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Functional properties of mildly fractionated soy protein as influenced by the processing pH

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ABSTRACT

In this study an alternative mild fractionation process for the extraction of soy protein is investigated; aqueous fractionation, in which oil extraction and intensive washing steps are omitted. Moreover, a pH adjustment is proposed instead of the conventional neutralization step. The mildly fractionated soy protein fractions (SPFs) showed higher protein and oil content compared to commercial soy protein isolate. The process retained the proteins' native state. SPFs adjusted at pH 4.5 and 5.5 (close to pI) formed a powdery texture, resulting in larger size particles after dispersion in water. Despite their low nitrogen solubility index, water holding capacity and viscosity, when mixed with flour these SPFs presented the highest G* values. A flaky texture and reversed properties were observed with SPF adjusted at pH away from the pI. The range of properties achieved exhibits new routes in creating soy protein ingredients with desired functionality, avoiding over-processing due to post-treatment modifications.

1. Introduction

Nowadays, plant-based protein demand is increasing rapidly around the world because of the awareness among the consumers of sustainable diets and food production (Reipurth et al., 2019; Rizzo and Baroni, 2018). To meet the market's demands, the industry is in search of protein-rich crops and more sustainable protein extraction methods. The primary use of several crops is reconsidered, and with the development of new technologies, their potential use is also broadened. An important source in this transition is soybean, which currently already presents a production of 350 million metric tons in the 2017/2018 market year. The primary reason for soy bean production is to produce oil for food and bioenergy, while the left meal is destined for animal feed (Stutte et al., 2018). Approximately 2% of soybean protein is nowadays consumed directly by humans (Nishinari et al., 2017), but its potential to produce protein-rich food in a palatable form is gaining attention (Kumar et al., 2017; Post, 2012). Hence, soybean protein ingredients, which were co-products from oil extraction before, are becoming gradually one of the main products of soybean processing for human consumption.

Novel products like soy-based protein-rich beverages and meat analogues have one thing in common, the use of soy protein products, isolates (SPI) or concentrates (SPC), as ingredients instead of whole soybeans or soybean flour, which are used for more traditional products like soy milk and tofu. It means that the fractionation process is indispensable in the processing chain of such modern foods. However, the conventional approach of producing SPI and SPC often involves oil extraction steps (using organic solvents) and/or several washing steps, which are energy and resource-intensive (Berghout et al., 2014). The large requirements of water and solvents reduce the sustainability potential of novel soy-based foods, sometimes even leading to the opposite effect than the initial purpose (Berghout et al., 2015a, 2015b). Moreover, except for the adverse effects of intensive fractionation, the functionalities of SPI and SPC are not targeting these novel products. Soy protein ingredients are mostly used as industrial materials like adhesives or as food additives (Nishinari et al., 2017; Vnučec et al., 2017). In case of food applications, functionality is focussed on their use in general applications for maximizing stability of liquid products and high-fat food systems (Rizzo and Baroni, 2018), which could be achieved best with high protein solubility However, for novel applications like soy burgers or soy sausages, soy ingredients are expected to form a gel or a fibrous structure. This means different functionalities like gelling, water holding capacity and viscoelastic properties are requested rather than only solubility. Overall, a fractionation process with minimal environmental impact is needed, while aiming in parallel for specific functional properties.

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To meet the requests from the new market, mild fractionation process with pH adjustment step is explored in this study. Mild fractionation process is based on conventional aqueous fractionation but with some steps modified. Soy oil is removed by centrifugation instead of organic solvent extraction, and intensive washing steps are omitted. The whole soybean is taken as the starting material, and water is used as an extracting medium. Although the oil is separated by simple centrifugation, such process performed in oilseeds was found to recover equally high oil yields as with organic solvent extraction (Campbell et al., 2011; Rosenthal et al., 1996). The oil extracted in the form of oil-bodies also can be further used as ingredients for emulsion stabilization (Karefyllakis et al., 2019b, 2019a). Furthermore, pH adjustment is applied instead of neutralization step during fractionation. It has been reported that the functional properties of soy protein can vary dramatically at different pH-values (Idris et al., 2003; Voutsinas et al., 1983). But most studies have investigated the effect of pH on the functional properties of plant protein by adding an extra pH adjustment step to dried isolates or concentrates (Benelhadj et al., 2016; Jiang et al., 2018, 2010; Kim et al., 2016), which introduces more salt in the system as well. Limited experiments were designed to achieve specific functional properties of plant protein by adjusting pH value directly during fractionation process.

In this study, soy protein fractions (SPFs) were obtained by mild fractionation and standardized on different final pH-values. The fractions were tested on their NSI, WHC, microstructure, denaturation and structural behaviours to evaluate their potential for use in multiple novel soy-food applications. The objective of this research was to gain insight on how the processing pH impacts the functional behaviour of mildly fractionated soy protein. We believe this will unlock new routes in creating protein-rich ingredients with desired functionality for specific applications, avoiding over-processing due to post-treatment modifications.

2. Materials and methods

2.1. Materials

Dry, full soybean was obtained from FRANK Food Products (the Netherlands) and was harvested in Canada in October/November of 2016. The HCl and NaOH used for pH adjustments during fractionation were purchased from Sigma Aldrich (Germany). The water used was purified in a Milli-Q Lab Water System (Milli-Q IQ 7000 Ultrapure Lab Water System, Merck KGaA, Darmstadt, Germany).

2.2. Methods

2.2.1. Soy flour preparation

Firstly, soybeans were pre-milled by using a pin mill LV 15M (Condux-Werk, Wolfgang bei Hanau, Germany) into grits. Then, the soy grits were further milled by a ZPS50 impact mill (Hosokawa-Alpine, Augsburg, Germany) into full-fat soy flour (FFSF). The impact mill was set according to the parameters described by Pelgrom (Pelgrom et al., 2015) with slight changes: feed rate was around 5 rpm, impact mill speed was 8000 rpm, airflow at 80 m^3/h and a classifier wheel speed of 2500 rpm. Full-fat soy flour was stored at 4 °C for further use.

2.2.2. Mild fractionation process and pH adjustments

The mild fractionation process used in this study was based on previous research (Geerts et al., 2018) with additional modification for a pH adjustment step. Five different soy protein fractions (SPFs) were produced using a final step with pH varying from 3.5 to 7.5. An overview of all processing conditions can be found in Fig. 1. Full-fat soy flour (FFSF) was firstly mixed with water (20% w/w). The pH of the mixture was adjusted between 8 and 9 with 1M NaOH solution. Then, the suspension was stirred for an hour to solubilize the proteins and, subsequently, centrifuged (10,000 rpm, 30 min, 25 °C). After centrifugation, the supernatant was poured through a cheese-cloth to separate the semi-solid cream layer from the soluble proteins. The cream layer and pellet were discarded. The pH of the protein-rich supernatant was adjusted between 4.5 and 5 by adding 1M HCl. The suspension was stirred for one more hour and subsequently centrifuged again (10,000 rpm, 30 min, 20 °C). The pellet, containing the soy protein fraction was collected for further pH adjustments.

Each protein-rich pellet was mixed with water and stirred for at least 1 h. The pH of the suspension was adjusted to various levels (3.5, 4.5, 5.5, 6.5 and 7.5) with 1M HCl or 1M NaOH, and was checked for every half an hour. Once the pH value became constant, the suspensions were stirred overnight and subsequently freeze-dried (Christ, Germany). The freeze-dried SPFs were milled (Rotormill Pulverisette 14, Fritsch Germany) into powder using a sieve ring with a perforation size of 0.5 mm and a rotation speed of 6000 rpm. The obtained SPFs were stored in the refrigerator for further analysis. All the SPFs were prepared in triplicate.

2.2.3. Composition analysis

The protein content was determined by using Dumas analysis (Nitrogen analyzer, FlashEA 1112 series, Thermo Scientific, the Netherlands) with a nitrogen-to-protein conversion factor of 5.7. The oil content was determined by using petroleum ether as an extraction solvent with a Buchi extraction system B-811LSV (Buchi Labortechnik AG, Flawil, Switzerland). The ash content was determined by AACC official method (AACC International, 1999). The carbohydrate content was calculated as the difference between the dry matter content and the other components that were measured before. The protein yield was calculated from the measured protein content in the SPFs relative to the total protein content of starting full-fat soy flour. All the measurements were performed in triplicate.

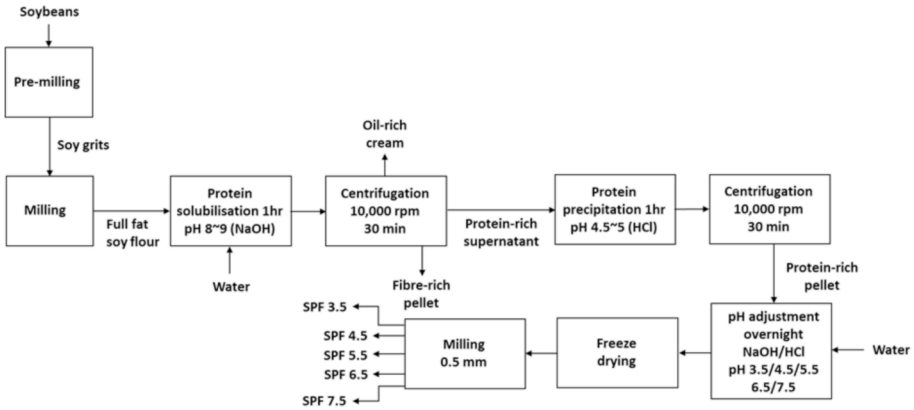

Fig. 1. Mild fractionation process for all the SPF samples.

2.2.4. Nitrogen solubility index (NSI) and water holding capacity (WHC)

The WHC-values of SPFs were determined using the method described by Geerts (Geerts et al., 2018) with slight modifications. For each SPF, a 2% (w/v) dispersion was placed in a centrifuge tube and shaken overnight. Then, the dispersion was centrifuged (10,000 rpm, 30 min, 25 °C) to separate the supernatant and pellet. The tubes were drained on tissue paper and the pellets were weighed. Subsequently, the pellets were oven-dried and weighed again. The nitrogen contents in the oven-dried pellets were measured by using Dumas analysis. The WHC was calculated by the ratio of the wet pellet weight over the dried pellet weight. 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 triplicates.

2.2.5. Microscopic analysis

Scanning electron microscope (SEM, Phenom Pure G2, Phenom-world BV, Eindhoven, The Netherlands) was performed for viewing the microstructures of the SPF powders with different processing pH. Each powder sample was evenly fixed on an aluminum sample holder by carbo tabs. In addition, 1% (w/v) protein dispersions were evaluated as well. For these measurements, an upright microscope Axioscope (Carl Zeiss Microscopy, LLC, USA) was used for the visualization. The protein dispersions were prepared by mixing different SPF powders with water for around 1 h under magnetic stirring. The images were captured by the connected video camera and acquisition software.

2.2.6. Particle size analysis

The particle size distribution of SPFs produced under different processing pH was measured with a laser light scattering instrument (Mastersizer 3000, Malvern Instruments Ltd, United Kingdom) and a wet module (Hydro SM, Malvern Instruments Ltd, United Kingdom). For these measurements, 1% (w/v) protein dispersions were prepared with Milli-Q water. 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 triplicates.

2.2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Diamond DSC, PerkinElmer, USA) was used to measure the denaturation temperature and the enthalpy of the transition of the SPF samples. Firstly the DSC was calibrated with indium, and an empty stainless pan was used as a reference. The SPF powders were mixed with water (20 g sample/100 g total). The samples were scanned from 20 °C to 150 °C with a heating rate of 5 °C/min. Measurements were analyzed with Start Pyris Software for denaturation temperature and enthalpy of transition. All the measurements were performed in triplicates.

2.2.8. Rheological behaviors

Two types of rheometers were used to analyze the rheological properties of SPF samples: stress-controlled rheometer (Anton Paar GmbH MCR502, Austria) and closed cavity rheometer (CCR, RPA elite, TA instrument, USA).

In the stress-controlled rheometer, a plate-plate geometry (PP-25/P2) was used to obtain the rheological properties of SPF dispersions. Each SPF powder was suspended in water (12 g sample/100 g total) and was stirred for 1 h before transferring to the rheometer. Then, the SPF dispersion was equilibrated for 5 min, and a shear rate sweep was performed at 25 °C in steady-state with an increasing shear rate. The range of the shear rate was set from 1 to 100 s^{-1} (Berghout et al., 2014).

Based on previous research, a mixture of SPF with FFSF in a ratio 70/30 was prepared, and the SPF-FFSF mixture was added to the NaCl solution (1 wt% NaCl in the total blend) to obtain a 44% dry matter (Dekkers et al., 2018; Geerts et al., 2018). The rheological properties of soy protein fraction-full fat soy flour (SPF-FFSF) blends were determined with a closed cavity rheometer (CCR). After the blends were hydrated in vacuum bags for 30 min, approximately 5 g sample was placed between two plastic films and transferred to a CCR. An oscillation time sweep was performed with frequency 10 Hz, strain 80% for 15 min under 140 °C. In order to prevent water evaporation during measurement, a down pressure of 4.5 bar was used to close the CCR.

3. Results and discussion

In this study, mildly fractionated SPFs were obtained with different processing pH values. The fractions were examined on their chemical composition, microstructure and functional properties. The connection between mild fractionation, processing pH value and functional properties of SPFs was analyzed, and the potential application of the produced SPFs was further discussed based on process parameters and properties.

3.1. Chemical composition

The chemical composition of all the SPFs was identical since the pH adjustment step was performed as the last step before drying. The results showed that mild fractionation process achieved SPFs with protein content around 85.3%, while the commercial SPI was 83.3% (Table 1). This was unexpected since the intensive washing steps during conventional fractionation were skipped in this study. The yield of mild fractionation process reached up to 55.8%, which was also higher than that reported yields of isolates produced from soy flour (30–40%) by the conventional processes (Alibhai et al., 2006; De Moura et al., 2011).

In terms of oil content, the start material, full-fat soy flour, contained around 21.8% of oil. During the mild fractionation process, after alkaline treatment, the centrifugation step was meant to separate protein-rich supernatant from the fibre-rich pellet, however the oil was separated as well because of the low density. Therefore, no extra defatting step was needed. 2.3% oil remained in SPFs while no residual oil was left in commercial SPI. Nevertheless, compared with organic solvent defatting step, mild fractionation can lead to soy protein ingredients with clean labels and less environmental impact, which is of great interest in the industry currently.

3.2. Nitrogen solubility index (NSI) and water holding capacity (WHC)

Protein solubility and water holding capacity (WHC) are fundamental functional properties of soy protein ingredients and can be influenced by many processing parameters during fractionation, such as pH, salt content and drying methods (Kinsella, 1976). Among those, the processing pH can influence the protein's net charge and conformation, which could lead to the exposure or burial of the water binding sites of the proteins, thus to different properties regarding NSI and WHC.

In this study, dispersions of SPFs (2% (w/v)) were used for the NSI and WHC measurements. The pH of the SPF dispersions was not adjusted further since it was quite close to the initial processing pH (results not shown). The plots of NSI and WHC values against the processing pH (Fig. 2A and Fig. 2B) resulted in characteristic U-shaped curves. The lowest values of NSI occurred at processing pH of 4.5 and 5.5, which is around the isoelectric pH (pI) of soy protein according to literature (Arrese et al., 1991). The NSI of SPF increased dramatically when

Table 1
Composition of a number of different SPFs, commercial SPI, and full-fat soy flour (mean value ± standard deviation (n = 3), dry basis).

	Protein (N × 5.7)	Oil	Ash	Carbohydrate	Protein yield (%)
Full-fat soy flour	37.0 ± 1.1	21.8 ± 0.4	6.7 ± 0.7	34.5 ± 2.2	
Commercial SPI	83.3 ± 0.7	0.0 ± 0.0	3.4 ± 0.0	13.3 ± 0.7	
SPFs	85.3 ± 6.2	2.3 ± 0.0	2.1 ± 0.1	10.3 ± 6.3	55.8 ± 6

adjusting the processing pH away from pI in both sides, for example in the case of pH 6.5 or higher, or pH below 3.5. Specifically, SPF 7.5 showed the highest NSI (mean value 91.6%), which was even higher than the commercial SPI (mean value 62.2%) measured in this study.

The WHC of the SPF 4.5 was the lowest as well, and the WHC increased when more basic or more acidic pH was used. This is because the net charge on the soy protein is around zero when the processing pH is around the isoelectric region. At that point the protein-protein interactions are maximal, and less water binding sites are exposed (Idris et al., 2003). Subsequently, with an increase in the net charge of protein, the polarity of protein increases, resulting in an increase in the amount of bound water. Thus, compared with other SPFs, SPF 7.5 exhibited the maximum value of the WHC (mean value 2.52 g/g SPF), while higher WHC (mean value 9.17 g/g SPI) was detected from the commercial SPI.

To further investigate the effect of the fractionation process in combination with the pH adjustment to the NSI and WHC, commercial SPI was dissolved and its pH was adjusted prior to drying similarly to the SPFs. In this case, the reducing trends of both NSI and WHC were observed instead of typical U-shaped curves when the pH of SPI adjusted from 7.5 to 3.5 (results are shown in Appendix 1). This argues that an extra pH adjustment step for commercial SPI could produce ingredients of varying specifications in terms of NSI and WHC, but different than preforming the pH adjustment during fractionation process. Moreover, this additional dissolution and drying steps also reduce the sustainability of the overall soy protein process and produced more salt in the ingredient, which is undesirable for future product development.

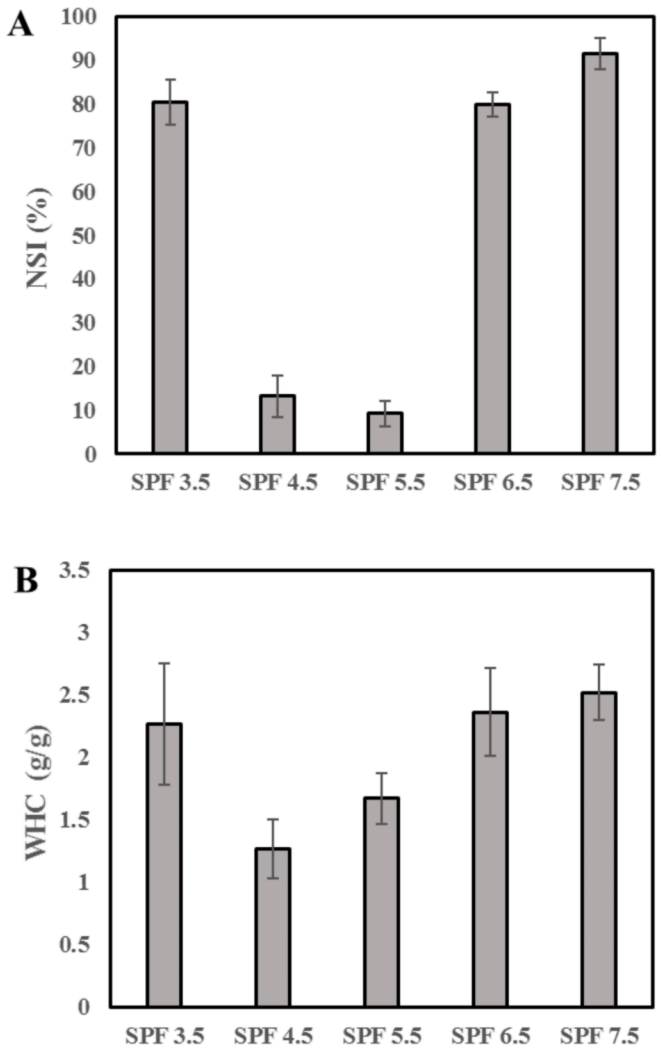

Fig. 2. (A) Nitrogen solubility index (NSI) and (B) water holding capacity (WHC) for SPFs with different processing pH.

3.3. Microstructure and particle size

At the end of the mild fractionation process, all the SPFs were freeze-dried and milled into powder. The processing pH strongly influenced the microstructure of SPFs, as revealed by microscopy and particle size distribution analysis. Visible inspection of the powders revealed that SPF 4.5 and SPF 5.5 form finer, sand-like powders, while SPF 3.5, SPF 6.5 and SPF 7.5 resulted in more glassy-like flakes. The structural differences between SPFs became more obvious under SEM (Fig. 3). Glass-like pieces were observed in the SEM images of SPF 3.5, SPF 6.5 and SPF 7.5 while more blocky and spherical pieces were seen in SPF 4.5 and 5.5. These differences can be explained by protein net charge and freeze-drying process. When the protein net charge was low (processing pH around pI), most of the soy proteins were present in highly aggregated and even precipitate state and, thus, large particles were formed in the system and spherical structures were observed. In contrast, at pH far away from pI, most of the proteins would be completely solubilized in the suspensions, as a result, a continuous structure was formed and glass-like structures were observed after milling. The pH adjustment step resulted in these microstructural differences, as evidenced also from the SEM image of SPF 5.5 (Fig. 3C), because both spherical and glass-like shapes were found, indicating that transformation happened when the processing pH increased from 4.5 (Figure 3B) to 6.5 (Fig. 3D). The glassy textures were also found in other freeze-dried plant protein materials such as mild-fractionated lupine protein isolate with neutralization step (Berghout et al., 2015a, 2015b).

The differences between SPFs due to pH adjustment were also observed after adding water. The microstructure and particle size distribution of 1% (w/v) SPF dispersions were shown in Fig. 4 and Fig. 5 respectively. The dispersions of SPF 4.5 and SPF 5.5 had larger particles under light microscopy, while no distinctive particles were detected in the images of SPF 3.5, SPF 6.5 and SPF 7.5. These differences can be further validated by the results of particle size distribution. SPF 3.5, SPF 6.5 and SPF 7.5 exhibited remarkably smaller particle size compared to SPF 4.5 and SPF 5.5, and also smaller than all the commercial SPI with adjusted pH (results are shown in Appendix 2). Moreover, as for commercial SPI with adjusted pH, we saw that the particle size of all the SPI dispersions did not change despite the pH difference and the lower solubility exhibited. Previous research reported that the free surface of the protein increases by reducing the size of particles, which further affects the protein functionalities such as solubility, turbidity, heat stability and gelation (Jambrak et al., 2014; Song et al., 2013). Therefore, pH adjustment during mild fractionation process has a significant influence on the particle size and morphology, leading to more variations in the properties.

3.4. Denaturation behaviors

The denaturation temperature (T_d) of SPFs and enthalpy of transition were summarized in Table 2. Two separate peaks (peak 1 and peak 2) were identified in the DSC measurements for all the SPFs, which corresponded to the denaturation behaviours of β-conglycinin (7 S) and globulins glycinin (11 S) respectively (Mujoo et al., 2003). On the contrary, no peak was detected from commercial SPI. This means mildly fractionated SPFs still kept their native state while commercial SPI was fully denatured after processing.

Despite the native state, different processing pH led to some differences in the denaturation behaviors of SPFs. SPF 3.5 had the lowest T_d with both 7 S (mean value 60.02 °C) and 11 S proteins (mean value 81.75 °C), which indicated that SPF 3.5 was the least thermal stable protein fraction as compared with other SPFs. For 7 S protein, T_d of SPF increased with the increase of processing pH. Hence, the highest T_d occurred with SPF 7.5 (mean value 75.60 °C). This might be explained

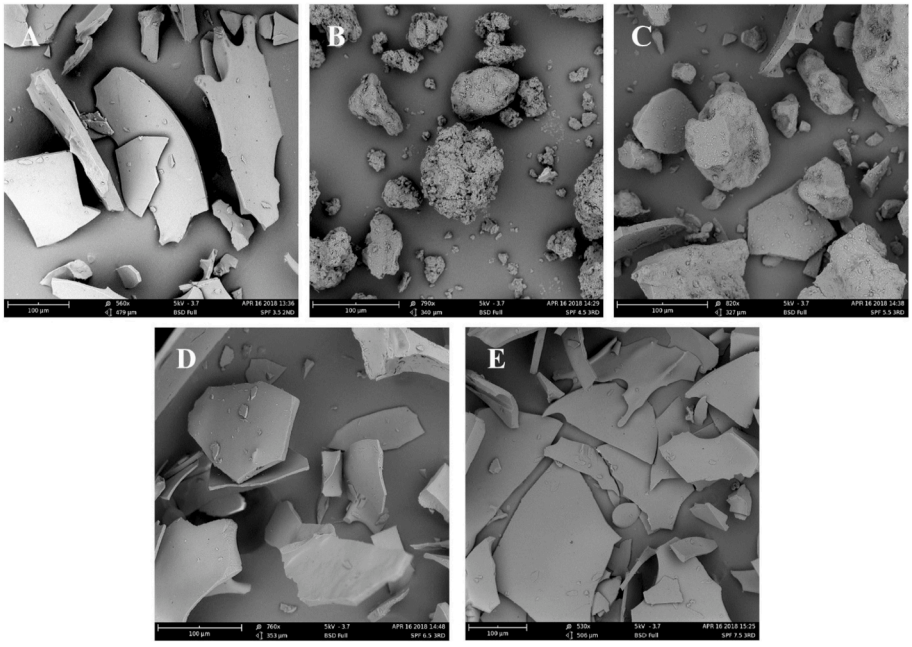

Fig. 3. SEM images of freeze-dried (A) SPF 3.5, (B) SPF 4.5, (C) SPF 5.5, (D) SPF 6.5 and (E) SPF 7.5.

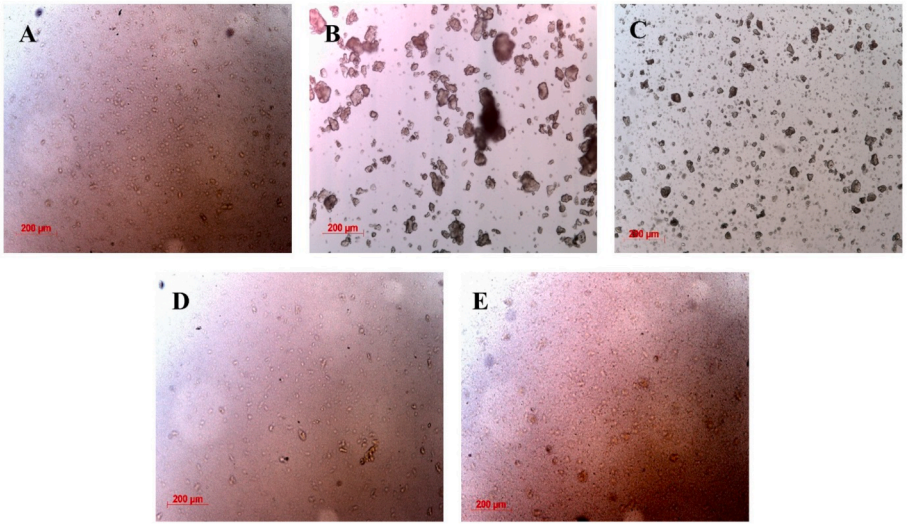

Fig. 4. Light Microscopy images of (A) SPF 3.5, (B) SPF 4.5, (C) SPF 5.5, (D) SPF 6.5 and (E) SPF 7.5 dispersions. The scale bar correspond to 200 μm.

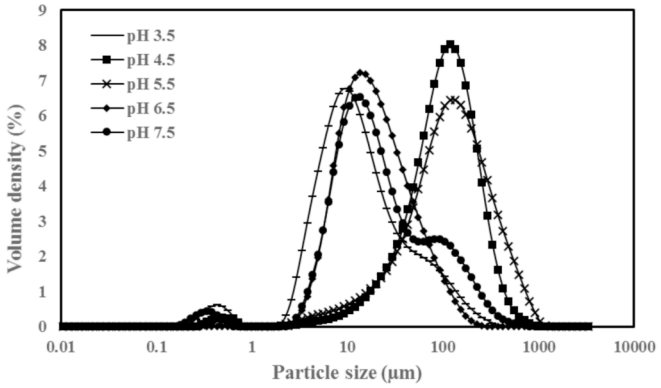

Fig. 5. Particle size distributions of SPFs with different processing pH.

Table 2
The denaturation temperature and enthalpy of transition of soy protein fractions processed at different pH, and commercial SPI (ND = not detected).

	Peak 1 T_d (°C)	Enthalpy (J/g)	Peak 2 T_d (°C)	Enthalpy (J/g)
SPF 3.5	60.02 ± 0.27	1.35 ± 0.64	81.75 ± 0.31	2.64 ± 0.29
SPF 4.5	68.73 ± 0.96	0.41 ± 0.06	92.96 ± 0.49	4.51 ± 1.47
SPF 5.5	68.77 ± 0.52	1.11 ± 0.23	95.22 ± 0.29	6.51 ± 0.64
SPF 6.5	73.11 ± 3.77	1.41 ± 0.23	93.60 ± 0.44	5.39 ± 0.94
SPF 7.5	75.60 ± 0.07	1.29 ± 0.29	91.88 ± 0.25	6.92 ± 0.76
Commercial SPI	ND	ND	ND	ND

by the lower molecule weight and higher flexibility in the structure of 7 S protein compared with 11S protein; the structure of 7 S protein is easier to be influenced by the net charge distribution. Previous research also found that increasing the pH from 3.8 to 7.6 caused the denaturation temperature of β-conglycinin shift to higher values (Renkema et al., 2000). However, as for 11 S protein, the highest T_d showed up with SPF 5.5 (mean value 95.22 °C) while the processing pH was closer to pI. This was also in line with the result reported previously that soy glycinin was found to be most stable against denaturation at a pH of 5, because hydrophobic interactions that favor the folded state of 11 S protein are weakened due to a low net charge, protecting the protein against denaturation (Hermansson, 1986, 1978). Moreover, the denaturation behaviors of SPFs after pH adjustment step are considered to be reversible, on the contrary to the adjustment of pH in commercial SPI (results not shown). However, any additional pH adjustment step introduces more salt in the system, which influences the ionic strength and thus the structure of soy protein (Jiang et al., 2015).

Overall, the mild fractionation process did not result in full denaturation of soy protein, but pH adjustment step during process led to different denaturation behaviors for SPFs, which could further link to the changes of protein functionalities.

3.5. Rheological behaviors

It is known that structure formation in food materials is influenced by the ingredient properties and processing conditions (van der Goot et al., 2008). For better soy-based product development, it is important to understand the food structure formed. In this study, the rheological behaviors of SPFs with different concentrations were determined by a stress-controlled rheometer and a closed cavity rheometer (CCR), which can provide information on the structuring potential of SPFs for different food applications.

Soy beverages such as soymilk smoothie and soy yogurt are novel soy-based products that developed as the alternatives to dairy drinks. For this liquid-like applications, the rheological properties of 12% SPF dispersions were analyzed in this study by a stress-controlled rheometer with shear rate sweep (Fig. 6A). Most SPFs were possible to be measured expect for SPF 4.5 due to its low solubility and limitation in its hydration process. Beyond that, all the examined SPF dispersions showed similarities in behaviour especially with respect to typical shear thinning behaviour; upon increasing shear rate from 1 to 100 s^{-1} the viscosity values of SPF dispersions decreased. Fig. 6A shows that, among the SPFs, SPF 7.5 had the highest viscosity, followed by SPF 6.5, SPF 3.5 and SPF 5.5 during the shear sweep measurements. However, when compared to commercial SPI dispersions, the latter had a much higher viscosity and a stronger shear thinning behaviour than all the SPF dispersions at similar protein concentrations. This means more SPF can be added into products than SPI to achieve certain level of viscosity, bringing more potentials for SPF to develop high-protein soy drinks.

Soy-based meat-replacer products are made by soy protein ingredients with an aim to mimic specific types of meat, including protein content, taste and structure as well. The creation of a fibrous structure from mildly fractionated SPFs, that could be used as a basis for meat replacer products has been reported earlier (Geerts et al., 2018). In that study, a specific blend recipe (SPF/FFSF, 70/30) was considered a successful recipe, since it exhibited certain rheological properties and lead to desired fibrous structure (Geerts et al., 2018). Therefore, similar rheological experiments were implemented to evaluate the properties of SPF blends with different processing pH. A high frequency and high strain treatment were performed under 140 °C and the complex modulus (G*) was used to describe the entire rheological behaviour of the SPF/FFSF blends (Fig. 6B). Similar trends were detected in the G*curve of all the SPF/FFSF blends, an apparent valley showed up in the first 2–3 min followed by a retarded steady-state creep. However, different processing pH also caused some variations in the curve, including the valleys and the G* values. For the valleys, it is hypothesized that the

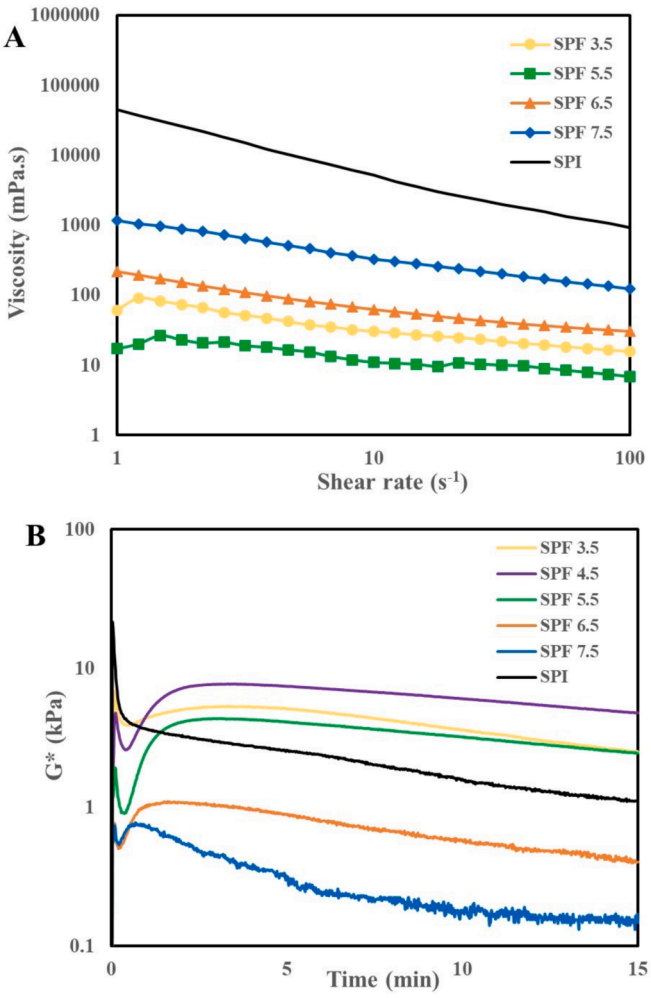

Fig. 6. (A) Viscosity measured as a function of shear rate and (B) complex modulus (G*) measured as a function of time at 140 °C of SPFs processed at different pH.

denaturation behaviour of SPF/FFSF blends might be responsible for the appearances since no valley was detected in the commercial SPI. This hypothesis was also particularly evident by the results of DSC. SPF 3.5 had the lowest T_d for both 7 S and 11S protein, while it also showed the smallest valley in the G* curve. For the G* values, SPF 4.5/FFSF blend and SPF 5.5/FFSF had higher G* while the SPF 7.5/FFSF blend had the lowest one, which indicated that SPFs showed more potentials for structure formation when the processing pH was close to pI.

To sum up, pH adjustment during the mild fractionation process can lead to a range of soy protein products with different rheological properties, which can be used in tailor-made applications according to the requirements.

4. Conclusion

The demand of novel soy-protein products is increasing, and also the need for sustainable soy-based ingredients. Therefore, the application of a mild fractionation process and pH adjustment was explored in this study. Mildly fractionated SPFs showed higher protein and oil content as compared to commercial SPI. Proteins were still in their native state after processing. The processing pH altered the functionality of the SPFs and two clusters could be distinguished; one close and one away from the pI. SPF 4.5 and SPF 5.5, processed around isoelectric point, formed a powdery texture with lower NSI, WHC and viscosity. However, in combination with FFSF, they presented higher G* value as compared to

other SPFs under high temperature, which brought more potentials for structure formation. SPFs processed away from pI in both sides, presented increased NSI, WHC, and viscosity, but decreased particle size and G* value. A glass-like microstructure was also observed. Recognising the difference this pH adjustment step can have on the functionality of the SPFs, it is recommended to adjust the mild fractionation based on the requirement on the functional properties of multiple soy-based products. Forthcoming studies could up-scale the quantities of SPFs and work with specific applications such as structure formation for meat analogue.

Author contribution

Yu Peng: Formal analysis, Investigation, Data Curation, Writing - Original Draft, **Natalie Kersten**: Investigation, Data Curation, Visualization, **Konstantina Kyriakopoulou**: Validation, Writing - Review & Editing, Supervision, **Atze Jan van der Goot**: Conceptualization, Writing - Review & Editing, Supervision.

Declaration of competing interest

None.

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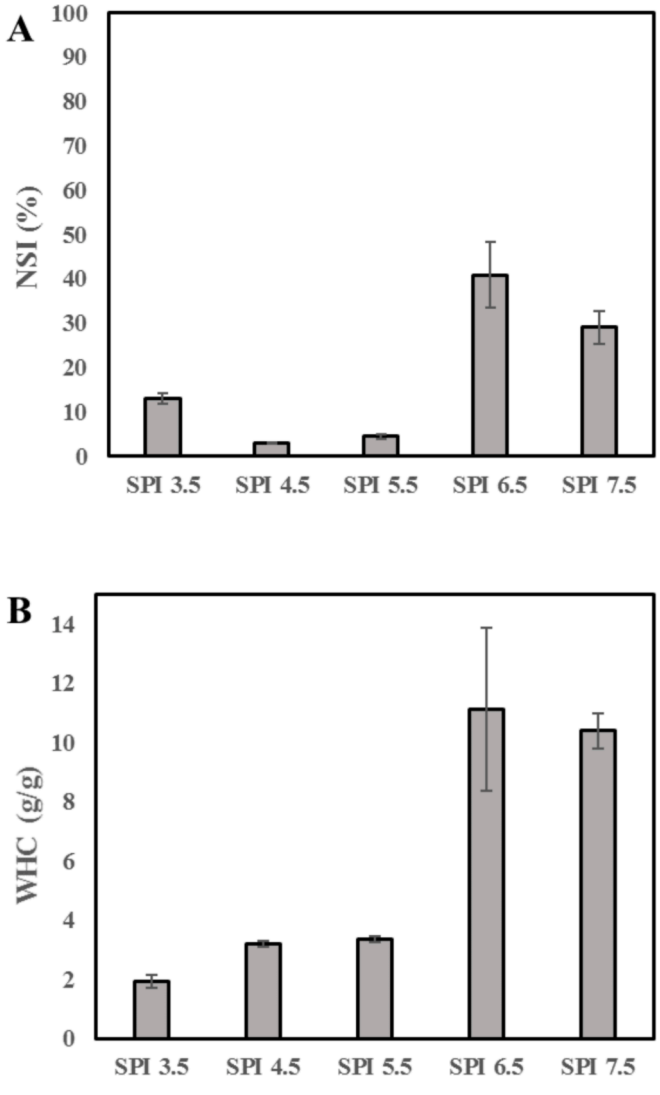
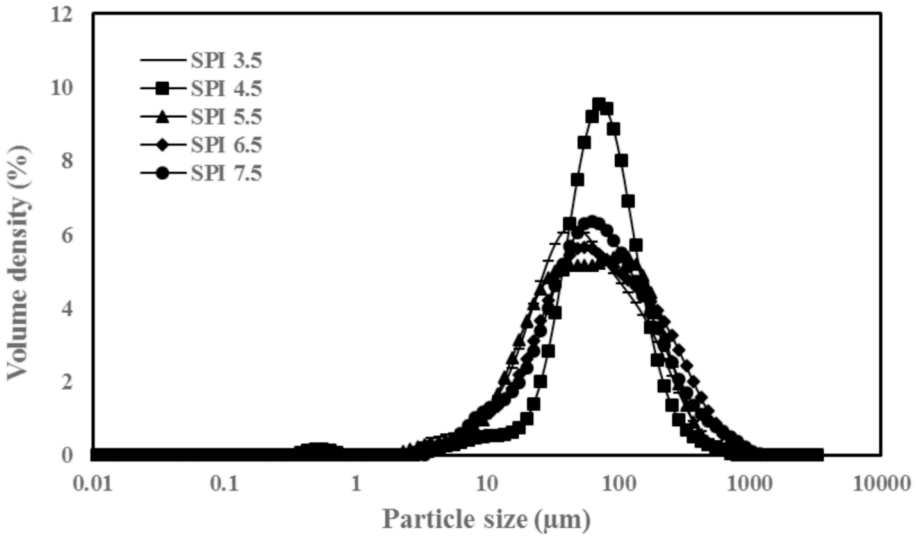
Appendix 1: (A) nitrogen solubility index (NSI) and (B) water holding capacity (WHC) for commercial SPI with adjusted pH



Appendix 2: Particle size distributions of commercial SPI with adjusted pH



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