Structural and mechanical anisotropy in plant-based meat analogues

Joel I. Zink\textsuperscript{a,1}, Viviane Lutz-Bueno\textsuperscript{b,1}, Stephan Handschin\textsuperscript{c}, Cathrina Dütsch\textsuperscript{a}, Ana Diaz\textsuperscript{b}, Peter Fischer\textsuperscript{a,1}*, Erich J. Windhab\textsuperscript{a}

\textsuperscript{a} Food Process Engineering, Institute of Food Nutrition and Health, ETH Zurich, Schmelzbergstrasse 7, 8092 Zurich, Switzerland
\textsuperscript{b} Scientific Center for Optical and Electron Microscopy, Department of Materials, ETH Zurich, 8093 Zurich, Switzerland
\textsuperscript{c} Paul Scherrer Institute, 5232 Villigen PSI, Switzerland

ARTICLE INFO

Keywords:
Meat analogues
Rheology
Small angle X-ray scattering
Anisotropy
Protein structuring

ABSTRACT

The rising demand for plant-based meat analogues as alternatives to animal products has sparked interest in understanding the complex interplay between their structural and mechanical properties. The ability to manipulate the processing parameters and protein blend composition offers fundamental insights into the texturization process and holds economic and sustainable implications for the food industry. Consequently, the correlation between mechanical and structural properties in meat analogues is crucial for achieving consumer satisfaction and successful market penetration, providing comprehensive insights into the textural properties of meat analogues and their potential to mimic traditional animal produce. Our study delves into the relationship between structural and mechanical anisotropy in meat analogues produced using high moisture extrusion cooking, which involves blending protein, water, and other ingredients, followed by a controlled heating and cooling process to achieve a fibrous texture akin to traditional meat. By employing techniques such as scanning small-angle X-ray scattering, scanning electron microscopy, and mechanical testing we investigate the fibrous structure and its impact on the final texture of meat analogues. We show that textural and structural anisotropy is reflected on the mechanical properties measured using tensile and dynamic mechanical techniques. It is demonstrated that the calculated anisotropy indexes, a measure for the degree of textural and structural anisotropy, increase with increasing protein content. Our findings have significant implications for the understanding and development of plant-based meat analogues with structures that can be tuned to closely resemble the animal meat textures of choice, thereby enabling consumers to transition to more sustainable dietary choices while preserving familiar eating habits.

1. Introduction

The increased public awareness on the relationship between the over-consumption of animal products and climate change, as well as animal welfare, sustainability and personal health, accelerate the demand for alternative meat-like products. In this context, plant-based meat analogues can act as a protein source replacement for consumers who do not want to change their eating habits drastically (Boukid, 2021; Malek et al., 2019; Michel et al., 2021). These meat alternatives are induced from proteins that form macro- and microstructures, and resemble the textural properties of animal meat. Such textures can be produced by shear cells (Krintiras et al., 2014; Kyrıakoğlu et al., 2021), electrospinning (Nieuwland et al., 2014; Manski et al., 2007) and high moisture extrusion cooking (HMEC) (Zink et al., 2023; Dekkers et al., 2019). HMEC is a highly versatile process for meat analogues that can be efficiently up-scaled for large industrial throughput, and involves the use of an extruder to mix protein, water and other ingredients into a mixture called the protein blend. Blending is followed by a heating step, resulting in a hot protein melt.

While the texturization occurs throughout the whole extrusion process (van der Sman and van der Goot, 2023), the final texturization into a fibrous extrudate occurs when this hot protein melt is cooled by flowing through a long, often slit- or annular-shaped die. The cooling temperatures must be below water evaporation to reduce moisture loss and uncontrolled restructuring of the protein melt. The shear and temperature gradients in the cooling die are keys for texturization. The center of the cooling die remains hotter than its wall, thus the extrudate develops a faster, and less viscous "core" flow, which is stretched by

\footnotesize
\textsuperscript{1} Corresponding author.
\textsuperscript{1} Equally contributed.

https://doi.org/10.1016/j.foodres.2024.113968
Received 28 July 2023; Received in revised form 21 December 2023; Accepted 2 January 2024
Available online 5 January 2024
0963-9969/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
shear and structured in layers along the die (Wittek et al., 2021; Zink et al., 2023). The flow profile within the die is believed to evolve from a parabolic shape similar to Hagen-Poiseille flow profile (Osen and Schweigert-Weisz, 2016) at the die entrance into a flat-tipped plug flow at the die exit as a result of increasing the melt viscosity at the cooling die wall. Apart from the difficulties of measuring in situ, this evolution is no trivial matter to predict, since it depends on aspects of heat transfer, mass transport, and phase transitions. The structuring by diffusion and advection is finally arrested by solidification of the extrudate when reaching critically low temperatures (Sandoval Murillo et al., 2019). The solidified protein fraction in the final extrudate is what defines the fibrous texture of meat analogues. In summary, the texture of the product is determined by the acting mechanical and thermal stresses during the cooling and solidification steps, as well as the composition of the protein blend, which determines the molecular and inter-particle interactions at the macro- and nanoscale.

Several techniques can determine the texturization in meat analogues. Mechanical stress–strain properties are often measured by uniaxial compression with a texture analyser (Lee et al., 2022; Kaleda et al., 2021; Zink et al., 2022). While stress–strain relationships provide textural hardness and cohesiveness, they usually fail to measure the mechanical anisotropy, i.e., the dependence of mechanical properties on the direction of the sample texture. For meat analogues, their textural anisotropy is essential to evaluate their resemblance to targeted animal produce. For example, tofu and soy protein-based meat analogues can have similar composition and firmness, but differ greatly regarding their fibrous structure. Tofu has a more isotropic structure in contrast to the fibrous structure. Tofu has a more isotropic structure in contrast to the fibrous structure of soy protein-based meat substitutes, which is easily sensed when chewing.

In this study, we investigate the relationship between structural and mechanical anisotropies of soy protein concentrate (SPC) meat analogue samples produced by HMEC with three different concentrations of solids in the protein blend. Higher protein concentration is known to improve the melt connectivity through disulfide bond formation during heating and electrostatic forces during cooling (Zink et al., 2016) and also impact the viscosity. As a first step, we investigate the texture extruded samples using a specially developed scanning electron microscope (SEM) technique allowing to obtain SEM images of the texture with very fine control of meat analogue texture, which is easily sensed when chewing.

To investigate the influence of the protein content on the texturability of the extrudate, we increased the SPC content from 30 to 40 wt% under the same processing parameters (Fig. 1B) by adapting the water fraction to uphold a total throughput of 35 kg/h. The range of concentrations are selected within the feasible processing of the extrusion setup and minimal conditions for protein blend texturization. We expect that concentration will mainly impact the viscosity, thus by keeping the same processing conditions and only increasing the protein content, we can generate higher shear stresses and potentially higher structuring of the meat analogues.

### 2.2. Sampling and reference system

The main flow direction within the cooling die is the reference for sampling, which is carried out longitudinal and transversal to it (Fig. 2A). The sampling is described in Cartesian coordinates, whereby the flow direction is represented by the x-axis, the width of the cooling die by the y-axis, and its height by the z-axis. Consequently, the transversal samples are on the xy-plane, and the longitudinal samples on the yz-plane. The protein concentration in the blend, as well as the position within the cooling die can have an impact on the internal structure of the extrudate. To observe and characterize these changes, longitudinal samples are collected transversely to the flow direction at the outer edge of the extrudate (x = 0, called L1, Fig. 2B), at its center (x = 25 mm, called L3), and in between these two positions (x = 12.5 mm, called L2). These are the “front view” of the extrudate flow or the xy-plane. We expect that the texturization mechanism that occurs in the cooling die will lead to measurable differences in the structure of these three longitudinal samples (xy-plane). These positions allow us to observe the impact of their positions relative to the flow profile, die wall, and the cooling front. Samples L1 capture the outer extremity of the parabolic-like profile near the die wall and are the most exposed to the heat exchange and the shear force exerted by the die walls. Samples L3 are at the tip of the flattened parabolic, i.e., plug flow profile, and probably the last to be affected by cooling. Fig. 2A also shows samples collected longitudinally to the flow direction at the longitudinal center-line (yz-plane, x = 0) with an interval of 10 mm corresponding to positions along the z direction of 0, 10, and 20 mm called transversal samples T1, T2, and T3, respectively. These samples are the side view of the extrudate under similar conditions, and their structure should not vary with the position along z, as they are under similar flow field and temperature gradient conditions.

### 2.3. Scanning Electron Microscopy

The sample was processed with a PELCO BioWave, Pro + microwave system (Ted Pella, USA), following a microwave-assisted fixation and dehydration procedure. Small rectangular pieces of sample were cut out of the extrudate with the longitudinal side parallel to the extruder-axis. Fixation was done in 2.5 % glutaraldehyde / 2 % paraformaldehyde in phosphate buffer solution on ice. After washing samples were post-fixed in 1 % osmium tetroxide OsO₄ in bi-distilled water, washed again and dehydrated in a graded series of ethanol (50, 75, 90, 98, and three times 100 %) on ice followed by critical point drying out of dry ethanol (CPD 931, Tousimis, USA). The resulting sample was cross-fractured and mounted on SEM aluminum stubs in conductive carbon paint (Plano, Germany) and then rotary sputter-coated with 5 nm of platinum/palladium (CCU-10, Safematic, Switzerland). SE-inlens and
Fig. 1. (A) Views of the flow direction of HMEC produced meat analogue exiting the cooling die. (i) and (ii) are isometric viewpoints, (iii) a transparent side view, and (iv) a front view of the cooling die dimensions. The flow direction is shown by a red arrow, the flow profile is depicted in blue and the shear profile in yellow. (B) Macroscopic structure of SPC extrudates during tearing. Left: 30 wt% SPC sample being peeled from the side. Right: 40 wt% SPC sample being peeled from the top. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. (A) Isometric viewpoint of longitudinal and transversal sampling for sSAXS measurements and the flow direction. (B) Front view of the extrudate exiting the cooling die and the detailed positions of the sSAXS samples.
Everhart–Thornley (ET) SE-images were recorded at a working distance of around 4–5 mm with a scanning electron microscope (Merlin, Zeiss, Germany) operated at an accelerating voltage of 1.5 kV.

### 2.4. Scanning SAXS and WAXS

The samples were cut with a manual microtome (Leica Microsystems, Germany) into sections of 5 x 10 x 0.5 mm. These sections were then immediately placed on a glass cover slip prepared ready with epoxy resin on the edges, sealed carefully with a second cover slip and avoiding contact of the resin with the sample (Figure S1). Samples were allowed to polymerize for about 2 h at room temperature and then stored at 4°Celsius to minimize moisture loss and microbial growth. Longitudinal and transversal samples were prepared for 40 wt% SPC. Only longitudinal samples were prepared for formulations containing 30 and 35 wt% SPC.

Scanning Small and Wide-Angle X-ray Scattering (sSAXS and sWAXS) experiments were conducted at the coherent small-angle X-ray beamline at the Swiss Light Source (Paul Scherrer Institut, Switzerland). The beam size was 20 x 50 μm and the energy was set to 12.4 keV corresponding to a wavelength of 1 Å. The principles of this technique is given by Bunk et al. (Bunk et al., 2009). Briefly, the sSAXS and sWAXS measurements record scattering patterns while scanning the samples. We first collected sWAXS data with a Pilatus 2 M detector with pixel size 172.6 μm placed at 237 mm downstream the sample in air. For sSAXS measurements, the same detector was placed at a distance of 7.106 m from the sample with a 7 m long evacuated flight tube in the beam path. The same identical samples were measured in sWAXS and sSAXS and we expect minor misalignment due to remounting the sample holder. The scanning of the sample was performed by translating the samples in the xy-plane in steps of 50 μm covering areas of 4 x 7 mm.

### 2.5. Tensile test

Tensile measurements of extruded meat analogue samples were conducted with a Z010/Th2s texture analyzer (Zwicker Roell Group, Germany) equipped with a 10 kg load cell. For statistical relevance, ten samples were tested for each SPC formulation and for each of the two orientations. The samples were cut out into bone-shaped pieces for optimal sample grip. The sample depth, corresponding to the thickness of the extruded HMEC strand, was measured prior to the tensile test, which was performed at a constant speed of 1 mm/s. The cut out tensile specimens had a total length of 50 mm, a gauge length of 20 mm with a gauge width of 10 mm and a grip section of 10 x 50 mm in length and width, respectively. Force $F$ was measured and the Young’s Modulus $E$ was calculated according to Eq. 1, whereby $F_0$ is the initial distance of 30 mm between the clamps holding the sample, $A$ the cross-section calculate with the sample depth, $ΔL$ the difference between $F_0$ and the pulled distance. Briefly, this approximation covered the applied strain $ε$ and the measured stress $σ$. The tensile anisotropy index $A_{I_{Tensile}}$ is calculated by Eq. 2, whereby $E_x$ and $E_y$ stand for the Young’s Modulus in longitudinal and transversal direction of the flow, respectively.

$$E = \frac{F_0}{AΔL} \frac{σ}{ε} \quad (1)$$

$$A_{I_{Tensile}} = \frac{E_x}{E_y} \quad (2)$$

whereby $AI \sim 1$ indicates isotropic orientation and $AI \neq 1$ indicates anisotropy. We attempt to measure the fracture stress and strain of the samples, but obtained inconclusive results, due slip and non-representative tearing of the samples near the holding clamps.

### 2.6. Dynamic mechanical analysis

Bar-shaped samples with a width of 5 mm and a length of 50 mm were cut out in longitudinal and in transversal direction to the extrudate flow (Fig. 1A). The flexural moduli of the HMEC meat analogue bars were measured with a Dynamic Mechanical Analyser (DME, DMA Q800, TA Instruments Inc., USA) using a dual cantilever system. The bars were bend by their thinnest side corresponding to 90° flipped cut out samples. Storage $G’$ and loss $G’’$ moduli were measured in amplitude sweeps at strains from 0.01 to 10%, keeping the frequency constant at 1 Hz. Frequency sweeps were measured in the linear viscoelastic region (LVR) obtained from the amplitude sweeps) at constant strain of 0.1% from 1 to 10 Hz. The anisotropy index $AI_{DMA}$ for all three longitudinal samples and for each meat analogue formulation were calculated from the amplitude and frequency sweeps according to Eq. 3. All measurements were conducted in triplicates.

$$AI_{DMA} = \frac{G’’}{G’} \quad (3)$$

Similar to the tensile testing, $AI \sim 1$ indicates isotropic orientation and $AI \neq 1$ indicates anisotropy.

### 3. Results

#### 3.1. Structural anisotropy

**3.1.1. SEM:**

Scanning electron micrographs (SEM) depict the texture of extrudates with 30 and 40 wt% SPC (Fig. 3), revealing features ranging from several micrometers down to a few nanometers. These samples were collected at the center of the cooling die (position L3 in Fig. 2A). We carefully cross-fractured the dried samples in the direction (xy-plane) transversal to the extrudate flow. The SEM images show clear structural differences at their surface. Samples produced with 30 wt% SPC exhibit a smoother surface, but with larger cracks than samples with 40 wt% SPC, which exhibits spotted irregularities. We hypothesize that these white spots are fibril solidified protein structures of approximately 10–30 nm in thickness, progressing upwards in the direction of the capturing lens, hence in the flow direction (z-direction). This hypothesis is supported by reports on meat analogues that have shown structures with similar structural dimensions (Chiang et al., 2019; Chiang et al., 2021). The solidified protein matrix is composed of flat surfaces formed by a network of thin sheets of about 100 nm. These sheets are mainly composed of soy proteins, and preferentially align along certain directions. We notice that the sample with 30 wt% SPC is coarser and less oriented in the z-direction than the 40 wt% SPC. This difference in smoothness is also observed macroscopically in the extrudate (Fig. 1B). Nonetheless, fine fibrillar network structures of a few nanometers in diameter are also observed. Due to the potential formation of artifacts during sample preparation, we ensured that the sample did not have pores from water evaporation or uneven cuts, which improves the quality of our data set. However, these SEM images are micrometric areas of a macroscopic sample that were purposefully selected, and they must be interpreted as such, avoiding generalizations. Furthermore, the SEM sample preparation might interfere with the structure of the sample at the surface, thus techniques that probe the structure as a bulk are essential. Here, we improve the statistical relevance of our study by complementing SEM with transmission-based scattering imaging, which probes and provides information of nanostructures in rather bulky and unmodified samples (only slicing is needed).

**3.1.2. sSAXS and sWAXS:**

sSAXS and sWAXS techniques are used to gather structural information at resolutions ranging from a few nanometers up to millimeter scales (Bunk et al., 2009; Berujon et al., 2012; Lutz-Bueno et al., 2016; Lutz-Bueno et al., 2022). SAXS data provides information on the nanometer range, such as particles sizes and alignment, while WAXS is sensitive for internal structures at the molecular level, consequently to
protein conformation and structure. Areas of 4 x 7 mm were scanned with a beam size of 17 x 50 μm² at motor step size of 50 μm. The combination of large sample area, high spatial resolution, and the possibility to still probe the nanometer length scale allows microscopy and scattering techniques to complement each other, allowing the structural characterization of complex heterogeneous samples such as meat analogues. For each sample, we scanned a total of 10’044 scattering patterns that are interpreted regarding their anisotropy and intensity to generate the maps shown in Figs. 4 A and B. Fig. 4 C compares the integrated scattering intensity \( I \) of 140 scattering patterns, which were collected along the vertical axis of the map shown as the dashed line for samples L1 and T1. The integrated scattering curves of all samples are shown in Figure S2, selecting every second pixel in \( x \) and \( y \) for longitudinal and \( y \) and \( z \) for transversal samples. The features of the integrated intensity curves such as the peak (Fig. 4 C, blue arrows) become less prominent with the increase in protein, which is clear by the broadening of such a peak. This broad correlation peak is related to \( q \approx 0.26 \) nm\(^{-1}\) and to a real space position \( d = 25 \) nm. We speculate that this \( d \) could be the main average distance between agglomerated proteins connected by electrostatic or covalent forces to form the fibrous structure of the meat analogue. This assumption is supported by the SEM images shown in Fig. 3, which show fibrous solidified protein structures distanced with similar length scales. Even though the distance between chains might be around 25 nm, we notice that the alignment of the fibers is highlighted in the range of 100–200 nm, which might be related to the fiber length. To enhance the contribution of the fiber length to the main orientation of the scattering pattern, we focus our SAXS analysis on this range in reciprocal space (Fig. 4C).

3.1.3. sSAXS maps:
To represent the intensity of anisotropy, the scattering signal is decomposed in three main scalars, the symmetric scattering intensity \( I_{\text{sym}} \), the asymmetric scattering intensity \( I_{\text{asym}} \) and the angle of orientation of the scattering signal. These values are obtained from the fitting of a cosine function to the integrated scattering intensity as a function of the azimuthal angle \( \chi \). \( I_{\text{sym}} \) is the base of the cosine, which is related to the total scattering of a sample: bright colors indicate higher concentration of sample, while dark color indicate lower concentrations. The angle \( \theta \pm 90^\circ \) is defined by the shift of the cosine function due to the alignment of the scattering pattern, and for simplicity, here we will rather represent the real orientation of the sample \( \theta \) in that pixel in the nanometer region. The maps in Fig. 4 exhibit the \( I_{\text{sym}} \) in the range from 100–200 nm in gray scale and the orientation of protein fibers \( \theta \) that follow the color wheel. We compare, longitudinal samples (xy-plane) for increasing concentration, positions along the transversal (yz-plane) die position and the anisotropy of 40 wt% SPC sample (longitudinal and transversal samples).

Concentration: For all longitudinal (xy-plane) sampling positions (L1, L2 or L3), the flow direction (z-axis) is oriented out of the plane. Hereby, following the color wheel, regions with horizontal alignment are
highlighted in red, whereas regions with vertical orientation are highlighted in cyan (Fig. 4A). It is clear that the orientation domains become more uniform, observed by the sharper transitions between colors with increasing protein content. The dashed black line between the blue and cyan regions, called the “transition region”, identifies where the orientation of the fibers is fully vertical, which should coincide with the tip of the plug flow. For samples with SPC content higher than 35 wt%, these transitions are clear and large domains with similar orientations are measured. We do not observe the same for the 30 wt% SPC sample, in which the orientation oscillates around the vertical directions, indicated by the cyan-blue-green colors. Only samples with 40 wt% SPC content were measured longitudinally (xy-plane, Fig. 4A) and transversely (yz-plane, Fig. 4B), which resulted in similar structures. Among these, the structure of sample L1 seems to be affected by its proximity to the outer edge of the die wall, and a large transition region is observed as highlighted by the relatively large cyan, green and blue fields that represent vertical orientations. Thus, we conclude that the protein content stabilizes the anisotropic orientation of the extrudate, since higher viscosities are reached upon cooling and consequently, higher stretching rates and structuring of the protein melt in the cooling die.

**Die position:** At the edge of the cooling die, fibers and aggregates with dimensions in the range of 100–200 nm are oriented with the flow lines, but not along the flow direction. We included white dashed lines in Fig. 4A, which follow the orientation obtained from sSAXS and can be interpreted as the orientation of the fibers in an parabolic flow profile, considering the front view of the extrudate (xy-plane). Note that we are also sensitive to the double orientation effect of extrusion to these fibers, as the transversal samples (Fig. 4B) are highly aligned along the yz-plane, as well as sample L3 (centre of the extrudate, xy-plane). The higher the protein concentration in the melt, the smaller is the radius of the parabolic shear profile, measured by the orientation of the extrudate. We suggest that the higher viscosity of the blend, due to the increase in protein content, could create higher stresses in the cooling die, thus flattening more the flow profile.

**Anisotropy Index:** sSAXS maps also provide the degree of orientation of the material per pixel. This asymmetric intensity \( I_\text{anym} \) is obtained from the fitting of the cosine amplitude on the scattering intensity as a function of the azimuthal angle (Bunk et al., 2009). The maps of the asymmetric intensity are shown in Figure S3, and follow the trends shown by the transition regions discussed previously. By averaging all \( I_\text{anym} \) values for each one of these maps, we obtain sample orientations that increase with concentration. We calculate the anisotropy index \( AI \) for the 40 wt% SPC sample by dividing the averaged longitudinal \( I_\text{anym} \) at three positions (L1, L2, and L3) by the averaged transversal \( I_\text{anym} \) from samples (T1, T2, and T3). Values of \( AI \sim 1 \) indicate that the samples are isotropic (same intensity on both directions), while \( AI > 1 \) indicates some degree of anisotropy, as the scattering signal is higher in one direction. All selected samples show anisotropy, with a mean \( AI \) around 1.65 (inset in Figure S4). The highest structural anisotropy is observed for the position L2. The lowest \( AI \) was calculated for L3, which confirms its proximity to the flow tip, and that the similarity between the transversal and longitudinal samples is indeed the highest for this central position. This conclusion indicates that the flow profile developed has indeed a rather flat parabolic shape, and that for regions under similar temperature and shear, the structure formed is similar.

### 3.1.4. sWAXS maps:

The WAXS signal is sensitive to length scales below the nanometer range, towards the secondary structure of the proteins, such as conformation and folding. We measured sWAXS maps of the same samples (Figure S5). After subtracting the background scattering coming from the microscope cover slips used to fix the extrudate slices for the measurements (Figure S1), we observe three main peaks in the WAXS signals, coming from the structure of the SPC (Figures S6 and S7). These peaks correspond to dimensions of \( d = 0.21 \) nm of the alpha-helix radius of gyration, \( d = 0.35 \) nm of the intra-beta-sheet distance, and \( d = 1 \) nm as the inter-beta-sheet distance (Zagrovic et al., 2005). We mapped the WAXS signals over all sample concentrations and sampling directions. The WAXS signals do not vary and remain very similar for all conditions in the observed \( q \)-range (1–50 nm\(^{-1}\)). We conclude that there are no clear structural changes in the protein structure upon processing, and that the protein conformation is independent on concentration, shear or temperature profile, as well as of the position relative to the flow direction. This can only be affirmed at the dimensions between 0.16–6.28 nm that correspond to the observed \( q \)-range. Considering that the raw SPC is already denatured, we confirm that no changes in the protein structure, conformation or degradation are caused by the extrusion process. In the sWAXS maps, we observe some regions with higher
scattering intensity (Figures S5 and S6). These regions have higher density, and are assigned to the crystallization of residual salts from the protein raw material, since they also show sharp Bragg peaks characteristic of crystals superposed with the protein signal.

3.1.5. Proposed flow fields:

Scanning SAXS and WAXS provide singular information for the mapping of flow fields and the impact on structure. Based on the SAXS maps, we propose the schematic described in Fig. 5 for the flow field in the cooling die, which is reflected by the protein alignment in the extrudate. When the extrudate is seen from a side view (mid row), the proteins are most probably aligned with a flatter V-shaped flow profile for the 30 wt% SPC sample (left) than for the 40 wt% SPC sample (right). In the transversal positions, the transition region occurs in the symmetry center of the cooling die and is independent on the \( z \)-position as confirmed by measurements of samples T1, T2, and T3 (Fig. 4B). Seeing the extrudate from the top (top row), we expect a sharper plug flow profile to be formed for the melt with higher protein concentration (40 wt% SPC, right). Here, the increase in viscosity at the cooling die wall is higher for more concentrated melts. If the flow rate of the extrusion is maintained constant, melts with higher viscosity will be more stretched and originate a more fibrous structure that resemble better the texture of meat. Seeing from the front (bottom row), a core layered flow is formed, while the melt solidifies in layers starting from the die wall and going towards the core of the extrudate. When an outer layer solidifies, the remaining liquid-like inner fraction slides along the viscous melt-solid interface and stretches the protein to form fiber-like structures that solidify. This onion-like layered structure is confirmed by sSAXS for samples L1, L2, and L3. The increase of flow profile curvature with increasing concentration (white dashed line in Fig. 4B) and with the closeness to the center of the cooling die is consistent for all measured samples. Furthermore, the 40 wt% SPC sample has similar structure for the L3 (center) and T positions, but differ for L1 (close to the die wall), which reinforces that a plug-flow profile is formed, and that the extrudate structure depends on the position within the cooling die. These clear changes in the structure at the nanometric length scale can only be observed by SAXS, however no changes in the structure was observed at the molecular level, i.e., WAXS region. These results indicate that the processing of such protein blends can influence the texturization process and the final product, however without changing the protein structure at the molecular level, i.e., no change in conformation, denaturation, or disaggregation.

3.2. Mechanical anisotropy

To link the characterized structure to the final texture of meat analogues, we assessed the mechanical anisotropy of SPC extrudates longitudinally (\( yz \)-plane) and transversally (\( xy \)-plane) to the flow direction (Fig. 6A), collecting new samples at the similar sampling positions as for the scattering experiments. The Young’s moduli of both the transversal \( E_T \) and longitudinal \( E_L \) sample sets increased with increasing protein concentrations (Fig. 6B). While the protein content increases, the water fraction that acts as a plasticizer decreases, and thus the \( E_L \) increases. The slope of the increase in \( E_L \) is higher than the one of \( E_T \), indicating that the extrudate is stiffer perpendicularly to the flow direction. We calculate the tensile anisotropy index \( AI_{Tensile} \), whose values lie around 1 for isotropic materials and above 1 for anisotropic
The 30 wt% SPC sample behaved nearly isotropically with \(A_{\text{Tensile}} \approx 1\), while \(A_{\text{Tensile}}\) increased to nearly 1.5 for the 40 wt% SPC sample (Krintiras et al., 2015; Jia et al., 2021; Nishinari et al., 2014; Kiiru et al., 2020). A higher anisotropy comes from the more heterogeneous structure formed for higher protein contents as confirmed by SEM and sSAXS.

Dynamic mechanical analysis (DMA) has been extensively used to relate the mechanical properties of polymers to their underlying molecular composition (Menard and Menard, 2020). Similarly to tensile tests, DMA yields information on directional mechanical properties (Jia et al., 2016). DMA setups are ideal for oscillatory measurements, in which a dual-cantilever setup measures bar-shaped samples (Fig. 6C) with medium stiffness, without applying static forces that can negatively impact the accuracy (Duncan, 2008). As for a rotational oscillatory rheological measurement, we can change the frequency and amplitude of the stress applied to a sample. However, in contrast to rotational measurements, the DMA setup has the benefit to enable the measurement of the directional mechanical properties of the sample, which is the main goal of this work. We measured the storage \(G'\) and loss \(G''\) moduli of samples taken in the transversal (yz-plane) and longitudinal (xz-plane) direction of the extrudate flow direction (z-axis) in the previously determined linear viscoelastic regime at an amplitude of 0.1 and a frequency of 1 Hz (Figure S8). As for the sSAXS measurements, the longitudinal bar-shaped samples of 10 mm in length were taken at three distinct positions namely, \(x = 0, 12.5, \text{and} 25\) mm, denoted \(L_1^c, L_2^c, \text{and} L_3^c\) (Fig. 6A). The anisotropy indices of \(G'\) and \(G''\) obtained by DMA measurements \(A_{\text{DMA}}\) follow the same trend observed with the tensile measurements. Higher protein contents resulted in more anisotropic networks, and in higher \(A_{\text{DMA}}\) values for both \(G'\) and \(G''\). Conversely, the 30 wt% SPC sample had \(A_{\text{DMA}}\) around unity, reflecting the coarser unstructured networks of those samples. Furthermore, mechanical differences between the longitudinal (xy-plane) samples taken at different distances from the cooling die channel center (\(x = 0, y = 0\)) are measured. The sample \(L_3^c\) (center of the cooling die) exhibits higher textural anisotropy than \(L_2^c\) and \(L_1^c\). Here, parallels between the mechanical properties measured via DMA and the structural characterization obtained by sSAXS can be clearly drawn. As discussed, larger domains of orientation were measured by sSAXS for high protein contents, and for samples at the center of the die.

4. Conclusions

This study sheds light on the interdependence between the structural properties of texturized meat analogues and their mechanical anisotropic behavior. Through the use of scanning X-ray scattering maps and mechanical stress measurements, it was found that the mechanical anisotropy arises from differences in the nanometric structure, which are controlled by protein concentration, viscosity, temperature gradient, and stresses applied during the passage in the cooling die. This finding has implications for the food industry, as it suggests that higher protein content stabilizes the anisotropic orientation of the extrudate, leading to improved structuring of the protein melt, and to products with higher quality that resemble animal meat. Furthermore, we confirmed that the raw soy protein concentrate is already denatured and that the extrusion process does not cause changes in protein structure, conformation, or degradation. Overall, this work provides valuable insights into the possibility of adjusting and tuning the texture and chewing properties of texturized meat analogues by changing the formulation and the processing conditions, which are important considerations for the development of sustainable food products.

Data availability

The data sets generated and/or analysed during the current study are available from the corresponding author on request. Source data are provided with this paper.

Code availability

The codes used for the analysis were developed by the Coherent X-ray Scattering group at the Paul Scherrer Institute in Villigen, Switzerland and can be found on the cSAXS web page at https://www.psi.ch/sls/csaxs/software.
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.

Acknowledgement

We acknowledge the Paul Scherrer Institut (Villigen, Switzerland) for provision of synchrotron radiation beamtime at the cSAXS beamline of the Swiss Light Source under proposal 20210413. We thank the beamline staff for technical support during the experiment.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodres.2024.113968.

References


Appendix A. Supplementary material

beamline staff for technical support during the experiment.

References