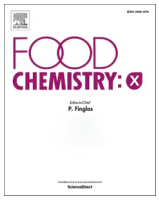
Food Chemistry: X 22 (2024) 101259

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



A novel approach has been developed to produce pure plant-based gel soy yogurt by combining soy proteins (7S/11S), high pressure homogenization, and glycation reaction

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ARTICLE INFO

Keywords:
Soybean 7S
Soybean 11S
High pressure homogenization
Glycation conjugate
Gelation

ABSTRACT

This research sought to examine how the physicochemical characteristics of soy globulins and different processing techniques influence the gel properties of soy yogurt. The goal was to improve these gel properties and rectify any texture issues in soy yogurt, ultimately aiming to produce premium-quality plant-based soy yogurt. In this research study, the investigation focused on examining the impact of 7S/11S, homogenization pressure, and glycation modified with glucose on the gel properties of soy yogurt. A plant-based soy yogurt with superior gel and texture properties was successfully developed using a 7S/11S globulin-glucose conjugate at a 1:3 ratio and a homogenization pressure of 110 MPa. Compared to soy yogurt supplemented with pectin or gelatin, this yogurt demonstrated enhanced characteristics. These findings provide valuable insights into advancing plant protein gels and serve as a reference for cultivating new soybean varieties by soybean breeding experts.

Introduction

Soy yogurt is increasingly being embraced as an alternative product by individuals who suffer from lactose intolerance, malabsorption, and health issues related to milk protein allergy. Research showed that the European plant-based yogurt market grew by 37 % from 2018 to 2020 (Boeck, Zannini, Sahin, Bez, & Arendt, 2021). This is primarily attributed to its lactose-free composition and hypoallergenic properties (Montemurro, Pontonio, Coda, & Rizzello, 2021). In addition, soy proteins have garnered significant attention due to their affordability. More significantly, it has the potential to provide supplementary health advantages to individuals due to its lipid-lowering, anti-cholesterol, and anti-atherosclerotic properties (Ramdath, Padhi, Sarfaraz, Renwick, & Duncan, 2017). Overall, it has become a rapidly developing category in the yogurt market. According to Gupta et al. (2022), plant-based products currently hold a market share of US$2.2 billion, which is significantly smaller compared to the global animal dairy market. Currently, there are only a limited number of soy yogurt products made solely from plant-based gels that have achieved significant success. This can be attributed to the inherent drawbacks in their gel and texture properties,

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https://doi.org/10.1016/j.fochx.2024.101259
Received 16 October 2023; Received in revised form 22 February 2024; Accepted 26 February 2024
Available online 27 February 2024

which ultimately influence consumer acceptance. Researchers found that soy yogurt lacking lipoxygenase had the lowest content of off-flavor compounds and the best sensory acceptance, which provided theoretical support to produce soy yogurt with good texture (Zhou et al., 2019). In addition, studies have shown that soy protein isolate forms a more uniform gel network and can improve the hardness, elasticity, water retention and toughness of the gel (Wang et al., 2020). However, soy yogurt still has the defect of rough texture. Therefore, there is an urgent need to enhance the gel and texture characteristics of soy yogurt, with the goal of producing a high-quality, plant-based gel soy yogurt.

In recent years, such as reinforcement of 7S and 11S proteins (Pang, Safdar, Wang, Sun, & Liu, 2021), modification of protein glycation (Bu, Ren, Zuo, & Zhao, 2022), and high-pressure homogenization (Bi et al., 2020) have become effective methods to enhance the gel properties. Previous studies have focused on enhancing the properties of gel in soy yogurt by incorporating various additives, including bulking agents like maltodextrin, fibers such as inulin, and thickeners like gelatin or pectin (Saavedra Isusi, Paz Puga, & van der Schaaf, 2022). With the evolving shift towards a greener and healthier consumption mindset, there is an increasing demand for pure plant-based gel soy yogurt. Soy protein serves as the primary gel component in soy yogurt, and its composition significantly influences the gel properties of the yogurt. The main constituents of soy protein are 7S and 11S, which make up approximately 65–80 % of its composition. Studies conducted by Pang et al. (2021) have demonstrated that the reinforcement of 7S and 11S proteins alone can lead to improvements in the gel properties of acid soymilk. However, further investigation is needed to determine the impact of incorporating mixtures with varying ratios of 7S/11S proteins on the gel properties of soy yogurt. Moreover, it should be noted that soy protein exhibits a high degree of sensitivity to external factors. Specifically, its properties can be significantly influenced by various processing conditions, such as acid and heat treatment. These treatments have the potential to diminish the gel properties of soy protein. A significant amount of research has been conducted on the utilization of chemical, physical, and enzymatic modifications to enhance the interfacial properties and emulsification properties of proteins. However, there is a dearth of comprehensive studies focusing on the application of these modifications to improve gel properties. Physical methods, such as heat treatment and ultrasonic treatment, are associated with certain drawbacks, including high costs and high energy consumption. Enzymatic modifications have the potential to generate specific bitter or other undesirable flavor compounds during the process of protein degradation, thereby influencing the sensory acceptability to some degree. While the glycation reflection is known as a green modification method using in food science widely, which may be an efficient method to improve the gel properties of soy yogurt (Bu et al., 2022). In addition, the homogenization pressure will also affect the gelation of soy protein in the process of producing soy yogurt. High-pressure homogenization has been shown to enhance the gel properties through the formation of a more stable isotropic network gel structure (Bi et al., 2020). It remains to be studied which homogenization pressure can effectively improve the gel characteristics of soy yogurt after adding 7S and 11S with different ratios. In conclusion, the reinforcement of 7S and 11S proteins with varying ratios, the modification of protein glycation, and the utilization of high-pressure homogenization techniques show promise as potential methods for enhancing the gel properties of soy yogurt. These approaches warrant further investigation and research.

This study aimed to develop a method for creating plant-based soy yogurt with enhanced gel properties and improved consumer appeal. The present study investigates the impact of the 7S/11S ratio, high-pressure homogenization, and the glycation reaction on the quality and characteristics of soy yogurt. And a comparison was made between pure plant-based soy yogurt developed and soy yogurt supplemented with thickeners such as gelatin and pectin. The comparison encompassed various aspects including physicochemical properties, texture, gastrointestinal digestion, and sensory attributes. This study would offer a theoretical foundation and practical implications for the production of plant-based gel yogurt.

Material and methods

Materials

Soybean was provided by the Institute of Soybean Research of Northeast Agricultural University (Harbin, China). Yogurt starter culture of YO-MIX300, containing Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus were provided by DANISO Co., Ltd. (Copenhagen, Denmark). Acetone was purchased from Kermel Chemical Reagent Co., Ltd (Tianjin, China). Phthalaldehyde was obtained from Macklin Biochemical Technology Co., Ltd (Shanghai, China). Glucose was purchased from Solarbio Technology Co., Ltd (Beijing, China). 1-anilinonaphthalene-8-sulfonic acid (ANS) was obtained from Sigma-Aldrich Biochemical Technology Co., Ltd (Jiangsu, China). β-mercaptoethanol was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Glycine was obtained from Bjbiotopped Technology Co., Ltd (Beijing, China). All other chemicals were of analytical grade.

Preparation of 7S and 11S globulins

The preparation of 7S and 11S globulins was according to the method of Pang et al. (2021) with a slight modification. The soybeans underwent a process of pulverization and subsequent sieving through a 60-mesh sieve to obtain soybean powder. The soybean powder was dispersed in acetone at a ratio of 1:5 (w/w) and subsequently reduced by magnetic stirring for a duration of 2 h. The defatted soybean powder was then dispersed in water at a ratio of 1:10 (w/w), and the pH of the suspension was adjusted to 8.5 using a 2 mol/L NaOH solution. The mixture was further stirred magnetically for 1 h. The resulting slurry was subjected to centrifugation at 10,000 g for a period of 20 min using a refrigerated centrifuge (3 K15, Sigma-Aldrich, Missouri, USA). The supernatant was collected and designated as supernatant 1. To fully obtain soybean globulins, the above precipitate was then washed. The precipitate was dispersed in water at a ratio of 1:5 (w/w) and subsequently subjected to centrifugation at 10,000 g for 20 min following a 10-min stirring period. The resulting supernatant was collected and designated as supernatant 2. The supernatants obtained from the two centrifugations were mixed, and $NaCO_3$ was added to the mixed solution so that its concentration was 0.98g/L. The pH of the resulting mixture was adjusted to 6.4 using a 2 mol/L HCl solution. The turbid suspension obtained was then refrigerated at 4 °C overnight. The suspension was subsequently subjected to centrifugation at 5,000 g for a duration of 15 min. The resulting precipitate, known as soy 7S globulin, was collected after undergoing lyophilization. The supernatant was then subjected to another round of centrifugation at 5,000 g for a duration of 30 min, with the addition of 0.25 g/L NaCl. Following this, the supernatant was collected and mixed with double ice water, and the pH was adjusted to 4.8 using a 2 mol/L HCl solution. The suspension was subsequently subjected to centrifugation at 5,000 g for a duration of 15 min. The resulting precipitate, identified as soy 11S globulin, was collected following the process of lyophilization. The molecular composition and purity of the two isolated globulin fractions were assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Fig. S1). Relatively high purity (above 80 %) was obtained for fractions 7S and 11S with purity values of 90.51 % and 91.23 %, respectively. Studies have shown that purities higher than 80 % are suitable for follow-up studies (Jia et al., 2022). The 7S and 11S used in this study were 7S and 11S with purity of 90.51 % and 91.23 %. The protein contents of the 7S and 11S globulin were determined using the Dumas method (N × 6.25) and found to be 92.74 ± 0.20 % and 94.28 ± 0.30 %, respectively.

Preparation of globulin-glucose conjugates

Soy 7S globulin-glucose conjugate and soy 11S globulin-glucose conjugate were synthesized following the procedure outlined by (Li et al., 2019) with minor adjustments. In this study, the 7S and 11S globulins were dissolved to a concentration of 8 mg/mL. Later, glucose was introduced to the 7S protein solution, establishing a ratio of 4:1 (w/w) between the 7S protein solution and glucose. Similarly, glucose was incorporated into the 11S protein solution, creating a ratio of 4:1 (w/w) between the 11S protein solution and glucose. The solution was agitated for a duration of 3 h in a water bath maintained at a temperature of 70 °C. To prevent evaporation of water, the beaker was securely sealed with parafilm. Subsequently, the prepared samples of soy 7S globulin-glucose conjugate (7S_G) and soy 11S globulin-glucose conjugate (11S_G) were cooled to room temperature. Finally, the powders containing SPI-G conjugates were subjected to freeze-drying and subsequently stored at a temperature of −20 °C. The grafting degree (GD) is quantified based on the extent of amino group depletion, and the concentration of free amino groups was measured using the o-Phthalaldehyde (OPA) method as described in the study conducted by (Xu, Han, Chen, Li, & Jin, 2018). The GD of the soy 7S globulin-glucose conjugate is 25.4 %, while the GD of the soy 11S globulin-glucose conjugate is 33.7 %.

Physicochemical analysis of pre-fermentation mixtures

Soybeans were immersed in a sodium bicarbonate solution (0.5 %) for a duration of 12 h. Subsequently, the soaked soybeans were milled with a solid to water ratio of 1:10 (w/v) to obtain soybean milk. Different ratios of 7S/11S content (1.5:1, 1:1, 1:1.5, 1:2, 1:2.5, and 1:3) were incorporated into forming mixtures prior to fermentation to investigate their impact on gel properties, specifically under a homogenizing pressure of 30 MPa. Different homogenizing pressures (30, 50, 70, 90, and 110 MPa) were applied to investigate their impact on gel properties, taking into consideration the optimal 7S/11S ratio. 7S globulin and 11S globulin underwent initial glycation. Four pre-fermentation mixtures were prepared by adding different combinations of 7S + 11S, 7S-glucose conjugate (7S_G) + 11S-glucose conjugate (11S_G), 7S + 11S_G, and 7S_G + 11S. These mixtures were used to investigate their effect on gel properties, taking into consideration the optimal 7S/11S ratio and optimal homogenizing pressure. Last the gelatin and pectin were added to soybean milk to form pre-fermentation mixtures to compare the gel difference with added soybean protein. In all research, pre-fermentation mixtures of adding different ratios 7S/11S, pre-fermentation mixtures treated with different pressure, and pre-fermentation mixtures of adding different globulin-glucose conjugates, which all owned the protein concentration of 6 % and the granulated sugar concentration of 7 %. After that, each of the above mixtures was homogenized for 2 min with a high-pressure homogenizer (SPCH-10, Stansted Fluid Power, UK) and pasteurized at 95 ∘C for 10 min. The pre-fermentation mixtures were allowed to cool to room temperature before being used for physicochemical analysis.

The particle size and zeta potential were analyzed at 25 °C using NANO ZS90. The determination of particle size, specifically the average particle size, is achieved through the utilization of dynamic light scattering (DLS). The measurement of surface hydrophobicity (H_0) of a protein is determined by analyzing the slope of fluorescence intensity in relation to protein concentration. This analysis was conducted using the method of (Du, Zhang, Zhao, & Chen, 2020). To ascertain the concentration of free sulfhydryl groups (-*SH*), an initial step involved the preparation of buffer A (pH 8.0). Buffer A consisted of 0.086 mol/L Tris, 0.09 mol/L glycine, and four mmol/L ethylene diamine tetraacetic acid (EDTA). Then Ellman reagent was prepared by dissolving 0.04 g 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in buffer A to 10 mL constant volume. Next, a protein sample of 1 mL (2 mg/mL) was suspended in 5.0 mL buffer A and 40 μL Ellman reagent and then incubated at room temperature in darkness for 15 min, while the absorbance was measured at 412 nm, and the water was set as the control. The free sulfhydryl group (-*SH*) content was calculated according to the Eq. (1):

$$-SH\,(\mu mol/L) = \frac{73.53 \times A_{412} \times D}{C} \quad (1)$$

A_{412}: the absorbance value of the sample at 412 nm; D: the dilution factor of the sample; C: protein concentration (mg/mL) of the sample.

Preparation of soy yogurt

The yogurt starter was inoculated into the pre-fermentation mixtures mentioned in section 2.4 with a concentration of 0.1 % w/v, and the mixture was fermented at 42 °C. At the pre-determined intervals, a bottle was taken out and the pH was monitored. The fermentation ended at pH 4.5, and then the prepared soy yogurt cooled to ambient temperature in an ice bath, and then stored at 4 °C for further analysis.

Gel strength and water holding capacity (WHC) analysis of soy yogurt

The texture of soy yogurt was assessed using a texture meter model TA-XT Plus C (Stable Micro System Ltd., Surrey, UK). A cylindrical cup was probed using a 35 mm diameter instrument, resulting in a final depth of 30 mm. The yogurt was allowed to ripen at a temperature of 4 °C for a duration of 24 h, after which it was placed on the table. The experimental conditions for the measurement were as follows: the test speed was maintained at a constant rate of 1 mm/s throughout the entire testing procedure. The contact point value was set at 5 gf, and the target displacement was set to 5 mm. Each sample was subjected to three parallel tests. Water holding capacity (WHC) was assessed using the methodology outlined by (Valdez-Hurtado et al., 2019).

Rheological measurement of of soy yogurt

A rotary rheometer (MARS40, Thermo, American) was used to measure the rheological properties of the yogurt (Lu, Zhang, Yuan, Gao, & Mao, 2021). The yogurt sample was applied onto the substrate and allowed to stabilize for a period of 5 min. A rheological analysis was performed, employing a frequency sweep spanning from 0.1 to 100 Hz, while maintaining a constant strain of 1 %. Viscosity was measured with a shear rate sweep test, where the shear rate was varied within the range of 0.01 to 100 rad/s.

Scanning electron microscopy (SEM) analysis of soy yogurt

The scanning electron microscopy (SEM) was used for microstructures analyzed of soy yogurt gels according to the method reported by (Lin et al., 2019). In summary, the gels were sectioned into small dimensions measuring 2 mm × 5 mm × 5 mm. Subsequently, they were submerged in a glutaraldehyde solution with a concentration of 2.5 % w/v and a pH of 7.2. The resulting mixture was then refrigerated for a duration of 1.5 h to facilitate fixation. Next, the samples underwent three washes, each lasting 10 min, with phosphate-buffered saline (PBS, 0.1 mol, pH 7.2), in order to eliminate the glutaraldehyde. Afterward, the dehydration of the samples was carried out using solutions of 50 %, 70 %, and 90 % ethanol for 10 min each, respectively. Subsequently, the samples were dehydrated twice with 100 % ethanol for 15 min each time. Before undergoing the drying process, the samples were immersed in a solution consisting of ethanol, *tert*-butanol (in a 1:1, v/v ratio), and *tert*-butanol for 15 min each to facilitate replacement. The samples underwent a drying process utilizing a freeze dryer for a duration of 4 h subsequent to being placed in a freezer at a temperature of −20 °C for a period of 30 min. Subsequently, the entire set of samples that were chosen were affixed onto the observation sample stage using conductive tape and inverted to ensure that the surface to be observed was facing

upwards. The samples were coated using a sputtering ion coater (E-1010, HITACHI, Japan), and the microstructure of the samples was then observed and recorded by SEM (S-3400 N, HITACHI, Japan) with an accelerating voltage of 5 kV.

Sensory analysis of soy yogurt

Sensory analysis was conducted according to the method reported by (Anuyahong, Chusak, & Adisakwattana, 2020) with some changes. A total of 82 healthy, non-smoking potential evaluators were recruited from students and staff in the College of Food Sciences at Northeast Agricultural University. A panel of 10 well-trained panelists (five male and five female with age of 20–42) performed sensory evaluations of soy yogurt. Prior to sensory evaluation, all group members were trained at least 2 h a day on the characteristics of soy yogurt and sensory evaluation requirements for one week. The yogurt samples were divided into different plastic tasting cups with a random code (three-digit numbers), and the samples were tasted in random order. Participants were asked to score yogurt samples according to color, odor, texture, flavor, and overall-acceptability using a 9-point hedonic scale (1: dislike extremely, 9: like extremely).

In vitro digestion analysis of soy yogurt

The in vitro simulated digestion assay of soy yogurt was determined according to the method of Zhao (Zhao, Su, Zhao, & Sun, 2019) with a slight modification. Gastric digestive juice was prepared by dissolving 2 g of NaCl in deionized water, adding 8.44 mL of hydrochloric acid (12 mol/L) and 0.32 % pepsin, and then adjusting the final volume to 1000 mL. Intestinal digestive juice was prepared by dissolving 0.0067 g of CaCl2, 0.359 g of NaCl, 1 g of bile salts, and 1.6 g of trypsin in 200 mL of solution. The yogurt samples were combined with gastric digestive juice and intestinal digestive juice, respectively. Samples were collected at 0.5 and 1 h intervals during the simulated gastric digestion process. Samples were collected at time intervals of 0.5, 1, 1.5, and 2 h during the simulated process of intestinal digestion. The aforementioned samples were thoroughly mixed with an equal volume of trichloroacetic acid (10 %, w/v) and subsequently subjected to centrifugation at 8000 g for a duration of 15 min. The supernatant was subsequently collected to determine the protein content using the Bradford method. The calculation formula was as Eq. (2):

$$Digestibility\ (\%) = \frac{Protein\ content\ after\ digestion}{Protein\ content\ befor\ digestion} \times 100 \quad (2)$$

Statistical analysis

All results were measured and analyzed with three parallel experiments. Origin software (version 9.65) was used to plot, and SPSS software (version 22) was used for significant difference analysis. The Tukey test was used for the differences among trials with a significance level of $p < 0.05$.

Results and discussion

Effect of globulin content ratio on the gel properties of soy yogurt

The findings pertaining to the fortification of soy yogurt with various 7S/11S proteins were depicted in Fig. 1. When the ratio of 7S/11S was 1:3, several parameters exhibited their highest values, including the average particle size, the absolute value of Zeta potential, the surface hydrophobicity (H_0), the hardness, the water holding capacity (WHC), the frequency sweeps dependence of interfacial elastic modulus G′ and viscous modulus G″, and the apparent viscosity. However, the content of free sulfhydryl group (*-SH*) was an exception to this trend. Fig. 1C demonstrated that the concentration of *-SH* groups initially increased

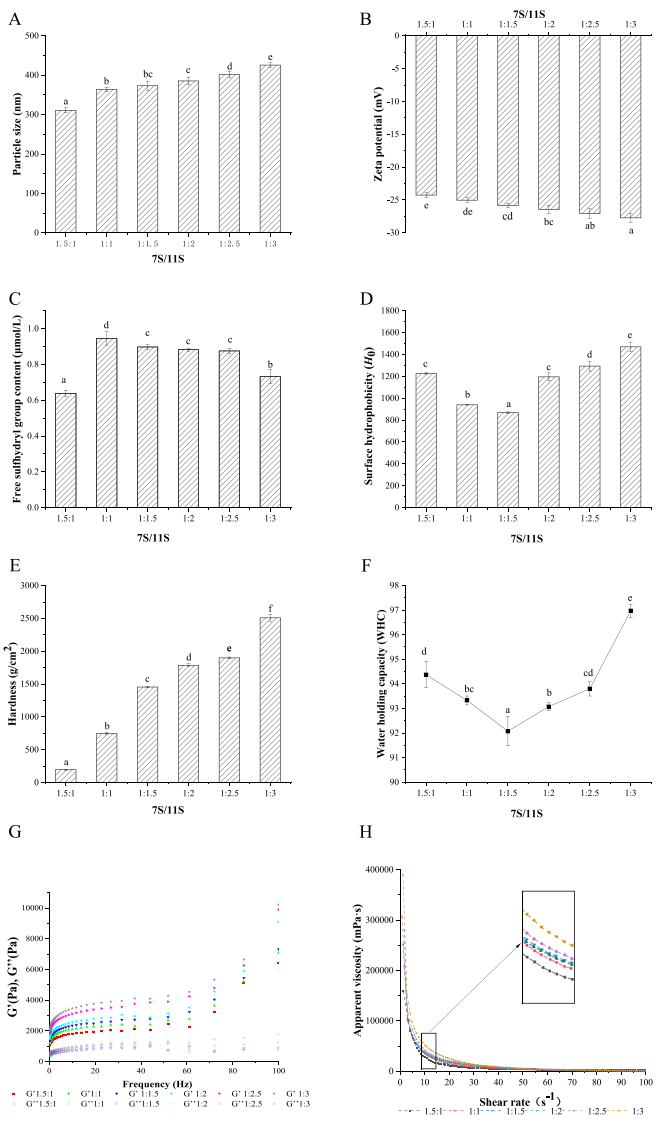

Fig. 1. Effects of different proportions of 7S and 11S globulin to pre-fermentation mixtures (A-D) and soy yogurt (E-H) when homogeneous pressure was 30 MPa and combination was 7S + 11S. A) Particle size (nm); B) Zeta potential; C) Free sulfhydryl group (*-SH*) content (μmol/L); D) Surface hydrophobicity (H_0); E) Hardness (g/cm^2); F) Water holding capacity (WHC); G) Frequency sweeps dependence of interfacial elastic modulus G′ and viscous modulus G″; H) Apparent viscosity (mPa·s).

and subsequently decreased as the 7S/11S ratio decreased. Given that the 11S globulin contains a higher concentration of *-SH* groups compared to the 7S globulin, it could be inferred that the *-SH* groups increased proportionally with the increase in 11S globulin. With the increased in particle size, there was a corresponding increase in the contact area between the protein and the air. This increased contact area facilitates oxidation, leading to the oxidation of *-SH* groups and the formation of disulfide bonds. Consequently, the content of free sulfhydryl groups decreased (Pang et al., 2021). Fig. 1A illustrated that with an increase in the content of 11S globulin, there was a corresponding tendency for the average particle size in the solution to increase. Disulfide bond formation between particles led to the aggregation of small-diameter particles, thereby facilitating the formation of larger aggregates that were stabilized by disulfide bonds. Consequently, this process resulted in an increase in particle sizes (Lu et al., 2022). Fig. 1D illustrated the variation of H_0 as the ratio of 7S/11S decreases, initially decreasing and then increasing. The reduction in exposure of

hydrophobic groups could be attributed to the formation of small-diameter particles and their subsequent mutual aggregation. However, as the content of 11S, which consisted of hydrophobic amino acids, increased, the hydrophobicity also increased accordingly (Lu et al., 2022).

The findings indicated that the soy yogurt exhibited the highest hardness value when the ratio of 7S/11S proteins was 1:3 (Fig. 1E). The study also indicated a positive correlation between gel hardness and the concentration of 11S globulin. Furthermore, it was observed that gels with a lower ratio of 7S/11S exhibited higher levels of hardness. This phenomenon could be attributed to the higher presence of S—S bonds in the 11S protein, which has the potential to create a more robust three-dimensional network structure (Zheng et al., 2021). Fig. 1F. showed that the water-holding capacity of soy yogurt exhibited a non-linear relationship with the increase in 11S globulin content, initially decreasing and subsequently increasing. This may be due to the partial formation of small-diameter particles disulphide bond aggregates and uneven particle distribution, which made the gel WHC decrease at first, then with the particle distribution became more and more uniform and the average particle size of protein aggregates increase led to a higher WHC of the gel (Yang, Ke, & Li, 2021). Fig. 1B showed that the higher the content of 11S globulin, the greater the absolute value of Zeta potential, which could cause mutual repulsion of the molecules to reduce aggregation and finally make the gel more stable (Zhang & Lu, 2015).

The result showed that the storage modulus (G')' and loss modulus (G“) of soy yogurt fermented with the six globulin proportions tended to increase, and the G' value was higher than the G'' value during the frequency range from 0.1 to 100 Hz (Fig. 1G). This phenomenon could be attributed to the gradual exposure of the sulfhydryl group in 11S globulin upon heating, leading to the formation of disulfide bonds. Consequently, the proteins undergo gelation, forming a network structure. Compared with the network gel structure formed by 7S mainly through hydrogen bonds, the network gel structure formed by 11S globulin has high hardness and strength characteristics (Wu, Hua, Chen, Kong, & Zhang, 2017). It could be seen from the results that increasing the content of 11S globulin could increase the apparent viscosity of the gel (Fig. 1H). This was because protein suspensions consisting of larger protein aggregates generally have higher viscosity (Ma et al., 2020).

According to sensory evaluation results of soy yogurt added different ratios of 7S/11S (Table S1), the overall-acceptability showed an increasing trend with the increase of 11S, while its overall acceptability started to show a decline when 7S/11S was 1:3. Besides, the content of -*SH* gradually reduced with the increase of 11S. These results may cause by the increase of the sample particle size and the gap between the particles. This would increase the risk of oxidation and negatively affect yogurt products. Considering this and combined other indicators, when the protein addition ratio of 7S/11S was 1:3, the sample owned the good gel strength and gel stability. Thus, the 7S/11S ratio was selected as 1:3 for the next step study.

Effect of homogeneous pressure on gel properties of soy yogurt

The result showed that the high-pressure homogenization treatment resulted a significant reduction of the sample particle size (Fig. 2A). This was because the cavitation will produce a strong shearing effect on the target protein, resulting in the structural change of the target protein and the destruction of the particle size. And last resulted more small and uniform particles, which made the gel more stable. Furthermore, the particles with smaller average diameter and uniform distribution easy connect to form a dense gel mesh structure to get high WHC gel (Liu et al., 2023). Fig. 2B demonstrated that the absolute value of the zeta potential exhibited an increase as the pressure was raised from 30 MPa to 110 MPa, and subsequently reached a steady state between 70 MPa and 110 MPa. The higher the absolute value of zeta potential, the gel was more stable (Wang & Guo, 2016). Fig. 2D showed that the surface hydrophobicity gradually increases. The observed phenomenon can be attributed to the disruption of protein's chemical bonds, resulting in an increased exposure of the hydrophobic surface area (Guo & Xiong, 2019). The concentration of -*SH* exhibited a significant decrease as the homogenization pressure increased (Fig. 2C). This finding was in line with the results reported in a previous study (Kang et al., 2022).

The findings indicated that subjecting the sample to homogenization treatment within a pressure range of 30 MPa to 110 MPa resulted in enhanced gel hardness (Fig. 2E). The increase in non-covalent bonds facilitated the formation of a protein gel network, leading to an enhancement in gel hardness (Kang et al., 2021). Fig. 2F demonstrated a significant increase in water holding capacity (WHC) as the homogenization pressure is increased, which aligned with the findings reported by Ferragut et al. (2009). Meanwhile, the higher the water holding capacity, the better the tissue state of yogurt.

Fig. 2G demonstrated a gradual increase in G' and G'' as the homogenization pressure is increased, which aligned with the findings reported by Bi et al. (2020). The enhancement of viscoelasticity could be attributed to the disruption of covalent bonds and non-covalent interaction forces, which was facilitated by the high-pressure homogenization treatment. The impact of various homogenization pressures on the apparent viscosity of soy yogurt was depicted in Fig. 2H. The results suggested that the apparent viscosity of soy yogurt decreased with increasing shear rate, which performed as shear thinning behavior and could be approved as a typical non-Newtonian. This was due to the reorientation and rearrangement of the molecules in the yoghourt gel in response to shear, which reduced the flow resistance, which was consistent with the findings of Chen et al. (2023). Soy yogurt had a high viscosity value at 110 MPa. This may be due to the production of lots of aggregates under high-pressure homogenization treatment, resulted in an increase in viscosity, which could be approved by Kang et al. (2022).

The sensory evaluation results of soy yogurt processed with different pressures were showed in the Table S2, where the texture and overall acceptability showed an upward trend with the increase of homogenizing pressure. When the homogenizing pressure was 110 MPa, the texture and overall acceptability did not change significantly, while the overall acceptability started to show a decline. Taking into the demand of consumers and the fact that it is not advisable to use too high pressure in production, 110 MPa was chosen for the follow-up study.

Effect of glycation reaction on the gel properties of soy yogurt

The impact of the glycation reaction on the gel properties of soy yogurt was illustrated in Fig. 3. The hardness, the water holding capacity (WHC), the frequency sweeps dependence of interfacial elastic modulus G' and viscous modulus G'', and the apparent viscosity were highest when the combination was 7S + 11_G. While the results indicated that there was no significant difference between the zeta potentials (Fig. 3B), suggesting that the interactions between protein molecules were not static, but rather influenced by hydrophobic interactions and steric hindrance (Nakayama et al., 2013). The protein suspension exhibited a notable decrease in surface hydrophobicity following protein glycation, with the 7S + 11_G protein suspension demonstrating the least reduction (Fig. 3D). Protein glycation had the potential to impact the hydrophilic/hydrophobic equilibrium of the protein surface, resulting in a reduction in protein surface hydrophobicity (Bielikowicz et al., 2012). Fig. 3A demonstrated that the addition of 7S + 11S_G resulted in a shift in the particle size distribution towards smaller particles. The alteration of the spatial structure of the 7S globulin and the disruption of protein–protein interactions, possibly caused by protein glycation, could lead to the aggregation of proteins. The covalent attachment of sugar groups to 11S globulin led to an increase in steric hindrance among protein molecules. Additionally, the presence of sugar chains weakened the interaction between protein molecules, resulting in a reduction in the extent of protein aggregation (Bu et al., 2022). This result showed that the addition of 7S + 11S_G could make the particle size of the suspension smaller and make the suspension more stable.

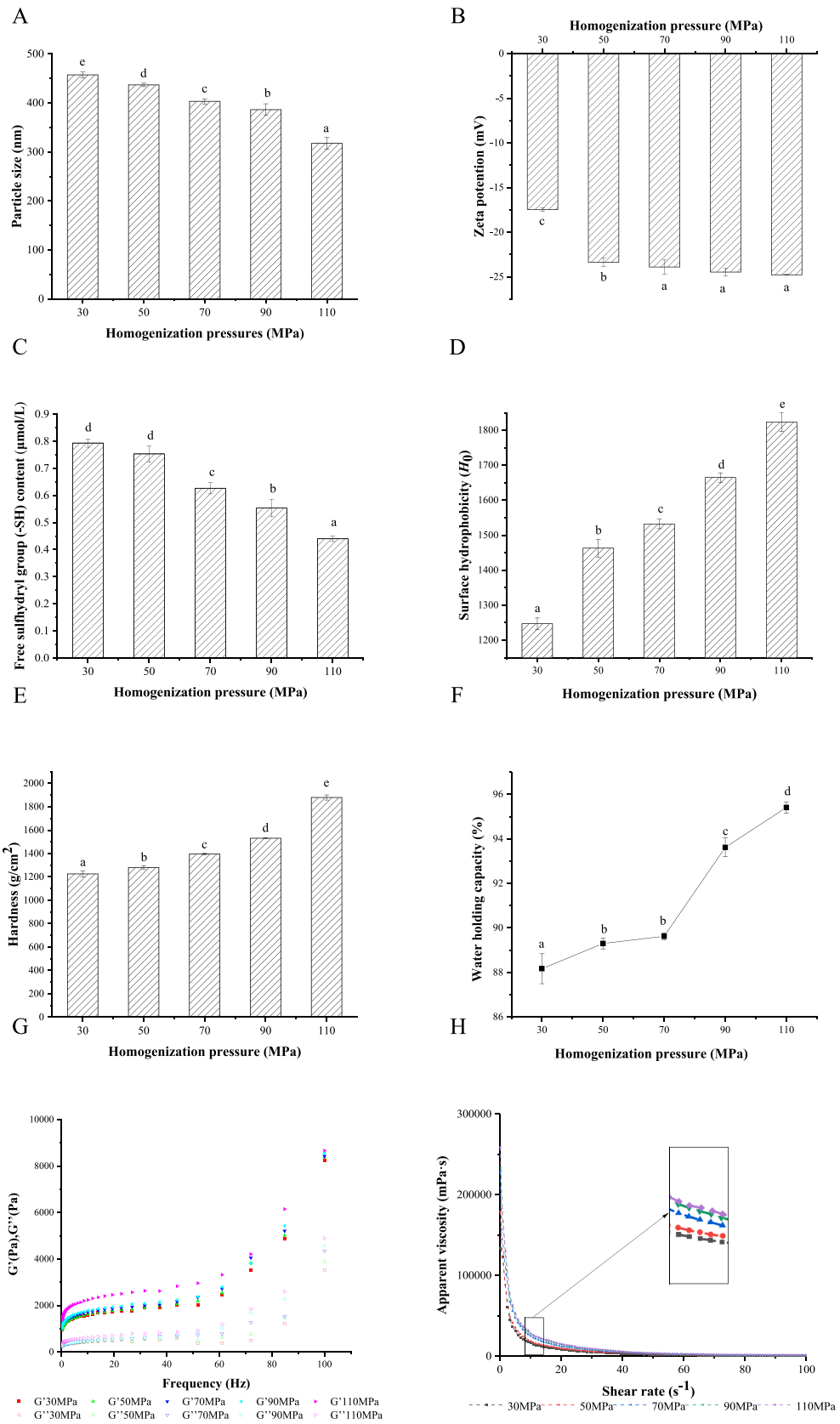

Fig. 2. Effects of different homogeneous pressure to pre-fermentation mixtures (A-D) and soy yogurt (E-H) when combination was 7S + 11S and ratio of 7S/11S was 1:3. A) Particle size (nm); B) Zeta potential; C) Free sulfhydryl group (-*SH*) content (μmol/L); D) Surface hydrophobicity (H_0); E) Hardness (g/cm^2); F) Water holding capacity (WHC); G) Frequency sweeps dependence of interfacial elastic modulus G′ and viscous modulus G″; H) Apparent viscosity (mPa·s).

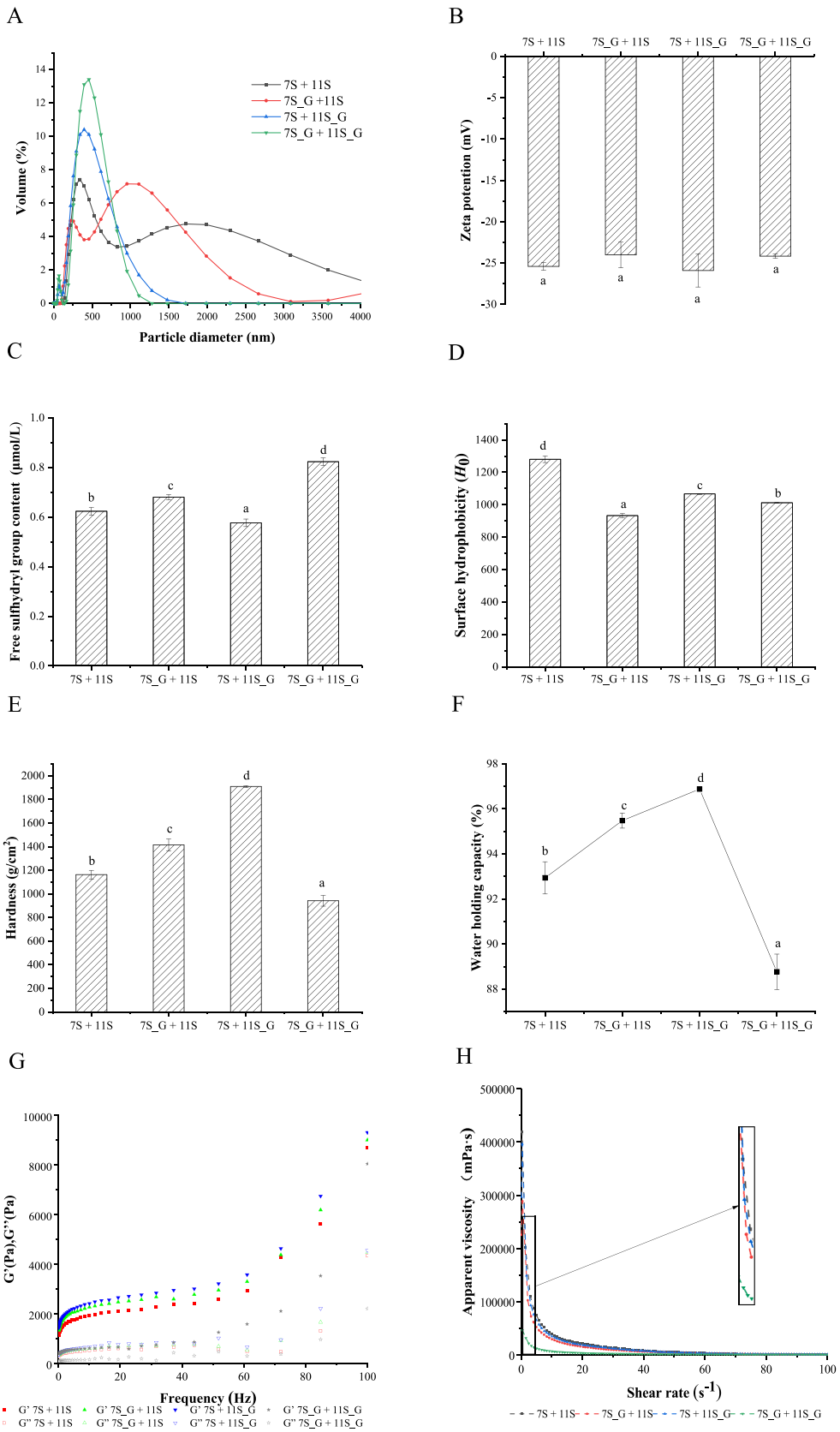

Fig. 3. Effects of protein glycation reactions to pre-fermentation mixtures (A-D) and soy yogurt (E-H). A) Particle size (nm); B) Zeta potential; C) Free sulfhydryl group (-*SH*) content (μmol/L); D) Surface hydrophobicity (H_0); E) Hardness (g/cm^2); F) Water holding capacity (WHC); G) Frequency sweeps dependence of interfacial elastic modulus G′ and viscous modulus G″; H) Apparent viscosity (mPa·s).

The suspension with the addition of 7S + 11S_G had the lowest *-SH* (Fig. 3C) and highest hardness (Fig. 3E). This phenomenon could be attributed to the increased accessibility of the sulfhydryl groups in 7S and 11S_G, facilitating their interaction and resulting in the formation of a greater number of disulfide bonds. The formation of disulfide bonds resulted in a decrease in the concentration of *-SH* groups, thereby enhancing the firmness of the gel and improving the stability of its structure., improving the gel hardness and structure's stability. Fig. 3F illustrated that the addition combination of 7S + 11S_G resulted in a significantly higher water holding capacity (WHC) of yogurt compared to other combinations ($p < 0.05$). This could be attributed to the glycation process, which enhanced the interaction between protein and water, thereby promoting the WHC of yogurt (Zhao et al., 2023). Fig. 3G showed that the G' and G'' were the highest when the soy yogurt added with 7S + 11S_G and the G' value was greater than the G" value. Furthermore, the apparent viscosity of yogurt supplemented with 7S + 11S_G decreased with increasing shear rate and had the highest value (Fig. 3H). The results indicating that the initial structure of the gel has been rapidly formed by the addition of 7S + 11S_G, and the gel performance as shear-dilution fluid behavior (Wang et al., 2020). The sensory evaluation results of soy yogurt under different globulin-glucose conjugates were showed in the Table S3, where the texture and overall acceptability obtained best value when the combination was 7S + 11S_G. Based on the above analysis, we could infer that the glycation treatment (7S + 11S_G) could improve the gel properties of yogurt.

Comparison between pure plant-based soy yogurt and thickeners addition soy yogurt

A plant-based gel soy yogurt with superior gel and texture properties was developed using a 7S/11S globulin-glucose conjugate at a 1:3 ratio and a homogenization pressure of 110 MPa. We compared the pure plant-based soy yogurt and thickeners (gelatin and pectin) addition soy yogurt in this study. The results showed that the soy yogurt with globulin possessed greater hardness and stronger water-holding capacity. In addition, it could be seen that the soy yogurts with globulin formed stronger gel through the slopes of G' and G'' (Fig. 4A) and had better apparent viscosity (Fig. 4B). This may be due to the formation of new interactions between protein particles, the rearrangement of protein networks, and the attachment of suspended gel chains to protein networks during gel formation (Yin, Yang, Lai, & Yang, 2021). Moreover, the soy yogurt with globulin exhibited a more homogeneous network structure, while some of the network structures were broken in the gelatin and pectin addition yogurts (Fig. 5) (Xia et al., 2022). This is consistent with the rheological results.

Combination the sensory evaluation presented in Table1, there was significant difference between these three yogurts concerning the texture. This finding implied that the texture of yogurt is a significant

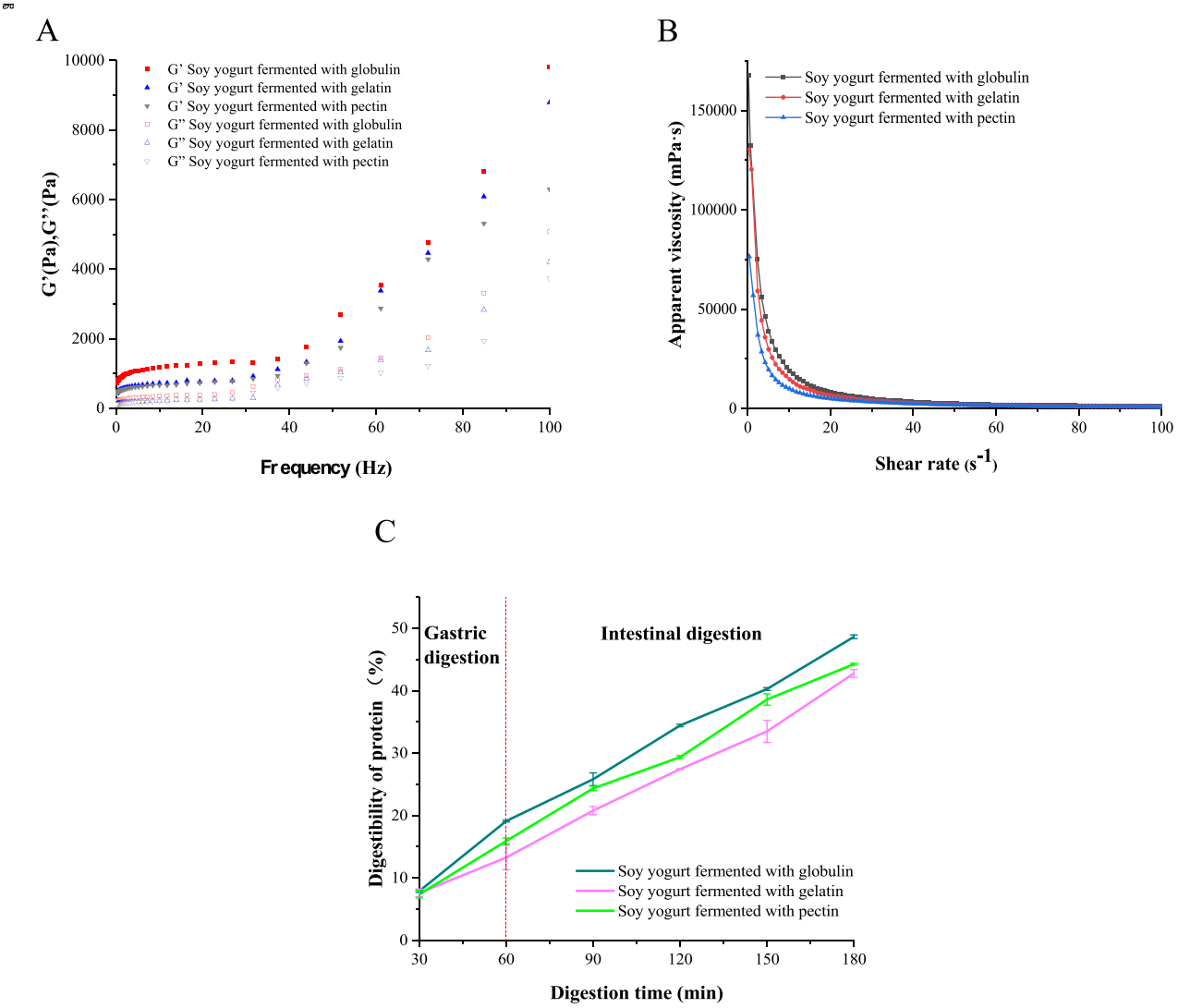

Fig. 4. Differences of three soy yogurt. A) modulus; B) Apparent viscosity (mPa·s); C) Protein digestibility.

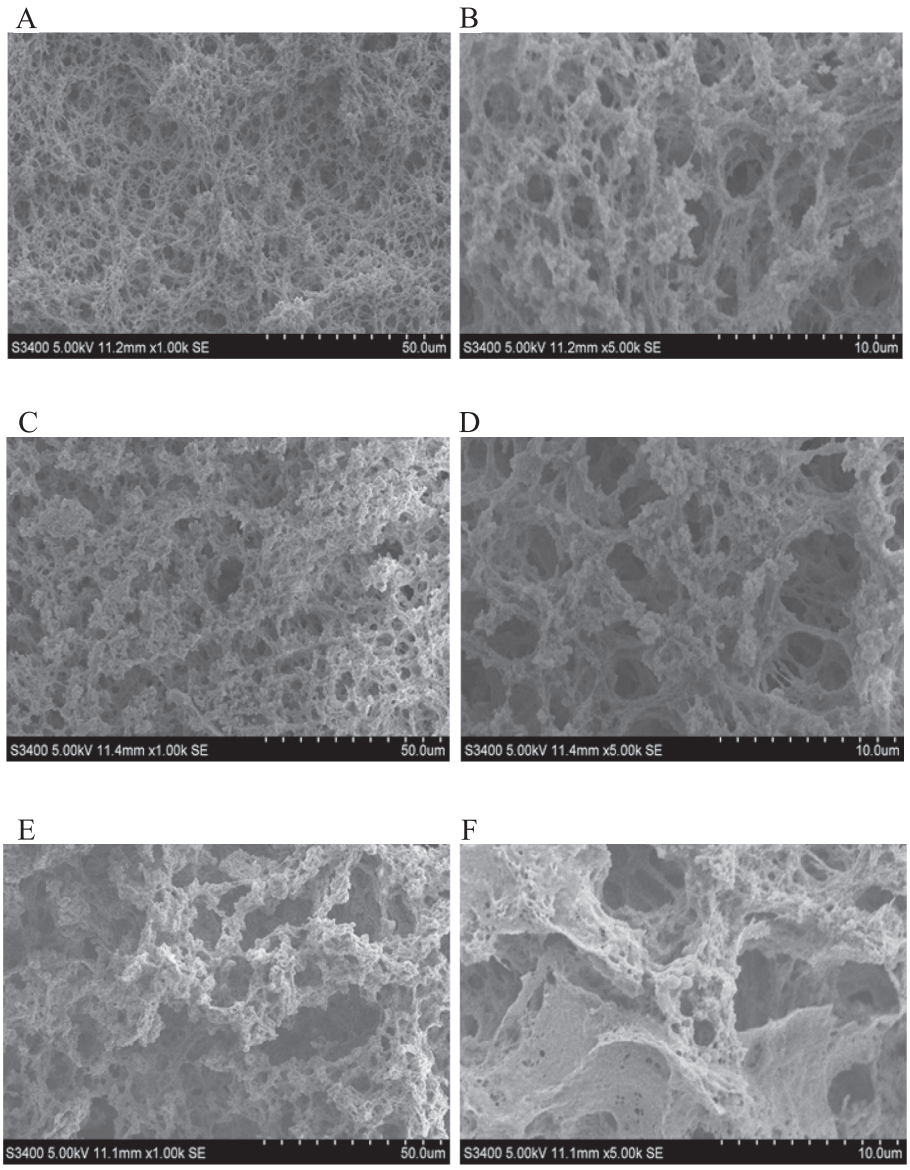

Fig. 5. Observation on microstructure of three soy yogurts A) soy yogurt with globulin-1000 times; B) soy yogurt with globulin −5000 times; C) soy yogurt with gelatin −1000 times; D) soy yogurt with gelatin-5000 times; E) soy yogurt with pectin-1000 times; F) soy yogurt with pectin-5000 times.

Table 1
Sensory evaluation of soy yogurt.

Soy yogurt	Color	Odor	Texture	Flavor	Overall-acceptability
Soy yogurt with addition of 7S and 11S_G	7.23 ± 1.16^{a}	6.27 ± 2.13^{a}	7.84 ± 1.05^{b}	6.87 ± 1.10^{a}	7.56 ± 0.54^{b}
Soy yogurt with addition of gelatin	6.58 ± 1.89^{a}	5.89 ± 2.34^{a}	4.69 ± 1.28^{a}	5.98 ± 1.56^{a}	4.33 ± 1.02^{a}
Soy yogurt with addition of pectin	6.24 ± 2.03^{a}	5.46 ± 2.79^{a}	4.47 ± 1.31^{a}	5.73 ± 1.81^{a}	4.12 ± 1.21^{a}

The results are expressed as mean ± SD (n = 10). Means with different lowercase letters are significant different (*p < 0.05).

factor in determining its acceptability (Greis et al., 2023). Overall-acceptability of soy yogurt with the addition of globulin was the highest, significantly differing from others. Additionally, we conducted further investigation into the digestive properties of these three yogurts. The nutritional value of yogurt was contingent upon the digestion of protein (Capriotti et al., 2015). In-vitro simulation of digestion played a crucial role in investigating the process of yogurt digestion (Rui et al., 2019). As shown in Fig. 4C, the protein in soy yogurt was continuously digested as the digestion time increased. And the result showed that the soy yogurt fermented with globulin had higher protein digestibility in gastric and intestinal juice than others. This may be because the glycation addition increased the in vitro digestibility of soy protein (Liu et al., 2021). Therefore, the consumption of soy yogurt fermented with globulin was found to be more favorable for the digestion and absorption processes in consumers.

This study investigates the scientific feasibility of developing a pure plant-based soy yogurt, which laid the foundation for bringing pure plant-based soy yogurt to market. Firstly, through this study we explored the influence of three factors on the development of a successful soy yogurt with pure plant-based gels, in response to the high cost mentioned, our study provides a reference for breeding experts to develop a specific proportion of globular protein soybeans, which makes the development of low-cost soy yogurts possible. In addition, the glycation reaction conditions were less demanding and have a higher

practical value compared to enzymatic modification and physical modification. In conclusion, the pure plant-based gel soy yogurt developed in this study has theoretical and practical significance.

Conclusion

This study presented a novel and efficient approach for producing soy yogurt made from pure plant-based gel, which exhibited favorable gel characteristics. The addition of soy protein in soy yogurt can affect the quality of soy yogurt, resulting in significant changes in its gel properties, rheological properties, microstructure, molecular structure, and digestibility. When the 7S/11S ratio was 1:3, the homogenization pressure was 110 MPa, and the combination was 7S + 11S_G, the quality of soy yogurt was the best than the soy yogurt added with gelatin or pectin. The sensory evaluation confirmed the compatibility of soy protein as a gel properties improver for soy yogurt. This study success developed a pure plant-based gel soy yogurt without thickeners (gelatin and pectin) addition. Additionally, this study provided innovative perspectives on the advancement and application of plant protein gels.

Ethical statements

Sensory evaluation is an important aspect of many research studies, and we recognize the ethical implications associated with conducting such evaluations. We hereby affirm our commitment to protecting the rights and privacy of all participants involved in this study. Throughout the execution of this research, we have implemented appropriate measures to ensure the well-being and autonomy of each participant.

Firstly, participation in this study is completely voluntary, and individuals have the right to decline participation without any negative consequences or coercion. Secondly, we have provided full disclosure regarding the requirements and potential risks associated with the research. Participants are fully informed about the purpose, methods, and expected outcomes of the study, enabling them to make an informed decision about their involvement. Thirdly, written or verbal consent has been obtained from each participant prior to their engagement in the study. This consent ensures that they understand the nature of the research, including any potential uses of their data, and grants permission for their participation. Furthermore, we assure that participant data will be treated with utmost confidentiality and privacy. Under no circumstances will participant-identifying information be published or shared without explicit consent. All data collected will be securely stored and only used for the purposes of this research study. Lastly, we emphasize that participants have the right to withdraw from the study at any time without penalty or explanation. Their decision to discontinue involvement will be respected, and any data collected up until the point of withdrawal will be handled according to the agreed-upon privacy protocols.

We are committed to upholding these ethical principles and ensuring the protection of participants' rights and privacy throughout the entirety of this research study.

CRediT authorship contribution statement

Hai-Bin Ren: Conceptualization, Methodology, Validation, Formal analysis, Visualization, Investigation, Writing – original draft, Writing – review & editing. **Bao-Long Feng:** Methodology, Validation, Visualization. **Hong-Yao Liu:** Methodology, Investigation. **Yu-Tang Wang:** Conceptualization, Supervision, Project administration, Writing – original draft, Writing – review & editing, Funding acquisition. **Hong-Tai Zhang:** Methodology, Writing – review & editing. **Zhi-Lu Li:** Methodology, Investigation. **Li Meng:** Writing – review & editing. **Jing-Jian Zhang:** Writing – review & editing. **Xiao-Sen Bai:** Methodology, Visualization. **Fei Gao:** Investigation, Methodology, Visualization. **Zhi-Peng Wang:** Methodology, Investigation, Writing – review & editing. **Bo-Wen Luo:** Methodology, Investigation. **Xiao-Lin Chen:** Methodology, Investigation. **Hong-Jie Song:** Methodology, Investigation. **Xin-Xu Yan:** Methodology, Investigation, Resources. **Jin-Yong Zhao:** Methodology, Investigation. **Ying-Hua Zhang:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Key Research and Development Program of China [2021YFD1600104]. We thank the High Performance Computing Platform of Northeast Agricultural University and Department of Modern Education Technology for providing computing resources and technical support. We acknowledge the anonymous referees for their contributions and comments to improve the paper.

Author contributions

Yu-Tang Wang designed the study. Hai-Bin Ren prepared, measured and analyzed the sample, Hong-Yao Liu, Zhi-Lu Li and Xiao-Sen Bai prepared, measured and analyzed the sample, Zhi-Peng Wang prepared, measured and analyzed the sample, Bo-Wen Luo prepared, measured and analyzed the sample, Xiao-Lin Chen prepared, measured and analyzed the sample, Hong-Jie Song prepared, measured and analyzed the sample and Xin-Xu Yan prepared, measured and analyzed the sample. Hai-Bin Ren wrote the manuscript. Bao-Long Feng and Hai-Bin Ren provided statistical advice. Bao-Long Feng, Hai-Bin Ren, Hong-Yao Liu, Hong-Tai Zhang, Jin-Yong Zhao, and Fei Gao designed the sensory evaluation. Ying-Hua Zhang, Jing-Jian Zhang, Li Meng, Hong-Tai Zhang, and Yu-Tang Wang were involved in critically reviewing the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101259.

References

Anuyahong, T., Chusak, C., & Adisakwattana, S. (2020). Incorporation of anthocyanin-rich riceberry rice in yogurts: Effect on physicochemical properties, antioxidant activity and in vitro gastrointestinal digestion. *LWT, 129*, Article 109571. https://doi.org/10.1016/j.lwt.2020.10957

Bi, C., Wang, P., Sun, D., Yan, Z., Liu, Y., Huang, Z., & Gao, F. (2020). Effect of high-pressure homogenization on gelling and rheological properties of soybean protein isolate emulsion gel. *Journal of Food Engineering, 277*(109923). https://doi.org/10.1016/j.jfoodeng.2020.109923

Bielikowicz, K., Kostyra, H., Kostyra, E., Teodorowicz, M., Rigby, N., & Wojtacha, P. (2012). The influence of non-enzymatic glycosylation on physicochemical and biological properties of pea globulin 7S. *Food Research International, 48*(2), 831–838. https://doi.org/10.1016/j.foodres.2012.06.028

Boeck, T., Zannini, E., Sahin, A. W., Bez, J., & Arendt, E. K. (2021). Nutritional and rheological features of lentil protein isolate for yoghurt-like application. *Foods, 10*(16928). https://doi.org/10.3390/foods10081692

Bu, G., Ren, M., Zuo, Y., & Zhao, C. (2022). Functional characteristics and structural properties of soybean protein isolate-maltose conjugates. *Cereal Chemistry, 99*(1), 100–110. https://doi.org/10.1002/cche.10454

Capriotti, A. L., Caruso, G., Cavaliere, C., Samperi, R., Ventura, S., Chiozzi, R. Z., & Lagana, A. (2015). Identification of potential bioactive peptides generated by simulated gastrointestinal digestion of soybean seeds and soy milk proteins. *Journal of Food Composition and Analysis, 44*, 205–213. https://doi.org/10.1016/j.jfca.2015.08.007

Chen, B., Zhao, X., Cai, Y., Jing, X., Zhao, M., Zhao, Q., & Van der Meeren, P. (2023). Incorporation of modified okara-derived insoluble soybean fiber into set-type yogurt: Structural architecture, rheological properties and moisture stability. *Food Hydrocolloids, 137*. https://doi.org/10.1016/j.foodhyd.2022.108413

Du, Y., Zhang, Q., Zhao, X., & Chen, F. (2020). Effect of reverse micelle on physicochemical properties of soybean 7S globulins. *Journal of Food Engineering, 282* (110026). https://doi.org/10.1016/j.jfoodeng.2020.110026

Ferragut, V., Cruz, N. S., Trujillo, A., Guamis, B., & Capellas, M. (2009). Physical characteristics during storage of soy yogurt made from ultra-high pressure homogenized soymilk. *Journal of Food Engineering, 92*(1), 63–69. https://doi.org/10.1016/j.jfoodeng.2008.10.026

Greis, M., Nolden, A. A., Kinchla, A. J., Puputti, S., Seppä, L., & Sandell, M. (2023). What if plant-based yogurts were like dairy yogurts? Texture perception and liking of plant-based yogurts among US and Finnish consumers. *Food Quality and Preference, 107*, Article 104848. https://doi.org/10.1016/j.foodqual.2023.104848

Guo, A., & Xiong, Y. L. (2019). Glucose oxidase promotes gallic acid-myofibrillar protein interaction and thermal gelation. *Food Chemistry, 293*, 529–536. https://doi.org/10.1016/j.foodchem.2019.05.018

Gupta, M. K., Torrico, D. D., Ong, L., Gras, S. L., Dunshea, F. R., & Cottrell, J. J. (2022). Plant and dairy-based yogurts: A comparison of consumer sensory acceptability linked to textural analysis. *Foods, 11*(4633). https://doi.org/10.3390/foods11030463

Jia, Y., Yan, X., Huang, Y., Zhu, H., Qi, B., & Li, Y. (2022). Different interactions driving the binding of soy proteins (7S/11S) and flavonoids (quercetin/rutin): Alterations in the conformational and functional properties of soy proteins. *Food Chemistry, 396* (133685). https://doi.org/10.1016/j.foodchem.2022.133685

Kang, Z., Bai, R., Lu, F., Zhang, T., Gao, Z., Zhao, S., … Ma, H. (2022). Effects of high pressure homogenization on the solubility, foaming, and gel properties of soy 11S globulin. *Food Hydrocolloids, 124*(107261A). https://doi.org/10.1016/j.foodhyd.2021.107261

Kang, Z., Zhang, X., Li, X., Song, Z., Ma, H., Lu, F., … Wang, Z. (2021). The effects of sodium chloride on proteins aggregation, conformation and gel properties of pork myofibrillar protein running head: Relationship aggregation, conformation and gel properties. *Journal of Food Science and Technology-Mysore, 58*(6), 2258–2264. https://doi.org/10.1007/s13197-020-04736-4

Li, R., Wang, X., Liu, J., Cui, Q., Wang, X., Chen, S., & Jiang, L. (2019). Relationship between molecular flexibility and emulsifying properties of soy protein isolate-glucose conjugates. *Journal of Agricultural and Food Chemistry, 67*(14), 4089–4097. https://doi.org/10.1021/acs.jafc.8b06713

Lin, D., Zhang, L., Li, R., Zheng, B., Rea, M. C., & Miao, S. (2019). Effect of plant protein mixtures on the microstructure and rheological properties of myofibrillar protein gel derived from red sea bream (Pagrosomus major). *Food Hydrocolloids, 96*, 537–545. https://doi.org/10.1016/j.foodhyd.2019.05.043

Liu, J., Wan, Y., Ren, L., Li, M., Lv, Y., Guo, S., & Waqar, K. (2021). Physical-chemical properties and in vitro digestibility of phosphorylated and glycosylated soy protein isolate. *Lwt-Food Science and Technology, 152*(112380). https://doi.org/10.1016/j.lwt.2021.112380

Liu, L., Huang, Y., Zhang, X., Zeng, J., Zou, J., Zhang, L., & Gong, P. (2023). Texture analysis and physicochemical characteristics of fermented soymilk gel by different lactic acid bacteria. *Food Hydrocolloids, 136*. https://doi.org/10.1016/j.foodhyd.2022.108252

Lu, Q., Zuo, L., Wu, Z., Li, X., Tong, P., Wu, Y., … Yang, A. (2022). Characterization of the protein structure of soymilk fermented by Lactobacillus and evaluation of its potential allergenicity based on the sensitized-cell model. *Food Chemistry, 366*. https://doi.org/10.1016/j.foodchem.2021.130569

Lu, Y., Zhang, Y., Yuan, F., Gao, Y., & Mao, L. (2021). Emulsion gels with different proteins at the interface: Structures and delivery functionality. *Food Hydrocolloids, 116*(106637). https://doi.org/10.1016/j.foodhyd.2021.106637

Ma, W., Wang, J., Wu, D., Chen, H., Wu, C., & Du, M. (2020). The mechanism of improved thermal stability of protein-enriched O/W emulsions by soy protein particles. *Food & Function, 11*(2), 1385–1396. https://doi.org/10.1039/c9fo02270h

Montemurro, M., Pontonio, E., Coda, R., & Rizzello, C. G. (2021). Plant-based alternatives to yogurt: State-of-the-art and perspectives of new biotechnological challenges. *Foods, 10*(3162). https://doi.org/10.3390/foods10020316

Nakayama, M., Shimatani, K., Ozawa, T., Shigemune, N., Tsugukuni, T., Tomiyama, D., … Miyamoto, T. (2013). A study of the antibacterial mechanism of catechins: Isolation and identification of Escherichia coli cell surface proteins that interact with epigallocatechin gallate. *Food Control, 33*(2), 433–439. https://doi.org/10.1016/j.foodcont.2013.03.016

Pang, Z., Safdar, B., Wang, Y., Sun, M., & Liu, X. (2021). Improvement of tribo-rheological properties of acid soymilk gels by reinforcement of 7S or 11S proteins. *Food Hydrocolloids, 110*(106173). https://doi.org/10.1016/j.foodhyd.2020.106173

Ramdath, D. D., Padhi, E. M. T., Sarfaraz, S., Renwick, S., & Duncan, A. M. (2017). Beyond the cholesterol-lowering effect of soy protein: A review of the effects of dietary soy and its constituents on risk factors for cardiovascular disease. *Nutrients, 9* (3244). https://doi.org/10.3390/nu9040324

Rui, X., Zhang, Q., Huang, J., Li, W., Chen, X., Jiang, M., & Dong, M. (2019). Does lactic fermentation influence soy yogurt protein digestibility: A comparative study between soymilk and soy yogurt at different pH. *Journal of the Science of Food and Agriculture, 99*(2), 861–867. https://doi.org/10.1002/jsfa.9256

Saavedra Isusi, G. I., Paz Puga, D., & van der Schaaf, U. S. (2022). Texturing fermented emulsion gels from soy protein: Influence of the emulsifying agent-soy protein vs. pectin microgels-on gel microstructure, rheology and tribology. *Foods, 11*(2943). https://doi.org/10.3390/foods11030294

Valdez-Hurtado, S., Lopez-Bermudez, L. S., Higuera-Barraza, O. A., Del Toro-Sanchez, C. L., Ruiz-Cruz, S., Suarez-Jimenez, M. G., & Marquez-Rios, E. (2019). Effect of ultrasonication time on the functional properties of giant squid (Dosidicus gigas) mantle protein concentrate. *Food Bioscience, 27*, 1–5. https://doi.org/10.1016/j.fbio.2018.11.003

Wang, J., Na, X., Navicha, W. B., Wen, C., Ma, W., Xu, X., … Du, M. (2020). Concentration-dependent improvement of gelling ability of soy proteins by preheating or ultrasound treatment. *Lwt-Food Science and Technology, 134*(110170). https://doi.org/10.1016/j.lwt.2020.110170

Wang, R., & Guo, S. (2016). Effects of endogenous small molecular compounds on the rheological properties, texture and microstructure of soymilk coagulum: Removal of phytate using ultrafiltration. *Food Chemistry, 211*, 521–529. https://doi.org/10.1016/j.foodchem.2016.05.086

Wu, C., Hua, Y., Chen, Y., Kong, X., & Zhang, C. (2017). Effect of temperature, ionic strength and 11S ratio on the rheological properties of heat-induced soy protein gels in relation to network proteins content and aggregates size. *Food Hydrocolloids, 66*, 389–395. https://doi.org/10.1016/j.foodhyd.2016.12.007

Xia, W., Zhu, L., Delahaije, R. J. B. M., Cheng, Z., Zhou, X., & Sagis, L. M. C. (2022). Acid-induced gels from soy and whey protein thermally-induced mixed aggregates: Rheology and microstructure. *Food Hydrocolloids, 125*, Article 107376. https://doi.org/10.1016/j.foodhyd.2021.107376

Xu, J., Han, D., Chen, Z., Li, M., & Jin, H. (2018). Effect of glucose glycosylation following limited enzymatic hydrolysis on functional and conformational properties of black bean protein isolate. *European Food Research and Technology, 244*(6), 1111–1120. https://doi.org/10.1007/s00217-018-3032-5

Yang, X., Ke, C., & Li, L. (2021). Physicochemical, rheological and digestive characteristics of soy protein isolate gel induced by lactic acid bacteria. *Journal of Food Engineering, 292*. https://doi.org/10.1016/j.jfoodeng.2020.110243

Yin, M., Yang, D., Lai, S., & Yang, H. (2021). Rheological properties of xanthan-modified fish gelatin and its potential to replace mammalian gelatin in low-fat stirred yogurt. *Lwt-Food Science and Technology, 147*(111643). https://doi.org/10.1016/j.lwt.2021.111643

Zhang, S. B., & Lu, Q. Y. (2015). Characterizing the structural and surface properties of proteins isolated before and after enzymatic demulsification of the aqueous extract emulsion of peanut seeds. *Food Hydrocolloids, 47*, 51–60. https://doi.org/10.1016/j.foodhyd.2015.01.007

Zhao, C., Chu, Z., Mao, Y., Xu, Y., Fei, P., Zhang, H., … Liu, J. (2023). Structural characteristics and acid-induced emulsion gel properties of heated soy protein isolate-soy oligosaccharide glycation conjugates. *Food Hydrocolloids, 137*. https://doi.org/10.1016/j.foodhyd.2022.108408

Zhao, J., Su, G., Zhao, M., & Sun, W. (2019). Physicochemical changes and in vitro gastric digestion of modified soybean protein induced by lipoxygenase catalyzed linoleic acid oxidation. *Journal of Agricultural and Food Chemistry, 67*(50), 13978–13985. https://doi.org/10.1021/acs.jafc.9b05843

Zheng, L., Wang, Z., Kong, Y., Ma, Z., Wu, C., Regenstein, J. M., … Li, Y. (2021). Different commercial soy protein isolates and the characteristics of Chiba tofu. *Food Hydrocolloids, 110*(106115). https://doi.org/10.1016/j.foodhyd.2020.106115

Zhou, Y., Li, X., Hua, Y., Kong, X., Zhang, C., Chen, Y., & Wang, S. (2019). The absence of lipoxygenase and 7S globulin of soybeans and heating temperatures on the properties of soymilks and soy yogurts. *Lwt-Food Science and Technology, 115* (108431). https://doi.org/10.1016/j.lwt.2019.108431