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ORIGINAL PAPER

Effects of Oligosaccharides and Soy Soluble Polysaccharide on the Rheological and Textural Properties of Calcium Sulfate-Induced Soy Protein Gels

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Abstract This study investigated the rheological and textural properties of calcium sulfate ($CaSO_4$)-induced gels formed by soy protein containing oligosaccharides (sucrose, raffinose, and stachyose) and soy soluble polysaccharide. The results showed that the hardness and water holding capacity of the gels were significantly strengthened ($P < 0.05$) by the incorporation of oligosaccharides at the 1, 3, and 5% (*w*/*v*) levels and soy polysaccharide at the 0.3 and 0.5% (*w*/*v*) levels. The storage modulus after the temperature cycle, frequency sweep, creep recovery tests, and large deformation properties demonstrated that the affixion of above cosolutes enhanced the rigidity (elastic properties) of gels. Confocal scanning laser microscopy (CLSM) analysis indicated that the cosolutes also improved the microstructures of the gels and herein the gels containing stachyose exhibited the greatest compactness. The gel solubility in different buffers verified the speculation that the protein-protein interactions were enhanced by the incorporation of cosolutes and hence accounted for the improvement of the gel properties. The results of this study would potentially promote the use of health-promoting raffinose, stachyose, or soy polysaccharide and facilitate the development of novel soy protein-based gel products with firmer texture and higher nutritional value.

Keywords Soy protein · $CaSO_4$-induced tofu-type gels · Oligosaccharides · Soy soluble polysaccharide · Texture · Health-promoting

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Introduction

Soy protein is widely used in the food industry for its unique functional properties such as emulsification, foaming, and gelation (Kinsella 1979). Generally, the functional characteristics of soy protein are connected with their physical, chemical, and structural properties, which are greatly influenced by their processing conditions and their interactions with other ingredients (Tseng et al. 2008). Hence, a thorough understanding of the effects of processing conditions and other ingredients on protein properties will provide valuable information for the manufacture of protein-based products, such as tofu and tofu-type gel induced by coagulant.

Two types of coagulant, acid (glucono-δ-lactone) and salts (e.g., $CaSO_4$), are commonly employed for the making of tofu or soy protein-based tofu-type gels (Kao et al. 2003; Murekatete et al. 2014a). The gelation mechanism of glucono-δ-lactone (GDL) has been widely accepted. But for the soy protein-based gels induced by $CaSO_4$, the coagulation mechanism is more complex. Combined with the previous studies, the gelation process can be summarized as follows. The first step is the heat denaturation of soy proteins accompanied by the increase of the degree of aggregation. During this process, some of the hydrophobic and SH groups initially located within the interior of protein molecules are exposed to the outside (Guo et al. 2015). Meanwhile, the soy protein molecules will aggregate with each other via the hydrophobic interactions and disulfide bonds. After the addition of $CaSO_4$, the electrostatic repulsive forces between protein molecules are neutralized and reduced, leading to the occurrence of the

further aggregation with Ca^{2+} acting as bridges between the charged carboxylic groups on adjacent soy protein isolate (SPI) molecules (Kao et al. 2003). During the second process, the SPI molecules in the aggregation state will interact more strongly with each other through protein-protein interactions and eventually promote the formation of the gel structures with the three-dimensional network (Zhao et al. 2016).

Oligosaccharides, especially sucrose, raffinose, and stachyose, are the major soluble carbohydrates in soy seeds (Obendorf et al. 2009). Sucrose is generally recognized to impart sweetness to soy-based products such as soy milk and tofu (Taira 1990). Raffinose and stachyose, referred to as raffinose family oligosaccharides (RFOs), are both α-galactosyl derivatives of sucrose; the former has one and the latter has two moieties of galactose attached to sucrose via α1 → 6 glycosidic linkage (Kumar et al. 2010; dos Santos et al. 2013). Given their growth-promoting effect on probiotics such as *Bifidobacterium* spp. and *Lactobacillus* spp., RFOs may improve the health of the colon, reduce the risk of colon cancer, and extend the lifespan. Accordingly, researchers in Japan have actually recommended that RFOs could be used as an alternative for common table sugar (Messina 1999; Mussatto and Mancilha 2007; Wongputtisin et al. 2015). Soy soluble polysaccharide (SSPS) is a dietary fiber extracted and refined from soy residue (okara), which is a by-product of soymilk, tofu, and SPI manufacturing (Maeda and Nakamura 2009). SSPS has been reported to possess potential health benefits to humans, such as improving laxation, reducing the risk of diabetes, and lowering blood cholesterol (Chen et al. 2010). Apart from its high nutritional value, SSPS is commonly used as a food ingredient for its physical properties.

As functional food ingredients, RFOs and SSPS have gained popularity in current years due to consumer demands for a healthier diet. They are now added to diverse products to create differentiated, value-added functional foods. Presently, limited information is available on the texture-modifying effects of RFOs and SSPS in soy protein-based gel products. To facilitate the use of these health-promoting saccharides in food processing, particularly for developing soy protein-based gel products, this study was conducted to investigate the influence of oligosaccharides and SSPS on the rheological and textural properties of $CaSO_4$-induced soy protein gels. The potential mechanism involved during the gelation process and protein-protein interactions in the retention of gel structures were also discussed. The microstructures of gels containing different cosolutes were characterized by confocal laser scanning microscopy.

Materials and Methods

Materials

Soybeans obtained from Xuelang Seed Station (Wuxi, Jiangsu, China) were ground and defatted at room temperature (25 °C). SPI was prepared from the resultant defatted soybean flour according to a modified method described by Puppo et al. (1995) and did not undergo any other drying treatment. The protein concentration of the SPI dispersion was determined by Kjeldahl analysis. $CaSO_4 \cdot 2H_2O$ and sucrose were purchased from Sinopharm Chemical Reagent Co Ltd. (China). Raffinose and stachyose were purchased from Xi'an Feide Biotech Co., Ltd. (China). SSPS was kindly donated by Fuji Oil Co., Ltd. (Japan). All other chemicals were of analytical grade.

Heat Treatment and $CaSO_4$-Induced Gel Formation

Oligosaccharides (sucrose, raffinose, and stachyose) were mixed with the SPI dispersion to give a final protein concentration of 7% (*w*/*v*) and oligosaccharide concentrations of 1, 3, and 5% (*w*/*v*), respectively. As the preliminary experiment indicated that SSPS had a destructive effect on the gel texture at concentrations of >0.75% (*w*/*v*), the concentrations were designated as 0.1, 0.3, and 0.5% (*w*/*v*), respectively. The dispersions (pH 7.0) were mixed using a magnetic stirrer for 1 h at room temperature (25 °C) and stored at 4 °C overnight. Then, the dispersions were heated in a water bath at 90 °C for 15 min to denature the soy protein. After cooling down to room temperature, the preheated dispersions were stored at 4 °C overnight. The control used in this study was 7% (*w*/*v*) SPI dispersion heated without cosolute under the same conditions. It should be noted that sucrose, raffinose, stachyose, and SSPS at the same concentrations as those mentioned above underwent the same heat treatment process kept in a solution state.

$CaSO_4$ was added at room temperature (25 °C) to the heated SPI-cosolute dispersions with gentle stirring for 1 min to mix well, and the final concentration of $CaSO_4$ in the dispersions was 0.35% (*w*/*v*). The dispersions were then subjected to heat treatment at 90 °C for 15 min and cooled down to room temperature to allow for gel formation. The gels were stored overnight at 4 °C.

Dynamic Rheological Measurements

Dynamic small-deformation oscillatory shear measurements were performed using an AR G2 rheometer (TA Instruments, New Castle, DE, USA) with parallel plates (40 mm in diameter and gap of 1 mm) based on the methods described by Gu et al. (2009) and Lee and Kuo (2011) with some modifications. Each sample was loaded in the rheometer, and the exposed rim of the sample was covered with a thin layer of silicon oil to prevent dehydration. The applied strain of 0.01 was within the linear viscoelastic region, and the frequency was 1 Hz. The temperature cycle was programmed to heat the samples from 25 to 90 °C at a heating rate of 5 °C/min, keep them at 90 °C for 10 min, and then cool them down to 25 °C at a cooling rate of 5 °C/min. After the

temperature cycle, a frequency sweep experiment was conducted on the same sample at 25 °C from 0.1 to 10 Hz at 0.01 strain, following the method described by Torres et al. (2014) and Kaewmanee et al. (2013) with slight modifications. The storage modulus (G'), which represents the energy stored due to elastic deformation in a cyclic deformation, was used to indicate the elastic nature of the gel.

A creep recovery test was performed according to the method of Bi et al. (2014), Cando et al. (2014), and Toker et al. (2013) with slight modifications under the shear stress of 8 Pa at 25 °C. The change in strain under constant stress was recorded for 5 min. After the removal of stress, recovery response was recorded for another 5 min. Burger's model, which is composed of Kelvin-Voigt and Maxwell models placed in series, was used to interpret the creep data because of its simplicity and providing acceptable results (Dolz et al. 2008). In food systems (proteins, polysaccharides and their composite gels, etc.), the model was applied to characterize the viscoelastic properties in many studies (Rabiey and Britten 2009; Yilmaz et al. 2012; Bi et al. 2014; Huang et al. 2016). The model was expressed as the following equation (Steffe 1996).

$$J(t) = \frac{1}{G_0} + \frac{1}{G_1}\left(1 - e^{-\frac{t}{\lambda}}\right) + \frac{t}{\mu_0} \quad (1)$$

where J is the creep compliance (1/Pa) which is the ratio of strain to stress, G_0 and G_1 are the instantaneous elastic modulus (Pa) and retarded elastic modulus (Pa), respectively, λ is the retardation time of Kelvin component (s), and μ_0 is the viscosity components (Pa s^{-1}) of Newtonian element.

The recovery rate (%) presented by Eq. (2) was used to quantify the percentage recovery of gel samples.

$$\text{Recovery}(\%) = \frac{\text{total strain-permanent strain}}{\text{total strain}} \times 100 \quad (2)$$

The large deformation properties of gels were measured based on the methods of Luyten et al. (1994) and Li et al. (2012) with some modifications. In this work, the determination was conducted in the oscillation mode at a frequency of 1 Hz up to the fracture point when the shear stress began to decrease.

Texture Analysis of $CaSO_4$-Induced Gels

A TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) was used to measure the hardness of the gels. The gel in the containers was 41 mm in diameter and 23 mm in height. Before measurement, the gels were not removed from the containers and were equilibrated at 25 °C for 30 min. The determination was performed using a cylindrical plunger with a diameter of 10 mm at a test speed of 0.8 mm/s. Gel hardness was defined as the maximum force in the force-distance curve.

Water Holding Capacity of $CaSO_4$-Induced Gels

The measurement of the WHC of the gels was conducted according to the method described by Maltais et al. (2005) with slight modifications. Equal volumes (3 mL) of gels were formed in the centrifuge tubes and stored at 4 °C overnight. Afterwards, the gels in the tubes were centrifuged at 10,000×g for 10 min at 4 °C. The supernatant was discarded and the tubes were inverted to drain for 30 min. WHC (%) was defined as the ratio of the weight of water remaining in the gels after centrifugation to the weight of the total water in the samples × 100.

Measurement of Gel Solubility

To investigate the noncovalent and covalent interactions involved in the $CaSO_4$-induced gels containing different cosolutes, the solubility of gels was measured in different buffers according to the method of Shimada and Cheftel (1988) with some modifications. Solvent systems consisted of distilled water (S1), 0.086 M Tris-0.09 M glycine-4 mM Na_2EDTA (pH 8.0) (S2), S2 containing 0.5% SDS (S3), S3 containing 8 M urea (S4), and S4 containing 2% β-mercaptoethanol (S5). Certain amounts of buffers were added to the same weight of gels and stirred on a Vortex Genius 3 mixer (IKA-Werke GmbH & Co., KG, Germany) for 1 min. The resultant solutions were incubated for 24 h at 25 °C in a shaking water bath and then centrifuged at 12,000×g for 30 min. The protein content of these supernatants was estimated by the biuret method using the BSA standard. The percentage of gel solubility was expressed as the protein content of the supernatant to the total × 100.

Confocal Scanning Laser Microscopy Analysis of $CaSO_4$-Induced Tofu-Type Gels

In all cases, a Leica TCS-SP8 confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany) was used to determine the microstructural properties of the $CaSO_4$-induced tofu-type gels according to the procedure of Tang et al. (2011). The gels were stained with a fluorescent dye, Rhodamine B, which stains the protein phase with the excitation wavelengths at 488 nm. Then, 20 μL of dye (0.01%, w/v) was mixed well with the 7% (w/v) heated SPI solutions containing different cosolutes. The mixtures were heated at 90 °C for 15 min for the formation of gels. The samples were then put on uncoated glass bottom dishes covered with coverslips and further sealed with nail polish to prevent evaporation. Images were taken with a ×40 objective lens at a certain depth below the coverslip surface.

Statistical Analysis

All experiments consisted of three independent trials, each with at least triplicate sample analyses. The results were expressed as means ± standard deviation. Data were subjected to analysis of variance (ANOVA) using the linear model procedure of the Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA). Significant differences ($P < 0.05$) between means were identified by the least significant difference procedure of all pairwise multiple comparisons. The creep data were modeled according to Burger's model, using the nonlinear regression feature of Statistix 9.0 (Analytical Software, Tallahassee, FL, USA).

Results and Discussion

Rheological Measurement

The values of final G' of the gels containing different cosolutes subjected to the heating-holding-cooling cycle after the gelation process are presented in Fig. 1. The cosolutes, oligosaccharides (sucrose, raffinose, and stachyose), and soy polysaccharide all improved the gelation of the SPI when compared with the control. The final G' order of the $CaSO_4$-induced SPI-oligosaccharide gels was SPI-stachyose gel > SPI-raffinose gel > SPI-sucrose gel at the same added level. The G' of the SPI-oligosaccharide gels increased as the concentration of sucrose, raffinose, and stachyose increased from 1 to 5%. However, the G' values of the above gels containing 3 and 5% cosolutes did not differ significantly ($P > 0.05$). For the SPI-SSPS gel, the G' value was relatively low in comparison with the SPI-oligosaccharide gels. It should be noted that although the amount of SSPS added was one tenth that of the other three cosolutes, the final G' of the gel containing 0.3 and 0.5% SSPS also improved significantly ($P < 0.05$) compared with the control gel.

The G' of the gels containing different cosolutes as a function of frequency is shown in Fig. 2. As is clearly demonstrated, the G' value of all of the samples increased as the oscillation frequency increased. The frequency dependence of G' suggests that the gels were in close proximity to the viscoelastic material (Ahmed et al. 2006). The tan δ of the gels ranged from 0.16 to 0.23, indicating that the gels belonged to the category of so-called weak gels or colloidal dispersions (Clark and Ross-Murphy 1987). In a weak gel system, G' is dependent on the frequency, exactly as the results in Fig. 2 show in this study. A power law model, which is $G' = a \times F^b$

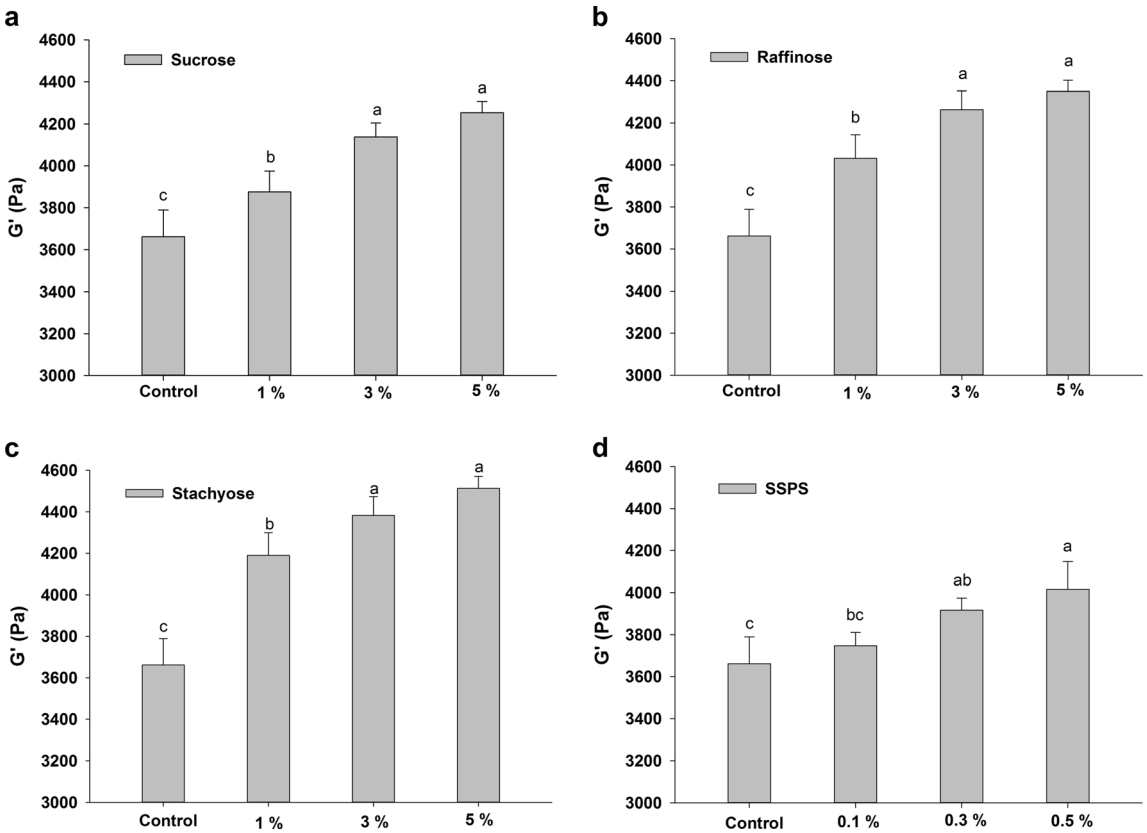

Fig. 1 Final G' of the $CaSO_4$-induced SPI gels containing different cosolutes. Means with different *superscript letters* (**a**–**c**) differ significantly ($P < 0.05$). The G' values represent the measurements at the final temperature of 25 °C after the heating-holding-cooling cycle

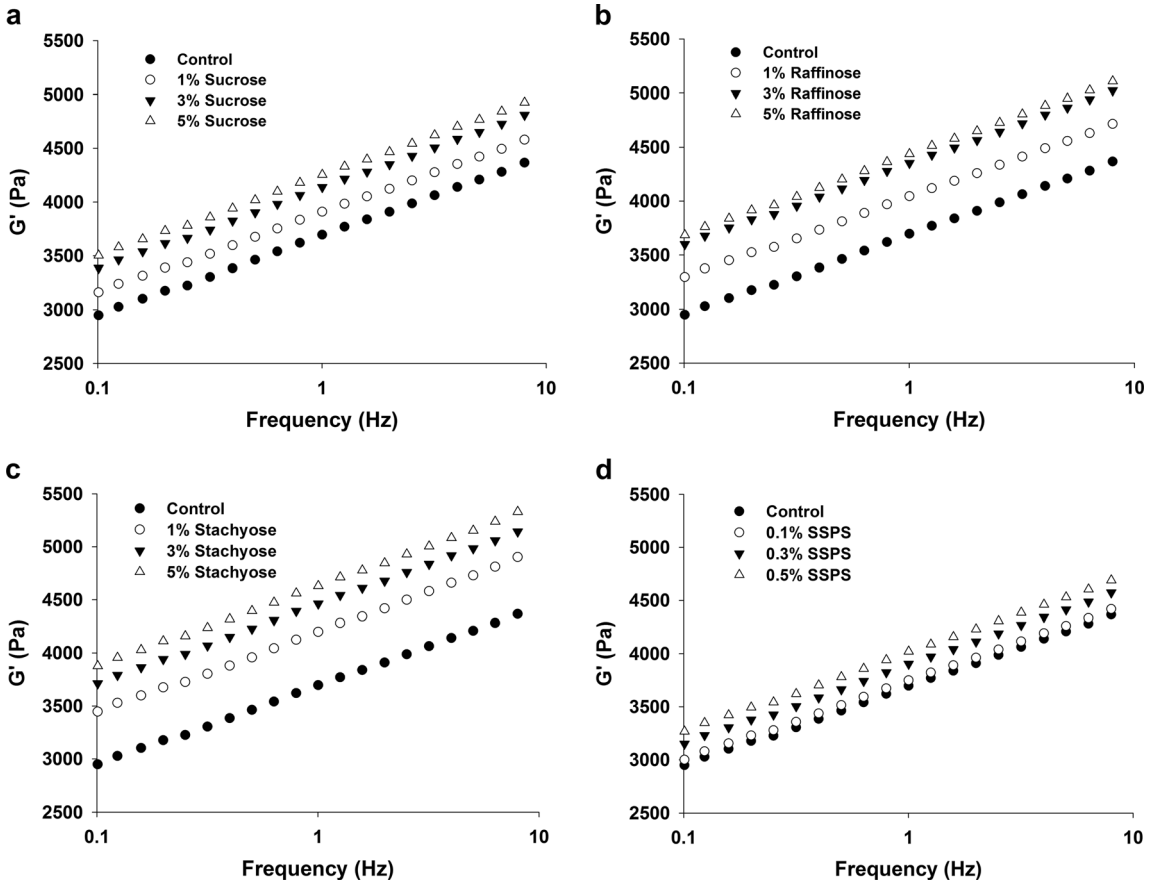

Fig. 2 Frequency sweep of the storage modulus (G') of $CaSO_4$-induced SPI gels containing different cosolutes at 25 °C

($R^2 > 0.99$), was employed to predict the relationship between the value of G' and frequency (F). The magnitudes of exponent (b) are in relevance to the strength of gel (Chang et al. 2009). The b value was 0.0877 for the control SPI gel (free of cosolute). In the case of the gels containing oligosaccharides in the ascending order of concentrations from 1 to 5%, b values were 0.0828, 0.0786, and 0.0765 for the SPI-sucrose gel; 0.0801, 0.0748, and 0.0733 for the SPI-raffinose gel; and 0.0789, 0.0732, and 0.0716 for the SPI-stachyose gel, respectively. The b values for the SPI-SSPS gel in the same ascending order of concentrations from 0.1 to 0.5% were 0.0864, 0.0833, and 0.0809 respectively. These results indicated that the incorporation of cosolutes all improved the gel strength (gel rigidity) of $CaSO_4$-induced SPI gels and the trends of promoted effects were inconsistent with the results of the final G' of the gels discussed above.

The creep recovery test, which belongs to small deformation, was used to evaluate the viscoelasticity of gels. The value of creep compliance (stress is divided by strain) which was recorded as a function of time can reflect the rigidity of gels (Bi et al. 2014). When a constant stress was loaded on the gel, compliance increased rapidly with time at the beginning and then more slowly. But after the removal of the stress, a constant deformation remained, which indicated that the gel was a viscoelastic material (Cheng et al. 2005). The creep behavior of gels containing different cosolutes is presented in Fig. 3 (the recovery data not shown). The results indicate that the structures of all the gels were strengthened by the addition of cosolutes. This is because the value of creep compliance (J) is closely related with the strength of the gel, which implies that a higher J value represents weaker structure and a lower J value shows stronger structure (Toker et al. 2013; Huang et al. 2016). The values of four parameters (G_0, G_1, λ, and μ_0) obtained by fitting the creep data to the Burger's model (Eq. 1) are summarized in Table 1. In all cases, the data was successfully fitted to Eq. (1) with the regression coefficients (R^2) > 0.99, indicating that the model is adequate to characterize the viscoelastic properties of the gels. As is clearly seen from Table 1, the addition of cosolutes all promoted the instantaneous elastic behavior (G_0) of $CaSO_4$-induced SPI gels. The trends of G_1 values were similar to G_0, which demonstrated prompt resistance to deformation as a result of a strengthened three-dimensional network structure of gels (Wang et al. 2009). The increase in the μ_0 value caused by the incorporation of oligosaccharides or soy polysaccharide indicated the promotion of the viscous component of gels. The retardation time λ is the time needed for the strain related to viscoelastic behavior to reach $1 - 1/e$ of the maximum strain (Wu et al.

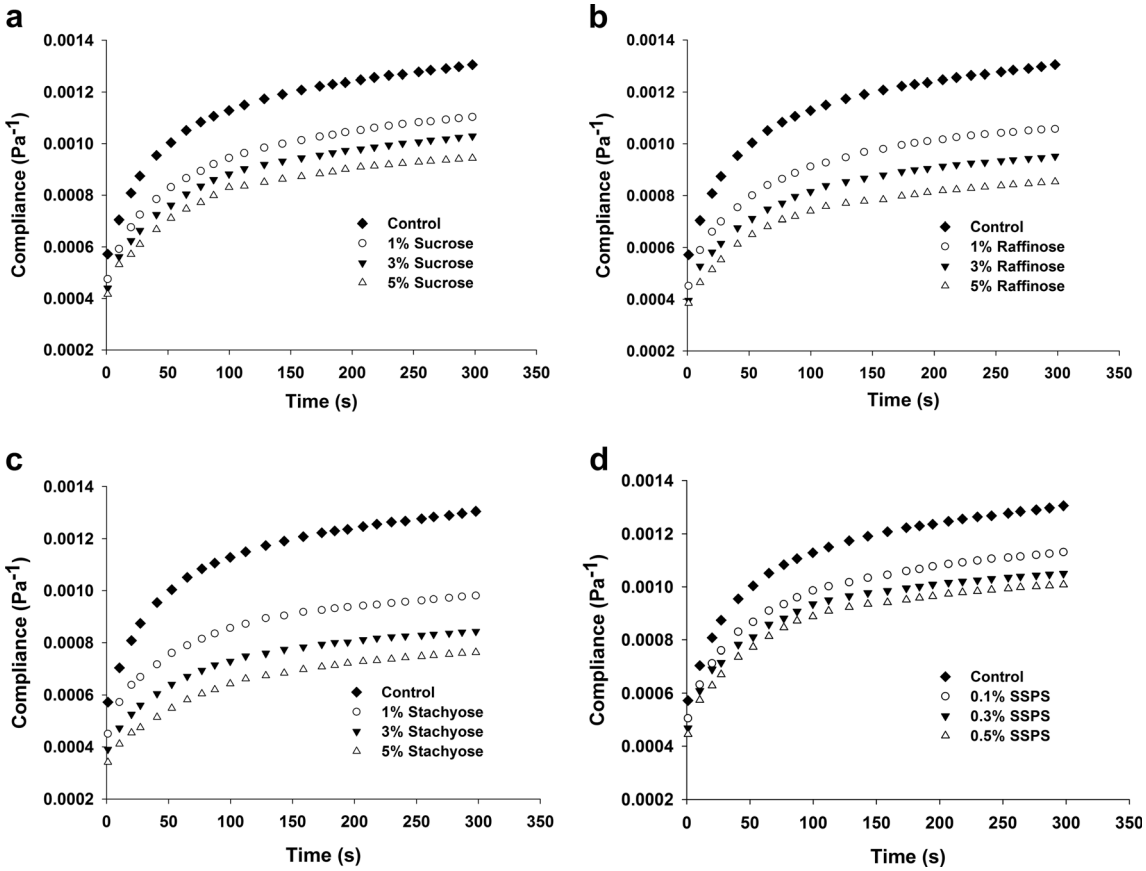

Fig. 3 Creep behavior of $CaSO_4$-induced SPI gels containing different cosolutes

Table 1 Parameters of Burger's model and recovery rate of $CaSO_4$-induced SPI gels containing different cosolutes

Treatment	Viscoelastic parameters				
	G_0 (10^3 Pa)	G_1 (10^3 Pa)	μ_0 (10^6 Pa s)	λ (s)	Recovery (%)
Control[a]	1.78 ± 0.07i	1.86 ± 0.04i	1.42 ± 0.06f	36.5 ± 0.9g	84.4 ± 1.1h
Sucrose					
1%	2.10 ± 0.06fg	2.28 ± 0.05gh	1.56 ± 0.06ef	39.2 ± 1.2ef	86.7 ± 0.6fgh
3%	2.24 ± 0.03e	2.39 ± 0.02f	1.77 ± 0.09de	42.7 ± 2.1c	88.6 ± 1.0cd
5%	2.38 ± 0.04d	2.50 ± 0.03e	2.41 ± 0.28b	47.0 ± 1.2b	89.2 ± 1.0bc
Raffinose					
1%	2.11 ± 0.04fg	2.35 ± 0.03fg	1.68 ± 0.12def	39.9 ± 1.5de	87.6 ± 0.6def
3%	2.42 ± 0.04d	2.52 ± 0.08de	1.94 ± 0.11cd	41.7 ± 1.7cd	89.6 ± 0.7bc
5%	2.64 ± 0.02b	2.80 ± 0.15c	2.49 ± 0.18b	43.8 ± 1.3c	90.5 ± 0.9b
Stachyose					
1%	2.17 ± 0.03ef	2.62 ± 0.08d	2.09 ± 0.18c	39.8 ± 1.2def	88.4 ± 0.6cde
3%	2.55 ± 0.03c	2.91 ± 0.08b	2.67 ± 0.20b	46.5 ± 1.2b	90.5 ± 0.9b
5%	2.89 ± 0.04a	3.11 ± 0.10a	3.05 ± 0.20a	59.1 ± 1.9a	92.2 ± 1.1a
SSPS					
0.1%	1.98 ± 0.04h	2.23 ± 0.04h	1.63 ± 0.10ef	37.5 ± 1.5fg	85.8 ± 0.8gh
0.3%	2.08 ± 0.04g	2.29 ± 0.03gh	2.13 ± 0.18c	38.4 ± 0.7efg	87.0 ± 0.8efg
0.5%	2.21 ± 0.04e	2.32 ± 0.04fg	2.43 ± 0.16b	42.5 ± 1.1c	88.0 ± 1.0cdef

Each value represents the mean ± standard deviation (n = 3). Values with different lowercase letters (a–i) in the same column differ significantly ($P < 0.05$)

[a] The gel formed by SPI with no cosolute served as the control

2010). Steffe (1996) indicated the gel with a high λ value to reach the full deformation slowly. The last column in Table 1 indicated the recovery rate of gels increasing with the increase of the concentration of cosolutes. These data well corresponded to the final G' discussed above. The increased recovery rate could be ascribed to the stronger network structures at higher concentrations of cosolutes. In brief, the creep recovery data reflected the enhancement of the viscoelastic behavior associated with the network structures of gels by the incorporation of cosolutes.

The large deformation measurement of gels containing different cosolutes was performed by investigating the change in shear stress as a function of strain, and the results are shown in Fig. 4. The maximum in the curve where shear stress began to decrease is identified as the fracture point (Murekatete et al. 2014a). As the deformation enlarged, the stress increased initially up to a certain point and then declined rapidly. In all cases, the gels exhibited smaller fracture modulus values than storage modulus values, indicating a decrease in gel rigidity with increasing strain or known as strain weakening (Vardhanabhuti et al. 2001). As is revealed in Fig. 4, the shear stress of the gels containing the same cosolute at the fracture point increased with its increasing concentration. The values of fracture stress of gels containing different oligosaccharides at the same added level displayed the same order as the final G'. Similarly, the incorporation of SSPS at lower concentration also remarkably enhanced the fracture stress. Moreover, the increase in shear deformation at the fracture point of gels resulted from the fact that the effects of cosolutes were not obvious but identifiable. Murekatete et al. (2014b) conducted a study on the fracture point of tofu-type gels induced by different concentrations of $CaSO_4$ and found a similar phenomenon. In general, the fracture point of a gel correlated with its elasticity. When the results were combined with the final G' discussed above, a gel with higher G' fractured at a higher stress was found. In brief, the incorporation of different concentrations of cosolutes enhanced the fracture stress (gel rigidity) of $CaSO_4$-induced SPI gels.

The rigidity of a protein gel depends on the concentration of protein particles incorporated into the gel structure and the strength of the protein-protein interactions (Narine and Marangoni 1999). In the present study, the presence of cosolutes in the aqueous phase altered the fraction of protein molecules that participated in the gel network and thus strongly influenced the gel rigidity. It could be interpreted that increasing the ratio of cosolutes in the continuous phase

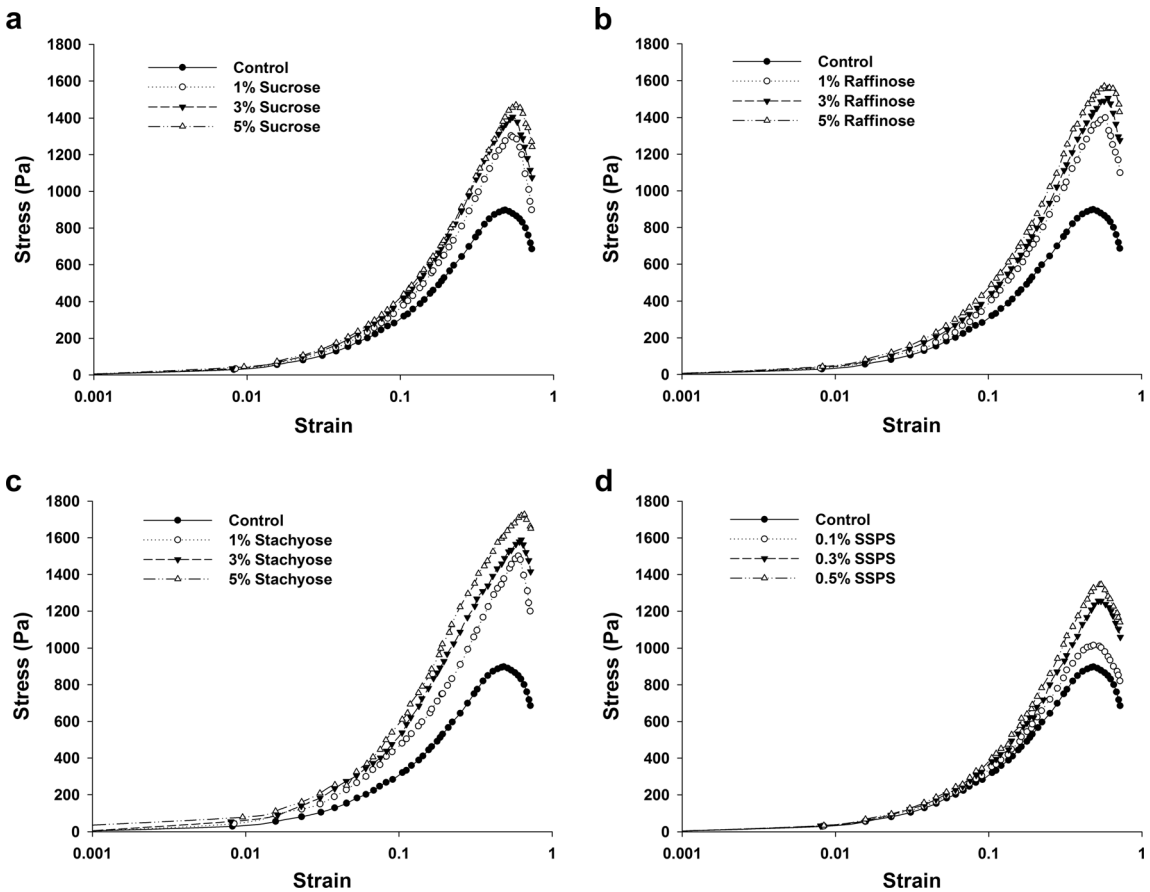

Fig. 4 Shear stress as a function of deformation of $CaSO_4$-induced SPI gels containing different cosolutes. The maximum in the *curve* is taken as the facture point

promoted the attraction between soy protein molecules due to preferential interaction effects (steric exclusion mechanisms), which are colligative, reduced the contact area between proteins and the surrounding solution, and favored the molecular arrangements of protein (Timasheff 1998). When soy protein molecules are adequately denatured and aggregated, the rigidity of the resultant gels should be stronger in the presence of cosolutes because the protein-protein interactions are enhanced (Baier and McClements 2001), as illustrated by the results of gel solubility.

Hardness and Water Holding Capacity of $CaSO_4$-Induced Gels

The gel hardness and WHC of the $CaSO_4$-induced tofu-type gels were obviously affected by the presence of cosolutes. The incorporation of cosolutes significantly increased ($P < 0.05$) both the hardness (except for the gel containing 0.1% SSPS) and the WHC of the gels (Figs. 5 and 6). For example, the addition of 5% sucrose increased ($P < 0.05$) the hardness and WHC of the gels by 12.9 and 9.5%, respectively, in comparison with the control gel, and adding the same level of stachyose enhanced ($P < 0.05$) the two characteristics of the gels by 29.9 and 12.3%, respectively, in comparison with the control gel. The gel hardness and WHC of the gels containing 0.5% SSPS also increased ($P < 0.05$) by 8.7 and 7.6%, respectively, compared with the control sample.

The improved hardness and WHC of the tofu-type gels treated with cosolutes can be explained by the mechanism of tofu curd formation, during which the textural properties of gels are greatly influenced by the excluded volume effects mentioned above. Before the addition of the coagulant, as the cosolute concentration increased, a more critical heating treatment was needed to unfold the protein molecules because the contact area of the proteins in the native state is lower than in the unfolded state. Once unfolded, the hydrophobic sites of the protein molecules are exposed and the hydrophobic interactions are enhanced by the excluded volume effects. Moreover, the unfolded protein molecules are more prone to aggregate (because aggregates have a lower contact area than individual molecules), which improves the capacity of the gel to resist puncture and compression (McClements 2002; Tseng and Xiong 2009). A plausible explanation for the WHC is that a higher concentration of cosolutes might become trapped within the interstitial spaces of the gels, thus entrapping more water within the gel. Our previous work also demonstrated that increasing the degree of aggregation, accompanied by the intensification of the degree of denaturation, favorably improved the hardness and WHC of the gels (Zhao et al. 2016).

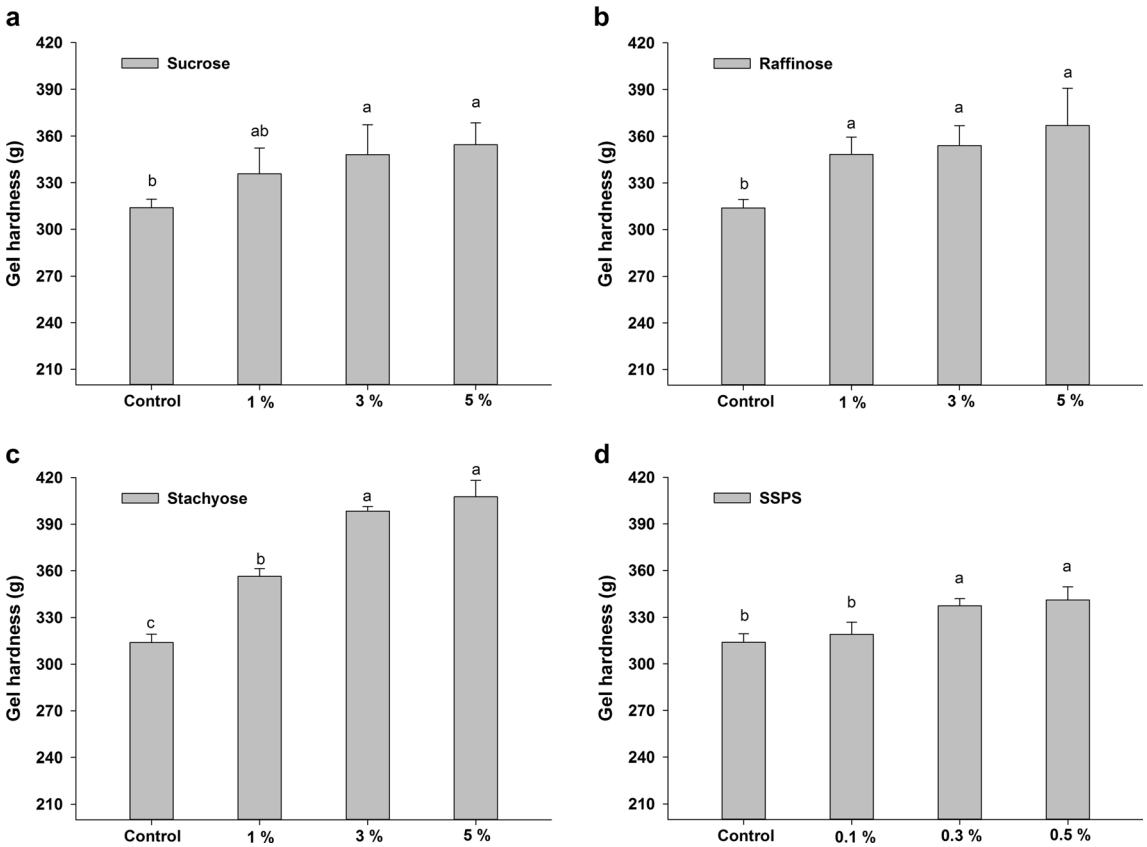

Fig. 5 Gel hardness of $CaSO_4$-induced SPI gels containing different cosolutes. Means with different *superscript letters* (**a**–**c**) differ significantly ($P < 0.05$)

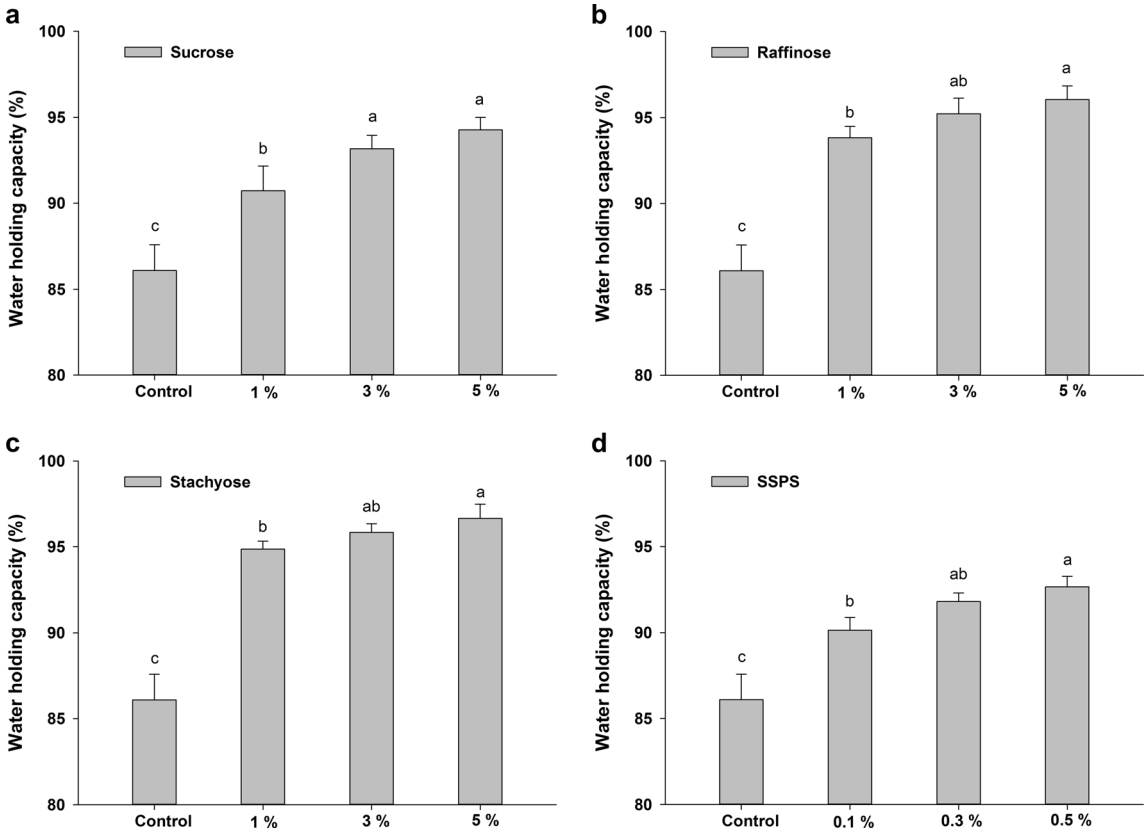

Fig. 6 Water holding capacity of $CaSO_4$-induced SPI gels containing different cosolutes. Means with different *superscript letters* (**a**–**c**) differ significantly ($P < 0.05$)

Force Involved in the Gel

To clarify the protein-protein interactions involved in the formation of the gel network, the protein solubility of $CaSO_4$-induced gels in five different solvents was measured. The differences in the protein solubility between pairs of adjacent solvents, S2-S1, S3-S2, S4-S3, and S5-S4, represent the interaction forces of electrostatic interactions, hydrophobic interactions, hydrogen bonds, and disulfide bonds (Puppo and Añón 1998; Tang et al. 2006). The results of the gel solubility tests are shown in Table 2. The gels in buffer S2 had slightly higher solubility than those in buffer S1, especially the gel formed by the incorporation of 0.3% (*w*/*v*) SSPS, indicating that electrostatic interactions are involved in maintaining the gel structure (Puppo et al. 1995; Gu et al. 2009). The increase in gel solubility from buffer S3 to S2 and from buffer S5 to S4 was more obvious than that in the other adjacent solvent pairs, demonstrating the important role of hydrophobic interactions and disulfide bonds in the retention of gel structures (Renkema et al. 2002; Campbell et al. 2009). The order of the differences in gel solubility from buffer S2 to S3 was SPI-SSPS gel > SPI-stachyose gel > SPI-raffinose gel > SPI-

Table 2 Protein solubility of $CaSO_4$-induced SPI gels containing different cosolutes in various solvents[a]

Gels	S1	S2	S3	S4	S5
Control[b]	2.00 ± 0.04a	4.70 ± 0.12c	32.90 ± 0.88d	49.27 ± 0.55c	81.28 ± 1.06d
3% Sucrose	1.91 ± 0.04b	6.03 ± 0.15b	37.25 ± 0.83bc	57.14 ± 1.07b	91.47 ± 1.27b
3% Raffinose	1.84 ± 0.03c	5.97 ± 0.09b	38.55 ± 1.00b	60.18 ± 1.68a	94.19 ± 1.92a
3% Stachyose	1.76 ± 0.03d	6.06 ± 0.08b	40.83 ± 0.59a	56.63 ± 0.74a	95.15 ± 0.82a
0.3% SSPS	1.91 ± 0.02b	8.46 ± 0.18a	43.54 ± 0.97c	55.80 ± 1.58b	88.64 ± 1.14c

Each value represents the mean ± standard deviation ($n = 3$). Values with different lowercase letters (a–d) in the same column differ significantly ($P < 0.05$)

[a] S1: distilled water; S2: 0.086 M Tris-0.09 M glycine-4 mM Na_2EDTA (pH 8.0); S3: S2 containing 0.5% SDS; S4: S3 containing 8 M urea; S5: S4 containing 2% β-mercaptoethanol

[b] The gel formed by SPI with no cosolute served as the control

sucrose gel > control SPI gel. These results demonstrate the corresponding order of the contribution of hydrophobic interactions to the retention of the gel network. The differences in solubility between buffer S4 and S3 suggest that the hydrogen bonds significantly influenced the gel structures. In summary, the overall differences in gel solubility from buffer S1 to S5 further confirmed the speculation that the incorporation of cosolutes enhances the protein-protein interactions in the retention of gel structures.

CLSM of $CaSO_4$-Induced Gels

The gel structures of $CaSO_4$-induced tofu-type gels with different cosolutes were investigated by CLSM. The images in Fig. 7 reveal that the gel network was relatively loose and porous when the gels were formed by SPI dispersions without cosolutes and with low concentrations of cosolutes (1% oligosaccharide and 0.1% SSPS). The addition of cosolutes at a somewhat higher level (3 and 5% for the oligosaccharides, 0.3

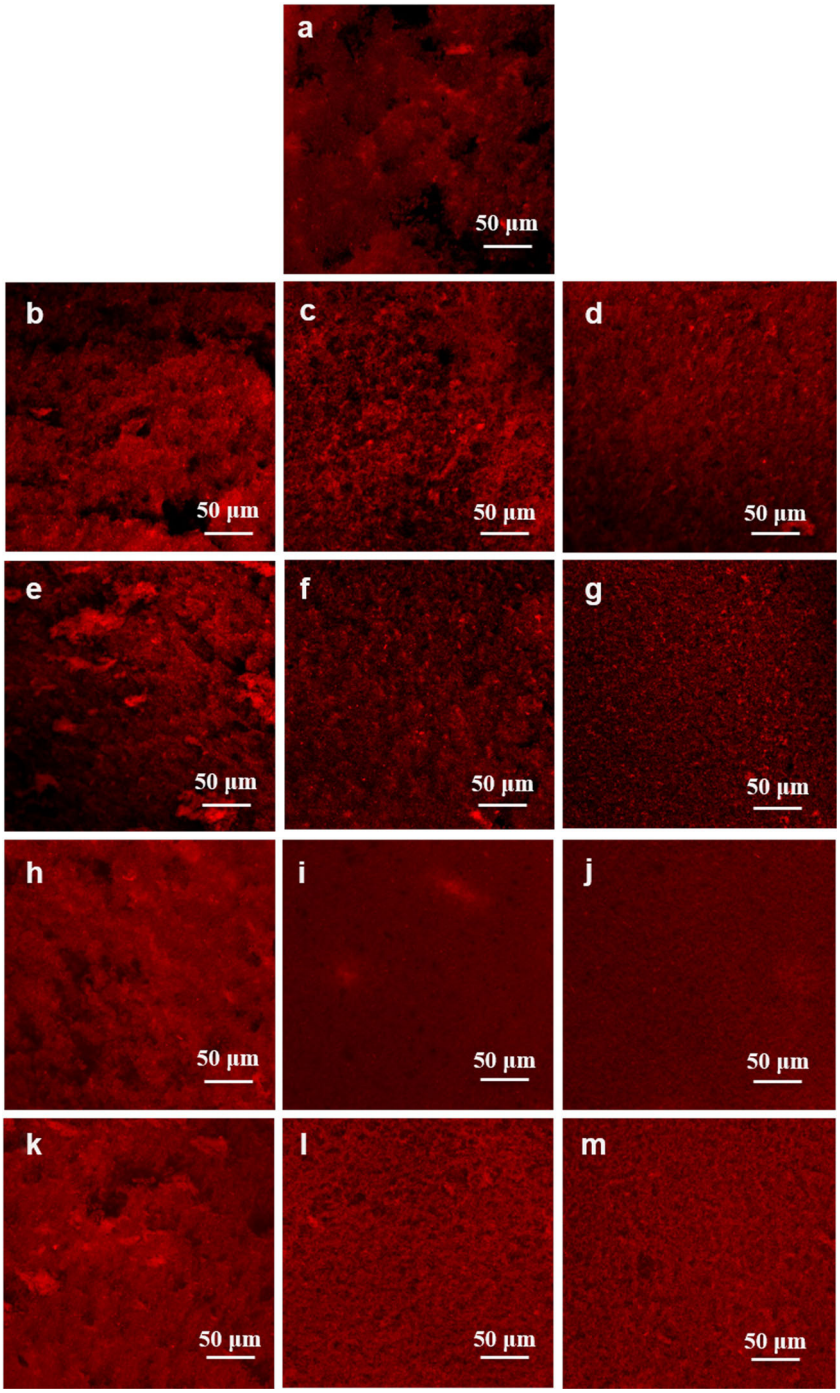

Fig. 7 CLSM images of $CaSO_4$-induced tofu-type gels formed by SPI containing different cosolutes. **a** Control (cosolutes free), **b** with 1% sucrose, **c** with 3% sucrose, **d** with 5% sucrose, **e** with 1% raffinose, **f** with 3% raffinose, **g** with 5% raffinose, **h** with 1% stachyose, **i** with 3% stachyose, **j** with 5% stachyose, **k** with 0.1% SSPS, **l** with 0.3% SSPS, **m** with 0.5% SSPS

and 0.5% for the SSPS) significantly improved the gel structures. As demonstrated in Fig. 7, the stachyose-treated gels (Fig. 7i, j) featured a more homogeneous and compact structure than the other treatments.

The results of the study indicated that the incorporation of cosolutes altered the aggregation state of the soy protein, and the improved density was probably due to the increased cross-linking of protein molecules. Tseng et al. (2008) demonstrated that the addition of cosolute (inulin) promoted the network formation of soy protein gels, generating SPI gels with smaller pore sizes and greater compactness. The microstructures of 11S and SPI gels coagulated with magnesium chloride were investigated, and the researchers concluded that the gel networks were closely related to the types of protein aggregates (Nagano and Tokita 2011). The results revealed by the CLSM images were generally consistent with the results for the rheological properties, hardness, and WHC of gels, the improvement of which eventually depended on the strength of the protein-protein interactions.

Conclusions

The results of this study revealed that the addition of sucrose, raffinose, stachyose, and SSPS improved the rigidity (elastic properties), hardness, WHC, and microstructures of $CaSO_4$-induced soy protein tofu-type gels. The gel solubility in different buffers indicated that the enhanced protein-protein interactions accounted for the improvement in the gel properties. The incorporation of raffinose, stachyose, or soy polysaccharide, which serves as a functional food ingredient, would facilitate the development of an innovative tofu-type gel with firmer texture and higher nutritional value. Future work studying on the influence of cosolutes on the sol-gel transition of soy protein at a molecular level as correlated with the texture of the composite SPI gel system would be highly desirable.

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