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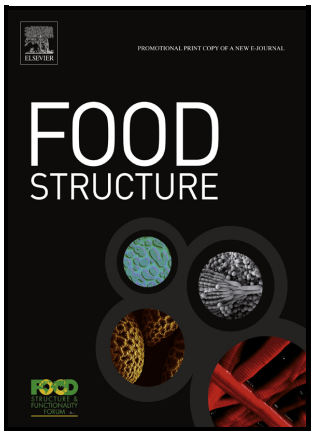
Effects of different soy protein concentrates on the structuring potential and extrudate quality of meat analogues via high moisture extrusion



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Effects of different soy protein concentrates on the structuring potential and extrudate quality of meat analogues via high moisture extrusion

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Abstract

Soy protein concentrate (SPC) is a common ingredient in high moisture extruded meat analogues. However, commercial SPCs can have diverse properties which can affect extrusion outcomes. Yet, the variations between commercial SPCs are often beyond the control of meat analogue manufacturers. Therefore, there is a need to understand how the properties of different commercial SPCs would affect extrudate quality so that manufacturers can screen their ingredients for the desired properties. By investigating a broad range of properties, this study served to study the SPCs on a holistic level and highlight the properties correlated to extrudate quality. The compositional, physicochemical and rheological properties of four SPCs were studied, and their properties were related to the macrostructure and mechanical properties of the extrudates. The study found significant differences between the SPCs for most of the studied properties. Correlation analysis revealed that compositional properties and pH correlated better with extrusion outcomes as compared to other physicochemical properties. Through simple regression analysis, the rheological behaviour of the SPC samples could be related to the fibrous structuring potential of the samples, suggesting that structural breakdown might be an important aspect of fibre formation. The study showed that commercial SPCs indeed varied based on suppliers, and these variations have an apparent impact on the extrudate quality that should not be overlooked, especially for quality control.

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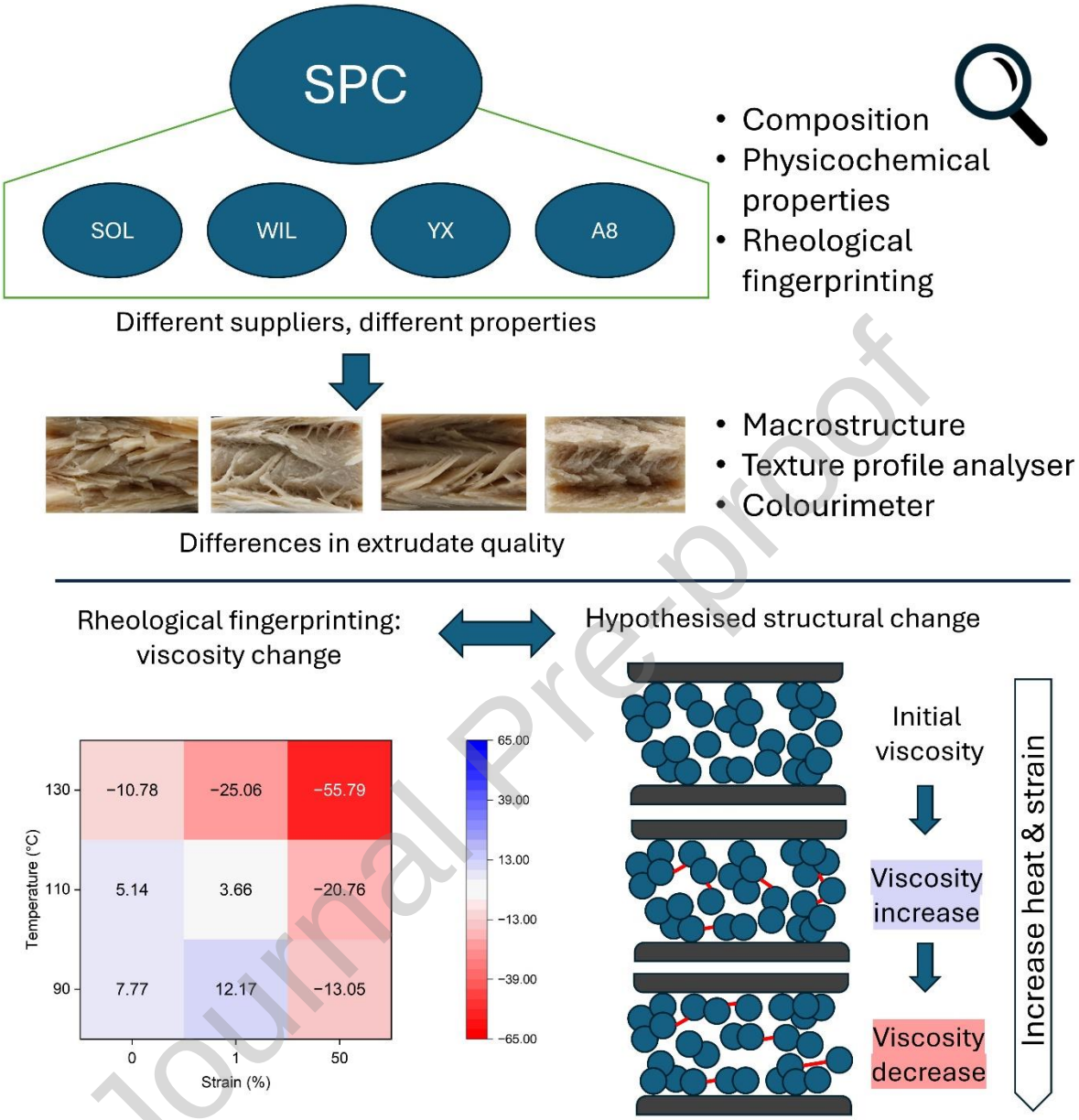
Graphical abstract



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Keywords: meat analogue, extrusion, soy protein, closed cavity rheometer, fibrous structure, protein-protein interactions

Abbreviations: SPC; soy protein concentrate, WHC; water-holding capacity, CCR; closed cavity rheometer

1. Introduction

In the past decade, plant-based diets have gained rapid momentum because they address some of humanity's most pressing issues – delivering sustainable forms of protein to a burgeoning global population (Langyan et al., 2021). To accelerate the adoption of plant-based diets, meat analogues are developed to offer consumers an option to substitute their current diets. Pertinently, high moisture extrusion has exhibited the ability to create fibrous whole pieces from plant protein, which can be potentially similar to meat cuts (Mateen et al., 2023).

However, high moisture extrusion technology is not without challenges. Its most notable challenge remains to be the intertwining complexity of various material and processing parameters that result in the unpredictability of the process (Emin & Schuchmann, 2017). A comparative study on eight different plant protein types found that high moisture extrusion yielded extrudates of different mechanical properties and microstructure, but correlations were difficult to establish (Nasrollahzadeh et al., 2023). Even within the same type of plant protein, there can be vast differences attributed to the cultivar type (Hendrawati et al., 2021; Zhu et al., 2020), environmental growth conditions, the processing involved in obtaining the concentrates or isolates (Burger et al., 2022). In the production process, soybeans are first de-hulled and de-fatted, then soluble carbohydrates are removed from the de-fatted soybeans through one of the three common methods: with acidification, with heat denaturation and water leaching, or with aqueous ethanol (Thrane et al., 2017). After spray-drying, the resultant concentrate or isolate powder is formed. There are many ways to vary the production process, which will lead to compositional differences. For example, using different fat extraction solvent and carbohydrate removal methods led to significant differences in protein, carbohydrate, fat and ash content of the extracted soy protein (L'Hocine et al., 2006; Peng et al., 2021). Zhang et al. (2024) studied 14 different commercial soy protein

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isolates and found large variations in protein composition, protein denaturation, solubility, and water- and oil-binding capacity. Concerning quality control, such differences could ultimately lead to differences in extrudate quality. Echoing Zhang et al. (2024), there are currently little avenues for food manufacturers to understand the commercial ingredients available to them "in terms of variability, functionality, and ways that the protein ingredients affect the final food product."

Considering the plethora of seemingly similar options manufacturers face, it would be imperative for them to know how to screen for protein ingredients that can lead to their desired outcomes. Natural and processing variations in commercial soy protein ingredients are usually beyond the control of meat analogue manufacturers, hence the goal is not to control for the protein powder production process, but rather to analyse and understand how powder properties of commercial soy protein ingredients can be related to extrusion outcomes so that the powders can be evaluated accordingly to ensure the correct selection of starting material. By focusing the scope on a particular plant protein type, it would be more plausible to establish such relationships relevant to that protein.

To this end, previous studies have attempted to relate the raw material qualities to the extrudate quality. Ferawati et al. (2021) found that the ash, fibre and fat content of pea and faba bean isolates and concentrates were positively correlated to the hardness of extrudates, while protein content and water-holding capacity were inversely correlated with the hardness of the extrudate. Osen et al. (2014) found that the functional properties of different pea protein isolates only had a minor effect on fibre formation in the extrudate because they were less relevant at the elevated processing temperature. To the best of our knowledge, despite soy-based meat analogues having the largest market share in Asia for meat analogues (GlobalData, 2022), no study compares different commercial sources of soy protein concentrate and their structuring potential and effect on extrudate quality. The closest study involved

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commercial soy protein isolates. However, the extrudates from the study had severe air cavitation, which suggests insufficient cooling, and that might have influenced the extrudate quality (Meng et al., 2023). Therefore, this study aims to compare how the properties of different commercial soy protein concentrates could affect extrudate properties and highlight key influential parameters.

2. Materials and methods

2.1 Materials

Four SPCs were obtained – Solcon S manufactured by Solbar Ningbo Protein Technology was supplied by KH Roberts (Singapore), Wilcon functional SPC was supplied by Wilmar (Singapore), ALPHA® 8 IP functional SPC by Solae was distributed by Connell Caldic (Selangor, Malaysia), and concentrated soy protein YX702 manufactured by Shandong Yuxin Bio-Tech Co. (China) was distributed by WWRC (Singapore). Table salt was obtained from the local supermarket NTUC Fairprice Co-operative Ltd (Singapore). For simplicity, SPCs, namely Solcon S, Wilcon, YX702, and ALPHA® 8 would be abbreviated to SOL, WIL, YX and A8, respectively. Other reagents and chemicals were obtained from Sigma-Aldrich (Singapore).

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2.2 Proximate and pH analysis of SPC

Moisture content was measured as the weight difference after the SPCs were dried in an oven at 105 °C for 24 h. Protein content was determined using the Dumas method (Dumatherm N Pro, Gerhardt, Germany) with a conversion factor of 6.25. The fat content of the SPCs was determined by acid hydrolysis based on AOAC method 922.06 and 954.02, while ash content was determined by AOAC method 923.03 (AOAC, 2005). Consequently, the remainder of the composition was taken as

carbohydrate content. The pH of the SPCs was determined by measuring a 1 wt% SPC dispersion in water, hydrated overnight.

2.3 Water-holding capacity (WHC) and solubility analysis of SPC

WHC and solubility were determined according to Bühler et al. (2020). 1 wt% SPC dispersion in water was hydrated overnight with a magnetic stirrer at room temperature. After which, the dispersions were centrifuged at 10,000 ×*g* for 10 min. The mass of the wet pellet was measured after removing the supernatant. The wet pellet was then freeze-dried and weighed again. The WHC was calculated according to Equation (1).

$$WHC = \frac{(m_{wet\ pellet} - m_{dry\ pellet})}{m_{dry\ pellet}} \quad (1)$$

Where $m_{wet\ pellet}$ refers to the mass of the pellet after centrifugation, $m_{dry\ pellet}$ refers to the mass of the pellet after centrifugation and freeze-drying.

Solubility was defined as the unsedimented component of the SPC powders after centrifugation. Overall solubility was determined by the difference between the original powder mass and the dry pellet mass. Since SPC contains both protein and non-protein components, the protein solubility ($Solubility_{protein}$) was further determined by measuring the protein content of the dried pellet using the Dumas method as described previously to find the mass of protein in the pellet, and calculated with Equation (2). Similarly, the solubility of the non-protein fraction ($Solubility_{non\text{-}protein}$) was determined by Equation (3). The non-protein masses were obtained by subtracting the protein mass from the total mass.

$$Solubility_{protein} = \frac{protein\ mass\ in\ SPC - protein\ mass\ in\ pellet}{protein\ mass\ in\ SPC} \times 100\ \% \quad (2)$$

$$Solubility_{non-protein} = \frac{non-protein\ mass\ in\ SPC - non-protein\ mass\ in\ pellet}{non-protein\ mass\ in\ SPC} \times 100\ \% \quad (3)$$

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2.4 Protein-protein interactions analysis of SPC

Since the insoluble component of SPC (ISPC) contains aggregated proteins, it was analysed for its protein-protein interactions using various extraction solvents. The insoluble component was prepared by dispersing 5 wt% of SPC in water overnight and centrifuging at 5,000 ×*g* for 10 min. Subsequently, the pellet was freeze-dried and ground into powder with a spice grinder. Protein extraction was carried out based on Chiang et al. (2019) and Pietsch et al. (2019) with modifications. 0.1 wt% ISPC was extracted in 0.1 M potassium phosphate buffer (pH 7.0) (P) with various selective agents. With P alone, it was used to extract and account for any residual soluble proteins that might not have been fully removed during the preparation of ISPC. When P was used with 8 M urea (PU), it solubilised protein aggregates by breaking both hydrogen bonding and hydrophobic interactions within the aggregates (Lee et al., 2006). When P was used with 0.5 M dithiothreitol (DTT) (PD), it disrupted the disulfide bonds between protein aggregates. When P was used with 8 M urea and 0.5 M DTT (PUD), both covalent and non-covalent bonds were disrupted, and it was assumed that the protein aggregates were completely solubilised. After vortexing the ISPC in their respective extractants, the samples were shaken at 350 rpm (PSU-10i, Biosan, Latvia) for 1 h at room temperature. After which, the samples were centrifuged at 10,000 ×*g* for 10 min. The extractable protein content in the supernatant was obtained by measuring the absorbance at 280 nm using a UV/Vis spectrophotometer (UV-2600, Shimadzu, Japan). The extractable protein content was calculated from a bovine serum albumin calibration curve. The extractable protein content for each ISPC in the various extraction solvents was expressed as a percentage of their respective extractable protein content in PUD.

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2.5 Rheological analysis of SPC

The closed cavity rheometer (CCR) (RPA elite, TA instruments, New Castle, Delaware, USA) was used to investigate the rheological properties of the hydrated SPCs. The SPCs were first hydrated to 40 wt% overnight in the fridge. After loading the sample into the CCR, a basic position with no oscillation was assumed at 60 °C for 2 min for temperature equilibration and relaxation of the material. Then, a 1 min time sweep of 1 % strain and 1 Hz was conducted to measure the initial viscosity. Then, a temperature sweep was conducted from 60 °C to the target temperature of either 90, 110, or 130 °C at 5 °C/min, and the sample was held at the target temperature for 2 min (isothermal period) before cooling down to 60 °C at 5 °C/min, throughout which oscillation was maintained at 1 % strain and 1 Hz. After the temperature sweep, another 1 min time sweep of 1 % strain and 1 Hz was conducted again to measure the final viscosity. The complex viscosity measurement at the target temperature was taken at the start of the isothermal period. Viscosity change was also measured by comparing the complex viscosity measured ~~during the time sweep~~ before and after the temperature sweep at 60 °C. The above test was repeated where the strain of the temperature sweep was changed to 0 and 50% strain to measure the viscosity change under different shear conditions.

2.6 High-moisture extrusion of SPC

The SPCs were texturised into meat analogues via high-moisture extrusion with a twin-screw co-rotating extruder (ZSE 27 HP PH, Leistritz, Nuremberg, Germany) with a cooling die of 48 × 10 × 540 mm (W × H × L). The screw diameter was 27 mm, and the length-diameter ratio was 40:1. SPCs mixed with salt were fed into the extruder, and water was injected through an inlet port such that the extrudates had an output of 10 kg/h with a moisture content of approximately 60 wt% and 1 wt% salt. The screw speed

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was 200 rpm, and the barrel had a 10-zone temperature profile for the three target temperatures of 90, 110 and 130 °C, as illustrated in **Figure 1**.

[Insert **Figure 1**]

2.7 Macrostructure quantification and colourimetric analysis

Macrostructure observation was done by cutting along the flow direction of the meat analogue extrudate and bending it open to reveal its internal structure. Pictures of the macrostructure were processed using the Fiberlyzer, a software developed for automated, quantitative, and standardised assessment of the visual fibrousness of meat analogues (Ma et al., 2023). The settings were set at 10 for the HSV step, 82% for the threshold and 0.045% for the minimal area. The Fiberlyzer generated fibre scores for the macrostructure. The colour of the extrudates was measured using a colourimeter (CM-5, Konica Minolta, Japan). Colours were expressed in Hunter-Lab parameters as L*, a*, and b* values. L* represents lightness (0 = black, 100 = white), -a/ +a represents greenness or redness, and -b/ +b represents blueness or yellowness (Hua et al., 2023).

2.8 Mechanical (double compression) analysis

Mechanical properties of the meat analogues were measured using a TA-XT plus C texture analyser (Stable Micro Systems, Surrey, UK) with reference to Chiang et al. (2019). Samples of size 2 × 2 cm (L × W) were prepared from the meat analogues. The height sample is the height of the extrudate. The samples were compressed using a P/75 probe to 50 % strain at 1 mm/s, then returned to the original position for 5 s, followed by a second compression to the same strain and speed. Hardness and chewiness were results obtained from the double compression test.

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2.9 Data analysis

All analyses were carried out in triplicates unless mentioned otherwise. Results are reported as mean ± standard deviation. The significance of the results was determined with SPSS 29 (IBM, Armonk, New York, USA) via one-way analysis of variance (ANOVA) and Tukey's post hoc test. In cases where equal variance could not be assumed, Welch's ANOVA with Dunnett's post hoc test was used. Pearson correlation analysis, simple regression analysis and analysis of covariance (ANCOVA) were also carried out with SPSS to quantify linear relationships between the SPC powder properties and their rheological and extrudate properties.

3. Results and discussion

3.1 Proximate composition and pH of SPC

Proximate and pH analysis revealed significant differences between the SPCs (**Table 1**). This highlights that SPCs varies by supplier. Across a few studies, a broad range of values was found for protein (46.5 – 79.0%), moisture (6.0 – 9.2%), carbohydrate (8.1 – 17.8%), fat (1.0 – 9.4%), and ash (2.6 – 5.1%) content in SPC (Chinma et al., 2013; Eldridge et al., 2002; Peng et al., 2021; Pietsch et al., 2019; Schmid et al., 2024). The differences in composition reinforced the notion that SPC as a category is still very diverse and cannot be treated as the same. Hence, their properties should be studied to understand how they would affect extrusion processing for protein structuring.

[Insert **Table 1**]

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3.2 Solubility and water holding capacity of SPC

Table 1 summarises the solubilities and WHC of the SPCs. WIL had the highest overall solubility, followed by SOL, A8 and YX. A closer inspection of the protein and non-protein components yielded insights that the component solubility differed across samples. WIL had a remarkably higher protein solubility compared to the other SPCs, while the converse was true for non-protein solubility, where WIL had the lowest. Similar to how extraction methods could affect SPC composition, the use of different extraction methods as well as processing conditions can also influence protein solubility (Burger et al., 2022; L'Hocine et al., 2006; Peng et al., 2021; Tirgar et al., 2017). Differences in protein solubility were most likely due to differences in protein conformation and intermolecular interactions rather than amino acid composition since the amino acid profiles between the SPCs were similar (**Supplementary Table 1**). Protein solubility is affected by surface charges and exposed non-polar groups (Grossmann & McClements, 2023; Kramer et al., 2012). Increased exposure to hydrophobic groups would induce greater hydrophobic interactions, leading to protein aggregation, while similarly charged groups do the opposite (Desantis et al., 2022). The zeta potential of the SPCs was found to be quite similar in the range of -29 to -36 mV (**Supplementary Table 2**), suggesting that surface charge was unlikely the main reason for the solubility differences observed. As found by Verfaillie et al. (2024), more extensive thermal processing would lead to a reduction in denaturation enthalpy and solubility, which could suggest that WIL may have been less denatured than the rest of the SPCs.

A8 had lower WHC compared to the other samples (**Table 1**). It can be seen that the WHC of the SPCs could not be directly correlated with the solubility profiles of the SPCs (**Table 1**). This has been similarly observed by Bühler et al. (2020), who found that the increase in the insoluble protein fraction of faba bean protein concentrate could not fully account for the increase in its WHC. Non-protein components, as well as the types of inter-molecular bonds present in the protein aggregates, were suggested as

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additional factors that alter water-binding properties (Bühler et al., 2020). On the other hand, Verfaillie et al. (2024) reported that a higher WHC was attributed to the decreasing solubility of soy protein, a correlation we did not observe in this study. Zhang et al. (2024) reported that increased surface hydrophobicity reduced WHC and concluded that the exposure of hydrophobic amino acid residues could interfere with water binding. The various conclusions found by different authors highlight that WHC is a complex functional property, which could be affected by protein aggregation, amino acid residue interaction with water and non-protein components in the SPCs.

3.3 Protein-protein interactions analysis of SPC

The WHC results indicated there could be differences in the type of interactions experienced by the protein aggregates, which prompted an investigation into the protein-protein interactions present in the insoluble component of the SPC. Results revealed that there were indeed significant differences between the SPCs in the amount of extractable proteins, highlighting differences in protein aggregate interactions (**Figure 2**). When PU was used, it could be seen that SOL had the highest amount of extractable protein, while A8 had the least amount of extractable protein. Since urea could solubilise protein aggregates by breaking both hydrogen bonding and hydrophobic interactions (Lee et al., 2006), this suggested that the proportion of aggregates with accessible non-covalent interactions was the highest in SOL and lowest in A8. Furthermore, for all SPCs, when the extractable protein content of PU and PD were summed up, they were not equal to 100 %. The extractable protein content when PUD was used was taken to be 100 % for the respective SPC. The phenomenon where the sum of individual extractants (PU and PD) not adding up to the effect of a combined extractant (PUD) was also observed by other authors (Chiang et al., 2019; Liu & Hsieh, 2007; Pietsch et al., 2019). Liu and Hsieh (2007) theorised that such observation was due to the synergy between covalent and non-covalent interactions, where disulfide bonds could have been buried within aggregates held together by non-covalent interactions, making them inaccessible to DTT in the

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absence of urea. The difference might also be due to variations in the non-protein component, such as polysaccharide composition and interactions, which could have hindered protein extraction. Regardless, the results point to A8 having a more restrictive and less accessible matrix than the rest of the SPCs. Uncannily, this trend mirrored that of WHC, where A8 had a much lower WHC than the rest (**Table 1**), and the two properties had a strong Pearson correlation of -0.997, where an increase in protein-protein interactions was almost linearly related to a decrease in WHC. Note that the values used for protein-protein interactions in the correlation were the inverse of the PU + PD results, so the correlation would be more intuitive. The strong correlation found could be due to competing interactions between protein-protein and protein-water (Chou & Morr, 1979).

[Insert **Figure 2**]

3.4 Rheological properties of SPC

Temperature sweeps were conducted to understand the rheological behaviour of the 40 wt% SPC samples and gain insights into their flow behaviour when they enter the cooling die during extrusion at different melt temperatures. As the temperature increased from 90 to 130 °C, complex viscosities decreased for all samples (**Figure 3**), consistent with previous literature (Pietsch et al., 2019; Wittek et al., 2021b). This would be expected since increasing temperatures reduce intermolecular interactions and increase molecular mobility (van der Sman & van der Goot, 2023). The differences in viscosity could affect the flow profile in the cooling die, as explored by Wittek et al. (2021a), where a lower viscosity due to higher temperature led to a more pronounced parabolic profile in the cooling die. This parabolic

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profile has been related to the degree of anisotropy or fibrousness in the final extrudate (Wittek et al., 2021a).

~~Remarkably, the~~ The SPC samples exhibited different complex viscosities, and the relative differences were consistent at all temperatures – A8 had the highest complex viscosity, followed by WIL, YX and SOL. Noticeably, the complex viscosity of A8 at 110 °C was higher than that of SOL and YX at 90 °C. The finding brings to attention that regardless of temperature, there were intrinsic differences between the nature of the SPC governing its rheological properties. Compositional differences were ruled out since none of the compositions mirrored the trend observed here, so further scrutiny was required. In one study, Fu et al. (2024) investigated how soluble and insoluble soy protein fractions would affect rheological properties in the CCR at extrusion-like conditions. They found that increasing insoluble protein content resulted in greater resistance to shear. Yet, such a correlation was not found in this study between the insoluble SPC content and their respective complex viscosities. One critique of the aforementioned study is that Fu et al. (2024) altered the insoluble protein content of their material through different ratios of soluble and heat-denatured soy protein. This meant that a singular source of the insoluble aggregated proteins was used – having undergone the same processing to be denatured. This could perhaps explain how the authors managed to find correlations between solubility and rheological properties. However, in reality, for commercial soy proteins, the different techniques and conditions used for protein extraction could have resulted in different types of aggregation (Monteiro & Lopes-da-Silva, 2018), which cannot be simply distinguished through protein solubility alone. Different types of aggregation could depend on the different protein sub-units involved, such as between the basic polypeptides of glycinin, which give rise to insoluble aggregates, or between the β subunit of β-conglycinin and basic polypeptide of glycinin that result in partially soluble aggregates (Meng et al., 2024). Other factors determining the type of aggregation include the ratio between glycinin and β-

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conglycinin in the soy protein (Meng et al., 2024). In this study, protein-protein interactions analysis revealed that there were indeed differences in aggregation behaviour between the SPCs. This means that the type of aggregation should also be an important consideration when evaluating the properties of the protein material. Considering the above statement, this could also be applied to the type of carbohydrate present in the SPC, which could exert the observed differences in viscosity. This is technically plausible since it has been reported that adding different polysaccharides to soy protein isolates changes their rheological properties (Snel et al., 2024; Taghian Dinani et al., 2023b).

[Insert **Figure 3** and **Figure 4**]

Additionally, we have measured the change in complex viscosity of the SPC samples after temperature sweeps at different combinations of temperature and shear (in the form of strain applied). Heat maps of the resultant rheological fingerprints of the SPC samples (**Figure 4**) highlighted distinct differences in the behaviour of the SPC samples in response to temperature and shear. It could be seen that WIL experienced a reduction in viscosity across all temperatures and strain conditions. SOL and YX experienced a small viscosity increase at only 90 °C at 0 and 1 % strain, while decreased at other condition combinations. A8 experienced an increase in viscosity at both 90 and 110 °C at 0 and 1 % strain. Among the samples, A8 also experienced the largest increase in viscosity.

Changes in viscosity could reflect structural changes such as aggregation and breakdown of molecules as the breaking and forming of bonds between biopolymers all contribute to the density of crosslinks and, thus, the rheology (van der Sman & van der Goot, 2023). As found by Fu et al. (2023), the size of protein aggregates correlates with complex viscosity. Other than protein aggregates breakdown, it is also possible for non-protein components, such as the pectin in SPC, to degrade (Voragen et al., 2009),

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leading to reduced viscosity. Changes in viscosity could also be due to shearing. It could be seen that the role of shear, in the form of different strains applied, had an impact on viscosity change. As the strain applied increased from 0 to 50 % at the corresponding temperature, the viscosity change became more negative for all samples. This could be due to the structural breakdown or the alignment of aggregates due to the high shear. It was observed in non-food polymers that the alignment of molecule chains under flow resulted in reduced bulk viscosity (Chan et al., 2001).

Temperature also affected viscosity change, where an increase in temperature generally resulted in more negative viscosity change. It can also be observed that in the absence of shear (0 % strain), viscosity change was positive at 90 °C for all the samples except WIL, and decreased to negative as temperature increased to 130 °C. This suggests that the samples had undergone aggregation as they were heated to 90 °C, but as temperature increased further, structural breakdown became more dominant, resulting in reduced viscosity, highlighting that the SPC samples' response to temperature is a dynamic process.

The importance of the interaction between temperature and shear was also noted. It could be seen that between 0 and 1 % strain, the difference in viscosity change was relatively small at 90 and 110 °C. However, at 130 °C, the effect of strain on viscosity change was apparent, where a small strain of 1 % resulted in a much more negative viscosity change. This could be due to the sufficient energy at 130 °C to overcome intermolecular forces, which allowed for a greater rearrangement of the biopolymer during shearing.

By combining the above observations and literature, we infer that the SPCs undergo a dynamic aggregation and breakdown process depending on temperature and shear conditions that could be roughly represented by schematic **Figure 5**. Aggregation would enhance resistance against flow, while

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breakdown and alignment would reduce resistance. This change in viscosity can allow us to infer the structural behaviour of the SPCs. When comparing the overall heat map profiles of the samples, WIL had the most negative change profile, while A8 had the least negative change profile. This might indicate that for WIL, it favoured breakdown behaviour across the various conditions, while A8 was more resistant to structural breakdown. It could also be possible that A8's rheological fingerprint might be related to its high protein-protein interactions and low WHC because these properties reflect A8's affinity to water, which acts as a plasticiser that weakens the material and improves flow. These differences in structural behaviour in response to heat and shear may subsequently affect the fibrous structuring potential of the SPC samples.

[Insert **Figure 5**]

3.5 Macrostructure of SPC extrudates

From the macrostructure and fibre scores of the extrudates (**Figure 6**), it could be observed that barrel peak temperature played an important role in the macroscopic fibrous structuring of high-moisture meat analogues. Initially, at 90 °C, all samples did not display fibrousness, which corresponded to low fibre scores. As the processing temperature increased, the extrudates developed fibrous macrostructures. SOL, WIL and YX showed fibrous macrostructure from 110 °C onwards, while A8 developed a fibrous appearance at 130 °C. The results corresponded well with the higher fibre scores obtained at higher temperatures. Högg and Rauh (2023) also found that fibre-like structures could only be observed in extrudates when the material temperature was above 110 °C, highlighting the importance of temperature on protein structuring. According to the authors, temperature is pivotal in influencing the phase transition of proteins into a completely denatured state, forming a protein melt that is crucial for fibre formation (Högg & Rauh, 2023). This sentiment was similarly shared by Osen et al. (2014) when the

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authors could not obtain fibrous structures for pea protein isolates extruded at temperatures below 120 °C.

It could also be observed that for the same temperature, the type of macrostructures developed and associated fibre scores were not the same. This was most evident at 110 °C, where A8 had a much lower fibre score than the other samples. This further suggests that there were inherent differences between the SPCs, which resulted in varied macrostructure. An example of inherent difference would be insoluble protein content, where Fu et al. (2024) found that increased insoluble protein content resulted in greater fibrous appearance, but this did not correlate with samples of this study. Instead, the rheological fingerprint of the samples seemed to explain the differences observed (**Figure 4**). By using viscosity change as an indication of structural changes, we could elucidate that A8's response to heat and shear was more resistant to structural breakdown. As posited by van der Sman and van der Goot (2023), liquefaction, a process where intermolecular bonds get broken to attain a liquid-like constitution, and alignment, a process where aggregates and biopolymers are arranged into anisotropic structures, are crucial for fibre formation. These processes involve structural breakdown, which might bridge the explanation between A8's rheological fingerprint and the low fibre score at 110 °C, where the thermal energy at 110 °C was unable to overcome A8's resistance to structural breakdown to allow for the above-mentioned fibrous structuring process. This highlights the potential of measuring viscosity change as a new tool to study the behaviour of SPCs under heat and shear.

In addition to fibrous macrostructure, we also noted differences in visual colour. WIL appeared and was measured to be the lightest in colour compared to the rest of the samples (**Table 2** and **Figure 6**). This might be related to its lower carbohydrate content, which lightness was strongly negatively correlated with (-0.87 to -0.96 across the three temperatures). Taghian Dinani et al. (2023a) also reported an

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increase in the browning index when the polysaccharide content increased in high-temperature shear cell products.

[Insert **Figure 6** and **Table 2**]

3.6 Mechanical properties of SPC extrudates

A double compression test was conducted, which revealed that an increase in barrel peak temperature increased both the hardness and chewiness of the extrudates (**Figure 7**). The increase in hardness due to increasing temperature was likely attributed to the greater degree of proteins unfolding, aggregation, and, subsequently, more extensive intermolecular interactions that form a network. This was further supported by the fact that the protein content of the samples mirrored closely to that of the hardness of their extrudates – WIL > YX > SOL > A8, possibly meaning that the protein content exerts a dominant effect on hardness. The influence of protein content on hardness has also been observed by Mateen et al. (2023), where an increase in protein content resulted in greater hardness. Other studies have also related the hardness of the extrudate to the solubility of initial protein material, where an intermediate protein solubility (~50 %) was found to give the highest hardness (Meng et al., 2023; Verfaillie et al., 2024). At first glance, this seemed to have corresponded with the literature since WIL also had a protein solubility of around 50 % and had the highest hardness, while the others had protein solubility between 20 – 24 %. However, the differences in hardness between WIL and the other samples were not proportional to their differences in protein solubilities, which makes it difficult to sufficiently convince that protein solubility was an influential factor in the hardness of the extrudates.

On the other hand, parallels could not be clearly drawn between protein content and chewiness, even though a previous study on a wide range of solid food products found an association between the two

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(Wee et al., 2018). This might indicate that chewiness as a complex parameter derived from hardness × cohesiveness × springiness could be influenced by more than one component in the SPC. Nonetheless, since chewiness has been associated with food elasticity (Wee et al., 2018), the general trend of increasing chewiness with temperature still indicates the contributions of protein network formation, which other components in the SPC could further modulate. The modulation of extrudates' mechanical properties through non-protein components has been previously observed (Chen et al., 2021; Zhang et al., 2016).

[Insert **Figure 7**]

3.7 Correlation matrix

[Insert **Figure 8**]

A correlation matrix (**Figure 8**) was plotted to screen for compositional and physicochemical properties of the SPC powders that could be related to the rheological properties of the SPCs and textural properties of the extrudates. We want to highlight to readers that the data was interpreted with caution due to the limited sample size. We focused on strong correlations, defined as the absolute correlation coefficient being more than 0.8.

Viscosity measured in the CCR at the target temperatures was strongly correlated to protein interactions and WHC of the SPC powder samples. As explored in the previous section, protein interactions and WHC were inversely correlated, which explained the difference in correlation direction with the CCR viscosity. The result could suggest that protein interactions could enhance resistance to flow, leading to higher measured viscosity.

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For viscosity change measurements, protein content and pH were negatively correlated, while carbohydrate and ash content were consistently positively correlated throughout the range of heat and shear conditions. The results suggest that these properties exert an effect on structural changes independent of test conditions. The correlations observed may indicate that increasing protein content and pH, or reducing carbohydrate and ash content can lead to negative viscosity changes, which seemed to favour fibrous macrostructure, as explored previously.

On the other hand, correlations between viscosity change and other physicochemical properties of the SPC seemed to be dependent on the test conditions. Correlations between viscosity change and the properties of protein interactions and WHC were strong at lower temperatures of 90 and 110 °C and at lower strains of 0 and 1 %. Subsequently, correlations became weaker at either 130 °C or 50 % strain. The loss of correlation was possibly because protein interactions and structure change more drastically at more intense conditions, to the point that the initial properties of the powder were no longer relevant. The correlations between viscosity change and overall solubility of the samples also seemed to be dependent on test conditions, where correlations got stronger with higher temperature and strain conditions. These results meant that the relevance of the physicochemical properties of the SPC samples to viscosity change was specific to the conditions the samples were subjected to.

For the extrudate mechanical properties, hardness had strong positive correlations with protein content and pH, and strong negative correlations with carbohydrate and ash content across all temperatures. On the other hand, the correlations between chewiness and protein, ash and carbohydrate content were dependent on temperature, where higher temperature had stronger correlations. When looking at the correlations between protein interactions (or WHC) and chewiness, they exhibited temperature

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dependence, where the correlations were weaker at higher temperatures. This would suggest that chewiness as a property could be more complex than hardness, which leads to the temperature-dependent correlation behaviour. The above was similarly observed when the properties of powders were correlated with the fibre scores – composition and pH had stronger and more consistent correlations across temperatures than the other physicochemical properties of the powders.

In summary, the correlation matrix revealed insights into how the SPC powder properties could be related to its rheological and extrudate properties. Correlations with compositional properties and pH seemed to be generally consistent across different test conditions of temperature and strain, while other physicochemical properties showed variations depending on the related property and the test conditions.

3.8 Regression analysis and analysis of covariance (ANCOVA)

Another approach we took was to relate the samples' rheology to the extrudate outcomes. We hypothesise that the influence of texturisation temperature and powder properties will manifest in the rheology of the samples, allowing for a singular independent variable to be used in simple regression analysis, as illustrated in **Figure 9**. This was supported by correlation analysis, which showed that the powder properties could be correlated with the rheological measurement (**Figure 8**).

[Insert **Figure 9**]

From the results in **Table 4**, the fit of the regression (R^2) varied for different properties and rheological variables used. For the hardness of extrudate, viscosity change at 0% strain had the best fit (0.88), while for chewiness and fibre score of the extrudate, viscosity change at 1% strain had the best fits (0.86 and

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0.94, respectively). These results were comparable to that in the multiple regression, suggesting that the use of sample rheology as a singular variable could be appropriate to correlate to extrudate properties. Among the variables, the viscosity at the target temperature has the poorest fit for all three extrudate properties, suggesting that it did not sufficiently capture the extent of heat and shear processing experienced by the samples. On the other hand, using viscosity change as the independent variable incorporates both aspects of heating and cooling and how the samples have been changed from their initial state. In particular, the high R^2 value for the regression with fibre score further supports our suggestion that measuring viscosity change has the potential to elucidate structural behaviour relating to fibrous structuring. In general, a reduction in viscosity was related to increased hardness, chewiness and fibre score.

[Insert **Table 3**]

In addition to the simple regression, we also conducted an analysis of covariance (ANCOVA) to determine if there were group differences exerted by the different samples used. A significant result meant that the relationship between the dependent and independent rheological variable was also dependent on the sample used. From **Table 4**, it can be seen that sample type did exert an effect depending on the independent variable used. This was most prominent for the variable of viscosity at target temperature and viscosity change at 0% strain. The significance of the sample effect implies that these variables could not fully capture the structural behaviour of the SPC powders, resulting in the inability to be generalised across the different sample types. From this, we could elucidate that just measuring the viscosity at the target temperature would be insufficient, which was consistent with its poor fit for the simple regression. Additionally, not having shear (viscosity change at 0% strain) or too much shear (viscosity change at 50% strain) would also cause the sample effects to become significant.

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The results suggest that the variable of viscosity change at 1% strain could be a useful measurement to relate to the extrudate properties that could be applied across all samples. Considering that these were four differently produced commercial SPC samples, this highlights the usefulness of using viscosity change as a measurement to potentially predict the outcomes of extrusion for other SPC samples. The advantage of this method lies in not having to know exactly how these commercial SPC samples were produced nor having to know their composition, but rather through a single measurement of its change in viscosity. The obvious limitation would be that it is not clear how changing the extruder, cooling die, or processing parameters other than temperature, such as screw speed, would impact the relationship between viscosity change and extrudate outcomes. Nonetheless, this serves as a good starting point for further research.

[Insert **Table 5**]

4. Conclusion

Four different commercial SPCs were investigated for their compositional, physicochemical and rheological properties and subsequently related to their extrudates' quality. Significant differences between the SPCs were found in the investigated properties. Correlation analysis found that compositional properties and pH were better correlated with the extrudate's mechanical and structural properties than the other physicochemical properties. The SPC samples also had different rheological properties measured in the CCR. The sample most resistant to viscosity reduction had the least fibre formation in the extrudate. This suggests that rheological behaviour and structural breakdown may be key to fibrous structuring in high moisture extrusion. Simple regression between viscosity changes and extrudate properties also showed a good fit, highlighting the potential of using viscosity change as a

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single measurement to predict extrudate quality. Overall, these findings have shown that different properties of the SPCs affect various aspects of their extrudates, revealing the nuances between SPCs that could change extrusion outcomes drastically. Consequently, manufacturers should carefully evaluate the properties of their SPC ingredients to ensure the desired quality.

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Declaration of interest

The authors declare that they have no conflict of interest.

Author contributions

Ding Xiang Chew: Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation. **Alicia Theng**: Methodology, Formal analysis, Investigation, Writing – Review & Editing. **Raffael Osen**: Writing-Review & Editing. **Jie Hong Chiang**: Conceptualisation, Methodology, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

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Figures

Zone	1	2	3	4	5	6	7	8	9	10
Temperature (°C)	30	50	60	70	90	90	90	90	90	90
	30	50	60	70	90	90	90	110	110	110
	30	50	60	70	90	90	110	130	130	130

Figure 1 Extruder barrel temperature profile.

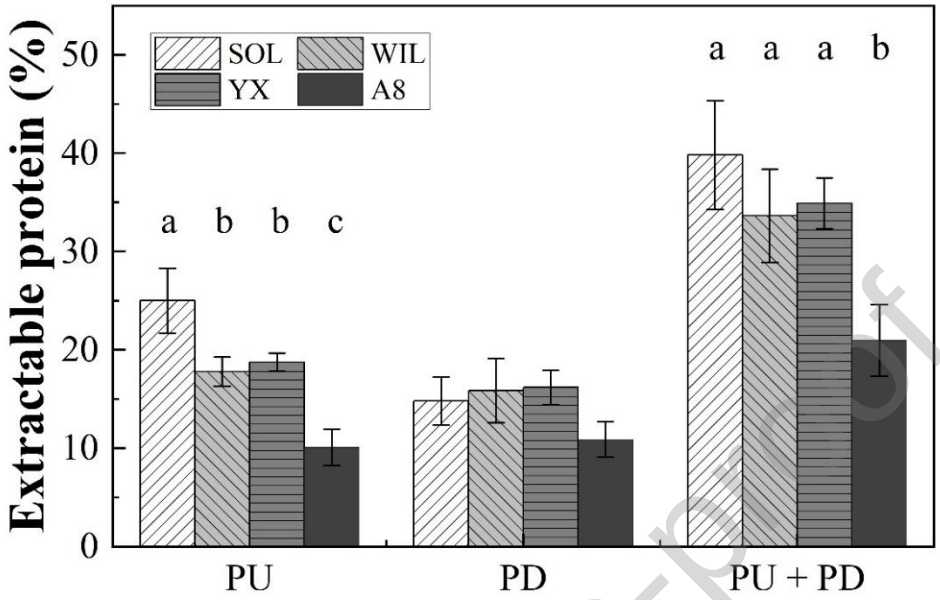

Figure 2 Protein extracted from the insoluble fraction of SPC expressed as a percentage of total proteins extracted with PUD. PU = phosphate buffer with 8 M urea, PD = phosphate buffer with 0.5 M DTT, PU + PD = summation of proteins extracted with PU and PD, PUD = phosphate buffer with 8 M urea and 0.5 M DTT. Different letters indicate a significant difference ($p < 0.05$) between samples in each category. No differences were observed for PD. Means are presented with standard deviation, where $n = 3$.

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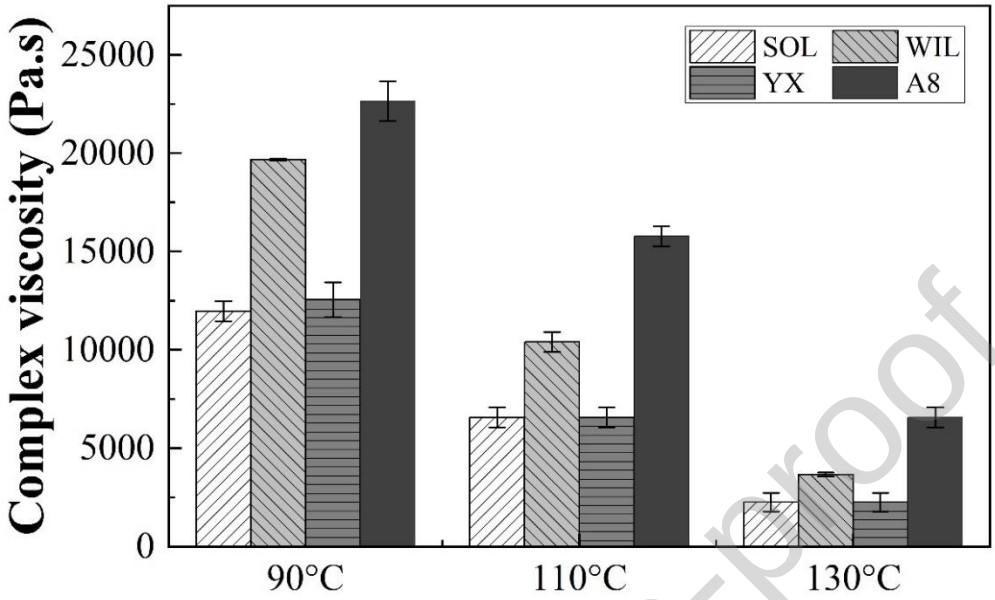

Figure 3 Complex viscosity at different temperatures corresponding to extrusion conditions. Means are presented with standard deviation, where *n* = 3.

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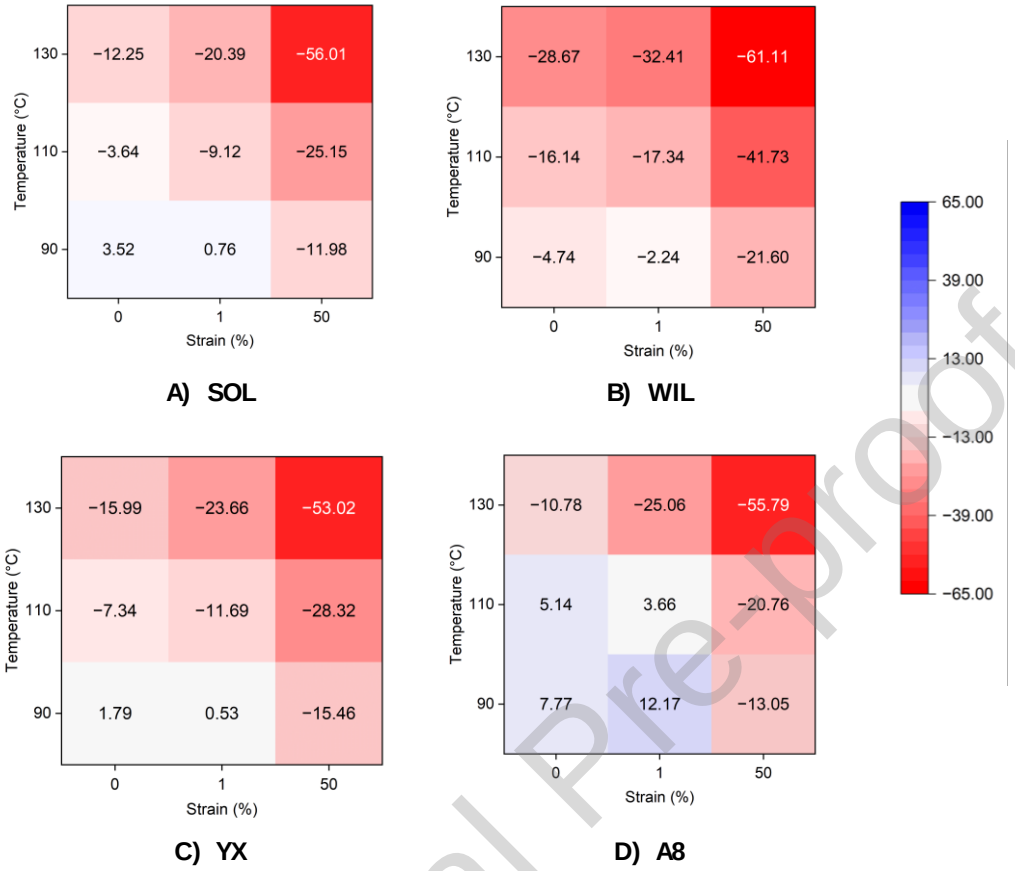

Figure 4 Rheological fingerprint of viscosity changes of 40 wt% SPC samples A) SOL, B) WIL, C) YX, D) A8 after being subjected to various combinations of temperature and strain conditions in the CCR. Shades of blue represent a percentage positive change; shades of red represent a percentage negative change from the initial viscosity.

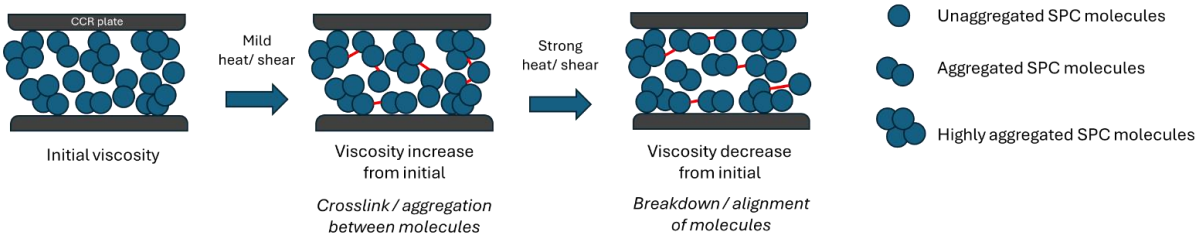

Figure 5 Schematic of structural changes occurring in the SPCs in relation to viscosity changes.

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Sample	90 °C	110 °C	130 °C
SOL			
	4.04	10.00	13.04
WIL			
	6.46	12.02	15.70
YX			
	6.13	10.70	13.90
A8			
	3.98	6.81	13.65

Figure 6 Macrostructure of extrudates made from different SPC at different temperature conditions.

Fiber scores of each sample are indicated below the images.

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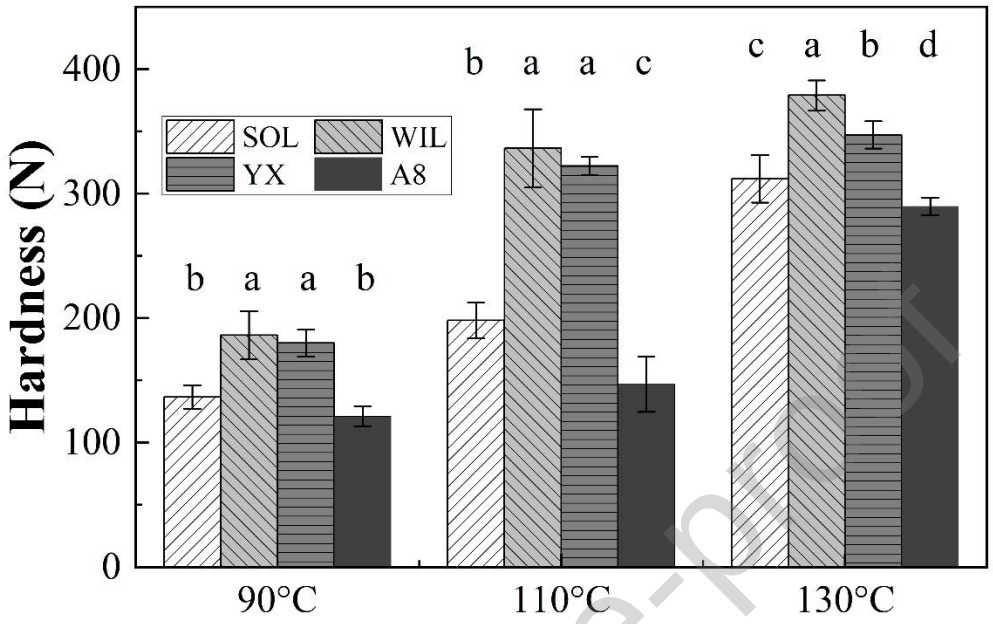
(A)



(B)

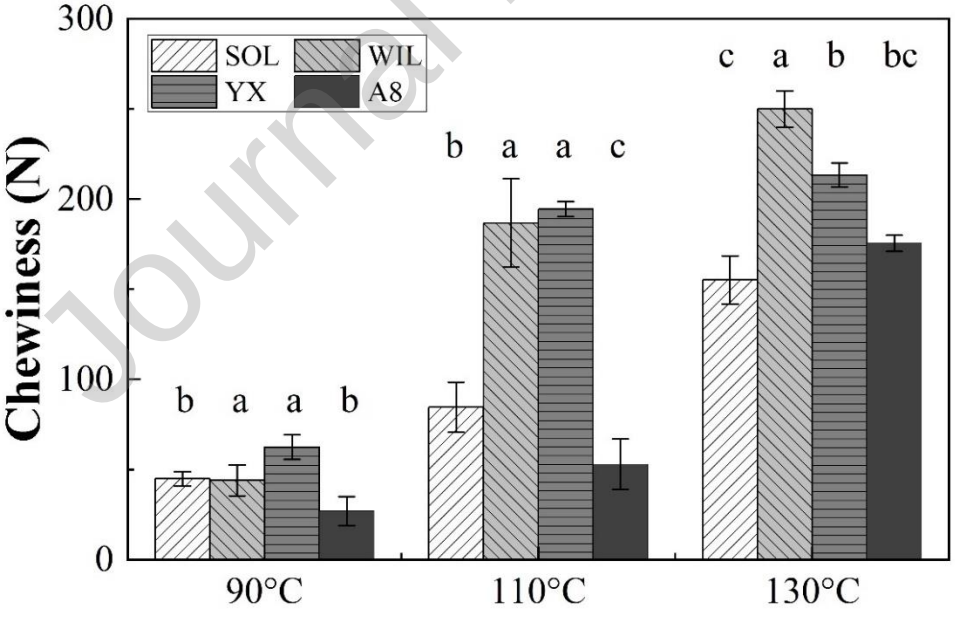


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Figure 7 Hardness (A) and chewiness (B) of extrudate samples from double compression test. Means are presented with standard deviation, where $n = 6$. Values bearing different lowercase letters in the same temperature category were significantly different ($p \leq 0.05$).

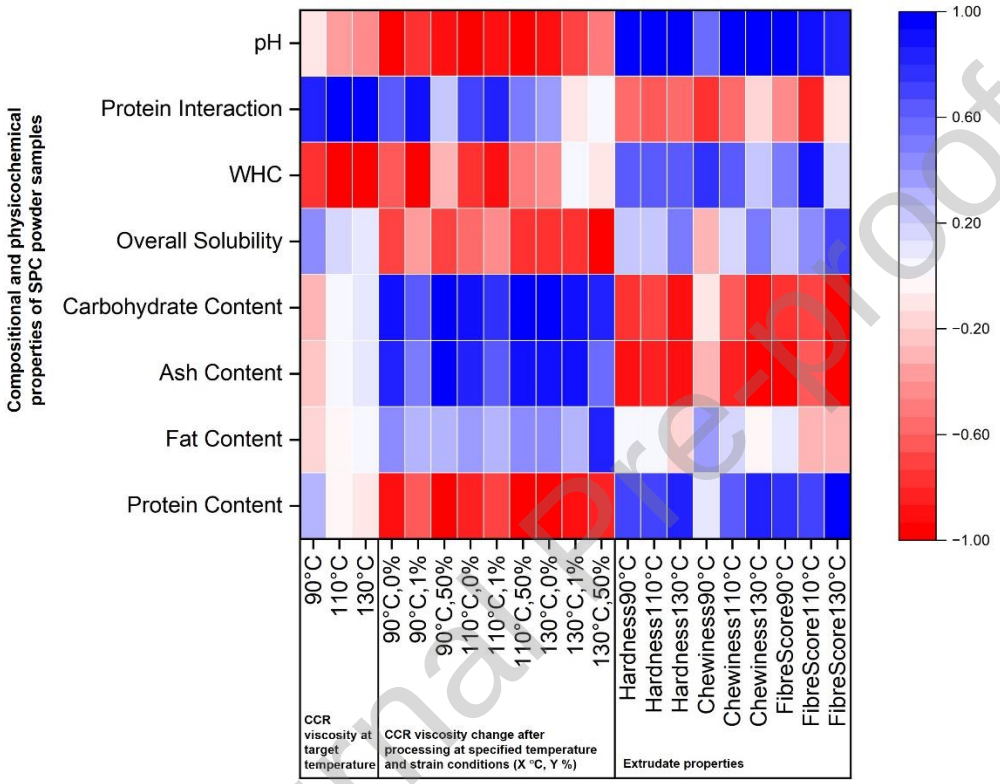

Figure 8 Correlation matrix for compositional and physicochemical properties of SPC powder samples and rheological properties and extrudate mechanical properties of 40 wt% SPC samples.

Figure 9 Illustration of the hypothesised relationship between powder properties, sample rheology and extrudate properties.

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Tables

Table 1 Proximate composition, pH and techno-functional properties of soy protein concentrates. Means are presented with standard deviation, where *n* = 3. Values bearing different lowercase letters in the same row were significantly different ($p \leq 0.05$).

	SOL	**WIL**	**YX**	**A8**
Proximate composition (g/ 100 g)				
Protein content	64.10 ± 0.23 ^{c}	71.53 ± 0.38 ^{a}	65.41 ± 0.29 ^{b}	62.56 ± 0.10 ^{d}
Carbohydrate content	26.30 ± 0.23 ^{a}	22.69 ± 0.49 ^{b}	26.07 ± 0.35 ^{a}	25.97 ± 0.45 ^{a}
Fat content	0.33 ± 0.06 ^{c}	0.20 ± 0.01 ^{c}	1.91 ± 0.10 ^{a}	1.13 ± 0.15 ^{b}
Moisture content	2.68 ± 0.33 ^{b}	1.83 ± 0.16 ^{c}	1.69 ± 0.22 ^{c}	4.51 ± 0.21 ^{a}
Ash content	6.58 ± 0.01 ^{a}	3.75 ± 0.02 ^{d}	4.92 ± 0.01 ^{c}	5.82 ± 0.03 ^{b}
pH	7.40 ± 0.01 ^{c}	7.85 ± 0.02 ^{a}	7.67 ± 0.01 ^{b}	7.32 ± 0.01 ^{d}
Techno-functional properties				
Solubility – overall (%)	39.50 ± 3.78 ^{abc}	52.92 ± 0.29 ^{a}	28.38 ± 1.66 ^{c}	36.05 ± 1.71 ^{b}
Solubility – protein fraction (%)	32.70 ± 4.08 ^{b}	60.93 ± 1.80 ^{a}	21.13 ± 1.35 ^{c}	30.67 ± 2.18 ^{b}
Solubility – non-protein fraction (%)	51.65 ± 3.45 ^{a}	32.80 ± 5.08 ^{c}	42.08 ± 2.61 ^{b}	45.05 ± 1.13 ^{ab}
Water-holding capacity (g/g pellet)	12.99 ± 0.16 ^{a}	12.32 ± 1.07 ^{a}	12.44 ± 0.63 ^{a}	7.77 ± 0.32 ^{b}

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Table 2 Lightness of SPC meat analogues extruded at various temperatures. Means are presented with standard deviation, where $n = 3$. Values bearing different lowercase letters in the same column were significantly different ($p \leq 0.05$).

L-value	90 °C	110 °C	130 °C
SOL	58.95 ± 1.83[b]	58.35 ± 0.25[b]	57.51 ± 1.91[b]
WIL	67.02 ± 0.24[a]	67.81 ± 0.57[a]	68.21 ± 0.40[a]
YX	54.33 ± 1.66[b]	55.49 ± 0.41[c]	56.10 ± 2.22[b]
A8	57.70 ± 0.75[b]	55.62 ± 0.40[c]	58.01 ± 1.68[b]

Table 3 Simple regression analysis of different rheological variables and extrudate properties.

Property	Rheological variable	R^2	Constant	Coefficient
Hardness	Viscosity at target temperature	0.63	357.90	-0.01
	Viscosity change at 0 % strain	0.88	181.60	-8.26
	Viscosity change at 1 % strain	0.86	180.05	-6.36
	Viscosity change at 50 % strain	0.78	97.08	-4.43
Chewiness	Viscosity at target temperature	0.66	220.52	-0.01
	Viscosity change at 0 % strain	0.85	70.44	-6.87
	Viscosity change at 1 % strain	0.86	68.18	-5.38
	Viscosity change at 50 % strain	0.77	-1.01	-3.72
Fibre score	Viscosity at target temperature	0.72	14.90	-0.00
	Viscosity change at 0 % strain	0.86	6.91	-0.36
	Viscosity change at 1 % strain	0.94	6.68	-0.29
	Viscosity change at 50 % strain	0.92	2.65	-0.21

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Table 4 ANCOVA results on whether the type of sample used had a significant effect on the relationship between the independent rheological variable and dependent extrudate variable

Independent variable	Is the sample effect significant?		
	Hardness	Chewiness	Fibre Score
Viscosity at target temperature	✓	✓	✓
Viscosity change at 0 % strain		✓	✓
Viscosity change at 1 % strain			
Viscosity change at 50 % strain	✓		

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Declaration of interests

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

Highlights

- Commercial SPCs have different compositions and physicochemical properties
- Hardness and chewiness of extrudates increased with processing temperatures
- Compositions and pH correlated better than functional properties to extrudate qualities
- Regression between viscosity and fibre score had an R^2 of up to 0.94