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Formation of Edible Soybean and Soybean-Complex Protein Films by a Cross-Linking Treatment with a New *Streptomyces* Transglutaminase

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Summary

Soybean protein isolate (SPI) was used in the investigation of the formation of edible protein films through an enzymatic cross-linking method with a purified microbial transglutaminase (MTG) produced from a new effective strain *Streptomyces* sp. WZFF.L-M1 preserved in our laboratory, followed by the addition of glycerol and suitable heating and drying treatments. Cheaper partially-purified skimmed soybean protein powder (SSP) and whey protein isolates (WPI) were used as the substitutes partially replacing the expensive SPI products, and purified β-lactoglobulin was taken as the positive control of WPI. As a result, the three alternatives could also form highly efficient edible films under the optimal operation conditions. The films made with SPI alternatives, about 50 μm thin, had homogenous network structures, without any holes by direct observation with the naked eye. The tests of the properties of these films showed that they had high water-keeping capacity and strong elasticity, that the ultimate tensile strength (TS) and the elongation at break (Eb) had been remarkably increased (TS>5 MPa, Eb>50 %), and that the prevention rates against the permeability of water vapour and oxygen in the air were also upgraded more than 85 and 70 %, respectively.

Key words: transglutaminase, edible films, soybean protein isolates, whey protein isolates, β-lactoglobulin, mechanical properties, barrier properties

Introduction

Edible coatings and films, acting as mechanical barriers, have the ability to protect the quality of food products from lipid oxidation, easy transfer of moisture, oxygen and carbon dioxide, and rapid loss of volatile flavours and aromas. Attempts using many kinds of natural or man-made biopolymers have been tried to prepare edible films (*1*). Among them, soybean proteins have been tested (*2*). Both chemical and enzymatic methods have been used to cross-link plant proteins to make films (*3*). Some chemical cross-linkers like formaldehyde and glutaraldehyde were reportedly effective in promoting film mechanical properties, and the results of the film formation were greatly dependent on the operating conditions such as glycerol/protein ratio (*4*). The enzymatic method, especially recently the use of transglutaminase (*5*) rather than peroxidase (*6*) to introduce covalent cross-links into protein films, has attracted much interests.

The transglutaminase (EC 2.3.2.13, TG, protein-glutamine γ-glutamyltransferase) catalyzes an acyl transfer reaction between the γ-carboxyamide group of glutamine residue of protein or polypeptide and a primary amine. The ε-amino group of lysine residue in a certain protein can perform as the amino donor and in a stoichiometric

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ratio forms an isopeptide bond of ε-(γ-glutamine)-lysine. This reaction introduces covalent cross-linkages between proteins as well as peptides, and therefore, significantly changes the structure, property and function of proteins. TG can be used to modify the functional properties of food proteins, including altering solubility and enhancing the mechanic properties. TG from liver of guinea pig and pig plasma and other animal tissues have been purified. However, because of low recovery and difficulty in isolation and purification from animals, more economic sources are needed to be developed for commercial utilization of TG. Japanese scholars first reported the production of microbial transglutaminase (MTG) by using microbial strains of *Streptoverticillium* sp. S-8112 (*7*), and achieved great economic benefit. Since then, the production, purification and application of MTG have attracted many researchers all over the world. Covalent bonding among protein molecules like gelatin proteins (*8*) and egg white proteins (*9*) by MTG catalysis was very effective in forming a self supporting network which then makes edible films. But the enzymatic cross-linking catalysis of MTG on soybean proteins alone or mixed with other proteins to form edible films has not been reported.

A new efficient MTG-producing strain, *Streptomyces* sp. WZFF.L-M1 was isolated from soil (*10*), and optimal conditions for production and purification of the MTG enzyme have been reported (*11*). For the purpose of developing the practical industrial applications of this MTG, and considering that soybean protein isolate (SPI) is a good material for food coatings with high nutrient values but only a small quantity (less than 10 %) of soybean protein is used directly in human food, we used SPI to prepare new kinds of functional SPI films by the MTG cross-linking treatment. Though SPI has reportedly a potential to form edible films with a valuable sensory evaluation, the SPI films with pure soybean globulins are usually defective in meeting the requirements for the practical food industry, and purified SPI is expensive for preparation in a large scale. Therefore, a partially purified skimmed soybean protein powder (SSP) was used and another economical material, partially purified whey protein isolate (WPI), was also tried to partially replace SPI. Whey is a coproduct of the casein and cheese industries, and more than 60 % of total proteins in whey are used for animal feed and low cost food applications. Here, purified β-lactoglobulin from whey proteins was comparatively tested with WPI as the positive control. Therefore, the objectives of this study are to develop a methodology to produce edible protein films by using MTG-catalyzed cross-linking reaction of SPI alone and with its cheaper substitutes through the investigation of mechanical properties and barrier properties of the formed films.

Materials and Methods

Chemicals

Bovine serum albumin (BSA), N-α-carbobenzoxyl-L--glutaminyl-glycine (CBZ-Gln-GLy), L-glutamic acid-γ--monohydroxamic acid (Glu-MHA) and phenyl methyl sulphonyl fluoride (PMSF) were purchased from Sigma. CM Sepharose CL-6B, Blue Sepharose CL-6B and Sepharose CL-6B were purchased from Pharmacia. All the reagents were at least of analytical reagent grade.

Preparation of base materials

SSP was prepared from ground soybean flour defatted three times at room temperature using hexane (soybean flour/hexane=1/4, by mass per volume). The extracted soybean protein was dissolved at 1/10 ratio (by mass per volume) in 50 mmol/L of phosphate buffer (pH=8.0) containing 10 % NaCl, 0.1 mmol/L of PMSF and 0.5 mmol/L of 2-mercaptoethanol, and then centrifuged at 4000 rpm for 20 min. Solid ammonium sulphate was added to the supernatant to 50 % saturation degree and then centrifuged. The precipitant was dissolved in phosphate buffer and dialyzed with distilled water overnight, then concentrated and dried. The protein content of SSP was (90.6±2.5) % on dry basis, moisture was 6.2 % and lipid content was less than 3.5 %.

SPI was purchased from a Chinese oil production company in Henan Province of China, or prepared from defatted flour by acid and salt precipitation, and separated by chromatography from a Sepharose CL-6B column as described previously (*12*). The SPI had a protein content of (90±2.1) % on dry basis. Moisture and lipid contents were 7.2 and 1.5 %, respectively.

WPI was prepared from skimmed milk powder at a ratio of 1/5 (by mass per volume) in 50 mmol/L of phosphate buffer (pH=6.5), by adjusting pH to 2.0 and adding 7.0 % NaCl, followed by cold stabilization, centrifugation and vacuum drying treatment. The WPI powder had a dry content of (91±1.5) % protein, 0.8 % lactose and sugars, 1.5 % lipid and 6.8 % moisture. The protein concentrations in WPI were 20 % α-lactoglobulin, 75 % β-lactoglobulin and 5 % bovine serum albumin.

β-lactoglobulin was prepared from skimmed milk powder as described previously (*13*), and had more than 90.2 % protein content.

Protein determination

Protein content was determined by the method of Lowry *et al.* (*14*) using BSA as a standard. For protein content of SPI standards, the Kjeldahl method was also used.

Microbial strain

The MTG-producing strain *Streptomyces* sp. WZZF.L--M1 (*10,11,15*) was newly isolated from the soil and preserved in our laboratories.

Production and purification of microbial transglutaminase

One loop of spore suspension of *Streptomyces* sp. WZFF.L-M1 picked from a slant containing organic salts--starch agar medium was activated in a 500-mL Erlenmeyer flask containing 100 mL of medium (starch 2.5 %, peptone 2.0 %, yeast extract 0.2 %, K_2HPO_4 0.2 %, NaH_2PO_4 0.2 %, $MgSO_4 \cdot 7H_2O$ 0.1 %, pH=7.0) and cultured with shaking (200 rpm) at 30 °C for 48 h. This culture suspension was then transferred at an inoculum ratio of 5 % to a 30-litre jar fermentor containing 20 L of fermentation

medium (K_2HPO_4 0.4 %, NaH_2PO_4 0.4 %, $MgSO_4 \cdot 7H_2O$ 0.1 %, glucose 1.5 %, starch 1.0 %, polypeptone 2.0 %, yeast extract 0.2 %, pH=7.0). After a 96-hour incubation at 30 °C with stirring at 300 rpm and aeration at 0.6–0.9 vvm (pH=6.6–6.8), the culture broth was filtered through a mechanical filter.

Solid ammonium sulphate was added into the filtered fluid. After stirring overnight, the filtered fluid was centrifuged. The precipitant was collected, dissolved in 50 mM phosphate buffer (pH=6.5), and further filtered through a 10-kD Amicon membrane ultrafiltration package unit. The supernatant was then put into a CM Sepharose CL-6B ion exchange column, and eluted with the buffer containing 0.2, 0.4 and 0.8 M NaCl using a stepwise method. The activity fractions containing MTG were collected, concentrated, then put into a Blue Sepharose CL-6B column, and eluted with 0.2–0.8 M NaCl-containing buffer using a linear gradient method. Finally, pure MTG product was isolated from the pooled eluates, and then dialyzed against 5 mmol/L of acetate buffer (pH= 5.0). After that, it was centrifuged using filter unit, and the pure MTG was stored at –4 °C.

Determination of enzyme activity

MTG activity was measured by the method described previously by Folk (*16*) with some modifications. Briefly, reaction mixture containing enzyme solution, Tris-acetate buffer (pH=6.0), hydroxylamine, and 0.1 mol/L CBZ-Gln--Gly was incubated for 15 min at 37 °C and then stopped by adding trichloroacetic acid containing 5 % $FeCl_3$. After 15 min of centrifugation, the supernatant was collected and the absorbance was measured at 525 nm. The standard curve was made by using Glu-MHA as a standard. One unit of MTG activity was defined as the amount of enzyme that can catalyze the formation of 1 μmol of hydroxamic acid within 1 min at 37 °C.

Formation of protein films

To prepare the edible films from soybean protein alone or with other three kinds of proteins enzymatically catalyzed by MTG purified above, all aqueous solutions of base proteins were first heat-denatured in an instant treatment (95 °C, 5 min in a water bath). These various fractions were cooled to room temperature, mixed at the necessary ratio, and adjusted to the pH at which MTG could effectively catalyze. MTG was added at 5–20 U/g protein, and allowed to react at the suitable temperature for certain time. To the enzymatically cross--linked solutions, 0.5–2.0 % (by mass per volume) of glycerol and 0.005 % (by mass per volume) of sodium azide were added, then adjusted to pH=8 and heated at 80 °C for 20 min in a water bath. Vacuum was then applied to remove dissolved air, and the film-forming solutions containing 2.5 g of solids were pipetted on a stainless steel plate (15×15 cm) to minimize thickness variations between treatments. The solutions were spread evenly with a glass rod and dried in a chamber at the conditions of 50 °C, (50±5) % relative humidity (RH) overnight, and the dried films were peeled carefully from the casting surface. Then these film products were left at 20 °C, (45±5) % RH for one day and used for property analysis.

Film thickness measurement

Film thickness was measured using a micrometer with a sensitivity of 0.15 μm. Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of the contact. Mean thickness of the films was determined from the average of measurements at five locations.

Tensile strength measurement

Mechanical properties were analyzed by three duplicates for each tested film. Tensile strength (TS) and elongation at break (Eb) of the film were measured using a texture analyzer QTS-25. Film samples were conditioned at ambient temperature and 50 % of RH for at least 24 h prior to textural analyses (ASTMD 882-1991) (*17*). TS measurements were performed by mounting the film strips (40×5.5 mm) on the texture analyzer. Initial grip separation was 25 mm and crossed speed was set at 2 mm/s in tension mode. TS was computed as peak force divided by cross sectional area of a specimen. Eb was expressed in percentage of the initial length (L_0=25 mm) of the piece tested.

Measurement of film transparency

The film prepared with the same material was stretched out on a clear A4 paper on which at least two or three ranks of Chinese words in No. 10 size were typed at standard intervals. Then the film was folded layer by layer until it was impossible to read the word clearly, and the number of film layers was recorded as a comparison of film transparency.

Measurement of water solubility of the film

Solubility was determined in a buffered solution by using small pieces of films (20–30 mg) placed in 5 mL of 0.1 mol/L buffered solutions (pH=6.8). Three randomly selected samples from each type of film samples were first conditioned at ambient temperature and 50 % RH for at least 24 h prior to solubility test, and the initial dry matter (dried at 105 °C) was weighed. After drying, films were immersed in 30 mL of the same buffered solution in a 50-mL beaker. Beakers were covered with Parafilm® wrap and stored in an environmental chamber at 25 °C or sometimes at room temperature (20–25 °C) for 24 h. Undissolved dry matter was determined by removing the film pieces from the beakers, gently rinsing them with distilled water, and then oven drying (50 °C, 12 h). The mass of solubilized matter (S) was calculated by subtracting the mass of unsolubilized dry matter from the initial dry matter and expressed as a percentage of the initial dry matter content. Dry mass (DM) was determined at least in triplicate for each film type. The percentage of solubility was calculated as follows:

$$S = \frac{(\text{initial film DM} - \text{final film DM}) \times 100}{\text{initial film DM}} / \% \quad /1/$$

Water vapour permeability (WVP) measurement

The gravimetric modification cup method based on ASTM E96-1995 (*18*) was used to determine WVP. A volume of 6 mL of deionized water was pipetted into test

cups made of polymethylmethacrylate. Films without pinholes and any defects were placed between the cup and the ring cover of each cup coated with silicon sealant, and held with 4 screws around the cup. After that, the cups were placed in cabinets with constant RH (0 %, using anhydrous calcium sulphate) containing fans, located in a room with temperature controlled at 25 °C. Once steady-state moisture transfer was obtained, masses for measurement were taken at 2-hour intervals. The WVP value of the film was calculated by multiplying the steady-state water vapour transmission rate with the film thickness and dividing by the water vapour partial pressure difference across the film.

$$WVP=WVTR\times thickness/(P_{a1}-P_{a2}) \qquad /2/$$

where WVTR is water vapour transmission rate, and P_{a1} and P_{a2} are water vapour partial pressure inside and outside the cup, respectively.

Oxygen permeability measurement

Oxygen transmission rate (OTR, $cm^3/(m^2\cdot day)$ was determined using an Ox-Trans permeability tester with a modified method (*19*). Samples were tested at 15, 25 and 35 °C with both sides of the film maintained at predetermined RH levels. In order to prevent the coulometric sensor from overloading, a 0.5 % O_2 in N_2 gas mixture was used as the upstream gas. Since the flow rate was low (10 mL/min), the partial pressure difference (ΔP) across the films was essentially 0.005 atm (507 Pa). Oxygen permeability coefficients, Po_2 $(cm^3\cdot cm)/(m^2\cdot day\cdot kPa)$ were calculated by multiplying OTR by film thickness and dividing by ΔP.

Statistical analysis

Each replication process was undertaken from individual film preparation to film formation. At least three replications were performed in a completely randomized design. A minimum of three observations was collected unless specified otherwise. Data were analyzed using the general procedure of a linear processing model to determine differences between treatment methods. Pairwise comparison of all treatment methods was performed using the least significant difference (LSD) procedure with significance defined at p=0.05.

Results and Discussion

Production and purification of microbial transglutaminase

After a 4-day incubation of *Streptomyces* sp. WZZF.L--M1 in a 30-litre jar fermentor containing 20 L of fermentation broth for the production of MTG, the MTG activity reached 2.73 U/mL. MTG was purified about 109.4--fold, and the final recovery ratio and specific activity were 26.2 % and 12.03 U/mg protein, respectively. The final purified MTG and partially purified enzyme products were yellow. They were used for the preparation of edible soybean protein films. The results are shown in Table 1.

Film formation of soybean proteins by MTG cross-linking catalysis

It has been suggested that after heat denaturation proteins are considered to be partially unfolded, more flexible and susceptible to MTG attack (*20*) due to heat treatment inducing structure denaturation, exposing internal sulphydryl groups to promote intermolecular disulphide bonds, and releasing both glutamine residues and lysine residues to promote MTG-catalyzed intermolecular cross-linking reaction. Thus, all the base protein solutions tested in this work were first treated thermally. The results shown below confirm that the protein preheating effectively enhanced MTG-catalyzed cross-linking and allowed formation of film with greater strength and barrier properties than expected.

As shown in Fig. 1, the films obtained from SPI with or without the other three proteins were very thin,

Fig. 1. Six layers of MTG-catalyzed SPI films over a Chinese identity card, indicating their clear transparent degrees

Table 1. Summary of the purification of MTG from the strain *Streptomyces* sp.

No.	Purification steps	Total protein absorbance ($A_{280\ nm}$)	Total MTG activity / U	Specific activity ($U/A_{280\ nm}$)	Purification / fold	Recovery / %
1	Culture broth	496800	54650	0.11	–	100.0
2	Filtration	38260	53560	0.14	1.27	98.0
3	$(NH_4)_2SO_4$ precipitation	75140	48070	0.64	5.82	87.9
4	Amicon ultrafiltration	11570	39350 (crude MTG)	3.40	30.90	72.0
5	CM Sepharose ion exchange chromatography	2880	24590 (partially purified MTG)	8.54	77.60	45.0
6	Blue Sepharose chromatography	1190	14310 (purified MTG)	12.03	109.40	26.2

flexible, glossy and translucent, and had a smooth shiny surface without any holes observed directly with the naked eye.

The analysis of the mechanical properties of SPI films as the single substrate in film-forming solution at different concentrations (1.5, 4.5 and 7.5 %) showed that the mean values of thickness of SPI were 44, 52 and 64 μm, respectively (Table 2), and that they increased with the amount of SPI added.

Mechanical properties of edible protein films provided an indication of expected film integrity under the conditions of stress that would occur during practical uses in processing, handling and storage. As a result of MTG catalysis, TS values of MTG-treated samples were much greater than that of control without the addition of MTG.

MTG cross-linked SPI films appeared to have much higher tensile force, and enhanced more than 80 % with the increase of SPI addition from 1.5 to 4.5 %, which was 2–3 times greater than that of films (*21*) made from wheat gluten catalyzed by MTG of Ajinomoto Co., Inc. (Tokyo, Japan). These results were also accompanied with the increase of Eb. However, the two parameters had no significant difference between 4.5 and 7.5 % of SPI. Therefore, 4.5 % of SPI was chosen for next experiments.

The solubility of MTG-treated films was also analyzed, and the data showed that the action of MTG induced a remarkable decrease in the water solubility of the films, and very little solubilized matter could be checked out. Therefore, the analysis of water solubility of films was not carried out further.

Formation of SPI with other proteins

Although SPI was good enough to form edible films with efficient values mentioned above, the properties of film preparations using only SPI are usually not enough to satisfy the requirements of food industry, and in particular, purified soybean globulins are expensive and difficult to be used in a large scale as required in food processing. Thus, economical SSP and WPI were tested as the substitutes to partially replace SPI, and β-lactoglobulin was used as a positive control for WPI. The results in Table 3 show that the purified MTG product could catalyze these protein materials to form efficient films. Some significant statistical differences ($p>0.05$) were found among different degrees of thickness of the prepared films. The results suggest that the film preparation method was suitable, resulting in films with reasonably uniform thickness.

The MTG catalytic action also increased both TS and Eb about twice compared to that without the addition of the enzyme, and these results were nearly consistent with the results reported previously (*8*). Although these results were further analyzed by using multiple range tests, which permitted the identification of groups among the different protein types, and their meanings

Table 2. Formation of films with different SPI concentration

Concentration of SPI/%	Thickness / μm	TS / MPa	Eb / %	Transparency / fold	Water solubility / %
1.5	44±1.4a	3.4±0.5b	19.6±2.1a	15±0.8a	2.8±0.9a
4.5	52±0.9b	6.2±0.9c	45.1±4.1b	17±1.1a	2.4±1.0a
7.5	64±1.9c	6.5±2.1c	51.6±3.7b	16±1.0a	3.7±1.4b
4.5-MTG	68±3.7c	2.4±0.9a	17.8±3.8a	14±0.8a	13.3±2.7c

Means of at least three replicates±standard deviation. TS: tensile strength, Eb: elongation at break. Enzymatic treatment conditions: MTG concentration 10 U/mg protein, pH=7.0, 30 °C, 1 h. Glycerol addition 1.5 %, films heated at pH=8.0, 80 °C, 20 min. 4.5-MTG – no MTG addition as a control. a, b, c – the different level of the data in the same lane with a significant difference ($p<0.5$) between a and b, b and c

Table 3. Formation of SPI films with other base materials

No.	Fraction of protein / %	Thickness / μm	TS / MPa	Eb / %	Transparency / fold
1	SPI 4.5	54±2.9b	5.7±1.1c	44.5±5.6a	18
2	SPI 3.0+SSP 1.5	53±3.4b	6.1±1.6c	44.6±7.8a	18
3	SPI 1.5+SSP 3.0	48±2.1b	4.5±0.7b	39.5±11.1a	16
4	SSP 4.5	57±4.3bc	3.7±0.7a	41.1±12.8a	14
5	SPI 3.0+WPI 1.5	61±5.6bc	7.8±2.2d	60.1±6.8b	17
6	SPI 1.5+WPI 3.0	49±3.0b	6.9±1.1cd	66.5±13.4b	18
7	WPI 4.5	60±4.4bc	5.6±1.2c	49.0±10.5a	16
8	SPI 3.0+βlg 1.5	50±5.1b	10.1±3.1e	74.2±22.3bc	20
9	SPI 1.5+βlg 3.0	41±2.5a	13.2±2.1f	82.4±19.9c	20
10	βlg 4.5	47±2.7b	8.7±2.3d	67.5±17.6b	18

Means of at least three replicates±standard deviation. Conditions of film formation were the same as in Table 2. SPI: soybean protein isolate, SSP: skimmed soybean protein powder, WPI: whey protein isolate, βlg: β-lactoglobulin

were still unclear, the general contributions of MTG catalytic function can suggest that the formation of covalent linkages reinforces the film and makes it more flexible for stretching. These results, obtained from various proteins characterized by different amino acid compositions and structures, clearly indicate that these effects on TS and Eb reflected the formation of new isopeptidic bonds in the films. In particular, the TS and Eb values in both SPI/SSP and SPI/WPI mixture films were at the same level or higher than those of single SPI films, which were better than expected. Both TS and Eb of MTG-treated films were higher than those of the non-cross-linked films, meaning that these mixed films were successfully endowed with high mechanical properties, which may have great economic potential in future.

Effect of MTG concentration on the properties of films

Statistical analysis of the results in Table 4 shows that MTG treatment had significant effects on TS and Eb ($p<0.05$). As a result of MTG catalysis function, TS values of MTG-treated samples were much greater than those of the control, and increased relative to the increase of the amount of the used enzyme, meaning that these films are endowed with high elasticity due to enzymatic cross-linking. Therefore, it is further clear that the introduction of covalent isopeptide bonds into the soybean protein structure increases the strength of films.

Table 4. Effect of MTG concentration on SPI film formation

MTG (U/g SPI)	TS MPa	Eb %	WVP	OP×10^{11}
0	2.5±0.7a	18.1±4.1a	112.3±13.3c	46.4±13.4c
5	6.5±0.9b	47.8±5.6b	14.4±2.6b	12.2±2.3d
10	12.3±1.1c	76.4±20.9c	7.4±0.9a	4.7±1.1a
20	14.5±1.0c	87.5±17.7c	6.1±1.1a	3.5±0.8a

Means of at least three replicates±standard deviation. Conditions of film formation were the same as in Table 2. SPI addition 4.5 %. WVP/((g·mm)/(m^2·day·kPa)): water vapour permeability, data from the measurement conditions: 25 °C, (55±5) % RH. OP/((cm^3·cm)/(cm^2·s·kPa)): oxygen permeability, data from the measurement conditions: 35 °C, (55±5) % RH

TS and Eb reflect the tension and extensibility of the material, respectively. Table 4 shows that the enhancing effect of MTG treatment on TS and Eb was significant compared to other results reported with animal transglutaminase (*22*). Because of the hydrophilic nature of SPI proteins, the WVP values of such films were usually much higher than those of standard packaging films. However, barrier properties of the protein films tested in this work, including WVP and OP, were good under low RH conditions.

WVP values of SPI films were compared first. In general, these kinds of protein films were not good enough as water vapour barriers, due to the inherent high hydrophilicity of proteins and the substantial amount of hydrophilic plasticizers added to protein films. However, MTG-catalyzed edible SPI films were different from the control ($p<0.05$), and the WVP considerably decreased 6.7 to 17.4 times.

The effects of MTG catalyzing time on the mechanical property (tensile strength) and barrier property (water vapour transmission) of SPI films are shown in Fig. 2. As the time of MTG enzymatic treatment went on, WVP value of the films went down sharply in 60 min, while TS increased almost in a straight line.

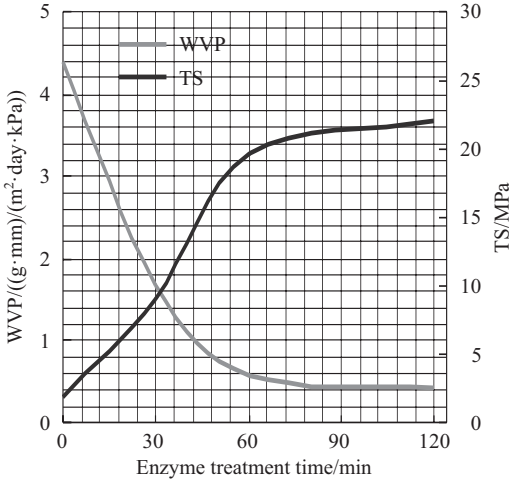

Fig 2. Changes of WVP and TS of 4.5 % SPI films formed at different MTG treatment times. Enzymatic treatment conditions were the same as in Table 2, except for the MTG treatment time

The MTG addition also resulted in the enhancement of the barrier property of oxygen permeability of SPI films. These SPI films had extremely low oxygen permeability values, showing a significant ability to restrict oxygen transmission, indicating a modification of the surface hydrophobicity of the films. The capacity of the SPI films and other protein films to prevent oxygen gas transmission was 40–80 % lower than of soybean protein-wheat gluten protein films (*23*) and they were more than ten times better barrier than gluten films treated with MTG (*9*). But at the same time, they were ten to hundreds of times higher than the results of the films reported to be made from β-lactoglobulin without enzymatic treatment but with high concentration of glycerol (*24*), where glycerol was indicated to play a very important role in improving film properties.

Effect of glycerol concentration on the properties of films

As commonly shown, biopolymer films from animal and plant proteins without any plasticizer are brittle and cannot be handled. Among the usual plasticizers used, glycerol was generally first to be chosen and added to improve their flexibility and stretchability through decreasing the intermolecular forces acting along polymer chains. When the proportion of glycerol was increased, a slow strengthening of the maximum stress (TS) and a fast enhancement in the strain at break (Eb) were observed (Table 5). This could be in agreement with a plasticizing effect of glycerol, but it is in contradiction in some way to other protein materials (*21*).

Increased levels of glycerol resulted in decreased barrier properties of films within each treatment. A signifi-

Table 5. Effect of glycerol concentration on film formation

No.	Film base materials and fractions	Thickness / μm	TS / MPa	Eb / %	WVP	OP
	Glycerol concentration 0.5 %					
1	SPI (4.5 %)	53	4.4±1.0a	26.3±9.6a	7.2±1.3b	8.9±1.9b
2	SPI/SSP=1/2	49	5.1±1.6a	31.5±10.5a	4.5±1.4c	13.4±2.1c
3	SPI/WPI=1/2	56	5.7±1.2a	38.3±8.1a	2.7±0.9ab	7.7±2.0b
	Glycerol concentration 1.5 %					
4	SPI (4.5 %)	59	7.8±1.8b	69.3±21.6b	1.9±0.4a	2.2±0.6a
5	SPI/SSP=1/2	52	8.7±6.2bc	78.2±19.5bc	1.1±0.3a	3.3±1.4a
6	SPI/WPI=1/2	57	9.5±4.5bc	85.5±30.1bc	1.0±0.3a	2.7±0.2a

Means of at least three replicates±standard deviation. Conditions of film formation were the same as in Table 2

cant interaction between MTG treatment and glycerol addition (1.5 %) was found in both WVP and OP. Every kind of film made from SPI only or mixed with SSP or WPI containing higher concentration of glycerol had better water vapour and oxygen barrier functions, which is suggested to be due to their highly cross-linked structures.

In addition, MTG cross-linking induced the different decreasing degrees of WVP and OP of various films, which means that a possible variation of the protein orientation after cross-linkage or a modification of the number or pore size has occurred and needs to be confirmed in the future.

Conclusion

In this work, edible protein films were prepared from soybean protein isolate with or without whey proteins by an enzymatic cross-linking catalysis with a purified Ca^{2+}-independent microbial transglutaminase produced from an effective strain *Streptomyces* sp. WZFF.L-M1. This study showed that MTG was effective in introducing covalent bonds into soybean proteins and whey proteins intermolecularly to form edible films. Instant preheating treatment could promote the catalysis reaction of MTG. Glycerol imparted flexibility, reduced brittleness, and increased toughness and strength of films by reducing intermolecular forces between adjacent polymer chains. It also showed that MTG was efficient in increasing the functional properties of the SPI films. The increase of MTG concentration and the time of the cross-linking reaction enhanced the functional properties of the films.

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