academicJournals

Vol. 9(9), pp. 391-399, 15 May, 2014
DOI: 10.5897/SRE2014.5951
Article Number: AA953F244570
ISSN 1992-2248 © 2014

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Characterization of WPI-NaCas composite films modified by transglutaminase

Lei Qiao[1,2], Pan Jiazhen[1]*, Huang Zhiying[2], Bao Jianqiang[2] and Xun Qiannan[2]

[1]School of Mechanical and Power Engineering, East China University of Science and Technology, Shanghai 200237, China.
[2]College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China.

Received 3 January, 2013; Accepted 2 March, 2013

Synergistic effects of additive amount, reaction time and temperature of transglutaminase on properties of WPI-NaCas composite protein films were discussed by orthogonal experiments. Results indicated that transglutaminase (TGase) modification decreased D50 and D90 of film-forming solution particles, resulting in decrease in water solubility and increase in both mechanical and water barrier properties of films under most conditions. Compared with control sample, scanning electron microscope analysis indicated that cross-section of enzyme modification sample was denser and more impermeable. However, there was no significant difference in both thermogravimetric analysis and solution apparent viscosity changes for all samples. Orthogonal experiment results also showed that TGase reaction time was the most effective factor on tensile strength (TS) and water vapor permeability (WVP) properties of film, and the secondary, tertiary factors were reaction temperature, TGase additive amount respectively. Films modified by 0.1 mg/ml TGase at 50°C for 30 min exhibited the optimum mechanical and water barrier performance.

Key words: Transglutaminase (TGase), mechanical properties, water vapor permeability (WVP), scanning electron microscopy analysis.

INTRODUCTION

Edible films receive great attention in recent years because of the increasing consumer demand for high quality foods, the need of food processing for new storing technology, dealing of non-renewable food packaging materials and the use of agricultural waste products for film-forming components (Ferreira et al., 2009; Liu et al., 2006). Among various edible films, great interest is given to protein based films because of their moderate barrier properties of water, oil volatile components, selective gas permeability and unique nutritional functions. Proteins are polymers with specific amino acid sequences and molecular structure. Depending on the sequential order of the amino acids, the protein will assume different structures along the polymer chain which will determine the secondary, tertiary, and quaternary structures. The secondary, tertiary, and quaternary structures of proteins can be easily modified to optimize the protein configuration, protein interactions, and resulting film

*Corresponding author. E-mail: jzpan@ecust.edu.cn

properties. Films based on proteins are edible or biodegradable, depending on formulation, formation method, and modification treatments. As long as food-grade proteins and additives are used only protein changes due to heating, pH modification, salt addition, enzymatic modification, and water removal occur, the resulting film is edible (Baldwin et al., 2012).

Whey protein isolate (WPI) obtained from whey-a co-product of cheese-making and casein manufacture in the dairy industry, consists of special nutritional composition and exhibits good film forming characteristics, while WPI-based films shows excellent oxygen barrier at low or intermediate RH (Oses et al., 2009).

Sodium caseinate (NaCas) is one kind of soluble calcium phosphate compound, which contains a variety of amino acids and trace elements that the human body needs, showing good film forming properties and water solubility (Yu et al., 2009). Caseins form films from aqueous solutions without further treatment due to their random-coil nature and their ability to hydrogen bond. Extensive hydrogen and electrostatic bonds and hydrophobic interactions facilitate the formation of intermolecular interactions to form casein edible films.

WPI-NaCas based edible films show better barrier properties of gas, optical properties, water solubility, but lower mechanical properties and barrier properties of water vapor than polyethylene (PE) due to a synergistic effect (Chen and Lei, 2011).

Transglutaminase (TGase) is a kind of bio-enzyme and can catalyze the formation of covalent linkage through an acyl-transfer reaction intra- or inter- protein molecular to changing structure of protein, forming high polymer and improving properties of protein, thereby ameliorating the rheology and sensory characteristics of food (Kuraishi et al., 2001; Mahmoud and Savello, 1993; Motoki et a., 1987; Nielsen et al., 1995). Research also showed that TGase could ameliorate the properties of edible films, for example improving tensile strength, elongation at break and decreasing moisture content, water vapor permeability of soy protein isolate- wheat gluten edible film (Wang et al., 2009). TGase has been used to decrease the solubility of the films (Carvalho and Grosso, 2004; Yildirim and Hettiarachchy, 1998). However, the results (regarding the effect of cross-linking), using TGase, on the water vapor permeability (WVP) and mechanical properties were inconsistent, probably due to different protein components of the films, various conditions of enzymatic reaction and preparation of films.

Our previous experiments by uniform design method showed that the edible WPI-NaCas films were smooth, transparent and showed acceptable mechanical properties and good barrier properties to gas higher than PE. The increases of WPI concentration resulted in a decrease in water solubility and gas barriers. Sodium caseinate was efficient for improving transparency and water solubility, though decreasing the barrier properties to some extent. The addition of glycerol provoked an increase of elongation, water solubility and flexibility, while film tensile strength exhibited a reverse trend. Films prepared with 5% whey protein isolate, 2% sodium caseinate, 50% glycerol at 50°C showed moderate mechanical properties, optical properties, water solubility and maximum barrier properties of gas and water vapor, with tensile strength=5.85 MPa, elongation=101.20%, transparency=91.4%, gas permeability rate=49.92 $cm^3 \cdot m^{-2} \cdot d^{-1} \cdot 0.1\ MPa^{-1}$ and water vapor permeability were $1.252 \times 10^{-11} g \cdot m^{-1} \cdot s^{-1} \cdot pa^{-1}$, (RH:10%/70%), respectively. It is possible that they may be alternatives to some plastics, as inner packaging materials for food with low water activity (Chen and Lei, 2011). The unfavorable properties of protein films are excessive water solubility and poor WVP compared with plastic. The possibility of using TGase as a modifying agent of WPI-NaCas films was estimated to improve their resistance to solubility and water vapor.

The objective of this work was to find the influence of additive amount, reaction time and temperature of TGase on properties of WPI-NaCas films, so as to design biodegradable material with good mechanical and barrier properties, suitable for packages of many kinds of food products with different acidities and contents of moisture.

MATERIALS AND METHODS

Whey protein isolate (WPI, protein > 92.0%, Hilmar Company, California, USA) and Sodium caseinate (NaCas, with 92.9% protein, Murray Goulburn Company, Australia) were used as film-forming components. Glycerol (GLY, analytical grade, SCRC, China) was used as a plasticizer to improve the flexibility properties of the films. Transglutaminase was obtained from Dongsheng Food Technological Company in China.

Orthogonal experiment of enzymatic modifications

Orthogonal experiment in three factors and three levels was designed based on the results of single-factor experiment, with the unmodified experimental group as control group. Orthogonal test schedule was shown in Table 1.

Films preparation

WPI solution was prepared by dispersing 5 g protein powder in 100 ml distilled water, stirring continuously at room temperature for 2 h with a magnetic stirring apparatus (ZNCL-T, Qiangqiang instrument corporation, Shanghai, China). Then the solution was placed in a water bath and kept at 85°C for 30 min to denature the protein and then cooled down to room temperature until the powders completely dissolved. NaCas solution was prepared by dispersing 5 g sodium caseinate powder in 100 ml distilled water at room temperature until the powders were completely dissolved. GLY was added as 30% (w/w) of the total polymers. The blending ratio of WPI solution and NaCas solution was 1:1 (v/v). WPI, NaCas and GLY were completely mixed, to achieving the composite protein solution. TGase was added into the solution with reaction parameters shown in Table 1, and then inactivated at 75°C for 15 min. After cooling down to room temperature, the composite solution was degassed by vacuum pump and casted on organic glass plates, then heated

Table 1. Orthogonal test schedule.

Test number	TGase reaction time/min (A)	TGase additive amount/mg/ml (B)	TGase reaction temperature/°C (C)
01	22.5(1)	0.10(1)	40(1)
02	22.5(1)	0.15(2)	50(2)
03	22.5(1)	0.20(3)	60(3)
04	30.0(2)	0.10(1)	50(2)
05	30.0(2)	0.15(2)	60(3)
06	30.0(2)	0.20(3)	40(1)
07	37.5(3)	0.10(1)	60(3)
08	37.5(3)	0.15(2)	40(1)
09	37.5(3)	0.20(3)	50(2)
10	0.0	0.00	0

at 50°C in air blow drying cabinet (GX-ZGF101, Hede Co. Ltd, shanghai, china). The films obtained were peeled from the plates and conditioned in a thermostatic and humidistatic chamber (GDS-100L, Suoyate Co. Ltd., Jiangsu, China) at 50 ± 5% RH and 23 ± 2°C for no less than 48 h prior to testing.

Properties testing

Particle size distribution

Particle size distribution of film-forming solution was measured using laser particle analyzer (BT-9300HT, BaiTe Co. Ltd, Dandong, China), with optical mode, analysis mode and medium set as Mie, multimodal and water. Four measurements of each sample were taken, then calculating the average value.

The apparent viscosity of film-forming solution

The apparent viscosities of film-forming solutions were measured by Rheometer (Anton Paar, Austria) at 25°C, with shearing rate: 0 to 100 rpm/s, testing system: CC27-SN23937, equipment TU1=C-PTD200-SN80910176. Each sample was 40 ml.

Mechanical properties

Tensile strength (TS) and elongation at break (EB) were measured using a computer control tensile testing machine (DCP-KZ300, CDMC Co. Ltd., Sichuan, China). Samples were cut into 15 × 100 mm pieces. According to ASTM D882-09 (ASTM, 2009), the initial grip separation was set at 50 mm, with cross-head speed 500 mm/min. At least ten samples of each type of film were measured. Tensile strength was calculated by this formula:

$$TS = F/S \quad (1)$$

Where, F is the force when the film was broken, S is the cross section area.

Water solubility

The method modified from Rhim (2004) was used to determine water solubility (WS). Samples were cut into 30 × 30 mm pieces and firstly dried at 105°C for 24 h in an air blow drying cabinet (GX-ZGF101, Hede Co. Ltd., Shanghai, China) to obtain the initial weight w_1. After drying, the films were submerged with 50 ml water in 100 ml breakers for 24 h at room temperature. Undissolved dry matters were removed from the breakers and dried at 105°C again until they reach a constant weigh (±0.0001 g). The final dry weight was taken as w_2. Each type of film was determined in triplicate.

$$WS(\%)=(w_1- w_2) / w_1\times100 \quad (2)$$

Water vapor permeability

The water vapor permeability (WVP) of films was measured according to ASTM E398 (ASTM, 2003), standardized by a water permeability tester (PERMATRAN-W Model 1/50G, MOCON, USA). Tank pressure was set to 30 ± 2 psig (approximately 0.2 MPa). The 100%RH test cell and saturated pad were used in correction test. Test mode was set to continuous, with test temperature 37.8°C, and RH at two sides of tested films was 10%/50% respectively.

Thermogravimetric analysis (TGA)

Thermogravimetric measurement was carried out on a thermogravimetric analyzer (TG209 F1, NETZSCN, Germany) in the temperature range 35 to 500°C at heating rate of 10 K/min. Mass of each tested sample was 3 to 5 mg, while flow-rate of sweep gas and protective gas (both were nitrogen) were 30 and 20 ml/min, respectively.

Scanning electron microscopy

Cross-section morphologies of films were obtained by scanning electron microscope (S3400N, Hitachi Co. Ltd, Japan) with gold-plating time under vacuum state, accelerating potential, amplification factor were 10 min, 10 kv, 2000, respectively.

RESULTS AND DISCUSSION

Particle size distribution of film-forming solutions

No significant difference was observed in film-forming solutions of 01-09 experimental groups (Figure 1). However, reductions in both median particle (D50) and

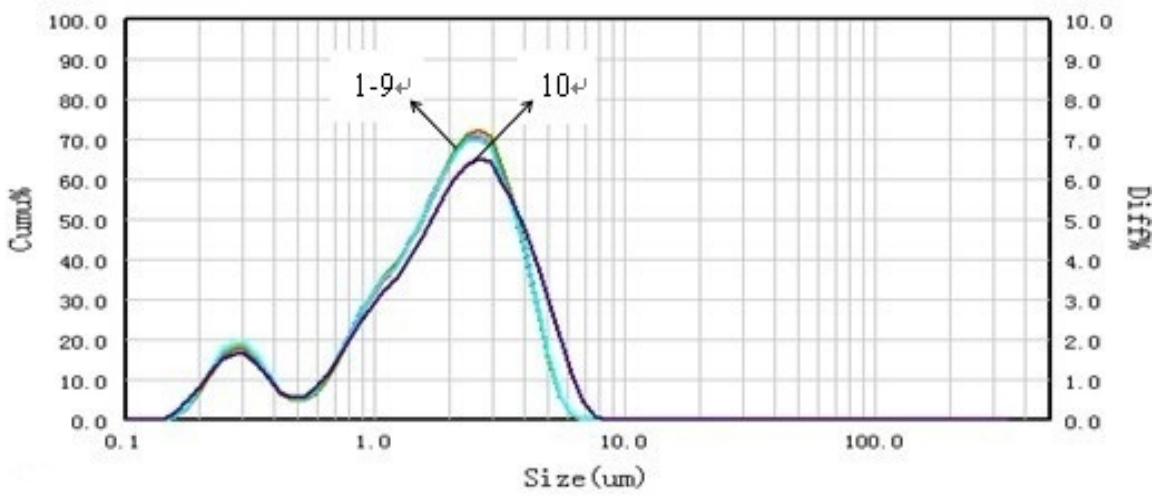

Figure 1. Particle size dispersion of film forming solutions.

Table 2. Particle size of film forming solutions.

Sample number	D10	D50	D90	D4.3 (um)	D3.2 (um)	AN (m^2/g)
01	0.426	1.971	3.783	2.079	1.125	1.719
02	0.401	1.960	3.776	2.067	1.109	1.744
03	0.415	1.950	3.762	2.060	1.114	1.737
04	0.442	1.968	3.777	2.077	1.132	1.709
05	0.385	1.948	3.774	2.057	1.091	1.772
06	0.428	1.961	3.769	2.070	1.123	1.721
07	0.435	1.971	3.777	2.079	1.131	1.710
08	0.408	1.982	3.762	2.079	1.122	1.724
09	0.383	1.943	3.768	2.052	1.088	1.777
10	0.417	2.080	4.305	2.271	1.136	1.702

volume average diameter (D4,3) were compared with control group (Table 2); the reduction resulted from the hydrolysis or cross-linking reaction of protein, causing the change of particle size value by the addition of TGase in the film preparation process.

Rheology characteristics of film-forming solutions

Intermediate concentration for film solutions will generally result in the highest cohesive strength as a compromise between optimal viscosity and polymer salvation. The functionality of the polymer is related to solvent characteristics. Maximum coating, solution salvation and polymer chain extension will produce the most efficient films. A high sol viscosity is a good indication of the adequacy of salvation and chain extension according to the formulation and method of preparation prior to film application. The apparent viscosity of WPI-NaCas composite film-forming solutions remained higher preventing deposition of solution particles at low shearing rates, while with the increase of shearing rates, molecule forces weakened and free space enlarged which directly caused a decrease of viscosity. Results indicated that experimental samples belong to non-Newtonian fluid with little lower viscosity than that of control sample due to the effects of TGase (Figure 2).

Effects of TGase on mechanical properties of films

Except for Group 1 and 8 (lower to control group for inadequate reaction time and TGase addition) and Group 9, TS of other groups significantly increased by about 25～50% after TGase modification, which associated to the cross-linking reaction of protein caused by TGase, and forming covalent bond, thereby improving TS and decreasing flexibility of films (Figure 3). On the other hand, the elongations at break of most of the enzymatic modified films were about 2～3 times higher than the

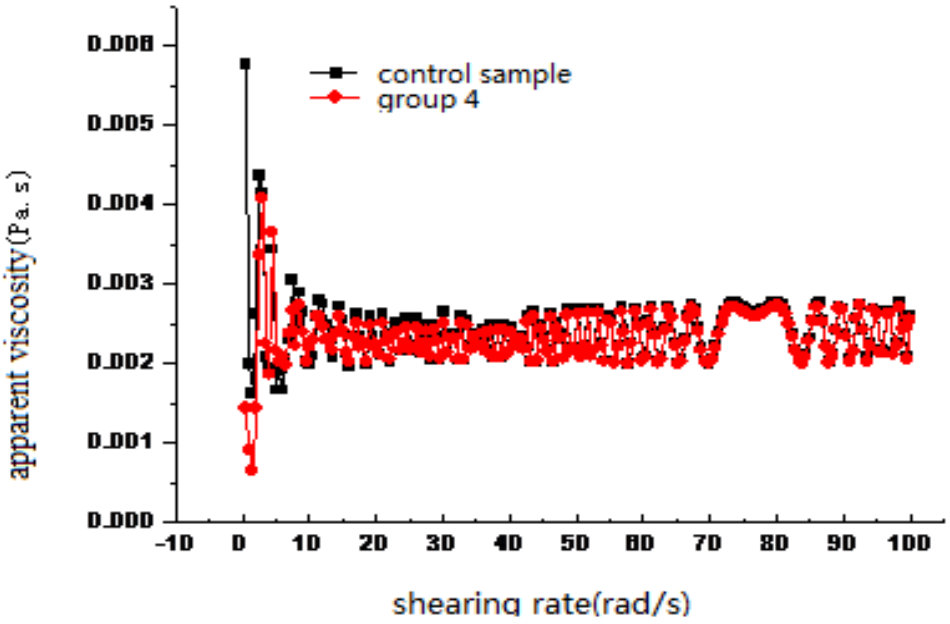

Figure 2. The apparent viscosity of film solutions.

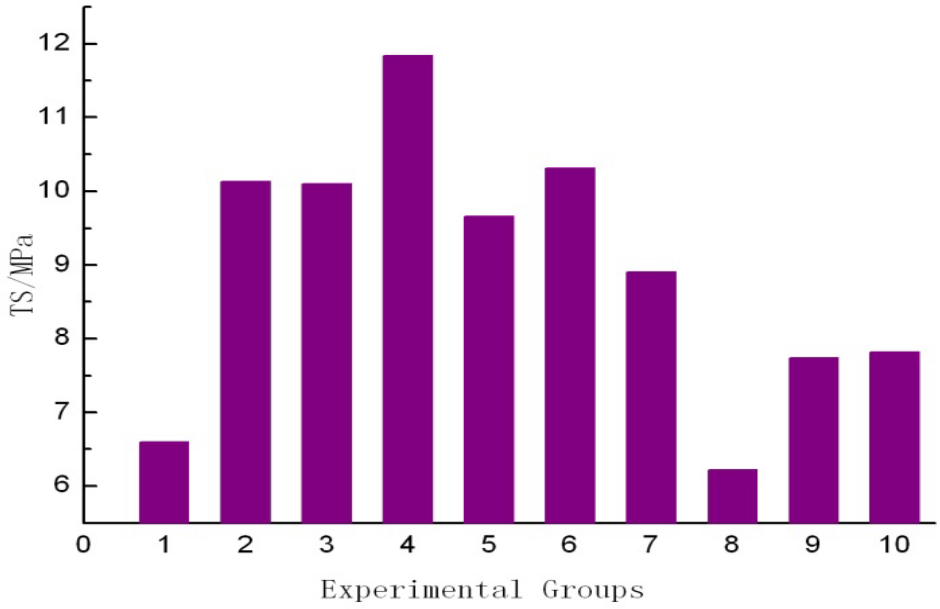

Figure 3. Tensile strength of different films.

control one except Group 2, 4 and 6, differently from TS (Figure 4). However, higher reaction temperature especially at 60°C allowed better improvements in both TS and elongation (Groups 3, 5 and 7).

Without adequate content of TGase or experimental reaction condition, the cross-linking reaction did not germinate intra- or inter-molecular, then forming heteropolymer, which resulted in a decrease in mechanical properties of films.

Effect of TGase on water solubility of films

The addition of TGase resulted in a marked decrease in WS of WPI-NaCas films (Figure 5), from 44.51% (unmodified samples) to 28.62% (Group 4), which

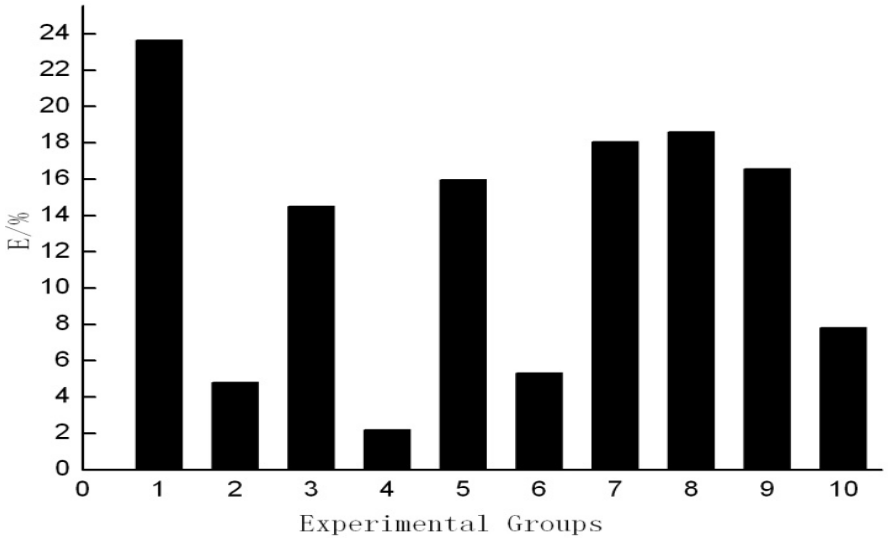

Figure 4. Elongation of different films.

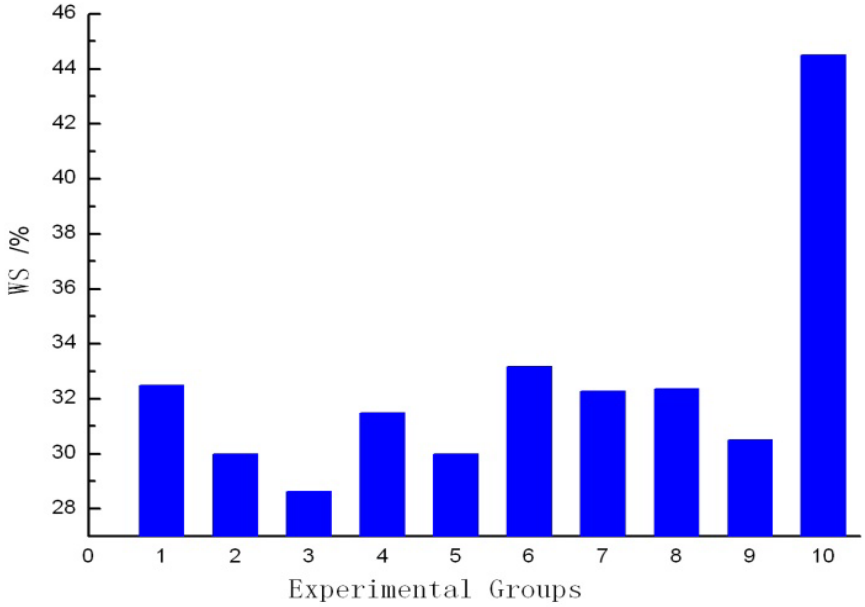

Figure 5. Water solubility of different films.

corresponded to the results of Kołodziejska' research (Kołodziejska and Piotrowska, 2007). This can be explained that cross-linking reaction of protein caused by TGase resulted in non exposition of hydrophilic groups, which decreased WS of films. And the unfolded structure of enzyme-heat-denatured proteins and the covalent S-S bonding during drying led to film insolubility in water and produced films that were stronger and could withstand higher deformations.

Water-resistance of modified films was improved, however, no statistically significant difference of WS was observed from Group 1 to 9, with the lowest value of 28.62% (Group 3) and the highest of 33.16% (Group 6). On the other hand, the higher enzyme reaction temperature and additive amount rather than enzyme reaction time caused further reduction in solubility of the films.

WVP of films modified by TGase

Protein based films generally embody poor vapor barrier

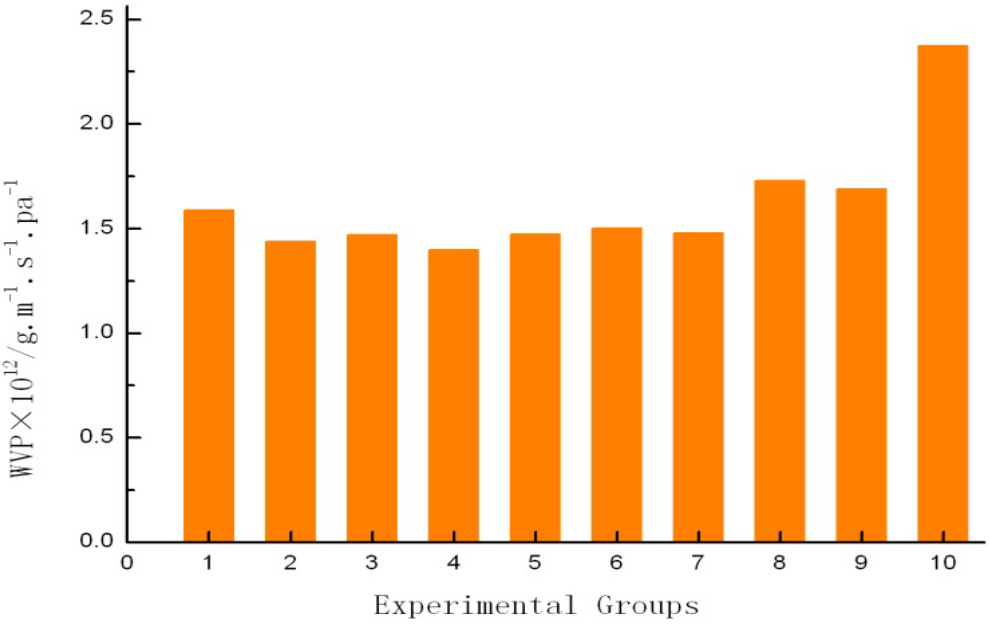

Figure 6. The WVP of different films.

Table 3. Scheme of range analysis.

Index	K	A	B	C	Major factor	Optimum group
TS	k_1	26.83	27.33	23.13	ACB	A2 C2 B3 30 min/50°C /0.20 mg/ml
	k_2	31.80	26.02	29.71		
	k_3	22.86	28.14	28.66		
	R	8.95	2.12	6.58		
WVP	k_1	4.50E-12	4.47E-12	4.82E-12	ACB	A2 C3 B1 30 min/60°C/0.10 mg/ml
	k_2	4.38E-12	4.64E-12	4.53E-12		
	k_3	4.90E-12	4.67E-12	4.43E-12		
	R	5.20E-13	1.96E-13	3.98E-13		

property, which becomes a limitation in their application. It was found that TGase addition reduced the moisture transmission coefficient of WPI-NaCas films to 1.400～1.732 × 10^{-12}g·m^{-1}·s^{-1}·pa^{-1}, about 42% lower than that of unmodified films (Figure 6). Cross-linking reaction of protein resulted in the reduction or diminution of free space intra- and inter-molecular, thereby impeding the penetration of water vapor molecules. So, the water barrier properties of WPI-NaCas films were improved by cross-linking of the components with TGase and increasing hydrophobicity, which widened the practical applications of modified films as packaging material.

Range analysis of orthogonal experiment

TS and WVP values of orthogonal experiments were selected as the objective parameters of range analysis for their significant differences and relative importance in properties and applications of films. Range analysis of orthogonal experiment results showed that reaction time of TGase was the most effective factor on both TS and WVP properties of film, and the secondary, tertiary factors were reaction temperature and TGase additive amount respectively (Table 3). Films modified by 0.1mg/ml TG at 50°C for 30 min (being optimized technological parameters) as a compromise showed the largest TS with 11.84 Mpa and the lowest WVP with 1.40×10^{-12}g·m^{-1}·s^{-1}·pa^{-1}.

Thermogravimetric analysis

TGA thermograms revealing thermal degradation

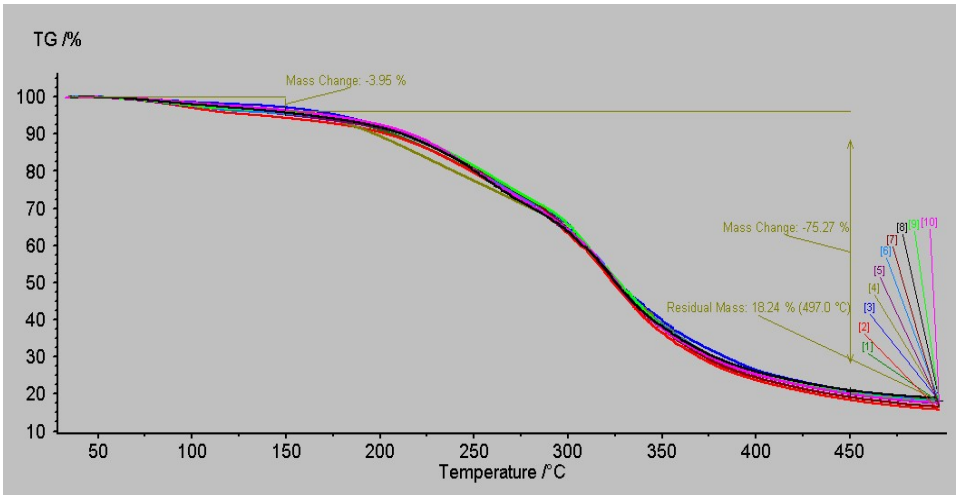

Figure 7. Weight loss profiles of control sample and films prepared by orthogonal test.

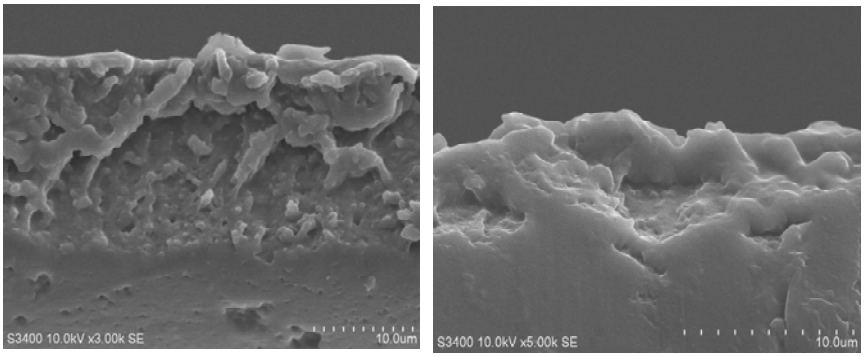

Figure 8. Cross-section morphology: (a) control sample, (b) sample in group 4.

behavior of composite protein films in the presence of transglutaminase at different levels were depicted in Figure 7. All films exhibited similar three main stages of weight loss. The first stage weight loss was observed approximately up to 3.95% at 35 ～ 150°C, possibly related to the loss of free water adsorbed in the film. The second stage weight loss gave a maximum degradation rate at 150～200°C, and was most likely associated with the loss of glycerol compound and structurally bound water. For the third stage, weight loss appeared at 200～450°C attributed to the degradation of protein chain. The high temperature step caused the decomposition of more thermally stable structures due to crosslinking reactions produced during heating (Hoque et al., 2011; Martucci and Ruseckaite, 2009; Martucci et al., 2007; Mu et al., 2012), when the non-covalent bonds (hydrogen bond, ionic bond), hydrophobic interactions and Van der Waals attraction of protein molecules were destroyed and oxidized into carbon dioxide, water, nitrogen oxide, sulfur dioxide and so on. However, no significant difference between control group and experimental groups was noticed. It was also suggested that degradation temperature of protein based films was relatively high and the temperature of 150°C could be considered as the critical temperature for use.

Scanning electron microscopy analysis

The results (Figure 8) showed that cross-section of unmodified films were relatively rough, unordered and porous, while those of the modified films became smooth and compact, which explained that enzymatic modification could be used to promote crystallinity and molecular order and caused greater changes in microstructure of WPI-NaCas composite films. This effect resulted from the decrease in the free volume between

polymer chains due to increasing attractive intermolecular forces made by the polymer network denser and more impermeable

Conclusions

TGase could ameliorate the properties of edible WPI-NaCas composite films. Synergistic effects of content, reaction time and temperature of TGase on WPI-NaCas edible films by orthogonal experiments indicated that D50 and D90 of film-forming solutions decreased by the enzyme modification, which also resulted in a decrease in water solubility and an increase in both mechanical and water barrier properties under proper conditions. Compared with control sample, scanning electron microscope analysis indicated that cross-section of enzyme modification sample was more compact and smooth. However, there was no significant difference in thermogravimetric analysis and solution apparent viscosity changes for all the samples. Orthogonal experiment results also showed that reaction time was the most effective factor on TS and WVP properties of film, and the secondary, tertiary factors were reaction temperature, TGase additive amount respectively. Films modified by 0.1 mg/ml TGase at 50°C for 30 min exhibited the largest TS 11.84 Mpa and lowest WVP $1.40\times10^{-12}g\cdot m^{-1}\cdot s^{-1}\cdot pa^{-1}$.

Enzyme modified and denatured protein films were rather more water resistant, rigid and impermeable but less flexible and transparent. Orthogonal experiments showed the potential of WPI-NaCas composite films modifided by TGase as alternative biodegradable polymers for practical applications.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This research project was financed by the Shanghai University Knowledge Service Platform and Shanghai Ocean University Aquatic Animal Breeding Center (ZF1206).

REFRENCES

ASTM (2003). Standard Test Method for Water Vapor Transmission Rate of Sheet Materials Using Dynamic Relative Humidity Measurement. E 398, Annual book of ASTM 2009, Philadelphia, PA: American Society for Testing and Materials.

ASTM (2009). Standard Test Method for Tensile Properties of Thin Plastic Sheeting. D 882-09, Annual book of ASTM (2009). Philadelphia, PA: American Society for Testing and Materials.

Baldwin E, Hagenmaier A, Robert D, Jinhe B (2012). Edible coatings and films to improve food quality, ed. Boca Raton, FL, Chap. 2, p. 15.

Carvalho RA, Grosso CRF (2004). Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocoll. 18:717-726. http://dx.doi.org/10.1016/j.foodhyd.2003.10.005

Chen GY, Lei Q (2011). Research on preparation and properties of edible composite protein films. Appl. Mech. Mater. .87:213-222. http://dx.doi.org/10.4028/www.scientific.net/AMM.87.213

Ferreira CO, Nunes CA, Delgadillo I, Lopes-da-Silva JA (2009).Characterization of chitosan-whey protein films at acid pH. Res. Int. 42:807-813.

Hoque MS, Benjakul S, Prodpran T (2011). Effects of partial hydrolysis and plasticizer content on the properties of film from cuttlefish (Sepia pharaonis) skin gelatin. Food Hydrocoll. 25:82-90. http://dx.doi.org/10.1016/j.foodhyd.2010.05.008

Kołodziejska I, Piotrowska B (2007). The water vapour permeability, mechanical properties and solubility of fish gelatin–chitosan films modified with transglutaminase or1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol. Food Chem. 103:295-300. http://dx.doi.org/10.1016/j.foodchem.2006.07.049

Kuraishi C, Yamazaki K, Susa Y (2001). Transglutaminase: Its utilization in the food industry." Food Rev. Int. 7:221-226. http://dx.doi.org/10.1081/FRI-100001258

Liu JT, Hong R, Yang Y (2006). The preparation of curdlan and its application on edible films. Mod. Food Sci. Technol. 22:57-60.

Mahmoud R, Savello PA (1993). Solubility and hydrolyzability of films produced by transglutaminase catalytic cross-linking of whey protein. J. Dairy Sci. 76:29-35. http://dx.doi.org/10.3168/jds.S0022-0302(93)77320-8

Martucci JF, Ruseckaite RA (2009). Tensile properties,barrier properties and biodegradation in soil of compression-molded gelatin-dialdehyde starch films. J. Appl. Polym. Sci. 112:2166-2178. http://dx.doi.org/10.1002/app.29695

Martucci JF, Vázquez A, Ruseckaite RA (2007). Nanocomposites based on gelatin and montmorillonite: Morphological and thermal studies. J. Therm. Anal. Calorimet. 89:117-122. http://dx.doi.org/10.1007/s10973-006-7454-0

Motoki M, Aso H, Seguro K, Nio N (1987). Asl-Casein film prepared using transglutaminase. Agric. Biol. Chem. 51:993-996. http://dx.doi.org/10.1271/bbb1961.51.993

Mu CD, Guo JM, Li XY (2012). Preparation and properties of dialdehyde carboxymethyl cellulose crosslinked gelatin edible films. Food Hydrocoll. 27:22-29. http://dx.doi.org/10.1016/j.foodhyd.2011.09.005

Nielsen GS, Petersen BR, Moller AJ (1995). Impact of salt, phosphate and temperature on the effect of a transglutaminase (F XIIIA) on the texture of restructured meat. Meat Sci. 41:293-299. http://dx.doi.org/10.1016/0309-1740(94)00002-O

Oses J, Fabregat-Vazquez M, Pedroza-Islas R, Tomas SA, Cruz-Orea A, Mate JI (2009). Development and characterization of composite edible films based on whey protein isolate and mesquite gum. J. Food Eng. 92:56-62. http://dx.doi.org/10.1016/j.jfoodeng.2008.10.029

Rhim JW (2004). Physical and mechanical properties of water-resistant sodium alginate films. Lebensmittel-Wissenschaftund-Technologie. 37:323-330.

Wang C, Zhang CH, Zhao QC (2009). Research on the composite films modified by transglutaminase. Cereal Feed Ind. 5:3-5.

Yildirim M, Hettiarachchy NS (1998). Properties of films produced by cross-linking whey proteins and 11S globulin using transglutaminase. J. Food Sci. 63:248-252. http://dx.doi.org/10.1111/j.1365-2621.1998.tb15719.x

Yu XL, Zhou GH, Li XB (2009). The application of Sodium alginate based edible films in food processing industry. Food Res. Dev. 9:181-184.