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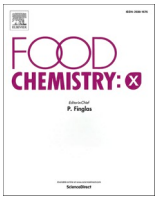

Structural, functional properties of protein and characteristics of tofu from small-seeded soybeans grown in the Loess Plateau of China

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ABSTRACT

The structural, functional properties of protein isolated from small-seeded soybeans were investigated and characteristics of tofu were studied. Small-seeded soybean protein had obvious α', α, β, acidic and basic subunits bands and two endothermic peaks (76.02–76.63℃ and 91.94–94.25℃). Small-seeded black soybean protein isolates (SBSPI) had more β-sheet (31.90–33.54%) structure, while small-seeded yellow soybean protein isolates (SYSPI) had more α-helix (18.89–20.72%) structure. SYSPI had higher fluorescence intensity (839.10–847.80) than SBSPI (482.70–565.10). SBSPI exhibited higher surface hydrophobicity (939.51–1252.75) and water absorption capacity (8.07–8.50 g/g). Tofu made from small-seeded yellow soybeans had higher yield (549.46–560.23 g/100 g soybean) and was brighter (L*, 74.61–77.48) and more yellowish (b*, 14.83–14.95) in color. Tofu made from Fugu small-seeded black soybean (FGSBS) had the highest hardness (178.52 g), adhesiveness (-25.77 g.sec), chewiness (87.45 g) and resilience (0.26), signifying a more compact structure.

1. Introduction

Soybean (*Glycine max*) is an annual dicotyledonous legume that originated in China, and it has become an essential part of the Chinese national diet for thousands of years (Ali, Tian & Wang, 2021). Being an essential source of protein in Asian countries, soybeans have been utilized in various forms, such as tofu, miso, natto, abura-age and soymilk (Nishinari, Fang, Guo & Phillips, 2014), mainly because of the excellent functional and nutritional properties of soybean protein isolates (SPIs) (Zhu et al., 2020). For instance, SPI contains all the essential amino acids with a good balance and is rich in physiologically beneficial components which are beneficial in reducing the risk of hyperlipidemia and cardiovascular diseases, also, SPI has exceptional processing abilities such as emulsifying properties and water/oil holding capacity (Nishinari et al., 2014). SPI can be utilized as an emulsifier to create oil-in-water emulsions with good diffusion and/or adsorption capabilities to stabilize the oil droplet surfaces due to its amphiphilic nature (Tang, 2017). Compared with animal protein, SPI has a lower cost, higher nutritional value and richer functional ingredients (Yan, Xu, Zhang & Li, 2021), which has attracted significant attention.

Soybeans can be classified by their 100-seed weight into extra-small grains of <10 g, small grains of 10–15 g, medium grains of 15–20 g, large grains of 20–25 g and extra-large grains of > 25 g. Small-seeded soybeans selected for the present research are grown in the Loess Plateau region, a part of China's dry farming area with a chronically dry climate and severe soil desertification. Growing in such an environment makes small-seeded soybeans highly resistant and adaptable. As a local characteristic legume resource, small-seeded soybeans primarily comprise small-seeded black soybeans (SBS) and small-seeded yellow soybeans (SYS), both of which have the widest distribution and highest yield. Some studies have pointed out that the phytochemical compositions of common beans may be influenced by their growing environment, genotype and interactions (Karaman, Bekiroglu, Kaplan, Çiftci, Yürürdurmaz & Sagdic, 2022). However, little research has been conducted on small-seeded soybeans, besides, as the main nutritional composition, the structural and functional properties of their proteins are still unclear, which severely limits the processing, application and consumption of small-seeded soybeans. Therefore, studies on the properties of protein

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isolated from small-seeded soybeans are necessary.

Tofu is a traditional soybean product that has existed in China since the western Han Dynasty (from 202 BC to 8 AD) (Ali et al. 2021). Due to its abundant beneficial lipids and bioactive compounds (especially isoflavones, saponins and phytosterols), tofu is classified as one of the greatest sources of plant-based protein (Ali et al. 2021) and is increasingly consumed in people's life. Traditional tofu processing mainly consists of the soaking, draining and grinding of soybean seeds, heating and filtering of soybean slurry, the addition of coagulants, pressing and others (Zhang et al. 2018). Tofu is a highly hydrated gel-type food, so that the formation of tofu is attributed to the gelation properties of soybean protein (Zhang et al. 2018). In general, the yield and quality of tofu can be affected by various factors, such as the composition of soybean seeds (protein, lipid, sugar, phytic acid and other chemical components), coagulant type and concentration, interactions between oil/protein and protein, pH and other factors (Ali et al. 2021). Amongst, the protein composition, which is mainly influenced by the variety, growing environment, and storage condition, is considered the most important factor for tofu production (Zhang et al. 2018). Therefore, we prepared small-seeded soybean tofu and investigated the relationship between protein properties and tofu quality.

Accordingly, the structural, functional properties of protein isolated from small-seeded soybeans were investigated and characteristics of tofu were studied. Five small-seeded soybean varieties were used, including three small-seeded black soybean (SBS) varieties and two small-seeded yellow soybean (SYS) varieties. Fen soybean 78 (FS) is a classical medium soybean and was used as the control. The differences in protein properties between SBS, SYS and FS were studied, and the relationship between protein properties and tofu characteristics was discussed. This work aims to reveal the characteristics and advantages of the protein isolated from small-seeded soybeans, and thus provide basic data and theoretic guidance for their deep processing and efficient utilization.

2. Materials and methods

2.1. Materials

Six soybean cultivars, namely, Fugu small-seeded black soybean (FGSBS), Dingbian small-seeded black soybean (DBSBS), Zizhou small-seeded black soybean (ZZSBS), Fugu small-seeded yellow soybean (FGSYS), Shenmu small-seeded yellow soybean (SMSYS) and Fen soybean 78 (FS, a classical medium soybean) were provided by the Shenmu Agricultural Technology Promotion Center (Shenmu, Shaanxi, China). The grains were ground, sieved through an 80 mesh and defatted thrice using petroleum ether (1:10, w/v) at 25℃ for 4 h each time to produce the defatted soybean powder. All chemicals used were of analytical grade.

2.2. Preparation of SPIs

SPIs were prepared using the alkaline extraction and isoelectric precipitation method reported by Zhu et al. (2020). The resulting SPIs from FGSBS, DBSBS, ZZSBS, FGSYS, SMSYS and FS were designated as FGSBSPI, DBSBSPI, ZZSBSPI, FGSYSPI, SMSYSPI and FSPI. The SPIs of six soybean varieties had over 90% protein content as measured by the Kjeldahl method.

2.3. Structural properties of SPIs

2.3.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of each SPI was performed according to the method of Shevkani, Singh, Kaur and Rana (2015) using a 12% separating gel and 5% stacking gel. Each SPI was dissolved in a sample buffer containing the stacking gel (2 mL), glycerin (2 mL), 20% SDS (2 mL), 0.1% bromophenol blue (1 mL), β-mercaptoethanol (1 mL) and distilled water (2 mL). After 5 min of boiling in a water bath, the mixture was cooled to room temperature. Then, 15 μL of the solution was loaded into each well. Electrophoresis was carried out at 60 V in the stacking gel for 50 min and then at 120 V in the separating gel for 120 min. After electrophoresis, the gel was stained with Coomassie Brilliant Blue (R-250) for 2 h and then destained (using glacial acetic acid: methanol: water = 3:2:35, v/v/v). The electrophoretic pattern was captured using a Gel Doc XR + system (Bio-Rad, Hercules, California, USA).

2.3.2. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra and secondary structures of the SPIs were measured using an FTIR spectrometer (Vertex 70, Bruker Co., Karlsruhe, Germany) according to the method of Shevkani et al. (2015). The spectra were recorded in the range of 400–4000 cm^{-1}.

2.3.3. Thermal properties

Differential scanning calorimeter (DSC, Q2000, Waters Co., Milford, Massachusetts, USA) was used to determine the thermal properties of the SPIs following modifications of the method of Shevkani et al. (2015). Each SPI (3 mg) was loaded onto aluminum pans with distilled water (9 μL). The sample pans were heated from 30 to 130℃ at a rate of 10℃/min. The Universal Analysis 2000 software (V3.8B, TA Inc., Newcastle, Delaware, USA) was used to calculate the onset temperature (T_o), denaturation temperature (T_d) and enthalpy of denaturation (ΔH).

2.3.4. Intrinsic fluorescence spectroscopy

The fluorescence spectrum was determined according to the method of Ma et al. (2018) using a fluorescence spectrophotometer (LS55, PE Co., Waltham, Massachusetts, USA).

2.3.5. Surface hydrophobicity (H_0)

H_0 was determined using an ANS-hydrophobic probe following modifications of the method of Zhu et al. (2020). Each SPI was dissolved in 0.01 M PBS (pH 7.0) to afford 0.2 mg/mL of the protein solution. After magnetic stirring for 1 h, the solution was centrifuged at 10610 × g (10000 r/min) for 30 min and diluted to different concentrations (0.0625, 0.125, 0.25, 0.5, 1, and 2 mg/mL) using PBS. The resulting solutions (5 mL) of varying concentrations were mixed with 8 mM ANS (25 μL). The fluorescence intensity (FI) of each solution was determined at 390 nm (excitation) and 470 nm (emission) with a slit width of 5 nm using a fluorescence spectrophotometer (LS55, PE Co., Waltham, Massachusetts, USA) after standing for 15 min in the dark.

2.4. Functional properties of SPIs

2.4.1. Protein solubility (PS)

The bovine serum albumin (BSA) standard curve was used to calculate the soluble protein content of the supernatant by the Lowry method reported by Yan et al. (2021). Each SPI was dispersed in distilled water to afford 10 mg/mL of the protein solution. Then, the solutions were magnetically stirred for 1 h and centrifuged at 1532 × g (3800 r/min) for 30 min. The absorbance of the supernatant was measured at 595 nm. PS was defined as the ratio of the protein content in the supernatant to the total protein content (Ma et al. 2018).

2.4.2. Water absorption capacity (WAC) and oil absorption capacity (OAC)

The WAC and OAC of SPIs were measured following the method of Ma et al. (2018). WAC (OAC) was expressed as the weight of water/oil adsorbed per gram of SPI.

2.4.3. Emulsifying properties

The emulsifying activity index (EAI) and emulsion stability index (ESI) values were measured using the method described by Du et al. (2018) with slight modifications. Each SPI solution (1%, w/v) prepared

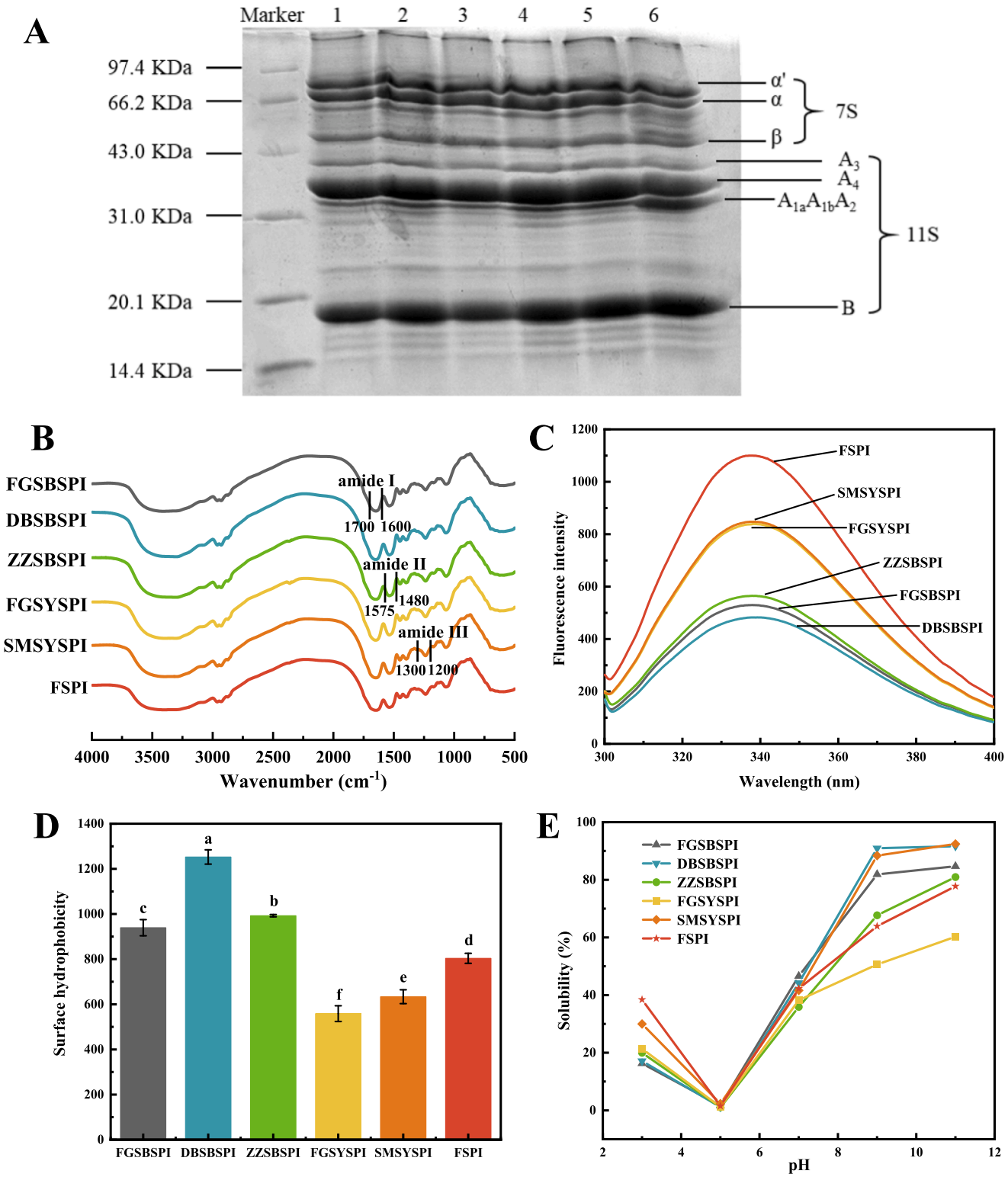

Fig. 1. (A) SDS-PAGE. Lanes 1–6 represent Fugu small-seeded black soybean protein isolate (FGSBSPI), Dingbian small-seeded black soybean protein isolate (DBSBSPI), Zizhou small-seeded black soybean protein isolate (ZZSBSPI), Fugu small-seeded yellow soybean protein isolate (FGSYSPI), Shenmu small-seeded yellow soybean protein isolate (SMSYSPI) and Fen soybean protein isolate (FSPI), respectively. (B) FTIR spectra of SPIs. (C) Intrinsic fluorescence spectra of SPIs. (D) Surface hydrophobicity of SPIs. (E) Solubility of SPIs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by dissolving SPI in 0.1 M PBS (pH 7.0) was blended with soy oil in a ratio of 3:1 (v/v) and homogenized at 9860 × g (10000 r/min) for 1 min. Then, 50 μL of the emulsion was diluted with 5 mL of 0.1% SDS solution. The absorbance of the diluted solution was recorded at 500 nm using 0.1% SDS solution as the blank. The EAI and ESI values were calculated as follows:

$$EAI\ \left(m^2/g\right) = \frac{2 \times 2.302 \times A_0 \times N}{C \times 0.25 \times 10^4}$$

$$ESI\ (min) = 10 \times \frac{A_0}{A_0 - A_{10}}$$

where N is the dilution factor; C is the mass fraction of SPI (g/ml); A_0 and A_{10} are the absorbance at 0 min and 10 min, respectively.

2.5. Preparation of tofu

For each variety, 100 g of soybeans were washed and soaked overnight in distilled water (1:3, w/v) at room temperature (25℃±1℃). The soaked soybeans were then drained and ground with distilled water (dry soybeans: distilled water = 1:8, w/v, excluding the absorbed water), and the slurry was filtered through a 120 mesh to obtain soymilk. The soymilk was boiled for 20 min to render the SPI fully denatured and then cooled to 80℃. Further, 0.3% glucono-δ-lactone (GDL) coagulant (of soymilk quality) was added to the soymilk. The coagulation was subsequently kept in a water bath at 80℃ for 20 min. After cooling to room temperature, the tofu was stored in a refrigerator at 4℃ for later use.

2.6. Characteristics of tofu

2.6.1. Determination of tofu yield

The tofu samples were weighed after being kept at room temperature (25℃±1℃) for 10 min, and the tofu yields were calculated as the weight of the tofu obtained from 100 g of soybeans.

2.6.2. Determination of the water holding capacity (WHC) of tofu

The WHC of the tofu samples was determined following the method of Ullah et al. (2019) with some changes. For each sample, 4 g of tofu was placed into a 50 mL centrifuge tube with defatted cotton at the bottom and then centrifuged at 152 × g (1200 r/min) for 8 min. The post-centrifugation sample was weighed and then dried at 105℃ to a constant weight. The WHC was calculated as follows:

$$WHC\ \left(\%\right) = \frac{w_1 - w_2}{w_1} \times 100\%$$

where w_1 represents the weight of the sample after centrifugation, g; w_2 represents the constant weight of the sample after drying, g.

2.6.3. Color properties of tofu

The color properties of tofu were measured using a Ci-7600 chromameter (X•rite Color Technology Co., Ltd, Shanghai, China). The tofu samples were dissected horizontally, and four different positions were selected on the cross-section. Each position was measured three times. The results were recorded as L*, a* and b* values.

2.6.4. Texture analysis of tofu

Following the method of Ullah et al. (2019) with some modifications, the texture profile analysis (TPA) of tofu was conducted using a texture analyzer (TA. XT PLUS/50, Stable Micro Systems, Godalming, Surrey, UK). The sample (2 × 2 × 2 cm) was collected from the center of the tofu and a P/0.5R probe was used. Trigger force and test distance were 5 g and 10 mm, respectively. The pre-test and post-test speeds were both 1.0 mm/s, the test speed was 0.5 mm/s. The hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience of tofu were analyzed.

2.6.5. Gelation and rheological properties of tofu

The gelling behavior of soymilk was determined using a rheometer (DHR-1, Waters Co., Milford, Massachusetts, USA) according to the method of Lee and Kuo (2011) with a few changes. The temperature sweep was performed from 25 to 80℃ at a heating rate of 5℃/min. The time sweep was then carried out at 80℃ for 30 min. The storage modulus (G') and loss modulus (G'') were recorded. The rheological property of tofu was measured based on the procedure of Lin, Lu, Hsieh & Kuo (2016). The frequency sweep of tofu was conducted from 0.01 to 10 Hz at 4℃.

2.6.6. Microstructure of tofu

The microstructures of tofu were observed using a scanning electron microscope (SEM, Nano SEM-450, FEI Co., Hillsboro, Oregon, USA) according to the method of Zuo, Chen, Shi, Wang and Guo (2016) with some modifications. Amongst, the tofu was dehydrated stepwise by 50%, 70%, 80%, 90%, 95% and 100% ethanol for 15 min each time.

2.7. Statistical analysis

All measurements were performed in triplicate, and the results were presented as mean ± standard deviation (SD). Significant differences of all experimental data were evaluated using SPSS 22.0 software (IBM, Armonk, New York, USA) by analysis of variance (ANOVA) with Duncan's multiple test ($p < 0.05$). All figures were produced using Origin 2022 software (Origin Lab Inc., Northampton, Massachusetts, USA).

3. Results and discussion

3.1. SDS-PAGE

Typically, SDS-PAGE is employed to examine the molecular weight distribution of protein subunits (Li et al., 2019). Glycinin (11S) and β-conglycinin (7S) make up the majority of SPI, and 11S globulin comprises acidic (~35 kDa) and basic (~20 kDa) subunits whereas 7S globulin contains α' (~72 kDa), α (~68 kDa) and β (~52 kDa) subunits (Ge, Sun, Sun, Zhang & Fang, 2022). The SDS-PAGE profiles of SBSPI (Lane 1–3), SYSPI (Lane 4–5) and FSPI (Lane 6) were shown in Fig. 1A. The SPIs of all soybean varieties showed similar molecular weight profiles with obvious α', α, β, acidic and basic subunits bands, whereas the content of each subunit is differently indicated by the width and depth of the bands. The acidic (especially A_4) and basic subunits bands of ZZSBSPI (Fig. 1A, Lane 3) were narrower and shallower than those of other varieties, indicating a lower 11S content and 11S/7S ratio. By contrast, FSPI (Fig. 1A, Lane 6) had higher 11S content with wider bands of acidic and basic subunits. The quality of tofu gels or tofu derivatives is highly correlated with both 11S and 7S globulins, in particular, the hardness of tofu is considerably influenced by the content of 11S, whereas its springiness is generally affected by the content of 7S (Zhang et al., 2018). In general, higher 11S content contributes to higher hardness of gels (Zheng, Regenstein, Teng & Li, 2020). Thus, ZZSBS tofu displayed the lowest hardness of 61.80 g (Table 4) owing to the lower 11S content in its SPI. This phenomenon may be attributed to the fact that 11S had a superior ability to form heat-induced gels with better texture properties than 7S, since under conditions of heating and coagulation, 11S gel can be produced in the formation of a stable three-dimensional network structure through disulfide bonds and electrostatic interactions, conversely, the formation of 7S gel is completed only by hydrogen bonding (Zhang et al., 2018). Besides, the protein subunit component of SPI is also important in determining the water holding capacity (WHC) of tofu. Yang and James (2013) indicated that soybean varieties with the absence of $11SA_4$ and low 11S/7S ratios in SPIs produced tofu with higher WHC, which is consistent with the highest WHC (80.34%) of ZZSBS tofu in our study (Table 3). On the contrary, FSPI with higher 11S content exhibited the lowest WHC (76.59%) of tofu (Table 3).

Table 1
Relative content of the secondary structure, WAC, OAC, EAI and ESI of SPIs from different soybean varieties.

Samples	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)	WAC (g/g)	OAC (g/g)	EAI (m^2/g)	ESI (min)
FGSBSPI	16.46 ± 0.30^{d}	31.90 ± 0.62^{bc}	27.47 ± 0.37^{e}	24.17 ± 0.76^{a}	8.31 ± 0.15^{a}	2.57 ± 0.08^{a}	38.57 ± 0.83^{a}	40.46 ± 0.90^{a}
DBSBSPI	13.42 ± 0.28^{f}	33.54 ± 0.71^{a}	29.71 ± 0.93^{c}	23.33 ± 0.32^{a}	8.07 ± 0.14^{b}	1.77 ± 0.38^{c}	32.36 ± 0.55^{bc}	38.94 ± 1.03^{ab}
ZZSBSPI	14.73 ± 0.89^{e}	32.53 ± 0.46^{ab}	31.96 ± 0.69^{a}	20.79 ± 1.66^{b}	8.50 ± 0.14^{a}	1.65 ± 0.11^{c}	31.79 ± 1.91^{bc}	30.81 ± 1.09^{d}
FGSYSPI	20.72 ± 0.42^{a}	29.86 ± 0.92^{d}	30.04 ± 0.18^{bc}	19.38 ± 1.06^{b}	7.11 ± 0.10^{d}	1.68 ± 0.08^{c}	32.62 ± 0.35^{b}	38.23 ± 0.39^{b}
SMSYSPI	18.89 ± 0.72^{b}	30.52 ± 1.26 ^{cd}	30.80 ± 0.21^{b}	19.79 ± 1.52^{b}	7.62 ± 0.07^{c}	1.86 ± 0.08^{bc}	30.52 ± 0.67 ^{cd}	28.43 ± 0.98^{e}
FSPI	17.58 ± 0.56^{c}	31.03 ± 1.01^{bcd}	28.65 ± 0.55^{d}	22.74 ± 0.50^{a}	5.92 ± 0.02^{e}	2.13 ± 0.01^{b}	28.97 ± 1.07^{d}	33.26 ± 1.10^{c}

Results are mean ± standard deviations of triplicate analysis. Values with different letters in the same column are significantly different ($p < 0.05$).

Table 2
Thermal properties of SPIs from different soybean varieties.

Protein samples	Peak I T_{o1} (℃)	T_{d1} (℃)	ΔH_1 (J/g)	Peak II T_{o2} (℃)	T_{d2} (℃)	ΔH_2 (J/g)
FGSBSPI	71.48 ± 0.50^{a}	76.02 ± 0.26^{b}	0.92 ± 0.07^{c}	86.77 ± 0.98^{ab}	93.32 ± 0.61^{ab}	5.74 ± 0.18^{bc}
DBSBSPI	71.33 ± 0.78^{a}	76.06 ± 0.38^{ab}	1.13 ± 0.25^{c}	85.64 ± 0.91^{b}	92.34 ± 0.15^{bc}	5.23 ± 0.29^{c}
ZZSBSPI	71.74 ± 0.71^{a}	76.11 ± 0.18^{ab}	0.95 ± 0.07^{c}	87.12 ± 2.39^{ab}	91.94 ± 1.23^{c}	5.35 ± 0.36^{bc}
FGSYSPI	71.80 ± 0.83^{a}	76.63 ± 0.55^{a}	1.42 ± 0.07^{b}	88.09 ± 0.26^{a}	93.95 ± 0.07^{a}	5.62 ± 0.50^{bc}
SMSYSPI	71.53 ± 0.19^{a}	76.07 ± 0.05^{ab}	1.18 ± 0.08^{c}	88.27 ± 0.09^{a}	94.25 ± 0.12^{a}	6.37 ± 0.15^{a}
FSPI	71.52 ± 0.37^{a}	76.30 ± 0.08^{ab}	1.72 ± 0.16^{a}	87.48 ± 0.30^{ab}	93.64 ± 0.27^{a}	5.90 ± 0.26^{ab}

Results are mean ± standard deviations of triplicate analysis. Values with different letters in the same column are significantly different ($p < 0.05$). T_o, onset temperature; T_d, denaturation temperature; ΔH, enthalpy of denaturation.

3.2. FTIR spectrum analysis

As shown in Fig. 1B, the infrared (IR) spectra of SPIs from different soybean varieties possessed obvious characteristic absorption peaks in the amide I (1600–1700 cm^{-1}, C═O stretching), amide II (1480–1575 cm^{-1}, N—H bending) and amide III (1300–1200 cm^{-1}, C—N stretching and N—H deformation) regions (Yu et al., 2018; Lu, Liu, Lee, Chan, Lee & Yang, 2023). The IR spectra of small-seeded black soybean protein isolates (SBSPI), small-seeded yellow soybean protein isolates (SYSPI) and FSPI were very similar, thereby indicating no differences in their functional groups. The amide I region (1600–1700 cm^{-1}) is most sensitive to protein secondary structures (Sow, Chong, Liao & Yang, 2018; Lu, Lee & Yang, 2022), including the α-helix (1648–1664 cm^{-1}), β-sheet (1615–1637 cm^{-1} and 1682–1700 cm^{-1}), β-turn (1664–1681 cm^{-1}) and random coil (1637–1648 cm^{-1}) (Li et al., 2019). Table 1 shows the relative content of each secondary structure unit of SPIs from different soybean varieties in the amide I band after curve fitting and area calculation. The mean relative proportions of the secondary structures of SBSPI and SYSPI were as follows: α-helix, 14.78% (SBSPI) and 19.81% (SYSPI); β-sheet, 32.66% (SBSPI) and 30.19% (SYSPI); β-turn, 29.71% (SBSPI) and 30.42% (SYSPI); and random coil, 22.76% (SBSPI) and 19.59% (SYSPI). These data suggest that the secondary structure of SBSPI was mainly β-sheet, while the secondary structure of SYSPI was mainly β-turn. Furthermore, SBSPI had relatively more β-sheet and random coil structures than FSPI and SYSPI, whereas SYSPI had relatively more α-helix and β-turn structures than FSPI and SBSPI. These outcomes may be related to the genetic characteristics of different soybean varieties.

The relative content of different secondary structures is correlated with the texture properties of protein gels (Zheng et al., 2021). DBSBSPI with the highest content of β-sheet structure (33.54%) exhibited a relatively lower hardness (92.38 g) of DBSBS tofu, while FGSYSPI with the lowest content of β-sheet structure (29.86%) showed a relatively higher hardness (129.50 g) of FGSYS tofu. It seemed that the relation between the content of secondary structures and tofu hardness was not clear in our study. Others (Gao, Kang, Zhang, Li, & Zhou, 2015; Zheng et al., 2021) showed a positive correlation between the β-sheet content and the hardness of different gel systems. This variation may be related to the soybean cultivars, the process of tofu production, coagulant type and concentration, oil-protein interactions, protein–protein interactions and other factors (Ali et al. 2021).

3.3. Thermal properties

The conformational and structural changes in proteins are reflected by DSC (Ma et al., 2018). T_d evaluates the thermal stability of proteins, and for globular proteins, a higher T_d value is typically indicative of greater thermal stability (Karaman et al., 2022). ΔH reflects the proportion of undenatured protein and the extent of the ordered structure (Shevkani et al., 2015). Thermal properties can also reflect the disruption of hydrogen bonds that maintain the tertiary conformation of proteins (Tang & Sun, 2011). The thermal properties of SPIs from different soybean varieties are presented in Table 2. Two endothermic peaks were observed and may correspond to the denaturation of 7S (T_{d1}) and 11S (T_{d2}) globulins (Wani, Sogi, Shivhare & Gill, 2015). Peak I for the SPIs had T_{o1}, T_{d1} and ΔH_1 in the ranges of 71.33–71.80℃, 76.02–76.63℃ and 0.92–1.72 J/g, respectively. Peak II had T_{o2}, T_{d2} and ΔH_2 in the ranges of 85.64–88.27℃, 91.94–94.25℃ and 5.23–6.37 J/g, respectively. Our outcome was in agreement with those of Zhang, Wang, Li, Guo and Lv (2022), who found two endothermic peaks in SPI with T_{d1} and T_{d2} ranging from 72–76℃ and 91–93℃, respectively. SYSPI exhibited higher T_d (T_{d1}: mean value 76.65℃; T_{d2}: mean value 94.10℃) than FSPI (T_{d1}: 76.30℃; T_{d2}: 93.64℃) and SBSPI (T_{d1}: mean value 76.06℃; T_{d2}: mean value 92.53℃), indicating a higher thermal stability. T_d is related to the amino acid composition, protein structure and conformation involved (Tang et al., 2011). DBSBSPI and ZZSBSPI with higher β-sheet content exhibited relatively higher T_{d1} and lower T_{d2}, while FGSYSPI with the lowest β-sheet content had the highest T_{d1} and higher T_{d2}. It seemed that the relation between β-sheet content and T_d was not obvious in our study, which is inconsistent with previous studies (Shevkani et al., 2015; Ma et al., 2018). Shevkani et al. (2015) reported a positive relationship between T_d and the relative proportion of β-sheet conformations. This difference may be due to soybean varieties and growing environment. The greater ΔH for FSPI (ΔH_1: 1.72 J/g; ΔH_2: 5.90 J/g) and SYSPI (ΔH_1: mean value 1.30 J/g; ΔH_2: mean value 6.00 J/g) indicated a more ordered secondary structure relative to SBSPI (ΔH_1: mean value 1.00 J/g; ΔH_2: mean value 5.44 J/g). SYSPI with higher T_d and ΔH indicates better thermal stability and a more ordered structure, which is in agreement with Shevkani et al. (2015), who reported that proteins with more organized structures had higher thermal stability given the positive correlation between T_d and ΔH.

3.4. Intrinsic fluorescence spectroscopy

The intrinsic fluorescence of tryptophan (Trp) residue is used to

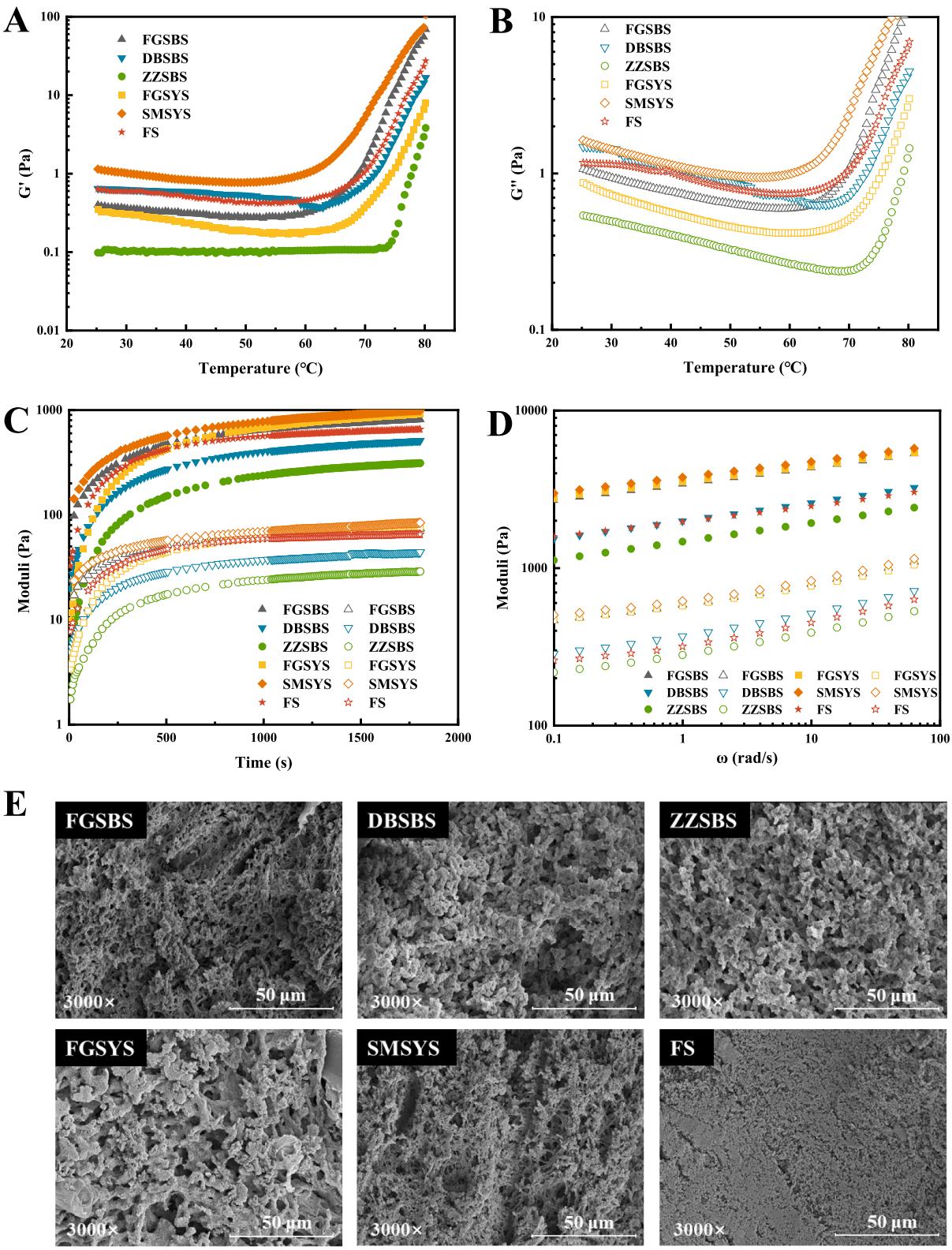

Fig. 2. Rheological properties (A-D) and SEM photographs (E) of tofu made from different soybean varieties. (A-B) The storage modulus (G') and loss modulus (G'') as a function of temperature. (C) G' (filled symbols) and G'' (unfilled symbols) as a function of time. (D) G' (filled symbols) and G'' (unfilled symbols) as a function of frequency. FGSBSPI represents Fugu small-seeded black soybean protein isolate; DBSBSPI represents Dingbian small-seeded black soybean protein isolate; ZZSBSPI represents Zizhou small-seeded black soybean protein isolate; FGSYSPI represents Fugu small-seeded yellow soybean protein isolate; SMSYSPI represents Shenmu small-seeded yellow soybean protein isolate; FSPI represents Fen soybean protein isolate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
The yield, WHC and color properties of tofu from different soybean varieties.

Samples	Tofu yield (g/100 g soybean)	WHC (%)	Color values L*	a*	b*
FGSBS	452.58 ± 15.51^{b}	77.20 ± 0.24^{b}	55.52 ± 0.68^{e}	3.81 ± 0.57^{b}	9.16 ± 0.45^{c}
DBSBS	395.75 ± 13.30^{c}	78.37 ± 0.44^{ab}	58.22 ± 0.07^{d}	3.56 ± 0.13^{b}	7.43 ± 0.38^{d}
ZZSBS	471.43 ± 11.20^{b}	80.34 ± 0.56^{a}	55.49 ± 0.51^{e}	4.44 ± 0.05^{a}	7.29 ± 0.34^{d}
FGSYS	560.23 ± 11.75^{a}	77.86 ± 1.81^{ab}	74.61 ± 1.11^{c}	−0.25 ± 0.21^{c}	14.83 ± 0.33^{a}
SMSYS	549.46 ± 8.01^{a}	77.41 ± 0.64^{b}	77.48 ± 0.43^{b}	−0.36 ± 0.27^{c}	14.95 ± 0.60^{a}
FS	553.34 ± 11.33^{a}	76.59 ± 2.69^{b}	83.17 ± 0.55^{a}	−1.22 ± 0.19^{d}	12.83 ± 0.88^{b}

Results are mean ± standard deviations of triplicate analysis. Values with different letters in the same column are significantly different ($p < 0.05$).

evaluate the polarity and conformation changes and characterize the tertiary structure of proteins (Ma et al., 2018). The emission peak (λ_{max}) is related to the microenvironment in which the Trp residue is located. As shown in Fig. 1C, all λ_{max} values of SPIs from different soybean varieties exceeded 330 nm, indicating that the Trp residue was in polar surroundings, most likely on the surface of the protein molecule (Zheng et al., 2021). Among the six soybean cultivars, FSPI had the highest fluorescence intensity (FI), followed by SMSYSPI and FGSYSPI, with DBSBSPI having the lowest FI (Fig. 1C). Both FSPI and SYSPI had higher FI than SBSPI, indicating a lower degree of denaturation and a more folded conformation (Ma et al., 2018), outcomes which were consistent with the result for thermal properties (Table 2). By contrast, DBSBSPI with the lowest FI had highly denatured protein molecules. The Trp residue was then extensively exposed to the hydrophilic environment as a result of that characteristic, which led to fluorescence quenching (Ajibola, Malomo, Fagbemi & Aluko, 2016). Moreover, the FI of small-seeded soybean proteins was significantly lower than that of other normal soybean proteins reported by Yan et al. (2021), which may be related to soybean varieties and growing environment.

3.5. Surface hydrophobicity (H_0)

The number of hydrophobic groups on a protein's surface and the propensity of protein molecules to aggregate can both be indicated by H_0, which also denotes the tertiary structures of proteins (Zhu et al., 2020). Moreover, H_0 influences the functional properties of proteins, for instance, their solubility and emulsifying properties (Shevkani et al., 2015). The H_0 values of the SPIs from different soybean cultivars varied significantly between 558.48 (FGSYSPI) and 1252.75 (DBSBSPI) (Fig. 1D), which was higher than that of other normal soybean proteins (129.6–511.5) reported by Zhu et al. (2020). This difference may be attributed to soybean varieties and growing environment. SYSPI (558.48–633.68) had significantly lower H_0 than SBSPI (939.51–1252.75) and FSPI (803.68), which may be attributed to the fact that SYSPI had higher α-helix but lower β-sheet content than SBSPI and FSPI (Table1). This result was consistent with the findings of Zhu et al. (2020), who reported that high β-sheet and low α-helix contents in protein structures caused an increase in surface hydrophobicity. Higher H_0 indicates a greater exposure of non-polar hydrophobic groups to the protein interface, which has a significant impact on the emulsifying abilities (Shevkani et al., 2015). SBSPI with higher H_0 had better EAI and ESI values (Table 1). This finding was in consistent with those of Ma et al. (2018), who claimed that the protein isolates from cottonseed meal had a positive correlation between H_0 and enhanced emulsifying capabilities.

3.6. Protein solubility (PS)

PS is an important feature as it is highly correlated to many functional characteristics of protein isolates including its emulsifying and gel abilities (Shevkani et al., 2015). Fig. 1E shows the PS of SPIs from six soybean varieties at different pH values. It can be seen that PS is significantly influenced by pH value. The six SPIs showed different PS profiles with similar trends: an initial decrease with the increase in pH until it reached the minimum solubility at the isoelectric point (pH 4.0–5.0), followed by an increase in solubility between pH 5.0–11.0. This result is consistent with the PS of other plant proteins (Du et al., 2018; Ma et al., 2018). In general, SPIs of six soybean varieties presented higher solubility in strong alkaline pH values. PS ranged between 35.92% and 46.57% at pH 7, whose highest value was for FGSBSPI. FSPI and SYSPI had higher PS in acidic pH, whereas SBSPI had higher PS in alkaline pH. In particular, SMSYSPI had higher PS in both acidic and alkaline pH. Amino acid compositions as well as the distribution of hydrophilic and hydrophobic amino acid residues on the molecular surface are significant factors of solubility pH dependence (Kimura, Fukuda, Zhang, Motoyama, Maruyama & Utsumi, 2008). Shevkani et al. (2015) reported a positive relationship between PS and the surface charge of kidney bean and field pea protein isolates and suggested a possible connection between PS and protein structure. Besides, PS is also affected by the ionic strength of the medium. The PS of barley protein isolates was generally adversely affected by increasing the ionic strength, which could be explained by a decrease in electrostatic repulsions and an increase in hydrophobic interactions caused by protein-protein and protein-solvent interactions (Yalçın & Çelik, 2007). Furthermore, Ghribi, Gafsi, Blecker, Danthine, Attia and Besbes (2015) pointed out that high solubility is a sign of native proteins and low denaturation.

3.7. WAC and OAC

WAC and OAC are two vital properties of SPI that are connected to the flavor retention, mouthfeel and texture of packaged foods (Shevkani et al., 2015). As shown in Table 1, significant differences were observed in the WAC of SPIs from six soybean varieties. Compared with SYSPI (7.11–7.62 g/g), SBSPI (8.07–8.50 g/g) exhibited higher WAC. The WAC of ZZSBSPI (8.50 g/g) and FGSBSPI (8.31 g/g) were significantly higher than that of other varieties, followed by DBSBSPI (8.07 g/g). Both SBSPI and SYSPI had significantly higher WAC than FSPI, which had the lowest WAC of 5.92 g/g. Besides, the SPIs of five small-seeded soybean varieties showed higher WAC (7.11–8.50 g/g) than the proteins from kidney bean (3.0 g/g), field pea (4.2 g/g) (Shevkani et al., 2015), chickpea

Table 4
Texture characteristics of tofu made from different soybean varieties.

Samples	Hardness (g)	Adhesiveness (g.sec)	Springiness	Cohesiveness	Chewiness (g)	Resilience
FGSBS	178.52 ± 9.65^{a}	−25.77 ± 3.29^{a}	0.89 ± 0.04^{ab}	0.55 ± 0.04^{ab}	87.45 ± 6.82^{a}	0.26 ± 0.03^{a}
DBSBS	92.38 ± 11.54^{d}	−126.06 ± 22.48^{c}	0.97 ± 0.01^{a}	0.50 ± 0.01^{bc}	44.95 ± 5.52^{c}	0.17 ± 0.01^{b}
ZZSBS	61.80 ± 0.74^{e}	−80.84 ± 15.31^{b}	0.96 ± 0.01^{a}	0.52 ± 0.01^{abc}	30.45 ± 1.15^{d}	0.16 ± 0.01^{b}
FGSYS	129.50 ± 8.07^{b}	−220.31 ± 7.73^{d}	0.83 ± 0.14^{b}	0.56 ± 0.05^{a}	72.69 ± 9.57^{b}	0.22 ± 0.06^{a}
SMSYS	83.90 ± 7.73^{d}	−120.41 ± 7.40^{c}	0.94 ± 0.03^{ab}	0.48 ± 0.03 ^{cd}	44.86 ± 13.11^{c}	0.13 ± 0.02^{b}
FS	113.90 ± 3.89^{c}	−132.20 ± 9.32^{c}	0.98 ± 0.01^{a}	0.44 ± 0.01^{d}	49.48 ± 2.46^{c}	0.16 ± 0.01^{b}

Results are mean ± standard deviations of triplicate analysis. Values with different letters in the same column are significantly different ($p < 0.05$).

(2.06–2.70 g/g) (Ghribi et al., 2015), soybean (3.55 g/g) and pea (2.52 g/g) (Ge, Sun, Mata, Corke, Gan & Fang, 2021). In general, WAC demonstrated significant source-dependent variance. This outcome may arise from the fact that WAC could be affected by amino acid composition, protein conformation and the ratio of surface polarity to hydrophobicity (Du et al., 2018).

As for OAC, FGSBSPI had the highest (2.57 g/g) and ZZSBSPI had the lowest (1.65 g/g) OAC. No significant differences in OAC were observed between SBSPI and SYSPI. The OAC of SPI reflects the hydrophobic ability of protein, and the high OAC of FGSBSPI may be attributed to the enhanced hydrophobic properties of the corresponding protein and the superior fat-binding performance of non-polar amino acid side chains (Ghribi et al., 2015). The OAC of the SPIs from five small-seeded soybeans (1.65–2.57 g/g) was lower than that of the proteins from kidney bean (5.9 g/g), field pea (6.4 g/g) (Shevkani et al., 2015), chickpea (2.28 g/g) (Ghribi et al., 2015), panda bean (7.65 g/g) (Ge et al., 2022), black bean (4.88 g/g), soybean (7.51 g/g) and pea (7.14 g/g) (Ge et al., 2021). Small-seeded soybeans with proteins of high WAC and low OAC are suitable for tofu processing.

3.8. Emulsifying properties

EAI and ESI, which stand for emulsifying properties, reflect how well proteins can form and maintain emulsions (Ghribi et al., 2015) and the ability to absorb to the oil–water interface (Zhu et al., 2020). The EAI and ESI values greatly varied among the SPIs from different soybean varieties (Table 1). EAI values ranged from 28.97 (FSPI) –38.57 (FGSBSPI) m^2/g, with SBSPI (mean value 34.24 m^2/g) having higher EAI values than SYSPI (mean value 31.57 m^2/g) and FSPI (28.97 m^2/g). The partial unfolding of the globular proteins, which exposed hydrophobic amino acid residues and increased surface activity and adsorption at the oil-water interface, may be the cause of the higher EAI of FGSBSPI (Ghribi et al., 2015). ESI values ranged from 28.43 (SMSYSPI) –40.46 (FGSBSPI) min, with FGSBSPI and DBSBSPI having higher ESI values relative to other varieties. FGSBSPI was confirmed to have better capability to reach the oil-water interface and stabilize the emulsion without coalescence, followed by DBSBSPI and FGSYSPI. PS and surface hydrophobicity are two important factors that determine the initial adsorption of proteins and their emulsifying properties (Shevkani et al., 2015). However, a wide range of additional factors, such as conformation state, protein composition and molecular flexibility, also influence the emulsifying abilities of proteins (Ge et al., 2021).

3.9. Tofu yield, WHC and color properties

The yields, WHC and color parameters of tofu from different soybean varieties are presented in Table 3. The tofu yields of SYS (549.46–560.23 g/100 g soybean) and FS (553.34 g/100 g soybean) were significantly higher than those of their SBS counterparts (395.75–471.43 g/100 g soybean). The tofu yield can be influenced by the protein content and composition of different soybean varieties (Zhang et al., 2018). The relatively lower tofu yield of ZZSBS may be attributed to the lower 11S content of ZZSBSPI (Fig. 1A). This outcome was in agreement with that of Mujoo, Trinh and Ng (2003), who reported a positive correlation between the soybean 11S protein and tofu yield. Thus, FS tofu had a higher yield because of the high 11S content of FSPI (Fig. 1A). Tofu yield is also affected by other factors in soymilk including fat content, the network structure of tofu and the type and content of coagulants (Li et al., 2021). Furthermore, the cultivar, genotype, growing environment and storage conditions of the soybean seeds affect the composition and structure of their protein and consequently influence tofu yield and other tofu-related characteristics (Zhang et al., 2018; Zheng et al., 2020).

The WHC of tofu samples from different soybean varieties were in the range of 76.59%–80.34%, with FS being the lowest and ZZSBS the highest. The highest WHC of the ZZSBS tofu may be attributed to its lack of 11S, especially the A_4 subunit of the protein (Fig. 1A), a finding which is in agreement with the result of Yang et al. (2013). SBS tofu exhibited higher WHC (mean value 78.64%) than its SYS (mean value 77.64%) and FS (76.59%) counterparts.

The color of tofu prepared from different soybean varieties varied significantly, with L* values ranging from 55.49 to 83.17, a* values varying from −1.22 to 4.44 and b* values varying from 7.29 to 14.95. SBS tofu had a higher a* value and lower L* value for a less bright and more reddish tofu. By contrast, SYS and FS tofu had higher L* and b* values but lower a* values for brighter and more yellowish tofu. This phenomenon is related to the different pigments contained in the seed coats of soybean varieties. The seed coats of black soybean are rich in three anthocyanins, namely, cyanidin-3-glucoside, delphinidin-3-glucoside and petunidin-3-glucoside (Choung et al., 2001), which, in turn, are dissolved in the soymilk and give black soybean tofu its dark color. Although these three anthocyanins affect the sensory quality of the product, they also confer a higher antioxidant value.

3.10. Texture properties of tofu

Texture is a direct and critical factor that affects consumer acceptance (Ran, Lou, Zheng, Gu & Yang, 2022). Significant differences in texture characteristics were observed in tofu from different soybean varieties (Table 4). Tofu had hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience in the ranges of 61.80–178.52 g, −220.31– −25.77 g.sec, 0.83–0.98, 0.44–0.56, 30.45–87.45 g and 0.13–0.26, respectively. FGSBS tofu had the highest hardness (178.52 g), adhesiveness (−25.77 g.sec), chewiness (87.45 g) and resilience (0.26). The differences in the texture properties of tofu may be associated with the differences in the microstructures of its cultivars, as well as their 11S and β-sheet contents. Zheng et al. (2021) reported a significant positive correlation between gel hardness and 11S subunits and a high correlation between β-sheet content and gel hardness. Poysa, Woodrow and Yu (2006) investigated the interaction between the protein subunits and the texture characteristics of tofu using different subunit-deficient soybean varieties and confirmed that a) the A_3 subunit of 11S played a major role in promoting tofu resilience, b) the A_1 and A_2 subunits also played a role in maintaining the texture properties of tofu and c) the A_4 subunit and α' subunit of 7S had a negative effect on the formation of the texture of tofu. For tofu products, their springiness was mainly affected by their 7S content, and their hardness was primarily determined by their 11S content (Zhang et al., 2018). Tofu prepared by Zuo et al. (2016) using $CaSO_4{\cdot}2H_2O$ as the coagulant had a hardness of 445.40–452.10 and springiness of 6.10–6.23, and these outcomes were significantly higher than those in our study. This variation is probably due to the fact that the type of coagulant is the most critical factor affecting the texture of the tofu. Using GDL as a coagulant usually generates a smoother and softer tofu texture in comparison to using traditional salts (Zhang et al., 2018). The gelling ability can be affected by the different relative ratios of constituents such as proteins, carbohydrates and lipids as well as the interactions between all components (Ghribi et al., 2015), which in turn affects their texture properties.

3.11. Gelation and rheological properties of tofu

The changes in dynamic moduli as a function of temperature of the heat-treated soymilk using GDL as the coagulant are illustrated in Fig. 2A-B. Soymilks made from different soybean cultivars showed similar trends for gelation. As temperature increased, the moduli (G' and G'') of the soymilk first decreased slightly and then increased rapidly at around 60℃. This phenomenon could be primarily related to the structural changes such as the denaturation (decrease), aggregation and rearrangement (increase) of SPI in the soymilk during the heat-induced gelling process (Ran & Yang, 2022; Sow et al., 2018; Lu et al., 2023). The G'' values for all samples were higher than the G' values during heating from 25 to 60℃, suggesting that the soymilk behaved as a viscous

solution. As heating continued, both G' and G'' increased significantly until at around 70℃, G' surpassed G'', which indicated a phase transition of viscous soymilk to viscoelastic tofu. This critical temperature point is defined as the gelation temperature (Shevkani et al., 2015). The gelation temperature of soymilks made from different soybean cultivars were 68.21℃ (FGSBS), 69.79℃ (DBSBS), 76.61℃ (ZZSBS), 72.95℃ (FGSYS), 60.27℃ (SMSYS) and 70.31℃ (FS), respectively. The gelation temperature of ZZSBS lagged somewhat relative to that of other varieties, which may be related to the insoluble fibers and polysaccharides remaining in the soymilk (Guan et al., 2021). A large number of insoluble dietary fiber particles and the presence of okara are embedded in the network structure, which destroys the continuity of the soy protein gel network (Guan et al., 2021; Sow et al., 2018). Therefore, the network structure of ZZSBS tofu was discontinuous and disorganized with large pores (Fig. 2E). Meanwhile, the loose structure of ZZSBS tofu contributes to the lowest hardness (61.80 g) and chewiness (30.45 g) (Table 4).

The dynamic moduli versus time for holding at 80℃ for 30 min is shown in Fig. 2C. Both G' and G'' values for all samples continuously increased with time and reached their maximum values with G' about 10 times higher than G'', indicating the formation of strengthened structures (Sow et al., 2018; Lu et al., 2023) and reflecting the firmness characteristics of the tofu samples (Lin et al., 2016). The denaturation process was a prerequisite for the gelation of globular proteins (Lee et al., 2011). During heating, the denaturation of soy proteins in soymilk was completed and the quaternary structure of soy proteins was dissociated into subunits (Lee et al., 2011). Then, the denatured proteins were associated into soluble aggregates with the hydrophobic regions and negative charges exposed outside by electrostatic attraction and disulfide linkage (Huang & Kuo, 2015). The addition of GDL with non-isothermal heating resulted in gradually generating protons in the solution, which neutralized the surface charges of soluble aggregates in heated soymilk (Lee et al., 2011). Consequently, the van der Waals attraction and hydrophobic interaction of neutralized soluble aggregates became predominant, and induced coagulation, leading to gel formation (Huang et al., 2015).

Fig. 2D illustrates the relationship between the frequency and dynamic moduli of tofu samples after complete gelation and letting the gel store at 4℃ for 1 h. G' was higher than G'' indicated that a continuous network structure was formed in tofu (Lin et al., 2016; Lu et al., 2022). Both G' and G'' increased slightly as the frequency increased, indicating that the prepared tofu samples were all close to viscoelastic materials (Lin et al., 2016; Sow et al., 2018). Besides, the rheological properties are also related to the secondary structure of the protein. Studies have shown that proteins with higher β-sheet content showed higher gelation temperatures, more excellent thermal stability and rheological properties (Ge et al., 2022; Shevkani et al., 2015).

3.12. Microstructure of tofu

The microstructure of tofu made from different soybean varieties is shown in Fig. 2E. The tofu samples showed a honeycomb-like structure with a homogenous network, probably due to the gel mechanism of GDL. FGSBS tofu performed relatively uniform internal flocculent aggregates, even pore size and a denser, finer and more ordered structure, thus indicating the highest hardness, adhesiveness, chewiness and resilience (Table 4). FS tofu showed the most continuous, homogeneous and dense network structure, which could trap more water and other soluble substances, leading to a higher yield (Table 3) and better springiness (Table 4). ZZSBS tofu had larger pores that were relatively evenly distributed. However, the network structure of FGSYS tofu was discontinuous and disorganized with large pores, exhibiting a looser form and poorer springiness (Table 4). It should be noted that the tofu sample was first frozen in liquid nitrogen and then freeze-dried before SEM observation, and the ice damage on the protein structure caused by the freezing procedure might lead to a slight increase in pore size (Huang et al., 2015).

4. Conclusion

The structural, functional properties of protein and characteristics of tofu from different small-seeded soybean varieties were investigated and the relationship between different parameters was analyzed. SBSPI had more β-sheet structure, higher H_0, and better WAC and emulsifying properties. Among the protein isolates, ZZSBSPI had lower 11S content, resulting in the highest WHC and the lowest hardness of tofu. FGSBS tofu had relatively uniform internal aggregation and a more ordered structure with higher hardness, springiness and chewiness. SYSPI had more α-helix structure and higher T_d, ΔH and FI, thereby indicating a lower degree of denaturation, a more ordered secondary structure and higher thermal stability. SMSYSPI had better PS in both acidic and alkaline pH. The results of this work provide useful information on the properties of small-seeded soybean protein for their improved utilization as functional ingredients.

CRediT authorship contribution statement

Yueyi Dang: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Jing Ren:** Methodology, Visualization, Data curation, Validation. **Ying Guo:** Software, Visualization, Data curation, Validation. **Qinghua Yang:** Validation, Investigation. **Jibao Liang:** Resources, Visualization. **Rui Li:** Resources, Visualization. **Rui Zhang:** Resources, Visualization. **Pu Yang:** Validation, Investigation. **Xiaoli Gao:** Supervision, Project administration. **Shuang-kui Du:** Funding acquisition, Project administration, 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.

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