



PEGylation may reduce allergenicity and improve gelling properties of protein isolate from black kidney bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Effects of PEGylation with methoxy polyethylene glycol (mPEG) on gelling properties of black kidney bean protein isolate (BKBPI) were investigated. PEG-protein conjugations were confirmed using SDS-PAGE, and a significant decrease of allergenicity was observed using an ELISA test. Gelling concentration and gelling properties of BKBPI and mPEG-BKBPI with various treatments, including the additions of NaCl and N-ethylmaleimide (NEM) as well as a heat pretreatment were evaluated. Results showed that the critical gelling concentration of PEGylated BKBPI was 75 mg/ml, and the PEGylation resulted in a higher gelling strength and a shorter gelling time compared with the native BKBPI. The increases in gel strengths were observed both in BKBPI and mPEG-BKBPI with the addition of NaCl, and no obvious variations in storage modulus (G') were found after PEGylation even at a high NaCl concentration of 1 mol/l. Meanwhile, retardation of the G' was observed in the PEGylated protein with the addition of NEM. Furthermore, the G' increased significantly after preheating. Therefore, the results suggested that PEGylation was beneficial to the safety and gelling properties of BKBPI and served to extend applications of BKBPI.

1. Introduction

In recent years, many legume proteins have been used in food processing, due to their good gelling properties (Graham & Vance, 2003). These proteins, especially the globular proteins, can form heat-denatured gels without any cross-linking agent (Renkema, Gruppen, & van Vliet, 2002). The textural properties of many food products, such as tofu, depend on the gelling properties of proteins during their preparations (Ayadi, Kechaou, Makni, & Attia, 2009; Hongsprabhas & Barbut, 1997; Madadlou, Khosroshahi, Mousavi, & Djome, 2006), and the formation of gels can be controlled by a number of factors, such as heating temperature, pH, and ionic strength (Hua, Cui, Wang, Mine, & Poysa, 2005). Meanwhile, the use of legume proteins in the food industry has stimulated research to fully use their gelling properties. There is a growing interest in developing proteins with better gelling properties at lower protein concentrations. Several physical, chemical and enzymatic strategies have been used to improve the gelling properties of protein isolates, such as high-pressure treatment,

transglutaminase modification, and acid and alkali treatments (Dondero, Figueroa, Morales, & Curotto, 2006; He, He, Chao, Ju, & Aluko, 2014; Zhang et al., 2010; Zhang, Yang, Zhou, Zhang, & Wang, 2017). However, isomerization, amino acid degradation, corrosion of equipment, introduction of toxic chemicals and the increasing complexity of production are issues of concern (Song & Zhao, 2014). Thus, easier and safer protein modifications to improve gelling properties are needed.

Black kidney beans (*Phaseolus vulgaris* L.) are a widely produced *Phaseolus* legume in Asian, African, and Latin American countries (Yin, Tang, Wen, Yang, & Li, 2008). According to previous studies, black kidney beans have similar protein content to soybeans, eggs and milk, and have a favorable balance of amino acids, vitamins, isoflavones, flavonoids and polyphenols (Lee, Hung, & Chou, 2008; Takahashi et al., 2005; Xu & Chang, 2008). However, compared with other legume proteins, the black kidney bean protein has not been well used because of its potential allergenicity (He et al., 2018). As a strong anti-nutritional factor, lectin has been found to be one of the major components

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of its globular proteins (Dan, Liu, & Ng, 2016), and a high content of lectin will be retained after protein isolation and might induce allergic reactions if unheated (Souza, Carvalho, Ruas, Ricci-Azevedo, & Roque-Barreira, 2013). Furthermore, the gelling properties of black kidney bean proteins need further study.

Polyethylene glycol (PEG) with molecular weight above 1000 Da is recognized as a non-toxic compound by the US Food and Drug Administration (FDA), and the PEG derivatives might have a wider range of applications due to their non-toxic, hydrophilic and highly flexible properties (Kim & Park, 2001; Roberts, Bentley, & Harris, 2012; Zhong et al., 2016). Over the past decades, various applications of PEG derivatives have been reported for biological macromolecule modifications, and gel and membrane formations (Chronakis, Kasapis, Richardson, & Doxastakis, 2010; Kurakazu et al., 2010; Roberts et al., 2012; Wongchitphimon, Wang, Jiraratananon, Shi, & Loh, 2011; Yoshida, Uchida, Ito, Takami, & Murakami, 2017). It has been found that the microporous network structure of gels and/or membranes can be enhanced with the addition of PEG derivatives (Chou, Yu, Yang, & Jou, 2007; Wongchitphimon et al., 2011). Furthermore, some hydrogels and chitosan gels had better properties in practical application when PEG was added, as the PEG derivatives could accelerate the coagulation and improve the hydrophilicity (Gayet & Fortier, 1996; Gong et al., 2009; Jeong, Kibbey, Birnbaum, Won, & Gutowska, 2000; Lin & Anseth, 2009; Yoshida et al., 2017). According to previous studies, the N-terminal amines of surface lysine residues could be modified using methoxy polyethylene glycol (mPEG) in neutral or mild alkali solutions with no other chemicals introduced, and thus would affect the protein structures and changed the functional properties and biological activities of proteins in a green way (Liu et al., 2014; Roberts et al., 2012). Several activated PEG derivatives, such as mPEG succinimidyl carbonate (mPEG-SC), succinimidyl succinate (mPEG-SS) and succinimidyl propionate (mPEG-SPA) have been developed with high selectivity in reactions with amino groups and are widely used in protein modifications (D'Souza & Shegokar, 2016; Mu, Tao, & Yu, 2013).

In the previous study, PEGylation was used in the modification of black kidney bean protein isolate (BKBPI), and the solubility, emulsifying and foaming properties of the proteins were successfully improved using mPEG-SC, mPEG-SPA, and mPEG-SS, respectively, and the hemagglutination activity was significantly decreased, indicating that the PEGylation might improve the safety of these protein isolates (He et al., 2018). Therefore, to broaden the application of BKBPI in food packages, medical dressings, and cosmetics, and so on, the allergenicity, gelling concentration, gel strength and gelling time of BKBPI during heat-induced gelation before and after PEGylation were investigated in this study, and the gelling properties of BKBPI and mPEG-BKBPI with various treatments, including the addition of NaCl, N-Ethylmaleimide (NEM) and heat pretreatment, were also studied.

2. Materials and methods

2.1. Materials

Commercial dried black kidney beans (*Phaseolus vulgaris* L.) were obtained from a local market (Hefei, Anhui, China). mPEG-SC, mPEG-SS and mPEG-SPA were obtained from YareBio Biotech, Inc. (Shanghai, China). ELISA testing kits for black kidney bean lectin were purchased from Senbeijia Biotechnology Co., Ltd. (SBJ-P193, Nanjing, Jiangsu, China). Precast gels (4–15%) used for the SDS-PAGE analysis were obtained from BioRad Laboratories, Inc. (Hercules, CA, USA). All other chemicals and reagents used were of analytical grade.

2.2. BKBPI preparation

BKBPI was prepared using the method of Fan and Sosulski (1974) with some modifications. The black kidney beans were ground using a domestic eccentric vibratory mill (XDW-6J model, Micro Machinery

Co., Jinan, Shandong, China). After degreasing using petroleum ether, the defatted bean flour was suspended in 10-fold deionized water (≥ 18.2 M Ω /cm, Millipore Co. Ltd., Billerica, MA, USA), and was adjusted to pH 7.0 with 2 M NaOH and then mixed at 25 °C for 6 h. The slurry was centrifuged at 8000g for 30 min (TG16G, Anting Scientific Instrument Co., Ltd., Shanghai, China), and then the supernatants were recovered, and adjusted to pH 4.5 with 2 M HCl. The precipitates obtained using centrifugation (8000g, 30 min) were adjusted to pH 7.0 and dialyzed using dialysis bags (nominal MW cut-off of 3000 Da, Aladdin Co., Shanghai, China, previously boiled in 1 mM EDTA for 10 min) against deionized water for 24 h at 4 °C, and then freeze-dried (FD-1A-50 model, Jiangsu Tianling Co. Ltd., Yancheng, Jiangsu, China) at –50 °C for 48 h, and stored at –20 °C and used within 6 wk. The final BKBPI product had a protein content of $82 \pm 1\%$ as determined using the Kjeldahl method ($N \times 5.8$) (AOAC, 2000; method 976.05).

2.3. PEGylation of BKBPI using mPEG

BKBPI was dissolved into sodium phosphate buffers (10 mM, pH 7.0) with a final protein concentration of 10 mg/ml. Afterwards, mPEG-SC, mPEG-SPA, or mPEG-SS was slowly added to the protein isolate solution at a weight ratio of 1:10. The resulting mixture was slightly stirred at room temperature (25 ± 0.5 °C) for 12 h, following an extensive dialysis at 4 °C for 48 h to remove the excess unreacted PEG. Finally, the aqueous solutions of the three PEG-modified BKBPI (mPEG-SC-BKBPI, mPEG-SPA-BKBPI and mPEG-SS-BKBPI) were obtained and stored in sealed vials at –20 °C for the following analyses for a maximum of 6 wk (He et al., 2018).

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was done using a precast 4–15% gradient gel in a Bio-Rad mini-protein electrophoresis cell (Bio-Rad Laboratories, Inc.) at a constant voltage of 100 V. Samples were prepared by mixing proteins with sample reducing buffer (2.0 ml glycerol, 1.6 ml 10% (w/v) SDS, 0.8 ml β -mercaptoethanol and 0.4 ml 1% (w/v) bromophenol blue) to a concentration of 2 mg/ml, and the solution was heated at 100 °C for 5 min and then centrifuged at 8000g for 1 min. Afterwards, 20 μ l of each sample was loaded in each well of the gel, and run for 75 min using the tris-glycine-SDS running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3). Gel images were captured using a gel imaging system (Tanon-2500, Tanon Science & Technology Co., Shanghai, China) after staining treatments as described below, and the relative motilities of the bands were measured to estimate the molecular weight. For Coomassie brilliant blue staining of the SDS-PAGE gels: Gel slabs were fixed and stained simultaneously using Coomassie blue R-250 stain solution (0.25% Coomassie brilliant blue R-250, 50% methanol, and 10% acetic acid) for 60 min, then destained using 5% methanol and 7.5% acetic acid for 5 h with 2–3 changes of destaining solution (He et al., 2014).

For barium iodide staining of the SDS-PAGE gels: Gel slabs were fixed and soaked simultaneously using 5% barium chloride solution for 20 min, followed by immersion 3 times with deionized water. Then, gel slabs were stained in 0.1 mol/l Titrisol iodine solution (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for another 5 min and destained using deionized water (Wang et al., 2017; Zhang et al., 2015).

2.5. Allergenicity determination of BKBPI and PEGylated BKBPI

Allergenicity was measured using the ELISA testing kit following the manufacturer instruction. Each diluted sample (50 μ l/well) and the standard curve solutions (50 μ l/well) were added to the 96 well plate, and incubated for 1 h at 37 °C with HRP tagged antibody (100 μ l/well). After washing five times using the cleaning solution in the kit, substrate A and B were added to each well of 50 μ l, respectively, and the plate was incubated for 15 min at 37 °C in the dark. The reaction was stopped

by the addition of stop buffer (50 μ l/well), and absorbance (OD) of each reaction well was measured using a microtiter plate reader (Eon model, Inc., Winooski, VT, USA) at a wavelength of 450 nm within 15 min.

2.6. Gelling concentration determination of BKBPI and PEGylated BKBPI

BKBPI and PEGylated BKBPI were dissolved in deionized water to a range of protein concentrations between 50 and 130 mg/ml with an interval of 5 mg/ml. Sample dispersions were transferred to the rheometer sample tray (TA Instruments Co., New Castle, DE, USA), and a layer of silicified oil was added around the edges to prevent drying. Afterwards, thermal gelation was measured using a TA rheometer (AH1000 model, TA Instruments Co.), and the storage modulus (G') and loss modulus (G'') of the samples were measured to obtain the possible gelation concentrations using the software provided with the instrument. The heat-induced gelation of proteins was done using a heating-cooling cycle as follows: heating from 25 to 95 °C at 2 °C/min, holding at 95 °C for 30 min, then cooling to 25 °C at 2 °C/min.

2.7. Gelling properties of PEGylated BKBPI in distilled water

Distilled water dispersions of BKBPI and mPEG-BKBPI at 90 mg/ml were transferred to the rheometer sample tray for thermal gelation analyses. The storage modulus (G') of the samples were obtained during the process of gelation, and the heating-cooling cycle was the same as described in Section 2.6.

2.8. Gelling properties of PEGylated BKBPI in the presence of NaCl

BKBPI and mPEG-BKBPI were first dispersed in distilled water at 90 mg/ml, and were then mixed with NaCl to obtain NaCl concentrations of 0, 0.2, 0.4, 0.6 and 0.8 mol/l. The storage modulus (G') of the samples were then measured using the TA rheometer following the heating-cooling cycle as described in Section 2.6.

2.9. Gelling properties of PEGylated BKBPI in the presence of NEM

NEM was added into the BKBPI and mPEG-BKBPI distilled water solution (90 mg/ml) to obtain the final NEM concentrations of 0, 10, 15, 20 and 25 mmol/l. Storage modulus (G') of the samples were then obtained during the process of gelation according to the heating-cooling cycle as described in Section 2.6.

2.10. Gelling properties of PEGylated BKBPI with heat pretreatment

BKBPI and mPEG-BKBPI were dissolved in deionized water to obtain a protein concentration of 90 mg/ml. Heat pretreatment was then carried out using the TA rheometer with the heating-cooling cycle as following, heating from 25 to 85 °C at 5 °C/min, holding at 85 °C for 20 min, and cooling to 25 °C at 5 °C/min. Afterwards, the preheated dispersions or formed gels were further treated with the conditions used for heat-induced gelation in 2.6, and the storage modulus (G') during gelation was obtained.

2.11. Rheological measurements

Rheological measurements were carried out on a TA rheometer using a parallel plate with 40 mm radius and 1.0 mm gap. The protein dispersions were placed between the two parallel plates, and the gap between the two plates was set at 1.0 mm. The storage modulus (G') was determined using a temperature sweep test with the following stress mode: auto stress, initial stress 0.8 Pa, target strain 0.02 (which is within the linear viscoelastic region), frequency 0.1 Hz. Relationships between the storage modulus (G') and the loss modulus (G'') were determined using a frequency sweep test and the sweep frequency range was from 0.1 to 10 Hz.

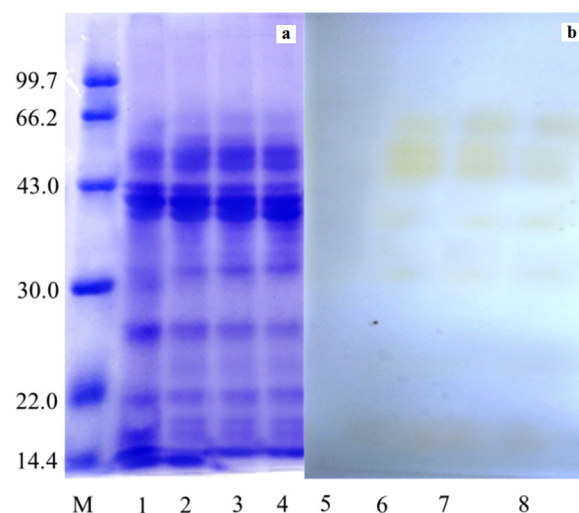


Fig. 1. SDS-PAGE analysis of the PEGylated BKBPI. Coomassie Brilliant Blue staining of the SDS-PAGE gel (a). Lane M: protein marker (14.4 kDa: lysozyme, 20.0 kDa: growth hormone, 30.0 kDa: carbonic anhydrase, 43.0 kDa: actin, 66.2 kDa: bull serum albumin, 99.7 kDa: PEGylated hemoglobin), Lanes 1–4: BKBPI, mPEG-SC-BKBPI, mPEG-SPA-BKBPI, mPEG-SS-BKBPI, respectively. Barium iodide staining of the SDS-PAGE gel (b). Lanes 5–8: BKBPI, mPEG-SC-BKBPI, mPEG-SPA-BKBPI, mPEG-SS-BKBPI, respectively.

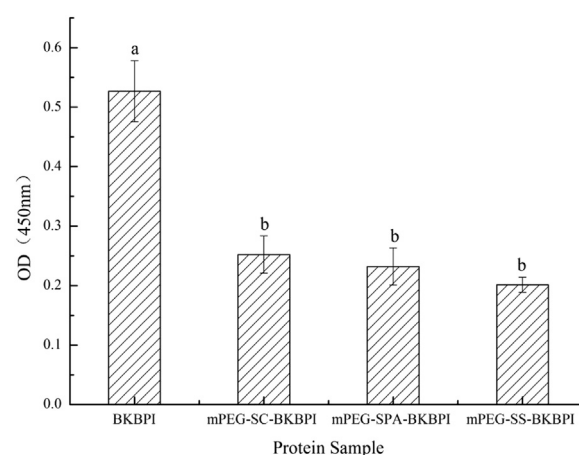


Fig. 2. Allergenicity of BKBPI and mPEG-BKBPI determined using the ELISA kit at 450 nm. Error bars denote standard error (SD, n = 6). Different letters indicate significant differences ($P < 0.05$) between samples.

2.12. Statistics analysis

All experiments were done at least in triplicates. Data was subjected to a one-way analysis of variance (ANOVA) using the SPSS 13.0 statistical analysis system student version (SPSS Inc. Chicago, IL, USA), and differences between pairs of means were compared using the least significant difference (LSD) at alpha of 0.05.

3. Results and discussion

3.1. PEGylation of BKBPI

The SDS-PAGE analysis of PEGylated BKBPI is shown in Fig. 1. As suggested in Fig. 1(a), compared with the native BKBPI, several new light bands with larger molecular weights were observed after PEGylation, which might result from the linking of PEG chains (Ginn, Choi, & Brocchini, 2016). To get a more visible characterization of PEGylated BKBPI, barium iodide staining SDS-PAGE was done with the selective staining for the successfully PEG modified proteins (Wang et al., 2017).

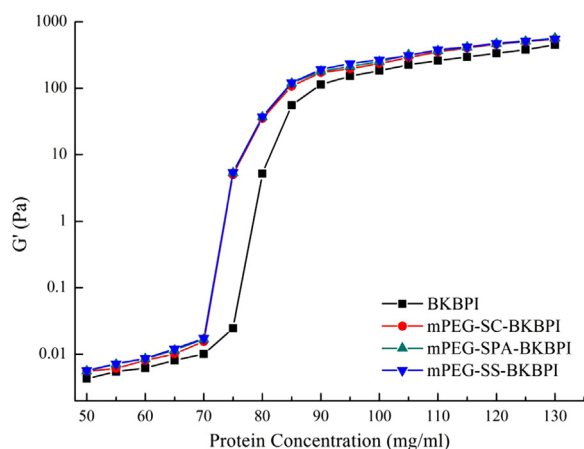


Fig. 3. Storage modulus (G') of heat induced gels of BKBPI (—■—), mPEG-SC-BKBPI (—●—), mPEG-SPA-BKBPI (—▲—) and mPEG-SS-BKBPI (—▼—) at different protein concentrations.

As observed in Fig. 1(b), no band was found in the BKBPI lane, however, several bands were seen in the lanes of mPEG-SC-BKBPI, mPEG-SPA-BKBPI and mPEG-SS-BKBPI, indicating that the conjugation of PEG and BKBPI was successful. In addition, the color densities of bands observed in barium iodide staining gel (Fig. 1) were consistent with the degree of PEGylated mPEG-BKBPI (mPEG-SC-BKBPI $6.5 \pm 0.8\%$,

mPEG-SPA-BKBPI $11 \pm 1\%$ and mPEG-SS-BKBPI $12 \pm 1\%$) measured in the previous study (He et al., 2018).

3.2. Allergenicity of BKBPI and PEGylated BKBPI

The allergic reaction will be triggered when immunologically active substances are released, normally induced by the specific binding between antigen epitopes and their corresponding antibodies or sensitized lymphocytes (Matsuo, Yokooji, & Taogoshi, 2015; Pomés, 2010). Fig. 2 shows the allergenicity changes of BKBPI before and after PEGylation. It was observed that the allergenicity of BKBPI was significantly decreased ($P < 0.05$) after PEGylation treatment, and mPEG-SPA-BKBPI and mPEG-SS-BKBPI would be less allergenic when used in products. The sharp decrease of allergenicity of PEGylated BKBPI also suggested that the effective modifications using PEG derivatives might help explain the screening effect induced by PEG chains (Roberts et al., 2012). After PEGylation, PEG chains could conjugate with proteins and shield a large area around the protein molecule, which would obstruct the access of antibodies or sensitized lymphocytes to the protein epitopes (Rajan et al., 2006). Furthermore, the sharp decrease in allergenicity suggested that the safety of BKBPI was enhanced using the PEG conjugations.

3.3. Gelling concentrations of BKBPI and PEGylated BKBPI

The effects of protein concentrations on the storage modulus (G') of

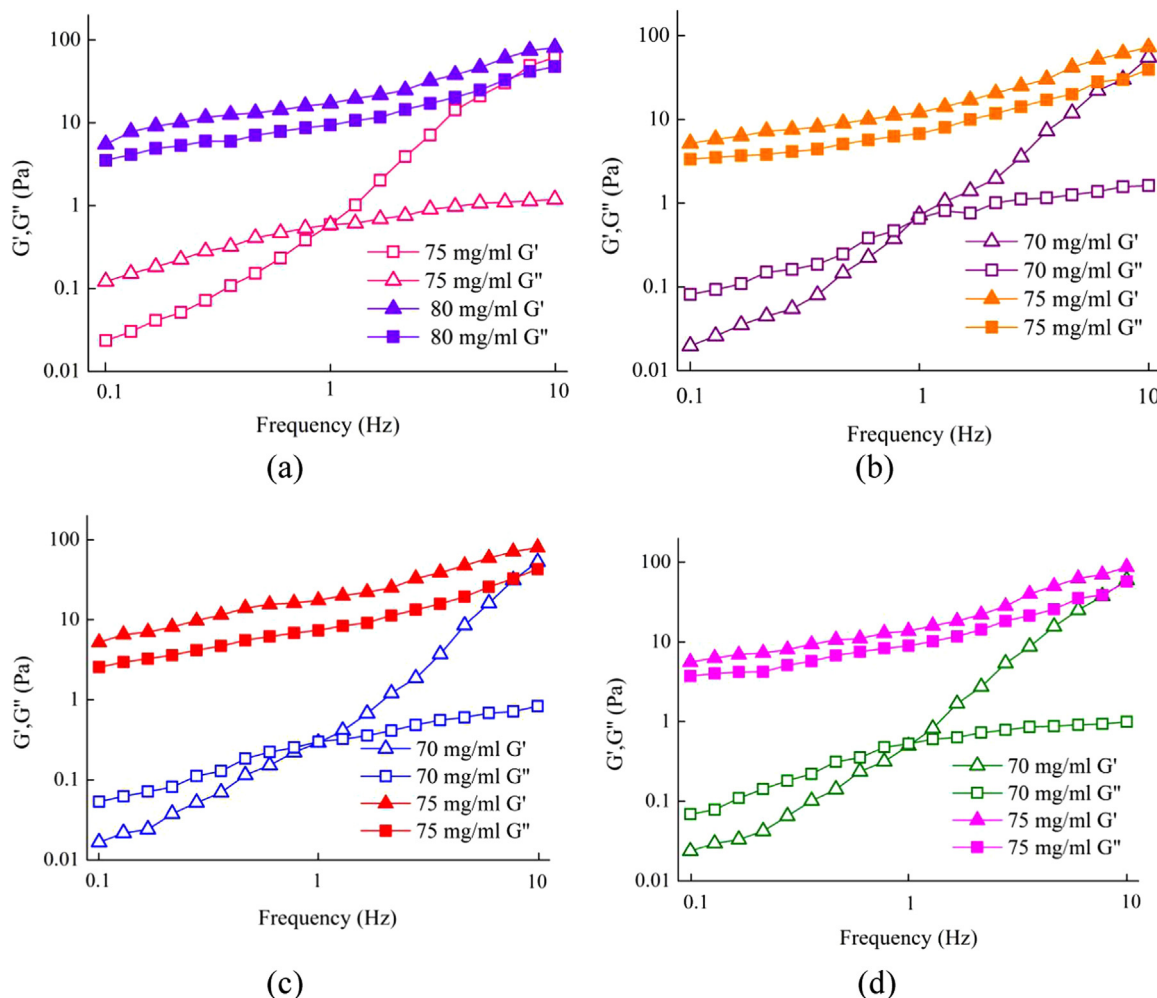


Fig. 4. G' and G'' curves of BKBPI (a), mPEG-SC-BKBPI (b), mPEG-SPA-BKBPI (c) and mPEG-SS-BKBPI (d), respectively, with frequency sweeping at different possible gelation concentrations.

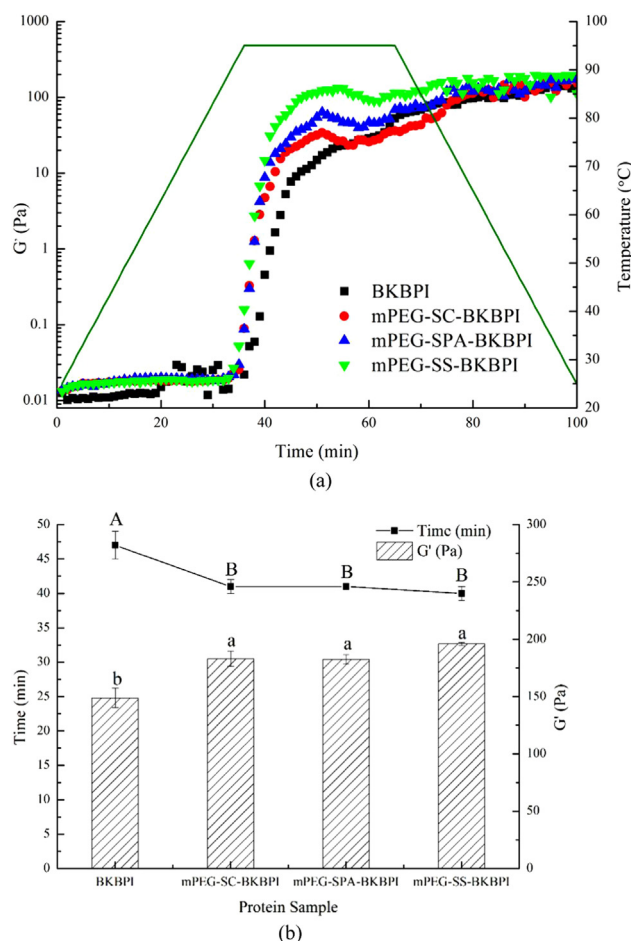


Fig. 5. Gelation process (a) and gel strength and gelation time (b) of BKBPI and mPEG-BKBPI in distilled water. Error bars denote standard error (SD, $n = 6$). Different letters indicate significant differences ($P < 0.05$) between protein samples within a parameter.

BKBPI and PEGylated BKBPI can be seen in Fig. 3. All samples showed very low G' s and behaved as a fluid at low protein concentrations (< 70 mg/ml). However, the storage modulus (G') increased quickly at 75 mg/ml, indicating the occurrence of a gel-like structure in the test samples, and a similar storage modulus (G')–concentration relationship was reported in a previous study of soy protein isolate gels (Chronakis et al., 2010). The storage modulus (G') of PEGylated BKBPI was higher than that of the native BKBPI over a wide concentration range from 50 to 130 mg/ml, and no significant differences ($P \geq 0.05$) in G' values were obtained among the PEGylated BKBPI samples conjugated with mPEG-SC, mPEG-SPA and mPEG-SS.

Fig. 4 shows the relationships between storage modulus (G'), loss modulus (G'') and shear frequency for each protein sample. As shown in Fig. 4, similar slopes for G' and G'' curves with frequency sweep can be obtained at the gel point, indicating the parallelism between G' and G'' , as well as the independence of phase angle to frequency (Winter & Chambon, 1986). As a result, the gelling concentration of BKBPI was 80 mg/ml, and mPEG-SC-BKBPI, mPEG-SPA-BKBPI and mPEG-SS-BKBPI had gelling concentrations of 75 mg/ml.

3.4. Gelling properties of PEGylated BKBPI in distilled water

At 90 mg/ml, which was higher than the gelling concentration in distilled water, BKBPI gels showed a shorter gelation time with a higher maximum G' after the PEGylation (Fig. 5), indicating better gelling properties compared with the native BKBPI. This can be explained by the reduced hydrophobic insoluble aggregation of mPEG-BKBPI during

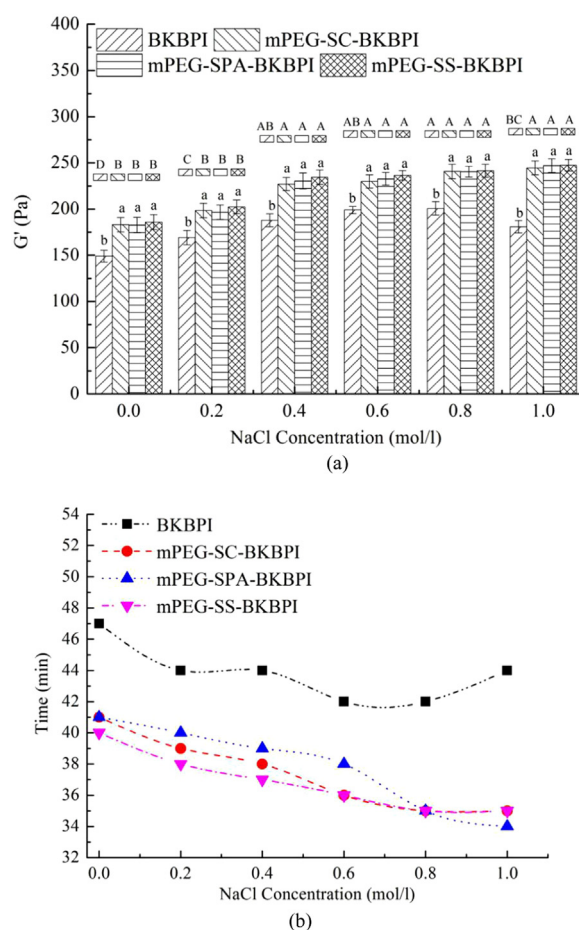


Fig. 6. Gel strength (a) and gelation time (b) of BKBPI and mPEG-BKBPI with different NaCl concentrations. Different capital letters mean significant differences ($P < 0.05$) among different additions of NaCl in the same protein gelation group, while different lowercase letters mean significant differences ($P < 0.05$) among different proteins in the same NaCl concentration group.

the gelation process, which was possibly due to the shielding effect of PEG chains on protein hydrophobic groups (Bryant & McClements, 1998; He et al., 2018). Moreover, there was a positive correlation between the gelation and the concentration of protein dispersions during the gelation process (Chen, Chassenieux, & Nicolai, 2018), better gelling properties could be expected with the higher solubility of mPEG-BKBPI (He et al., 2018).

3.5. Gelling properties of PEGylated BKBPI in the presence of NaCl

The effects of salt concentrations on gelling properties of BKBPI and mPEG-BKBPI are shown in Fig. 6. Increasing salt concentration in the range of 0–0.8 mol/l at pH 7.0 would lead to better gelling properties of BKBPI and mPEG-BKBPI. Electrostatic repulsion between protein molecules would be reduced by shielding the electric charges on protein surfaces. According to the previous study, the superior gelling properties of proteins were observed in the presence of salt, because more proteins were incorporated into the gel network caused by the reduction of electrostatic repulsion (Renkema et al., 2002). It should be noted that the change of NaCl concentrations would lead to various G' values and gelling times of BKBPI and mPEG-BKBPI (Fig. 6). With the addition of NaCl, G' of mPEG-BKBPI increased to a higher value near its maximum around 0.4 mol/l, and the maximum G' of BKBPI was obtained at a higher NaCl concentration of 0.6 mol/l. Besides, G' decreased as well as the gelling time increased at 1.0 mol/l NaCl for the native BKBPI samples, while no obvious changes were observed for mPEG-BKBPI gels

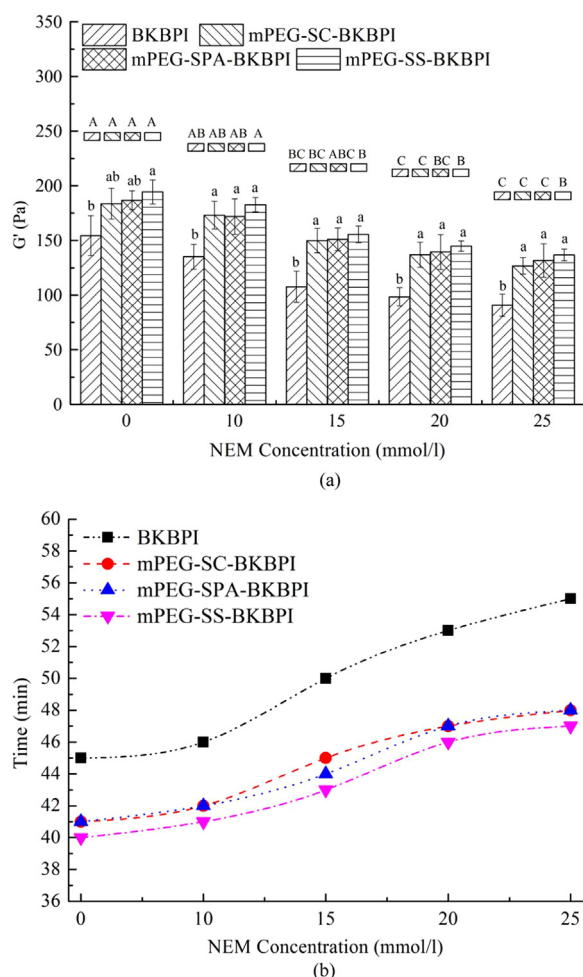


Fig. 7. Gel strength (a) and gelation time (b) of BKBPI and mPEG-BKBPI with different NEM concentrations. Different capital letters mean significant differences ($P < 0.05$) among different additions of NEM in the same protein gelation group, while different lowercase letters mean significant differences ($P < 0.05$) among different proteins in the same NEM concentration group.

at the same concentration. The differences may be explained by the high surface hydrophobicity of BKBPI, which would result in a stronger hydrophobic interaction between protein molecules, which predominated in the presence of NaCl. This would lead to the formation of insoluble protein aggregates or salting out of protein (Dill, 1990). On the other hand, insoluble aggregations of mPEG-BKBPI were reduced as the result of linking of PEG chains to shield the hydrophobic groups on protein surfaces (He et al., 2018), and the unfolding of protein molecules was not affected even at 1.0 mol/l NaCl.

3.6. Gelling properties of PEGylated BKBPI in the presence of NEM

Fig. 7 shows the decrease of G' and increase of gelling time of all proteins with the increase of NEM concentrations. It has been reported that NEM could inhibit or weaken the gelation of proteins (Monahan, German, & Kinsella, 1995; Mori, Nakamura, & Utsumi, 1982; Wang & Damodaran, 1990). As a sulfhydryl-blocking reagent, NEM can prevent disulfide-sulfhydryl (SS-SH) interchange and inhibit the formation of disulfide bonds (Alting, Hamer, Kruij, & Visschers, 2003; Meng & Ma, 2002). Because of the role of disulfide bonds in gel network formation, the inhibition of disulfide bonds formation could weaken the formation of the gel network and decreased G' as well as increase gelling time of proteins with the addition of NEM (Monahan et al., 1995). Furthermore, the attachment of NEM to the SH groups in proteins would

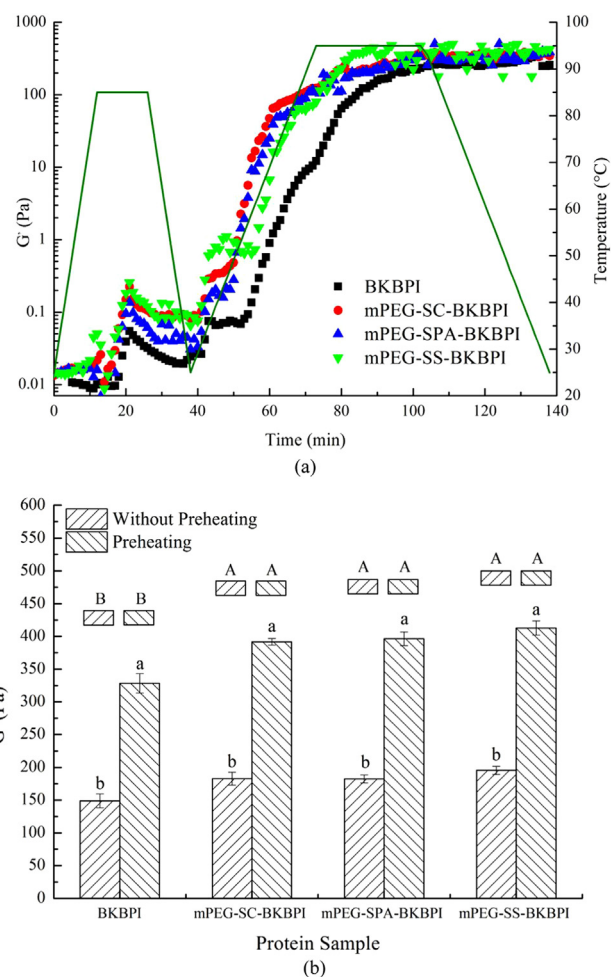


Fig. 8. Gelation process (a) and gel strength (b) of BKBPI and mPEG-BKBPI with heat pretreatment. Different capital letters mean significant differences ($P < 0.05$) among different protein samples in the same treatment group, while different lowercase letters mean significant differences ($P < 0.05$) between both treatments in the same protein gelation group.

expose more hydrophobic groups on the molecular surface, which could promote the further aggregation of protein and the formation of insoluble aggregations (Hua et al., 2005). Compared with the changes of BKBPI gels, it is interesting to observe that G' and gelling times of mPEG-SC-BKBPI, mPEG-SPA-BKBPI and mPEG-SS-BKBPI changed with the changes of NEM concentrations (Fig. 7). Furthermore, a lower gelation drop of mPEG-BKBPI was obtained with the addition of NEM, which was probably because of the masking effects of the hydrophobic groups induced by the PEG chains (Hinds & Kim, 2002). Based on the results, the masking of the hydrophobic groups might impair the NEM-induced hydrophobic group exposure, and prevent hydrophobic-induced aggregation, which would lead to the decrease of gelling properties.

3.7. Gelling properties of PEGylated BKBPI with the heat pretreatment

The heat induced gelation of BKBPI and mPEG-BKBPI combined with the heat pretreatment is shown in Fig. 8. During the subsequent cooling of the pretreatment and heating with the normal heating-cooling cycle, the G' of BKBPI and PEGylated BKBPI increased exponentially, indicating the occurrence of gelation. Specifically, compared with the normal heating-cooling cycle, the G' increased by 116% after the heat-induced gelation combined with preheating of BKBPI and mPEG-BKBPI, which could be explained by the differences of

extent of denaturation and the degree of soluble protein aggregation (Shand, Ya, Pietrasik, & Wanasundara, 2007). In the case of proteins with preheating, greater amounts of soluble protein aggregates were stabilized predominantly by the formation of disulfide bonds, and resulted in a higher level of proteins aggregation, which would accelerate the protein gelation (Rodrigues et al., 2015). Furthermore, more unfolding and interaction of the protein formations could be obtained after the heat pretreatment, which would be favorable for formation of three-dimensional gel networks and thus improve the gelling properties (Hongsprabhas & Barbut, 1996; Jian et al., 2014). In addition, because of the higher solubility, mPEG-BKBPI always showed better gelling properties before and after the heat pretreatment.

4. Conclusions

The low allergenicity and good gelling properties of BKBPI were obtained after PEGylation, which might be attributed mainly to the shielding effect of the hydrophobic groups by the PEG chains. Compared with the native BKBPI, the resistances of mPEG-BKBPI to the different environmental factors were improved with significant enhancements in G' and a reduction in gelling time. Thus, the PEGylated BKBPI would have useful properties when used for gel and film manufacture, and PEGylation could be considered as a simple and effective pre-processing method for use by the food industry.

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Conflict of interest statement

The authors declare no competing financial interest.

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