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Addition of Salt Ions before Spraying Improves Heat- and Cold-Induced Gel Properties of Soy Protein Isolate (SPI)

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Abstract: Spray drying is used in the food industry to convert liquids into dry powders. The effect of the addition of salt ions before spray drying to improve the heat- and cold-induced gel properties of soy protein isolate (SPI) was investigated. Certain concentrations of Na^+ (0.005–0.01 M), Mg^{2+} (0.005 M), and Ca^{2+} (0.005 M) significantly increased the hardness, springiness, cohesiveness, chewiness, gumminess, resilience, and water holding capacity of the heat- and cold-induced gels. This effect arises predominantly due to the functional groups buried in the protein matrix that are partially exposed to improve the interactions between the protein molecules. The main interactions that promoted gel formation and maintained the three-dimensional structure of the heat- and cold-induced gels were hydrophobic and disulfide interactions. Analysis using scanning electron microscopy showed that the heat- and cold-induced gels were uniform, had smooth surfaces, and had smaller pores with added Na^+ (0.01 M), Mg^{2+} (0.005 M), and Ca^{2+} (0.005 M). The results indicate that we might broaden the applications of SPI by simulating the industrial gel manufacturing process for products such as fish balls and chiba tofu. Overall, adding salt ions before spray drying could offer great potential for the development of SPI with enhanced functionality suitable for comminuted meat products.

Keywords: soy protein isolate; sodium; magnesium; potassium; spray drying; heat-induced gels; cold-induced gels

1. Introduction

Soy protein isolate (SPI) is an important food ingredient and is often used in human foods due to its nutritional value and technological properties, as well as its relatively low price [1]. The gel properties are important functional properties of SPI. SPI has the ability to form a gel when heated. Traditionally, SPI is added in the industrial production of emulsified muscle foods to improve product texture and yield [2,3]. However, this increases the cost because of the gel softening of natural soy protein isolate (N-SPI) with the extension of the storage period [1,4]. Furthermore, Wang et al. (2015) reported that N-SPI had a negative effect on the gel properties of some meat proteins. The limitations

on the functional properties of N-SPI affect its application in food processing. Therefore, research on how to improve gel strength and delay gel softening might be potentially beneficial.

The literature identifies many factors that affect the gel properties of soy protein isolate, e.g., protein concentration, drying methods (spray drying, freeze drying, and vacuum drying), and environmental factors (pH, temperature, time, and salt ions) [5–8]. Spray drying has been shown to be a simple, low-cost, reproducible process. It is widely used in the food industry [9]. Joshi et al. (2011) reported that spray-dried lentil protein isolates (LPI) powder had the highest solubility and better gelling properties when compared with that produced using freeze drying and vacuum drying [10]. Wang, Shen, and Liu (2018) reported that the addition of Ca^{2+} and Na^{+} (0.005–0.01 M) increased gel strength, elasticity, viscosity, water holding capacity (WHC), and thermal stability [11]. Wang, Zeng, and Qin (2018) reported that when the Ca^{2+} concentration during pre-aggregation was increased from 0 to 7.5 mM, the elastic modulus of the gels increased substantially. Compared with the normal "one-step" process, pre-aggregation promoted the formation of stronger gels with thicker strands and denser structure [12]. Moreover, Zhou, Zhang, and Gao (2015) reported that stiffer gels of SPI were formed at lower salt concentrations (0.2 M NaCl) [13]. Chen, Chassenieux, and Nicolai (2018) reported that during the NaCl-induced gelation of aggregates, the activation energy was much smaller and the gels were more homogeneous than native soy proteins [14]. Thus, spray drying and the ionic strength have roles in the formation of gel products with an appropriate texture and WHC [15]. SPI gels can be classified into heat- and cold-induced gels depending on differences in gel formation conditions, and SPI heat-induced gels have been widely used in the food industry. The formation of cold-induced gels of SPI is relatively mild, which provides a way to develop new foods. The current research focuses on the formation of heat-induced gels in the presence of salt ions or gels induced with salt addition and their properties after spray drying. Little research has been done on the modifications of salt addition before spray drying on SPI and its heat- and cold-induced gel properties or on efforts to explain the mechanism of action of salt ions.

Thus, the objectives of the presented research were to compare the heat- and cold-induced gel properties of SPI as affected by salt ions (Na^{+}, Mg^{2+}, Ca^{2+}) at various concentrations (0.005, 0.01, 0.015 M) before spray drying. The properties measured included the texture, WHC, and microstructure of the SPI gels to hopefully improve SPI's gelation properties. The experiments were also designed so that we may begin to understand the mechanism(s) of any improvements.

2. Materials and Methods

2.1. Materials

Materials: Soy protein isolate (≥90.0% protein, ≤1.5% fat, ≤5.0% ash, and ≤6.0% moisture) was purchased from Beijing Hua Mei Polymer Co., Ltd. (Beijing, China). Bovine serum albumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), and other required reagents not otherwise specified were of analytical grade and purchased from Sinopharm Chemical Reagent Shenyang Co., Ltd. (Shenyang, Liaoning, China). Tris-HCl, sodium dodecyl sulfate (SDS), urea, and β-mercaptoethanol (β-ME) were of analytical grade and were purchased from Suobaolai Technology Group Co., Ltd. (Beijing, China).

2.2. Soy Protein Isolate Spray Drying

SPI powders (1.00 g) and 20.0 mL of deionized water (Lefred Ltd., Harbin, Heilongjiang, China) at room temperature (20 ± 1 °C) were mixed using a constant-temperature magnetic stirrer (X85-2, Meiyingpu Ltd., Shanghai, China) for 30 min, and the pH was adjusted to 7.0 with 0.1 M NaOH and 0.1 M HCl solutions. Then, the salt ions Na^{+}, Mg^{2+}, and Ca^{2+} (0.005, 0.01, 0.015 M) were added and the solution was centrifuged at 9000*g* for 30 min at 4 °C (GL-21M, Xiangyi Ltd., Changsha, Hunan, China); the supernatant was then placed in a spray dryer (MDR.P-5 model, Modern Spray-drier Ltd., Wuxi, China) with an air inlet temperature of 180 ± 5 °C and an air outlet temperature of 80 ± 5 °C.

2.3. Preparation of Heat- and Cold-Induced Gels

SPI powders and deionized water 1:4 (w/v) were put into a Bruun mixer (K600, Bruun, German), mixed for 1 min at low speed, then stopped. A scraper was used to clear the wall (the time taken for this was shorter than 30 s), then the product was mixed again for 1 min at low speed, turned off, and scraped again. This operation was repeated a second time. The gel was centrifuged at 3500*g* for 10 min at 4 °C, after which it was heated in a constant-temperature water bath (DK-S12, Sanfa Scientific Ltd., Shanghai, China) at 85 °C for 20 min after centrifugation to obtain a pre-gel, then cooled at room temperature and chilled at 4 °C for 8 h to get cold-induced gels. After centrifugation, the other samples were heated in a constant-temperature water bath at 85 °C for 45 min, and then refrigerated at 4 °C for 8 h to obtain heat-induced gels. The gels were equilibrated at room temperature for 30 min before each measurement and then used to determine the WHC, whiteness, and texture of the gel.

2.4. Determination of Gel Texture

The gel product was cut into a cube-shaped gel block having a thickness of 30 mm at room temperature, and texture determination analysis of the heat- and cold-induced gels was conducted using a Model TA-XT2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) attached to a 5 kg load cell and with a P/0.5 flat-surface cylindrical probe (12 mm in diameter) with a trigger-type button [11,16]. The operating conditions were as follows: pre-test speed, 2.0 mm/s; test speed, 0.5 mm/s; trigger force, 5g; up-test speed, 2.0 mm/s; puncture distance, 5.0 mm. Hardness, springiness, chewiness, cohesiveness, gumminess, and resilience were calculated using Texture Expert software version 1.22 (Stable Micro Systems). Hardness was defined as the peak force (N) required for first compression. Cohesiveness was defined as the area of work during the second compression (Area 2) divided by the area of work during the first compression (Area 1). Springiness: Springiness is now expressed as a ratio or percentage of a product's original height relative to the distance of the detected height during the second compression (Distance 2) divided by the original compression distance (Distance 1). Gumminess: Gumminess applies to semi-solid products and is Hardness × Cohesiveness (which is Area 2/Area 1). Chewiness: Chewiness applies to solid products and is calculated as Gumminess × Springiness (which is Distance 2/Distance 1). Resilience is calculated by dividing the upstroke energy of the first compression by the downstroke energy of the first compression, Area 4/Area 3. Tests were done on three different areas of the same gel block.

2.5. Determination of Gel Whiteness

Whiteness analysis of the heat- and cold-induced gels was carried out using a ZE-6000 color meter (Nippon Den-shoku, Inc., Tokyo, Japan) as described by Sochaya and Soottawat (2017) with slight modifications [17]. The gel product was cut into a 30 mm cube-shaped gel block. The L^* value (brightness), a^* value (red to green), and b^* value (yellow to blue) of the gels were measured on three different areas in the same gel block. The formula for calculating the whiteness value was as follows:

$$Whiteness = 100 - \sqrt{(100-L)^2 + a^2 + b^2}. \quad (1)$$

2.6. Measurement of Water Holding Capacity

The WHCs of heat- and cold-induced gels were measured based on the method described by Valdez-Hurtadoa et al. (2019), slightly modified [18]. Protein gels (10.00 g) were wrapped in Whatman#1 filter papers (Maidstone, UK), placed in 50 mL centrifuge tubes, and centrifuged at 1600*g* for 15 min at 4 °C using an Allegra 25-R centrifuge (Beckman Coulter, Fullerton, CA, USA). The weight of the centrifuge tubes was M_0, the weight of the gel samples before centrifugation was M_1, and the

weight of the gel samples after centrifugation was M_2. The WHC was expressed as the weight of the gel after centrifugation relative to the weight of the initial gel.

$$WHC(\%) = \frac{M_2 - M_0}{M_1 - M_0} \times 100\% \quad (2)$$

2.7. Determination of the Molecular Forces in the Gels

Non-covalent and covalent interactions in SPI gels were studied using the method described by Wang and Arntfield (2016) [19]. The heat- and cold-induced gels were placed in five different buffers to measure the solubility of the gel. The buffer series is shown in Table 1. A sample (0.50 g) and 10.0 mL of one of the five buffer solutions were added together, shaken by hand for 1 min, and then kept in a 40 °C water bath for 4 h. However, S1 was kept at 4 °C for 4 h instead, and S5 was first heated in boiling water for 2 min and then kept in a 40 °C water bath for 4 h. After the incubation, the supernatant after centrifugation at 12,000*g* for 30 min (GL-21M) was mixed with 2.00 mL of 50% (w/v) TCA (Trichloroacetic acid), left at 4 °C for 18 h, and centrifuged at 10,000*g* for 20 min at 20 °C, and the precipitate was washed with 10% TCA and was centrifuged at 15,000*g* for 15 min. The supernatant was discarded, and the precipitate was dissolved in 0.5 mol/L NaOH. The concentration was determined using the Biuret method with bovine serum albumin used as a standard assuming 100% purity [20].

Table 1. The five different buffers used to measure the solubility of the gel.

Buffer Series	NaCl	Tris-HCl, pH 8.0	SDS	Urea	β-ME
S1	0.6 mol/L				
S2		20 mmol/L			
S3		20 mmol/L	1%		
S4		20 mmol/L	1%	8 mol/L	
S5		20 mmol/L	1%	8 mol/L	2%

2.8. Determination of the Gel Microstructure

The microstructures of the gels were measured according to the procedure by Lin et al. (2018) [21]. Briefly, the gel samples with better hardness, springiness, and WHC were selected: Ca^{2+} (0.005 M), Mg^{2+} (0.005 M), and Na^{+} (0.01 M). The gel samples were cut with a knife into small pieces ($3 \times 3 \times 3$ mm^3), fixed in a 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 24 h. The sample was immersed in 50% sulfoxide (freeze protectant) for 24 h, rapidly frozen with liquid nitrogen, and then freeze-dried (Critical Point Dryer CPD03 Balzers, Alzenau, Germany). The freeze-dried samples were plated with gold using a sputter coater (SCD 050, Balzers, Liechtenstein) and subjected to electron microscopy at 5 keV (JSM6701F, JEOL Ltd., Tokyo, Japan) [22].

2.9. Statistical Analysis

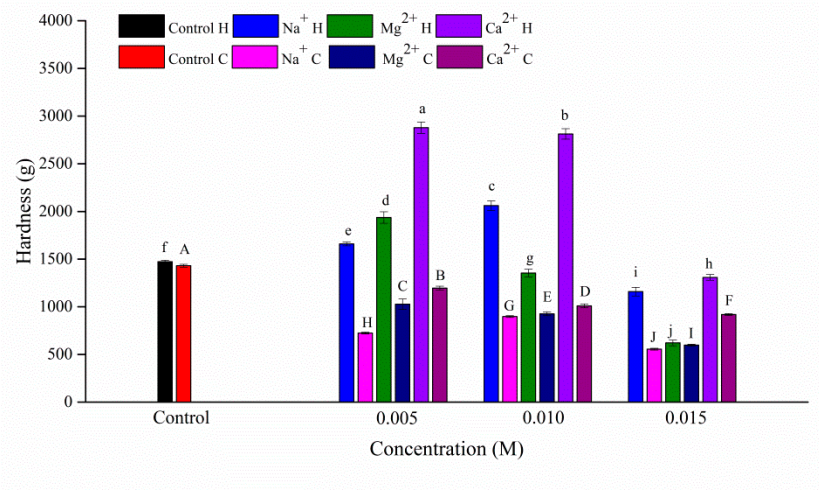
Triplicate runs were carried out for each experiment, and the data are expressed as means with standard deviations. The results were evaluated using one-way analysis of variance (ANOVA) to evaluate the significance of differences ($p < 0.05$) using the Statistical Package for the Social Sciences version 18.5 software (SPSS Inc., Chicago, IL, USA). Figures were prepared using Origin 8.6 software (Origin Lab Corp., Northampton, MA, USA).

3. Results and Discussion

3.1. Texture Properties of the Gels

The gel textures of the heat-induced and cold-induced gels are shown in Figure 1. As can be seen from Figure 1a, an increase in hardness of the heat-induced gel was observed at lower concentrations, while the gel hardness was decreased at higher concentrations of Na^{+} (0.01–0.015 M),

Mg^{2+} (0.01–0.015 M), and Ca2^{+} (0.015 M). At the same concentration, Ca^{2+} resulted in the highest gel strength. The hardness of the cold-induced gel was decreased. These results might be due to the gel strength being related to the amount of protein involved in the formation of the network structure and the forces forming the network structure [23–25]. The addition of Na+ diminishes repulsive forces, and protein–protein association occurs, forming a self-supporting gel [26]. Mg^{2+} and Ca^{2+} at lower levels may act as a bridge between negatively charged residues, which would promote protein aggregation with increased gel strength [27]. Additionally, excessive salt ions may result in excessive aggregation of proteins, which would decrease the gel strength. Salts at high levels might also compete for water, causing the "salting out" effect, with gels in turn being less soluble and weaker [28]. The strengths of gels containing Ca^{2+} and Mg^{2+}, which had the same divalent ions, were very different. This difference resulted from dissociation of the two salts in aqueous solution. The strength of gels is affected by the type of cation [29]. Thus, the effect of Ca^{2+} on the strength of the heat-induced gel was stronger than those of Na+ and Mg^{2+}.



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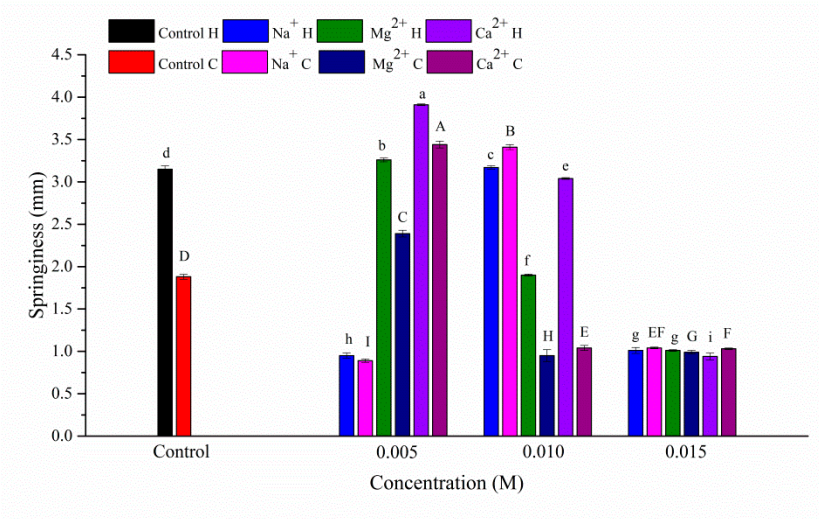
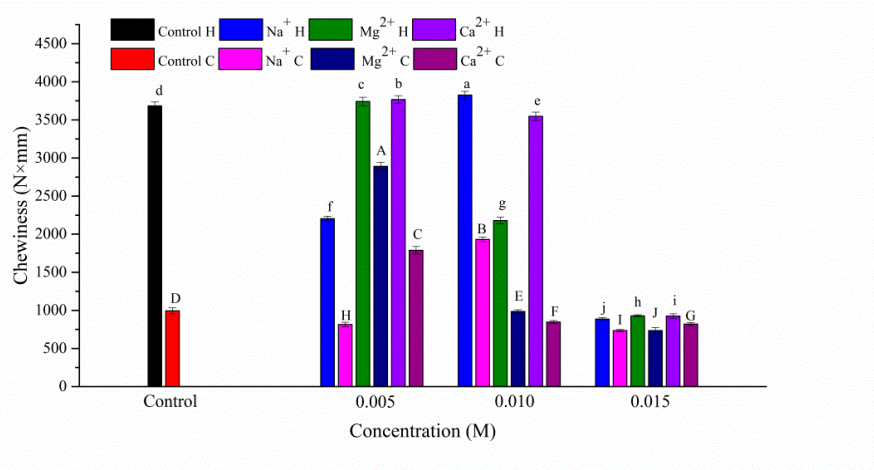


(**b**)

Figure 1. *Cont.*



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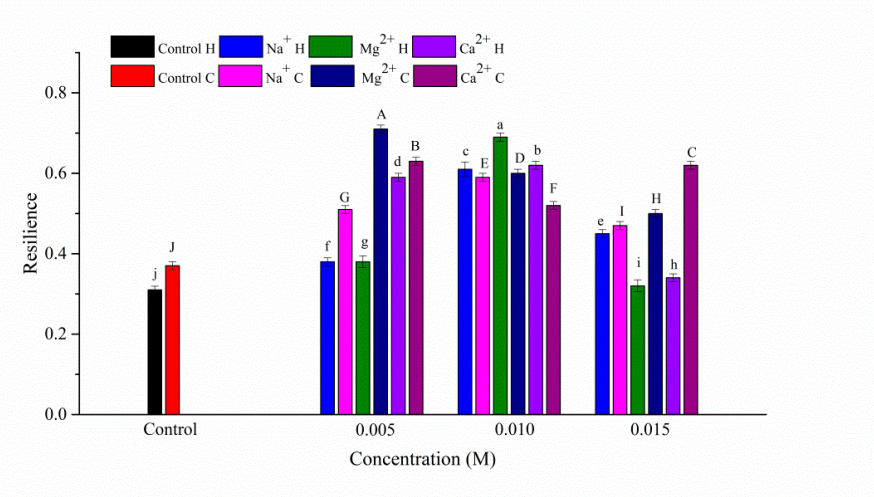

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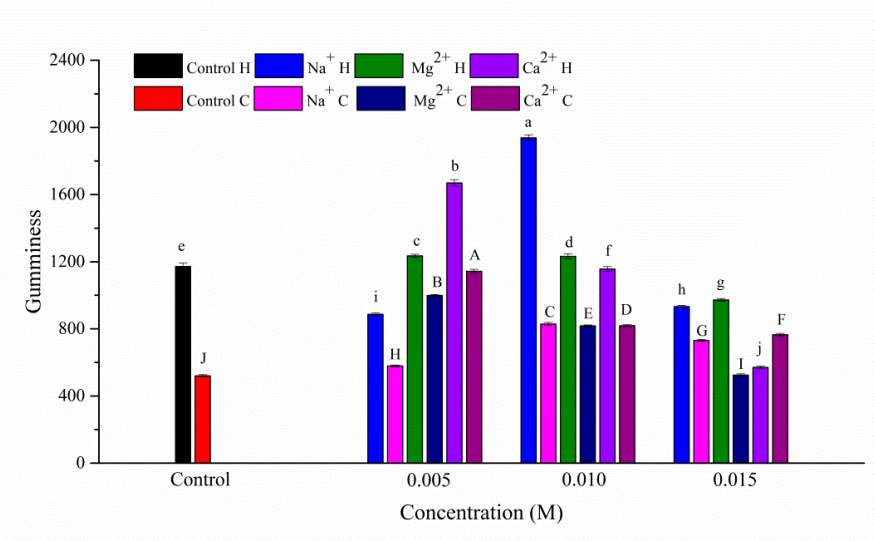
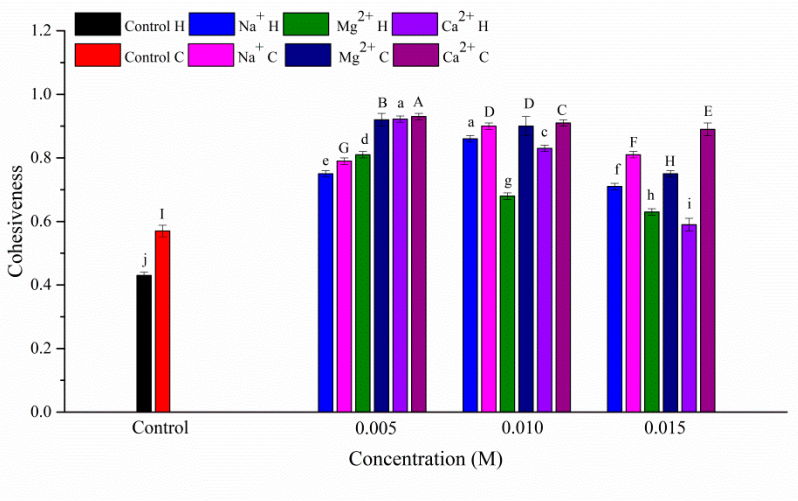


(**e**)

Figure 1. *Cont*.



(**f**)

Figure 1. Heat- and cold-induced gel textures from salt ions applied before spray drying. (**a**) Hardness; (**b**) Springiness; (**c**) Chewiness; (**d**) Resilience; (**e**) Gumminess; (**f**) Cohesiveness. All samples are average values ± standard deviation (n = 3), and different letters in the same figure represent a significant difference between samples ($p < 0.05$). Capital letters represent cold-induced gels and lower case letters represent heat-induced gels. C represents cold-induced gels; H represents heat-induced gels.

The springiness and chewiness of heat-induced and cold-induced gels both increased at 0.01 M Na^+, 0.005 M Mg^{2+}, and 0.005 M Ca^{2+} as shown in Figure 1b,c. Springiness measures the degree of springing back after being deformed under the first compression [21]. Chewiness signifies how much energy is needed to crunch a semi-solid food [21]. If hardness and springiness are high, it requires more mastication energy in the mouth. High springiness will be obtained when the gel structure is broken into a few large pieces during the first TPA (texture profile analysis) compression, while low springiness results from the gel breaking into many small pieces [30]. The resilience and gumminess of heat-induced and cold-induced gels both increased as shown in Figure 1d,e. Resilience is the ratio of recoverable energy when the first compression is relieved and gumminess is the product of stress at 70% compression [21,26]. The results indicated that disulfide bonds played an important role in the network formation of heat- and cold-induced gels. Gel cohesiveness is often used as an index of the ability of a gel to maintain an intact network structure; it indicates how well the product withstands a second deformation compared to the first deformation [31]. The results showed that salt-induced aggregates of gels with different divalent and monovalent cations showed decreased cohesiveness as the concentration increased to 0.01 M, as shown in Figure 1f. The results were in good agreement with those of Kaewmanee, Benjakul, and Visessanguan (2009) who reported that cohesiveness was slightly decreased when the salting time was increased in salted eggs [32]. Based on the texture data, the addition of Na^+ (0.01 M), Mg^{2+} (0.005 M), and Ca^{2+} (0.005 M) before spray drying led to stronger gels, with the hardness, springiness, gumminess, and chewiness of the heat-induced gels being greater than those of the cold-induced gels, while the cohesiveness and resilience were smaller than those of the cold-induced gels.

3.2. Whiteness of the Gels

The whiteness results are shown in Figure 2 and the appearance of the gels is shown in Figure 3. The gels' appearance can be considered a good indicator of the type of network formed. Gels formed with an ordered structure (linear aggregation) are translucent, while gels formed with aggregated particles are opaque due to larger and more random aggregation of particles [33]. Through visual analysis, it was possible to observe that the different salts added before spray drying led to the formation of opaque systems. As the salt ion concentrations increased, no significant differences

were observed in the whiteness of SPI gels compared to control ($p \geq 0.05$). However, the whiteness of cold-induced gels was slightly higher than that of heat-induced gels. The results were in good agreement with those of Soottawat, Wonnop, and Yuwathida (2004) who reported that the addition of Ca^{2+} did not result in a marked increase in the whiteness of surimi [34]. In addition, it was reported that color did not affect the functional properties of gels [35].

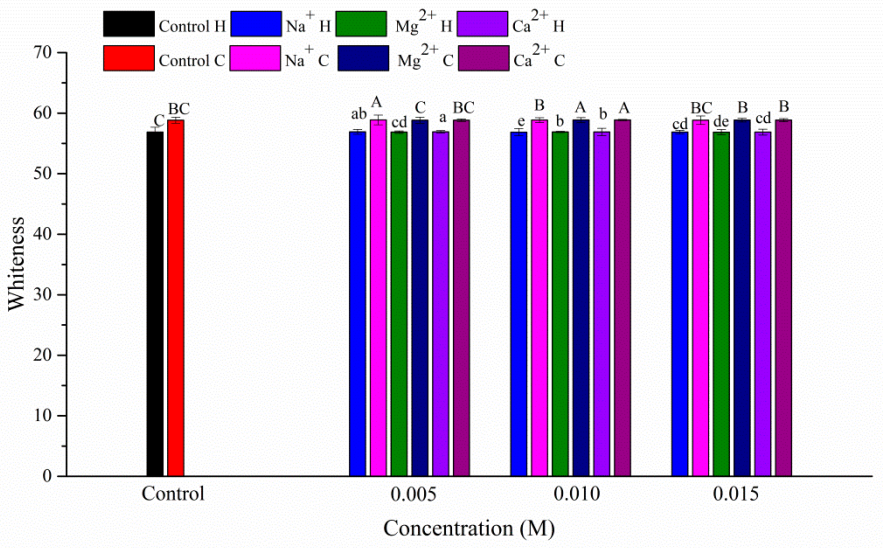


Figure 2. Effect of salt ions before spray drying on the whiteness of soy protein isolate heat- and cold-induced samples. All sample values are average values ± standard deviation (n = 3), and different letters in the same figure represent a significant difference between samples ($p < 0.05$). Capital letters represents cold-induced gels and lower case letters represents heat-induced gels. C represents cold-induced gels; H represents heat-induced gels.

Figure 3. Effect of salt ion addition before spray drying on the gel whiteness of soy protein isolate: (**a**) heat-induced gels; (**b**) cold-induced gels.

3.3. WHC

The WHC of gels is one of the most important functions of the protein gel system [36]. The WHC values are shown in Figure 4a,b. The WHC of gels was the highest when Na^+, Mg^{2+}, and Ca^{2+} were added at concentrations of 0.01, 0.005, and 0.005 M, respectively. The effect of Ca^{2+} on the WHC of heat-induced and cold-induced gels was the greatest. However, increased salt ion concentrations

(0.01–0.015 M) led to decreased WHC. In addition, the WHC values of heat-induced and cold-induced gels were higher at various concentrations ($p < 0.05$) compared to that of the control.

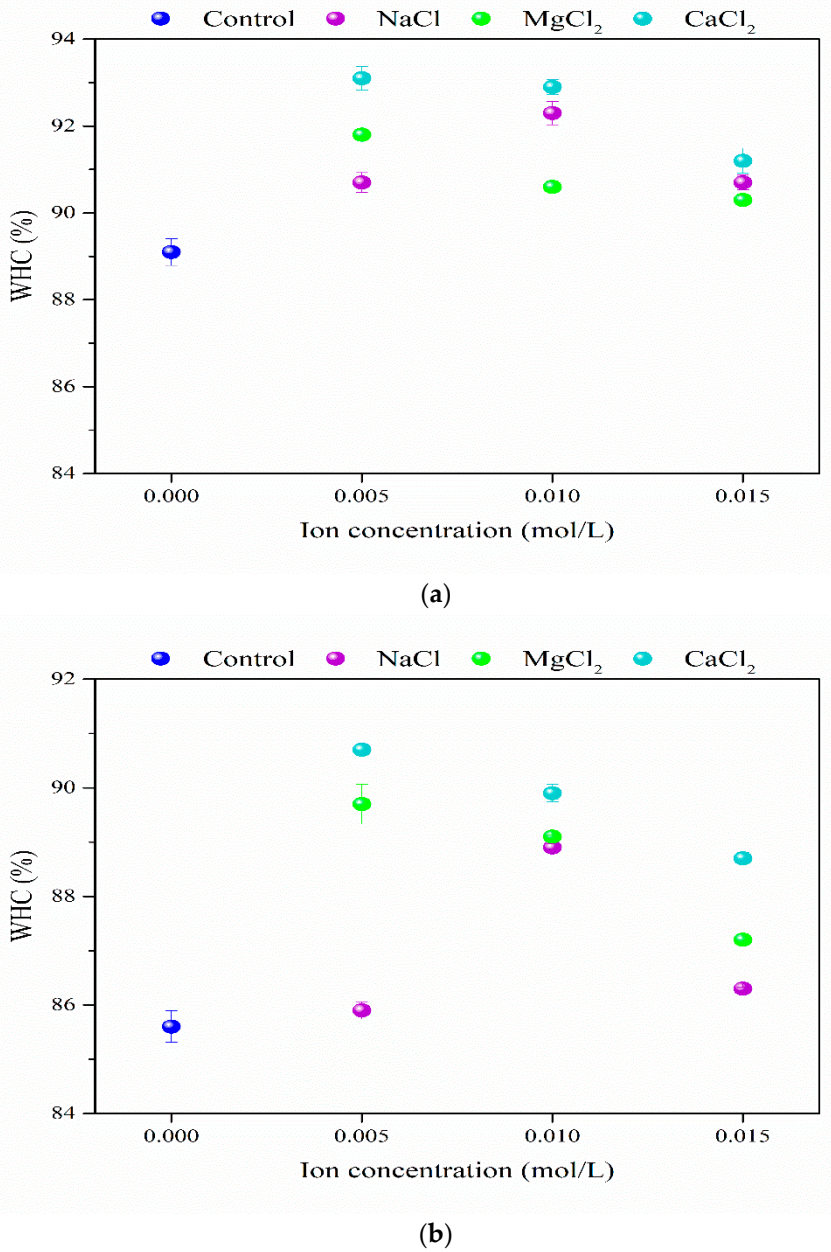


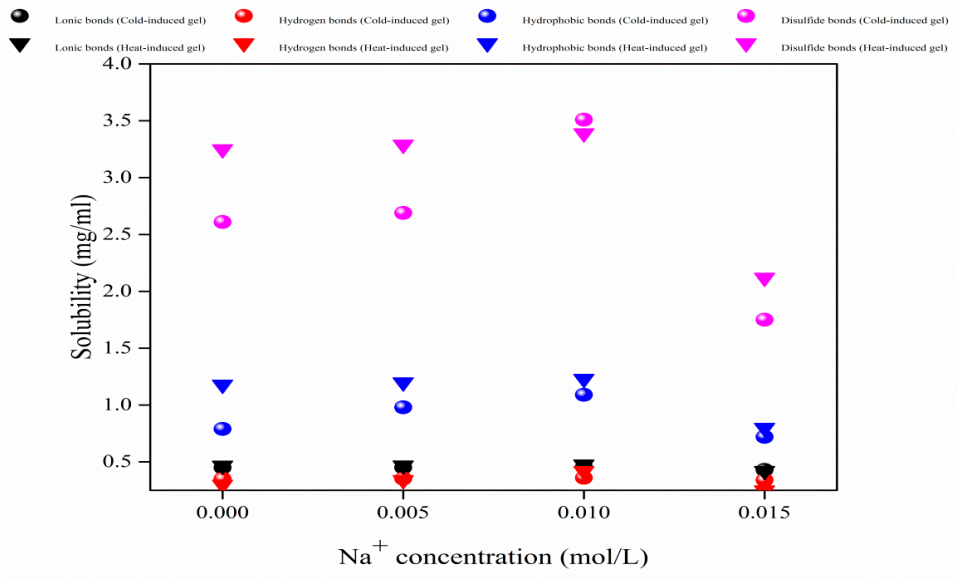
Figure 4. Effect of salt ion addition before spray drying on the gel water holding capacity (WHC) of soy protein isolate: (**a**) heat-induced gels; (**b**) cold-induced gels.

These results were similar to those for texture, which suggested that the small-size aggregates formed at low salt contents were most likely responsible for creating high capillary forces which, in turn, resulted in high WHC [37]. The WHC of the gels began to decrease with increased salt. This may be due to the gel dehydration resulting from higher concentrations of salt ions [36,38]. The Ca^{2+} bridges might also help retain water. Thus, the effect of Ca^{2+} on the WHC of the gels was greater than those of Na^{+} and Mg^{2+}, and the WHC values of heat-induced gels were higher than those of cold-induced gels at various salt ion concentrations (0.005–0.015 M).

3.4. Chemical Interaction Forces Analysis

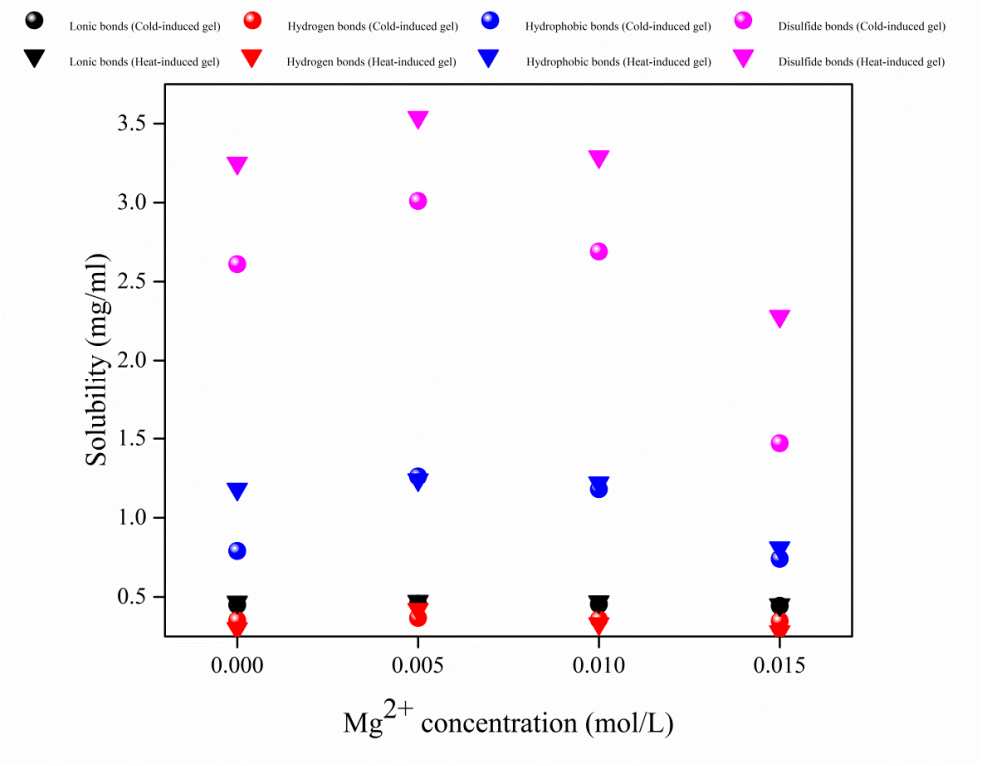
The solubility of heat- and cold-induced gels in five different solvents was determined to obtain information about the types of bonding between protein molecules in the two gel types. The differences in gel solubility between solvents S2 and S1 mainly represent ionic bonds; the differences in solubility between S3 and S2 mainly represent the hydrogen bonds; the differences between S4 and S3 mainly represent the hydrophobic interactions; and the differences between S5 and S4 mainly represent the disulfide bonds [39].

With the addition of Na^+, the bonding for both types of gels was in the order of disulfide bonds > hydrophobic interactions > ionic bonds > hydrogen bonds with increasing concentration (Figure 5a). Disulfide bonds and hydrophobic interactions increased at low ion concentrations (0.005–0.01 M). The addition of Mg^{2+} (Figure 5b) had an effect similar to that of Na^+, but the disulfide bonds decreased with increased concentration (0.01–0.015 M). The addition of Ca^{2+} (Figure 5c) significantly increased ionic bonds. These results may be due to the functional groups buried in the protein matrix becoming sufficiently exposed to improve the interactions between the protein molecules at low ionic strength; Ca^{2+}, Mg^{2+}, and Na^+ ions could bind negatively charged groups on the protein molecules, which leads to conformational changes that make more hydrophobic groups available at the surface [40]. Thus, the hydrophobic interactions and disulfide interactions were increased at low ion concentrations, while the high ionic strength resulted in salting-out as previously discussed, thus affecting the gelation of the protein [41]. Therefore, hydrophobic interactions and disulfide bonds were reduced at high ion concentrations (0.01–0.015 M). In addition, at the same ion concentration, the effect of Ca^{2+} was stronger than those of Mg^{2+} and Na^+. This may be due to the Ca^{2+} forming more hydrophobic interactions and salt bridges, which lead to increased hydrophobic interactions and disulfide interactions [42]. Furthermore, Ca^{2+} may be used as a crosslinking agent in the gel [43]. The hydrophobic interactions and disulfide bonds of gels containing Ca^{2+} and Mg^{2+}, which are both divalent ions, were very different. These results were consistent with the hardness of the gels. Other examples of Ca^{2+} and Mg^{2+} differences are common in biological systems. However, the mechanism accounting for these differences has not yet been completely clarified. Liu et al. (2015) studied the effect of a pH shift combined with a heat treatment on the SPI gel structure, and the results showed that SPI formed disulfide bonds during heating [44]. O'Kane et al. (2004) suggested that the formation of the soy protein gel network relies mainly on hydrogen bonds and hydrophobic bonds, and heat treatment can increase these forces [45].



(**a**)

Figure 5. *Cont.*



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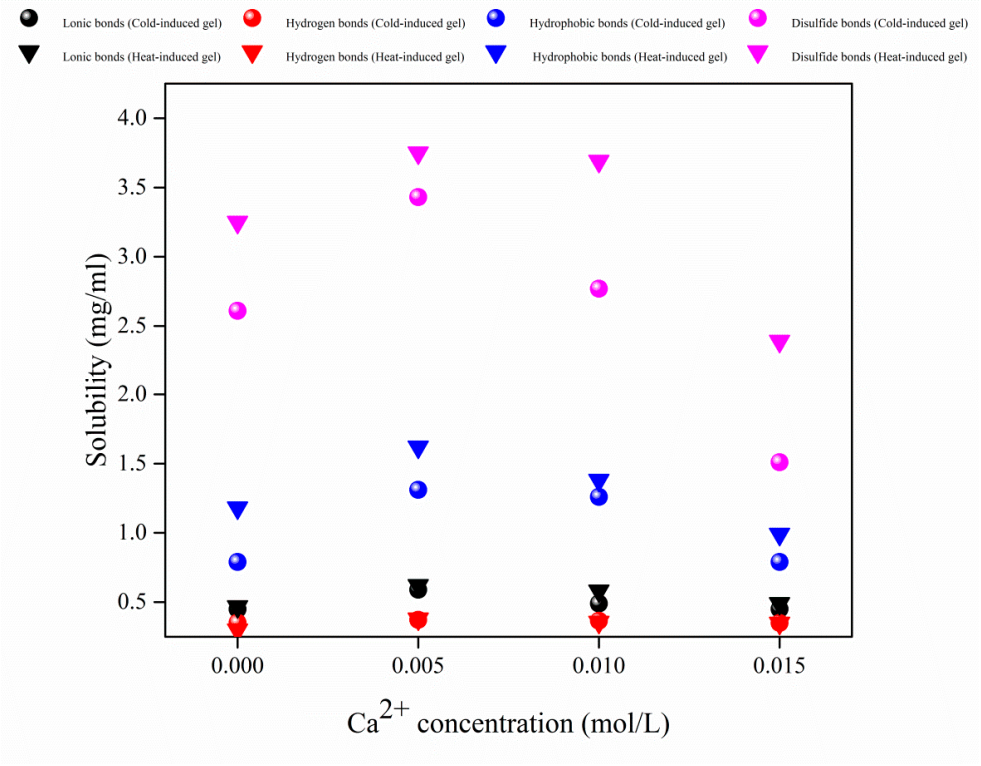

(**c**)

Figure 5. (**a**) Forces in heat- and cold-induced gels formed with Na^+ (0.005, 0.01, 0.015M) before spray drying; (**b**) Forces in heat- and cold-induced gels formed with Mg^{2+} (0.005, 0.01, 0.015M) before spray drying; (**c**) Forces in heat- and cold-induced gels formed with Ca^{2+} (0.005, 0.01, 0.015M) before spray drying.

Ions could enhance the interactions within the gel system when the ionic concentrations were from 0.005 to 0.01 M, and the hydrophobic interactions and disulfide bond interactions were the main interactions within the gels [23,46]. Heat-induced gels had stronger interactions than cold-induced gels. This was consistent with previous research on gel texture and WHC [47]. The interaction between salt ions and SPI was increased at low ion concentrations and this interaction was decreased at high ion concentrations.

3.5. Microstructure Analysis of the Gels

The microstructures of the heat- and cold-induced gels at different ion concentrations are shown in Figure 6. Compared with the cold-induced gels (Figure 6A–D), the heat-induced gels had a more uniform, smoother surface, with smaller pores for all three ions (Figure 6a–d). Compared with Na^+ and Mg^{2+}, Ca^{2+} promoted a better gel microstructure, though with each ion, protein interactions were maximized and aggregation minimized [48–50]. In addition, the dense network structure promotes water retention within the gel network structure. Therefore, the WHC values of the gels were higher.

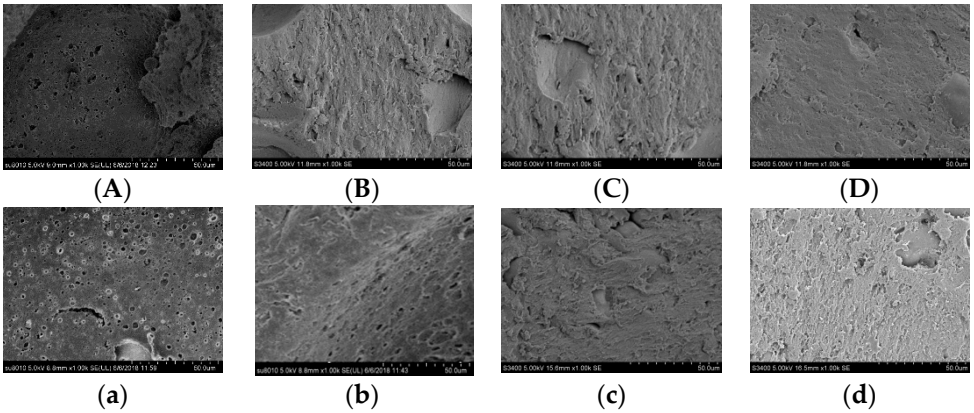

Figure 6. Scanning electron microscopy of heat- and cold-induced gels with 1000× magnification: (**A**) Cold-induced control gels; (**B**) Cold-induced gels with Na^+ (0.01 M); (**C**) Cold-induced gels with Mg^{2+} (0.005 M); (**D**) Cold-induced gels with Ca^{2+} (0.005 M); (**a**) Heat-induced control gels; (**b**) Heat-induced gels with Na^+ (0.01 M); (**c**) Heat-induced gels with Mg^{2+} (0.005 M); (**d**) Heat-induced gels with Ca^{2+} (0.005 M).

4. Conclusions

The effect of the addition of salt ions before spray drying to improve heat- and cold-induced gel properties of soy protein isolate was investigated. The results showed that appropriate concentrations of Na^+, Mg^{2+}, and Ca^{2+} (0.005–0.01 M) significantly increased the hardness, springiness, cohesiveness, chewiness, gumminess, resilience, and WHC of the gels. Presumably, this effect arises predominantly due to the increased exposure at the surface of functional groups buried in the protein matrix, which leads to improvement of the interactions between the protein molecules. Disulfide and hydrophobic interactions were the major forces in maintaining the gel network structure. The hardness, springiness, gumminess, and chewiness of the heat-induced gels were greater than those of the cold-induced gels. However, the cohesiveness and resilience were smaller than those of the cold-induced gels. Moreover, Ca^{2+} addition had a greater effect compared with Mg^{2+} and Na^+. This effect is probably due to Ca^{2+} forming more hydrophobic interactions, possibly through increased salt bridge formation. Therefore, Ca^{2+} (0.005 M), Mg^{2+} (0.005 M), and Na^+ (0.01 M) can be used to improve the hardness, springiness, and structural uniformity of heat- and cold-induced gel products. This study is useful for the industrial production of gels. Adding salt ions before spray drying could easily improve the gel properties of SPI. In addition, other ions such as anions can also be considered. Overall, adding salt ions before spray drying could offer great potential for the development of SPI with enhanced functionality suitable for comminuted meat products.

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