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Effect of mixing and hydrating time on the structural properties of high-temperature shear cell products from multiple plant-based ingredients

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ABSTRACT

Mixing and hydrating plant-based ingredients to form a dough is an essential step to produce meat analogues using high-temperature shear cell (HTSC) technology. In this study the effect of mixing and hydrating time on the structural properties of soy protein concentrate (SPC), pea protein isolate (PPI)-wheat gluten (WG) and soy protein isolate (SPI)-WG doughs and HTSC products was investigated. Our results showed mixing and hydrating time minimally affected the structural properties of the dough and corresponding HTSC products of SPC. For both PPI-WG and SPI-WG mixtures, longer mixing resulted in tougher doughs. Additionally, for PPI-WG, mixing the dough to the optimal dough development time led to higher tensile strength of HTSC products. The same effect was not observed for SPI-WG, which showed the importance of ingredient properties in determining structural properties of plant-based meat analogues. These findings offer insights for optimizing processes for meat analogue production through tailored mixing strategies.

1. Introduction

Mixing is an essential unit operation during food processing. Its main purpose is to combine multiple ingredients, but it is also used to modify the structure of food products such as (bread) dough, cream, and margarine (Cullen and O'Donnell, 2009). Mixing and hydrating are vital to produce meat analogues from plant-based ingredients using high-moisture extrusion (HME) or high-temperature shear cell (HTSC) technology (Cornet et al., 2021b). However, for both processes the effect of mixing on the final product properties remains unclear. In case of HME this is because mixing occurs in the first part of the barrel, which cannot be separated from the thermomechanical treatment in the subsequent part of the barrel. It should be noted though that mixing can also occur before the extruder barrel through pre-mixing of the dry ingredients, possibly in combination with pre-hydrating (Giezen et al., 2014). In case of HTSC processing, mixing is always separated from thermomechanical processing, as mixing is done outside of the HTSC. The standard mixing method used before HTSC processing is hand-mixing (Taghian Dinani et al., 2023b). Mixing could therefore be influenced by operator changes, though by experience we know these effects are limited. For the upscaled version of the HTSC, the Couette Cell, a z-blade mixer was already successfully applied to prevent this (Krintiras et al., 2016). A similar mixer, such as a farinograph, can therefore be used to mix the proteinaceous doughs systematically before HTSC processing. This makes HTSC an interesting tool to study the effect of mixing and hydrating during thermomechanical processing of meat analogues.

One of the ingredients that is commonly used to produce meat analogues using HME or HTSC is gluten (Sha and Xiong, 2020). It is well known that gluten network formation in bread doughs is heavily impacted by the initial mixing step of the bread making process. During mixing of bread dough, the mechanical input results in a distribution of the flour components and enables new interactions between these components resulting in an increase in the dough cohesion. However, its shearing effect also results in the fracturing of organized structures, which reduces the dough cohesion, especially during longer mixing times. As a result, it is known that bread dough can be under- and over-mixed, which both results in a lower bread volume (Cuq et al., 2003; Dobraszczyk and Morgenstern, 2003). The optimum kneading time lies at the equilibrium of the effects of creating new interactions and fracturing of structures (Cuq et al., 2003). The cohesive structure that is formed during bread kneading is formed by the gluten in the dough (Dobraszczyk and Morgenstern, 2003). Gluten network formation is also important for the creation of fibrous structures in HTSC products produced from a protein isolate-wheat gluten (WG) mixture, in which the protein isolate acts as a filler (Cornet et al., 2021a). We therefore

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expect that mixing intensity would influence the final product properties of protein isolate-WG HTSC products in similar way at it does in bread doughs and both under- and overmixing were expected to be detrimental for the formation of fibrous structures. However, the protein isolate that is used affects the properties of the final HTSC products. The elastic modulus of the protein isolate influences the visual fibrousness of the final product (Schlangen et al., 2023; van der Sman and van der Goot, 2023). Furthermore, the used protein isolate determines the mechanical strength of the meat analogue (Schreuders et al., 2019). The protein isolate that is used in combination with WG was therefore expected to have an influence on the effect of mixing on the final HTSC product.

Hydrating the protein dough is also an important step during HTSC processing. One of the protein ingredients is hydrated with a NaCl or $CaCl_2$ solution for 30 min after which a second protein or carbohydrate is added (Schreuders et al., 2022; Taghian Dinani et al., 2023a). Up till now, it is assumed that the ingredients form a dispersion with two or more phases upon mixing and that molecular mixing is not likely to occur under these mixing conditions (van der Sman and van der Goot, 2023). Schreuders et al. (2020) described how water distributed among the two different phases in pea protein isolate (PPI)-WG and soy protein isolate (SPI)-WG mixtures. The pea and soy protein phases take up more water, which makes the WG gluten phase more concentrated. This influenced the rheological behaviour of these mixtures at temperatures relevant for HTSC processing and is therefore expected to influence the structuring properties of the mixture during HTSC processing. Taghian Dinani et al. (2023b) also found an influence of hydrating and hypothesized that hydrating iota-carrageenan before adding it to PPI and WG was detrimental for fibrous structure formation because of the reduced amount of water available for gluten network formation. Hydrating of WG after mixing could therefore influence the final products structure.

Nevertheless, the effect of mixing and hydrating of the proteinaceous dough before HTSC processing has not been systematically investigated yet. Therefore, the effect of mixing and subsequent hydrating time on the structural properties of proteinaceous doughs and the corresponding HTSC products were investigated in this study. Three different protein doughs were investigated: soy protein concentrate (SPC) to investigate the effect of mixing and hydrating on non-gluten containing doughs and PPI-WG and SPI-WG to explore the effect on gluten containing doughs. Systematic mixing of the protein doughs was performed in a farinograph after which the doughs were processed in a HTSC. The rheological properties of the dough were analyzed in a closed-cavity rheometer (CCR). The quality of the HTSC products was analyzed by visual observations of their fibrousness and tensile testing. Finally, this study describes the effect of mixing time on the properties of proteinaceous doughs and the structural properties of HTSC products.

2. Materials and methods

2.1. Materials

Soy protein concentrate (SPC) (ALPHA 8 IP) and soy protein isolate (SPI) (SUPRO EX 37 HG IP) were purchased from Solae (St. Louis, USA). Pea protein isolate (PPI) (Nutralys S85F) and vital wheat gluten (WG) were purchased from Roquette (Lestrem, France). The dry matter contents of SPC, SPI, PPI and WG were 93.9 wt%, 94.6 wt%, 94.2 wt% and 93.4 wt%, respectively. The protein contents of SPC, SPI, PPI and WG as measured with the Dumas combustion method were 62.0 wt% (Nx5.71), 80.2 wt% (Nx5.71), 72.2 wt% (Nx5.52) and 77.4 wt% (Nx5.7) based on dry matter, respectively. Sodium chloride was obtained from Sigma-Aldrich (Zwijndrecht, Netherlands).

The water holding capacity (*WHC*) was measured according to the method described by Jia et al. (2021). To determine the *WHC* of the insoluble fraction 1 g of material and 49 g of deionized water were placed in a 50-mL Falcon tube and hydrated for 24 h while rotating at a speed of 20 rpm in a rotator (Bibby Scientific Stuart Rotator Disk SB3, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the dispersion was centrifuged at a speed of 15,000×*g* at 25 °C for 10 min. The pellet was transferred dried in an oven at 105 °C for 24 h. The mass of the wet pellet and dry pellet were determined and denoted as *Mwet pellet and Mdry pellet*. The *WHC* was calculated using Equation (1).

$$WHC = \frac{M_{wet\ pellet} - M_{dry\ pellet}}{M_{dry\ pellet}} \quad \text{Equation 1}$$

2.2. Preparation of proteinaceous doughs

Three different types of proteinaceous doughs were prepared, SPC, PPI-WG and SPI-WG. These blends were used, because these are well-known to form fibrous products, contrary to blend containing only SPI or PPI (Grabowska et al., 2016; Schreuders et al., 2019; Snel et al., 2024). All proteinaceous dough had total dry matter content of 40 wt% consisting of 39 wt% protein ingredient(s) and 1 wt% sodium chloride (NaCl). The PPI-WG and SPI-WG mixtures had a 1:1 mass ratio. The preparation of the mixtures was adapted from Schreuders et al. (2019) and Jia et al. (2021). The total weight of one mixing batch in the farinograph was 300 g. For all materials the NaCl was first dissolved in distilled water to prepare a saline solution.

In case of SPC, the protein ingredient was placed in a farinograph (Brabender GmbH &Co KG, Duisburg, Germany) before the saline solution was added and the material was mixed for 2, 4, 5, 7, 10, and 30 min at 50 rpm at 30 °C. Mixing of SPC in the farinograph before HTSC processing was performed once for every mixing time to validate that mixing of a single protein ingredient with water did not have a large effect on the dough and HTSC product properties.

In case of the PPI-WG and SPI-WG mixtures, the PPI and SPI were hand-mixed with the saline solution and hydrated for 30 min to mimic the standard high-temperature shear cell (HTSC) process (Schreuders et al., 2019). After hydrating the PPI or SPI paste was placed in the farinograph. Subsequently, WG was added, and the dough was mixed for an additional 2, 4, 5, 7, 10, and 30 min at 50 rpm at 30 °C. Mixing of PPI-WG and SPI-WG before HTSC processing was performed in duplicate to get a more reliable insight in the effect of mixing and hydrating on the dough and final product properties.

The specific mechanical energy (*SME* in J/kg) required for mixing in the farinograph was calculated from the torque recorded by the WinMix software (version 3.2.14, Brabender GmbH &Co KG, Duisburg, Germany).

$$SME = \frac{\int_{t=0}^{t_f} \omega . M(t) . dt}{m} \quad \text{Equation 2}$$

In which t_f is the final time, $t = 0$ is the start time, ω is the rotor speed (s^{-1}), M is the torque (Nm) and m is the mass of the material loaded in the farinograph (kg) (=0.3 kg) (Peighambardoust et al., 2005).

To investigate the effect of hydrating after mixing and to allow water migration from PPI or SPI to WG the protein dough was further hydrated in a zip-lock bag after mixing for 60, 140 or 220 min starting from the moment WG was added to the protein isolate before it was used in the HTSC. This means total hydrating times of 90, 170 or 250 min for PPI or SPI. To ensure that all protein doughs had the same total hydrating time, the SPC dough was hydrated for an additional 30 min resulting in the same total hydrating times of 90, 170 or 250 min from the moment of water addition to SPC until HTSC processing. In the rest of this paper the total hydrating time will be indicated as hydrating time. Hand-mixed samples were prepared as a control sample for all product compositions used and all hydrating times.

2.3. Closed-cavity rheometer

The rheological properties of the protein materials were measured with a closed-cavity rheometer (CCR) (RPA Elite, TA instruments, New Castle, DE, USA) (Emin et al., 2017). However, the preparation of the proteinaceous doughs slightly differed from what was described in section 2.2. Approximately 50 g of the dough was removed from the farinograph after 2, 5, 10 and 30 min of mixing. The doughs were hydrated until the total hydrating time was 90 min, which means that the PPI-WG and SPI-WG doughs were hydrated for 60 min after WG addition. The protein doughs were vacuumized to prevent the effect of air in the sample on the rheological measurements (Wittek et al., 2021). Approximately 6 g was placed in between two plastic films in the closed cavity, which was sealed with a closing pressure of 4.5 bar to prevent water evaporation at high temperature.

The geometry of the CCR has a radius of 22.5 mm, the maximum height of the inner cavity is 4 mm and a biconical opening has an angle of 3.35° to allow homogeneous transmission of the shear stress to the protein materials. The grooves on the surface of the cones prevent slippage. In this setup, the lower cone oscillates in strain-controlled mode while the upper cone remains stationary.

First, the protein material was heated for 2 min at the measurement temperature (i.e., 30 °C, 120 °C or 140 °C) without a shear treatment. Subsequently, strain sweep (1%–1000%) experiments were performed at 30 °C (all doughs), 120 °C (PPI-WG and SPI-WG) or 140 °C (SPC) at a constant frequency (1 Hz). All strain sweeps were performed in duplicate. The strain sweep experiments were used to determine the yield stress and the flow stress of the materials. The yield stress is defined as the value of the shear stress at the end of the linear viscoelastic (LVE) regime. Here, we define this stress as the point where G' 'differs more than 5% from its strain-independent value in the LVE regime (Schreuders et al., 2021). The flow stress is defined as the value of shear stress at the crossover point where the storage modulus is equal to the loss modulus (G' = G") (Schreuders et al., 2021).

2.4. High-temperature shear cell

The protein doughs were treated in a high-temperature shear cell (HTSC) (Wageningen University, Netherlands) that was developed in house (Grabowska et al., 2016). Heating and cooling were done using an external oil bath. The hydrated protein doughs were transferred to the pre-heated HTSC and sheared for 15 min at 30 rpm at 140 °C for SPC and 120 °C for PPI-WG and SPI-WG. The different temperatures for SPC and PPI-WG, SPI-WG mixtures was used to enable fibrous structure formation (Grabowska et al., 2016; Schreuders et al., 2019). The SME of the mechanical treatment was calculated from the torque measured by the DO-Corder E330 (Brabender GmbH &Co KG, Duisburg, Germany) using Equation (2). After the shear treatment the material the products were cooled down until the temperature measured at the centre of the upper cone reached 35 °C using an external oil bath set to 25 °C. SPC products were prepared once to validate that mixing with water did not have a large effect on the properties of products produced from a single ingredient. PPI-WG and SPI-WG products were prepared in duplicate to get a more reliable insight in the effect of mixing and hydrating on gluten network formation.

2.5. Visual observations

The fibrousness of the products produced in the HTSC was assessed visually by bending the products parallel to the shear flow direction to obtain a tear at the middle of the product. The bent pieces were placed onto a metal pin and the fracture surface was photographed using a digital microscope with 36 × magnification using the extended depth of field (EDF) option (Smartzoom 5, Carl Zeiss, Germany).

2.6. Tensile test

Tensile tests were performed with a Texture Analyzer (TA.XTPlusC, TA Instruments, USA) using a trigger force of 0.001 N. A uniaxial tensile test was performed at room temperature with a displacement rate of 1 mm/s. Tensile bars were taken from the product with a dog bone-shaped mould in parallel and perpendicular to the shear flow direction. The measured tensile strength was normalized based on the dimensions of the tensile bar. The ends of the tensile bars were placed into the two clamps such that 15.5 mm was the initial length of the sample. Tensile stress and tensile strain at fracture were calculated from the force and displacement measured using the equations for the Hencky stress and strain for uniaxial extension as explained in Macosko (1994) and used by Schreuders et al. (2019). The strain (ϵ) and tensile stress (σ) were be calculated using the following equations:

$$\epsilon = ln\frac{h(t)}{h_0} \qquad \text{Equation 3}$$

$$\sigma(t) = \frac{F}{A} \qquad \text{Equation 4}$$

In which:

$$A = \frac{h_0}{h(t)} * A_0 \qquad \text{Equation 5}$$

In which $h(t)$ (m) is the sample length at fracture, h_0 (m) is the sample length at the start of the measurement, F (N) is the force at fracture, A (m^2) is the cross-section area, A_0 (m^2) is the cross-section of the sample at the start of the measurement and l and w (m) are the length and width of the sample cross-section measured before the start of the measurement. The Young's modulus (Pa) was calculated at the initial slope of the stress-strain curve until a 1.5 mm extension was achieved.

For every product, three parallel and three perpendicular specimens were taken.

2.7. Statistical analysis

All values are represented as the mean ± standard deviation. A factorial ANOVA was performed to evaluate the effect of mixing time, hydrating time and their interaction a significance level of 95% ($P < 0.05$) analyzed using SPSS statistics Version 28.0.1.1 (IBM, Armonk, NY, USA). A one-way ANOVA per hydrating time and ingredient combination was used to test the effect of mixing time. Tukey HSD was used to find differences between samples at a significance level of 95% ($P < 0.05$) Levene's test ($P < 0.05$) was used to check the assumption of equal variances. The results of the statistical analysis can be found in Tables S1-S15.

3. Results and discussion

3.1. Dough formation

The torque profile of the different proteinaceous doughs during 30 min of mixing in the farinograph is shown in Fig. 1. The torque profile of wheat gluten (WG) containing doughs had a similar shape to the typical farinograph curves during mixing of bread doughs. In these curves first, the torque increases suggesting the development of the gluten network. Subsequently, the optimal dough development time is reached, and the dough has its maximal strength. Finally, the torque decreases, which is generally interpreted as a disruption of the gluten network (Létang et al., 1999; Li et al., 2020). The torque profile of pea protein isolate (PPI)-WG showed similarities to this typical farinograph pattern of bread doughs with respect to having an optimum and continued breakdown after the maximum peak. The time to peak, which the case of wheat dough is referred to as optimal dough development time, was around 4.5 min. Mixing the soy protein isolate (SPI)-WG dough resulted in a higher torque and longer optimal dough development time around 8 min compared with the torque and development time of PPI-WG doughs. The higher energy input required to deform the SPI-WG dough is probably due to the more elastic and tougher nature of SPI-WG blends compared with PPI-WG (Schreuders et al., 2021). Part of the explanation for the

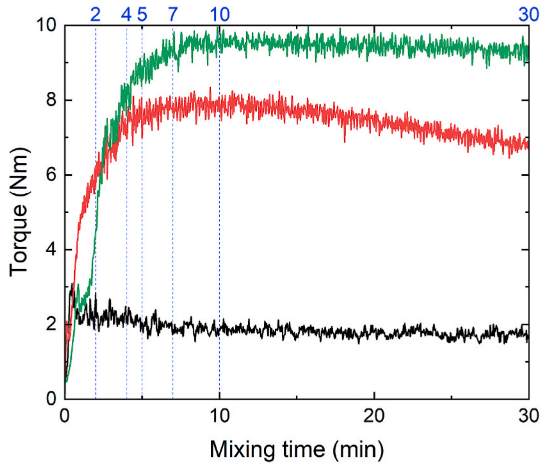

Fig. 1. Torque profile during mixing of SPC (black), PPI-WG (red) and SPI-WG (green). The blue dotted lines indicate the mixing times that were used in the high-temperature shear cell (HTSC).

shorter dough development time and dough stability of PPI-WG compared with SPI-WG is the lower WHC of PPI compared with SPI (Table 1). This leaves more water available for the WG in the PPI-WG dough compared with the WG in the SPI-WG dough (Schreuders et al., 2020). Jia et al. (2022) found that a higher water content in the wheat flour doughs made it more susceptible to mixing and thus resulted in a faster development and breakdown of the gluten network. However, the opposite effect of the water content on the development of the gluten network is also reported in literature (Dufour et al., 2024; Koksel and Scanlon, 2012). We expect these conflicting results were caused by the relatively low water to gluten protein ratio used in the study of Jia et al. (2022) (4–6) compared to those of Dufour et al. (2024) (8–15) and Koksel and Scanlon (2012) (7–31). The water:gluten ratio used by Jia et al. (2022) was more comparable to the one used in this study (4) and we therefore expect the effects reported in that study to be the most relevant for our doughs. However, other differences between PPI and SPI, such as their protein content, gelling capacity, amino acid composition or non-protein components (Shrestha et al., 2023), also play a role in the observed difference in the torque profile during farinograph mixing.

Mixing of soy protein concentrate (SPC) resulted in a different torque profile, probably due to the absence of WG. Because of the low water holding capacity of SPC (Table 1) network formation does not occur upon hydrating, which explains the low torque measured. During the initial mixing phase (<1 min), a minor torque increase was observed, which can be attributed to the initial force required to start the mixing process.

3.2. Rheological properties of the protein doughs

Strain sweeps were performed in a closed-cavity rheometer (CCR) to study the effect of mixing on the rheological properties of the protein doughs. The obtained rheological properties were summarized in texture maps in which the shear stress is plotted against the strain at the end of the linear viscoelastic (LVE) regime or the cross-over point (Fig. 2). The different corners in these texture maps indicate different textural properties of the material (Schreuders et al., 2021).

The rheological properties measured at 30 °C provide information about the behaviour of the dough during and after mixing. It was found that the rheological properties at the end of the LVE were not influenced by mixing for all protein doughs (Fig. 2A). In case of the WG containing doughs, this implies that changes in the gluten network are not reflected in the rheological properties in the LVE regime (Meerts et al., 2017). However, at the cross-over point longer mixing times resulted in tougher behaviour of the WG containing doughs and slightly mushier behaviour of SPC (Fig. 2B). The increased toughness could indicate that mixing induced aggregation of the gluten (Jia et al., 2022). The effect of mixing on the rheological properties of the PPI-WG and SPI-WG doughs at the cross-over point showed a similar trend. However, a longer mixing time resulted in a larger shift in the texture map for PPI-WG doughs compared with SPI-WG doughs (Fig. 2B). This indicates that PPI-WG doughs were affected by mixing more than SPI-WG doughs. This difference was also visible in the torque profile during farinograph mixing (Fig. 1), in which it was shown that SPI-WG had a higher dough stability. As explained in section 3.1 the reason for this different behaviour could be the difference in water distribution between the protein isolate and WG for these two doughs, but other differences between PPI and SPI could also play a role.

The WG containing doughs were rubberier than SPC at the end of the LVE and the cross-over point (Fig. 2A&B). This can be explained by the gluten network that is present in these doughs. SPI-WG had a slightly higher stress compared with PPI-WG at the end of the LVE. However, at the cross-over point SPI-WG was tougher compared with the PPI-WG dough. The same trend was seen by Schreuders et al. (2021).

The rheological properties measured at 120 and 140 °C provide information about the behaviour of the dough during HTSC processing. Mixing did not influence the rheological properties at the end of the LVE (Fig. 2C). As can be seen, the hand-mixed control showed different behaviour compared with the doughs in the farinograph for the doughs containing gluten. In case of PPI-WG the hand-mixed dough was mushier than the farinographs doughs while, in case of SPI-WG the hand-mixed dough was tougher compared with the doughs that were mixed in the farinograph. The PPI-WG dough showed one deviating point after 2 min mixing, which turned out to be very mushy compared with the other mixing conditions. At the cross-over point no consistent effect of mixing was seen for SPC and SPI-WG (Fig. 2D). However, mixing increased the toughness of PPI-WG.

At 120 or 140 °C more differences between the different ingredients were observed at the end of the LVE than at 30 °C (Fig. 2A&C). Clearly SPI-WG was tougher than PPI-WG. For SPC no clear LVE could be determined, which is why no data points at the end of the LVE are given for this dough. At the cross-over point the same trend was visible but the clusters were closer together. SPC was the mushiest and SPI-WG the toughest (Fig. 2D). PPI-WG lied in the middle of these two extremes.

It remains difficult to link these fundamental rheological properties of the doughs to the HTSC product properties. However, previous studies show that rheology can be used to obtain more insights in differences between proteins and effects of processing such as extrusion and HTSC processing (De Angelis et al., 2023; Ji et al., 2023; Schreuders et al., 2020; Snel et al., 2023). The effect of mixing at the end of the LVE and cross-over point was small compared with the effect heating and cooling of the PPI-WG or SPI-WG blends that was reported by Schreuders et al. (2021). However, the changes induced by mixing PPI-WG were similar to those induced by heating mixtures of PPI or SPI with cellulose or pectin (Schreuders et al., 2022). Mixing can therefore be considered to have a relevant influence on the rheological properties of the protein doughs and is therefore be expected to influence the final HTSC product properties.

3.3. Specific mechanical energy (SME) of mixing and thermomechanical processing

As was discussed in the previous section (section 3.2), mixing

Table 1
Water holding capacity of the ingredients used in this study. Letters indicate homogeneous subgroups at $p < 0.05$ using Tukey's HSD.

	SPC	PPI	SPI	WG
Water holding capacity (g/g)	6.73 ± 0.26b	9.34 ± 0.09c	20.99 ± 0.27d	1.69 ± 0.13a

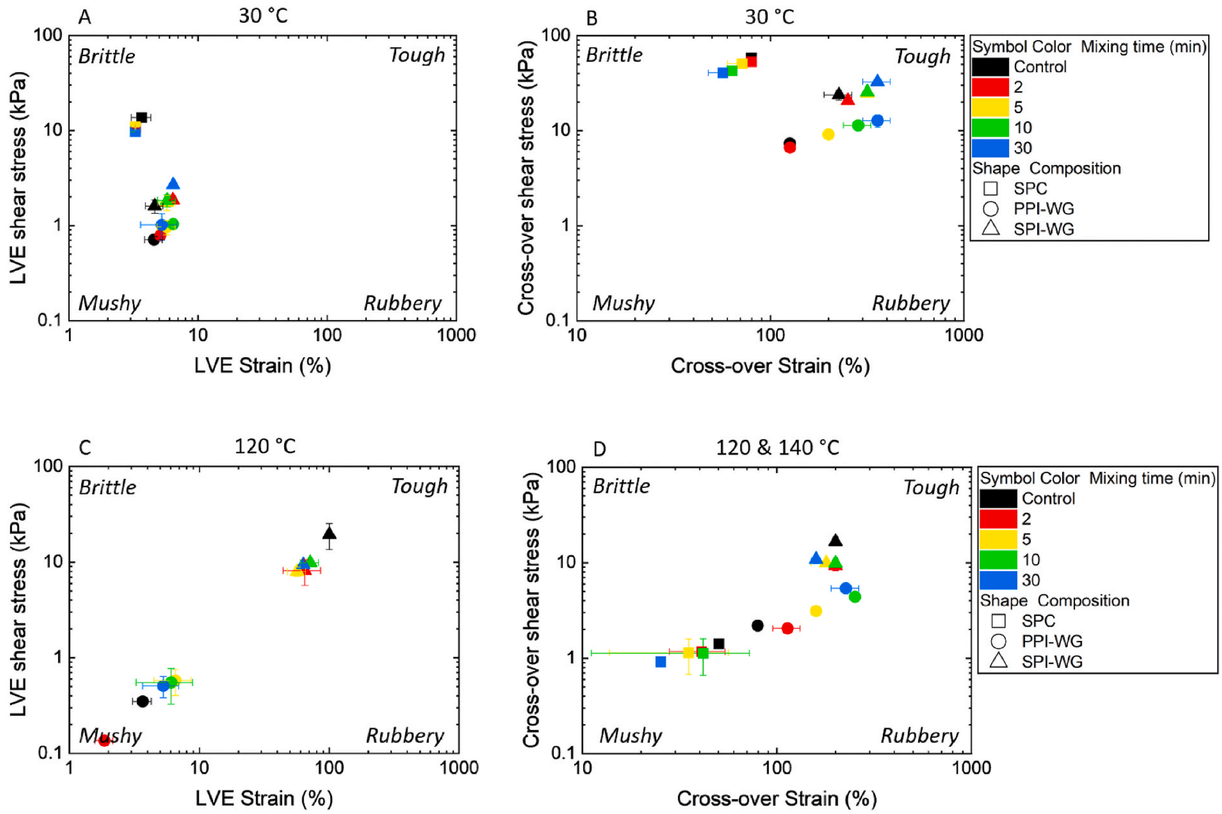

Fig. 2. Texture maps of at the end of the linear viscoelastic regime (LVE) (A&C) and at the cross-over (B&D) point of SPC (■), PPI-WG (●) and SPI-WG (▲) at 30 °C (A&B), 120 (PPI-WG&SPI-WG) and 140 °C (SPC) (C&D). The colour of the datapoints indicates the mixing time in the farinograph. The control treatment is shown in black.

affected the rheological properties of PPI-WG at the cross-over point measured at temperatures relevant for HTSC structuring. It was therefore hypothesized that mixing can affect the mechanical input required to deform the material in the HTSC. The hydrating time after mixing might also influence the behaviour of the dough in the HTSC. Fig. 3 shows the specific mechanical energy (SME) input during mixing in the farinograph and thermomechanical treatment in the HTSC for the different combinations of ingredients and mixing conditions investigated in this study. Separate figures with only mixing SME or HTSC SME including statistical differences can be found in Figures S1 and S2.

The thermomechanical SME ranged from 23.2 kJ/kg (PPI-WG 4 min mixing 250 min hydrating) to 50.0 kJ/kg (SPC 10 min mixing 90 min hydrating) (Fig. 3). The mixing and hydrating times used in this study did not significantly affect the thermomechanical processing SME in the HTSC, even though different rheological properties were observed at the cross-over point in the CCR in cases of PPI-WG (Fig. 2).

It is interesting to compare the SME of mixing to the SME during the thermo-mechanical treatment in the HTSC. The mixing SME ranged from 0.7 kJ/kg (SPC 2 min mixing) to 43.6 kJ/kg (SPI-WG 30 min mixing). The SME of mixing was lower compared with the SME of thermomechanical processing, except for PPI-WG mixed for 30 min. The combined SME of the mixing and thermomechanical processing ranged from 27.0 kJ/kg (PPI-WG 5 min mixing 250 min hydrating) to 93.0 kJ/kg (SPI-WG 30 min mixing 250 min hydrating). This is on the low end of the SME during high-moisture extrusion (HME) 42.8–1276 kJ/kg (Maung et al., 2021; Wang et al., 2022; Zahari et al., 2021; Zhang et al.,

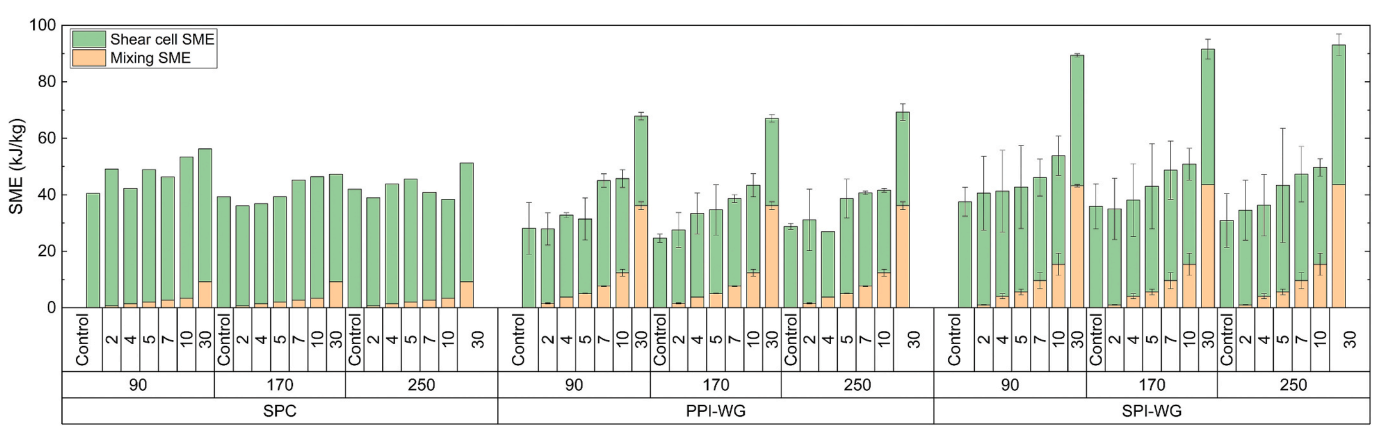

Fig. 3. Specific mechanical energy (SME) of mixing in the farinograph (orange) and thermomechanical processing in the HTSC (green) of the SPC, PPI-WG, and SPI-WG mixed for 2, 4, 5, 7, 10 or 30 min the control treatment and a hydration time of 90, 170 or 250 min.

2023). This means that HTSC processing including the mixing step is relatively mild compared with HME.

3.4. Visual observations of HTSC products

Figs. 4–6 show the macrostructures of the HTSC products produced after different mixing conditions. The use of the different ingredient formulations and mixing conditions led to a wide range of (fibrous) structures including small-fibres, thick fibres, layers and flaky structures. Some products showed combinations of these structural elements.

In case of SPC, anisotropic structures were visible for all mixing and hydrating combinations (Fig. 4). Surprisingly, the hand-mixed control treatment with a hydrating time of 90 min appeared the least fibrous and longer hydrating of the hand-mixed control led to thicker fibres. No consistent effect of mixing or hydrating time was observed, although 7 and 10 min of mixing led to many small fibres and 5 min mixing time showed the least fibrous structures. A longer hydrating after mixing of 4–7 min appears to be favourable for fibrous structure formation, but this effect was not observed for other mixing times.

The WG containing products had structures that can best described as layered and were less clearly fibrous compared with the SPC products (Figs. 5 and 6). The hydrating times used in this study had no clear effect on the structures of both PPI-WG and SPI-WG products. Additionally, the composition of the HTSC products appeared to have a larger effect on

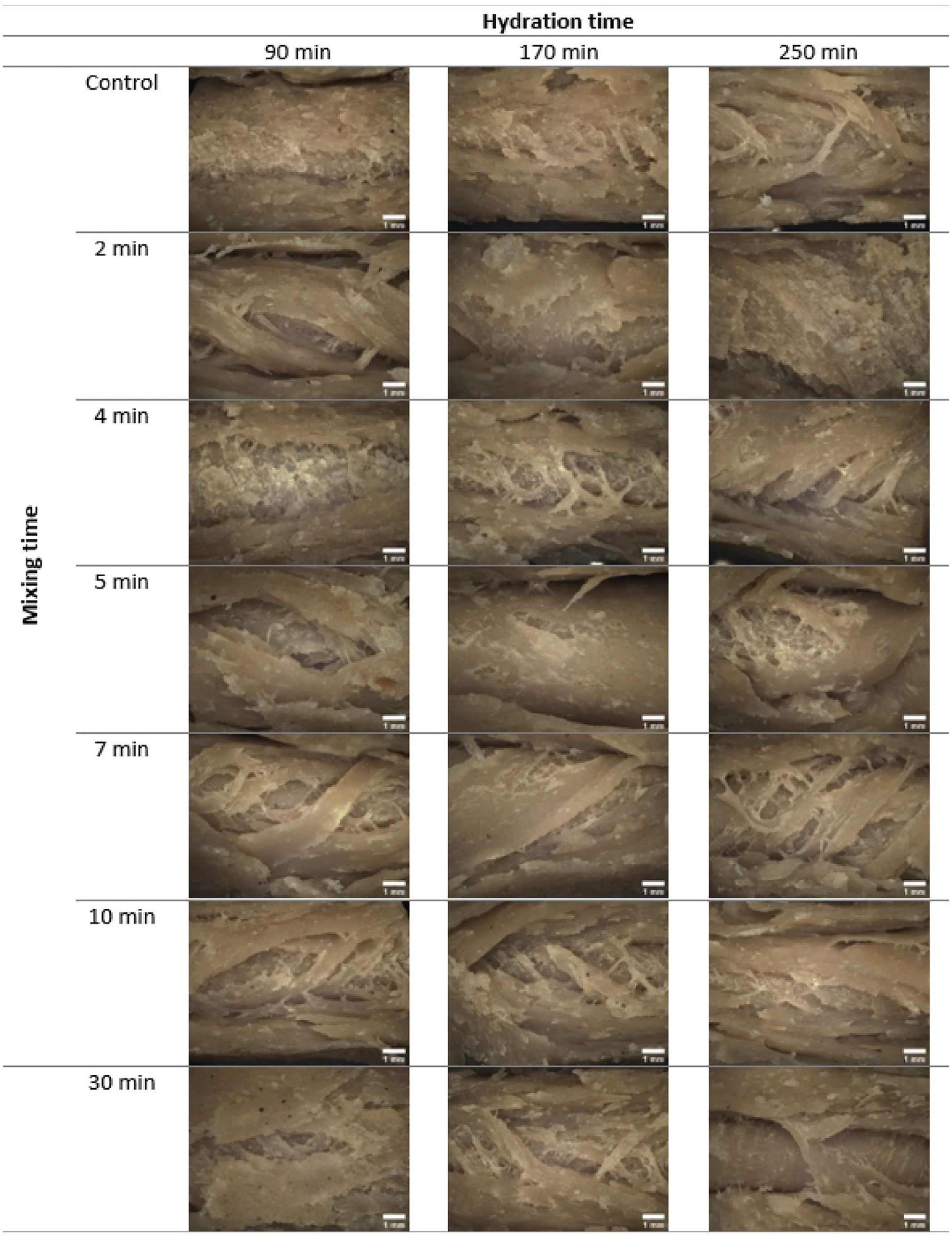

Fig. 4. Representative extensive depth of field (EDF) images of SPC HTSC products processed after different mixing and hydration times taken with a SmartZoom digital microscope at 36× magnification.

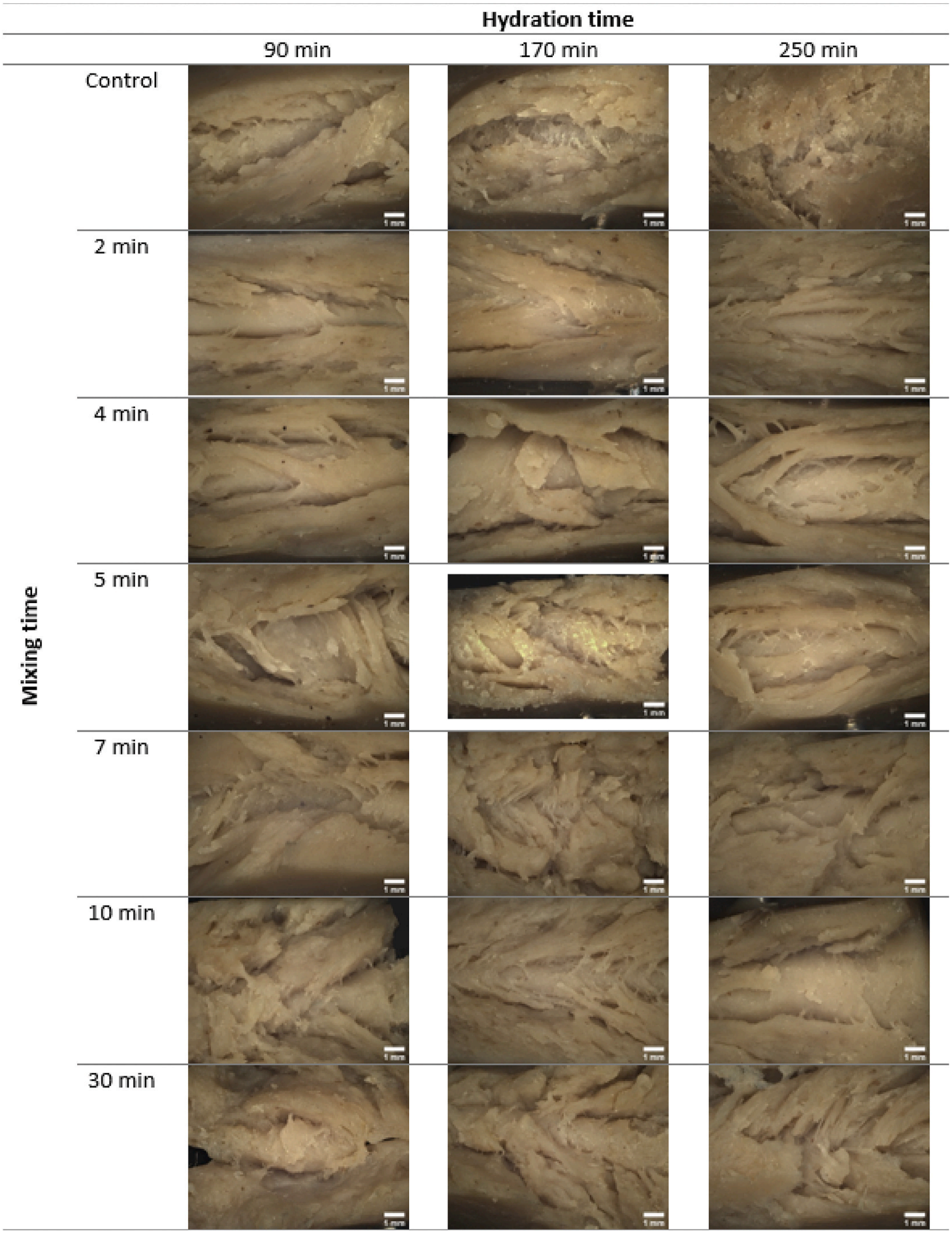

Fig. 5. Representative extensive depth of field (EDF) images of PPI-WG HTSC products processed after different mixing and hydration times taken with a SmartZoom digital microscope at 36× magnification.

the visual fibrousness compared to the mixing and hydrating conditions.

The limited visual fibrousness of PPI-WG products corresponds to our previous results with this protein mixture where the optimal shear rate for fibrous structure formation in the HTSC of this protein mixture was lower than the rotational speed of 30 rpm used in this study (Köllmann et al., 2023). Mixing time appeared to have an influence on the visual macrostructure of the PPI-WG products. At mixing times up to 5 min the structures were clearly layered, whereas at longer mixing times this layered structure became less clear (Fig. 5).

The visually observed macrostructure of the SPI-WG products was less fibrous than expected from the results of Schreuders et al. (2019) and Cornet et al. (2021). Here, the control did not show a clearly fibrous structure. This difference with previous research might be caused by batch-to-batch variations in the ingredients used, the higher process temperature (140 °C) and lower dry matter content (30 wt%) that were used by Cornet et al. (2021). Although the SPI-WG products appear to be more gel-like compared with SPC or PPI-WG products, they still were visually anisotropic (Figs. 4–6). Longer mixing times, for example 10 min mixing and 250 min hydrating and 30 min mixing 90 min hydrating did result in the formation of small fibres (Fig. 6).

3.5. Tensile test

To determine the effect of mixing and hydrating time on the

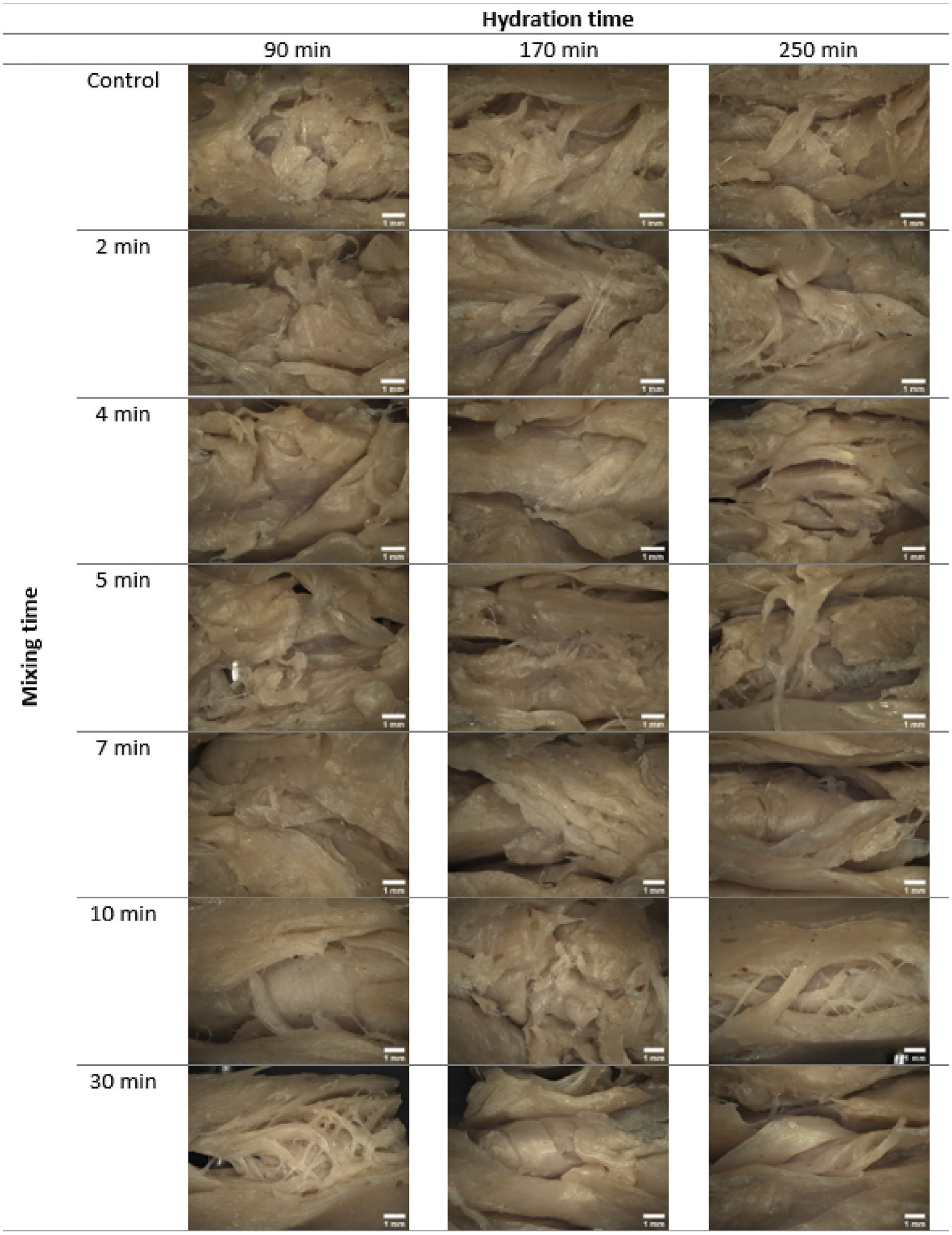

Fig. 6. Representative extensive depth of field (EDF) images of SPI-WG HTSC products processed after different mixing and hydration times taken with a SmartZoom digital microscope at 36× magnification.

mechanical properties of the products a tensile test was performed (Fig. 7). In case of SPC mixing time did not influence the tensile test properties to a large extent (Fig. 7A1, 7A2, 7A3). This limited effect of mixing was also seen in the rheological properties (Fig. 2) and visual observations (Fig. 4). This is probably caused by the absence of network formation in SPC during mixing, because of its low WHC and the absence of WG.

However, in case of PPI-WG mixing influenced tensile test properties (Fig. 7B1, 7B2, 7B3). The strain and stress in parallel and perpendicular direction were higher after 5 min of mixing compared with other products. The Young's modulus was not influenced by mixing. The increase of the tensile stress and strain that was observed for a mixing time of 5 min at corresponds with the optimal dough development time observed in the torque profile (Fig. 1). This indicates that the optimal dough development corresponds with an increased tensile strength, suggesting that how gluten network is formed during mixing remains relevant when processing the blend in the HTSC, despite the higher SME of the thermomechanical treatment (Fig. 3). This corresponds with the work of Peighambardoust et al. (2005) who showed that the glutenin macropolymer was broken down during mixing of bread dough in a farinograph, but not during shear cell treatment. Thus, the dough properties measured during mixing in a farinograph type mixer could be used to control the mechanical properties of the final meat analogue product. However, the maximum in the tensile stress and strain did not

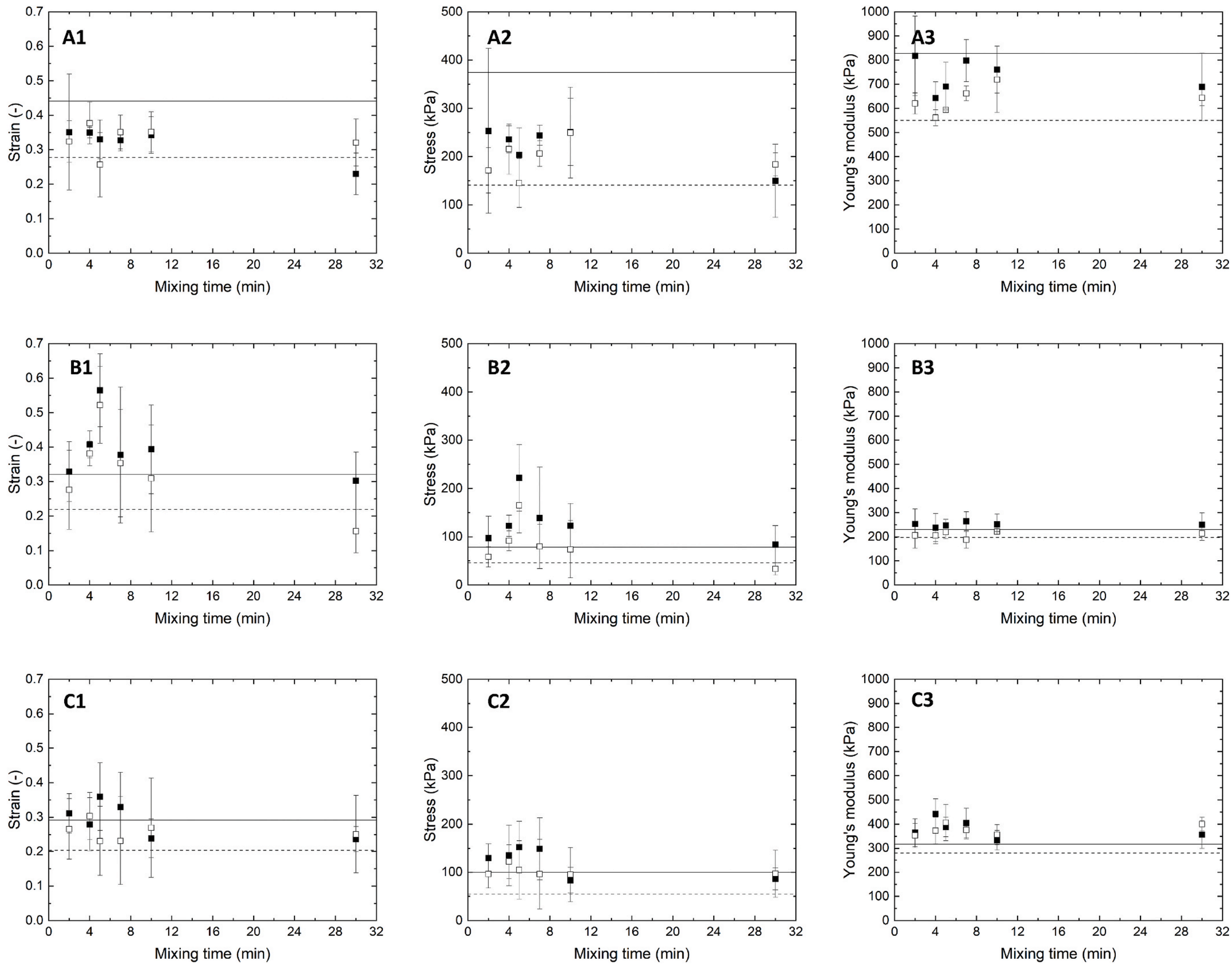

Fig. 7. Tensile strain (1), stress (2) and Young's modulus (3) in parallel (closed symbols) or perpendicular (open symbols) direction for SPC (A), PPI-WG (B) and SPI-WG (C) products after 90 min hydration. The horizontal lines represent the control treatment at the same hydration time in parallel (filled line) and perpendicular (dashed line) direction.

correspond with clear changes in the visual macrostructure (Fig. 5). For the most constant product properties a mixing time longer than the optimal dough development time might be beneficial, as the product properties are expected to be less affected by small changes in the mixing conditions. Optimization of the mixing process could be used to produce stronger HTSC products without the use of additional ingredients or more intensive processing in the HTSC, which could reduce the cost and energy usage of the process.

Mixing time only significantly influenced the Young's modulus in case of SPI-WG (Fig. 7C1, 7C2, 7C3). The Young's modulus in parallel direction was higher after 4 min of mixing compared with the control. In perpendicular direction the Young's modulus after 4,5,7 or 30 min was higher compared with the control. Unlike for the PPI-WG no maximum was measured for the tensile test properties. This corresponds with the more subtle effect of mixing in the torque profile and rheological properties of SPI-WG compared with PPI-WG (Figs. 1 and 2). As was already speculated in section 3.1, a possible explanation is a difference in available water for gluten in the two doughs. However, Schreuders et al. (2019) reported the fibrous structure formation was less affected by temperature for SPI-WG in comparison with PPI-WG and attributed this the higher gelling capacity of SPI compared with PPI. This could also influence the effect of mixing on these products.

In the range of hydrating times used in this study, hydrating time did not have a significant effect on the tensile test properties of the different ingredients used, except for the Young's modulus in parallel direction for PPI-WG (Tables S3-S5). Because of this only the tensile test results of a hydrating time of 90 min were discussed here. The results for the other hydrating times can be found in Figures S3 and S4. The limited effect of hydrating time on the tensile test properties indicates that the water distribution from PPI or SPI to the WG phase was already completed within 60 min after the addition of WG. However, at shorter hydrating times, water distribution could still have an influence on the structuring properties of the protein dough, which we would like to exclude in this study. Further research on the effect of hydrating at shorter hydrating times therefore remains necessary.

Fig. 8 summarizes the textural properties of the HTSC products based on the measured tensile stress and strain. For all product compositions changes in mixing intensity resulted in a shift in behaviour on the diagonal line between the bottom left corner, which indicates mushy behaviour, to the top right corner, which indicates tough behaviour.

The PPI-WG products show a wider range of textural properties compared with SPC and SPI-WG products. The cluster of SPI-WG products fell in the middle of the range for the PPI-WG products. This indicates that the mechanical properties of SPC and SPI-WG were hardly

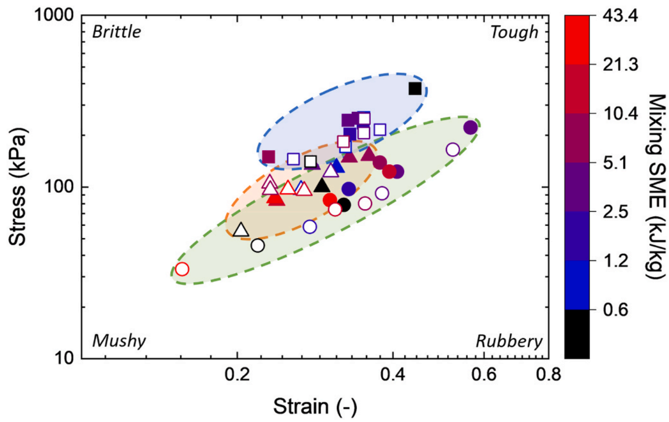

Fig. 8. Texture map tensile stress plotted against tensile strain for SPC (■), PPI-WG (●) and SPI-WG (▲) in parallel (closed symbols) and perpendicular (open symbols) direction for products of which the dough was hydrated for 90 min. Shades indicate are of tensile test properties of SPC (blue), PPI-WG (green) and SPI-WG (orange).

influenced by the mixing time compared to those of PPI-WG products. This aligns with the observation that the rheological properties of SPC and SPI-WG doughs and visual fibrousness of SPC and SPI-WG HTSC products were also hardly different as a result of different mixing times (Fig. 2). Additionally, Schreuders et al. (2019) found the structure of SPI-WG products was less affected by the processing temperature in the HTSC than PPI-WG. Furthermore, in our previous study (Köllmann et al., 2023), varying rotational speed (0–100 rpm) or shearing time (8 or 15 min) during HTSC processing of PPI-WG yielded similar variations in the tensile stress and strain of the products, as the variations presented in this study. This could indicate that SPI-WG is generally more robust to changes in the process conditions compared with PPI-WG.

This study gives more insight in the effect of mixing and hydrating time on mixtures of protein isolates with WG and protein concentrates mixed with water. However, this information is not sufficient to optimize the mixing strategy before HTSC processing, as additional variables such as mixer type, mixing speed and mixing temperature should be further studied to do so. Furthermore, protein isolates can also be mixed with carbohydrates such as dietary fibres (e.g. pectin or cellulose) instead of gluten, to create fibrous structures in the HTSC (Schreuders et al., 2022), but these ingredients were not included in this study. We expect that mixing of the dough will also influence the final product properties for protein-carbohydrate HTSC products. Mixing might lead to break up of the dispersed carbohydrate phase, like the break-up of the pectin phase in a SPI-pectin mixture at higher shear rates in the HTSC that was observed by Dekkers et al. (2018). However, the mechanical deformation during HTSC processing might overshadow the effect of mixing in this case. Furthermore, we do not expect a clear optimal dough development time to be visible in the farinograph curves of these mixtures since no network formation takes place. We therefore expect a similar torque profile as was seen for SPC in this study (Fig. 1) and for other pulse ingredients (Bresciani et al., 2022). However, to the best of our knowledge the farinograph properties of pulse ingredients with the addition of fibres or hydrocolloids has not been investigated previously. Therefore, further research on the effect of mixing on protein-carbohydrate doughs and their resulting properties remains necessary before general mixing guidelines can be developed.

Additionally, this research provides more insight in the effects of mixing in the extrusion process. However, the total SME of mixing and HTSC processing was low compared with HME processing (section 3.3). We therefore expect the subtle effects of mixing on the final product structure that were found in this study to be overshadowed during HME. This corresponds with previous research in which screw speed was found to have a minor effect compared to for example moisture content or barrel temperature (Omohimi et al., 2014; Samard et al., 2019).

4. Conclusions

This study described the effect of mixing and hydrating soy protein concentrate (SPC), pea protein isolate (PPI)-wheat gluten (WG) and soy protein isolate (SPI)-WG doughs and on the properties of the properties of HTSC products. The effect of mixing depended on the composition of the dough. For SPC mixing had a limited effect on the rheological properties of the doughs and the visual and tensile test properties of the HTSC products. In case of SPI-WG, prolonged mixing resulted in a slightly tougher dough, but prolonged mixing had no effect on the final product properties. However, mixing did influence the final product properties in case of PPI-WG, for which a highest tensile strength was observed when applying a mixing time of 5 min. This could be related to the limited formation of a gluten network at short mixing times and break-down of the gluten network when using mixing times longer than 5 min. Hydrating the protein doughs after mixing did not result in large changes in the final HTSC product properties.

The effect of mixing on final product was remarkable as the specific mechanical energy input of the subsequent thermomechanical treatment in the HTSC is substantially higher than the SME input during mixing. However, the different effects of mixing on the final product properties of SPC, PPI-WG and SPI-WG products highlights the importance of considering the ingredient properties when optimizing the processing parameters for HTSC products.

CRediT authorship contribution statement

Nienke Köllmann: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Kasidaj Sivakul:** Investigation. **Lu Zhang:** Conceptualization, Supervision, Writing – review & editing. **Atze Jan van der Goot:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfoodeng.2023.111911.

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