Journal of Food Engineering 350 (2023) 111490

Contents lists available at ScienceDirect

Journal of Food Engineering

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

On the importance of cooling in structuring processes for meat analogues

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ARTICLE INFO

Keywords:
Shear cell
Meat analogue
Fibrous structure
Plant protein
Cooling
Extrusion

ABSTRACT

High moisture extrusion (HME) is a common method to produce meat analogues. This process requires cooling of the product in the die and is associated with a high mechanical energy input. Here, we use high-temperature shear cell (HTSC) technology to better understand the importance of cooling while shearing for the formation of fibrous products upon thermomechanical processing. The maximal rotational speed for fibrous structure formation from a pea protein isolate-wheat gluten (PPI-WG) blend was found to be the same (<30 rpm) when shearing during heating or cooling. However, shearing during cooling was not beneficial for making visually fibrous products in the HTSC and it weakened the products, while simultaneously inducing mechanical anisotropy. Additionally, we found that the mechanical energy necessary to produce these fibrous structures is only a fraction of the mechanical energy input currently used for HME. We therefore conclude that the HME process for production of meat analogues could still be improved.

1. Introduction

The development of plant-based meat analogues with a fibrous structure could stimulate the transition towards a more plant-based diet among consumers (Michel et al., 2021). High moisture extrusion (HME) and high-temperature shear cell (HTSC) technology rely on thermomechanical processing to create fibrous products from plant-based ingredients. Both processing techniques have similarities in their basic three unit operations, which are mixing/hydration, thermomechanical treatment and cooling (Cornet et al., 2021b). Unfortunately, even today, the structure formation process in HME is still poorly understood because parameters such as the shear rate, temperature and pressure in the extruder are highly correlated and their effects on food materials' behaviours are difficult to disentangle. In the HTSC though, these parameters can be controlled separately (van den Einde et al., 2004). Therefore, HTSC was developed as a useful tool to study the effects of processing parameters on the quality of products, to better understand the effects of thermomechanical treatment on materials.

The mechanism of fibrous structure formation during HME and HTSC processing has been explained with several hypotheses and is thought to have a similar basis. Most of these hypotheses consider the presence of two different phases in the heated protein-rich material as a prerequisite for structure formation. However, researchers hold different opinions on the nature and origin of the formation of two phases and why such multi-phase material is needed (Cornet et al., 2021b). In case of extrusion, fibrous structure formation is thought to mostly occur during cooling when materials pass through a long die and are subjected to shear flow (Cheftel et al., 1992; Sandoval Murillo et al., 2019; Tolstoguzov, 1993). For structuring in the HTSC it is often assumed that the fibrous structure formation is a result of deformation of the dispersion of biopolymers upon shearing (Dekkers et al., 2018). For the pea protein isolate (PPI)-wheat gluten (WG) blend used in the current work Cornet et al. (2021a), hypothesized PPI acted as the dispersed phase while WG acted as the continuous phase. However, Schreuders et al. (2020) found indications that the PPI-WG blend formed a bi-continuous phase using the polymer blending law.

As stated above, cooling is thought to be essential to make fibrous products with HME. Therefore, it is also interesting to explore whether shear treatment during cooling can also lead to fibrous structure formation in plant-based materials using the HTSC. During cooling, the material is expected to become more viscous and might even solidify as the temperature decreases sufficiently. Shear rate or stress applied to material will influence structure formation in HTSC, which is related to the processing parameter of rotational speed (rpm). The application of shear during cooling might enhance structure formation through shear-induced deformation because mechanical forces can be better transferred to the two phases in the product as the material solidifies. However, the application of too high shear stresses during cooling will result in structural breakdown. Therefore, shear-induced deformation in a two-phase system needs to be well in balance with the extent of

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https://doi.org/10.1016/j.jfoodeng.2023.111490
Received 3 October 2022; Received in revised form 17 February 2023; Accepted 26 February 2023
Available online 7 March 2023

solidification for optimal fibrous structure formation during cooling.

This study aims to better understand the importance of cooling during shearing for thermomechanical processing of plant-based proteins. To investigate the effect of cooling on structure formation, PPI-WG blends were processed using different shearing time and shear rate combinations during heating and cooling. Subsequently, the obtained products were evaluated in terms of fibrous structure formation by visual observation and textural properties by tensile test analysis.

2. Materials and methods

2.1. Materials

Pea protein isolate (PPI) (Nutralys S85F) and vital wheat gluten (WG) (Vitens CWS) were both purchased from Roquette (Lestrem, France). The manufacturer's specifications indicated the PPI contained, 83–88% protein, 1 wt% dietary fibre, 9 wt% lipids, 5 wt% ash; and WG contained 10 wt% starch, 0.5 wt% cellulose fibre, 3 wt% lipids and 1 wt % ash. PPI and WG had an average dry matter content of 93.8 wt% and 92.8 wt%, respectively. Sodium chloride was obtained from Sigma-Aldrich (Zwijndrecht, Netherlands).

2.2. Preparation of proteinaceous materials

A protein blend of PPI–WG at mass ratio 1:1 was prepared with a final dry matter content of 40 wt%. The sample preparation procedure has been previously described by Grabowska et al. (2014) and Schreuders et al. (2019). First, 1 wt% sodium chloride was dissolved in distilled water to prepare a saline solution. Then, PPI was added to this solution, followed by mixing this dispersion by hand using a spatula until a homogeneous paste was obtained. The protein material was then hydrated at room temperature for 30 min. Finally, WG was mixed into the hydrated PPI dispersion with a spatula immediately before use.

2.3. High-temperature shear cell

The PPI-WG blends were treated in a high-temperature shear cell (HTSC) (Wageningen University, Netherlands) that was developed in house (Grabowska et al., 2016). For all treatment conditions, the blends were heated at 120 °C for 15 min in the pre-heated HTSC and were sheared at 5–100 rpm (corresponding to shear rates of 6.5–130 s^{-1}). This shearing was performed at various shear rates i) for 15 min during heating, ii) from the 8th minute onwards during heating and iii) for 8 min during cooling. After the shear treatment the products were cooled until they reached a temperature of around 30 °C, using an external oil bath of which the temperature was set to 25 °C. An overview of the experimental design can be seen in Fig. 1.

The average temperature of the protein blend during shearing was measured at the upper non-rotating cone of the HTSC and was 110–115 °C when shearing during heating (Figures A1 and A2). The temperature depended on the shear rate used. A higher shear rate led to a higher temperature, probably due to increased viscous dissipation. The average temperature during the shear treatment when shearing during cooling was 80–90 °C (Figure A3).

All products were left at room temperature in a closed plastic bag for at least 1 h before further measurements were performed. All products were prepared in triplicate.

The torque measured during the HTSC treatment by the Haake Polylab QC drive (Germany) was used to calculate the specific mechanical energy (SME in J/kg) of the process (Equation (1)).

$$SME = \frac{\int_{t=0}^{t_f} \omega . M(t) . dt}{m} \tag{1}$$

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 HTSC (kg) (=0.1 kg) (Peighambardoust et al., 2004).

2.4. Visual observation of macrostructure

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 34× magnification (Smartzoom 5, Carl Zeiss, Germany).

2.5. 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:

$$\varepsilon = ln\frac{h(t)}{h_0} \tag{2}$$

$$\sigma(t) = \frac{F}{A} \tag{3}$$

In which:

$$A = \frac{h_0}{h(t)} * A_0 \tag{4}$$

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.

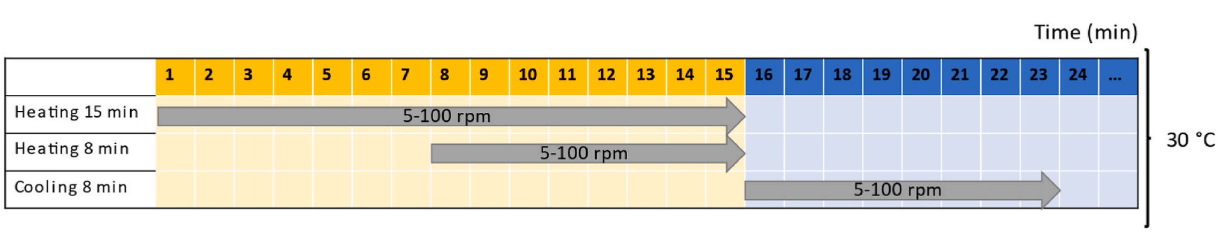

Fig. 1. Experimental design of the current study: the heating and cooling periods were indicated by yellow and blue colour, respectively, and the material was sheared in a range from 5 to 100 rpm.

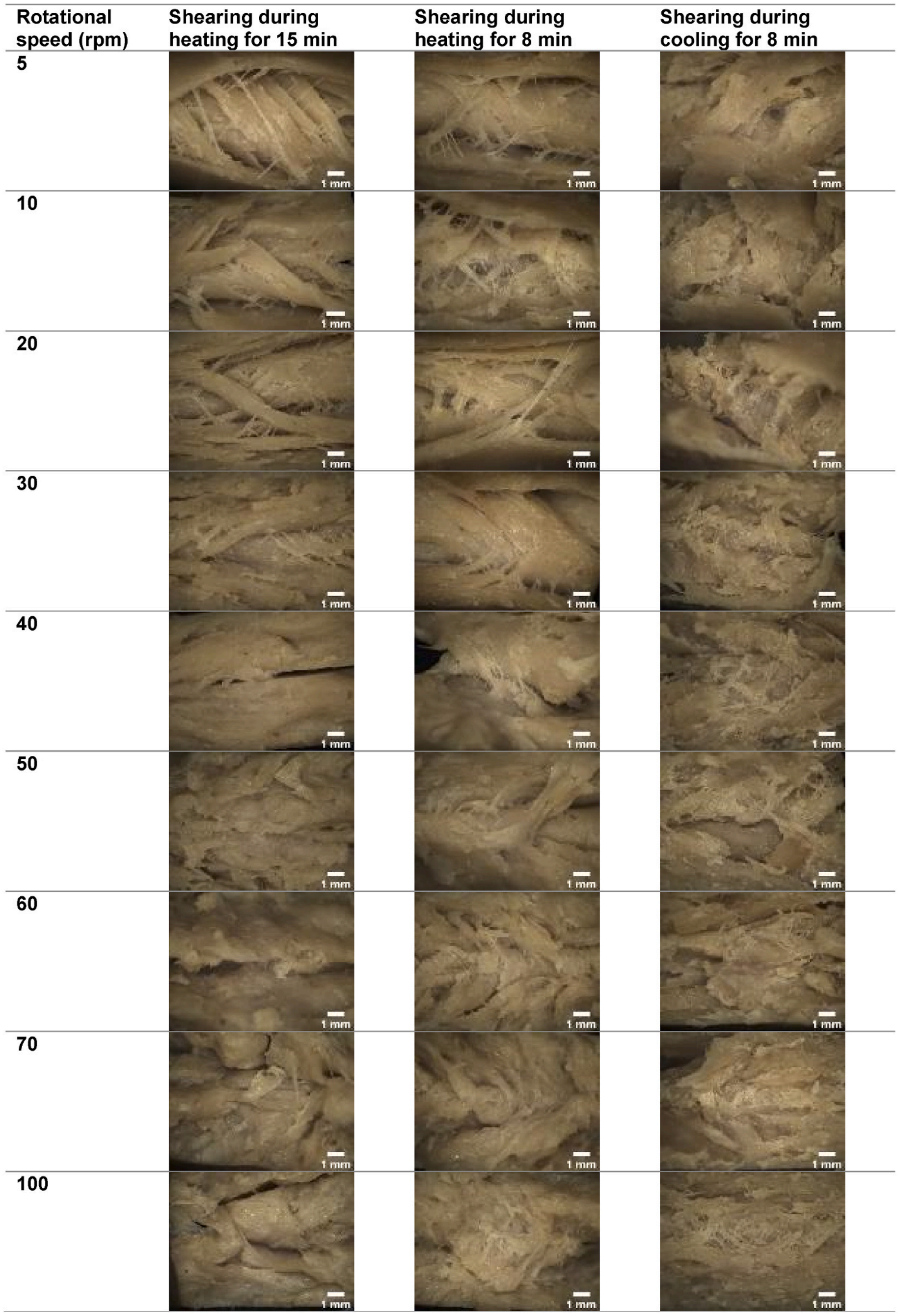

Fig. 2. Representative digital microscopy pictures with 34× magnification of HTSC product structures sheared at different shear rates for 15 min during heating, 8 min during heating or 8 min during cooling.

For every product, three parallel and three perpendicular specimens were taken. Therefore, in total nine parallel and nine perpendicular specimens per treatment were measured.

2.6. *Statistical analysis*

All experiments were performed in triplicate unless stated otherwise. Values are represented as the mean ± standard deviation. Tukey's HSD test was performed to evaluate the statistical significant differences between samples at a significant level of 95% ($P < 0.05$) analysed using SPSS statistics Version 25.0.0.2 (IBM, Armonk, NY). The results of the statistical analysis can be found in Tables A.1, A.2 and A.3.

3. Results and discussion

3.1. *Visual observations*

Anisotropy in a product can be defined in two manners: visual fibrousness and mechanical anisotropy. Visual observations of the HTSC products were used to assess the visual fibrousness of the HTSC products. HTSC products produced at rotational speeds below 30 rpm were more fibrous than products prepared at higher shear rates (Fig. 2). This effect was visible for all temperature conditions used. However, the fibrous structures formed during cooling appeared to be more "flaky" and showed less distinguishable fibres compared to the products sheared during heating. For treatments with rotational speeds above 30 rpm texturization of the products was still visible, but no clear fibres could be

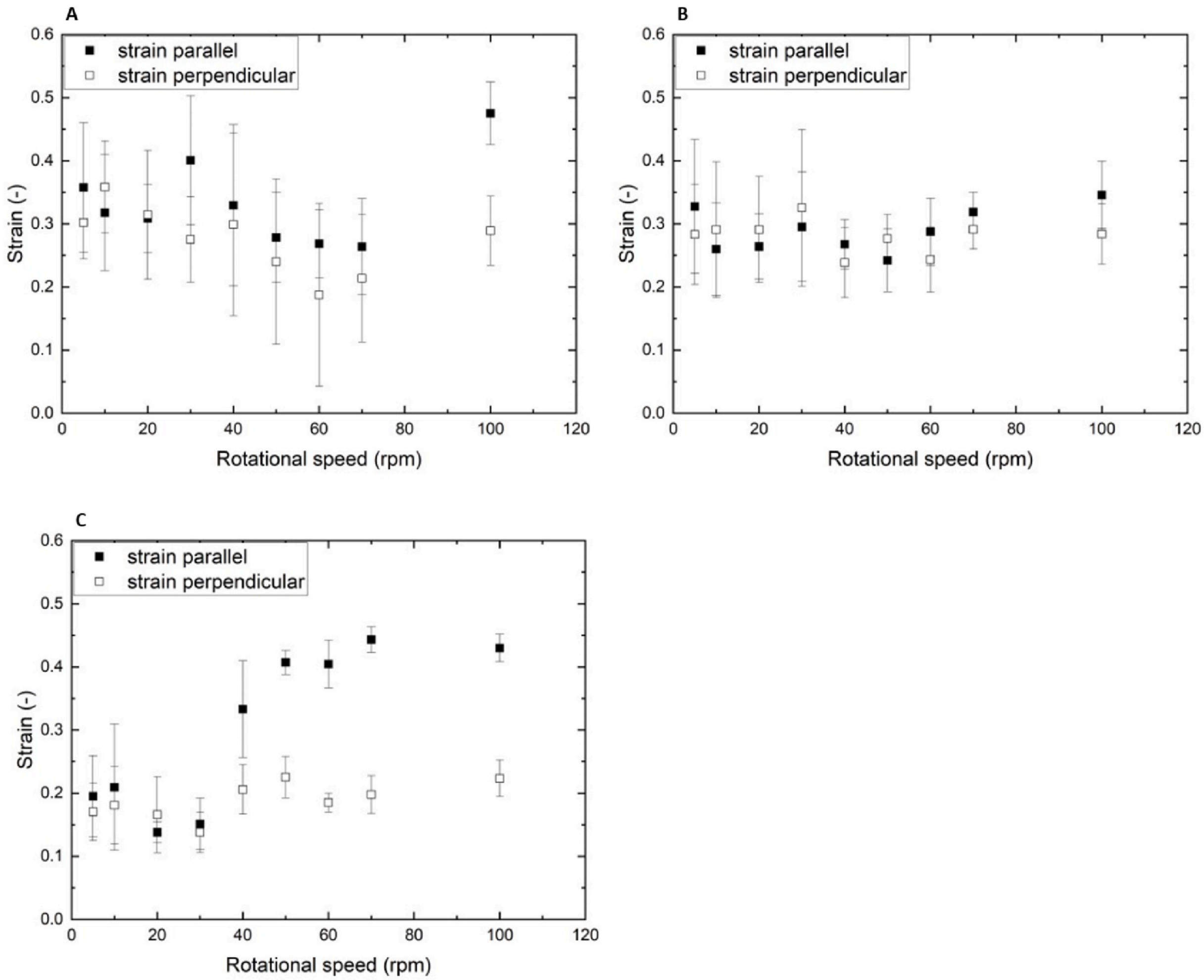

Fig. 3. Tensile strain at fracture plotted against shear rate in parallel (■) and perpendicular (□) direction for products sheared for A. 15 min during heating, B. 8 min during heating, C. 8 min during cooling.

observed.

3.2. Tensile test

Tensile tests in the parallel and perpendicular direction to the shear flow were used to quantify the anisotropy in the textural properties of the different HTSC products. Generally, HTSC products are stronger in parallel direction than they are in perpendicular direction. Products sheared during cooling had a lower tensile strain compared to products sheared during heating, especially in perpendicular direction at rotational speeds below 30 rpm (Fig. 3). The tensile strain of these products sheared at a rotational speed below 30 rpm and above 30 rpm during cooling clearly differed (Fig. 3C). Above 30 rpm parallel tensile strain of products sheared during cooling increased when the rotational speeds (Fig. 3C). In contrast, the perpendicular tensile strain only increased slightly with an increasing shear rate. As a result, the difference between the tensile strain in parallel and perpendicular direction was larger for products sheared at a rotational speed of 40 rpm or higher. This indicates that the HTSC products reached a higher mechanical anisotropy at higher rotational speeds, while not showing visual fibrousness (Fig. 2). This increase in tensile strain anisotropy at high rotational speeds was only observed for products sheared during cooling. Remarkably, the effect of rotational speed on the tensile strain of products sheared during heating was small, even though the products' structures were visually very different and only showed visual fibrousness when processed using rotational speeds below 30 rpm (Figs. 2 and 3A,B).

It is remarkable that the variation in strains is higher for samples that were sheared during heating, especially when shearing at high rotational speeds for 15 min. Obviously, the structure of these products is more heterogeneous at the length scale of the measurement. This corresponds to the more clearly fibrous structures that were observed for the product sheared during heating. The variation in the tensile properties of HTSC products might therefore be a better indicator of fibroussness compared to the anisotropy index that does not always correspond with visual fibrousness (Schreuders et al., 2022).

Similarly to the tensile strain, the tensile stress was lower for products sheared during cooling, especially in perpendicular direction (Fig. 4). These products had a higher tensile stress in parallel direction for rotational speeds above 40 rpm (Fig. 4C). This means that similar to the tensile strain also the tensile stress indicated a more anisotropic structure at higher shear rates, while no clear fibrous structures were visible. Both visual fibrousness as mechanical anisotropy can therefore be obtained when shearing during cooling depending on the rotational speed that is used, which gives new opportunities to obtain a HTSC product with desired visual or mechanical properties. This was not observed for the products sheared during heating. The parallel tensile stress of the products sheared for 8 min during heating slightly increased at rotational speeds higher than 30 rpm (Fig. 4B). The tensile stress in perpendicular direction did not change significantly for these products. However, for products sheared for 15 min during heating, the tensile stress was not affected by rotational speed, except for a slight increase at

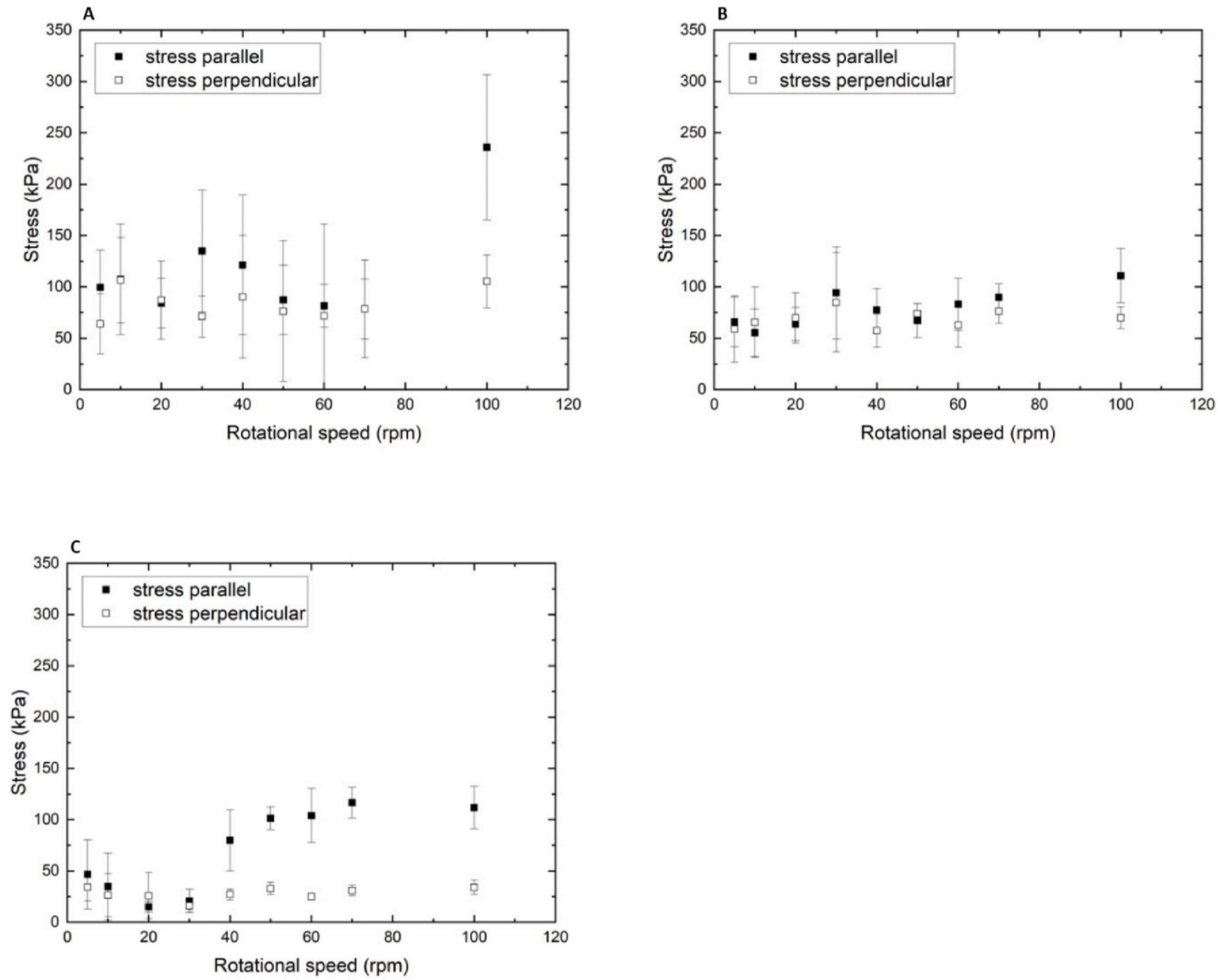

Fig. 4. Tensile stress at fracture plotted against rotational speed in parallel (■) and perpendicular (□) direction for products sheared for A. 15 min during heating, B. 8 min during heating, C. 8 min during cooling.

100 rpm compared to the other rotational speeds (Fig. 4A). The tensile stress results overall showed the same trend as the tensile strain results. The standard deviation of the tensile stress and strain was higher for products sheared for 15 min during heating compared to the other temperature conditions. This could be an indication that the products sheared for 15 min had a more heterogeneous structure at the length scale of the measurement.

The Young's modulus indicates the elasticity of the HTSC products. The Young's moduli of products sheared during cooling were lower compared to those during heating (Fig. 5). The Young's modulus of products sheared during cooling was not affected by rotational speed. However, for the products sheared for 8 min during heating the Young's modulus slightly increased with an increasing rotational speed (Fig. 5B). The Young's modulus of the products sheared for 15 min during heating increased with an increasing rotational speed in perpendicular direction, while in parallel direction no significant changes were found (Fig. 5A). An increase in Young's modulus can be linked to an increase in rigid structures both on molecular as on macroscopic levels (Cuq et al., 2000). WG polymerization can lead to more rigid protein chains because of the formation of disulfide bonds. The higher rigidity of HTSC products sheared at increasing rotational speeds during heating could therefore be the result of the more extensive deformation and orientation during WG polymerization (Pietsch et al., 2019).

From these observations we can conclude that shearing during cooling weakened the HTSC products as indicated by a lower tensile stress and strain and Young's modulus and was detrimental for visual fibrousness. Furthermore, it was possible to create HTSC products from the same PPI-WG blend with different visual and mechanical characteristics, by changing the processing conditions. Especially during cooling structures with either visual fibrousness or mechanical anisotropy could be created depending on the rotational speed during shearing, resulting in a wider range of product properties. However, it remains difficult to predict the product properties based on a single processing parameter (i.e. rotational speed). As mentioned in the introduction, we hypothesized that the application of shear during cooling would either enhance structure formation, because mechanical forces can be better transferred to the product or could cause structural breakdown as a result of deforming a solidified material. The weaker products that were produced when shearing during cooling compared to shearing during heating indicated that structural breakdown was important for the PPI-WG system.

In a previous study, fibrous products using a PPI-WG blend were formed in the HTSC at a rotational speed of 30 rpm (Cornet et al., 2021; Schreuders et al., 2019). Here, we observed the most pronounced fibrous structure in products produced at a rotational speed of <20 rpm (Fig. 2) which was somewhat lower. We attribute this observation to the slightly different functional properties of PPI used in the current study (Nutralys® S85F) and the previous one (Nutralys® F85M). Nutralys® S85F has a smaller particle size and is more soluble than Nutralys® F85M according to the manufacturer's specifications. This difference in functional properties of the raw ingredients may have influenced the overall behaviour of the blend during thermomechanical treatment.

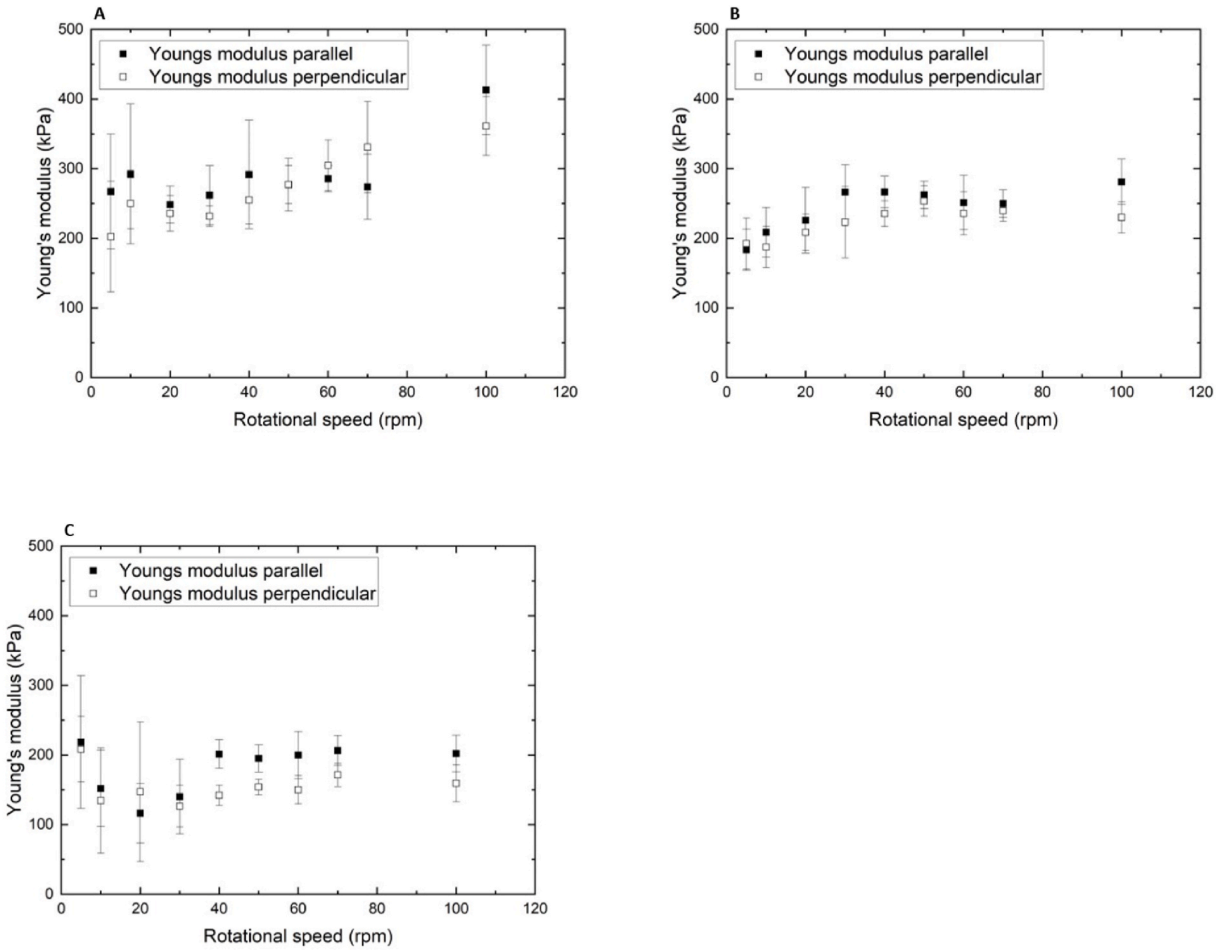

Fig. 5. Young's modulus plotted against rotational speed in parallel (■) and perpendicular (□) direction for products sheared for A. 15 min during heating, B. 8 min during heating, C. 8 min during heating.

3.3. Specific mechanical energy (SME)

The total mechanical input to HTSC products during the process can be quantified by calculating the specific mechanical energy (SME). The SME is often considered a measure to describe the intensity of HTSC processing and high moisture extrusion (HME) (Cornet et al., 2021b; Peighambardoust et al., 2004). SME is indirectly affected by temperature through the rheological properties of the material. An increased temperature is known to reduce viscosity, which results in a reduced resistance to deformation and therefore a lower torque and SME (Palanisamy et al., 2019). We used SME to compare the different processing conditions. In all processes, the total time and overall temperature profile were kept constant, but rotational speed, shearing time and moment of shearing were varied. The temperature during shearing was therefore different in case shearing was applied at constant temperature during heating or during cooling. SME of the different shear treatments ranged from 1.6 to 163 kJ/kg (Fig. 6). The SME was not much affected by cooling at low rotational speeds. At higher rotational speeds, the SME was slightly higher during cooling. This was probably due to a higher viscosity of the protein blend inside the HTSC as the temperature dropped.

The SME used to create visually fibrous products ranged from 1.6 to 16.8 kJ/kg (Figs. 2 and 6) which is lower compared to what was previously reported for structuring with the Couette cell device (8.6–59.9 kJ/kg) and HME (32–1276 kJ/kg) (Krintiras et al., 2016; Maung et al., 2021; Pietsch et al., 2019; Wang et al., 2022; Zahari et al., 2021). The low SME required for structuring in the shear cell could mean that the

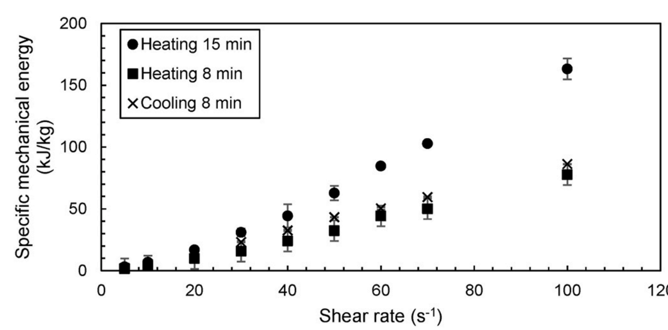

Fig. 6. Specific mechanical energy of the shear treatments under different conditions.

intensity during HME processing could be lower, while still giving good products.

To better correlate the SME during processing and the textural properties of the HTSC products, texture maps were created (Fig. 7). Texture maps are used to describe the structure of food gels and correlate the tensile strain and stress with textural properties (Ersch et al., 2015; Schreuders et al., 2021; Shand et al., 2008). Products that fall in the top left and right corner of the texture map are classified as tough and brittle, respectively. Products that fall in the lower left and right corners can be classified as mushy and rubbery, respectively (Kim et al., 2005; Schreuders et al., 2021). At first glance, all the data points were located in the diagonal line of the texture map that connects the "mushy"

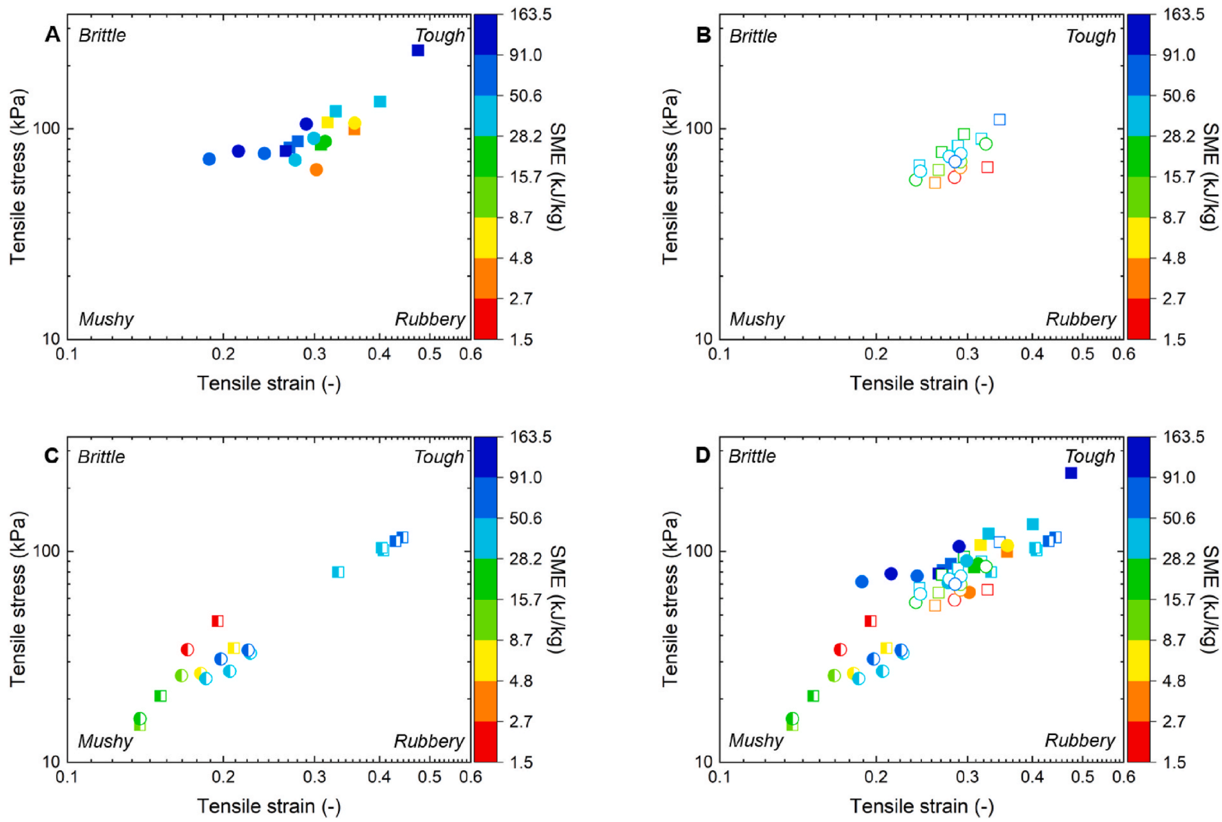

Fig. 7. Texture map with tensile stress plotted against tensile stress in parallel (□) and perpendicular direction (○) for shear cell products sheared for A. 15 min during heating (filled symbols), B. 8 min during heating (open symbols), C. 8 min during cooling (half-filled symbols) and D. All treatment conditions.

and "tough" corners (Fig. 7D). This indicates that the HTSC products produced varied in texture, i.e. more towards "mushy" or "tough", depending on the temperature or SME during processing. For comparison: the middle region of the diagonal line lies in the same range as the tensile stress (100 kPa perpendicular, 44 kPa parallel) and strain (0.3 perpendicular, 0.2 parallel) of cooked chicken reported by Schreuders et al. (2019). Data points of products sheared during cooling are spread out over the diagonal, while the data points of samples sheared during heating were more clustered in the middle of the texture map and were slightly tougher (Fig. 7A, B, C). No clear correlation between SME and the position of the samples in the texture map was found. This indicates that SME cannot completely explain the variation in product characteristics that can be achieved by using different processing conditions.

3.4. Implications for the importance of cooling during extrusion

Deformation during cooling is often thought to be essential for fibrous structure formation using HME (Cheftel et al., 1992; Sandoval Murillo et al., 2019; Tolstoguzov, 1993). However, our research revealed that shear application during cooling is not a requirement for visual fibrous structure formation. We found that visually fibrous products could be produced using a low shear rate (<30 rpm or <39 s^{-1}). The apparent shear rates in the cooling die range from 1 to 46 s^{-1} (Cornet et al., 2021b), which is comparable to the lower shear rates at which visual fibrous structures were found in this study. Additionally, products sheared during heating were visually more fibrous and had a higher tensile strain and stress, which suggests that a higher temperature during shear application in the cooling die could lead to stronger extrudates. Ramos Diaz et al. (2022) indeed showed that a higher cooling die temperature (40–80 °C) resulted in increased hardness, as measured using texture profile analysis (TPA), and cutting strength of HME extrudates made from oat fibre concentrate and PPI. However, Kantanen et al. (2022) did not find this correlation for mixtures of faba protein concentrate and isolate, possibly because it was overshadowed by the effect of mixture composition. Even though increasing the cooling die temperature could be beneficial for fibrous structure formation, the product temperature at the end of the cooling die should remain below 100 °C to prevent product expansion (Maningat et al., 2022). Furthermore, the use of a long cooling die remains imperative for HME as it enables upstream pressure generation, the use of high temperature, channel filling and the continuity of the extrusion process (Cheftel et al., 1992).

4. Conclusions

In this study, we report on the importance of cooling to produce fibrous products using HTSC and HME. It was found that shearing during cooling can be used to create product structures that differ from structures obtained without shearing during cooling. Cooling of PPI-WG in the HTSC was not beneficial for visual fibrous structure formation. The structure of the product could be described best as "flaky". Besides, products sheared during cooling were weaker compared to products sheared during heating. However, shearing at higher rotational speeds during cooling did lead to increased mechanical anisotropy and could therefore be used to obtain HTSC products with a specific desired texture. It was therefore concluded that deformation during cooling is not essential for structure formation. When overviewing all results, it was found that fibrous products could be obtained at low SME conditions in the HTSC. This finding suggests that it is still possible to further optimise the HME process towards lower SME-inputs.

Credit author statement

Nienke Köllmann: Investigation, Writing – original draft, Writing –

review & editing. Floor K.G. Schreuders: Investigation, Writing – review & editing. Lu Zhang: Conceptualization, Writing – review & editing. Supervision. Atze Jan van der Goot: Conceptualization, Writing – review & editing. Supervision.

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.

Acknowledgments

The authors would like to thank Sophia Oebel and Hanhong Lu for their contribution to the explorative work leading to this study and Jarno Gieteling for his assistance with the shear cell.This research is part of the PlantPromise project, which is co-financed by the Top Consortium for Knowledge and Innovation Agri & Food by the Dutch Ministry of Economic Affairs. The project is registered under contract number LWV-19027

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfoodeng.2023.111490.

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