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Differences in physicochemical properties of high-moisture extrudates prepared from soy and pea protein isolates

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ABSTRACT

This study aimed to compare the physicochemical properties of high-moisture extrudates prepared from soy protein isolate (SPI) and pea protein isolate (PPI) at different barrel temperatures (BTs) of 120 °C, 140 °C, and 160 °C. Increasing BT promoted more anisotropic structures and darker extrudates. PPI extrudates produced more fibrous structures than SPI extrudates under the same BT. Textural and rheological properties of SPI extrudates elevated significantly with increasing BT. Compared to SPI extrudates at the same BT, PPI extrudates displayed apparently weaker shear thinning (gel-like) behavior and showed less textural properties of hardness, chewiness, and tensile strength. PPI extrudates showed no significant differences in hardness and chewiness to the cooked chicken breast. Heat-treatment during low-field nuclear magnetic resonance (LF-NMR) measurements promoted the release of intra-space water of the fibrous structure for both SPI and PPI extrudates. Water in PPI extrudates was more prone to migrate compared to SPI extrudates. Hydrogen bonds and hydrophobic interactions played marginally more essential roles than the disulfide bonds to stabilize protein structure for both SPI and PPI extrudates. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that most protein subunits were still present after extrusion, except two bands over 100 kDa in SPI extrudates extracted by phosphates buffer. This study provided valuable information for improving the quality of high-moisture extrudates based on SPI and PPI.

1. Introduction

High-moisture extrusion processing (HMEP) has been applied for plant-based proteins for many years to create long fibrous structures that can mimic small pieces of muscle-type meat products (chicken, pork, or beefsteak) (Kyriakopoulou, Keppler, & van der Goot, 2021; van der Weele et al., 2019). HMEP is typically performed at high-moisture contents (40–80%) with a long cooling die attached at the end of the extruder barrel (Osen, 2017; Pietsch, Bühler, Karbstein, & Emin, 2019; Samard et al., 2019). During HMEP, plant-based proteins are mixed with water and subjected to denaturation and molecular transformation by the rotating screws and the barrel heating, finally realigning in the flow direction to form an anisotropic structure in the cooling die (Wittek, Ellwanger, Karbstein & Emin, 2021). The performance of high-moisture meat analogs varies depending on protein sources and processing conditions (Pietsch, Emin, & Schuchmann, 2017; Zhang et al., 2020).

Soy proteins have been gaining popularity to generate plant-based meat analogs due to their high availability and ability to texturize (Chiang, 2007; Chiang, Loveday, Hardacre, & Parker, 2019; Zahari et al., 2020). Pea proteins are increasingly used as alternatives to soy proteins during HMEP because of their low potential for allergic reactions (Chen, Zhang, Zheng, Meng, & Wang, 2021; Osen, Toelstede, Wild, Eisner & Schweiggert-Weisz., 2014). Very few studies focused on the application of pea proteins for high-moisture meat analogs, limited information was seen to clarify the texturization of pea proteins during HMEP. In addition, based on the gelation properties, where pea proteins showed weaker heat-induced gel capacities than their soy counterparts (Hua, Cui, Wang, Mine, & Poysa, 2005; Shand, Ya, Pietrasik, & Wanasundara, 2007), some researchers illustrated that pea proteins had structural limitations to generate high-moisture meat analogs in

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provide helpful guidance for the industry when choosing protein in meat analogs (Chen, Wei, Hsieh, 2007; Osen, Store Heddinge, Denmark). The screw speed was 200 rpm, and the water was pumped to the third section of the extruder channel. The extruder is divided into 7 internal (Zone 2 to Zone 8) and 1 external zone with different equal-length heating zones (die zone). The protein powders were metered into the first section of the extruder with the feed rate of 0.3 kg h⁻¹ by a gravimetric twin-screw feeder (MT-S, MiniTwin, Brabender Technologie, Duisburg, Germany). Water was pumped to the third section to maintain the extrudates with the water content of 60 ± 3% (w/w) using a peristaltic pump (Fillmaster Type 421, Delta Scientific Medical, Store Heddinge, Denmark). The screw speed was 200 rpm, and the cooling temperature was set to 20 °C by a refrigerated circulator (Polystat, Buch & Holm A/S, Denmark).

| Setting no. | Temperature (°C) |
|------------|------------------|
|            | Zone 2 | Zone 3 | Zone 4 | Zone 5 | Zone 6 | Zone 7 | Zone 8 | Die zone | Cooling die |
| 1          | 40     | 60     | 90     | 105    | 120    | 120    | 120     | 90       | 20        |
| 2          | 40     | 60     | 90     | 110    | 140    | 140    | 140     | 90       | 20        |
| 3          | 40     | 60     | 90     | 120    | 160    | 160    | 160     | 90       | 20        |

2. Material and methods

The experimental design is illustrated in Fig. 1.

2.1. Materials

Soy protein isolate (SUPRO® EX 37 HG IP) was obtained from Solae (Europe S.A.). Pea protein isolate (PISANE™ M9) was supplied by the Cosucra Groupe (Warcoing, Belgium). The specification from the suppliers indicated that the protein contents on the dry basis of SPI and PPI were 90% and 86%, respectively. The initial pH of SPI and PPI in 5% (w/v) aqueous solutions were 7.77 and 7.79. Chicken breasts were purchased from a local supermarket in Copenhagen, Denmark.

2.2. Extrusion process and samples preparation

Extrusion experiments were carried out using a laboratory-scale co-rotating and intermeshing twin screw extruder (Process 11, Thermo Fisher Scientific, Karlsruhe, Germany) with a cooling die (H 5 mm × W 20 mm × L 200 mm) attached to the end of the extruder channel. The extruder is divided into 7 internal (Zone 2 to Zone 8) and 1 external equal-length heating zones (die zone). The protein powders were metered into the first section of the extruder with the feed rate of 0.3 kg h⁻¹ by a gravimetric twin-screw feeder (MT-S, MiniTwin, Brabender Technologie, Duisburg, Germany). Water was pumped to the third section to maintain the extrudates with the water content of 60 ± 3% (w/w) using a peristaltic pump (Fillmaster Type 421, Delta Scientific Medical, Store Heddinge, Denmark). The screw speed was 200 rpm, and the cooling temperature was set to 20 °C by a refrigerated circulator (Polystat, Buch & Holm A/S, Denmark).

BT profiles are listed in Table 1, and the effect of BT was investigated by setting different temperatures from zones 5 to 8 of the extruder. Samples with H 5 mm × W 20 mm × L 150 mm were manually cut and sealed in plastic bags once BTs and the cooling temperature were stable. The sealed fresh samples (stored at room temperature (22 ± 2 °C) within 2 h before analysis) and frozen samples (stored at −18 °C) were collected for further analysis. SPI extrudates under BTs at 120 °C, 140 °C, and 160 °C were named SPI120, SPI140, and SPI160, while the PPI counterparts were designated as PPI120, PPI140, and PPI160, respectively. All extrusion trials were repeated at least twice.

2.3. Preparation of cooked chicken breasts

Cooked chicken breasts used as a reference for texture profile analysis (TPA) were prepared according to Chiang et al. (2019) with slight modifications. Chicken breasts were packaged in plastic bags and cooked to an internal temperature of 75–80 °C in a heated water bath.
2.5. Microstructure

The microstructure analysis of frozen extrudates (stored at −18 °C) was conducted using a scanning electron microscope (SEM) (Quanta FEI 3D, FEI, Eindhoven, The Netherlands). The method was based on Osen et al. (2014) with some modifications. Frozen extrudates were thawed for 30 min at room temperature before samples preparation, and cut into small pieces with H 2 mm × W 5 mm × L 5 mm. After being mounted in a Karnovsky’s fixative for 48 h, the samples were rinsed with a sodium cacodylate buffer (0.1 M), followed by a secondary fixation in 1% osmium fixative (1%) for 2 h. The samples were then dehydrated for 80 min in a graded series of ethanol solutions (10%, 30%, 50%, 70%, 100%) and dried to critical point. The samples were then coated with gold particles with a sputter coater (ACE200, Leica Microsystems, Vienna, Austria). Images of the samples were taken with the SEM at magnifications of 10,000×.

2.6. Textural properties

The texture profile analysis (TPA, including hardness and chewiness) and tensile strength of fresh extrudates were determined within 1 h after extrusion using a TA.XT2 Texture Analyzer (Stable Micro System, UK) with a probe at a speed of 0.5 mm s⁻¹ for 5 s. Next, a sample shaped like a dog bone (Fig. 2) was stretched using an A/TC probe at a speed of 0.5 mm s⁻¹ until the strip broke, and the tensile strength results were recorded. The data of hardness and chewiness from 7 pieces of each treatment were recorded. The tensile strength experiment was performed in triplicate.

2.7. Rheological measurements

The rheological behaviors of extruded samples within the linear viscoelastic region were assessed using dynamic oscillatory measurements by a stress-controlled rheometer (Discovery Hybrid Rheometer HR-2, TA Instrument, Elstree, UK). The method was based on Osen (2017) with some modifications. Before rheological analysis, fresh extrudates were cut using a circular cutting probe with a diameter of 20 mm and loaded into the measuring system. The measurements were conducted using a plate-plate-geometry with a diameter of 25 mm (PP25, TA Instrument, Elstree, UK). A constant normal force of 5 N was applied to compensate small variations of the sample height of the extruded samples. Experiments were performed at 22 °C, and each measurement was carried out in duplicate.

The samples were conducted the amplitude sweep test within the strain range of 0.01–100% at an angular frequency (ω) of 10 s⁻¹, where the changes of elastic modulus (G’) and viscous modulus (G’’) as the function of strain (γ), were recorded. The frequency sweep was conducted at a constant strain of 1.0% (within the linear viscoelastic region), and the angular frequency varied from 500 to 0.05 s⁻¹, G’ and G’’ were obtained, and G’ was modeled as a power-law function of ω (Rao, 2007):

\[
G’ = K \cdot \omega^n
\]

Where the behavior index n’ means slope of G’, and the consistency index K’ indicates intercept with ordinate.

2.8. Low-field nuclear magnetic resonance (LF-NMR) determination during home cooking conditions

The water distribution analysis of extruded samples when simulating home cooking was carried out according to Bertram, Wu, van den Berg, and Andersen (2006) and Bosmans et al. (2012), with some modifications. LF-NMR measurements were performed at 0.47 T magnetic field strength using a MQR Spectro-P spectrometer (Oxford Instruments, Oxfordshire, UK), controlled by the Oxford Instruments NMR Application Developer software. Each PP160 and SPI160 sample was cut into five fractions with H 5 mm × W 10 mm × L 40 mm, placed in 18-mm tubes, and inserted in the LF-NMR probe. The five samples from each SPI160 or PP160 were heated in-situ to 25 °C, 60 °C, 80 °C, and 100 °C, respectively. Carr-Purcell-Meiboom-Gill (CPMG) sequences were used to investigate spin-spin relaxation times with the parameters recycle delay 8 s, τ-delay 200 μs, 32 scans averaged, 10,000 echoes, and a receiver gain of 5. Transverse relaxation times (T2m) and relative abundances (M2m) of different proton populations were obtained using in-house MATLAB (R2019a, MathWorks, MA, USA) scripts designed for fitting the relaxation curves to a sum of exponential decays according to Equation (2):

\[
I(t) = \sum_{n=1}^{N} M_{2m} \cdot e^{-t/T_{2m}}
\]
Here \( I(t) \) corresponds to intensity as a function of time, and \( N \) represents the number of relaxation components, determined by visual inspection of the residuals after model fitting. Each measurement was performed in triplicate.

### 2.9. Protein solubility

The protein solubility of raw SPI and PPI ingredients, and their extrudates was analyzed to assess the protein-protein interactions during HMEP. According to Liu and Hsieh (2007) and Chiang et al. (2019), four buffer systems were used to disintegrate specific chemical and non-chemical interactions within the protein networks:

1. 0.1 M phosphate buffer consisting of \( \text{NaH}_2\text{PO}_4 \) and \( \text{Na}_2\text{HPO}_4 \) with a pH of 7.5 (P).
2. 8 M Urea in P (PU).
3. 0.05 M dithiothreitol (DTT) in P (PD).
4. 8 M Urea + 0.05 M DTT in P (PUD).

Samples weighing from 0.05 to 0.30 g depending on protein contents were extracted in a centrifuge tube with 15 mL of each buffer system. Mixtures were blended using a homogenizer (T18 digital ULTRA TURRAX, IKA, Germany) at 12,000 rpm for 1 min. The mixtures were then agitated for another 40 min, followed by centrifugation at 12,400 g for 20 min at 25 °C. The soluble proteins in the supernatant were determined using the Bradford protein assay at 595 nm with a multimode microplate reader (SpectraMax M2, Molecular Devices, CA), and using bovine serum albumin (BSA) as standard. The total nitrogen in the original samples was measured using an Elementar vario MACRO cube CHNS Analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Hanau, Germany) with helium as the carrier gas, and the protein content was calculated with nitrogen conversion factors of 6.25 for both SPI and PPI. The protein solubility was calculated as the ratio of soluble protein in the supernatant to total protein in the samples. Each measurement was conducted in triplicate.

### 2.10. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted to evaluate the differences in protein distribution according to Chen, Wei, and Zhang (2011) and Chiang (2007), with some modifications. Protein extracts, collected from the protein solubility tests, were individually diluted to 0.8 mg mL\(^{-1}\) of protein content before mixing with four volumes of sample buffer without DTT (Laemmlli sample buffer, Bio-Rad, Hercules, CA) at a 3:1 ratio (v/v). After being heated in a boiling water bath for 5 min, aliquots of 10 μL solution were loaded on a 4–15% Bis-Tris gel (Mini-PROTEIN TGX Gels, Bio-Rad, USA). The gel was running at 80 V for 20 min before adjusting to 150 V for about 40 min with running buffer (Tris/Glycine/SDS, Bio-Rad, Hercules, CA). After separation, gels were stained with 40% (v/v) ethanol and 10% (v/v) acetic acid, followed by staining with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 25% (v/v) ethanol and 8% (v/v) acetic acid, and de-stained by the same solution without Coomassie Brilliant Blue R-250. A pre-stained marker that ranged from 10 to 250 kDa (Precision Plus Protein Standards, Broad Range, Bio-Rad, Hercules, CA) was used to identify the molecular weight of relevant protein bands.

### 2.11. Data analysis

Results were treated and plotted using GraphPad Prism version 8.0 (San Diego, USA). Analysis of variance (ANOVA) between BT and physicochemical properties of extruded samples were analyzed by SPSS Statistics 27.0 (SPSS, Chicago, IL). Turkey’s test was applied to evaluate the statistical significance between samples at a significant level of 95% (\( P < 0.05 \)).

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**Table 2**

| Sample      | Sample Type | \( L^* \) | \( a^* \) | \( b^* \) |
|-------------|-------------|----------|----------|----------|
| SPI-R       | Raw SPI     | 81.23 ± 0.19 \( a \) | 0.63 ± 0.03 \( a \) | 15.39 ± 0.06 \( a \) |
| SPI120      | 120°C SPI   | 53.98 ± 0.68 \( b \) | 0.83 ± 0.24 \( b \) | 10.49 ± 0.49 \( b \) |
| SPI140      | 140°C SPI   | 51.16 ± 0.08 \( c \) | 1.74 ± 0.01 \( c \) | 12.20 ± 0.37 \( c \) |
| SPI160      | 160°C SPI   | 50.71 ± 0.06 \( d \) | 1.77 ± 0.57 \( d \) | 13.25 ± 0.11 \( d \) |
| PPI-R       | Raw PPI     | 73.53 ± 0.51 \( a \) | 4.16 ± 0.16 \( a \) | 22.50 ± 0.51 \( a \) |
| PPI120      | 120°C PPI   | 54.89 ± 0.28 \( b \) | 6.67 ± 0.09 \( b \) | 23.11 ± 0.92 \( b \) |
| PPI140      | 140°C PPI   | 53.41 ± 0.65 \( c \) | 6.52 ± 0.19 \( c \) | 22.77 ± 0.31 \( c \) |
| PPI160      | 160°C PPI   | 50.65 ± 0.01 \( d \) | 5.66 ± 0.03 \( d \) | 20.85 ± 0.12 \( d \) |

Note: Data represent the mean and error values represent the standard deviation. Different lowercase letter in the same column means significant differences (\( P < 0.05 \)). SPI-R: raw SPI powder. SPI120, SPI140, and SPI160: extruded SPI with the barrel temperature at 120 °C, 140 °C and 160 °C. PPI-R: raw PPI powder. PPI120, PPI140, and PPI160: extruded PPI with the barrel temperature at 120 °C, 140 °C and 160 °C.
3. Results and discussion

3.1. Product color

Fig. 3 presents the visual impression of the raw, and extruded SPI and PPI using representative samples, and Table 2 demonstrates their color parameters. SPI ingredient showed significantly higher L* but lower a* and b* values compared to raw PPI. It could be also observed from Fig. 3 that SPI powder represented off-white while PPI powder showed a cream color. Compared with the raw proteins, SPI and PPI extrudates showed a decrease of L* value but an increase of a* value during extrusion (Table 2). On the other hand, HEMP reduced the b* value in the SPI extrudates while did not change the b* value of PPI extrudates ($P > 0.05$).

BT affected the color parameters of the extrudates during extrusion. As BT rose from 120 °C to 160 °C, L* showed a significant drop (6.06%) for SPI extrudates and an evident reduction for PPI extrudates (7.72%, $P < 0.05$), indicating that higher BT resulted in darker extrudates. In contrast, as BT increased, b* values decreased for SPI extrudates but showed no apparent difference for PPI extrudates during extrusion (Table 2). On the other hand, HEMP reduced the b* value in the SPI extrudates while did not change the b* value of PPI extrudates ($P > 0.05$).

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3.2. Microstructure

The micrographs of representative samples taken by SEM are depicted in Fig. 4. All samples had visible anisotropic structures where more fibers were generated after extrusion when increasing BT. This could be explained that higher temperature promoted more disassembled protein subunits, which could realign more efficiently by the directional shear force inside the cooling die (Chiang, 2007). In addition, SPI extrudates showed a compact structure, while PPI extrudates were overall loose and contained more air inclusion (Fig. 4).

Grabowska et al. (2016) reported that the prerequisite for the anisotropic structure during extrusion was the presence of two (or more) separate phases, e.g., proteins-proteins or proteins-macromolecular substances. These two or more thermodynamically incompatible phases could prevent the transversal aggregation of proteins and be beneficial to the longitudinal arrangement to form the fiber. Our previous extrusion experiments showed that compared to SPI, soy protein concentrate (SPC) could generated more obvious fibrous structures due to the two separate phases of proteins-starch in SPC (not shown here). However, a single-ingredient of SPI (Fang et al., 2014) or PPI (Osen, 2017) could form a fibrous structure during HMEP. The generation of fibrous structures by SPI and PPI alone may be due to a “suspension model” proposed by Osen (2017). Protein isolate contained insoluble and non-melting components, which could be used as a second phase and promote formation of fibrous structures during HMEP. Fig. 4 shows PPI extrudates had more obvious fibrous structures than SPI extrudates at the same BT. This may be because of more non-protein fractions, including lipids and ash (amounting to more than 10% of the dry mass), in PPI compared to SPI powders (less than 6%). Moreover, Day and Swanson (2013) indicated that lipid-protein interactions were non-covalent and may be important for structural stabilization with low lipid concentrations during extrusion.

3.3. Textural characterizations

As displayed in Fig. 5, the textural properties, including hardness, chewiness and tensile strength of extrudates, increased when increasing BT. Compared to SPI extrudates, the textural performance of PPI extrudates was less dependent on BT, where no significant differences of hardness, chewiness and tensile strength in PPI extrudates were found at BT from 120 °C to 160 °C. The textural properties of SPI extrudates were
significantly higher \((P < 0.05)\) than PPI extrudates at the same BT between 140 °C and 160 °C. In contrast, no apparent differences in textural attributes between SPI and PPI extrudates produced at 120 °C were found (Fig. 5). The texture differences could be reflected by the microstructure from SEM results. Compared to PPI extrudates, the more compact structure of SPI extrudates was beneficial to generate a harder rubber-like texture (Fig. 4).

The hardness and chewiness of the chicken breasts cooked at 70–80 °C can be used as a reference to characterize the fiber formation in meat analogs (Schreuders et al., 2019). As shown in Fig. 5 (a) and (b), the hardness of the cooked chicken breasts (2408 g) was comparable to the values of SPI140 (2794 g) and PPI160 (2053 g) \((P > 0.05)\). In addition, the chewiness of the cooked chicken breasts (1274 g) showed no apparent difference to the values of SPI120 (1272 g), PPI120 (1317 g) and PPI140 (1389 g). These findings indicated that PPI extrudates showed more significant potential to mimic chicken breasts in terms of hardness and chewiness than SPI extrudates.

### 3.4. Rheological properties

#### 3.4.1. Amplitude sweep

Fig. 6 depicts the rheological properties of the SPI and PPI extrudates under small amplitude sweep experiments, where \(G'\) and \(G''\) were plotted as the function of shear strain. Table 3 shows the average \(G'\) and \(G''\) values in the linear viscoelastic range, the modulus and the flow stress \(\gamma_f\) at the flow point when \(G' = G''\). All extrudates displayed \(G'\) dominated over \(G''\) within the linear viscoelastic range, indicating the shear thinning (gel-like) behavior (Bertram et al., 2006; Osen, 2017). After a critical strain range (4–10%), \(G'\) decreased and \(G''\) increased non-linearly for all samples, implying that the extrudates structure was irreversibly changed. With the further rise of strain, a crossover of \(G'\) and \(G''\) was found (except for PPI120, shown in Table 3), indicating the material started to flow and became disintegration (Osen, 2017).

Based on Fig. 6 and Table 3, the viscoelastic values of SPI160 and SPI140 were much greater than SPI120. However, BT had no evident effect on \(G'\) and \(G''\) of PPI extrudates, and all PPI extrudates showed similar rheological values to SPI120 (Fig. 6). These findings indicated that SPI extrudates showed more strength in structure than PPI extrudates, which were consistent with the TPA results (Fig. 5).

#### 3.4.2. Frequency sweep

The results of frequency sweep experiments with angular frequency from 500 to 0.05 s\(^{-1}\) at a constant amplitude (1%) are shown in Fig. 7, where \(\log G'\) and \(\log G''\) values were plotted against \(\log \omega\) at different BTs. All the extruded samples were predominantly elastic with \(G' > G''\), implying the typical characterization of cross-linked networks with a gel-like behavior (Osen, 2017). \(G'\) and \(G''\) raised gradually with increasing frequencies, which could be due to the disentangled molecular chains during the short period of oscillation (Osen, 2017; Rao, 2007). The rheological values of SPI160 and SPI140 had no apparent difference and they were much higher than the values of other extrudates (Fig. 7). All PPI extrudates showed no evident difference in rheological properties, which were in similarity with the values of SPI120.

Based on the shape of the scatter plots (Fig. 7), the function of \(\log G'\) and \(\log \omega\) was fitted with power-law model (Equation (1)), with the \(R^2\) greater than 0.97 (not shown). According to Rao (2007) and Osen...
The rheological properties of fresh SPI and PPI extrudates under different barrel temperatures (BTs) by amplitude sweep experiments (with the strain between 0.01% and 100%) at a constant angular frequency of 10 s\(^{-1}\). Notes: Data represent the mean and error values represent the standard deviation. 

### Table 3

| Sample                  | G’ (Pa)       | G” (Pa)       | G’ - G” (Pa) | Flow stress (Pa) |
|-------------------------|---------------|---------------|--------------|------------------|
| SPI120                  | 15,268 ± 1518\(^a\) | 2633 ± 78\(^a\) | 4282 ± 22\(^a\) | 6144 ± 77\(^a\)  |
| SPI140                  | 36,420 ± 1413\(^a\) | 6515 ± 182\(^a\) | 9352 ± 264\(^a\) | 5812 ± 131\(^a\) |
| SPI160                  | 35,926 ± 6170\(^a\) | 6539 ± 1354\(^b\) | 9670 ± 2016\(^a\) | 5913 ± 31\(^b\)  |
| PPI120                  | 17,653 ± 26\(^a\) | 3943 ± 31\(^a\) | Not detected | Not detected     |
| PPI140                  | 18,314 ± 1029\(^b\) | 3373 ± 43\(^b\) | 4893 ± 510\(^b\) | 6299 ± 283\(^b\) |
| PPI160                  | 17,569 ± 98\(^b\) | 3196 ± 35\(^a\) | 4432 ± 46\(^a\) | 6144 ± 77\(^a\)  |

Note: Data represent the mean and error values represent the standard deviation. Different lowercase letter in the same column means significant differences (P < 0.05). SPI120, SPI140, and SPI160: extruded SPI with the barrel temperature at 120 °C, 140 °C and 160 °C, respectively. PPI120, PPI140, and PPI160: extruded PPI with the barrel temperature at 120 °C, 140 °C and 160 °C. G’, the viscous modulus; G” = G’; the modulus at the crossover of G’ and G”. Flow stress, the stress at the flow point when G’ = G”.

The behavior index n’ (the slope of log G’ versus log ω) in the power-law model (Equation (1)) of the rheological properties was supposed to be zero for pure elastomers, with positive slopes for weak gels and highly concentrated solutions. The n’ values of all extrudates were around 0.10, indicating that SPI and PPI extrudates performed like an elastomer (Osen, 2017). The consistency index K’ can be used to evaluate viscoelastic properties. K’ showed a high positive correlation with the chewiness (r = 0.97, P < 0.05). Table 4 shows that the consistency index K’ of SPI140 and SPI160 were significantly higher than other extrudates. These findings may explain that the higher viscoelastic properties resulted in greater structural strength in terms of chewiness.

### 3.5. Water distribution by LF-NMR during home cooking conditions

Water migration during home cooking conditions influences the products quality, including water holding capacity, textural properties of tenderness and juiciness (Pearce, Rosenvold, Andersen, & Hopkins, 2011). LF-NMR has been widely used to investigate the moisture distribution in meat (Bertram et al., 2006; Shaarani, Nott, & Hall, 2006) and meat analogs (Chen et al., 2010; Guo et al., 2020). The water distribution of the extrudates under different heating temperatures (incremental, 25 °C–100 °C) using LF-NMR was measured. Previous studies revealed that BT (140 °C–160 °C) had no particularly critical effect on water distribution of high-moisture SPI extrudates (Chen et al., 2010). According to the microstructure in our study, extrudates under BT of 160 °C had a more evident fibrous structure (Fig. 4). To reduce the experiments, only SPI160 and PPI160 in this study were selected for LF-NMR measurements by heating the samples at the home cooking temperatures.

Table 5 shows that three spin-spin relaxation time constants (T\(_{21}\), T\(_{22}\), and T\(_{23}\)) and their corresponding relaxation signal components (M\(_{21}\), M\(_{22}\), and M\(_{23}\)) were identified from the LF-NMR experiments, indicating that three fractions of water with different mobility degrees existed in extrudates. Higher degrees of freedom correspond to longer transverse relaxation time, while lower mobility degrees are associated with a shorter transverse relaxation time. T\(_{21}\) (below 5 ms) represented bound water with compact hydrogen protons inside the samples. In comparison, T\(_{22}\) (around 20 ms) and T\(_{23}\) (above 60 ms) showed the protons in the moderately and weakly bound water with the proteins (Guo et al., 2020; Srikantha & Rahman, 2018). SPI160 and PPI160 showed similar water distribution in T\(_{21}\) and M\(_{21}\), where water distribution were not distinctly affected until 100 °C (Table 5). The findings reflected that cooking temperatures less than 100 °C had no evident influence on the bound water. T\(_{22}\) values remained stable (18.8–22.1 ms) while the M\(_{22}\) decreased gradually from 93.6 to 86.6% as the cooking temperature increased from 25 °C to

![Fig. 6](image1.png)

![Fig. 7](image2.png)

**Fig. 6.** Rheological properties of SPI and PPI extrudates under different barrel temperatures (BTs) obtained by frequency sweep (with the angular frequency from 500 to 0.05 s\(^{-1}\) ) experiments at a constant shear strain of 1.0%. Note: Data represent the mean and error bars represent the standard deviation. SPI120, SPI140, and SPI160: extruded SPI with the barrel temperature at 120 °C, 140 °C and 160 °C, respectively. PPI120, PPI140, and PPI160: extruded PPI with the barrel temperature at 120 °C, 140 °C and 160 °C.

**Fig. 7.** Viscoelastic properties of SPI and PPI extrudates under different barrel temperatures (BTs) obtained by frequency sweep (with the angular frequency from 500 to 0.05 s\(^{-1}\) ) experiments at a constant shear strain of 1.0%. Note: Data represent the mean and error bars represent the standard deviation. SPI120, SPI140, and SPI160: extruded SPI with the barrel temperature at 120 °C, 140 °C and 160 °C, respectively. PPI120, PPI140, and PPI160: extruded PPI with the barrel temperature at 120 °C, 140 °C and 160 °C.
and $T < 0.05$. SPI160, extruded SPI with the barrel temperature at 160 °C.

100 °C (Table 5). Guo et al. (2020) indicated that water molecules in the fibrous protein structure were present in the extrudates’ internal holes and were bound with proteins. These results displayed that more water in the intra-space of fibrous structure existed at elevated cooking temperatures. $T_{23}$ presented water associated with other macromolecules (Shaarani et al., ). As displayed in Table 5, $T_{23}$ value of PPI160 was significantly higher than that of SPI160 at the same cooking temperature, indicating that PPI160 showed a higher degree freedom of water between the protein macromolecules than SPI160. The structure tightness affected the water distribution in meat analogs, where tighter structure resulted in weaker water mobility and stronger water holding capacity (Guo et al., 2020). The findings implied that water in PPI extrudates could easily migrate, and it can be proved by the large amounts of water on the surface of fresh PPI extrudates (not shown).

### 3.6. Protein solubility

The protein-protein interactions during HMEP were determined by protein solubility test. The amount of protein solubilized in four different solvents (represented as a percentage of total protein) from raw and extruded SPI and PPI samples at different BT is shown in Fig. 8. Protein solubility was between 4% and 80%, and the percentage of extracted proteins dropped significantly in all solvents after extrusion. It could be explained that the extracted solvents could not solute the protein aggregation formed during extrusion. Increasing BT from 120 °C to 160 °C had no significant effect on protein solubility of extrudates in all extract solvents. These findings were similar to the study of Prudencio-Ferreira and Areas (1993), where the protein solubility of SPI extrudates showed no evident differences with BT between 140 °C and 160 °C.

Phosphate buffer (P) extracted the least protein in all samples because P alone is known to only dissolve protein molecules in their native states (Liu & Hsieh, 2007). As expected, high temperature and chemical reagents treatment during processing commercial preparation for PPI and SPI could cause protein denaturation (Adebiyi & Aluko, 2011; Liu & Hsieh, 2007). Raw PPI with protein solubility around 12.80% had significantly higher amounts ($P < 0.05$) of extractable protein by P than raw SPI (10.50%, Fig. 8). This was because the different source led to the different functionalities between SPI and PPI.

Urea can break non-covalent interactions, such as hydrogen bonds and hydrophobic interactions, while DTT was used to cleave disulfide bonds (Lin, Huff, & Hsieh, 2000; Liu & Hsieh, 2007). Fig. 8 shows that as one of the other reagents (Urea, DTT) combined with P, the amount of protein solubilized increased compared with P alone, indicating that more than one type of chemical bonding existed in the raw materials and extrudates (Chiang et al., 2019). PU (8 M Urea in P) extracted more proteins (30–40%) than PD (0.05 M DTT in P, around 20%) in SPI and 

![Fig. 8. Protein solubility of SPI, PPI, and their respective extrudates obtained at different barrel temperatures (BTs) induced by extracting solutions P, PU, PD, and PUD. Note: Data represent the mean and error bars represent the standard deviation. Values bearing different lowercase letters were significantly different ($P < 0.05$). P: 0.1 M phosphate buffer consisting of Na$_2$HPO$_4$ and NaHPO$_4$ with a pH of 7.5; PU: 8 M Urea in P; PD: 0.05 M dithiothreitol (DTT) in P; PUD: 8 M Urea +0.05 M DTT in P. SPI120, SPI140, and SPI160, extruded SPI with the barrel temperature at 120 °C, 140 °C and 160 °C. PPI120, PPI140, and PPI160, extruded PPI with the barrel temperature at 120 °C, 140 °C and 160 °C.](image-url)
PPI extrudates, indicating that hydrogen bonds and hydrophobic interactions played marginally more important roles than the disulfide bonds in stabilizing protein structure during extrusion (Chiang et al., 2019).

The protein extracted by PUD (8 M Urea + 0.05 M DTT in P) had the most incredible amount (around 60–85%), indicating that the fiber structure of the extrudates was supported by disulfide bonds, hydrogen bonds, and hydrophobic interactions, and their combinations (Chiang et al., 2019; Liu et al., 2000; Liu & Hsieh, 2007). Compared to the sum of PU and PD, all samples showed a noticeable increase ($P < 0.05$) of extracted protein in PUD due to the synergistic effect of the Urea and DTT. It could be explained by that some of the tertiary and quaternary structures formed by non-covalent interactions were disrupted by urea and disulfide bonds inside become exposed and have easier access to reduction by DTT (Chiang et al., 2019; Lin et al., 2000; Liu & Hsieh, 2007; Osen, 2017). Additionally, no legumin (L) subunits were observed in any PPI samples, but more L subunits by PD (Osen, 2017).

3.7. SDS-PAGE

The SDS-PAGE result of the SPI, PPI, and their extrudates, extracted from the protein solubility test, was shown in Fig. 9. Protein subunits of all samples extracted by P (Fig. 9a) showed the lowest intensity on the SDS-PAGE compared with other extraction solvents, indicating that most native proteins were denatured and participated in the formation of texturization during extrusion (Chiang, 2007). Most protein bands were still present after extrusion for SPI and PPI samples, with no distinct changes on the bands of PPI samples before and after extrusion. However, two bands above 100 kDa in raw SPI samples extracted by P disappeared after extrusion, Chen et al. (2011) explained that extrusion processing could cause proteins to aggregate and further form sizeable molecular weight proteins or protein-non-protein macromolecules on top of gels (in the area of combs). These aggregations could be a reason for fiber formation with protein alone during extrusion (Fig. 4). BT had no essential effect on the distribution of protein subunits under each extracted solvent, which was in agreement with the protein solubility tests.

Most SPI subunits distribution demonstrated no significant difference between raw and extruded samples (Fig. 9). When using PD solvents, the β subunits of the 7 S globulin disappeared, and acidic polypeptides of the 11 S fraction from SPI samples showed more intense (Fig. 9c). This may be because the non-covalent bond contribution was essential to aggregation and texturization during extrusion (Chiang, 2007; Hua et al., 2005). Vicilin (V) and convicilin (CV) of PPI subunits were observed on the gel before and after extrusion by all extracted solvents, indicating that these protein fractions may not contribute to the formation of texturization (Osen, 2017). Additionally, no legumin (L) subunits were observed in any PPI samples, but more Lα subunits were seen in the gel extracted by PD, implying that L existed in the insolubility cross-linked by disulfide bonds, and L was disrupted to Lα subunits by PD (Osen, 2017).

4. Conclusions

To better understand the performance of soy and pea proteins at the same high-moisture extrusion processing (HMEP) conditions, this study investigated the effect of barrel temperature (BT) on physicochemical properties of high-moisture extrudates prepared from soy protein isolate (SPI) and pea protein isolate (PPI). Higher BTs (120 °C–160 °C) improved the generation of anisotropic structure but had no evident effect on the protein solubility and profile for extrudates. The influence of BT on rheological properties was consistent with the textural properties, namely that greater viscoelastic properties resulted in higher structural strength in terms of hardness and chewiness. Comparison with cooked chicken breasts indicated that PPI extrudates were in a similar range of hardness and chewiness as the cooked chicken breasts. In contrast, SPI extrudates showed tougher and greater chewiness. For both
SPI and PPI extrudates, higher cooking temperatures promoted the release of intra-space water within the fibrous structure, and hydrogen bonds and hydrophobic interactions played more critical roles than disulfide bonds to stabilize protein structure. Compared to the SPI counterpart, PPI extrudates had a more fibrous structure, while the water inside PPI extrudates was more prone to migrate. This study offered a good theoretical basis for researchers and industry to select raw protein materials for high moisture plant-based meat analogs, based on the different performance of SPI and PPI extrudates.

Authors contributions

Hong Wang: Experimental design, Methodology, Investigation, Data analysis, Writing-Original draft preparation;
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Birthe Moller Jespersen: Conceptualization, Writing-Review & Editing;
René Lametsch: Experimental design, Writing-Review & Editing.

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