LWT - Food Science and Technology 150 (2021) 111979

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Isochoric moisture heating as a tool to control the functionality of soy protein

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ARTICLE INFO

Keywords:
Soybean
Hydro-heating
Mild fractionation
Viscoelastic behaviors
Gelling

ABSTRACT

In this study, we investigated the effect of isochoric moisture heating on the functionalities of simply fractionated soy protein. Soy protein-rich fractions (SPFs) were produced through a simplified wet fractionation process, in which the defatting and intensive washing steps were omitted. After a neutralization step, the protein-rich dispersions were heated up to various temperatures ranging from 60 to 100 °C and subsequently freeze-dried. It was found that the denaturation level, the water holding capacity and the viscosity of SPFs increased as the processing temperature rose, whereas the solubility of SPFs decreased. Additionally, among the different conditions, an intermediate heating temperature of 70 °C resulted in SPFs having a gel network with the highest elasticity. Overall, isochoric moisture heating coupled to simplified fractionation can be used to precisely control the functionality of soy protein ingredients with mild heating conditions. This work provides insights on the production of ingredients with desired functionality for novel soy-based applications like soy meat analogues.

1. Introduction

Currently, diets containing more plant protein are stimulated due to reasons of environmental concerns, vegetarianism trends and increased consumer acceptance of plant-based products (Pojić, Mišan, & Tiwari, 2018). These changed diet habits lead to a higher demand for plant-based ingredients with the proper functionality to make new products. To meet the demands, multiple novel technologies have been explored to improve the yield, functional and nutritional properties of plant-protein ingredients (Liu, Gasmalla, Li, & Yang, 2016). Conventional wet fractionation based on alkaline solubilization and isoelectric precipitation is the industrialized and widely applied process for soy protein production because the process provides highly purified protein isolate at a low price (Alibhai, Mondor, Moresoli, Ippersiel, & Lamarche, 2006; Nazareth, Deak, & Johnson, 2009). There is a growing interest to develop simplified wet fractionation to avoid the use of organic solvents (especially during the oil extraction step) and intensive washing steps (Berghout, Boom, & Van Der Goot, 2014; Campbell et al., 2011; Peng, Dewi, Kyriakopoulou, & van der Goot, 2020). Therefore, simplified fractionation can be rated as applicable, effective and potentially as a more sustainable alternative enabling solvent-free label status (Geerts, van Veghel, Zisopoulos, van der ; Nazir, Asghar, & Aslam Maan, 2016).

Soy-based ingredients are getting a lot of attention, because of their potential to be used in many novel products, such as meat analogues (Kyriakopoulou, Keppler, & van der Goot, 2021). Traditionally, high solubility of soy-based ingredients is considered as the most important functionality for food and industrial applications (Wang & Johnson, 2001). However, new generations of soy-based products require different ingredients functionalities. For example, the water holding capacity (WHC) of soy protein gels is a significant parameter for soy-based yogurt manufacturing, because it is related to syneresis and water may be lost due to passive diffusion after extensive storage time (Cruz et al., 2009; Kovalenko & Briggs, 2002). For soy cheese analogues, high water retention properties coupled with increased viscoelasticity improve the quality attributes of final products (Jeewanthi & Paik, 2018). In the case of meat analogue products, soy protein ingredients are

Abbreviations: SPF(s), soy protein-rich fraction(s); FFSF, full-fat soy flour; SEM, scanning electron microscopy; NSI, nitrogen solubility index; WHC, water-holding capacity; WHC_P, water-holding capacity of the pellet; OAC, oil absorption capacity; DSC, differential scanning calorimetry; SPI, soy protein isolate; SPC, soy protein concentrate; PDD, pre-denaturation degree.

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https://doi.org/10.1016/j.lwt.2021.111979
Received 21 January 2021; Received in revised form 6 May 2021; Accepted 19 June 2021
Available online 21 June 2021

desired to have intermediate solubility, superior absorption of water and oil, and to form a meat-like fibrous structure (Geerts, Dekkers, van der Padt, & van der Goot, 2018; Jones, 2016).

Heating is a commonly used method to alter the functionalities of protein, and it can be introduced with different treatments, such as boiling, autoclaving, ultra-heating and toasting (Hickisch, Bindl, Vogel, & Toelstede, 2016; Obatolu, Fasoyiro, & Ogunsunmi, 2007; Zhang, Yang, & Acevedo, 2020). Heating can be applied in different stages during the protein fractionation process as well. For example, the starting materials (soybean or full-fat soy flour) can be toasted before the protein extraction, the dispersions can be heated during extraction, or the obtained fractions can be processed after extraction (Alavi, Chen, Wang, & Emam-Djomeh, 2021; Das, Anjaneyulu, & Kondaiah, 2006; Deak & Johnson, 2007). However, heating before or during the protein extraction stage has an impact on the purity, yield and functionality of the obtained protein fractions. Deak and Johnson (2007) heated the soy protein during the solubilization step and found that soy protein isolates extracted at 80 °C had a lower yield and purity as compared to room temperature extraction. Therefore, to modify the functionalities of protein, but minimize the influences on the purity and yield, it is suggested to perform the heating step after the protein extraction stages, before or after the final drying process.

Our previous study observed that simply fractionated SPF presents high solubility and low WHC (Peng, Kersten, Kyriakopoulou, & van der Goot, 2020). Generally, those properties are highly desired for functionalities like foaming and emulsification but are less desired for meat analogue-like applications (Moure, Sineiro, Domínguez, & Parajó, 2006). WHC and gelling properties are more important. Unfortunately, the WHC-value of SPF is lower than commercial SPI and SPC reported in the literature (Geerts, Dekkers, et al., 2018), which can be attributed to differences in drying methods. Freeze drying was performed for the production of SPF while spray drying was applied industrially. Spray drying probably leads to higher WHC due to the denaturation caused by the heat applied to the soy ingredients, and limited impacts can be observed for protein isolates on the functional behaviors before and after freeze-drying (Berghout, Venema, Boom, & Van der Goot, 2015). To modify the functionalities of protein-rich fraction, toasting is a heating process performed in a dry state after the drying step. Toasting treatment requires a high temperature (up to 150 °C) to change the functional properties of the protein in the desired direction. However, this severe heating could induce additional protein changes, which are less favorable, such as color formation (Bühler, Dekkers, Bruins, & van der ; Geerts, Dekkers, et al., 2018). In this study, moisture heating treatment was explored with milder temperature (60–100 °C) after the protein neutralization step and before the freeze-drying. The heating treatment is relatively easy to apply in the industry, while no extra step was required after the drying and the heating. The novelty of our approach lies in the introduction of a mild heating step in a simplified fractionation process. It will be investigated how, the functionality of the ingredients can be tuned through physical means, as an alternative for chemical modification or the use of additional ingredients. The objective of this research is therefore to better understand how moisture heating influences the functional behavior such as protein solubility, viscosity and gelling properties of simply fractionated SPF. It is expected that the results of this study can aid in the development of soy protein ingredients processed under mild conditions, thereby expanding the current market of novel soy-based products for sustainable diets.

2. Materials and methods

2.1. Materials

Dry, full soybeans were obtained from FRANK Food Products (the Netherlands). The Ultrapure water was used in the whole procedure and was purified by a Milli-Q Lab Water System (Milli-Q IQ 7000 Ultrapure Lab Water System, Merck KGaA, Darmstadt, Germany). NaOH and HCl were analytical grade and purchased from Sigma Aldrich (Sigma, USA).

2.2. Methods

2.2.1. Full-fat soy flour preparation

The full-fat soy flour (FFSF) was prepared according to the parameters developed earlier (Peng, Kersten, Kyriakopoulou, & van der Goot, 2020). Briefly, full soybeans (without de-hulling) were pre-milled to soy grits by a pin mill LV 15M (Condux-Werk, Wolfgang bei Hanau, Germany), and the soy grits were further milled into FFSF by a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany). Around 5 kg of FFSF was produced and stored in a cooling room (4 °C) for further analysis and processing.

2.2.2. Simplified fractionation process and isochoric moisture heating

The simplified fractionation process performed in this study is presented in Fig. 1. In more detail, FFSF was used as the starting material that was mixed with water in a ratio of 1:5 (w/w). The dispersions were adjusted to pH 8.5–9 by adding 1 M NaOH solution. The dispersion was stirred for 1 h and centrifuged at 18,670 g for 30 min. After centrifugation, the semi-solid oil-rich cream layer, the protein-rich supernatant and the fiber-rich pellet can be easily separated by a cheese-cloth. The protein-rich supernatant was further purified by isoelectric precipitation at pH 4.5–5 with 1 M HCl, stirred for 1 h and then centrifuged (18,670 g, 30 min). The protein-rich pellet was then mixed with water and neutralized at pH 6.5–7. Once the pH value became constant, the dispersion was transferred to a closed flask to avoid water evaporation, and subsequently heated on a heating plate to a set temperature (60, 70, 80, 90 and 100 °C), where it remained for 30 min. A reference sample was prepared without any moisture heating. The abbreviations of the obtained soy protein fractions were referred in this study as SPF WH (without heating), SPF 60, SPF 70, SPF 80, SPF 90 and SPF 100. All the soy protein dispersions were cooled to room temperature before the flask was opened. After that, the soy protein dispersions were freeze-dried (Freeze dryer, Epsilon 2-6D, Martin Christ, Germany) and milled (Rotormill Pulverisette 14, Fritsch, Germany) into powder by using a sieve ring with a perforation size of 0.5 mm and a rotation speed of 11,202 g. The obtained SPFs were stored in a cooling room (4 °C) till further analysis. Each procedure was performed in duplicate, and all the mass balances were recorded for the yield calculations.

2.2.3. Composition and yield analysis

The protein content of the FFSF and SPFs was determined by using a Nitrogen Analyzer (Flash EA 1112 series Dumas, Thermo Scientific, The Netherlands). A nitrogen-to-protein conversion factor of 5.7 was used (Krul, 2019). The oil content was measured through the Soxhlet extraction procedure (B-811 Buchi Extractor, Buchi, Switzerland). In short, the petroleum ether was heated up and evaporated, after which the vapor was condensed and dropped into the chamber with the sample inside. Subsequently, the oil in the sample was extracted, collected and measured. All measurements were performed in triplicate.

The product yield was calculated using Eq. (1), while the protein yield was calculated according to Eq. (2):

$$\textit{Product yield}\ (\%) = \frac{\textit{Weight of dried SPF}}{\textit{Weight of starting FFSF}} \times 100\% \quad (1)$$

$$\textit{Protein yield}\ (\%) = \frac{\textit{Total protein in dried SPF}}{\textit{Total protein in starting FFSF}} \times 100\% \quad (2)$$

2.2.4. Color analysis

The color was measured using a Chroma Meter (CR-400, Konica Minolta, USA) in terms of L^* (lightness), a^* (redness and greenness) and b^* (yellowness and blueness). The instrument was calibrated with a standard black and white tile followed by measurement of samples. A glass cell containing the SPF was placed above the light source and L, a

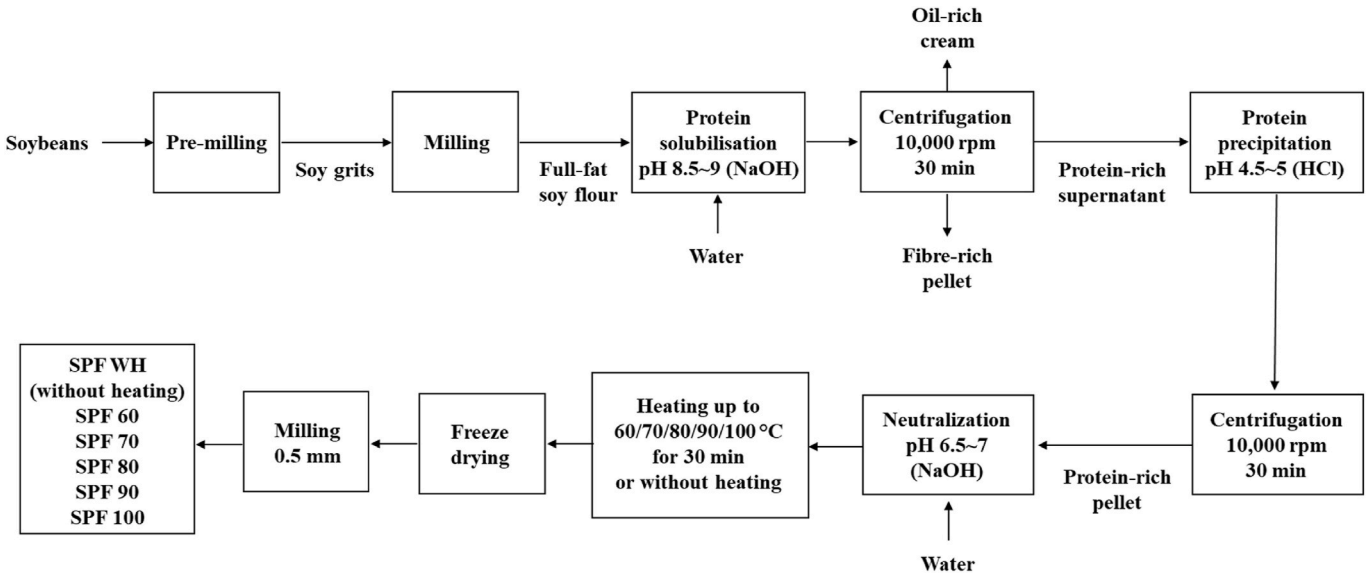

Fig. 1. Simplified fractionation process with isochoric moisture heating step and abbreviations for all the soy protein fractions (SPFs).

and *b* values were recorded. Color measurements were taken in triplicates.

2.2.5. Denaturation behavior analysis

The denaturation temperature and the enthalpy of the transition of the SPF samples were determined by a differential scanning calorimetry (Diamond DSC, PerkinElmer, USA). A 20% (w/w) protein dispersion of each SPF was prepared, placed in high volume aluminum pans (100 μL) and well-sealed. The DSC was calibrated with indium, and an empty high-volume aluminum pan was used as a reference. The sample was scanned at 5 °C/min from 20 °C to 150 °C, and the heating cycle was repeated two times. Measurements were analyzed with Start Pyris Software (PerkinElmer, Shelton, USA) and taken in triplicates.

2.2.6. SEM analysis

A scanning electron microscope (SEM, Phenom Pure G2, Eindhoven, The Netherlands) was used to view the powder morphology of the SPFs. Each SPF was evenly fixed with double-sided adhesive conductive carbon tabs on an aluminum sample holder. The accelerating voltage was 5 kV.

2.2.7. Particle size distribution analysis

A 1% (w/v) dispersion of each SPF sample was prepared with Milli-Q water and divided into two parts: one part was stirred under room temperature for 1 h, and one part was heated at 95 °C for 30 min. The particle size distribution was determined by a static laser light scattering (Malvern Master Sizer 3000, Malvern Instruments, UK) with a wet module. To determine the size of small particles (0.5 nm - 5 μm) in the dispersions, another 1% (w/v) dispersion of each SPF sample was well mixed with Milli-Q water and centrifuged at 2000 g for 10 min. After centrifugation, the supernatant was collected and divided into two parts: one part was measured directly by a Zetasizer (Zetasizer Ultra, Malvern Instruments, UK), while the other part was heated at 95 °C for 30 min, and subsequently cooled down and measured by the Zetasizer. A refractive index of 1.45 was used for the dispersion phase and 1.33 for the water continuous phase. All the measurements were performed in triplicate.

2.2.8. Nitrogen solubility index (NSI)

The nitrogen solubility index (NSI) is routinely used to evaluate protein solubility (Wang & Johnson, 2001). A 2% (w/w) dispersion of each SPF sample was placed in a centrifuge tube and moderately shaken overnight. Subsequently, the sample was centrifuged at 18,670 g for 30 min to separate the supernatant and pellet. The pellet was oven-dried at 105 °C and weighed. The nitrogen contents in the oven-dried pellets were measured by using Dumas analysis. The NSI was calculated by the ratio of soluble nitrogen over the total initial nitrogen content present in the SPFs. All the measurements were performed in triplicate.

2.2.9. Water holding capacity (WHC) and oil absorption capacity (OAC)

The water holding capacity (WHC) of SPFs was measured using the method described by Peters (Peters, Vergeldt, Boom, & van der Goot, 2017) with slight modification. A 2% (w/v) SPF dispersion was prepared with Milli-Q water and shaken overnight. Then, the dispersion was centrifuged at 18,670 g for 30 min to separate the supernatant and the wet pellet. The wet pellet was collected and weighed. Subsequently, the wet pellet was oven-dried overnight at 105 °C and weighed again to determine the mass of the dry pellet. The WHC was calculated according to Eq. (3), and the WHC of the pellet (WHC_P) was determined according to Eq. (4).

$$WHC\ (\frac{g\ water}{g\ dry\ SPF}) = \frac{M_{wet\ pellet} - M_{dry\ pellet}}{M_{dry\ SPF}} \quad (3)$$

$$WHC_P\ (\frac{g\ water}{g\ dry\ pellet}) = \frac{M_{wet\ pellet} - M_{dry\ pellet}}{M_{dry\ pellet}} \quad (4)$$

The oil absorption capacity (OAC) of SPFs was measured according to a previously described method (Lin, Humbert, & Sosullski, 1974) with minor modifications. Around 0.5 g of the SPF and 10.0 ml of sunflower oil were added to a centrifuge tube, and mixed for 3 min with a vortex mixer to disperse the sample into the oil. After a holding period of 30 min, the tube was centrifuged for 25 min at 3050 g. The separated oil was then removed with a pipette, and the tube was inverted for 25 min to drain the oil before reweighing. The OAC was expressed according to Eq. (5). All the measurements were performed in triplicate.

$$OAC\ (\frac{g\ oil}{g\ dry\ SPF}) = \frac{M_{pellet} - M_{dry\ SPF}}{M_{dry\ SPF}} \quad (5)$$

2.2.10. Viscosity analysis

The viscosity of the SPFs at different concentrations (5–30%, dry basis) was analyzed by a rheometer (Anton Paar Physica MCR301, Graz, Austria) with a concentric cylinder (CC-17) or a cone-plate geometry (CP-20-2). The samples were equilibrated for 5 min. Viscosity was determined at 25 °C using a shear rate range from 1 to 100 s^{-1}. The

viscosity at a shear rate of 28 s^{-1} was used to quantify the effect of SPF concentration. The lowest shear rate reported corresponded with the shear rate at which the torque fell within the minimum torque of the rheometer to measure accurately. All the measurements were performed in triplicate.

2.2.11. Gelling behavior analysis

Oscillatory rheological measurements of SPF samples were carried out using a rheometer (Anton Paar Physica MCR301, Graz, Austria) with a plate-plate geometry (PP-50). SPF with 15% concentration (on a dry basis) was selected due to their consistency and requirements for gel formation. Samples were prepared and kept for 30 min before transferring to the rheometer.

Strain sweep experiments (0.01–100%) were performed at a constant frequency of 1 Hz to determine the limit of the linear viscoelastic region (LVR) of each SPF. Temperature and frequency sweeps were carried out afterward within the determined range. A thin layer of high-temperature-resistant silicone oil was covered on top of the samples to prevent water evaporation during heating. After an initial equilibration of samples at 40 °C for 3 min, ramp heating occurred at 2 °C/min to an endpoint of 95 °C at a strain of 1% and frequency of 1 Hz. The samples were then immediately cooled back to 25 °C at a rate of 5 °C/min and equilibrated for 5 min. Subsequently, a frequency sweep was carried out from 0.01 to 10 Hz at a strain of 1%. All the rheological measurements were carried out in triplicate.

2.2.12. Statistical analysis

Data were collected from triplicate experiments or in specified cases from more experiments. IBM SPSS Statistics Version 23.0 was used to analyze the variance, and Duncan's test was performed to determine the statistical significance between samples at an α level of 0.05. All the results were displayed by mean values ± standard deviations.

3. Results

3.1. Composition, yield and color analysis

In this study, the protein and oil content of all the SPFs were quite similar since the isochoric moisture heating step was carried out after all the separation steps before freeze-drying (Table 1). The simplified fractionation process resulted in protein-rich fractions with approximately 75% protein and 2% oil (dry basis). The product yield was an average of 27% while the protein yield reached up to 56%. These results were in line with the yields reported by our previous research (Peng, Dewi, Kyriakopoulou, & van der Goot, 2020). All the SPFs obtained in this study presented lower protein content and higher oil content than commercial SPI, as commercial SPI contains around 83.3% of protein and 0% oil. This is due to the simplification of the fractionation processes compared to the conventional wet procedure. However, the protein yield of SPFs (55.7 ± 2.8%) was higher than that reported yields of lab-produced SPI (30–50%) (Jung, Lamsal, Stepien, Johnson, & Murphy, 2006; L'hocine, Boye, & Arcand, 2006).

SPFs processed at different heating temperatures showed changes in their color values (Table 1). Visually, all the SPFs were yellowish, but with increased heating temperature from 60 to 100 °C, the color parameter L^* of SPF decreased from 79.0 to 72.3. No regular pattern was observed in the color values of a^* and b^* when changing temperature.

Table 1
Protein, oil content and color analysis of FFSF, commercial SPI and all the SPFs in dry basis.

Sample names	Protein, % (N × 5.7)	Oil, %	L^*	a^*	b^*
FFSF	36.8 ± 1.3^{a}	21.9 ± 0.1^{a}	–	–	–
Commercial SPI	83.3 ± 0.7^{b}	0.0 ± 0.0^{b}	–	–	–
SPF WH	75.0 ± 0.4^{c}	2.6 ± 0.7^{c}	80.2 ± 0.2^{a}	1.5 ± 0.0^{a}	20.4 ± 0.2^{ab}
SPF 60	77.8 ± 0.7^{d}	2.4 ± 0.6^{c}	79.0 ± 0.4^{b}	2.3 ± 0.1^{b}	20.6 ± 0.3^{abc}
SPF 70	75.0 ± 1.4^{c}	1.5 ± 0.2^{d}	76.5 ± 0.2^{c}	2.5 ± 0.0^{c}	21.1 ± 0.1^{c}
SPF 80	73.2 ± 0.4^{e}	2.0 ± 0.3^{cd}	78.5 ± 0.2^{b}	1.9 ± 0.1^{d}	19.5 ± 0.5^{d}
SPF 90	75.9 ± 1.1^{c}	2.6 ± 0.4^{c}	75.4 ± 0.2^{d}	2.3 ± 0.1^{b}	20.7 ± 0.4^{bc}
SPF 100	75.1 ± 1.0^{c}	2.4 ± 0.1^{c}	72.3 ± 0.8^{e}	1.8 ± 0.2^{d}	20.1 ± 0.3^{ad}

* The values in the table are compared in columns and different lower case letters indicate a significant difference ($P < 0.05$).

3.2. Degree of protein denaturation

DSC analysis was performed to assess the effect of moisture heating on SPF denaturation levels (Table 2). SPF WH and SPF 60 exhibited two heat absorption peaks, though SPF WH had a higher enthalpy value of 7S than SPF 60. The results obtained in this study were in line with the previously reported data on the peak denaturation temperatures of 7S (68.0–79.4 °C) and 11S (88.0–96.4 °C) (Damodaran, 1988; Guo et al., 2012). Only one peak was detected in SPF 70 and SPF 80, suggesting that the 7S was fully denatured when the moisture heating temperature was 70 °C or higher. Also, here, the enthalpy of denaturation of the remaining peak became lower when the sample was treated at a higher temperature. No peaks were identified anymore when measuring SPF 90 and SPF 100, meaning that all protein was fully denatured. Thus, SPFs were gradually denatured with the increase of the moisture heating temperature.

Table 2
The denaturation temperature and enthalpy of transition of all the SPFs (ND = not detected).

Sample names	7 S T_d (°C)	Enthalpy (J/g)	11 S T_d (°C)	Enthalpy (J/g)
SPF WH	71.25 ± 0.99^{a}	1.31 ± 0.55^{a}	94.52 ± 0.81^{a}	3.22 ± 0.71^{ab}
SPF 60	72.82 ± 0.75^{b}	0.65 ± 0.17^{b}	93.57 ± 0.06^{b}	3.54 ± 0.79^{ab}
SPF 70	ND	ND	95.05 ± 0.83^{a}	4.15 ± 1.00^{b}
SPF 80	ND	ND	96.07 ± 0.25^{c}	2.87 ± 0.25^{c}
SPF 90	ND	ND	ND	ND
SPF 100	ND	ND	ND	ND

* The values in the table are compared in columns and different lower case letters indicate a significant difference ($P < 0.05$).

3.3. Powder morphology and particle size distribution

All the SPF powders were obtained by freeze-drying followed by milling, using a sieve ring with a perforation size of 0.5 mm. The application of the same procedure to all samples is probably the reason that similar powder particle sizes were obtained. SEM images revealed further that moisture heating treatment played a minor role in the powder morphology of SPF, as can be seen from Fig. 2. Irregular and compact flakes, surrounded by small fragments were observed for all the SPFs. These are typical particle structures obtained after freeze-drying for proteins (Dehnad, Jafari, & Afrasiabi, 2016; Zhao et al., 2013). In contrast, commercially available SPI and soymilk powder are mainly processed with spray drying and present individual particles with spherical shapes (Porras-Saavedra et al., 2015).

Differences between SPFs became clear when dispersing the powders in water for 1 h. The dispersion of SPF 100 exhibited the largest particle size while the SPF WH the smallest. The particle size of SPF dispersion

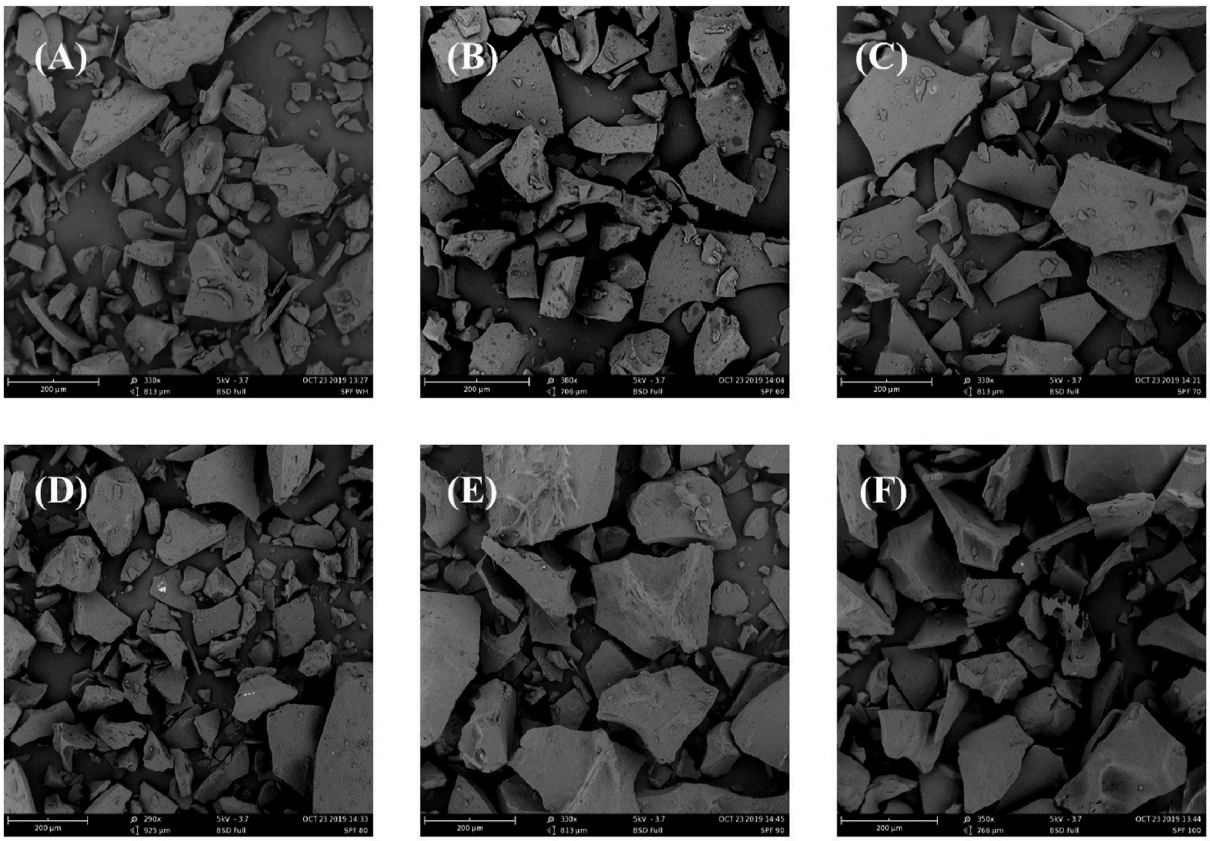

Fig. 2. SEM images of freeze dried (A) SPF WH, (B) SPF 60, (C) SPF 70, (D) SPF 80, (E) SPF 90 and (F) SPF 100. The scale bar is 200 μm.

increased with an increase in the moisture heating temperature applied during fractionation (Fig. 3A). After additional heating of the SPF dispersions to 95 °C for 30 min (Fig. 3B), the particles became smaller for all the SPF dispersions. However, this heating step did not alter the size sequence between samples. The dispersion of SPF 100 still presented the largest particle size among all the SPF dispersions. In addition, both SPF WH and SPF 60 showed a bimodal distribution in Fig. 3B. SPF WH presented a small fraction of particles ranging from 0.01 to 0.6 μm and a large volume percentage with particles from 2.4 to 454 μm, while for SPF 60, the ranges for two peaks were 0.04–0.46 μm and 4.58–516 μm respectively.

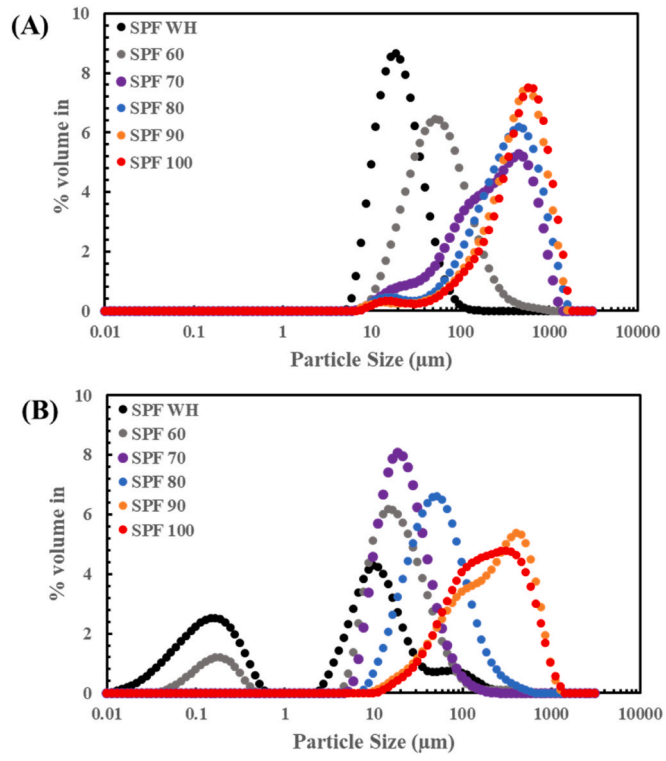

Fig. 3. Particle size distribution (0.01–10,000 μm) of all the SPFs (A) mixed and stirred after 1 h and (B) heated under 95 °C for 30 min.

After centrifugation, as observed from Fig. 4, the particles left in the supernatant of SPF WH showed the largest size, while these remained in the supernatant of SPF 100 had the smallest size. In this particle size range, the particles of all the SPF supernatants grew upon heating but the change of size in SPF 90 and SPF 100 were less noticeable (Fig. 4B).

3.4. Nitrogen solubility index (NSI), water holding capacity (WHC) and oil absorption capacity (OAC)

The nitrogen solubility index (NSI) is routinely used to evaluate protein solubility (Wang & Johnson, 2001). SPF WH presented the highest NSI-value among all the SPFs, which was around 83%, as shown in Fig. 5A. The NSI of SPF was reduced by the application of moisture heating during fractionation. The greatest reduction occurred when the heating temperature increased from 60 to 70 °C; where the NSI of SPF decreased from 75 to 38%. A small change in NSI occurred when the heating temperature increased from 90 to 100 °C.

Water holding capacity (WHC) is defined as the ability of a protein sample to hold water while the water holding capacity of the pellet (WHC_P) is defined as the ability of the insoluble part in a protein sample to hold water (Peters et al., 2017). Fig. 5B shows that the moisture heating step had limited impacts on the WHC of SPF when the heating temperature was 60 °C or below. The WHC of SPF increased

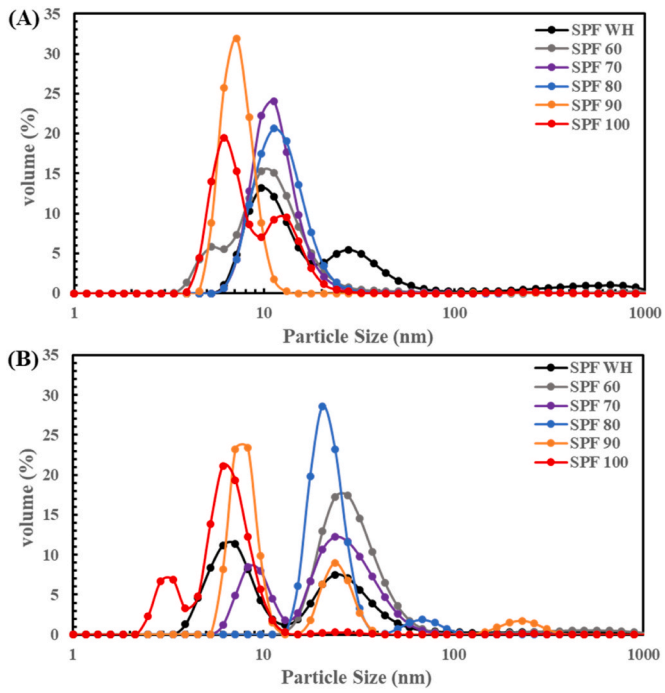

Fig. 4. Particle size distribution (1-1000 nm) of all the SPFs after the centrifugation at 2000*g* for 10 min (A) mixed and stirred after 1 h and (B) heated under 95 °C for 30 min.

dramatically from 3.1 to 10.0 g water/g SPF when the heating temperature changed from 60 to 70 °C, and reached the highest value of 13.2 water/g SPF when the heating temperature approached 100 °C. However, the difference of WHC_P values became smaller between SPFs and ranged from 12.8 to 16.8 g water/g pellet.

Regarding the OAC, the difference between SPFs was not distinct when the moisture heating temperature was 90 °C or below (Fig. 5C), and the highest OAC value of 2.4 g oil/g SPF was observed for the SPF 100.

3.5. Viscosity

All the SPFs presented the typical shear thinning behavior when dispersed in water with concentrations ranging from 5 to 30% (dry basis). The increase of the shear rate from 1 to 100 s^{-1} led to a decrease of the viscosity values. Fig. 6A showed an example of SPF dispersions with 10% of concentration. The greatest reduction in viscosity took place for all the SPFs when the shear rate was lower than 10 s^{-1}, and the change was less steep with a higher shear rate. To quantify the effect of SPF concentration on the viscosity changes of all the SPFs, a shear rate of 28 s^{-1} was used and the viscosity values for other SPF samples were determined (Fig. 6B).

For all the SPF samples, the viscosity became larger with the increase of SPF concentrations in the range of 5–30%. A tendency to the plateau was observed for SPF samples as the moisture heating temperature was increased, especially when the moisture heating temperature was 80 °C and above. Additionally, with a fixed concentration, the SPF dispersions became more viscous when the moisture heating temperature was increased, which can be beneficial for food applications that require a certain viscosity without further heating.

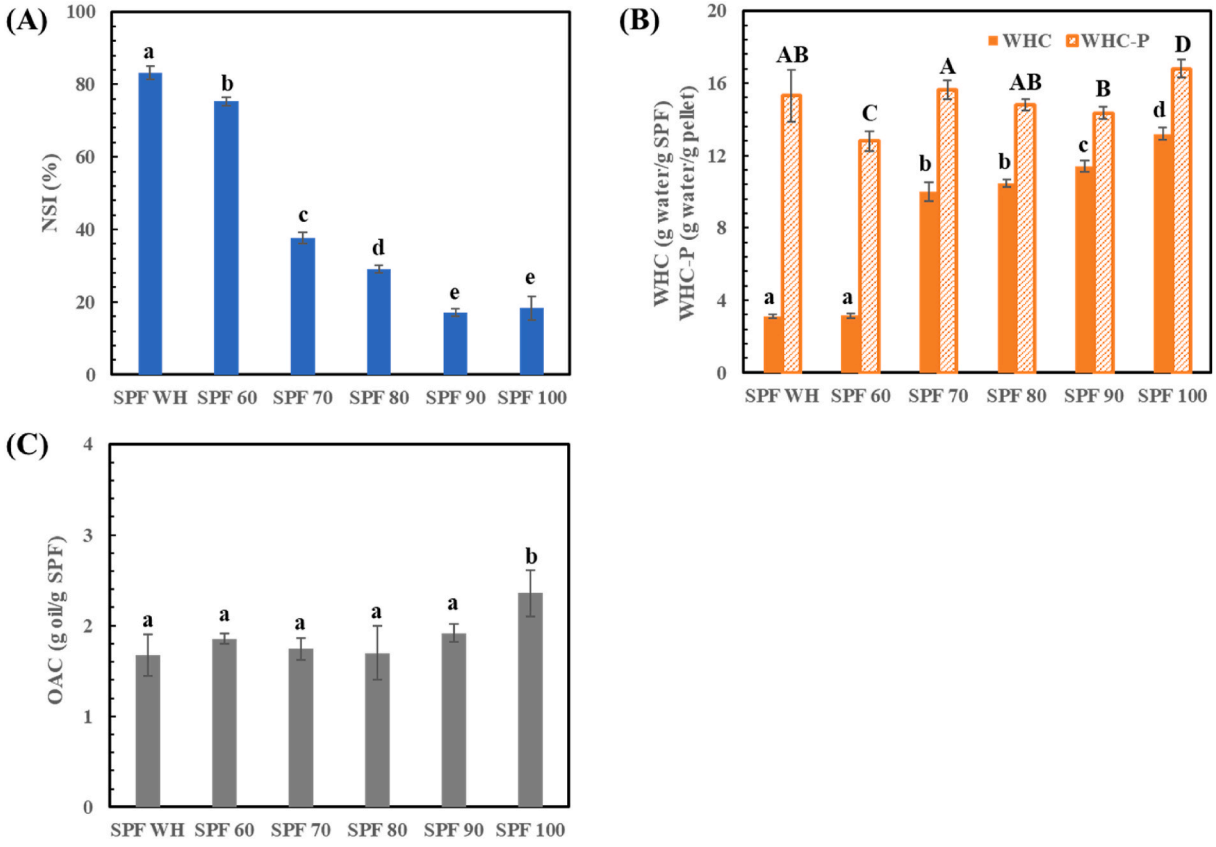

Fig. 5. (A) Nitrogen solubility index (NSI), (B) water holding capacity (WHC) and (C) oil absorption capacity (OAC) of all the SPFs.
* The values in the figures with different top letters indicate a significant difference ($P < 0.05$).

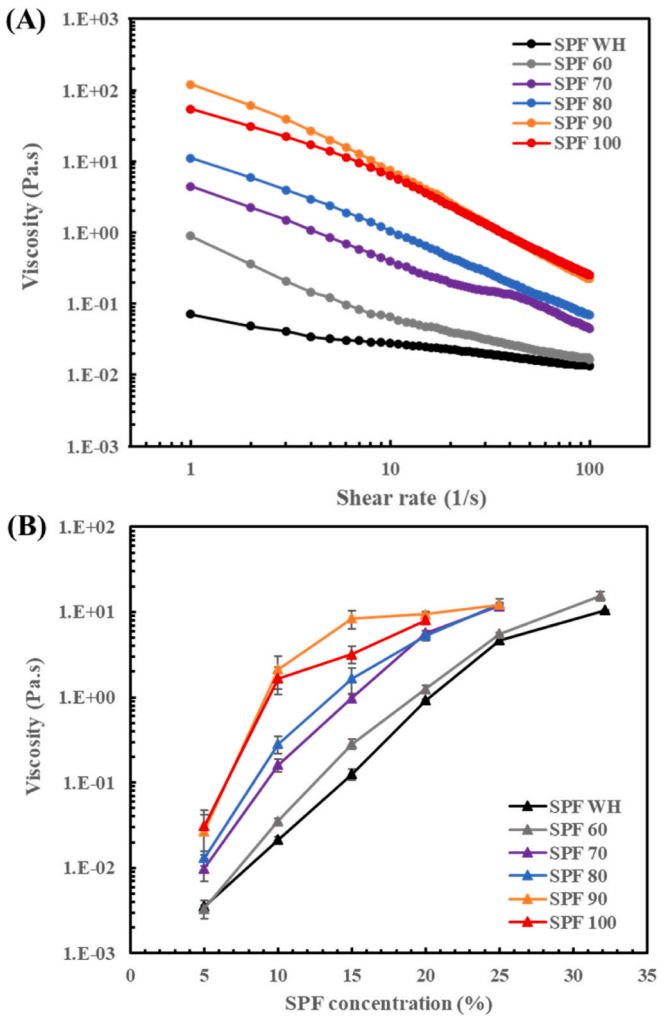

Fig. 6. (A) Viscosity measured as a function of shear rate with the 10% SPF concentration, and (B) viscosity as a function of the SPF concentration at a shear rate of 28 s^{-1}.

3.6. Gelling properties

All SPF dispersions had a constant storage modulus (G′) up to a certain strain, after which the modules decreased (Appendix 1). The strain point at which G′ began to drop by 5% from its maximum value was determined as a critical strain limit (Rueb & Zukoski, 1997), indicating a transition from linear to non-linear behavior. The results showed that the critical strain limit of SPF dispersions was between 1.7 and 10.3%, and it increased with the increasing temperature. SPF 100 presented the highest value, which indicated that the dispersion of SPF 100 contained a more stable and less easily fractured network than other SPF dispersions (Li, Xia, Zhou, & Xie, 2013). Overall, the strain was selected as 1% for our following temperature and frequency sweep measurements.

The results of G′ and G″ from temperature sweeps are presented in Fig. 7A and B respectively. During the temperature sweep from 40 to 95 °C, the G′ values of all the SPF dispersions were consistently higher than G″, indicating that 15 % wt was sufficient to induce solid-like behavior in all SPF-dispersions before heating already. After heating, SPF WH showed the lowest G′ and G″ values while SPF 70 presented the highest one. Additionally, with the increase of temperature, the G′ and G″ values of SPF 90 and SPF 100 decreased. The G′ values of SPF WH and SPF 60, though, increased when the temperature exceeded 70 °C, while for SPF 70 and SPF 80, the uptrend started around 85 °C.

After the temperature sweep, the SPF samples were immediately cooled back to 25 °C and the frequency sweep was performed. Fig. 7C and D shows that the G′ and G″ of all the SPFs increased after cooling and were relatively independent of the frequency applied. Also after cooling, SPF WH had the lowest G′ and G″ values among all the SPFs respectively, while SPF 70 gave gels with the highest values for G′ and G''. Table 3 showed the G′ and tan δ value of all the gelled soy protein samples obtained at a frequency of 1 Hz after temperature sweep. The gel formed by SPF 70 showed the highest G′-value, while the tan δ value of all the samples were comparable and below 0.15, indicating that all the SPF gels exhibited solid-like behaviors.

4. Discussion

In this study, a simplified fractionation process was used to obtain SPFs. Without additional processing, an SPF was obtained with high purity, high solubility and low WHC. For modern applications though, like meat analogues, other functionalities are required, such as high WHC and the ability to form strong gels. An isochoric moisture heating treatment was performed following the extraction stage and before the final freeze drying to drive functionality in the desired direction. We studied isochoric moisture heating as this step can be easily applied in an existing industrial fractionation process, since no additional process equipment is required. Besides, soy ingredients with a broad range of functionalities can be created without using severe heating temperatures, like in the case of toasting, chemicals or other additives.

The results showed that higher moisture heating temperature contributed to higher denaturation levels of SPFs. The hydrophobic groups located in the interior of the molecule could be exposed for interaction with water, and therefore, causing the reduction of NSI for SPFs (Arrese, Wagner, Añón, & Sorgentini, 1991). The SPF formed new aggregated structures in the dispersion. This aligns with the outcomes of the WHC-measurements and the viscosity measurements. WHC_P of all the SPFs remained comparable, which hinted that the insoluble protein aggregates newly formed as a result of protein denaturation had similar properties. This means that the higher WHC observed can be attributed to a higher amount of insoluble aggregates rather than altered water-binding properties of that insoluble phase.

The viscosity of all the SPFs increased with the increase of SPF concentration, but all samples showed a tendency to a plateau value for the viscosity. This plateau-value can be related to the phase transition of the dispersion. Once the concentration of SPF approached the point, the dispersion with colloidal protein aggregates might undergo a liquid-solid transition to the soft glass phase state (Xu, Atrens, & Stokes, 2018). Above this concentration, the flowability of SPF particles wares measured rather than the viscosity of the SPF dispersions. This result can be correlated to the particle size distribution under room temperature, while larger aggregates are more efficient in obstructing the flow than the ones that are heated at lower temperatures or without heating and formed smaller aggregates, thus, the plateaus differ. Besides the concentration, the increased viscosity caused by higher moisture heating temperature might correspond with the NSI and WHC_P of SPF. The SPFs processed with higher temperature mainly consisted of large protein aggregates that obstruct the flow, thereby increasing the viscosity. In addition, the WHC_P results showed that the insoluble fractions in SPFs could take up water from the aqueous phase. This lowered the water in the aqueous phase and increased the hydrodynamic volume in the system, and thus the effective protein concentration (Purwanti, Moerkens, Van der Goot, & Boom, 2012; Rickert, Johnson, & Murphy, 2004).

For the gelling properties, the G′ values were higher than G″ of all the SPF dispersions within the linear viscoelastic region (LVR), showing that a kind of network was formed for all the SPF samples with 15% concentration already at room temperature. The results from the temperature sweep were correlated to the denaturation level of the SPFs and the onset T_d of 7S and 11S. In thermal gelation, the protein unfolds, exposes hydrophobic patches that produce a gel network, while the further

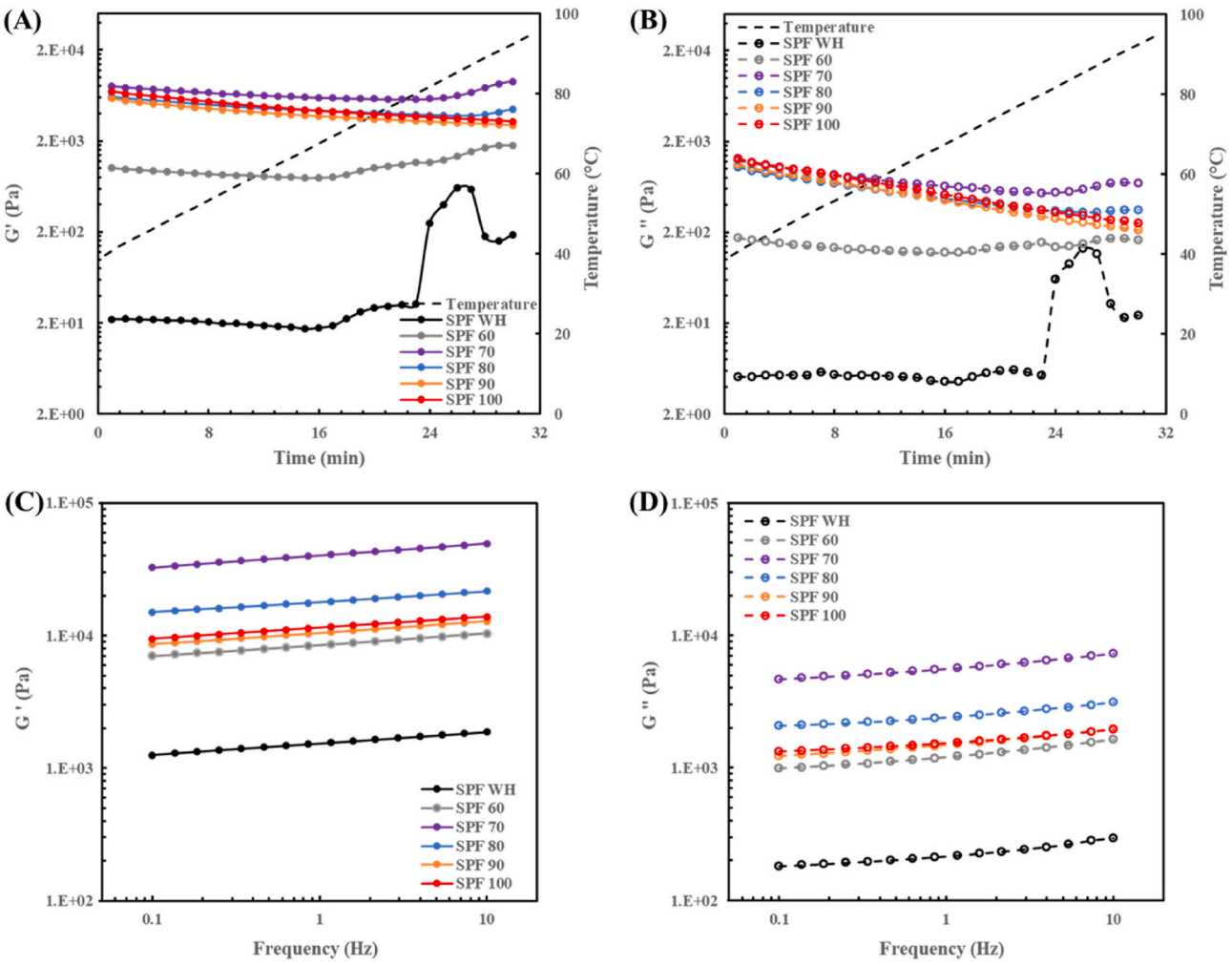

Fig. 7. (A) G′ and (B) G″ values during the temperature sweep; (C) G′ and (D) G″ values during the frequency sweep after heating for all the SPF dispersions (15% wt.) at a strain of 1%.

Table 3
Average elastic moduli (G') and loss factors (tan δ) of the gelled soy protein fractions obtained at a frequency of 1 Hz after temperature sweep.

Sample names	G' (Pa)	tan δ
SPF WH	1296 ± 368	0.14 ± 0.00
SPF 60	8406 ± 280	0.13 ± 0.01
SPF 70	37813 ± 4193	0.14 ± 0.00
SPF 80	17942 ± 247	0.13 ± 0.00
SPF 90	14265 ± 5218	0.14 ± 0.00
SPF 100	11778 ± 177	0.13 ± 0.00

association of the proteins follows (Hermansson, 1986). After the onset of thermal gelation, more denatured proteins become incorporated into the initial network leading to a further build-up of structure, and therefore, an increase in G′ can be observed on further heating (Renkema & Van Vliet, 2002). SPF WH and SPF 60 were still in their native state; when the heating temperature was above the onset T_d of 7S, the structure of 7S proteins was able to change thereby contributing to the gel formation. Similarly, when the temperature was approaching the T_d of 11S, the G′ of SPF 70 and SPF 80 started to increase. The decreased G′-values found for SPF 90 and SPF 100 might be caused by the fact that no additional protein interactions could be formed due to the fact that the proteins were fully denatured by the moisture heating with high temperature already. Commercial SPI and SPC also showed a similar decreasing trend in G′ value during temperature sweep measurement as SPF 90 and SPF 100, and they were fully denatured as well before the measurements (Ahmed, Ramaswamy, & Alli, 2006; Li, Yeh, & Fan, 2007).

The G′ and G″ of all the SPFs increased after cooling. The gel formed by SPF 70 showed the highest elastic behavior among all the SPFs, followed by SPF 80, which could be considered as the result of partial denaturation. The gel network is formed by cross-linked soy proteins and their aggregates, while the participation of 7S and 11S subunits in the gel network is different (Wu et al., 2018). In both SPF 70 and SPF 80, the 7S protein was denatured while 11S remained native before the measurements. This means the dissociated subunits of 7S probably have unfolded, aggregated and networked evenly with themselves. Subsequently, the temperature sweep till 95 °C caused the denaturation of 11S, allowing the dissociated subunits of 11S to associate with the networks of 7S to form a more ordered and strong gel structure (Liu, Chang, Li, & Tatsumi, 2004; Peng, Ren, & Guo, 2016). Unlike SPF 70 and SPF 80, both 7S and 11S denatured simultaneously during the process for SPF 90 and SPF 100, lacking the ability of 11S proteins to interact with the network of 7S proteins formed during the moisture heating treatment (Tang, 2007). This hypothesis can be partially proven by the results of particle size distribution. The changes in the particle size of SPF 90 and SPF 100 were relatively small as compared to other SPFs while additional heating was applied. Besides, the results presented in this paper are aligned with previous studies, which have demonstrated that SPI with pre-denaturation degree (PDD) of 22.28% and 86.11% had higher G′ than the native SPI (PDD 0%) and the completely

pre-denatured SPI (PDD 100%) during gel formation process, and the formed gel was denser with more uniform gel networks (Li et al., 2020). Moreover, practically, researchers have reported that separate denaturation of 7S and 11S by two-step heating significantly increased the tofu's apparent Young's modulus and breaking strength during tofu production (Liu & Chang, 2007; Liu, Chang, Li, & TeTatsumi, 2004; Lu, Lu, Yin, Cheng, & Li, 2010). Overall, soy proteins with selective denaturation levels could form a network with high gel elasticity, while complete denaturation was not absolutely required. This concept can be used optimally when developing fractionation processes that use mild conditions that allow controlled protein denaturation, which can be used to create a much broader range of functional properties using SPF.

5. Conclusion

Modern and new food products require soy ingredients with different functionalities than originally aimed for. Simply fractionated SPF without heating treatment (SPF WH) presented properties that are very suitable for applications like emulsification and foaming, but less optimal for modern applications such as meat analogue, while isochoric moisture heating step with mild conditions investigated in this study can be used to precisely control properties of soy ingredients without impacting the composition, yield and powder morphology. With increasing temperature, proteins in SPFs became more denatured, with complete denaturation at the highest temperature applied. In addition, WHC and viscosity of SPFs gradually increased correlated with the lower NSI. Besides, SPF 100 exhibited the highest OAC value, while the use of partial denaturation led to gels with the highest elastic behaviors. Overall, mild heating conditions allow the possibility to tune properties of simply fractionated SPFs through the moisture heating step, and therefore, can be potentially desired for specific novel applications.

CRediT authorship contribution statement

Yu Peng: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Visualization, Validation, Writing – original draft. **Konstantina Kyriakopoulou:** Conceptualization, Validation, Writing – review & editing, Supervision. **Afina Rahmani:** Investigation, Data curation, Visualization. **Paul Venema:** Validation, Writing – review & editing. **Atze Jan van der Goot:** Conceptualization, Writing – review & editing, Supervision.

Acknowledgments

The authors would like to thank the China Scholarship Council (CSC) and Plant Meat Matters project for financial support. The Plant Meat Matters project is co-financed by the Top Consortium for Knowledge and Innovation Agri & Food by the Dutch Ministry of Economic Affairs; the project is registered under contract number TKI-AF-16011.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111979.

References

Ahmed, J., Ramaswamy, H. S., & Alli, I. (2006). Thermorheological characteristics of soybean protein isolate. *Journal of Food Science, 71*, E158–E163. https://doi.org/10.1111/j.1365-2621.2006.tb15629.x

Alavi, F., Chen, L., Wang, Z., & Emam-Djomeh, Z. (2021). Consequences of heating under alkaline pH alone or in the presence of maltodextrin on solubility, emulsifying and foaming properties of faba bean protein. *Food Hydrocolloids, 112*, 106335. https://doi.org/10.1016/j.foodhyd.2020.106335

Alibhai, Z., Mondor, M., Moresoli, C., Ippersiel, D., & Lamarche, F. (2006). Production of soy protein concentrates/isolates: Traditional and membrane technologies. *Desalination, 191*, 351–358. https://doi.org/10.1016/j.desal.2005.05.026

Berghout, J. A. M., Boom, R. M., & Van Der Goot, A. J. (2014). The potential of aqueous fractionation of lupin seeds for high-protein foods. *Food Chemistry, 159*, 64–70. https://doi.org/10.1016/j.foodchem.2014.02.166

Berghout, J. A. M., Venema, P., Boom, R. M., & Van der Goot, A. J. (2015). Comparing functional properties of concentrated protein isolates with freeze-dried protein isolates from lupin seeds. *Food Hydrocolloids, 51*, 346–354. https://doi.org/10.1016/j.foodhyd.2015.05.017

Bühler, J. M., Dekkers, B. L., Bruins, M. E., & van der Goot, A. J. (2020). Modifying faba bean protein concentrate using dry heat to increase water holding capacity. *Foods, 9*, 1077. https://doi.org/10.3390/foods9081077

Campbell, K. A., Glatz, C. E., Johnson, L. A., Jung, S., De Moura, J. M. N., Kapchie, V., et al. (2011). Advances in aqueous extraction processing of soybeans. *JAOCS, J. Am. Oil Chem. Soc.*. https://doi.org/10.1007/s11746-010-1724-5

Cruz, N. S., Capellas, M., Jaramillo, D. P., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009). Soymilk treated by ultra high-pressure homogenization: Acid coagulation properties and characteristics of a soy-yogurt product. *Food Hydrocolloids, 23*, 490–496. https://doi.org/10.1016/j.foodhyd.2008.03.010

Damodaran, S. (1988). Refolding of thermally unfolded soy proteins during the cooling regime of the gelation process: Effect on gelation. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf00080a007

Das, A. K., Anjaneyulu, A. S. R., & Kondaiah, N. (2006). Development of reduced beany flavor full-fat soy paste for comminuted meat products. *Journal of Food Science*. https://doi.org/10.1111/j.1750-3841.2006.00045.x

Deak, N. A., & Johnson, L. A. (2007). Effects of extraction temperature and preservation method on functionality of soy protein. *JAOCS, J. Am. Oil Chem. Soc., 84*, 259–268. https://doi.org/10.1007/s11746-007-1035-7

Dehnad, D., Jafari, S. M., & Afrasiabi, M. (2016). Influence of drying on functional properties of food biopolymers: From traditional to novel dehydration techniques. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2016.09.002

Geerts, M. E. J., Dekkers, B. L., van der Padt, A., & van der Goot, A. J. (2018). Aqueous fractionation processes of soy protein for fibrous structure formation. *Innovative Food Science & Emerging Technologies, 45*, 313–319. https://doi.org/10.1016/J.IFSET.2017.12.002

Geerts, M., van Veghel, A., Zisopoulos, F. K., van der Padt, A., & Jan van der Goot, A. (2018). Exergetic comparison of three different processing routes for yellow pea (Pisum sativum): Functionality as a driver in sustainable process design. *Journal of Cleaner Production, 183*, 979–987. https://doi.org/10.1016/j.jclepro.2018.02.158

Guo, J., Yang, X. Q., He, X. T., Wu, N. N., Wang, J. M., Gu, W., et al. (2012). Limited aggregation behavior of β-conglycinin and its terminating effect on glycinin aggregation during heating at pH 7.0. *Journal of Agricultural and Food Chemistry, 60*, 3782–3791. https://doi.org/10.1021/jf300409y

Hermansson, A. M. (1986). Soy protein gelation. *Journal of the American Oil Chemists Society, 63*, 658–666. https://doi.org/10.1007/BF02638232

Hickisch, A., Bindl, K., Vogel, R. F., & Toelstede, S. (2016). Thermal treatment of lupin-based milk alternatives – impact on lupin proteins and the network of respective lupin-based yogurt alternatives. *Food Research International, 89*, 850–859. https://doi.org/10.1016/j.foodres.2016.10.013

Jeewanthi, R. K. C., & Paik, H. D. (2018). Modifications of nutritional, structural, and sensory characteristics of non-dairy soy cheese analogs to improve their quality attributes. *Journal of Food Science & Technology*. https://doi.org/10.1007/s13197-018-3408-3

Jones, O. G. (2016). Recent advances in the functionality of non-animal-sourced proteins contributing to their use in meat analogs. *Curr. Opin. Food Sci.*. https://doi.org/10.1016/j.cofs.2015.08.002

Jung, S., Lamsal, B. P., Stepien, V., Johnson, L. A., & Murphy, P. A. (2006). *Functionality of soy protein produced by enzyme-assisted extraction 83*.

Kovalenko, I. V., & Briggs, J. L. (2002). Textural characterization of soy-based yogurt by the vane method. *Journal of Texture Studies, 33*, 105–118. https://doi.org/10.1111/j.1745-4603.2002.tb01338.x

Krul, E. S. (2019). Calculation of nitrogen-to-protein conversion factors: A review with a focus on soy protein. *Journal of the American Oil Chemists Society, 96*, 339–364. https://doi.org/10.1002/aocs.12196

Kyriakopoulou, K., Keppler, J. K., & van der Goot, A. J. (2021). Functionality of ingredients and additives in plant-based meat analogues. *Foods, 10*, 600. https://doi.org/10.3390/foods10030600

L'hocine, L., Boye, J. I., & Arcand, Y. (2006). Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction procedures. *Journal of Food Science, 71*, C137–C145. https://doi.org/10.1111/j.1365-2621.2006.tb15609.x

Li, X., Chen, L., Hua, Y., Chen, Y., Kong, X., & Zhang, C. (2020). Effect of preheating-induced denaturation during protein production on the structure and gelling properties of soybean proteins. *Food Hydrocolloids, 105*, 105846. https://doi.org/10.1016/j.foodhyd.2020.105846

Lin, M. J. Y., Humbert, E. S., & Sosullski, F. W. (1974). Certain functional properties OF sunflower meal products. *Journal of Food Science, 39*, 368–370. https://doi.org/10.1111/j.1365-2621.1974.tb02896.x

Liu, Z. S., & Chang, S. K. C. (2007). Soymilk viscosity as influenced by heating methods and soybean varieties. *Journal of Food Processing and Preservation, 31*, 320–333. https://doi.org/10.1111/j.1745-4549.2007.00128.x

Liu, Z. S., Chang, S. K. C., Li, L. Te, & Tatsumi, E. (2004). Effect of selective thermal denaturation of soybean proteins on soymilk viscosity and tofu's physical properties. *Food Research International, 37*, 815–822. https://doi.org/10.1016/j.foodres.2004.04.004

Liu, J. J., Gasmalla, M. A. A., Li, P., & Yang, R. (2016). Enzyme-assisted extraction processing from oilseeds: Principle, processing and application. *Innovative Food Science & Emerging Technologies*. https://doi.org/10.1016/j.ifset.2016.05.002

Li, Q., Xia, Y., Zhou, L., & Xie, J. (2013). Evaluation of the rheological, textural, microstructural and sensory properties of soy cheese spreads. *Food and Bioproducts Processing, 91*, 429–439. https://doi.org/10.1016/j.fbp.2013.03.001

Li, J. Y., Yeh, A. I., & Fan, K. L. (2007). Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. *Journal of Food Engineering, 78*, 1240–1247. https://doi.org/10.1016/j.jfoodeng.2005.12.043

Lu, X., Lu, Z., Yin, L., Cheng, Y., & Li, L. (2010). Effect of preheating temperature and calcium ions on the properties of cold-set soybean protein gel. *Food Research International, 43*, 1673–1683. https://doi.org/10.1016/j.foodres.2010.05.011

Moure, A., Sineiro, J., Domínguez, H., & Parajó, J. C. (2006). Functionality of oilseed protein products: A review. *Food Research International*. https://doi.org/10.1016/j.foodres.2006.07.002

Nazareth, Z. M., Deak, N. A., & Johnson, L. A. (2009). Functional properties of soy protein isolates prepared from gas-supported screw-pressed soybean meal. *JAOCS, J. Am. Oil Chem. Soc., 86*, 315–321. https://doi.org/10.1007/s11746-009-1360-0

Nazir, A., Asghar, A., & Aslam Maan, A. (2016). Food gels: Gelling process and new applications. In *Advances in food rheology and its applications* (pp. 335–353). https://doi.org/10.1016/B978-0-08-100431-9.00013-9

Obatolu, V. A., Fasoyiro, S. B., & Ogunsunmi, L. (2007). Processing and functional properties of yam beans (sphenostylis stenocarpa). *Journal of Food Processing and Preservation*. https://doi.org/10.1111/j.1745-4549.2007.00112.x

Peng, Y., Dewi, D. P. A. P., Kyriakopoulou, K., & van der Goot, A. J. (2020a). Effect of calcium hydroxide and fractionation process on the functional properties of soy protein concentrate. *Innovative Food Science & Emerging Technologies, 102501*. https://doi.org/10.1016/j.ifset.2020.102501

Peng, Y., Kersten, N., Kyriakopoulou, K., & van der Goot, A. J. (2020b). Functional properties of mildly fractionated soy protein as influenced by the processing pH. *Journal of Food Engineering, 275*, 109875. https://doi.org/10.1016/j.jfoodeng.2019.109875

Peng, X., Ren, C., & Guo, S. (2016). Particle formation and gelation of soymilk: Effect of heat. In *Trends in food science and Technology*. https://doi.org/10.1016/j.tifs.2016.06.005

Peters, J. P. C. M., Vergeldt, F. J., Boom, R. M., & van der Goot, A. J. (2017). Water-binding capacity of protein-rich particles and their pellets. *Food Hydrocolloids, 65*, 144–156. https://doi.org/10.1016/j.foodhyd.2016.11.015

Pojić, M., Mišan, A., & Tiwari, B. (2018). Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2018.03.010

Porras-Saavedra, J., Palacios-González, E., Lartundo-Rojas, L., Garibay-Febles, V., Yáñez-Fernández, J., Hernández-Sánchez, H., et al. (2015). Microstructural properties and distribution of components in microparticles obtained by spray-drying. *Journal of Food Engineering, 152*, 105–112. https://doi.org/10.1016/J.JFOODENG.2014.11.014

Purwanti, N., Moerkens, A., Van der Goot, A. J., & Boom, R. (2012). Reducing the stiffness of concentrated whey protein isolate (WPI) gels by using WPI microparticles. *Food Hydrocolloids, 26*, 240–248. https://doi.org/10.1016/j.foodhyd.2011.05.015

Renkema, J. M. S., & Van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*, 1569–1573. https://doi.org/10.1021/jf010763l

Rickert, D. A., Johnson, L. A., & Murphy, P. A. (2004). Functional properties of improved glycinin and β-nglycinin fractions. *Journal of Food Science, 69*, FCT303–FCT311. https://doi.org/10.1111/j.1365-2621.2004.tb06332.x

Rueb, C. J., & Zukoski, C. F. (1997). Viscoelastic properties of colloidal gels. *Journal of Rheology, 41*, 197–218. https://doi.org/10.1122/1.550812

Tang, C. H. (2007). Effect of thermal pretreatment of raw soymilk on the gel strength and microstructure of tofu induced by microbial transglutaminase. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 40*, 1403–1409. https://doi.org/10.1016/j.lwt.2006.09.006

Wang, C., & Johnson, L. A. (2001). Functional properties of hydrothermally cooked soy protein products. *JAOCS, J. Am. Oil Chem. Soc., 78*, 189–195. https://doi.org/10.1007/s11746-001-0242-y

Wu, C., Navicha, W. B., Hua, Y., Chen, Y., Kong, X., & Zhang, C. (2018). Effects of removal of non-network protein on the rheological properties of heat-induced soy protein gels. *Lebensmittel-Wissenschaft & Technologie, 95*, 193–199. https://doi.org/10.1016/j.lwt.2018.04.077

Xu, Y., Atrens, A. D., & Stokes, J. R. (2018). "liquid, gel and soft glass" phase transitions and rheology of nanocrystalline cellulose suspensions as a function of concentration and salinity. *Soft Matter, 14*, 1953–1963. https://doi.org/10.1039/c7sm02470c

Zhang, M., Yang, Y., & Acevedo, N. C. (2020). Effects of pre-heating soybean protein isolate and transglutaminase treatments on the properties of egg-soybean protein isolate composite gels. *Food Chemistry, 318*, 126421. https://doi.org/10.1016/j.foodchem.2020.126421

Zhao, Q., Xiong, H., Selomulya, C., Chen, X. D., Huang, S., Ruan, X., et al. (2013). Effects of spray drying and freeze drying on the properties of protein isolate from rice dreg protein. *Food and Bioprocess Technology, 6*, 1759–1769. https://doi.org/10.1007/s11947-012-0844-3