

Mechanical properties and sorption characteristics of stearic acid-cysteine-soy protein isolate blend films

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Abstract: The soy protein isolate(SPI) blend films which have good mechanical properties and better barrier to moisture were prepared by using stearic acid as plasticizers and cysteine as cross-linking reagent. The tensile strength(TS) and percentage elongation(E %) of SPI blend films were determined after conditioning blend film specimens at 25°C and 50% relative humidity(RH) for 2 days by using Texture Analyzer. Moisture sorption characteristics of the SPI blend films at 25°C were studied for water activities ranging from 0.10~ 0.90. The moisture sorption rate and sorption isotherm data were mathematically fitted to Peleg's Equation and GAB(Guggenheim-Anderson-de Boer) model, respectively. The results indicated the TS, E % and moisture sorption rate of SPI blend films changed remarkably with the ratio of stearic acid to cysteine in blend films. The optimum ratio of stearic acid to cysteine was 40 : 60 (w/w) (10g blend additive /L protein solution) in SPI blend films. The experimental results showed the TS value of the SPI blend films with ratio of 40 : 60 was 2 times higher than that of the original SPI films. In addition, the blend films with ratio of 40: 60 had the optimum sorption rate. The moisture sorption data of the SPI blend films were well fitted to GAB model. GAB model had the highest R²(0.99).

Key words: soy protein isolate blend film; sorption characteristics; tensile strength

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0 Introduction

Approximately 40,000,000 tons of plastic packaging is used annually worldwide. As we known, plastic materials are non-degradable in nature and result in continuous accumulation in environment and causes severe pollution^[1]. So the films made from agricultural products have received more attention for less environmental pollution and biodegradation, such as soybean protein isolate(SPI) films, corn zein films, protein films and wheat gluten films^[2-4]. SPI has excellent film forming abilities and superior barrier properties against oxygen and oil movement in relative humidities than that of the other films^[5-7]. Unfortunately, the films of SPI do not provide an efficient barrier to water transfer, swell easily as well as low mechanical strength, which limits its application in food packaging material industry. In recent years, considerable efforts are currently being made in the development of biomaterials based on natural macromolecules in order to obtain films with characteristics similar to those of

synthetic polymers commonly used in food protection and preservation. The research on biological macromolecule films have been focused on its mechanical properties and moisture sorption characteristics in particular^[8,9].

Previous studies showed that cysteine residues play an important role in the formation of wheat protein networks of both gliadin and glutenin films. The cysteine groups can undergo polymerization via sulfhydryl-disulfide interchange reactions during heating to form a continuous covalent network upon cooling and increase TS of SPI films, and shorten the film forming time^[8,10]. Among the lipid materials investigated, long-chain saturated fatty acids were most able to effectively improve the moisture barrier properties of hydrophilic films. It increased significantly not only the water barrier properties of SPI films to 15.0%~ 16.6% than that of control films also conferred the desired flexibility and extensibility to films^[11]. In present study, stearic acid and cysteine were used as a plasticizer and cross-linking agent for SPI blend films, respectively, which have a dual effect for increasing TS and reducing moisture transport of SPI films.

On the other hand, moisture sorption characteristics of hydrophilic protein films can also be significantly influenced by relative humidity condition and additive

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type as well as additive ratio^[12-14]. Any modification in the composition of food or material may influence its sorption isotherm. Seung et al^[15] reported the sorption isotherms of SPI films influenced by plasticizer concentration and concluded that the equilibrium moisture content of the films was largely determined by content and type of plasticizer in films. It has not been reported with regard to the effect of stearic acid-cysteine blend additive on sorption characteristics. In present study, the GAB model was selected for fitting to experimental data. The objective of the present study was to investigate the effect of stearic acid to cysteine ratio on kinetic moisture sorption characteristics and TS properties of SPI blend films and to study GAB model's application for SPI blend films as well as to develop a suitable SPI blend films.

1 Material and methods

1.1 Soybean protein isolate

Soy protein isolate(95.5% protein, dry basis) was kindly donated by Shenghua Plant Protein Co. Ltd. (Anyang, Henan). All chemicals used were reagent grade.

1.2 Preparation of blend films

Soy protein isolate film solutions were prepared by dissolving 40g of SPI in 1.0 L of distilled water, stirred for 20 min and adjusted pH value to 9.5 with 0.1 mol/L sodium hydroxide. The ethanol was added to film-forming solutions of SPI at levels of 10% (v/v) protein solution(as a emulsifier and dehydration). The stearic acid-cysteine blend additive was added to film-forming solutions of SPI at level of 10 g/L protein solution, w/v; the ratio of stearic acid to cysteine was at level of 100 : 0, 80 : 20, 60 : 40, 40 : 60, 20 : 80, 0 : 100, respectively. The solutions were stirred with a magnetic stirrer for 20 min and heated for 20 min in 70°C hot water bath, cooled, filtrated, degassed for 20 min, and then the film solution was poured on the coated glass plate, dried at 60°C and stored in a desiccators.

1.3 Thickness measurement

Film thickness was measured with a hand-held micrometer (SM-112, Teclock Co, Japan). Measurements for testing mechanical properties were taken at five different locations on the moisture-equilibrated film specimen. The mean thickness was used to calculate the mechanical properties of film specimen.

1.4 Tensile strength measurement

Tensile strength[TS(MPa)], percentage elongation[E(%)] at break of SPI blend films were determined at (25 ± 1) °C, using Texture Analyzer (TA-XT 2,

Stable Micro System, England) according to ASTM Standard Method D882-00^[16]. Five film specimens of each formulation, 80 mm × 25 mm strips, were cut from each preconditioned film at (25 ± 1) °C and 50% relative humidity (RH) for 48 h in a desiccator over saturated [Mg(NO₃)₂] solution and mounted between the tensile grips. The initial grip separation and crosshead speed were set to 50 mm and 30 mm/min, respectively. A microcomputer was used to record the force and percentage elongation at which each film was broken. The measurement was performed immediately when a sample was removed from a desiccator.

The tensile strength(TS, MPa) is calculated by

$$TS = F \times 10^{-6} / S$$

Where F is the maximum tensile of film specimens at break, N; S is area, m².

1.5 Moisture sorption characteristics

Water absorption was determined by Seung method^[15]. Stearic acid and cysteine-SPI blend film specimens (25 mm × 25 mm) were conditioned in a desiccator for 3 d at 11% RH and (25 ± 1) °C to reduce the initial moisture content. Blend film specimens were then placed at (25 ± 1) °C over saturated salt solutions in the desiccators having desired relative humidity (35% and 75% RH) conditions. Weights of blend film specimen as a function of time were measured, and moisture contents of the blend film were determined by an oven drying method at 113°C. Moisture adsorption curves of blend films with time were fitted to a mathematical model suggested by Peleg's Equation. Determinations were run in triplicate for each ratio level of stearic acid to cysteine.

$$M_t = m_0 + \frac{t}{k_1 + k_2 t} \quad (1)$$

Where M_t is the moisture after time t ; m_0 is the initial moisture and k_1 and k_2 are parameters. The sorption rate dM_t/dt was derived from Eqn^[11].

1.6 Moisture sorption isotherm curve and models

The sorption isotherms for the SPI blend films were determined gravimetrically^[17] at a temperature of (25 ± 1) °C. Each formulation film specimens(0.9~1.2 g) were equilibrated in saturated salt solutions having desired RH (10, 20, 35, 40, 53, 62, 75, 85 and 95% RH) conditions. Saturated salt solutions of different aw were prepared as table 1. These saturated solutions were taken in different desiccators. The film specimens were weighed every 6 h, and when the two consecutive weight were equal, it was assumed that an equilibrium condition was reached. The equilibrium moisture content was calculated after the samples reached constant weight.

Table 1 Standard saturated salt solutions of different a_w

Saturated salt solutions	a_w	Saturated salt solutions	a_w
LiCl	0.11	NaNO ₂	0.64
CH ₃ COOK	0.22	NaCl	0.75
MgCl ₂	0.33	KCl	0.86
K ₂ CO ₃	0.44	(NH ₄) ₂ HPO ₄	0.92
Mg(NO ₃) ₂	0.54		

The sorption isotherm data were fitted to Guggenheim-Anderson-de Boer (GAB) model^[18,19]. GAB isotherm model can be expressed as follows

$$M = \frac{m_0 C k a_w}{(1 - k a_w)(1 - k a_w + C k a_w)} \quad (2)$$

Where M is the equilibrium moisture content at a water activity a_w ; m_0 is the monolayer value; C , k are the constants. To determine the m_0 , C and k constants, the GAB equation^[2] transformed into the following parabolic expression^[20]

$$\frac{a_w}{M} = \alpha + \beta a_w + \gamma a_w^2 \quad (3)$$

where

$$\alpha = \frac{1}{m_0 C k} \quad (4)$$

$$\beta = \frac{(C - 2)}{m_0 C} \quad (5)$$

$$\gamma = \frac{(C - 1)k}{m_0 C} \quad (6)$$

The three constants α , β and γ are readily determined by a least-square regression of this second-degree polynomial and from these, the three GAB constants are calculated by

$$K = (f^{1/2} - \beta)/2\alpha \quad (7)$$

$$m_0 = 1/(\beta + 2k\alpha) \quad (8)$$

$$C = 2 + \beta/k\alpha \quad (9)$$

Where

$$f = \beta^2 - 4\alpha\gamma \quad (10)$$

The sorption data were analyzed according to the models. The goodness of fit of each model was computed in terms of coefficient of determination (R^2) from the plot of experimental (M_{exp}) and predicted (M_{pre}) sorption moisture and root mean square error (RMSE) values, as follows^[11]

$$RMSE = \left[\sqrt{\frac{\sum [\frac{M_{exp} - M_{pre}}{M_{exp}}]^2}{N}} \right] \times 100 \quad (11)$$

Where M_{exp} is the experimental equilibrium moisture content value, % db; M_{pre} is predicted equilibrium moisture content value, % db; N is the number of experimental points.

1.7 Statistical analysis

Measurements were replicated five times for formu-

lation of SPI blend film. Experimental data was analyzed using Excel (Microsoft Inc) and SPSS 10.0 for window. Duncan's multiple-range test ($P \leq 0.050$) was used to determine significance of differences between means.

2 Results and discussion

2.1 Mechanical properties

Tensile strength expresses film strength, whereas elongations at break exhibits film stretch ability prior to break. TS and E at break of control and stearic acid-cysteine SPI blend films are shown in Table 2.

Table 2 Mechanical properties of stearic acid-cysteine-SPI blend films*

AC: cysteine(% , w/w)	TS/MPa	E/%
100 : 0	4.5 ± 0.43	110.7 ± 8.6
80 : 20	5.2 ± 0.34	102.6 ± 4.2
60 : 40	6.8 ± 0.65	80.2 ± 3.8
40 : 60	10.9 ± 0.74	50.7 ± 4.7
20 : 80	12.7 ± 0.71	40.2 ± 2.9
0 : 100	14.5 ± 0.51	30.2 ± 1.9
0 : 0	5.7 ± 0.60	128.6 ± 6.3

Values (means + SD, n = 5) with different letter within a column significantly difference ($P < 0.05$).

* [10 g additive (stearic acid/cysteine)/L SPI solution].

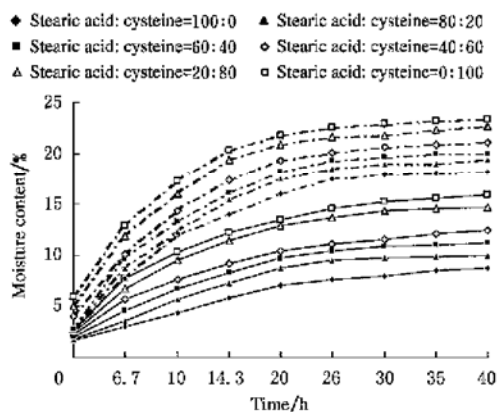
The results showed the TS value increased with the increase of cysteine concentration, whereas increasing stearic acid concentration resulted in decrease of TS values. For example, the SPI blend films with the ratio of stearic acid to cysteine (0 : 100) showed higher TS (14.5 MPa) and its TS value was 2~3 times higher than the original SPI films (5.7 MPa), whereas the SPI blend films with the ratio of stearic acid to cysteine (100 : 0) had much lower values (4.5 MPa), which indicated the cysteine addition can improve the mechanical properties of soy protein-based films, whereas stearic acid can lower the mechanical properties. Similar results have been reported for cysteine added-soy protein-wheat gluten film^[8]. Cysteine in the SPI blend film solution broke the intramolecular disulphide bond of protein, increasing sulphhydryl contents, which was beneficial for forming new disulphide bond between protein intermolecular and formed more network structures during coating film progress, these networks or aggregates were responsible for TS of blend films. The negative effect of stearic acid on mechanical properties of stearic acid-cysteine SPI blend films may have resulted from the partial replacement of protein polymers in the film matrix. The interactions between stearic acid molecules and protein polymer via hydrogen bond is weaker than the interaction

between protein and protein through not only hydrogen bond but also disulphide bond, this behavior may be attributable to the less of intermolecular covalent bonds between stearic acid and protein, so that the TS value of SPI blend film decreased with the increase in stearic acid addition ratio. However, when the addition ratio of AC to cysteine reached 40:60 in blend film solution, the cysteine-SPI films became opaque, so the optimum addition ratio of stearic acid to cysteine was 40:60 in SPI blend film solution.

Table 2 showed elongation at break of SPI blend films decreased with the concentration of the cysteine increased, whereas elongation of SPI blend films increased with the addition of hydrophobic substances—stearic acid. For example, the E value of SPI blend with ratio of 100:0 was 110.7%, whereas with the ratio of 0:100 were 30.2%, which indicated E value was determined by additive type and ratio. Similar results have been reported by Shellammer and Krochta^[21]. As you known, the cysteine enhanced protein intermolecular cross-linking and increase resistance for movement so that reduce E values of SPI blend films, however, stearic acid, a plasticizer, can increase protein molecular flexibility, movement space and reduce action force between protein and protein, so with increasing the concentration of stearic acid, E values gradual become large.

2.2 Moisture adsorption kinetics of SPI blend films

Moisture sorption curves of SPI blend films are presented in Fig. 1. The figure clearly showed moisture adsorption of SPI blend films were more rapid with time in initial stage and then slow adsorption trend emerged and moisture content of SPI films reached a plateau. This is for the lower water content of the SPI blend films, the faster adsorption.



Dash dot lines and broken lines correspond to RH 35% and RH 75%, respectively. The lines were derived from Eqn[1]
Fig. 1 Effects of additives on moisture sorption curves of SPI blend films at different relative humidities

Moisture equilibration time was mainly influenced by storage relative humidity and additive ratio. Films stored at higher relative humidity and contained cysteine required more time to reach their equilibrium, whereas at low relative humidity and contained stearic acid required less time to reach their equilibrium. This is attributed to increase stearic acid content limited water absorbed of SPI blend films in this range, this results agreed with Lia et al^[22-24]. So the time reached moisture equilibration at 75% RH was longer than that of at 35% RH. For example, the blend films stored at 35% RH reached relative equilibrium after 20 h, where the films stored at 75% RH needed 26 h for reaching moisture equilibrium.

Measured sorption curve data were fitted to Eqn[1] to obtain sorption curve equations. The constants k_1 and k_2 , which were derived from the linear fit, are shown in Table 3.

Table 3 Constant values (k_1 , k_2) and coefficient of determinations (r^2) for sorption curve equations (Eqn[1]) of SPI blend films at selected RH

AC: cysteine (%, w/w)	35% RH				75% RH			
	k_1	k_2	r^2	RMSE	k_1	k_2	r^2	RMSE
0:100	52.89	4.78	0.99	2.33	27.00	3.39	0.96	4.26
20:80	63.89	4.79	0.98	4.24	31.05	3.423	0.96	4.52
40:60	74.90	6.013	0.99	2.08	40.38	3.40	0.96	5.31
60:40	101.29	5.717	0.98	5.07	42.45	3.60	0.98	4.30
80:20	139.46	5.31	0.96	8.07	50.23	3.50	0.97	5.5
100:0	207.54	4.86	0.96	9.83	57.76	3.56	0.95	6.6

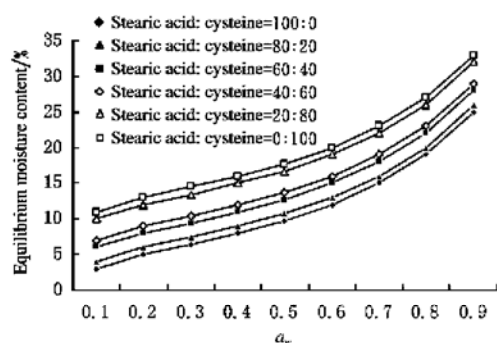
The coefficients of determination are found to be very high in all cases ($r^2 > 0.95$), this indicated a good fit to the experimental data. In general, blend films stored at a higher relative humidity tend to have lower k_1 and k_2 values. The blend films with cysteine had lower k_1 and k_2 values than did stearic acid-containing films at any humidity. For example, k_1 and k_2 values of blend films with the ratio of 40:60 stored at 75% RH were 40.38 and 3.40, respectively, whereas those of blend films with the ratio of 60:40 stored at 35% RH were 101.29 and 5.72. The low k_1 value in the blend films and stored at 75% RH indicated the higher initial moisture adsorption rate and low k_2 indicates large amount of moisture adsorbed until the blend films reached its equilibrium.

Moisture adsorption rates of blend films were influenced by the blend additive ratio and storage relative humidity. Films with cysteine showed a higher adsorption rate than stearic acid-containing films in the early stage of moisture adsorption. Moisture affinity differences between cysteine and stearic acid are main-

ly responsible for the different sorption rates of additives containing soy protein films. Cysteine molecules have more hydrophilic groups than stearic acid (Stearic acid was hydrophobic molecule), thus, films with cysteine have more sites to which moisture molecules could be absorbed.

2.3 Moisture sorption isotherm model

The moisture adsorption data of SPI films were fitted to GAB models and isotherms are showed in Fig. 2.



The lines were derived from the Smith equation (Eqn [2])

Fig. 2 Sorption isotherm of stearic acid-cysteine-SPI blend films

The moisture sorption displayed sigmoidal curves and indicated that the equilibrium moisture content increased slowly with an increase in environmental a_w up to 0.5, beyond which the slope increased rapidly. For example, the equilibrium moisture content (% db) of SPI blend films with ratio of 40 : 60 increased from 7 to 13.7 at a_w range from 0.1 ~ 0.5, whereas a_w changing from 0.5 to 0.9, the equilibrium moisture content (% db) of blend with same ratio increased sharply from 13.7 to 29.

The sorption isotherms for SPI blend films were influenced by the ratio of stearic acid to cysteine, the moisture content in blend films increased with increase addition amount of cysteine in the additive. Cysteine films absorbed more moisture at a given a_w than that of stearic acid films. As Fig. 2 showed the equilibrium moisture content (% db) of SPI blend films with ratio of 80 : 20 was 20, whereas with ratio of 20 : 80 was 26 at a_w 0.8.

These results could be attributed to the higher moisture affinity of cysteine compared to stearic acid. Because cysteine was a hydrophilic material, whereas stearic acid had long hydrophobic chain. Similarly, Kim and Ustunol reported butter fat-containing films had higher equilibrium moisture contents than films with candellilla wax at all a_w investigated.

The moisture adsorption data of SPI blend films

were fitted to the GAB models and isotherms are showed in Fig. 2. Calculated the GAB model constants and coefficient of determination (r^2) are shown in Table 4. Higher r^2 values and lower RMSE values confirmed that the equation was a good model for experimental data.

Table 4 Sorption isotherm model (GAB) constants and coefficient of regression (R^2) values for stearic acid-cysteine-SPI blend films when water activity ranged from 0.1~ 0.9

Stearic acid-Cysteine (% ratio, w/w)	Constants of linear fitting			R^2	RMSE
	m_0	C	k		
0 : 100	11.51	133.67	0.72	0.99	1.2
20 : 80	10.80	89.79	0.74	0.99	0.90
40 : 60	8.81	32.38	0.78	0.99	0.39
60 : 40	8.21	24.42	0.79	0.99	0.59
80 : 20	7.15	11.61	0.82	0.99	1.05
100 : 0	6.85	7.50	0.82	0.99	1.19

3 Conclusions

Effect of addition of stearic acid-cysteine on sorption characteristics and TS values of SPI films were studied at different RH conditions. It was demonstrated that additive type and adding ratio were the most important factors in determining the moisture affinity of SPI films and its TS. The sorption rate and sorption isotherms of SPI blend films showed that films with higher ratio cysteine absorbed more moisture rapidly at a given RH condition compared to films with the less hydrophilic stearic acid, in the contrast, the addition of cysteine increased TS of blend films. The TS value of blend films with ratio of 0 to 100 was 3 times than that of the films with ratio of 100 to 0. Comprehensively considering the effect of stearic acid and cysteine on TS and moisture sorption characteristics of blend films, the optimum addition ratio of stearic acid to cysteine was 40 : 60 (w/w) in blend SPI films.

Moisture adsorption and sorption isotherm data were mathematically fitted to Peleg's Equation and GAB model, respectively. High r^2 values and low RMSE values confirmed that the both equations were a good model for experimental data.

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硬脂酸-胱氨酸-大豆分离蛋白复合膜的机械特性和吸湿性研究

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摘要: 以硬脂酸作为增塑剂, 胱氨酸作为交联剂制备具有一定力学性能和良好抗湿性能的大豆分离蛋白复合膜。将膜放在 25℃, 相对湿度为 50% 的干燥器中平衡两天, 用质构仪测定膜的抗拉强度(TS), 延伸率(E(%))。在水分活度 a_w 为 0.10~ 0.90 的范围内研究了复合膜在 25℃ 的吸湿特性。吸湿速率和吸湿等温线数据分别拟合到 Peleg's 方程和 GAB(Guggenheim-Anderson-de Boer) 模型。结果表明: 大豆蛋白复合膜的 TS、延伸率 E(%) 以及吸湿速率随着硬脂酸和胱氨酸的添加比率显著地变化。硬脂酸和胱氨酸的最佳添加比率为 40:60(w/w) (每升蛋白质溶液中加入 10 g 混合添加剂), 此时, 大豆蛋白膜的强度比原来提高 2 倍, 并且有最佳的吸湿速率。吸湿数据和 GAB 模型有很高的拟合度, 拟合系数最高达 0.99。

关键词: 分离大豆蛋白复合膜; 吸湿性质; 抗拉强度