Carbohydrate Polymers 82 (2010) 958–964

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effects of chitosan on the gel properties of salt-soluble meat proteins from *silver carp*

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ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 27 May 2010
Accepted 14 June 2010
Available online 19 June 2010

Keywords:
Salt-soluble meat proteins
Chitosan
Gel properties
Chemical forces

ABSTRACT

Chitosans (CHI) with different degree of deacetylation (DD) (60.5%, 65.4%, 70.8%, 77.3%, 86.1%) and different molecular weight (MW) (299, 410, 600, 706, 880 kDa) were obtained. Heat-induced composite gels were prepared from 4% salt-soluble meat proteins (SSMP) with chitosans at 0.6 mol/L NaCl, pH 7.0. Penetration forces and storage modulus of SSMP–CHI composite gels increased proportionally with increasing amount of molecular weight. Gel containing chitosan with DD 77.3% exhibited the highest penetration force and storage modulus. The SEM observations showed that SSMP–CHI incorporated gels were compact and fine compared with that of SSMP. Chemical forces involved in SSMP–CHI composite gels were investigated by determining the effects of NaCl, urea, propylene glycol, and 2-MeSH on penetration forces. Results of these studies demonstrated that interaction between CHI and SSMP contributed to the enhancement of texture of the SSMP–CHI composite gels by electrostatic interactions and hydrogen bonds.

1. Introduction

Chitosan [β-(1,4)-2-amino-2-deoxy-D-glucopyranose] is a modified, natural carbohydrate polymer derived by deacetylation of chitin, which is the second most abundant biopolymer in nature next to cellulose and is found in the exoskeleton of crustaceans, fungal cell walls and other biological materials (No, Meyers, Prinyawtwatkul, & Xu, 2007; Shahidi, Arachchi, & Jeon, 1999). During the past several decades chitosan has received increased attention for its commercial applications in food industry, including preservation of food, formation of edible-biodegradable film, recovery of proteins from waste water and clarification of fruit juice. Furthermore, chitosan exhibits the potential to be used as food supplements with antitumor, antiulcer, antiuricemic and hypocholesterolaemic properties (Koide, 1998; Liu, Zhang, & Xia, 2008; Muzzarelli, 1996; Qin, Du, Xiao, Li, & Gao, 2002). And biological safety of chitosan has been demonstrated by trial-feeding with domestic animals (Hirano et al., 1990). Thus, chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983 respectively (KFDA, 1995; Weiner, 1992). In 2005, shrimp-derived chitosan was submitted to the US FDA to be considered as GRAS (generally recognized as safe) based on the scientific procedures for use in foods, including meat and poultry for multiple technical effects (USFDA/CFSAN, 2006). In China chitosan has been approved as a food thickener in meat products since 2007 (GB2760, 2007).

Silver carp is the most abundant fresh water fish in China, due to its fast growth rate, easy cultivation, high feed efficiency ratio as well as high nutritional value. Because of the strong earthy/musty taste and odour as well as containing too much intramuscular small bones, the consumption of *silver carp* has been limited and the price of the fish is low (Xu, Xia, Yang, Kim, & Nie, 2010). Surimi processing an effective way of utilizing fish species with low commercial value, but *silver carp* is difficult to process in this way. There is substantial recent interest in improving the quality of surimi products prepared from *silver carp*.

A few studies have been conducted on the use of chitosan to enhance gelation of surimi. Kataoka, Ishizaki, & Tanaka (1998) reported the strength of gels was nearly doubled by the addition of 1.5% chitosan when salted surimi pastes were set below 25 °C. Benjakul et al. (2000) observed that addition of chitosan with DD 65.6% at the level of 15 mg/g resulted in the maximum increases in both breaking force and deformation of suwari and kamaboko gels compared to the control and gels containing chitin or chitosan with other DD, Furthermore they found that breaking force of surimi gels from the heat-induced gelation of fish myofibrillar proteins added with 1.0% prawn shell chitosan increased (Benjakul, Visessangua, Phatchrat, & Tanaka, 2003). Mao and Wu (2007) also reported that whiteness, hardness, springiness, cohesiveness, chewiness, adhesiveness, TBA value of grass carp gels increased, while expressible water and peroxide value decreased when 1% chitosan were added into the surimi. Zhang and Xia (2010)

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doi:10.1016/j.carbpol.2010.06.026

have recently shown that chitosan could significantly enhance the texture properties and water-binding capacity of surimi from *silver carp* and the gel strength increased by approximately 34% and water loss rate decreased by 29.1%.

Salt-soluble meat proteins (SSMP) determine the texture and yield of processed meat products because of their ability to produce three-dimensional gels upon heating and subsequent cooling. The degree of deacetylation and polymerization are two important parameters used to characterize chitosan. It is very important to understand the effects of chitosan on the gel properties of SSMP when chitosans are added into meat products to improve their functional properties. But few studies have reported how the gel properties of SSMP were influenced by chitosan's degree of deacetylation and polymerization, and there is minimal information on the possible participation of physical/chemical forces in SSMP–CHI composite gels. Therefore, the objective of this study was to study the effects of chitosan on the gel properties of SSMP from *silver carp* and to determine any molecular forces involved in SSMP–CHI composite gels.

2. Materials and methods

2.1. Preparation of salt-soluble meat proteins (SSMP)

Fresh *silver carp* (2–3 kg/fish) was purchased from a local market (Wuxi, Jiangsu, China), and immediately gutted and scaled. Prepared fish was then manually filleted. Aliquots of 200 g muscle were vacuum-sealed in plastic bags, and stored at −20 °C for less than two weeks before use.

SSMP were prepared essentially in accordance with the procedure described previously (Chen, Xu, & Wang, 2007; Defreitas, Sebranek, Olson, & Carr, 1997) with slight modification and all preparation steps were carried out at 2–4 °C. The meat was homogenized in a Blender in three volumes isolation buffer (0.5 M NaCl, 17.8 mM $Na_5P_3O_{10}$, pH 8.3) for 60 s at maximum speed. The slurry was kept at 4 °C for 1 h and then centrifuged at 10,000 g using freezing centrifuge (Sigma 4k15 Germany) for 20 min. The protein extract was strained through three layers of cheesecloth and diluted with deionized water to precipitate the SSMP which was collected by centrifugation. The extraction step was repeated more than three times. Protein concentrations were determined by the Biuret method (Gornall, Bardawill, & David, 1949) using bovine serum albumin (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) as the standard. The final pellets were standardized to 4% protein concentration by diluting with the isolation buffer for use.

2.2. Preparation of chitosans

Commercial chitosan A was prepared from crab shell. Chitosans with the same deacetylation degree but different molecular weight were obtained by ultrasonic degradation (Gao & Wan, 2006; Tsaih, Tseng, & Chen, 2004). Chitosans were dissolved in acetic acid aqueous solution (1%, v/v), and then ultrasonically degraded (JY88-II, China) for different time (0–2 h) at 30 °C. After degradation, chitosans were precipitated with NH_4OH solution and washed with deionized water until neutral, then lyophilized.

Chitosans with various degrees of deacetylation but same molecular weight were prepared by homogeneous re-acetylation (Berth & Dautzenberg, 2002; Vachoud, Zydowicz, & Domard, 1997). The N-acetylation of chitosan was performed by means of acetic anhydride as the reactive medium, in a water/alcohol solution. Thus, 10 mL of an aqueous acetic acid solution (0.5%) containing chitosan (1%) was mixed with 8 mL of ethanol. Different amounts of pure and fresh acetic anhydride were added slowly to the above solution and stirred for 24 h in stoichiometric conditions to reach the desired %DD. At the end of reaction, the polymer was fully precipitated by addition of NH_4OH and washed several times with deionized water at pH 7.5 in order to maintain the amino groups in the $-NH_2$ form. Whatever the %DD, the final product was then lyophilized.

2.3. Characterization of chitosans

The weight-average molecular weight (Mw) and molecular weight distribution (Mw/Mn) of chitosan were measured by gel permeation chromatography (GPC). The GPC equipment consisted of Ultrahydrogel 2000 (7.8 mm × 300 mm) combined with Ultrahydrogel 250 (7.8 mm × 300 mm), Waters 600 pump and RI 150 refractive index detector. The eluent was 0.2 M CH_3COOH/0.15 M CH_3COONH_4. Eluent and chitosan solutions were filtered through 0.45 μm Millipore filters. The flow rate, the temperature of the column and the sample concentration were maintained at 0.9 mL/min, 30 °C and 4 mg/mL. Dextran standards (2000, 133.8, 41.1, 21.4, 4.6, 2.5 kDa) were used for a calibration curve. All data provided by the GPC system were collected and analyzed using the Waters Workstation software package.

The potentiometric method was used to determine the degree of deacetylation of these chitosan (Lin, Jiang, & Zhang, 1992). Dried chitosans of 0.5 g were accurately weighted and dissolved in 0.1 M HCl. The solution was titrated with 0.1 M NaOH. The degree of deacetylation was calculated as follows:

$$NH_2\% = \frac{(C_1V_1 - C_2V_2)}{G(1-W)} \times 0.016 \times 100 \quad DD\% = \frac{NH_2\%}{9.94} \times 100$$

where C_1 is the concentration of HCl (mol/L); C_2, the concentration of NaOH (mol/L); V_1, the volume of HCl (mL); V_2, the volume of NaOH; G, the sample weight (g); W, the water percentage of sample (%); and 0.16 is the weight of NH_2 equal to 1 mL 0.1 M HCl (g).where 9.94% is the theoretical NH_2 percentage of chitosan.

2.4. Preparation of gels

Different chitosans were dispersed with a little 1% acetic acid and added to the mixture. Gels without chitosan but containing the same amount of acetic acid were served as control. Before chitosan solution was added to the protein solution, the pH value of chitosan solution was controlled between 5 and 6 to avoid flocculation. When the chitosan solution was added in the basic protein solution, the pH value of mixture was about 6.0, and chitosan and SSMP were mixed and interacted with each other sufficiently. All formulations were standardized at 4% protein concentration, 0.6 mol/L NaCl and pH 7.0. All treatments were stirred and homogenized 30 s at medium speed by a blender. SSMP suspensions (sols, 5 g each) were transferred to 16.5 (inside diameter) × 50 mm (length) glass vials, covered with aluminum foil, and heated in a water bath at 90 °C for 30 min. After being heated, the formed gels were cooled and stored at 2–4 °C for 12 h before test.

2.5. Determination of rheological characteristics

Dynamic rheological experiments were performed using an AR1000 Rheometer (TA Instrument Ltd., England). Parallel plate geometry (diameter 40 mm) was used with gap size of 1.0 mm. A cover, treated with liquid paraffin, was applied to prevent evaporation. TA Rheometer Data Analysis software (version V1.0.74) was used to obtain the experimental data. The linear viscoelastic region was determined for each sample through stress sweeps at 1 Hz. The mechanical spectra were made using temperature sweeps from 15 to 90 °C at a constant frequency of 1 Hz. Storage modulus (G') were recorded every 2.6 °C within the linear viscoelastic region. The G' were presented to demonstrate viscoelastic changes during the

sol–gel transition. Three experimental replications were conducted and the reported result was an average of three measurements.

2.6. Determination of texture characteristics

Prior to the gel strength measurement, gel samples were allowed to equilibrate at room temperature (25 ± 1 °C) for 30 min. Gels were penetrated with a flat-faced stainless steel probe (10 mm dia) attached to a Model TAXT2 texture analyzer (Stable Micro Systems Ltd., Surry, U.K.) at a crosshead speed of 0.3 mm/s, Pre- and post-test speed were controlled at 1.00 mm/s. The distance of the compression was set at 10 mm. The penetration force which was the peak force required to rupture the gels was expressed as the gel strength (Xiong & Brekke, 1991).

2.7. Determination of water holding capacity (WHC)

After the textural analysis, gel samples (5 g each) were subjected to WHC measurement. Gel samples were transferred to 50 mL centrifuge tubes and centrifuged at 10,000 g for 15 min at 4 °C. Tubes were inverted to drain and collect the supernatant fluid for 15 min. WHC (%) was defined as the ratio of the pellets weight to the original gel weight then multiplying by 100 (Wu, Xiong, Chen, Tang, & Zhou, 2009).

2.8. Scanning electron microscope (SEM)

Samples were fixed in 2.5% (w/w) glutaraldehyde solution and 1% (w/w) osmium peroxide solution at 4 °C and successively. Fixed samples were rinsed in phosphate buffer (pH 7.2, 0.1 M) over 10 times and then dehydrated through a graded series of alcohol concentrations (30%, 50%, 70%, 90% and 100%). Samples were then immersed in isoamyl acetate and dried in a critical point driver (model CPD-030, BAL-TEC Company). Specimens were coated with a layer of gold/palladium, 200 Å thick by use of an ionic sputter (model SCI-005, BALTEC company) and observed in the scanning electron microscope (model Quanta-200, FEI company, acceleration voltage was 10 kv).

2.9. Molecular forces in SSMP–CHI composite gels

Treatments consisted of destabilizing reagents as follows: control (no reagent), NaCl (to neutralize charges on large molecules), 2-MeSH (to reduce disulfide bonds), urea (to disrupt hydrogen bonds) and propylene glycol (to disrupt hydrophobic forces) (Xiong, Means, & Decker, 1992). Reagents were solubilized in SSMP–CHI composites. All treatments were stirred and homogenized for 30 s at medium speed using a Blender. The gels were prepared and the penetration force and WHC (%) were determined as described before.

2.10. Statistical analysis

All experiments were repeated 3 times each with a new batch of SSMP isolate and chitosan. Least significant differences ($P < 0.05$) were used to determine differences between treatments.

3. Results and discussion

3.1. Characteristics of chitosans

Clean and white chitosans flour with desirable %DD and MW were obtained. Molecular weight (MW), molecular weight distribution (Mw/Mn) and degree of deacetylation (%DD) of chitosans were presented in Tables 1 and 2.

Table 1
Chitosans with the same degree of deacetylation but different molecular weight.

	Molecular weight (MW) (kDa)	Molecular weight distribution (Mw/Mn)	Degree of deacetylation (%DD)
Chitosan-A1	880	5.57	76.1
Chitosan-A2	706	4.63	76.4
Chitosan-A3	600	4.25	76.7
Chitosan-A4	410	3.71	76.9
Chitosan-A5	299	3.00	75.9

3.2. Effects of chitosans on rheological properties of set gels

Protein gels are distinguished by their viscoelastic characteristics. The changes in dynamic viscoelastic properties of SSMP and SSMP–CHI composites during thermal scanning from 15 to 90 °C were indicated by monitoring the storage modulus (G′). Similar patterns in gelation were observed in Fig. 1.The G′ which described the elastic property of a gelling system could be characterized into three main transitions. The first transition, shown by a steady increase in G′ and reaching the first peak at 30.5 °C, indicated that proteins formed gel networks. Heating of SSMP at low temperature generally resulted in dissociation of some myofibrillar components, for example tropomyosin from the F-actin backbone and F-actin from its super helix structure (Ziegler & Acton, 1984). However, conformational changes of these proteins had no significant contributions on the changes of gel modulus (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001; Xiong, 1997). It was postulated that partial unfolding of the protein structure initiated by the dissociation of myosin light chain subunits from the heavy chains could lead to an interfilamental junction of myosin and form a three-dimensional structure (Benjakul et al., 2001). In the second transition, G′ decreased drastically as heating proceeded from 33.1 to 48.7 °C, the decrease in G′ up to 48.7 °C was possibly caused by dissociation of actin–myosin complex and the denaturation of myosin tail (Egelandsdal, Fretheim, & Samejima, 1986). It was assumed that the helix-to-coil transformation of myosin leaded to a large increase in the fluidity of semi-gels and could disrupt some protein networks that had already been formed, resulting in a declined storage modulus (Xiong & Blanchard, 1994). The third transition characterized by the second increase in G′ after 48.7 °C was attributed to aggregation of unfolded protein domains to form irreversible gel networks (Lou et al., 2000). This was because major structural changes in myosin had already occurred above 48.7 °C, and continuing molecular interactions and cross-linking (which was responsible for a high elasticity) would predominate in the viscoelastic gel system (Xiong, 1997).

The addition of chitosans markedly increased G′ (Fig. 1a) over the entire temperature range when SSMP–CHI sols were converted into gels, and G′ generally increased with the increasing dosage of the chitosan. The result indicated that chitosans participated in the development of the SSMP gel networks. The higher G′ values brought about by addition of chitosans prior to heating suggested that chitosans interacted with proteins in the continuous phase to produce an amorphous network possessing an elastic charac-

Table 2
Chitosans with various degrees of deacetylation but the same molecular weight.

	Molecular weight (MW) (kDa)	Molecular weight distribution (Mw/Mn)	Degree of deacetylation (%DD)
Chitosan-B1	326	4.25	86.1
Chitosan-B2	326	4.25	77.3
Chitosan-B3	326	4.25	70.8
Chitosan-B4	326	4.25	65.4
Chitosan-B5	326	4.25	60.5

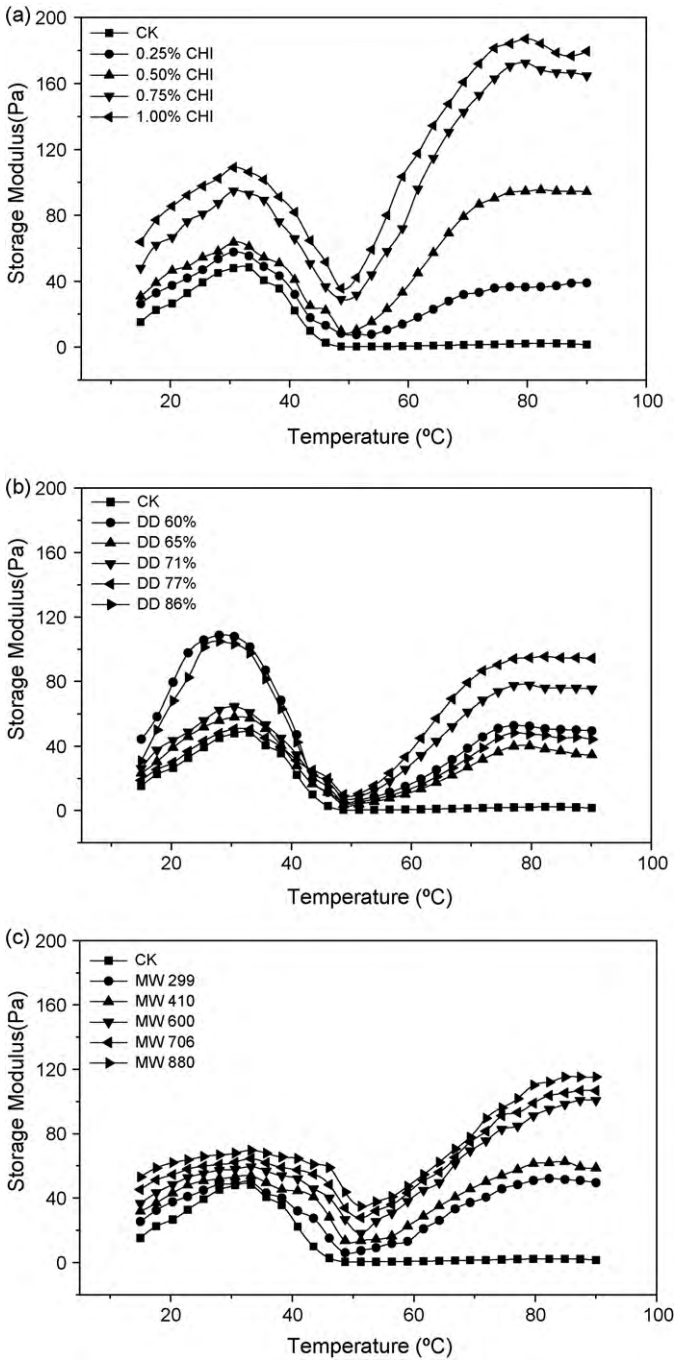

Fig. 1. Effects of chitosan on shear storage modulus of SSMP–CHI composites during thermal gelation: (a) concentration, (b) degree of deacetylation and (c) molecular weight.

teristic. These results were consistent with the previous reports (Kachanechai, Jantawat, & Pichyangkura, 2008), in which chitosans as cold-set binder could enhance the gelation of salt-soluble proteins by forming various molecular forces.

The degree of deacetylation and polymerization are two important parameters to characterize chitosans, which have prominent roles in the biochemical significance of chitosans. When chitosans with the same molecular weight but different degree deacetylation were added into the SSMP sols, G′ (Fig. 1b) increased over the entire temperature range. Chitosan of DD 77.3% had the highest G′, which meant this system had the most elastic property and the best texture. These observations accorded with results of texture characteristic. When chitosans with the same degree deacetylation but different molecular weight were added into the SSMP sols, G′ increased over the entire temperature range (Fig. 1c). G′ were raised with the molecular weight increasing, and gel containing chitosan with Mw 880 kDa had the highest G′. These results indicated that more chitosans contributed to the development of the SSMP gels networks with increasing molecular weight.

3.3. Effects of chitosan on textural and water holding properties of set gels

Dynamic rheological testing during heating provided an insight into the interaction of protein and chitosan leading to the formation and development of a composite viscoelastic gel network. To further elucidate the role of chitosans on the SSMP gel properties, gels prepared with various concentration levels of chitosans were evaluated by the penetration force and water holding capacity (WHC). Significant differences between SSMP and SSMP–CHI gels were also found. In Fig. 2, SSMP–CHI gels were several-fold harder than SSMP gels ($P < 0.05$), which suggested possible protein–chitosan interactions. Penetration force of composite gels increased proportionally with increasing amount of chitosan incorporated in gels (Fig. 2a). Therefore, it was presumed more chitosans participated in the interaction between chitosan and protein with increasing addition of chitosan. In the presence of chitosan, protein–polysaccharide conjugates would be formed between the reactive amino group of glucosamine and the glutaminyl residue of myofibrillar proteins. Bonds between chitosans and myofibrillar proteins could be associated with improving of texture properties in gels with the final structure formed by both covalent and non-covalent interactions (Benjakul et al., 2000; Kataoka et al., 1998).

At the same concentration added (0.5%), gel containing DD 77.3% chitosan exhibited the highest penetration force (Fig. 2b) ($P < 0.05$), with an approximate threefold increase compared to the control, while a gel containing DD 86.1% chitosan exhibited the lowest penetration force ($P < 0.05$). However, no distinct differences were observed among gels with added chitosans with DD 60.5%, DD 65.4% and DD 70.8%. Benjakul et al. observed that the breaking force of suwari gel with added chitosan decreased with increasing degree of deacetylation. Chitosans with higher degree of deacetylation had more amino groups and underwent more cross-linking with glutamyl residues of myofibrils, resulting in less polymerization of myofibrils inter- or intramolecularly. For chitosans with lower degree of deacetylation, some amino groups partially cross-linked with myofibrils and also worked as an effective binder, whereas the polymerization of myofibrils still occurred effectively. It was postulated that amino groups of chitosan molecules partially cross-linked with myofibrils and also worked as filler in the gel matrix (Benjakul et al., 2000).

Chitosan consists of 70–90% D-glucosamine and 10–30% N-acetyl-D-glucosamine units, connected through β-(1 → 4) glycosidic linkage. With the molecular weight increasing, the degree of polymerization added and the free amino groups of chitosan increased. Kataoka et al. (1998) summarized the free amino groups of chitosan play an important role in producing a strong gel. Therefore penetration forces of setting gels increased proportionally with increasing amount of molecular weight of chitosan incorporated in the gels (Fig. 2c).

A well-structured gel is characterized by its ability to effectively immobilize water through capillary effects of its matrices. Indeed, the SSMP–CHI composite gels, which had an improved penetration force, also had the higher WHC ($P < 0.05$) over the control gels that showed considerable syneresis (Fig. 2a). The WHC increased simultaneously with the raise of penetration force. The WHC improvement was not surprising because chitosan-added gels had a more compact structure due to the interaction between

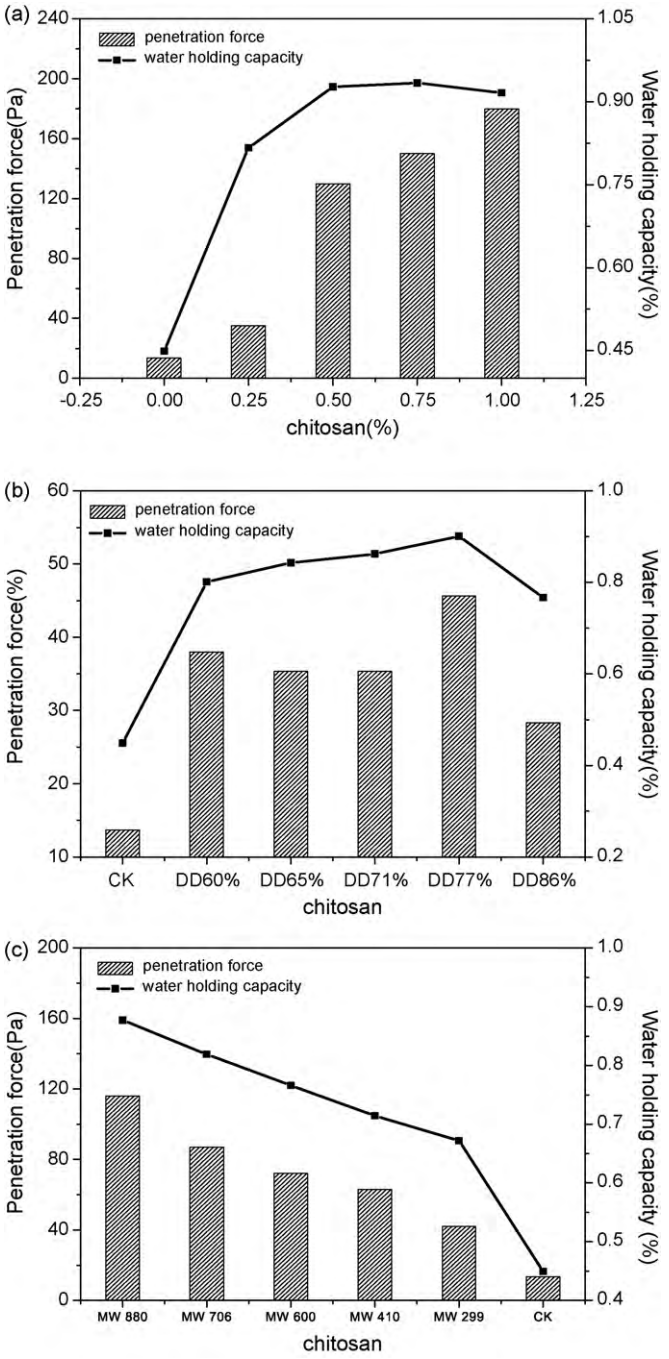

Fig. 2. Effects of chitosan on penetration forces and WHC of SSMP–CHI composite gels: (a) concentration, (b) degree of deacetylation and (c) molecular weight.

chitosan and protein. Therefore it was ascribed to the ability of chitosan to hold water and the interaction between protein and chitosan.

3.4. Effects of chitosan on microstructure of set gels

SEM provided structural information about gels. As shown in Fig. 3, distinctive differences between gels were observed, which corresponded to the differences in gel strength and WHC. The structure of SSMP gels (Fig. 3a) showed a granular aggregated structure within the matrix, with limited connectivity between the protein strands. The micrograph of SSMP–CHI gels (Fig. 3b) showed a well-structured matrix with a highly interconnected network of strands

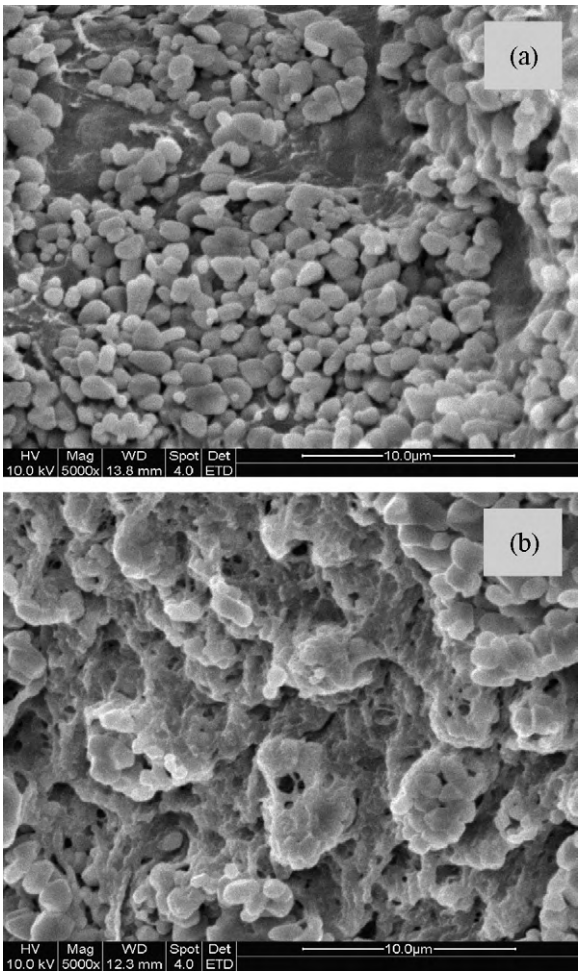

Fig. 3. Scanning electron micrographs (5000× magnifications, bar 10 μm) of SSMP gels (a) and SSMP–CHI composite gels (b).

that may cause more resistance to applied stress and great water holding capacity. These microstructural changes helped to explain these differences between SSMP and SSMP–CHI gels. A fine, uniform structure would probably result in more absorptive capacity and better retention of water compared to coarse structure with large pores. These results showed that interaction possibly existed between chitosan and meat protein. Chitosans dispersed uniformly and were tightly associated into the gel network and enhanced the gelation ability of SSMP by forming various chemical interactions.

3.5. Molecular forces in SSMP–CHI composite gels

The effect of various stabilizing/destabilizing reagents on formation, hardness and syneresis of gels may be correlated with molecular forces contributing to formation or maintenance of the three-dimensional network structure (Bernal, Smajda, Smith, & Stanley, 1987). As shown in Fig. 4, the addition of destabilizing reagents to SSMP–CHI changed the properties of composite gels.

NaCl was capable of modifying the electrostatic interactions between chitosan and myofibrillar proteins because of its ionic nature. Penetration forces of SSMP–CHI gels containing NaCl were much stronger than that of the control, and WHC of SSMP–CHI gels containing NaCl were more than that of the control (Fig. 4a). A few studies had been reported that meat proteins could interact with anionic polysaccharides such as flaxseed gum, alginate and carrageenan, to enhance the texture of meat products. The interactions were weaker with the addition of NaCl (Chen et al., 2007;

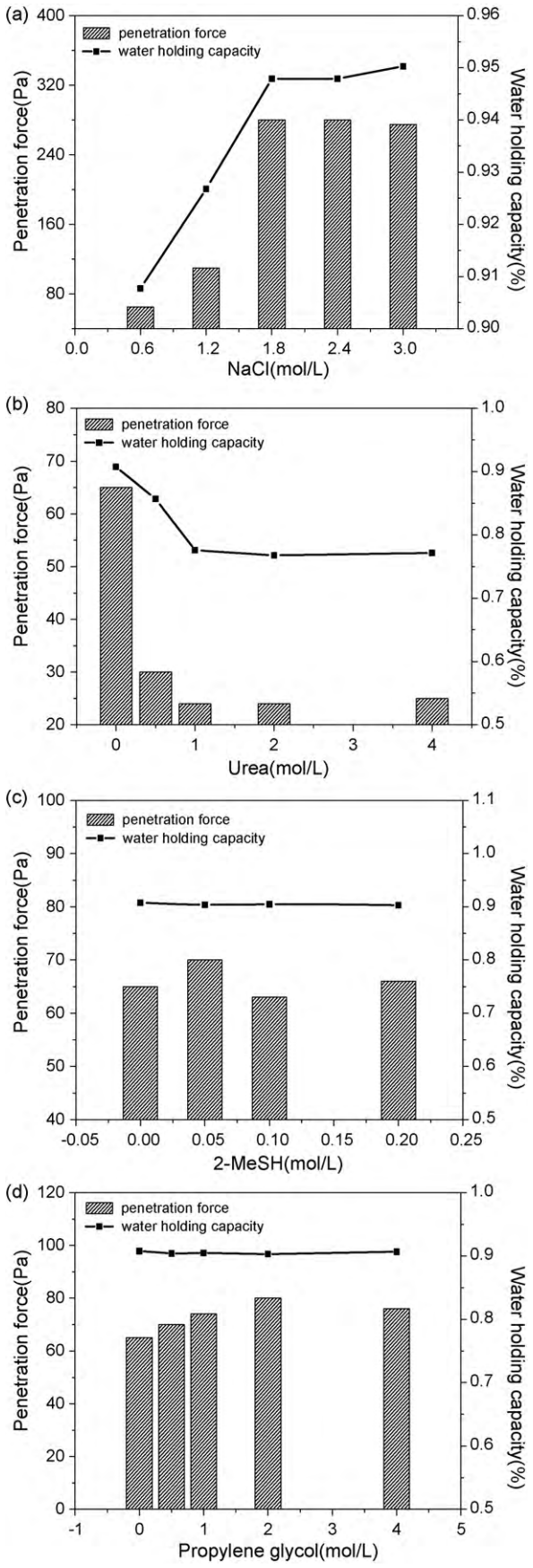

Fig. 4. Effects of NaCl (a), urea (b), 2-MeSH (c) and propylene glycol (d) on penetration forces and WHC of SSMP–CHI composite gels.

Defreitas et al., 1997; Xiong et al., 1992). Chen et al. (2007) reported that interactions between flaxseed gum and meat protein probably involved negatively charged carboxyl groups in the flaxseed gum molecules and positively charged side chains of the amino acids in the protein. However, since chitosan is a cationic polysaccharide, mutual repulsion could be expected between positively charged amino groups of chitosan and positively charged amino groups of proteins. Penetration forces of SSMP–CHI gels containing NaCl were much stronger than that of the control and composite gel containing DD 86.1% chitosan exhibited the lowest penetration force. Therefore electrostatic interactions were the main forces involved in SSMP–CHI gels.

Urea at high concentrations was known for its ability to diminish hydrogen bonds and weaken hydrophobic interactions by altering water structure and increasing solubility of hydrophobic amino acid side chains (Lapanje, 1978). Penetration forces of SSMP–CHI gels that contained urea were weaker than that of the control, and WHC of composite gels that contained urea were less than that of the control (Fig. 4b). The marked reduction in SSMP–CHI gel strength suggested the involvement of hydrogen bonds and/or hydrophobic interactions in gel networks. Hydroxyl groups in chitosan could interact with side-chain polar amino acids or peptide bonds via hydrogen bonding, imparting additional binding strength.

Propylene glycol could disrupt hydrophobic forces and enhance hydrogen bonds and electrostatic interactions by lowering the dielectric constant (Utsumi & Kinsella, 1985). The addition of propylene glycol caused little increase in gel strength (Fig. 4d), which suggest that hydrogen and electrostatic bonding were more important than hydrophobic forces in the SSMP–CHI systems.

The added 2-MeSH acted as a reducing agent to cleave inter- and intramolecular disulfide bonds to facilitate protein unfolding and increased the exposure of reactive groups that could be involved in hydrogen bonding and hydrophobic interactions (Utsumi & Kinsella, 1985). No differences ($P > 0.05$) in gel strength were observed among gels treated with 2-MeSH (Fig. 4c). The disulfide bonds within the sample had slight effects on the stability of SSMP–CHI gels.

Our results suggested that electrostatic interactions and hydrogen bonds were major forces cooperatively maintaining mixed SSMP–CHI gel networks.

4. Conclusions

The addition of chitosan markedly increased the G′ over the entire temperature range when the SSMP sol was converted into a gel. Chitosan could markedly enhance the gel strength of salt-soluble meat proteins. Gel containing DD 77.3% chitosan exhibited the highest penetration force and storage modulus and penetration forces of gels increased with increasing amount of molecular weight of chitosan incorporated in the gels. The interaction between chitosan and salt-soluble meat proteins was mainly stabilized by electrostatic interactions and hydrogen bonds.

Acknowledgements

This research was financially supported by the earmarked fund for 863 Project (No. 2007AA100401), Modern Agro-industry Technology Research System (NYCYTX-49), the guidance project of State key Laboratory of Food Science and Technology (SKLF-MB-200805), PCSIRT0627, BA2009082 and 111 project(B07029).

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