Characteristics of Soy Protein Isolate Films Modified by Glycerol and Oleic Acid

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Abstract: The aim of this study was to develop soy protein isolate (SPI) edible films that can be used as food refreshing materials. The SPI edible films modified by different concentrations of glycerol and oleic acid (OA) were prepared. The film-forming dispersions were characterized in terms of the group changes by FT-IR analysis. The appearance, tension strength (TS), water vapor permeability (WVP), optical properties, peroxide value (POV) and contact angle of different films were evaluated. The addition of OA promoted changes in the arrangement of groups on the surfaces of film-forming dispersion (FFD) particles. Microstructure analyses indicated that the OA-treated SPI films had a rougher surface. As regards the film properties, the addition of OA caused a decrease in TS and WVP. The mechanical and optical properties of the films were closely related with their microstructure. In addition, both pH value and temperature had direct effects on the film-forming features of FFD.

Key words: soy protein isolate; oleic acid; glycerol; water vapor permeability; contact angle; opacity; mechanical properties; microstructure

甘油和油酸修饰大豆蛋白复合膜的研究

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摘 要：利用大豆分离蛋白制备可食性的食品保鲜材料。用不同浓度的甘油和油酸对可食性大豆分离蛋白(SPI)薄膜进行修饰，通过FT-IR分析成膜粒子的结构变化，对不同薄膜的形态、拉伸强度、水蒸气透过率、光学特性、过氧化值和接触角进行了分析，结果表明：油酸改变了溶液中粒子的基团组成，从超微结构可以看出油酸处理的薄膜表面比较粗糙，同时拉伸强度和水蒸气透过率有所降低，机械强度和光学特性与超微结构有密切的关系。另外，pH值和温度对溶液的成膜特性有直接影响。

关键词：大豆分离蛋白；油酸；甘油；水蒸气透过率；接触角；透光值；机械性能；超微结构
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The problems of food safety have aroused worldwide attention. In order to prolong the food shelf-life, different preservatives have been used, such as sodium benzoate and potassium sorbate. However, these preservatives may have some harmful effects on human health[1]. In spite of the government's endeavor, many cases related to the use of preservatives still occur constantly. On the other hand, the so-called "white-pollution" caused by the use of non-degradable plastics as packaging material has led to the use of biodegradable packaging materials.

In view of the problems mentioned above, the use of edible film has drawn much attention. Edible film is a kind of membrane which can be eaten and has protective function. The film can be used as a barrier to control the permeability of water, prolong the oxidation of fatty acids, thus preserving the food features and prolong the shelf-life. The materials used to form edible films are mainly natural protein, polysaccharides, and fat[1]. These materials, being biodegrad-

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able without pollution, can form membranes by interactions among molecules or cross-linking. Protein-derived films have become a focus because they have good mechanical and water-barrier features. Much research evidence has proved that soy protein isolates have good film-forming feature and high oxygen-barrier capacity. Among the compositions of soybean, glycinin is a major storage protein of soybean seeds, upon film-forming[2]. Soy protein isolates also have high nutritional value and have been used as nutritional enhancers and emulsifiers. SPI films have been used in the drug delivery systems[3]. Previous studies on degradation behavior of SPI films have showed that the composition of amino acids affects the degradation rate of SPI films as higher content of disulfide contents may delay the degradation of protein molecules[4]. During the formation of SPI films, the disulfide bonds, hydrogen bonds and hydrophobic interactions exist which affected the mechanical features of the films[5]. In order to improve film flexibility and their manipulation in hydrocolloid films, many plasticizers such as saturated fatty acids, can be added to enhance the functional properties of films[6]. In the framework of composite films, protein can be used as supporting matrix, while the hydrophobic substances such as fatty acids are used to increase the water barrier characters.

In this study, SPI films were prepared using glycerol and oleic acid as modifiers. The aim of this work was to develop edible films which can be used as packaging materials and preservative. The materials of these films are the byproducts from the production of soybean oil.

1 Materials and methods

1.1 Materials

SPI were extracted from soybean cake in our laboratory by the methods as described by Tian et al[7] with protein content > 95%. Glycerol was obtained from Tianjin Xinbote Chemical Engineering Co. Ltd. (Tianjin, China). Oleic acid (OA) was purchased from Tianda Chemical Engineering Co. Ltd. (Tianjin, China). All other chemicals are of analytical grade.

1.2 Methods

1.2.1 Preparation of film-forming dispersion (FFD)

Four percent SPI (*m/V*) as dispersed in water with stirring for 20 min. Glycerol was added to the SPI solution with final concentration of 2%, 2.5%, 3% (*m/V*), followed by 0.3% sodium sulfite (*m/V*) as a reducer. As temperature and pH value have significant effects on the solubility feature of SPI, they and glycerol content were optimized using orthogonal array design. Factors and levels in the orthogonal array design are shown in Table 1.

Table 1 Factors and levels in orthogonal array design for SPI-glycerol FFD optimizing preparation

Levels	Factors		
	A temperature (℃)	B pH	C glycerol content (%)
1	70	8	2.0
2	80	9	2.5
3	90	10	3.0

Based on the preparation of SPI-glycerol FFD, 1 %, 2 % and 3 % oleic acid was added as plasticizers. 0.2 % Tween-20 (*m/V*) was added to the solutions as a surfactant. The pH value of the solutions was adjusted using 1mol/L NaOH. Table 2 shows factors and levals in the orthogonal array design for optimizing the FFD preparation. The FFD were emulsified magnetically for 20 min in water bath at different temperatures, and then cooled to room temperature.

Table 2 Factors and levels of orthogonal array design for optimizing SPI-glycerol-oleic acid FFD preparation

Levels	Factors		
	A SPI content (%)	B oleic acid content (%)	C pH
1	3	1	8
2	4	2	9
3	5	3	10

1.2.2 FT-IR analysis of FFD

FT-IR was used to characterize the chemical functional groups of FFDs with different preparation conditions. The spectrum was collected between 4000 and 370 cm^{-1} with a resolution of 1 cm^{-1} (100 scans per sample).

1.2.3 Preparation of SPI films

FFDs were poured onto a framed and leveled polytetrafluorethylene (PTFE) plate with an area of 40 cm^2 at a final SPI content of 0.5 g SPI/100 cm^2 and were dried at 55 ℃. Dried films were peeled off from the casting surface and preconditioned in desiccators at 50% relative humidity (magnesium nitrate saturation) prior to testing.

1.2.4 Measurements of film thickness and contact angle

Film thickness was controlled at less than 0.1 mm by pouring onto the PTFE plate with determined amount of FFD and measured using a hand-held digital micrometer (± 0.001 mm). At least, five different points of the same sample were selected randomly and measured. The results were expressed as $\bar{x} \pm s$.

The SPI films were cut into 1 cm×2 cm pieces. Contact

angle were obtained by analyzing the shape of a sessile drop after it had been placed over a candelilla wax plate for 60 s. Three points were selected randomly from each sample for the measurement.

1.2.5 Determination of tensile strength (TS)

A texture analyzer TA-XT-plus (Stable Micro Systems, Surrey, UK) with a 50 N load cell equipped with tensile grips (A/TG model) was used to measure the TS of different films according to the ASTM D-882 standard[8]. Grip separation was set at 50 mm, with a cross-head speed of 5.00 mm/s. TS was measured for eight samples from each type of film and was calculated by the following formula:

$$TS=\frac{F}{L\times W}$$

Where *TS* represents the tensile strength (MPa); *F* is the tension at break; *L* and *W* represent the length (mm) and width (mm) of film respectively.

1.2.6 Determination of water vapor permeability (WVP)

Five gram of dry anhydrous calcium chloride was put into dry weighting bottles, and the mouth of the bottles were then sealed with SPI films. Subsequently, the bottles were put into desiccators containing saturated $BaCl_2$ solutions (85 % relative humidity, RH) and weighed once every 24 h. After 48 h, the WVP was calculated as follows[9-10]:

$$WVP=\frac{\triangle m\times L\times 24}{A\times t\times \triangle p}$$

Where WVP is water vapor permeability (g • mm/m^2 • d • kPa), $\triangle m$ is the migration amount of water vapor (g); *A* is the area of film (m^2); t is the measure time(d); *L* is the thickness of film (mm); and $\triangle P$ is the vapor pressure difference (kPa).

1.2.7 Measurement of opacity

SPI films were cut into suitable strips and sticked onto both sides of cells. The opacity was measured at 600 nm using a UV-2550 spectrometer. Cells without SPI films were used as blanks.

1.2.8 Determination of peroxide value

Covered with the SPI films, 10 g fresh lard was put into 100 ml triangular flasks, which was sealed with parafilm. Fresh lard which only covered with refreshing films was used as a control. All the samples were put into thermostat oven at 50 ℃. After one week, 4 g of sample was taken out to measure POV using GB/T5538 － 2005 method[11]. The POV was calculated by the following formula:

$$POV=\frac{1000\times(V-V_0)\times C}{m}$$

Where V and V_0 are the volume of natrium hyposulfurosum used for titrating samples and the blank(ml), respectively; *C* is the concentration of natrium hyposulfurosum (mol/L); and *m* is the mass (g) of samples.

1.2.9 SEM analysis

Microstructure analysis of the surface and cross sections of dry SPI films were carried out using SEM (S-3400, Hitachi Science Systems, Ltd). Pieces of (5×1) mm^2 were cut from films and mounted in copper stubs. Samples were gold-coated and observed under an accelerating voltage of 10 kV.

1.2.10 Statistical analysis

Results were analyzed by multifactor analysis of variance with 95% significance level using Origin 6.0. Multiple comparisons were performed through 95% LSD intervals.

2 Result and Analysis

2.1 Group analysis of the FFDs by FT-IR

Fig.1 shows the IR spectra of soy protein isolates, glycerol, oleic acid, 4% soy protein isolates mixed with 2% glycerol, and 4% soy protein isolates modified with 2% glycerol and 4% oleic acid. The spectra of glycerol, SPI and SPI-glycerol composites exhibited clearly clearly broad peaks in the range of 3450－3300 cm^{-1}. The peaks were assigned to the －OH stretching, indicating intermolecular hydrogen bonding. However, this peak does not exist in OA. Compared with SPI-glycerol composites, the －OH peaks of SPI-glycerol-OA composites were less strong. This reduction of peak magnitude may attribute to the partial covering of SPI-glycerol composites by OA, and it was supported by SEM analysis (Fig. 2). The C － H stretching peaks near 3000 － 2800 cm^{-1} represent the structures of CH_2 － and － CH_3 and RCH_2 － in soy protein isolates. Glycerol and oleic acid were hardly observed in SPI-glycerol and SPI-glycerol-OA composites, indicating the rearrangement of groups on the surfaces of these composite films. However, the characteristic C=O stretching peak near 1650 cm^{-1} which was stronger in SPI-glycerol-OA composites than that in all other compounds in this study indicated the intermolecular rearrangements in SPI-glycerol-OA composites. Upon the formation of SPI films, the inter-molecular H-bonds played an important role.

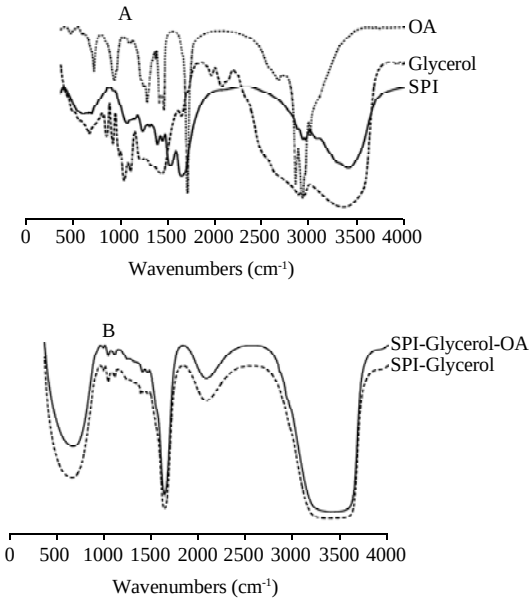


Fig.1 FT-IR spectra of soy protein isolates (SPI), glycerol, oleic acid (OA)(A) and 4% SPI modified with 2% glycerol (SPI-glycerol), 4% soy protein isolates modified with 2% glycerol and 4% oleic acid (SPI-Glycerol-OA) (B).

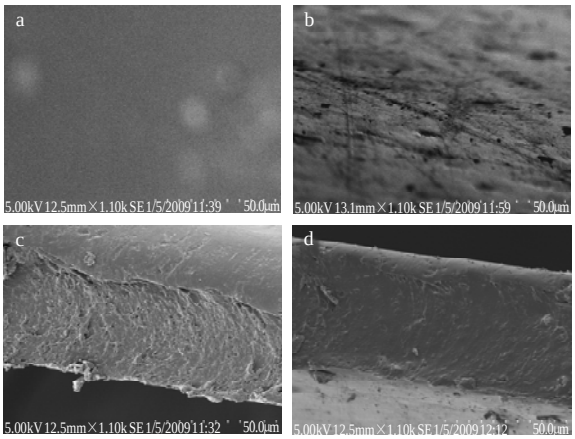
2.2 Characterization of the films

TWO orthogonal array designs were conducted as outlined in table 1 and 2 to study the effects of glycerol content, pH value, temperature and content of oleic acid on the film-forming features of SPI. The thickness of all films was controlled to be less than 0.1 mm. In the preparation of SPI films containing glycerol as described in table 1, the pH values at 8, 9 and 10 were selected, as SPI was more soluble in alkaline solutions; While in order to promote inner- or intermolecular interactions thus improving film-forming properties, higher temperature was chosen to treat the SPI.

The addition of OA caused nonhomogenous effect on the FFD system and therefore 0.2% Tween-20 was also added into the dispersions. As a large number of hydrogen bonds, disulfide bonds and hydrophobic interactions exist in natural protein molecules, anhydrous sodium sulfite was added into the system as a reductant to break the disulfide bonds in SPI and form new bonds. Then 40 ml of the FFD was poured onto the plates with an area of 40 cm^2.

2.2.1 Microstructure

The microstructure of the films may be affected by many factors, such as the concentration of the film-forming materials[12], pH value, and temperature. In this study, the SPI films developed from FFD were observed under SEM. Fig. 2 shows the SEM micrographs of surface of the SPI films modified with glycerol and OA. Compared with SPI-glycerol films (Fig.2a), SPI-glycerol-OA films (Fig. 2b) showed remarkable differences in terms of surface microstructure. The SPI-glycerol film surface showed a smooth and continuous structure, while the presence of OA caused discontinuities associated with the formation of two phases (lipid and polymer) in the matrix: lipid droplets embedded in a continuous polymer phase. Fig. 2c and 2d show the SEM micrographs of cross sections of the films, where differences were still appreciated in the formulations containing OA. The SPI-glycerol films had many small cavities as shown in Fig 2c, which was not exist when OA was added (Fig.2d). In fact, an almost continuous layer of partially coalesced droplets can be observed on the FFD surface which may explain the exceptional gloss of these films and the lower WVP. These differences in microstructures also can explain the values of contact angles. With the addition of OA into the SPI films, the contact angles also increased. These changes in the appearance may also reveal the conformational changes in the molecular levels. In the film network, intermolecular hydrogen bonding between segments of β-sheet may act as junction zones[2].



a. SPI-glycerol surface; b. SPI-glycerol-oleic acid surface; c. SPI-glycerol cross section; d. SPI-glycerol-oleic acid cross section.

Fig.2 SEM micrographs of the films

2.2.2 The WVP of SPI films plasticized with glycerol and OA

Since the main function of food packaging is often to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product, WVP should be as low as possible[9]. Recently, some studies have been published on the improvement of water barrier properties of films by blending the FFD with fatty acids, natural waxes,

surfactants and resins[13-14]. The effects of glycerol and OA with different concentrations on WVP of SPI film are displayed in Table 3 and Table 4. It could be concluded that the temperature has the most significant effects on the WVP of SPI films. Glycerol has many − OH groups, which has strong water absorption ability. As any factors can affect the WVP of SPI films[15], in this study, the OA was added as plasticizers to investigate the WVP changes. As expected, the SPI-glycerol-OA film has significantly lower WVP than that of the SPI-glycerol film.

2.2.3 Tensile strength (TS)

Previous studies have showed that the mechanical features of SPI are affected by many factors, such as addition of calcium salts and glucono-delta-lactone[15], cross-linking by formaldehyde vapors, and UV irradiation[16]. The plasticizing effects of glycerol and OA with different contents on mechanical properties of SPI films are also displayed in Table 3 and Table 4.

It could be observed that pH value, temperature and glycerol concentration were the main factors that affect the TS of SPI films, and pH value was the most significant one. An increase in temperature may lead to the changes of protein structures and rearrangements of molecules. Polar groups (−OH) along plasticizer chains (glycerol) were believed to develop polymer-plasticizer hydrogen bonds replacing the protein-protein interactions in biopolymer films as shown in the FT-IR analysis (Fig.1). In the case of SPI-glycerol films, the addition of OA led to the decrease of TS. The optimal combination of the parameters was 4.0 % SPI, 2 % glycerol, 2 % OA, and pH 9.0. When the concentration of SPI is lower than 3%, the films is not easy to form; while the SPI concentration of higher than 5 % may lead to the formation of SPI particles. Therefore, 4 % SPI was selected in this study.

2.2.4 Effects of glycerol and OA addition on visual appearance of SPI films

In order to increase the prevalence of the edible films, the opacity is the most important feature if a film is to be used as a food coating or as food packaging[17]. In this study, the transparence was used as one of the indexes for evaluating the SPI films. The color of SPI films was affected by many factors in this study. The color and appearance of SPI films were evaluated by both naked eye and UV methods. Results showed that pH value had the greatest effects on the opacity of the films, while glycerol had the least effects. Tempera-

Table 3 Results of orthogonal tests on preparation of SPI-glycerol films

	No	*A*	*B*	*C*	TS (MPa)	WVP(g·mm/m²·d·kPa)	Opacity	POV (mol/g)
	1	1	1	1	7.482 ± 2.352	72.284 ± 8.352	79.131 ± 1.268	2.093 ± 0.032
	2	1	2	2	8.197 ± 1.262	85.541 ± 7.359	83.512 ± 2.149	2.207 ± 0.056
	3	1	3	3	12.802 ± 1.347	91.713 ± 7.466	70.335 ± 4.483	2.160 ± 0.123
	4	2	1	2	8.836 ± 0.772	63.695 ± 5.554	77.199 ± 0.914	2.239 ± 0.121
	5	2	2	3	7.618 ± 0.509	72.749 ± 5.354	77.315 ± 3.455	2.272 ± 0.165
	6	2	3	1	11.768 ± 1.349	64.180 ± 6.411	70.974 ± 3.825	2.128 ± 0.064
	7	3	1	3	6.000 ± 1.691	89.389 ± 7.542	83.848 ± 2.096	2.062 ± 0.098
	8	3	2	1	8.533 ± 0.178	72.017 ± 6.358	78.648 ± 4.635	2.635 ± 0.089
	9	3	3	2	11.373 ± 2.134	93.279 ± 7.678	69.904 ± 2.548	2.702 ± 0.078
TS	K_1	28.483	22.318	27.783				
	K_2	28.222	24.348	28.406			Range analysis: $R_B>R_A>R_C$	
	K_3	25.906	35.946	26.420			Optimal combination: $A_1B_3C_2$	
	R	2.577	13.625	1.986				
WVP	K_1	249.538	225.368	208.481				
	K_2	200.624	230.307	242.515			Range analysis: $R_A>R_C>R_B$	
	K_3	254.695	249.172	253.851			Optimal combination: $A_2B_1C_1$	
	R	53.99	23.804	45.37				
Opacity	K_1	232.978	240.178	228.953				
	K_2	225.488	239.675	230.615			Range analysis: $R_B>R_A>R_C$	
	K_3	232.600	211.213	231.498			Optimal combination: $A_1B_1C_3$	
	R	7.49	28.965	2.545				
POV	K_1	6.46	6.394	6.856				
	K_2	6.639	7.114	7.148			Range analysis: $R_A>R_B>R_C$	
	K_3	7.399	6.990	6.474			Optimal combination: $A_1B_1C_3$	
	R	0.939	0.72	0.674				

Table 4 Results of orthogonal tests on preparation of SPI-Glycerol-OA films

	No	*A*	*B*	*C*	TS (MPa)	WVP (g·mm/m²·d·kPa)	Opacity	POV (mol/g)
	1	1	1	1	3.143 ± 0.354	45.168 ± 3.269	60.148 ± 0.140	2.308 ± 0.021
	2	1	2	2	5.275 ± 1.241	50.109 ± 5.369	50.472 ± 1.201	1.908 ± 0.131
	3	1	3	3	3.965 ± 0.734	39.609 ± 2.536	55.392 ± 1.883	1.637 ± 0.012
	4	2	1	2	9.395 ± 2.266	31.915 ± 9.362	39.847 ± 8.248	1.987 ± 0.054
	5	2	2	3	9.943 ± 2.131	31.030 ± 3.564	30.944 ± 5.124	1.865 ± 0.088
	6	2	3	1	5.421 ± 1.243	22.719 ± 1.265	42.753 ± 4.903	2.146 ± 0.065
	7	3	1	3	8.102 ± 1.819	42.806 ± 5.362	57.384 ± 2.481	2.074 ± 0.245
	8	3	2	1	10.773 ± 2.523	31.934 ± 2.356	27.046 ± 2.632	1.973 ± 0.162
	9	3	3	2	7.199 ± 2.152	23.924 ± 2.135	25.994 ± 2.343	1.900 ± 0.142
Ts	K_1	12.383	20.322	16.345				
	K_2	24.610	22.879	21.869			Range analysis: $R_A > R_B > R_C$	
	K_3	22.793	16.585	21.543			Optimal combination: $A_2B_2C_2$	
	R	12.227	6.294	5.524				
WVP	K_1	134.886	119.889	99.821				
	K_2	85.664	113.076	105.948			Range analysis: $R_A > R_B > R_C$	
	K_3	98.124	86.252	113.445			Optimal combination: $A_2B_3C_1$	
	R	49.222	33.637	13.624				
Opacity	K_1	166.012	157.379	129.947				
	K_2	113.544	108.462	116.313			Range analysis: $R_A > R_B > R_C$	
	K_3	110.424	124.139	143.720			Optimal combination: $A_1B_1C_3$	
	R	55.588	48.917	27.407				
POV	K_1	5.853	6.369	6.427				
	K_2	5.998	5.746	5.795			Range analysis: $R_C > R_B > R_A$	
	K_3	5.947	5.683	5.576			Optimal combination: $A_1B_3C_3$	
	R	0.145	0.686	0.851				

ture and pH value affect the solubility of SPI, thus also affecting the opacity. The increase of temperature below 100 ℃ may change structure of protein molecules and increase their activities. While higher temperature above100 ℃ may cause protein denaturation and decrease opacity. In addition, the OA addtion can cause the film to be less transparent. There was a white oil layer on SPI films containing OA indicating the migration of OA molecules which was also confirmed by SEM images (Fig.2b).

2.2.5 Effects of glycerol and OA addition on SPI film antioxidant activity

Antioxidant activity is another important index for evaluation the usefulness of the films when used for the preservation of vegetables and oil. It is mainly affected by the permeability of oxygen. SPI has oxidative stability but this stability is less than that of casein and whey protein isolate[18]. In this study, the antioxidant ability was evaluated by determining the POV of lard of filled into a triangular flask sealed with different SPI films. The POVs in SPI-glycerol film groups were larger than 2 mol/g in most cases, while in the SPI-glycerol-oleic acid film groups, the POVs were below 2 mol/g in most cases except for the sixth and the seventh combination, indicating that the OA addition can increase the antioxidant ability of SPI film, which can extend its prospective use in the preservation of lard. We have also prepared SPI-glycerol-OA films containing small amount of propyl gallate (PG), which is one of the antioxidants used in lipid preservation. However, the addition of PG may cause significant browning effect (data not shown).

3 Conclusion

The interactions among SPI, glycerol and OA in FFD and in the development of films were affected by pH value, temperature, and concentrations of different components. The optimal conditions for the development of SPI-glycerol-OA films were 4.0 % SPI, 2.0 % glycerol, 2.0 % OA and pH 9. The addition of OA changed the structure of film indicating in the changes of SEM images. The decrease of TS value, WVP and POV and the increase of contact angel indicate the possibility of modification of SPI film by OA.

To our knowledge, this is the first report on the modification of SPI films using oleic acid. As soy is a staple product in the world, the use of soy protein which is a byproduct, from the production of bean oil is of great

importance. There are many reports on the modification of chitosan films using glycerol and oleic acid in order to increase the film-forming features of this polymer[17]. However, few studies have been reported on the modification of SPI using oleic acid as a plasticizer. In this study, we found that the modification of SPI using glycerol and oleic acid led to a great increase of the water vapor permeability (WVP) and the antioxidatnt activity. These modified films have promising application in the preservation of meat and vegetables. This work may also be helpful for the syudy of drug delivery system.

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