (Schmid et al., 2015). Most of these plastic materials are non-recyclable and non-biodegradable, leading to waste accumulation and environmental issues. The packaging and food industries are collaborating to explore sustainable alternatives to petroleum-based plastics, aiming to develop environmentally friendly options.Research towards developing environmentally safe and biodegradable materials based on natural biopolymers is growing in an effort to replace petroleum-based plastics and films. Polysaccharides, proteins, lipids, and/or a combination of these substances

are all included in these biopolymers (Swain et al., 2004). Until now, many studies have been conducted on proteins used in films and coatings, such as whey protein (Diaz et al., 2016), soy proteins (Shakil&Thanachan, 2022a), sesame protein (Fathi et al.,2018), peanut proteins (Liu et al., 2004), gelatin (Cao et al., 2006), wheat gluten (Micard et al., 2000), corn zein and egg albumin (Rhim et al., 1999).

Soy protein has received the most attention among proteins used for film preparation due to its exceptional film-forming capabilities.Nevertheless, for practical applications, especially in high humidity circumstances, soy protein film has inferior mechanical strength and moisture barrier properties (Ou& Kwok, 2004). Several studies have focused on enhancing the mechanical and barrier qualities of soy protein films via physical, chemical, or enzymatic treatment. These studies included UV irradiation(Shakil, 2022b), Phenolic Acid Addition (Insaward et al., 2015), heat curing (Gennadios et al.,1996), and enzymatic cross-linking (Stuchell&Krochta, 1994). Heat curing, one of these modification techniques for soy protein film, has been promoted as an effective way to enhance protein film characteristics (Insaward et al., 2014; Gennadios et al.,1996; Kim et al., 2002). Proteins unfold during heat curing to reveal their hydrophobic interior, particularly the sulfhydryl group. The formation of inter- and intra-molecular disulfide connections is caused by the further induction of the thiol-disulfide exchange reaction by heating (Chiralt et al., 2018). Most previous studies applied heat curing on cast (preformed film) soy protein films. From the literature search, few studies found the application of heat curing on the film-forming solution of soy protein isolate films.

Therefore, this study aimed to evaluate the impact of different heat curing temperatures and times on the properties of soy protein isolate films, including their tensile strength, elongation at break, water solubility, color, and surface hydrophobicity.

2. MATERIALS AND METHOD

2.1 Materials

Soy protein isolate (90% protein, wet basis) and glycerol, Sodium Phosphate Dibasic Heptahydrate,and Sodium Phosphate Monobasic Monohydrate were purchased from a scientific chemical store in Dhaka, Bangladesh.

2.2 Film formation procedure

With some adjustments, the technique reported by Shakil and Mahawanich, 2022awas used to produce soy protein isolate (SPI) films. The control film (without heat curing) was obtained

by dissolving 5 g of soy protein isolate and 2.75 g of glycerol in 92.25 g of phosphate buffer at pH 7.4 (prepared from Sodium Phosphate Dibasic and odium Phosphate Monobasic Monohydrate) and homogenizing the mixture at 22,000 rpm for 2 min. The solution was heated at 70°C for 30 min for partial protein denaturation and homogenized at the same conditions. The air bubble from the solution was removed using cheesecloth and cast on level Teflon®- coated glass plates. The film-forming solution was at 40°C for 24 h, and the film sample was stored at 50% RH for 48 h before analyses.

2.3 Heat curing of SPI film

Film-forming solution of SPI was subjected to heat curing at 65, 75, and 85 °C for 2, 4, or 6 h. After that, the solution was cooled and homogenized at 22,000 rpm for 2 min. Other film preparation steps were the same as the control film.

2.4 Thickness of film

A hand-held micrometer (B.C. Ames Co., Waltham, MA, USA) was used to measure the film thickness to the closest 0.15-0.17 mm. Five thickness measurements were taken along the length of each specimen to determine the film's tensile strength, and the average value was utilized in the calculation.

2.5 Tensile strength and elongation at break

Tensile strength (TS) and elongation at break (EAB) were measured using an Instron Universal Testing Machine (Model 5566, Instron Corp., Canton, MA, USA) in accordance with ASTM Standard Method D 882-91. (ASTM, 1995b). The initial gap separation and cross-head speed were both set to 50 mm and 1 mm/s. The peak load (N) was divided by the initial cross-sectional area (m^2) of the specimen to calculate TS in MPa. EAB was measured as a percentage by dividing the end length of the point of sample rupture by the initial length of the specimen (50 mm). The TS and EAB tests were repeated three times for each type of film.

2.6 Color

To determine the color values of films, a portable colorimeter (CR-300 Minolta Chroma Meter; Minolta Camera Co., Osaka, Japan) was used. The HunterLab color scale was employed to measure color in the CIELAB system with a 10° observer and D65 illuminant. The film specimens were positioned on a white plate for measurement. Color measurements

were taken at five randomly selected positions on each film sample and then averaged to obtain the color values for each replicate.

2.7 Transparency

The transparency of a film sample was evaluated by determining its %transmittance based on the ASTM D1746 method (ASTM, 2015). To do this, a film sample was cut to precise dimensions (10 mm × 40 mm) and fixed to the interior of a glass cuvette. A visible spectrophotometer (model GENESYS20, Thermo Scientific, Waltham, MA, USA) was used to measure %transmittance at 500 nm

2.8 Film solubility

The method outlined by Insaward et al. (2015) was employed to determine the water solubility of the film samples. The total soluble matter was calculated using the equation:

% Total soluble matter = (initial dry weight - final dry weight)/initial dry weight × 100.

2.9 Statistical Analysis

The experiments were carried out in three replicates, utilizing a completely randomized design. The data were analyzed using Analysis of Variance, and to determine the difference between sample means at p=0.05, Duncan's new multiple range tests were employed. These tests were conducted using SPSS Statistics 27.0 (IBM, Armonk, NY, USA).

3. RESULT AND DISCUSSION

3.1 Mechanical properties

The tensile strength (TS) of Unheated (control) SPI films was 2.10 ± 0.15 MPa.Heat curing temperature significantly affected the TS of SPI film, as shown in Figure 1. TS increased with increasing heating curing temperature. TS of films heated at 65°C and 75°C showed an increasing trend with increasing heat curing time, whereas film heated at 85°C exhibited a decreasing trend.The decreased TS with increasing heat curing time of film-forming solution may be due to more protein denaturation.Across all heating time intervals, films subjected to heat treatment at 85°C exhibited higher TS than those treated at 65°C and 75°C.The films treated at 85°C for 2h demonstrated a significantly greater tensile strength thanthose treated and untreated control ($p \leq 0.05$).The increase in tensile strength may be attributed to promoting inter- and intra-molecular disulfide cross-links between protein chains through heat curing. These covalent cross-links facilitate the formation of stronger bonds, leading to the observed

enhancement in tensile strength (Liu et al., 2004). A similar effect of heat curing on the film-forming solution has also been reported by Tanjina et al., 2023.

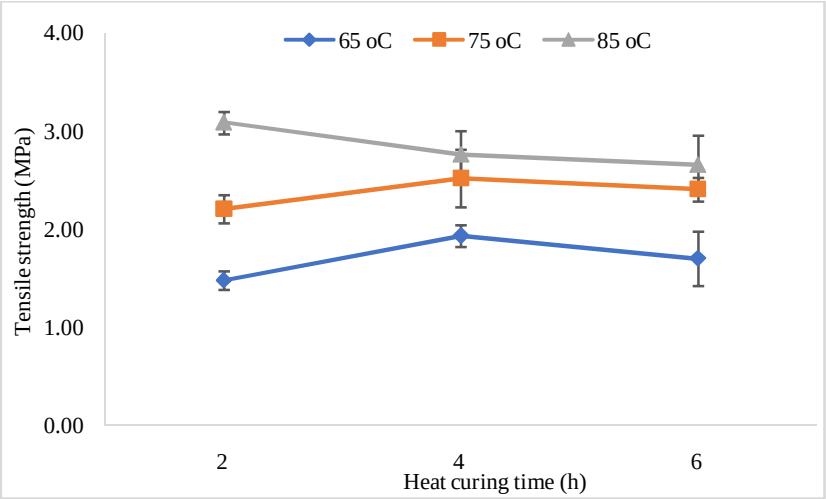

Figure 1: Tensile strength of SPI film heated at 65, 75 and 85 °C for 2, 4 and 6 h.

The elongation at break (EB) is also significantly affected by heat curing temperature and time, as depicted in Figure 2. The EB values fluctuated within the heat curing time with a decreasing trend corresponding with the heat curing time. Heating the film-forming solution at 85℃ significantly reduced the %EB of the film with increasing heat curing time. The highest %EB was obtained from the film-forming solution heated at 75℃ for 4 h. The current finding is consistent with the study conducted by Weng et al. in 2007, which examined the impact of heat curing on Surimi Film, an edible film-forming solution. It is believed that exposing the film-forming solution to high temperatures for extended periods can lead to protein denaturation, thereby affecting the physical properties, protein-protein interaction, and polymer morphology of the protein film.

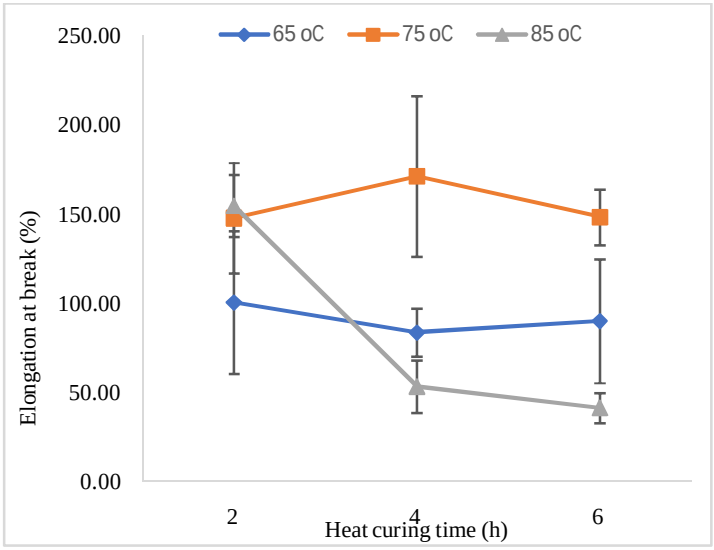

Figure 2: Elongation at break (%) of SPI film heated at 65, 75 and 85 ℃ for 2, 4 and 6 h.

3.2 Transparency and color value

The transparency and CIELAB color value (+b) of the films are summarized in Table1.Heating temperature and time significantly affected the transparency of SPI film. All heat-treated films were more transparent than the control (43%) except the film heated at 65°C for 2 h. Transparency of all heat-treated films increased with increasing heating curing temperature and time except for the film heated at 75°C for 4 h. The highest (71.14%) and lowest (38.67%) transparency was demonstrated by the films heated at 85°C for 6h and 65°C for 2h. The increase of transparency upon increasing the heat-curing temperature of the film-forming solution may be due to the formation of a more transparent film-forming dispersion.

The CIELAB color value +b* indicates the yellowness of the film, as presented in Table 1. Heat curing of the film-forming solution at 65 and 75°C did not show any effect on the +b* value of the film. However, the film-forming solution heated at 85°C significantly affected the +b* value with increasing heat curing time, producinga more yellowish film. Heat curing at 85°C for 6 h demonstrated more yellowish color of the film. According to the findings, it appears that subjecting the solution to heat treatment may promote the development of a yellow pigment, particularly through the Maillard reaction. Manzocco et al. (2000) observed that any alterations in color resulting from the Maillard reaction are invariably linked to heat-induced processes.

Table 1: Transparency and color values (+b) of heat-cured SPI film.

	Temperature (°C)	Heat curing time (h)			
		0	2	4	6
Transparency	65	43.00±1.75^{f}	38.67±0.59^{h}	56.67±1.32^{d}	53.08±0.75^{e}
	75	43.00±1.75 ^{f}	57.90±0.29 ^{cd}	52.07±0.98^{e}	59.40±0.65^{c}
	85	43.00±1.75 ^{f}	66.64±0.37^{b}	56.43±2.96^{cd}	71.14±0.18^{a}
+b* value (yellowness)	65	8.34±1.63^{c}	9.22±0.95 ^{bc}	9.49±2.05^{bc}	10.35±2.05^{bc}
	75	8.34±1.63 ^{c}	8.31±1.58^{c}	8.92±2.15^{c}	8.83±2.90^{c}
	85	8.34±1.63 ^{c}	10.95±2.71^{b}	15.19±2.85^{ab}	19.80±2.99^{a}

3.3 Film Solubility

The solubility of a film can serve as an indicator of the film's integrity and resistance to water.The solubility of heat-treated soy protein film is presented in Table 2.Changes in heat curing time and temperature affected the films' solubility. The solubility of the control sample was 49.42%. Heat curing of film-forming solution at 75 and 85°C showed a lower water solubility of film compared to the control. The lowest solubility (29.69%) was found for the film heated at 85°C for 6 h.The reduction in water solubility of the heat-cured films can be attributed to the formation of covalent cross-links, which creates a higher molecular weight network. This increase in molecular weight contributes to decreased water solubility, resulting in the observed reduction (Gan et al., 2021). The higher water solubility of the film indicates a weaker structure.A similar decreasing trend of solubility with increasing heat curing time has also been reported for edible surimi film may be higher denature of protein which results in less crosslink formation between proteins (Weng et al. in 2007).

Table 2: Film solubility of heat-treated SPI film.

	Temperature (°C)	Heat curing time (h)			
		0	2	4	6
Film solubility (%)	65	49.42±3.70 ^{b}	58.54±2.30^{a}	48.65±2.17^{b}	52.66±2.85^{b}
	75	49.42±3.70 ^{b}	43.75±2.34^{c}	40.14±2.29^{cd}	37.81±2.27^{d}
	85	49.42±3.70 ^{b}	34.04±1.25^{de}	32.78±2.09^{e}	29.69±2.28^{e}

4. CONCLUSION

This study examined the impact of heat treatment on the properties of soy protein isolate film, with film-forming solutions being heated at 65, 75, and 85°C for 2, 4, or 6 hours. Results showed that increasing the heat-curing temperature led to higher tensile strength, film transparency, and yellowish color (positive b*) of the films but lower water solubility and elongation at break. Among the tested conditions, the film cured at 85°C for 2 hours

demonstrated the highest tensile strength, film solubility, and elongation at break, surpassing the control and other heat-cured films. Based on these findings, heat curing of film-forming solutions within the 75-85°C range can potentially enhance the properties of soy protein isolate films.

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