A Thesis for the Degree of Master of Science

Influence of sucrose and acid on the structure and rheological properties of salt-induced soy protein isolate gel

대두 단백질 cold gel의 구조 및 유변학적 특성에 대한 자당과 산의 영향

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농학석사학위논문

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지도교수 문 태 화
이 논문을 석사학위 논문으로 제출함

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Influence of sucrose and acid on the structure and rheological properties of salt-induced soy protein isolate gel

by

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Abstract

In this study, structural, rheological properties and the fractal dimension of soy protein isolate (SPI) cold-set gels made with different concentrations of sucrose and acid were determined.

SPI dispersions were heated above those denaturation temperatures (97 °C) in the presence or absence of sucrose. And then gelation was induced by 20 mM $CaCl_2$ and 21.55 mM HCl. Zeta-potential, sulfhydryl group content, rheology, water holding capacity (WHC), microstructure and fractal dimension of soy protein gels were measured.

Zeta potential showed that sucrose blocks the revealed net charge of protein. And the sample added acid formed aggregation and its vale close to zero. Rheological measurement revealed that all SPI-sucrose gels were softer than gels without sucrose. As increasing of sucrose contents, storage modulus decreased. And acid treated gels represented larger storage modulus than without acid sample. Gels of SPI with 30% sucrose had largest water holing capacity. And gels of SPI with acid had smallest water holing capacity because they made more compact structure than sample without acid. The image of TEM showed that denser flocs were formed as well as increased aggregation of protein molecules as the concentration of sucrose decreased. Acid and salt

combined-treated gels without sucrose showed the most compact microstructure. D_f values determined from rheological data using scaling models of Wu and Morbidelli, were the highest (2.80) for combined-treated gel in absence of sucrose among all SPI gels.

These results showed that sucrose has protective effect on the protein denaturation. And combination of acid treatment with salt has synergetic effect on improving the properties of cold-set soy protein gel. Rheological properties indicated that acid and salt treatment also has beneficial effect on the rheology of gels. Therefore these results together represented that combination of acid and salt to forming gel may be an important approach to produce cold-set gels with improved rheological and textural properties.

Key words : Soy protein, Cold-set gelation, Fractal dimension, Sucrose, Acid, Combined-treated gel

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Introduction

Soy protein isolate (SPI) is generally used as functional ingredient in food industry due to their nutritive and functional characteristics, i.e. emulsification, water holding capacity, foaming and gelation (Renkema et al., 2002).

The ability to make gels is especially desirable character indicating the texture of the food products (LÓ PEZ-DÍAZ, 2003). Soy protein gels are commonly prepared by heat treatment at different pH and ionic strengths (Zhu et al., 2011). Heating a protein dispersion makes protein molecules unfolding, which leads to aggregation of protein molecules and then to gelation, when the number of aggregated protein exceeds a critical concentration (Maltais et al., 2008). However, a high temperature to induce gelation is not desirable to some protein products. Therefore, making gels at ambient temperature or near ambient temperature (cold-set gelation) is advantageous, which is favorable to protect non heat resistant food ingredient, such as some nutrition, flavours, and pigments etc (Maltais et al., 2005). Tofu is a gel of soybean proteins that is produced by adding coagulants to heat denatured soybean protein dispersions (Saio, 1979). Cacium chloride, calcium sulfate and glucono-δ-lactone (GDL) are common

coagulants used on an industrial scale for the formation of soybean protein gels (DeMan et al., 1986).

Recently, it has been revealed that soy protein isolate (SPI) can form a gel structure under cold conditions (Maltais et al., 2005). After soy protein dispersion is denatured by heat treatment, gels can be prepared by different means such as pressure treatment, enzyme treatment (MTGase) and by adding GDL or salt (Eissa et al., 2004; Jaros et al., 2006; Orlien et al., 2006). When salt is added or the pH is reduced towards the pI, the electrostatic repulsive forces between the filaments is reduced and they make aggregates (Barbut, 1995; Bryant et al., 1998).

Some studies revealed the effect of sugars on soy protein isolate gels. The presence of low molecular weight co-solutes such as sugars in the continuous phase of soy protein food systems can change the conformational properties and interactions of proteins by binding to protein surface, or they may indirectly affect these characteristics by altering the physicochemical properties of solvent (Gu et al., 2009). The interactions and their influence on protein functionality depend on the type and concentrations of co-solutes present (Baier et al., 2001). Sugars can stabilize proteins against heat denaturation affecting gel strength by increasing the onset temperature of heat denaturation and altering bond formation during gelation(Baier et al.,

2001; KulmyrzaevBryant et al., 2000; KulmyrzaevCancelliere et al., 2000; Rich et al., 2000). Therefore, adding sugar to SPI dispersion makes soft gels. To make up for this phenomenon, some research used combined cross-linking methods. To further improve the functionalities of cross-linked protein, it is possible to combine a method with an additional cross-linking treatment (Gan et al., 2008).

The objective of this study was to examine the effects of sucrose and acid on the structural and rheological characteristics of cold-set soybean protein gel. Base on the data, the final goal of this research was to apply to the food industry by providing the products with desired structural and textural attributes.

Materials and Methods

1. Materials

Commercial soy protein isolate (SPI, SUPRO® 120 IP) was purchased from Solae (St. Louis, MO, USA). According to the manufacturer's specifications, SUPRO® 120 IP has a minimum protein content of 90%, maximum moisture of 6%, 5.5% fat, 1.4% sodium, and 5% ash. Hydrochloric Acid (1N HCl solution) and calcium chloride (anhydrous $CaCl_2$) were purchased from Duksan Pure Chemicals Co.(Duksan, Ansan, Korea) and Sigma(Fisher Scientific, Springfield, NJ, USA), respectively. All other chemicals were of analytical reagent grade.

2. Methods

2-1. Cold-set gelation

Appropriate amount of sucrose was mixed with an SPI dispersion to give a final concentration of 8 % protein (w/w) and sucrose concentrations of 10 %, 20 % and 30 % (w/w). The dispersion was stirred using a magnetic stirrer for at least 1 h at room temperature to ensure complete hydration, and preheated in a water bath at 97 ℃ for 30 min. Then, it was cooled down to

room temperature for 2 h. Afterwards, for gelation, distilled water containing $CaCl_2$ (0.5 M) was added into the SPI dispersion to a final salt concentration of 20 mM, and 1N HCl solution was added into the SPI dispersion to a final acid concentration of 21.55 mM. The dispersion was very gently vortexed immediately. The temperature during development of gel-network was maintained at 25 ℃ in a water bath overnight, and analyses were performed.

2-2. Zeta-potential measurement

Electrical charge (ζ potential) of the dispersion was measured with a commercial dynamic light scattering instrument. A 1 mL aliquot of heated SPI dispersion (9% w/w) was prepared and diluted to 0.9% with distilled water, and this dispersion was homogenized at 1,100 rpm for 3 min with a homogenizer. Then it was filtered through a 5 μm HA Millipore membrane (Millipore, MA, USA) prior to analysis.

2-3. Determination of sulfhydryl groups

The sulfhydryl group content of SPI gels was measured using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) according to Ellman (1959) as modified by Shimada and Cheftel (1988). The gel (1 mL) was mixed with 3 mL of buffer T (0.086 M Tris - 0.09 M glycine - 4 mM Na_2EDTA buffer, pH

8.0). The gel suspension was homogenized at room temperature for 3 min and centrifuged at 10,000×g for 10 min.

For determination of free SH groups, 300 μL of supernatant was mixed with 2 mL of buffer T, and 200 μL of 20 mM DTNB (dissolved in buffer T) was added. Samples mixed with 2 mL buffer T without DTNB were used as blanks.

For determination of total SH groups, buffer T was replaced by buffer U (buffer T containing 6 M urea and 0.5 % SDS) in the method described above. The sample was vortexed, left stand at room temperature for 15 min, and the absorbance was measured at 412 nm using a UV-Vis spectrophotometer (V-530, Jasco, Tokyo, Japan). A molar extinction coefficient of $1.36\times10^{4}\,M^{-1}cm^{-1}$ was used for calculating micromoles of SH/g of protein. Samples mixed with 2 mL buffer U without DTNB were used as blanks.

2-4. Circular dichroism spectroscopy (CD) of SPI dispersions

The conformational changes of the acid treated and non-treated SPI-sucrose dispersion were inspected by circular dichroism (CD) analysis. All circular dichroism experiments were performed using a Chirascan plus (AppliedPhotophysics, UK) at 25 ℃. CD measurements were performed at a

protein concentration of 0.01 % (w/v) in distilled water. CD spectra of all samples were recorded at 25 ℃ in the range of 180-350 nm with a spectral resolution of 0.2 nm. The scan speed was 100 nm/min, and the response time was 0.5 sec with a bandwidth of 1 nm. Quartz cells with an optical path of 0.1 mm were used. Typically, three scans were accumulated and averaged.

2-5. Dynamic rheological measurements of SPI gels

The rheological measurement was performed using an oscillatory rheometer (Rheostress 1, ThermoHaake, Karlsruhe, Germany) with a cone/plate geometry (35mm Ø, 1°) and a designated gap of 1 mm. The sample was brought into the lower plate using a plastic spatula and filled up the gap by lowering the upper cone down to the designated gap (1 mm). The extra sample around the edge of the plate was trimmed with tissue. The storage modulus (G´) was recorded as a function of frequency of oscillation. The elasticity value was determined from the frequency sweep analyses according to the method of Maltais et al. (2008) with some modifications.

2-6. Water-holding capacity

Water-holding capacity (WHC) of gels was measured according to Kocher and Foegeding (1993) with some modifications. SPI gel (2 g) was

formed and transferred into the inner tube of a centrifugal ultrafiltration unit (Millipore) supporting an ultrafiltration membrane of 3,000 nominal molecular weight limit. Samples were centrifuged at 5,000×g for 20 min. After centrifugation, the water released in the outer tube was weighed. WHC was calculated according to Maltais et al. (2005) as follows ;

$$\text{WHC } (\%) = \frac{W_t - W_r}{W_t} \times 100$$

where W_t is the total quantity (g) of water in the sample, and W_r is the quantity (g) of water released from the gels.

2-7. Transmission electron microscopy (TEM)

Microstructures of SPI-sugar cold-set gels were observed by TEM. Gels were primarily fixed in 0.05 M sodium cacodylate buffer (pH 7.2) containing 2% paraformaldehyde and 2% glutaraldehyde for 2 h at 4 ℃ and washed three times in 0.05 M sodium cacodylate buffer (pH 7.2) for each 10 min at 4 ℃. Then post-fixation was performed using 0.05 M sodium cacodylate buffer (pH 7.2) containing 1% osmium tetroxide at 4 ℃ for 2 h. Samples were briefly washed two times at room temperature using distilled water. Afterwards, en bloc staining was performed in 0.5% uranyl acetate at 4 ℃ for 30 min. Dehydration was performed using a series of solutions of

increasing ethanol concentration from 30 % to 100 %. Transition was conducted using 100% propylene oxide two times at room temperature for 10 min each. Each sample was embedded using a mixture of Spurr's resin and propylene oxide for infiltration step. For polymerization, samples were placed in the oven at 70℃ for 24 h. Then samples were sectioned with an ultramicrotome (MT-X, RMC, Tucson, AZ, USA) and stained with 2% uranyl acetate for 7 min and Reynolds' lead citrate for 7 min. Specimens were observed with a transmission electron microscope (LIBRA 120, Carl Zeiss, German).

2-8. Calculation of mass fractal dimension from rheological data

The scaling model of Wu and Morbidelli (2001), which relates the microscopic structure parameters of colloidal gels to their macroscopic elastic properties, was used. The elasticity of gels (G') and their limit of linearity (γ_0) are described as proportional to a power of particle concentration (Φ) :

$$G' \sim \Phi^{\beta/(3-D_f)} \quad (1)$$

$$\gamma_0 \sim \Phi^{(2-\beta)/(3-D_f)} \quad (2)$$

$$\beta = 1 + (2 + x)(1 - \alpha) \quad (3)$$

The fractal dimension of the gel is designed as D_f. D_f is the fractal dimension;

β is an exponent that depends on α and x; x is the fractal dimension of the floc backbone ($1 \leq x < D_f$) ,and α is a microscopic elastic constant ($0 \leq \alpha \leq 1$). Using the auxiliary parameter β and assuming the backbone fractal dimension x in the range [1, 1.3], one can compute the corresponding α value and identify the prevalent gelation regime in the colloidal gel system.

Fractal dimension values were calculated for the 30 mM salt and 21.55 mM acid gels (protein concentration from 6 to 9 % w/w) using the Wu and Morbidelli scaling model. Eqs. (1) and (2) were resolved using the slopes of their log-log plots (G' vs Φ and γ_0 vs Φ) as the power-law exponents of the relations. Parameters D_f and β were obtained and used to determine α values with Eq. (3).

2-9. Statistical analysis

All experiments were repeated three times, and mean values and standard deviations are reported. Mean comparisons were conducted by Duncan's multiple range test using significant difference level of 0.05. All statistical analyses were performed using SPSS 12.0 for windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

1. Zeta potential

Zeta potentials of SPI dispersions with various concentration of sucrose are shown in Figure 1. The zeta potential value of native SPI dispersion was -13.8 mV, and decreased to -32.7 mV when the dispersion was heated without sucrose at 97 ℃ for 30 min. When the SPI dispersion is heated above denaturation temperature, this heating process induces unfolding of the protein globular structure. Thereby heated proteins expose reactive groups and protein surface has negative charges (Mulvihill et al., 1987; 2006). The zeta potential gradually increased to zero with the increase of sucrose concentration from 10 % to 30 %. For the SPI treated with 30 % sucrose, it increased to -26.8 mV showing the highest value among the non-acid treated groups. In comparison, the zeta potential of the control that was heat-treated without sucrose was significantly lower than that of SPI heated in the presence of sucrose. The zeta potential values of acid treated SPI dispersions were higher than those of non-acid treated SPI dispersions. The acid treated SPI with 30 % sucrose showed the most similar value to that of the native. Adding acid to an SPI dispersion caused the change in protein charges and decreased intra-molecular electrostatic repulsion, thereby

promoting the aggregation of the molecules and cross-linking of proteins resulting in small zeta-potential values.

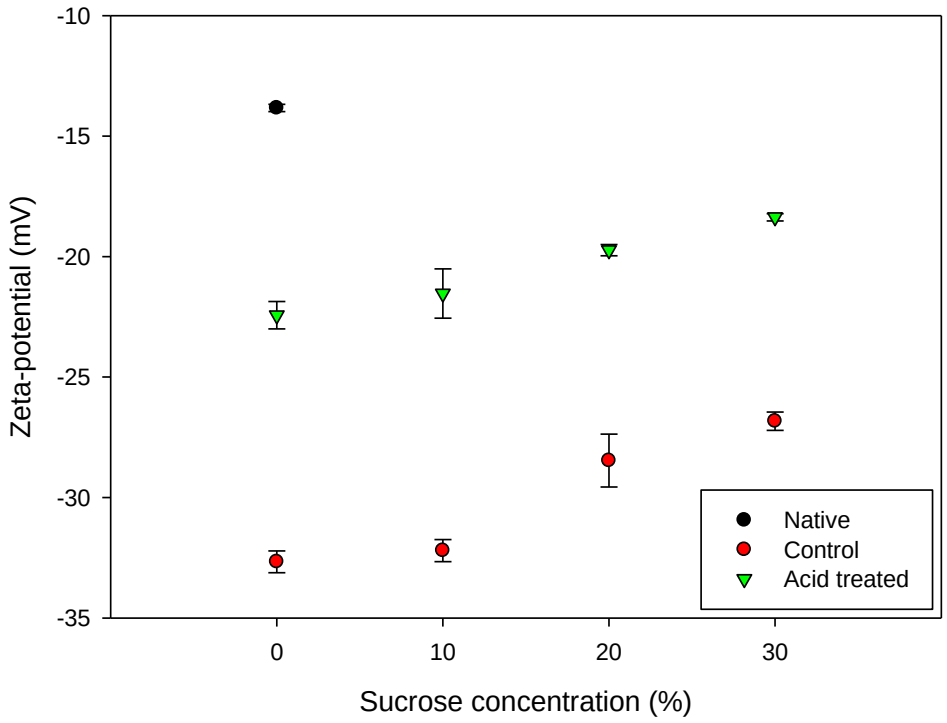

Figure 1. Zeta potential of native, heated, and acid treated SPI dispersions with different concentrations of sucrose.

2. Sulfhydryl group

Figure 2 demonstrates the values of free SH and total SH group ratio in native, heated, and acid treated SPI dispersions with different concentrations of sucrose. Nakamura et al. (1984) described free SH groups as the SH groups on the surface of native soy 11S globulin and the total SH groups was defined as the sum of free SH groups as well as SH groups inside 11S globulin that are exposed by buffer containing denaturants such as urea and SDS. This means that the number of free SH groups will increase as proteins unfold during heat denaturation, whereas the total SH groups remains constant. On the other hand, the number of free as well as total SH groups will decrease if covalent disulfide bonds are formed during gelation (Gu et al., 2009). In the current study, no difference was observed in total SH groups, but free SH groups showed a significant difference. Heating a 9 % SPI dispersion above protein denaturation temperature raised the content of free SH groups but had no significant effect on the content of total SH groups, as compared with those of unheated SPI. Free SH/total SH value in the native SPI dispersion was 40 % and increased to 61 % in the heated SPI dispersion. As the SPI dispersion was heated above denaturation temperature, free SH group of SPI was more exposed and free SH/total SH group value increased. All gels showed gradually decreasing free SH/total SH values with

the increase of sucrose concentration from 10% to 30%. Sucrose surrounded the protein molecule and impelled protein denaturation and exposure of free SH groups. Meanwhile, all acid treated gels showed decreased free SH/total SH values, and the value was the greatest for the sample containing 30% sucrose (36%). These results could be explained that the protein molecules were aggregated by the addition of acid to have a small quantity of reactive free SH groups. In comparison, non-acid treated gels hardly made aggregates, and thus free SH/total SH value was higher than that of acid treated one.

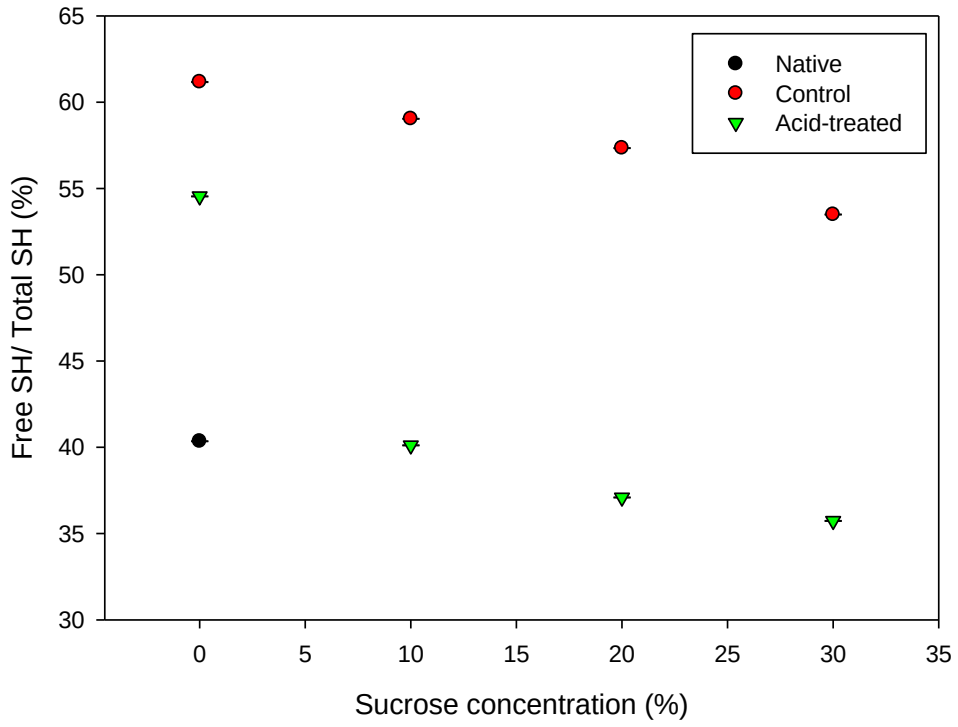

Figure 2. Free SH and total SH group ratio in native, heated, and acid treated SPI dispersions with different concentrations of sucrose.

3. Circular dichroism spectroscopy

CD data can be used to confirm the integrity of expressed domains of a multi-domain protein. In addition, the loss of CD signals either on addition of denaturing agents (such as urea and guanidinium chloride) or by an increase in temperature can be used to provide quantitative estimates of the stability of the folded state of the native protein (Kelly et al., 2005). CD analysis is divided into two spectra of near-UV ranging 250-340 nm and far-UV ranging 180-250 nm. CD measurements in the far-UV give quantitative estimates of secondary structure. Optical activity of α-helix in far-UV permits the use of CD studies for investigations of conformational change in protein solution, and the far-UV CD gives information on the backbone structure of a protein and is used to characterize the secondary structure and changes therein (Chen et al., 1972). Native SPI is known to be consisted of 5.5 % α-helix, 66.0 % β-sheet, 0 % β-turn, and 28.5 % random coil (Wang et al., 1991). Figure 3 shows far-UV CD spectra of acid treated and non-treated SPI dispersions in the absence or presence of sucrose denatured by heating 97 °C for 30 min. The positive peak from 260 nm to 280 nm, shown in native SPI, disappeared in all heated SPI dispersions. The disappearance of the spectrum in the near-UV region 250-340 nm indicated a loss in tertiary structure, suggesting that heat treatment caused a complete loss in the tertiary

structure of the proteins. The near-UV CD signal is due to the chirality of the environment of the side chains of aromatic amino acids such as tryptophan, tyrosine and phenylalanine, as well as of disulfide bonds (Chevalier et al., 2002). In the present study, there were no significant differences between samples. The CD spectrum in the far-UV ranging 180-250 nm of the denatured SPI with different concentrations of sucrose and acid showed a little reduced ellipticity compared with the native SPI dispersion. It suggested that the secondary structure of SPI heated with sucrose and acid changed to a great extent. The increase of α-helix and disordered structure and the decrease of β-sheet structure have been revealed after heating (Nagano et al., 1995). In case of heat denatured SPI, there was no significant difference compared to the control.

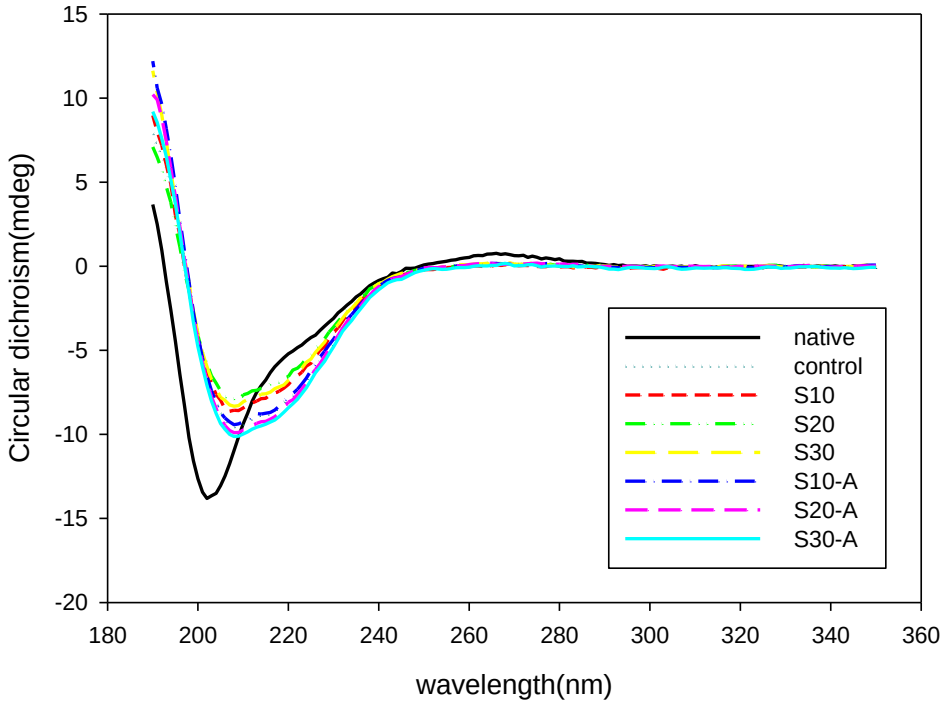

Figure 3. Circular dichroism spectra of native, heated, and acid treated SPI dispersions with different concentrations of sucrose.

4. Rheological characteristics

Figure 4 displays G' evolution over frequency of 9 % (w/w) protein gels with different concentrations of sucrose and acid. G' exhibited a very weak frequency dependence, with G' higher than G'' over the entire frequency range (G" data not shown). It indicated that the elastic component was much more than the viscous component of the gels confirming the solid-like character of the material. The G' values of SPI gels decreased with an increase of the sucrose concentration. The G' value was the largest for the gels of SPI with no sugar. The gel of SPI-30% sucrose was the softest among the samples. The initial G' of control gel (salt-induced gel without sucrose) is around 800 G, but the SPI-30% sucrose gel showed the value reduced by half. This trend could be due to the protective effects of sugars against protein denaturation when heat treatment occurred, which resulted in decreased protein precipitation upon adding salt leading to the formation of softer gels (Gu et al., 2009). The sugar molecules are preferentially excluded from the regions immediately surrounding the proteins. And an increase in viscosity of continuous phase causes a decrease in the frequency of protein-protein encounters (Semenova et al., 2002). The gels induced by acid showed the lowest G' values and the combined-treated gels with acid and salt showed the highest G' values. Acid slowly reduces pH and allows enough protein

denaturation to cause interactions and the formation of a network structure (Totosaus et al., 2002). Aggregation of denatured protein is enhanced at the iso-electric point where the net charge is zero due to the absence of stabilizing electrostatic repulsive force. Theses fractal clusters may be regarded as the building blocks of the gel (Lucey et al., 1998). High storage modulus value of acid treated sample suggests the presence of additional cross-link because storage modulus is related to the degree of cross-linking. (Zhu et al., 2011). Therefore, the combined-treated gel with acid and salt improved mechanical properties.

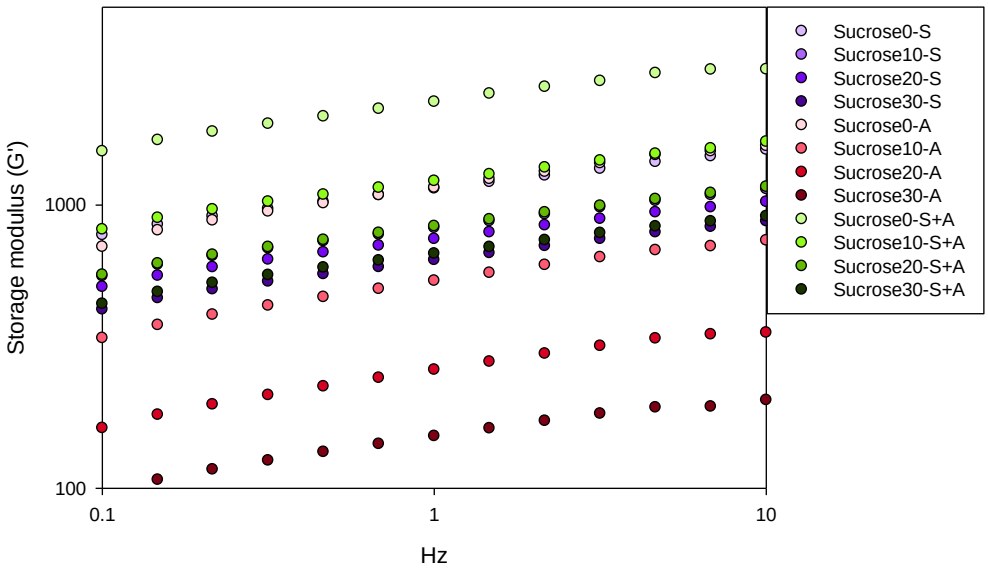

Figure 4. Frequency sweep profile of acid and non-acid treated SPI gel with different sucrose concentrations. Sucrose0, 10, 20, and 30: concentrations of sucrose. S, A, S+A: gelling agents used; salt, acid, and combined-treated with salt and acid, respectively.

5. Water-holding capacity (WHC)

The WHC data of the formed gels are shown in Figure 4. The most important functional properties of SPI are related to their interaction with that physically held within a protein matrix (Gu et al., 2009). As expected, diversely formed gels exhibited considerable differences in WHC. Although the combined-treated gels without sucrose had much higher mechanical strength than the other gels, the WHC (60%) of the former was considerably lower than that of the latter gels (around 85%). The acid-induced gels showed a higher WHC than the others. And WHC values of combined-treated gels with acid and salt were the smallest among the gels prepared with 3 different gelling agents. Van Kleef (1986) attributed the decreased WHC as the SPI gels approached the pI to the formation of a coarse gel structure with large pores. Puppo et al. (1995) also showed that the WHC of SPI gels decreased as the pH increased from pH 2.5 to 3.5, indicating the WHC decreased as the pH approached the pI. Thus, adding acid to an SPI dispersion reduced the pH to pI and made a coarse gel trapping more water in its structure. On the other hand, combined-treated gels with acid and salt made more compact structure than the others leading to a greater expulsion of water from the gel network. In other words, proteins so strongly aggregated themselves that having little space for trapping water, and thus

the gel had a low WHC value. Regarding sucrose concentration, all gels with 30 % sucrose showed the highest WHC value. Since the sucrose interrupts the aggregation between protein molecules, the SPI gels with higher sucrose concentrations presented higher WHC values. These results were similar to the findings of Boye et al. (1997) for whey protein gels. They reported that the WHC of gelled whey protein concentrate heated at neutral pH increased with increasing sucrose concentrations. The increased WHC could be attributed to increased solvation of sucrose trapped within interstitial spaces of the whey protein gel.

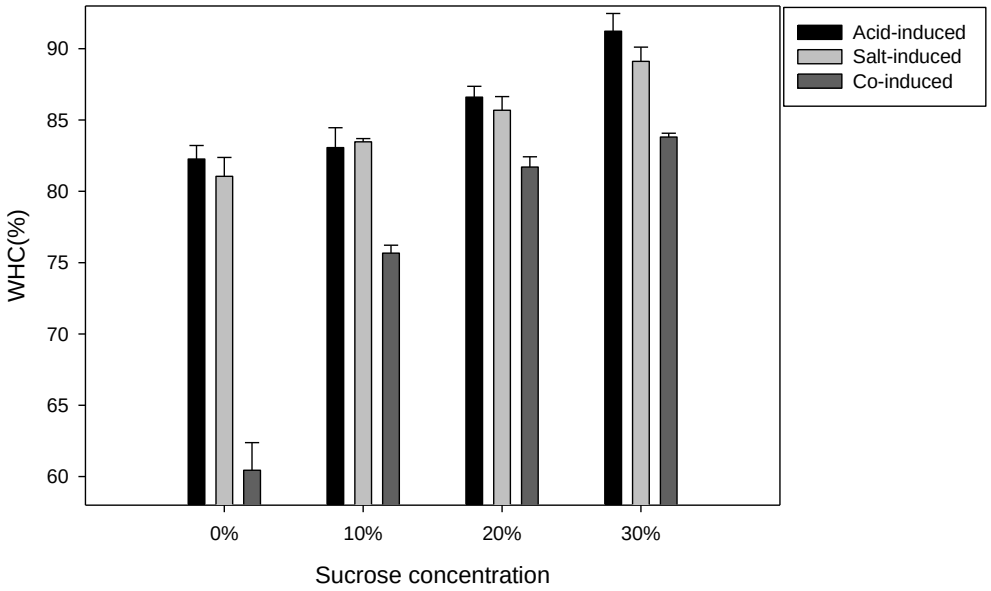

Figure 5. Water holding capacity (WHC) of acid and non-acid treated SPI gels with different sucrose concentrations.

6. Microstructure of SPI cold-set gels

Figure 6 shows transmission electron micrographs (TEM) of SPI cold-set gels with 9 % w/w protein and different gelling agents in the presence and absence of sucrose. Black areas in the picture represented protein, while light and gray areas represented the pores of the network containing the continuous phase. At this level of magnification (40,000×), the gel seemed to be composed of small spherical particles. Combined-treated gel prepared in the absence of sucrose displays a dense and a homogeneous structure composed of evenly sized particles and pores compared to the gel made with sucrose, which showed larger pores and a more disordered structure. As the sugar concentration increased, the gel structure became coarse and less homogeneous, and the pore size was increased. Sucrose addition resulted in a less homogeneous distribution of the protein aggregates. This phenomenon is probably because sucrose has an effect on slowing down the movement of the protein aggregates, thereby decreasing protein aggregation and gelation making more pores in protein matrix. And only acid treated gels showed a coarser structure than the others. As a result of the pH approaching the iso-electric point of protein, the network structures may be predicted to coarsen (Doi, 1993). On the other hand, a denser and finer network was obtained in the combined-treated SPI

gels. As a result of charge modification, the networks of the combined-treated gels were made up of highly cross-linked protein structures. More compact structures have less space to trap water molecules, thus having the low WHC values as well as the high storage modulus. Therefore, combined-treated SPI gel showed a denser and finer network having the lowest WHC and the highest storage modulus because of its compact structure.

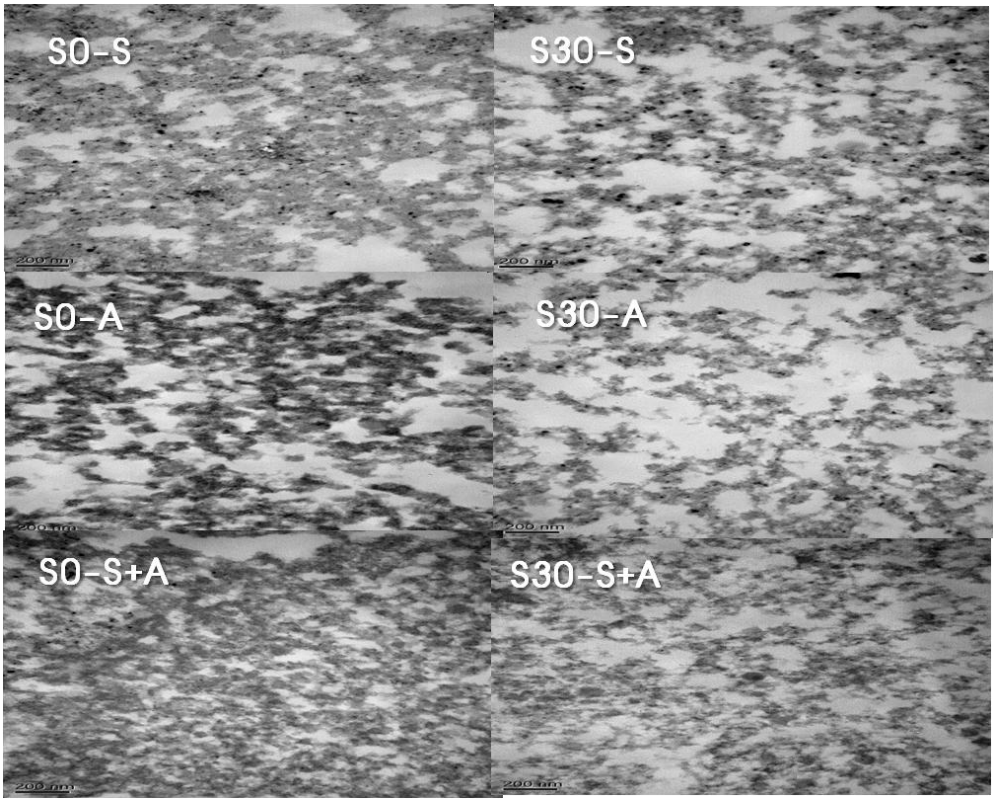

Figure 6. Representative TEM images (40,000×) of soy protein cold-set gels formed with different types of gelling agents in the absence and presence of sugar. Scale bar: 0.5 μm. S0 and 30: concentrations of sucrose. S, A, S+A: gelling agents; salt, acid, and combined-treated with salt and acid, respectively.

7. Fractal analysis

In order to investigate the effect of sucrose and acid on the gel properties, rheological analysis as a function of applied frequency was carried out to calculate the mass fractal dimension of gel networks (Figure 7 and 8). The power-law dependence of gel hardness (characterized by G' values) on protein concentration Φ was exploited for this purpose (Hagiwara et al., 1998). Prior to fractal dimension estimation, inter- and intra-floc link regime for each type of gel was determined using stress sweep analyses. For all protein concentration, G' remained almost constant as strain increased and then suddenly decreased, indicating bond breakage within the gel network and a transition from linear to non-linear behavior (Ould Eleya et al., 2004). The strain amplitude at which G' just decreased by 5 % from its maximum value was determined and taken as a measure of the limit of linearity of the gel (Shih et al., 1990). Figure 7 showed the G'-strain profiles of 6-9 % (w/w) soy protein cold-set gels which were prepared with different gelling agents in the absence and presence of sucrose. Figure 8 represented the limit of linearity-strain profiles of 6-9 % (w/w) soy protein cold-set gel. Each slope was used for calculation of D_f and β. For all SPI gels, γ_0 increased with the increase of protein concentration from 6 % to 9 %.

A previous study demonstrated that if $\alpha = 0$ for a strong inter-floc

link gel and $\alpha = 1$ for a strong intra-floc link, a value between these two extremes indicates that the gel is in the transition regime (Maltais et al., 2008). Table 1 clearly showed that the gels prepared with salt and acid were in the weak-link regime, indicating that intra-floc bonding was the major mode of link in the gels prepared with salt and acid. For all gels, gel systems appeared to be a weak-link regime gel in which intra-floc links were stronger than inter-floc links. For the combined-treated SPI gel without sucrose, D_f was 2.80. This value was the highest of the six D_f values, indicating the most complex structure of gels which corresponded to the transmission electron micrographs. The D_f decreased slightly with an increase of sucrose concentration. It could be explained that sucrose surrounded the protein molecules disturbing the protein-protein interaction.

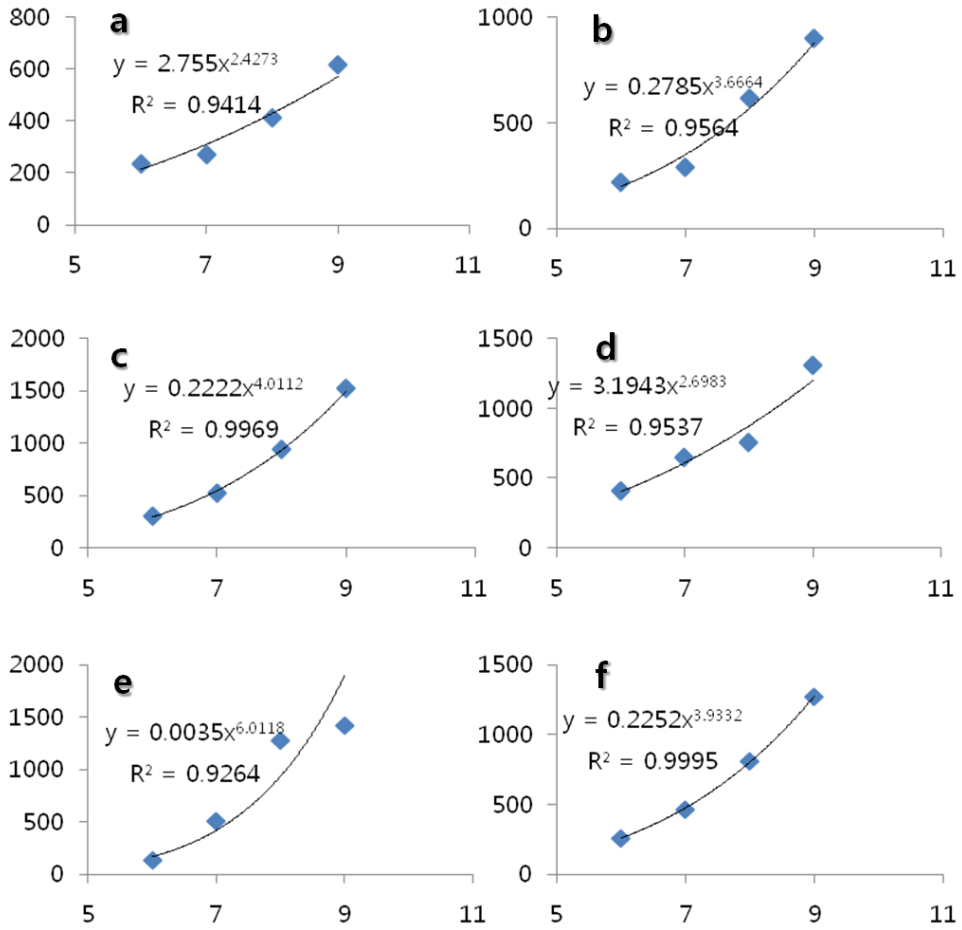

Figure 7. Storage modulus-protein concentration profile of soy protein gels. a: acid induced gel without sucrose. b: acid induced gel with 30 % sucrose. c: salt induced gel without sucrose. d: salt induced gel with 30 % sucrose. e: combined-treated gel without sucrose. f: combined-treated gel with 30 % sucrose.

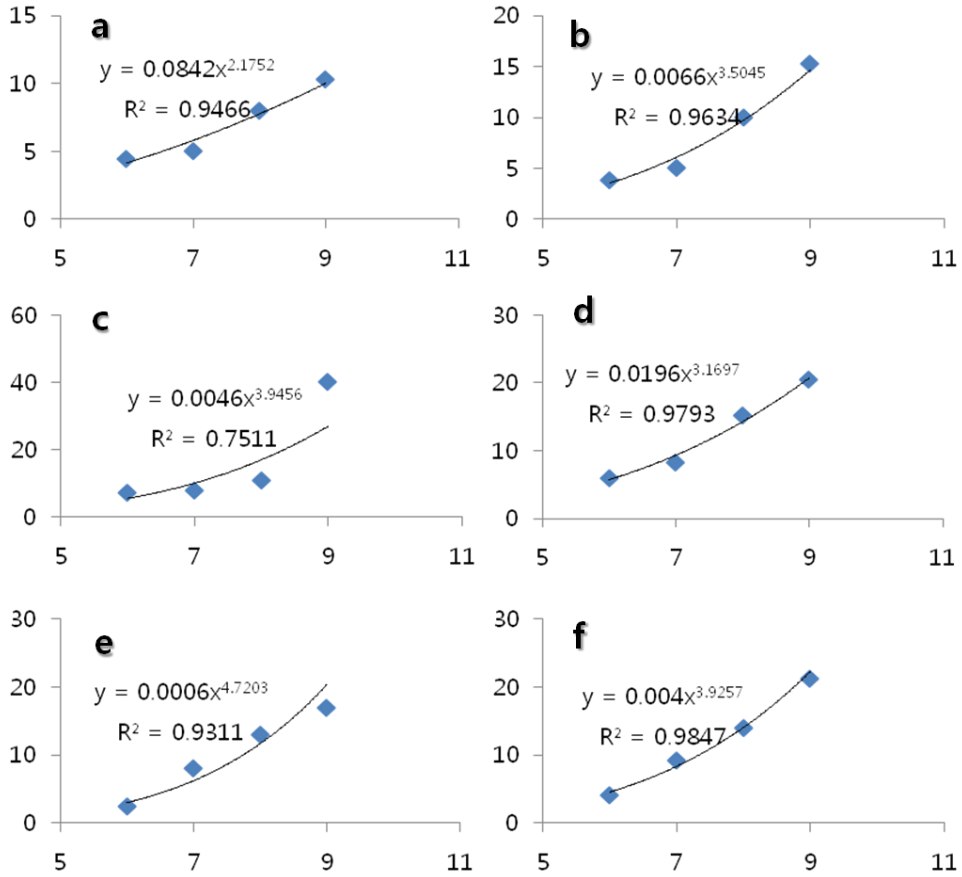

Figure 8. Limit of linearity-protein concentration profile of soy protein gels. a: acid induced gel without sucrose. b: acid induced gel with 30 % sucrose. c: salt induced gel without sucrose. d: salt induced gel with 30 % sucrose. e: combined-treated gel without sucrose. f: combined-treated gel with 30 % sucrose.

Table 1. Fractal dimension (D*f*) and elastic constant (α) of soy protein cold-set gels.

Sample	Power-law exponents		Model of Wu and Morbidelli		
	A[a]	B[b]	D*f*	α at x=1.0	Regime
Sucrose0-A	2.66	2.47	2.61^{D}	0.99	Weak-link gel
Sucrose30-A	3.71	3.48	2.72^{BC}	0.99	Weak-link gel
Sucrose0-S	4.12	3.87	2.75^{B}	0.99	Weak-link gel
Sucrose30-S	3.21	3.27	2.69^{C}	1.00	Weak-link gel
Sucrose0-S+A	5.61	4.21	2.80^{A}	0.95	Weak-link gel
Sucrose30-S+A	3.97	3.88	2.74^{B}	1.00	Weak-link gel

Sucrose0 and 30: concentrations of sucrose. S, A, S+A: gelling agents used; salt, acid, and combined treated with salt and acid, respectively.

[a] Slope from log-log plot of G' vs Φ.

[b] Slope from log-log plot of γ_0 vs Φ.

Conclusions

Gel properties of salt-induced soy protein isolate (SPI) gels were investigated and the influences of sucrose and acid were characterized. Cold-set SPI gels are significantly affected by sucrose. Structural and rheological properties were influenced by the concentration of sucrose.

Zeta-potential and free SH/total SH group ratio showed that sucrose has protective effect against protein denaturation. During the heating process, sucrose surrounded the protein molecules and increased the viscosity of continuous phase. The SPI gels heated in the presence of sucrose showed that sucrose led to softer SPI gels than the SPI gels prepared in the absence of sucrose, indicating inhibition of denaturation of SPI. D_f value of the SPI gels prepared in the presence of sucrose slightly decreased compared with that of SPI gels prepared in the absence of sucrose. It could be explained that sucrose surrounded the protein molecules disturbing the protein-protein interaction.

Soy protein gels are also influenced by the acid. The acid treated SPI dispersion showed a high zeta-potential value and a low sulfhydryl group content. Additional add of sucrose to SPI dispersion made aggregates shield negative charges and free sulfhydryl groups of proteins. Combined-treated

using acid and salt gels presented the most compact microstructure showing the highest storage modulus and the lowest water holding capacity.

Therefore, based on these results, the gelation in food protein systems can be controlled by sucrose and gelling agents. And these results have important implications for the application of SPI in food products with high sugar contents such as deserts and tofu.

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국문초록

대두 단백질은 다양한 방법에 의해 3차원적 망상 구조의 젤을 형성하고 이러한 단백질 젤 구조에 당류나, 향기성분, 색소 등 여러 가지 식품 첨가물을 함유할 수 있다. 이러한 젤 형성 방법에는 열을 이용하는 방법인 heat-set 젤화와 이온이나 효소, 압력 등을 이용하는 방법인 cold-set 젤화로 나뉘어 진다. Cold-set 젤화는 heat-set 젤화에 비해 열손상이 적고 최종 젤의 형성 전에 응집물의 특성을 조절함으로써 최종 젤의 특성을 조절할 수 있어서 최근 식품 산업에서 다양하게 이용되고 있다. 따라서 이 연구에서는 식품의 가장 주요한 첨가물인 자당과 산이 염을 이용해서 형성시킨 대두 단백질 젤의 구조적, 유변학적 그리고 프랙탈 차원 등에 대해 규명하였다.

9 %의 단백질 현탁액에 다양한 농도의 자당을 가하여 혼합한

후 97 ℃ 에서 30분간 열을 가하고 상온에서 2시간 동안 식힌 후 22 mM의 염산과 30 mM의 염화칼슘을 가하여 젤을 제조하였다. 당과 단백질의 마이야르 반응에 의한 산의 영향을 방지하기 위해 비환원당인 자당을 사용하였다. 표면전하량을 통하여 자당과 단백질을 함께 가열 시 자당이 단백질 입자의 표면을 감싸서 표면전하량을 감소시킨다는 것을 확인하였다. 자당의 농도가 증가함에 따라 표면전하가 드러나는 것을 막아 좀 더 0에 가까워지는 것을 확인하였다. 또한 산을 첨가하였을 경우 작은 입자들이 형성되어서 드러났던 표면전하가 다시 가려지면서 산이 첨가되지 않았을 때 보다 그 값이 더 0에 가까워지는 것을 확인하였다. 이러한 결과는 단백질 표면에 존재하는 자유 황화수소기 (free SH) 그룹 측정값에서도 동일하게 나타났다. 저장탄성률 측정 결과에서는 자당의 농도가 증가함에 따라 낮은 저장탄성률을 나타내었고, 염화칼슘과 산을 함

께 처리하였을 때 두 가지를 따로 처리했을 때 보다 높은 저장탄성률을 나타내는 것을 확인하였다. 또한 전자현미경 사진과 수분보유력 측정 결과 당이 30 % 첨가되었을 때 많은 공극을 나타내며 많은 수분을 함유하여 높은 수분보유력을 나타내었다. 그에 비해 염화칼슘과 산을 함께 처리하였을 때는 공극이 거의 없는 조밀 조밀한 구조를 나타내며 가장 낮은 수분보유력을 나타내었다. 이러한 결과를 프랙탈 기하학을 이용하여 살펴본 결과 전자현미경에서 볼 때 복잡하고 조밀 조밀한 구조를 나타내었던 염화칼슘과 산을 함께 처리한 젤의 경우 큰 값의 프랙탈 차원을 나타내는 것을 확인하였다.

이러한 자당의 대두 단백질의 열 변성 저해는 농도에 비례하여 나타났고 열 변성 저해에 의해 젤 형성 방해 또한 농도에 비례하여 나타나는 것을 확인하였다. 또한 젤 형성에 사용된 염화칼슘과

함께 산을 추가적으로 사용할 경우 공극이 적고 더 높은 프랙탈 차원을 나타내며 견고한 젤을 형성하는 것을 확인하였다. 따라서 이 연구 결과는 다양한 단백질 식품에 유변학적 특성을 조절하는 데 이용 될 수 있을 것이다.

주요어 : 콩 단백질, 콜드셋젤화, 자당, 물성, 프랙탈 차원

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