一般論文

Property Improvement of Fish Water Soluble Protein Films by Dialdehyde Starch (DAS) and / or Sodium Dodecyl Sulfate (SDS) Treatments

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Transparent biodegradable films were successfully prepared from fish water soluble proteins (FWSP) in our laboratory, but FWSP films generally have the inferior mechanical and water vapor barrier properties. The objective of this study was to improve physical properties of FWSP using treatments with dialdehyde starch (DAS) and/or sodium dodecyl sulfate (SDS). Film forming solution containing 3% FWSP from blue marlin (*Makaira mazara*) flesh was mixed with DAS (2.5, 5. 7.5 and 10% of FWSP) or SDS (10, 20, 30 and 40% of FWSP) or the combination of DAS with SDS (2.5/40, 5/40, 7.5/40 and 10/40% of FWSP) prior to pH adjustment to 10.5. Glycerol at a level of 50% of FWSP was used as a plasticizer. Film forming solution was cast and dried at 25 C, 50% relative humidity for 24 h. Increasing DAS concentration enhanced tensile strength (TS) of FWSP films, while increasing SDS concentration improved elongation at break (EAB). Optimum ratios of DAS/SDS were 5/40 or 7.5/40%, which increased EAB by 82 or 56 % with decreasing water vapor permeability (WVP) by 41 or 26%, respectively. Thus, biodegradable FWSP films with various strength and flexibility were successfully prepared by combining DAS with SDS.

Keywords : Biodegradable film, Fish water soluble proteins, Sodium dodecyl sulfate, Dialdehyde starch

1. Introduction

The interest of biopolymer films has increased during the last decade, because they are environmentally friendly alternatives to synthetic, non-biodegradable films. Although it is not feasible to entirely replace synthetic packaging films, biopolymer films have the potential to reduce and replace synthetic films in some applications. Basically, biodegradable/edible films are prepared with polysaccharides, proteins, and lipids. Among these materials, proteins have been extensively utilized for the development of biodegradable/edible films because of their relative abundance, film-forming ability, and nutritional qualities.

Effluents from seafood processing plants have become a crucial issue due to the presence of a large amount of organic matters. Furthermore, the cost of waste water treatment represents a major expenditure for processors. Recovery of water soluble proteins from effluents and their

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reutilization as the novel products should be a promising means to reduce the cost of waste water treatment and expand potential for the utilization of seafood by-products. In our laboratory, fish water soluble proteins (FWSP) were used for the preparation of biodegradable films[1]. However, FWSP films generally have the inferior mechanical and water vapor barrier properties to synthetic films.

Various physical, chemical and enzymatic treatments have been used to modify properties of protein films. Such treatments mainly promote cross-linking within protein film network. To enhance tensile strength of films aldehydes such as formaldehyde, glutaraldehyde, and glyoxal were added into film-forming solutions. Aldehydes can promote inter- and intramolecular cross-linking in proteins[2]. In particular, the ε-amino group of lysine is considered the primary reactive site between protein and aldehyde. Cross-linking with formaldehyde, glutaraldehydes. and glyoxal has been reported for protein films from collagen, gelatin, corn zein, and soy protein isolate[3]. However, the inherent toxicity of the aldehydes restricts their use in edible/biodegradable films.

Dialdehyde starch (DAS) is a polymeric aldehyde obtained by reacting native starch with periodic acid[4]. The cross-linking effect of DAS on various proteins such as soy protein isolate[3], collagen[5], casein[6,7], wheat gluten[8], and corn zein[9] has been documented. In contrast to low molecular weight aldehydes, DAS has low toxicity to rats by oral, dermal, and respiratory routes of introduction[10]. On the other hand, ionic surfactants such as sodium dodecyl sulfate (SDS) are powerful denaturing and dissociating agents for proteins[11,12]. Strength reduction and even re-solubilization of protein gels in SDS buffers have been reported[13-15]. Therefore, an incorporation of DAS and/or SDS into protein based film forming solutions would affect the structure and properties of protein films.

The objective of this study was to improve selected various properties (tensile strength, elongation at break, water vapor permeability, film solubility, color, light transmission, transparency, and protease digestibility) of FWSP films using treatments with DAS or SDS in separate and a combination of DAS with SDS in different ratios.

2. Materials and Methods

2.1 Preparation of film forming solution

FWSP were extracted from the flesh of blue marlin *Makaira mazara* according to Iwata *et al*[1] Freeze-dried powders were dissolved in distilled water at the protein content of 3% and pH was adjusted to 10.5. After glycerol was added as a plasticizer at 50% (w/w) of FWSP, the film forming solutions were heated at 70°C for 15 min and air bubbles were removed by a Hybrid

Mixer (HM-500, Kyence, Tokyo). In addition to the control films prepared in this manner, other films were prepared by adding DAS at 2.5, 5, 7.5, or 10%, SDS at 10, 20, 30, or40% and DAS/SDS at 2.5/40, 5/40, 7.5/40, or 10/40% (w/w) of FWSP prior to pH adjustment. All reagents used in this study were purchased from Wako Pure Chemical Ind., Tokyo.

2.2 Preparation of FWSP films

The prepared film forming solutions were cast by pipetting 4 ml onto a rimmed silicone plate (50×50 mm) placed on a level surface and dried in a ventilated oven (Environmental Chamber Model HllOK-30DM, Seiwa Riko Co., Tokyo) at 25 C and 50% relative humidity (RH). After the water was evaporated, resulting films were manually peeled off. All film samples were conditioned for 3 days in the Environmental Chamber maintained at 25°C and 50% RH. Synthetic polymer films for household wrap such as low density polyethylene (LDPE,), oriented polypropylene (OPP), polyethylene terephthalate (PET), and polyvinylidene chloride were purchase from Tokyu Hands Department Store (Tokyo) in June, 2002 and their mechanical properties, water vapor permeability, and light transmission were also determined for comparison.

2.3 Film thickness

Film thickness was measured using a micrometer (Dial Pipe Gauge, Peacock Co., Tokyo) to nearest 0.005 mm at 8 random locations around the film. Precision of thickness measurement was ±5%.

2.4 Mechanical property

Tensile strength (TS) and percentage elongation at break (EAB) were determined using a Texture Analyzer (TA.XT2 Stable Micro System, UK) operated according to the ASTM standard method D 882-22 (ASTM, 1989). Two rectangular strips (width 20 mm; length 45 mm) were cut from each FWSP film to measure the mechanical properties. Initial grip separation and mechanical crosshead speed were set at 30 mm and 0.5mm・s^{-1}, respectively. TS (MPa) was calculated by dividing the maximum load (N) necessary to pull the sample film apart by the cross-sectional area (m^2). Average thickness of the film strip was used to estimate the cross-sectional area of the sample. Percentage EAB was calculated by dividing film elongation at the moment of rupture by the initial grip length of sample and multiplied by 100%. A total of 10 samples were tested for each film type.

2.5 Water vapor permeability (WVP)

WVP was measured using a modified ASTM method reported by Gontard *et al*[16]. FWSP film was sealed on a glass permeating cup containing silica gel (0%RH) with silicone vacuum grease and a plastic band to hold the film in place. The cups were placed at 30°C in a desiccator with distilled water (100% RH). The cups were weighed at 1 h intervals over 12 h periods and WVP of the films was calculated as follows : $WVP=w \cdot x \cdot A^{-1}\text{-}t^{-1} \cdot (P_2\text{-}P_1)^{-1}$, where w is the weight gain (g), x is film thickness (m), A is the area of exposed film(m2), t is time of gain(s), and (P?-Pi) is the vapor pressure differential across the film (Pa). This entire procedure was repeated twice, for a total of 10 tests on each film type.

2.6 Film solubility in water

Film solubility in water which was defined as the percentage of dry weight solubilized after 24 h immersion in water was determined according to Gontard *et al*[16]. The percentage of initial dry matter of each film was determined by drying at 105°C for 24 h. Film samples weighing 0.13 to 0.14 g were immersed in 10 ml of distilled water containing 0.02 % sodium azide. After immersion for 24 h at 30°C with continuous gentle agitation, undissolved films were removed and dried at 105°C for 24 h to determine the loss of dry matter. Total protein content was determined using a Bio-Rad DC Protein Assay (Lowry method, Bio-Rad Lab., USA).

2.7 Protease digestibility

Ground film (50 mg) was suspended in 50 ml of enzyme solution (α-chymotrypsin 40 mg/rnl in 40 mM Tris-HCl buffer, pH 7.6). The protease-substrate suspension was incubated at 37°C for 120 min[17]. Hydrolysis was terminated on a 5 ml aliquot by heating in boiling water for 3 min. After standing at room temperature for 30 min, the precipitate was removed by centrifugation (2,000×g for 15 min). The amount of protein in the supernatant was determined by a Bio-Rad *DC* Protein Assay method.

2.8 Color measurement

Color values of FWSP films were measured using CIE L*, a*, b* color system. Following spectral scanning, the values of L* (lightness), a* (redness), b* (yellowness) of all films were determined by a Spectrum Color Sensor CLR-7100F (Shimadzu Co., Kyoto). Color measurements of each type of film were replicated ten times.

2.9 Light transmission and transparency

The ultraviolet and visible light barrier properties of the films were measured at selected

wavelengths from 200 to 800 nm using a UV-Visible Recording Spectrophotometer UV-160 (Shimadzu Co., Kyoto). The transparency of film was measured by a modified method of ASTM method D 1746-92 (ASTM, 1987)[18]. The transparency of the films was calculated as follows : transparencyA600/x or (-log T_{600})/x, where A_{600} is absorbance at 600 nm, where T_{600} is transmittance at 600 nm and x is film thickness (mm).

2.10 Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli[19]. A 4.5% stacking gel and a 10% separating gel were used. Prior to SDS-PAGE, the samples were heated at 100°C for 3 min in the presence of two different dissolving solutions ; 8 M urea, 2% SDS, 20 mM Tris-HCl (pH 8.8.) with or without 2% mercaptoethanol. Electrophoresis was performed at constant current 10 mA for 30 min and 20 mM for 1.5 h. Gels were stained with 0.05% Coomassie Brilliant Blue R-250 in isopropanol/acetic acid/water (25 : 10: 65%, v/v/v) and were destained in isopropanol/acetic acid/water (10 : 7: 83%, v/v/v). The standard protein mixture (Sigma Chemical Co., USA) ranged in molecular mass from 14.2 to 205 kDa.

2.1 1 Statistical analysis

Statistical analysis on a completely randomized experimental design was performed using the General Linear Models procedure in SPSS computer program (SPSS Statistical software, USA). One-way analyses of variance (ANOVA) were carried out and mean comparisons were run by Duncan's multiple range tests[20]

3. Results and discussion

3.1 Mechanical properties

Table 1 summarizes the mechanical properties of FWSP films treated with DAS and/or SDS together with some synthetic polymer films for household wrap. TS of FWSP films increased and EAB decreased significantly when DAS was incorporated in the film solution at 2.5 to 10% (w/w) of FWSP. DAS is a polymeric aldehyde which can cross-link between protein molecules[21]. Fig. 1 depicts SDS-PAGE patterns of FWSP films in the absence and presence of mercaptoethanol. FWSP films treated with 7.5 and 10% DAS could not be dissolved in the film dissolving solution, suggesting a strong structural film formation induced by DAS at higher concentration. It is obvious from Fig. 1 that the cross-linking effect of DAS resulted in increased film TS and decreased EAB. However, the effect of DAS on the mechanical

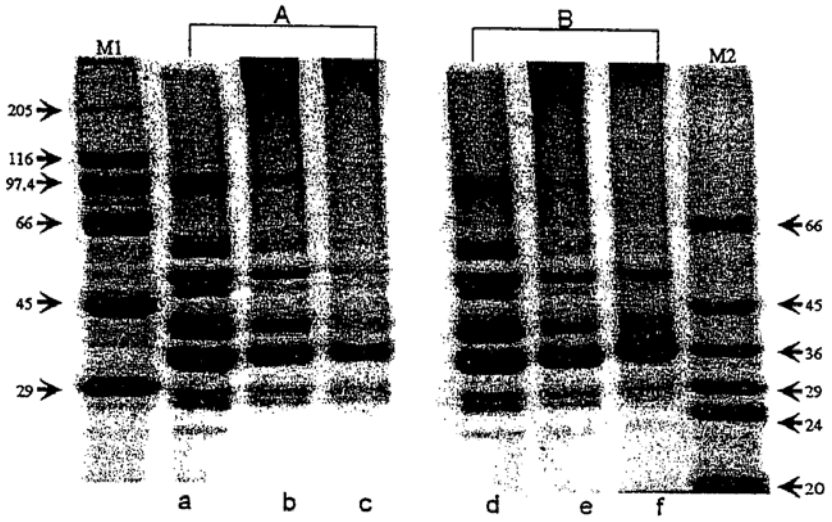

Fig. 1. SDS-PAGE patterns of fish water soluble protein films treated with dialdehyde starch DAS).
A: containing mercaptoethanol, B: not containing mercaptoethanol
Ml : high molecular weight standard, M2: low molecular weight standard
a-c: DAS films(0, 2.5, 5 %), d-f: DAS films (0, 2.5, 5 %)

Table 1. Tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP), film solubility (FS), and protease digestibility(PD) of fish water soluble protein (FWSP) films treated with different levels of dialdehyde starch (DAS), sodium dodecyl sulfate (SDS), and DAS combined with SDS, together with synthetic polymer films for household wrap.

		TS (MPa)	EAB (%)	WVP ($\times 10^{-10}$ g・m^{-1}・s^{-1}・Pa^{-1})	FS (%)	PD (%)
Control (FWSP)		4.8 1±0.93	81±27	1.73±0.12	1 9.2±0.2	66.4
DAS(%)	2.5	4.97±0.45	83±36	1.67±008	21.1±1.2	61.9
	5.0	5.20±0.80	71±29	1.5l±0.24	22.8±0.7	59.8
	7.5	6.39±1.74	41±16	1.39±0.09	19.7±1.0	56.8
	10	6.77±1.02	25±8	1.25±0.14	20.5±0.3	52.6
SDS(%)	10	3.16±1.13	79±27	1.86±0.26	92.4±8.4	52.6
	20	3.13±0.40	1 03±26	1.67±0.49	95.4±1.8	52.1
	30	3.45±0.77	123±29	1.32±0.28	99.0±2.0	48.5
	40	3.51±1.08	1 59±26	1.12±0.19	1 00±0.0	46.0
DAS/SDS (%)	2.5/40	4.08±1.16	124±26	0.92±0. 1 8	58.0±8.7	58.8
	5/40	5.44± 1.06	1 47±20	1.02±0.28	54. 1±3.4	64.0
	7.5/40	5.18±0.93	126±11	1.08±0.14	61.7±12.0	55.7
	1 0/40	4.29±1.12	82±19	1.024=0.24	40.8±9.5	51.6
Synthetic polymer films for household wrap						
Low density polyethylene		1 6.5±0.9	>1000	0.020±0.006	0	
Oriented polypropylene		50.7±8.2	73±27	0.03 8±0.035	0	
Polyethylene terephthalate		81.6±3.2	1 9±6	0. 198±0.015	0	
Polyvinylidene chloride		65.6±10.8	1 8±5	0. 002±0.000	0	

properties of protein films seems to be dependent on proteins used and the moisture content of films[3,21,22].

On the other hand, the addition of SDS in the film solution reduced TS of FWSP films, but its reduction was independent on SDS concentration up to 40% (Table 1). It is well-known that covalent bonds including disulfide bonds as well as hydrogen bonds and hydrophobic interactions are attributed to the formation of protein films[23,24]. Since SDS, powerful ionic surfactants, can denature and dissociate proteins, the TS reduction of FWSP films by SDS is expected. It is agreeable with other reports on whey protein isolate film[25] and soy protein isolate film[26]. This was also confirmed by the SDS-PAGE patterns of the films treated with SDS, where there were no polymerized protein bands observed (data not shown). In contrast to TS, EAB of FWSP films increased significantly by the addition of SDS and its increment was proportional to SDS concentration up to 40% (Table 1). This could be due to the unfolding of the protein molecules induced by SDS as pointed out by Rhim *et al*[26]. SDS disrupts hydrophobic interactions in proteins, resulting in the linear orientation of protein molecules in FWSP films and the reduction of film EAB.

From the above results, it is clear that DAS and SDS work in different ways toward the mechanical properties of FWSP films. Therefore, we tried to examine the effect of DAS and SDS combination on the mechanical properties of the film. In this experiment, the concentration of SDS was fixed to 40% and DAS concentration was altered from 2.5% to 10%. The results are also given in Table 1. The maximum TS and EAB were obtained by the combination of 5/40% and the increased ratio of DAS above 5% brought about the gradual reduction of the mechanical properties.

Any FWSP films prepared had smaller TS than synthetic polymer films for household wrap examined in this study. PET film had the strongest strength among the tested synthetic films, followed by polyvinylidene chloride, OPP, and LDPE films. On the other hand, most of FWSP films, especially those treated with SDS, were more flexible than synthetic polymer films for household wrap except LDPE.

3.2 Water vapor permeability

WVP of FWSP films decreased with increasing amount of DAS (Table 1). This is agreeable with the result reported by Rhim et al. on soy protein isolate[3] Although the bulky structure of DAS molecule should increase the diffusion of water vapor through the films, WVP of FWSP films was reduced by the increased incorporation of DAS. The reason for this phenomenon cannot be explained at this moment. SDS incorporation in the film solutions also decreased WVP (Table 1). This is likely due to the hydrophobic portions of SDS molecules,

which reduce the rate of sorption and diffusion of water vapor molecules through the film structures as pointed out by Rhim *et al*[26]. Generally, protein films have poor water vapor barrier property, limiting their use as packaging materials. The reduction of WVP by SDS is desirable for the functionality of FWSP films. The combination of DAS and SDS brought about the significant decrease in WVP of FWSP films, but the level of WVP did not vary with the ratio of DAS incorporated (Table 1). However, the WVP values of FWSP films treated with DAS and/or SDS were two or three orders of magnitude greater than those of synthetic polymer films for household wrap examined in this study (Table 1).

3.3 Film solubility and protease digestibility

Film solubility can be viewed as a measure of the water resistance and the integrity of a film[3]. According to the studies on soy protein isolate3) and egg white[27], their film solubilities decreased with increasing amount of added DAS due to the cross-linking treatments which can improve moisture resistance of protein films. However, the film solubility of FWSP films was not affected by the addition of DAS in this study (Table 1). Control FWSP film itself had lower film solubility than soy protein isolate film[3] and egg white film[27]. On the contrary, the incorporation of SDS in FWSP markedly increased the film solubility and the film with 40% SDS was completely dissolved in water. The large solubility of the FWSP films with SDS was probably attributed to the weaker structure of such films as evidenced by their smaller TS and larger EAB values compared with control films (Table 1). All of synthetic polymer films used in this study were not dissolved in the distilled water as shown in Table 1.

Protein digestibility of FWSP films was determined by α-chymotrypsin hydrolysis at 37°C after 2 hours (Table 1). Hydrolysis of the films by α-chymotrypsin increased with time and plateaued out towards the end of the digestion period (data not shown). The addition of DAS and/or SDS slowed down the digestion at the beginning, but did not apparently affect the protein digestibility of films, implying that neither DAS nor SDS interfered with the protein digestion of FWSP films by α-chymotrypsin.

3.4 Color

Color value was recorded as L* (lightness, 0=black, 100=white), a* (-a*=greenness, +a* = redness), and b* (-b* =blueness, +b* =yellowness). Addition of DAS in FWPS film solutions up to 7.5% caused yellowness as indicated by a drastic increase in +b* values (Table 2). Furthermore, the films containing DAS had lower -a* values (increased greenness). This was visually perceived as browning of the films[3]. DAS level of 7.5% of FWSP appears to be a saturation point in the FWSP-DAS reaction. The yellow/brown color associated with protein-

aldehyde interaction is mostly due to the various intermediate or final products of the Maillard reaction and this result is agreeable with other reports on several protein films[3,21,27-29]. On the other hand, SDS addition to FWSP film solutions did not affect L* and a* values of the films, but their + b* value (increased yellowness) increased with increasing amount of SDS used (Table 2). This increased yellowness of SDS-containing FWSP films was not remarkable enough to consider visually detrimental. The FWSP films treated with a combination of DAS/SDS tended to have yellow color, because the effect of DAS was more significant than that of SDS.

Table 2. L*, a*, b* color values of cast films from fish water soluble proteins (FWSP) treated with different levels of dialdehyde starch (DAS), sodium dodecyl sulfate (SDS), and DAS combined with SDS.

		L*	a*	b*
Control (FWSP)		95.6O±0.84	- 1.42±0.22	3.46±0.68
DAS(%)	2.5	95.12±0.25	-2.50±0. 14	7.07±0.43
	5	95.25±0.25	-3.28±0.20	9.64±0.67
	7.5	94.23±0.28	-5.23±0.38	18.33±1.79
	10	94.24±0.36	-5.06±0.54	1 8.05±2.43
SDS(%)	10	95.42±1.12	-2. 1 6±0.34	5.72±1.12
	20	95.69±0.49	-2.48±0.39	6.93± L33
	30	95.68±0.25	-2.66±0.22	7.40±0.78
	40	98.34±0.50	-2.70±0.47	7.44±1.79
DAS/SDS(%)	2.5/40	95.29±0.59	-3.70±0.43	13.20±2.27
	5/40	95. 12±0.68	-3.60±0.36	14.55±2.30
	7.5/40	95.32±0.31	-4.00±0.23	15.02±I.41
	1 0/40	94.55±1.75	-4.35±0.45	17. 19±3.30

3.5 Light transmission and transparency

Table 3 lists the light transmission at selected wavelengths for the FWSP films as influenced by DAS and/or SDS concentrations in comparison to some synthetic polymer films. The control FWSP film had excellent barrier properties to UV light in the 200-280 nm region regardless of composition, suggesting that FWSP films can prevent the lipid oxidation induced by UV light in

Table 3. Light transmission (%) and transparency (absorbance/mm) of cast films from fish water soluble protein (FWSP) treated with different levels of dialdehyde starch (DAS), sodium dodecyl sulfate (SDS), and DAS combined with SDS, together with synthetic polymer films for household wrap.

	Wavelength (nm)							
	200	280	350	400	500	600	800	Transparency
Control (FWSP)	0.3	0.3	55.4	74.5	79.9	81.7	83.5	2.7
DAS (%) 2.5	0.3	0.3	49.7	71.7	80.1	82.3	83.3	2.3
5.0	0.3	0.3	40.2	67.4	81.2	84.0	85.5	1.9
7.5	0.3	0.3	16.7	49.8	76. 1	82.2	84.5	2.4
10	0.3	0.3	15.1	49.0	75.7	82.0	84.0	2.7
SDS (%) 10	0.3	0.3	65.3	72.6	77.9	79.5	80.6	2.6
20	0.3	0.3	0.3	26.1	29.5	39.0	51.0	11.1
30	0.3	0.3	0.3	16.3	29.0	37.6	51.5	17.0
40	0.3	0.3	0.3	25.8	34.9	42.1	51.5	13.0
DAS/SDS (%) 2.5/40	0.3	0.3	0.3	18.0	34.8	46.5	61.9	10.4
5/40	0.3	0.3	0.3	18.4	34.9	46.5	61.6	9.8
7.5/40	0.3	0.3	0.3	18.7	43.0	55.8	68.9	5.8
1 0/40	0.3	0.3	0.3	20.8	39.5	50.5	63.2	7.2
Synthetic polymer films for household wrap								
Low density polyethylene	13.1	67.5	79.9	83.4	85.6	86.9	83.6	3.1
Oriented polypropylene	4.6	81.0	86.2	87.9	88.8	89.1	89.6	1.7
Po lyethylene terephthalate	0.3	0.3	68.3	73.6	82.1	83.5	84.9	1.5
Polyvinyl idene chloride	0.3	79.1	83.8	86.6	87.5	90.0	84.9	4.6

the food systems. This result is in the agreement with other studies[30]. It is interesting to note that the SDS-containing films blocked most light in the UV-visible range form 200 to 800 nm. On the other hand, synthetic polymer films for household wrap did not prevent the passage of UV light above 280 nm except PET film.

The control FWSP film showed a transparency of 2.7 (Table 3), indicating that the film is fairly transparent. The transparency of FWSP films was not affected by the addition of DAS, whereas the addition of SDS made the FWSP film semitransparent. The transparency of FWSP film and DAS-containing films was fairly close to that of synthetic films for household wrap examined in this study (Table 3). Those data obtained in this study seem to indicate that the FWSP films treated with DAS/or SDS are clear enough to be used as see-through packaging or coating materials[31].

4. Conclusion

In general, increasing DAS concentration enhanced TS of FWSP films, while increasing SDS concentration improved EAB. Optimum ratios of DAS/SDS were 5/40 or 7.5/40% which increased EAB by 82 or 56% with decreasing WVP by 41 or 26%, respectively. Thus, biodegradable FWSP films with various strength and flexibility were successfully prepared by

combining DAS with SDS. Continuing interest in increasing food quality, reducing use of limited resources, and reducing the environmental impact of synthetic polymers will likely result in increased use of edible/biodegradable films in the future. To achieve this potential, advances in information, properties, and economics of edible/biodegradable protein films are necessary.

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