



Hybrid extrusion printing as a novel strategy to engineer structurally complex extrusion-based meat analogues with enhanced liquid release

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ABSTRACT

Meat analogues continue to gain relevance, yet the replication of whole cut meat products still presents substantial challenges, particularly regarding texture and juiciness.

In this study, we introduce Hybrid Extrusion Printing (HEP), a novel co-extrusion-based approach that employs a specially designed bi-stream breaker plate system for high moisture meat analogue (HMMA) production. The system divides the protein-mass from the extruder's screw section into individual streams, while simultaneously distributing a secondary binder-stream between of them. Feasibility was assessed on the model system soy protein isolate for the protein-mass and gelatin for the binder-stream, examining different process parameters such as temperature and binder-stream flow rate. Optical and mechanical methods are used to evaluate the resulting product properties. Furthermore, rheological properties, including viscosity and relaxation time, were elucidated under extrusion-relevant conditions for a better process understanding with a focus on flow stability.

The findings demonstrate the strong potential of using HEP for production of complex structured protein-based food systems. While an increased protein-mass temperature led to a stronger healing of adjacent macroscopic strands and thus to a fourfold decrease in anisotropy, the binder-stream decoupled the effect of protein-mass temperature from inter-strand healing. Moreover, the addition of the binder-stream resulted in a significant increase in compressive liquid release, independent of protein-mass temperature, with values up to 13 wt% liquid release.

1. Introduction

The environmental and ethical concerns associated with industrial meat production have boosted research and development of meat analogue products. Despite growing consumer interest, acceptance remains limited due to sensory and structural challenges, particularly in texture and juiciness (Sun et al., 2021; Vallikkadan et al., 2023; Yan et al., 2024).

A key characteristic of conventional meat is its complex structure. Skeletal muscle spans multiple length scales, from nanometer-sized myofibrils to macroscopic muscle bundles. Groups of myofibrils (1–2 µm in diameter) form muscle fibers, cylindrical cells typically 10–100 µm in diameter (Slayven & Matthews, 2024). Multiple muscle fibers are organized into different levels of muscle fiber bundles (Albrecht et al., 2006). According to literature, typical diameters can range from 0.5 mm to a few up to 30 mm in diameter (Hena et al., 2017; Wilhelm et al., 2025). However, it has to be noted that size of muscle fibers and muscle

fiber bundles depends on many factors such as breed and age of the animal (Albrecht et al., 2006). Importantly, as shown by (Lillford, 2011), this level of organization, rather than the individual fibers, largely determines the fibrous texture perceived by consumers. Muscle fibers and fiber bundles are encased by collagen-rich connective tissue layers, known as endomysium and perimysium, respectively, with the perimysium contributing over 90% of the total collagen content. (McCormick, 1994).

Regarding consumer perception, higher collagen content as well as a higher amount of intercollagen cross-links can lead to a lower tenderness by sensorial investigation (Chriki et al., 2013; Xie & Grossmann, 2025). Under thermal treatment, degradation of native collagen occurs, with an increasing conversion to gelatin already starting around 50 °C (Duconseille et al., 2015; Wang, Tian, et al., 2022). As a thermoreversible gel, gelatin is liquid at higher temperature and sets upon cooling via formation of gelatin triple helices (Tang et al., 2022). Intramuscular fat (IMF) affects meat quality and plays an important role

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in regulating tenderness, juiciness, and flavor, which are key sensory attributes typically evaluated through sensory analysis (Corbin et al., 2015; O'Quinn et al., 2012; Zhang et al., 2022). Primarily located in the perimysium, IMF occurs as adipocytes (Lawrence et al., 2012). Factors such as species, sex, age, and growth stage significantly affect collagen crosslinking, IMF distribution, and muscle fiber size, thereby influencing meat properties. (McCormick, 1994; Slayven & Matthews, 2024).

When meat analogues are to be produced that mimic the textural characteristics of muscle meat as closely as possible, it is essential to consider the multiscale organization of muscle, including protein- and gelatin-rich phases. A variety of processes have been developed to replicate meat products by structuring protein-rich plant-based raw materials. Among these, high moisture twin-screw extrusion is the most widely used industrial method for producing intermediate meat analogue products. In this process, plant-based blends are fed into the extruder, where they are mixed with water and plasticized under controlled heat and shear in the screw section. The resulting mass is then conveyed through an adapter into the cooling die attached to the screw section, where the protein-mass solidifies, forming a characteristic parabolic microstructure. (van der Sman & van der Goot, 2023).

Structure formation can be influenced by formulation (Q. Chen et al., 2022; Dahal et al., 2025; Ellwanger et al., 2024) and process conditions (Ellwanger, Pernice, et al., 2023; Kaunisto et al., 2024), as demonstrated in numerous studies. High moisture twin-screw extrusion offers great potential for replicating meat structures at the level of individual muscle fibers, since the resulting structures operate on comparable length scales. However, conventional high moisture extrusion technology remains limited in its ability to generate larger-scale structures to imitate muscle fiber bundles (Wilhelm et al., 2025). Additionally, difficulties remain in enhancing juiciness and aroma in plant-based analogs (Godschalk-Broers et al., 2022; Oppen et al., 2024; Xu & Falsafi, 2023). Previous attempts to enhance the juiciness in high moisture meat analogues (HMMA) relied on the addition of oil in the screw section (Akdogan, 1999; Kendler et al., 2021; Opaluwa et al., 2023, 2024, 2025; Saavedra Isusi et al., 2023; Wang, Zhang, et al., 2022). However, this can negatively impact the stability of the extrusion process and affect the fibrous structure and mechanical properties of the resulting HMMA (Akdogan, 1999).

Among alternative structuring approaches, 3D printing has emerged as a promising tool due to its high precision in macroscopic structuring (Chakravorty & Das, 2024; Cheng et al., 2024; Lombardi et al., 2025). The use of tailored inks enables the targeted design of product properties such as juiciness (Demircan et al., 2023; Domžalska & Jakubczyk, 2025). In addition, alternating protein-rich and fat-rich phases allows the imitation of marbling structures (Park et al., 2023). However, the scalability of layer-by-layer deposition remains a major bottleneck, burdening industrial meat analogue production. Extrusion-based structuring, by contrast, offers high scalability but is traditionally limited in its ability to produce macroscopic fibrous structure and resembling the dimensions of muscle fiber bundles. (Dekkers et al., 2018) Recent studies have explored the potential for controlled macroscopic structuring by incorporating breaker plates at the outflow of the screw section during extrusion. Zheng et al. demonstrated that this modified die design led to the formation of macroscopic, parallel-aligned protein strands and enhanced the degree of texturization (Zheng et al., 2024). In our previous study, we demonstrated that geometrically varied breaker plates enable a wide spectrum of macroscopic structures. We further found that extrusion with a breaker plate can function as a multi-scale structuring process, in which the characteristic parabolic microstructure is superimposed by parallel macroscopic strands (Pernice et al., 2025). While both studies effectively highlight the potential of using breaker plates in extrusion to facilitate macrostructure formation, strategies for enhancing juiciness in an inline process remain limited.

This study introduces Hybrid Extrusion Printing (HEP) technology, employing a novel co-extrusion-based bi-stream breaker plate system as

an approach for the continuous production of multi-component meat analogues. In this configuration, the protein-mass is divided into individual streams, while a binder-stream is simultaneously distributed through dedicated channels between the protein streams. This approach simultaneously addresses the scalability limitations of 3D printing and the common shortcomings of meat analogues in terms of juiciness and flavor.

This is the first study investigating the application of a bi-stream breaker plate in high moisture extrusion to generate complex structures in concentrated protein systems while enhancing compressive liquid release. Gelatin was selected as a readily processable thermo-reversible gelling agent for the binder-stream. A central focus of this work is the influence of the binder-stream on the resulting texture. Structural characterization was conducted using imaging-based methods, including cryogenic imaging, tearing images, and confocal laser scanning microscopy (CLSM) for selected samples. These qualitative observations were complemented by quantitative evaluation of mechanical anisotropy and compressive liquid release.

We hypothesize that the incorporation of a gelatin-based binder-stream downstream of the bi-stream breaker plate suppresses the recombination of adjacent protein-mass streams, thereby promoting the formation of a more pronounced anisotropic structure. With increasing binder-stream proportion, this separation effect is expected to intensify, resulting in progressively higher mechanical anisotropy. Additionally, we propose that the integration of a gelatin-based binder-stream via the bi-stream breaker plate increases compressive liquid release compared to a control without binder incorporation, with the magnitude of liquid release rising as the binder proportion increases. Such behavior would demonstrate the potential of HEP technology to enhance juiciness in meat analogue products without compromising structural anisotropy.

1.1. Rheological background

Due to the co-extrusive nature of the proposed processing method, three main factors can influence flow stability: the flow rates of the individual streams, the viscosity ratio between streams, and the elasticity of each stream.

First, the flow rates of the participating fluids play a critical role in maintaining stable co-extrusion (Tzoganakis & Perdikoulis, 2000). Second, the viscosity ratio can significantly affect stability, as large contrasts in viscosity tend to destabilize the flow (Borzacchiello et al., 2014; Vynckier et al., 2014). Third, elasticity and relaxation behavior are major factors governing flow stability (K. Chen, 1991; Hinch et al., 1992; Martyn et al., 2009), influencing instabilities across a range of disturbance wavelengths (Su & Khomami, 1992), and can either stabilize or destabilize the flow depending on other fluid and process parameters (Hazra et al., 2024).

To gain insights into the rheological behavior of these fluids, rotational measurements of shear viscosity $\eta(\dot{\gamma})$, where $\dot{\gamma}$ is the applied shear rate, can be complemented by oscillatory measurements. The complex viscosity $|\eta^*(\omega)|$ from the storage modulus G' and the loss modulus G'' using Eq. (1), with ω being the angular frequency (Mezger, 2020).

$$|\eta^*(\omega)| = \sqrt{G'^2 + G''^2} \quad (1)$$

For certain materials, the complex viscosity can be converted into the shear viscosity with Eq. (2) according to Cox and Merz (Cox & Merz, 1958).

$$\eta(\dot{\gamma}) = |\eta^*(\omega)| \mid \dot{\gamma} = \omega \quad (2)$$

The literature provides strong indications that Eq. (2) is applicable not only to classical polymers (Ellwanger, Georgantopoulos, et al., 2023), but also to concentrated protein systems under conditions relevant to high-moisture extrusion (Van Der Sman et al., 2023). The wall shear rate $\dot{\gamma}_{\text{wall}}$, representing the maximum shear rate in laminar flow channels, can be estimated using Eq. (3). Here, v is the mean flow

velocity and D is the inner channel diameter (Macosko, 1994).

$$\dot{\gamma}_{wall} = \frac{8v}{D} \quad (3)$$

The characteristic relaxation time of a material τ_{relax} can be calculated according to Eq. (4) as the reciprocal of the angular frequency at which G' equals G'' (Mezger, 2021).

$$\tau_{relax} = \frac{1}{\omega} \mid G' = G'' \quad (4)$$

Temperature dependence of the relaxation time can be described using the Williams-Landel-Ferry (WLF) equation (Eq. (5)), fitting experimental relaxation time data (Mavridis & Shroff, 1992; Mezger, 2020). c_1 and c_2 are empirical determined parameters and T_{ref} is the reference temperature where the relaxation time $\tau_{relax}(T_{ref})$ is known.

$$\frac{\tau_{relax}(T)}{\tau_{relax}(T_{ref})} = \exp\left(\frac{-c_1(T - T_{ref})}{c_2 + (T - T_{ref})}\right) \quad (5)$$

Finally, the dimensionless Deborah number (De) can be used to quantify the relaxation state of viscoelastic masses, defined according to Eq. (6) as the ratio of the material's relaxation time τ_{relax} to the characteristic time scale of the applied deformation τ_{res} .

$$De = \frac{\tau_{relax}}{\tau_{res}} \quad (6)$$

For $De \gg 1$ a solid-like state of the material is expected, whereas for $De < 1$, the material behaves as a liquid.

2. Materials

Soy protein isolate (SPI) (90% protein content, dry basis) with a moisture content of 3–6% was supplied by Solae LLC (St. Louis, MO, USA). Tap water was used for all experiments. Gelatin (240 Bloom) was used as a gelling agent and purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). FSC 22 Frozen Section Medium (Leica Biosystems GmbH, Nussloch, Germany) was used as sectioning medium. For sample fixation, polyvinyl alcohol (Mowiol, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) was used.

3. Methods

3.1. Bi-stream breaker plate

The bi-stream breaker plate was constructed from stainless steel in the institute's own workshop. It consists of a plate carrier, two Swagelok connectors, a sealing ring, and plates 1 and 2, as shown in Fig. 1a. The Swagelok connectors serve as inlets into the plate carrier, each leading

to a through-hole that spans the thickness of the carrier for the binder phase. Plate 1 is inserted into the cut-out of the plate carrier, while the sealing ring ensures a tight joint between plate 2 and the plate carrier, on which the sealing ring is mounted.

Plate 1 contains holes with a diameter of 1 mm, designed to divide the protein-mass phase into individual streams. Plate 2 incorporates all of these protein-mass holes and additionally features channels originating at the exit of the two through-holes in the plate carrier connected to the Swagelok inlets. These channels run between the protein-mass holes, guiding the binder-stream to its intended locations. Smaller binder-stream holes of 0.5 mm diameter, aligned with the channels, allow the binder phase to exit plate 2 downstream in the extrusion direction, as illustrated in Fig. 1b. Both plates, 1 and 2, have a thickness of 2 mm.

3.2. Extrusion trials

A co-rotating twin-screw extruder (Process 11, Thermo Fisher Scientific GmbH, Karlsruhe, Germany) with a length-to-diameter (L/D) ratio of 40 was used for the extrusion experiments. The screw section is divided into eight barrels, with independent temperature control in the last seven barrels. The configuration of the intermeshing screws was adopted from Pernice et al. (Pernice et al., 2025).

While SPI was fed gravimetrically into the first barrel using a feeder (Brabender Technologie GmbH & Co. KG, Duisburg, Germany), water was introduced into the third barrel via a peristaltic pump (Masterflex L/S, Cole-Parmer, Vernon Hills, IL, USA). A heatable adapter reduced the flow cross-section from that of the screw section to that of the plate carrier. Protein-mass temperature and pressure were measured within the adapter. The bi-stream breaker plate was positioned directly downstream of the adapter. The binder-stream was dosed using a High-Performance Liquid Chromatography (HPLC) pump (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany) following volumetric calibration. Downstream of the breaker plate, an 8 mm polytetrafluoroethylene insulation layer was installed upstream of the cooling die to minimize heat loss within the bi-stream breaker plate. The 125 mm cooling die was temperature-controlled using a water-cooled process circulator (Presto Plus LH 47, Julabo GmbH, Seelbach, Germany). The downstream assembly of the screw section is illustrated in Fig. 2. Further geometric details of the adapter and cooling die are provided in (Pernice et al., 2025).

The screw speed was set to 600 rpm, and the protein-mass flow rate was maintained at 0.76 kg/h, keeping the water content constant at 61 wt% (neglecting the moisture of the protein powder). The binder-stream flow rates were set to 0, 2, and 4 ml/min, corresponding to 0 wt%, 14 wt%, and 24 wt% binder-stream of the total feed rate, respectively. The protein-mass temperature was adjusted from 90 to 140 °C in 10 K

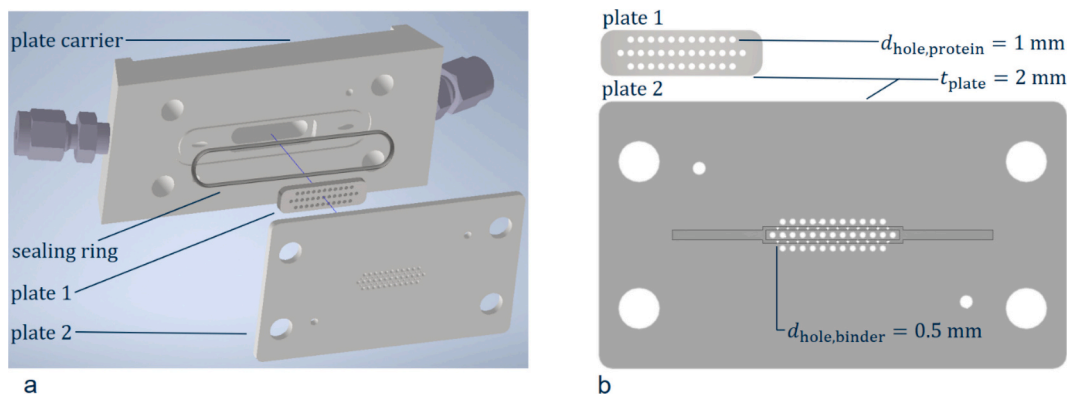


Fig. 1. a and b: Visualization of the bi-stream breaker plate, showing (a) an exploded view of the connectors, plate carrier, plate 1, plate 2, and sealing ring, and (b) a schematic representation of plates 1 and 2 with the respective hole diameters indicated as $d_{hole,protein}$ for the protein-mass stream and $d_{hole,binder}$ for the binder-stream; the plate thickness in extrusion direction is denoted as t_{plate} .

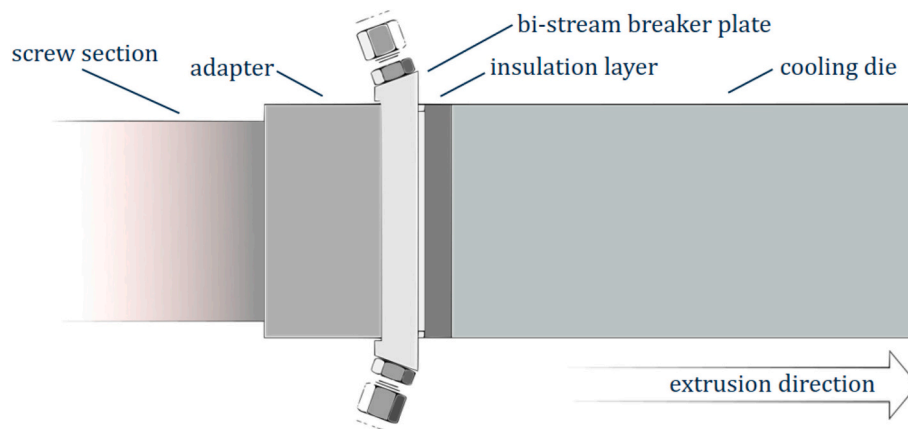


Fig. 2. Schematic representation of the extrusion setup, including the screw section, adapter, bi-stream breaker plate, insulation layer, and cooling die, with the extrusion direction indicated from left to right.

increments. Temperature control was achieved by adjusting the set temperatures of the last three barrels and the adapter, while the first four barrels of the screw section were kept at 30, 50, 70, and 90 °C, respectively. The temperature of the cooling die was maintained at 40 °C for all extrusion trials.

Samples were collected 5 min after reaching a steady state, which was defined as temperature fluctuations below 1 K and pressure variations within 2 bar at the extruder interface. Samples were then vacuum-sealed and frozen for subsequent analysis.

3.3. Binder-stream preparation

For the preparation of the binder-stream, gelatin powder was dissolved in water at 50 °C under continuous stirring for 10 min using a temperature-controlled heating plate with an integrated magnetic stirrer. The gelatin-to-water ratio was maintained at 0.1 on a weight basis. After the initial dissolution step, the mixture was incubated in a water bath at the same temperature for 1 h to ensure complete homogeneity. For readability, the water–gelatin solution is hereafter referred to as the gelatin solution, and the corresponding water–gelatin gel as the gelatin gel.

3.4. Rheological measurements

Rheological measurements were performed to characterize the relaxation behavior and complex viscosity of the protein-mass under extrusion-relevant conditions using a closed-cavity rheometer (CCR) (RPA Elite, TA Instruments Inc., New Castle, DE, USA). Temperature-dependent relaxation times were determined from individual frequency sweeps conducted at each temperature between 140 and 160 °C in 5 K increments. Each data point was obtained as the inverse of the angular frequency at the crossover point of the storage and loss moduli (G' and G''), approximated via linear regression. Each frequency sweep was constructed from a series of time sweeps performed at five discrete frequencies ranging from 0.03 to 3.16 Hz, with each measurement carried out in triplicate. To fit the data, a slightly modified base-10 logarithmic form of the WLF equation was employed instead of the exponential form described by Mezger (Mezger, 2020). Oscillatory frequency sweeps from 50 to 0.5 Hz were also conducted to gain insight into the shear-dependent viscosity. Using the Cox-Merz relation, complex viscosity was converted into shear viscosity (Cox & Merz, 1958).

3.5. Mechanical analysis

In all methods used for mechanical analysis, a Haake Mars 60 rheometer (Thermo Fisher Scientific GmbH, Karlsruhe, Germany) was

configured with different geometries under varying experimental conditions, as detailed in the following subsections.

3.5.1. Tensile analysis

For tensile testing, extrudates were stamped to create defined rupture locations. The rheometer was equipped with horizontally adjustable gripping clamps, allowing secure fixation of the samples without inducing premature fracture. Tensile tests were conducted in triplicate using an axial ramp until sample rupture at a speed of 2 mm/s. The rupture tension σ_{rupture} , was calculated from the rupture force F_{rupture} , using Eq. (7).

$$\sigma_{\text{rupture}} = \frac{F_{\text{rupture}}}{A} \quad (7)$$

Anisotropy (AI) was determined as the ratio of the mean rupture tensions measured orthogonal (transversal tension) and parallel (longitudinal tension) to the extrusion direction according to Eq. (8).

$$AI = \frac{\sigma_{\text{rupture,trans}}}{\sigma_{\text{rupture,long}}} \quad (8)$$

3.5.2. Test for compressive liquid release

As a qualitative indicator of perceived juiciness, compressive liquid release was determined by weighing the expressible liquid obtained during compression. Prior to testing, samples were stamped into cylindrical shapes with a diameter of 15 mm and a height of 4 mm and heated to 80 °C in a water vapor phase to prevent drying. Samples were then compressed at a speed of 0.25 mm/s to 75% of their initial height between two filtration papers, with the lower compression plate equally heated to 80 °C. Sample weights were recorded before and after compression. A plastic dome over the measurement setup was used to minimize drying during the experiment. Post-measurement, the liquid release was calculated as the relative difference in sample weight before and after compression. All trials were conducted in triplicate.

3.6. Optical analysis

Tearing analysis and cryo-imaging were used as optical methods.

3.6.1. Tearing analysis

Prior to tearing, samples were thawed at room temperature for 4 h. They were then torn open along the extrusion direction to reveal the internal structure, and top-view images were captured. This method has previously been applied by Pernice et al. (Pernice et al., 2025).

3.6.2. Cryo-imaging

To evaluate the microstructure of the samples, cryo-imaging was

performed, following the procedures described by Wittek et al. (Wittek et al., 2021) and Pernice et al. (Pernice et al., 2025). Frozen samples were mounted on a sample holder using a sectioning medium. Thin slices were cut from the samples using a cryo-microtome (Leica Biosystems GmbH, Nussloch, Germany). The sample holder was cooled to -20°C , while the surrounding chamber was maintained at -24°C . Once the internal structure of the macroscopic strands was revealed as a smooth surface, top-view images were captured.

3.7. Statistical analysis

In this study, extrusion experiments were carried out as single trials. A one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) was performed using OriginPro 2023 (OriginLab Inc., Northampton, USA) to evaluate differences in tensile strength resulting from variations in protein-mass temperature. The same statistical approach was applied to assess the significance of differences in liquid release among the control, 2 ml/min, and 4 ml/min samples.

4. Results and discussion

To evaluate how protein-mass temperature affects structure formation downstream of the bi-stream breaker plate, extrusion trials were conducted at temperatures ranging from 90°C to 140°C in increments of 10°C . Since protein-mass temperature governs molecular mobility, relaxation behavior, and network formation, it directly influences the recombination of adjacent strands after passing the breaker plate. In addition, the binder-stream flow rate was varied from 0 ml/min (control) to 4 ml/min. By separating adjacent protein-mass strands, increasing binder incorporation is expected to progressively suppress strand recombination and thereby enhance structural anisotropy. As a thermo-reversible gel, gelatin is further anticipated to promote liquid release upon compression at 80°C , potentially contributing to increased juiciness.

4.1. Cryo-imaging

Fig. 3 shows top-view images of the extruded samples: the control without binder-stream (top row), and samples with gelatin solution as a binder-stream (middle and bottom rows). The middle row corresponds to a binder-stream flow rate of 2 ml/min, equivalent to a mass flow of 0.12 kg/h , or $14\text{ wt\%}$ of the total mass flow. The bottom row represents a binder-stream flow rate of 4 ml/min, corresponding to a mass flow of

0.24 kg/h , or $24\text{ wt\%}$ of the total mass flow.

For the control samples without a binder-stream, extrusion at 90°C results in the formation of parallel macroscopic strands oriented along the extrusion direction and delineated by thin boundaries. Within these strands, an irregularly marbled microstructure of bright and dark regions is visible. As the protein-mass temperature increases up to 130°C , the dominance of the macroscopic strands gradually diminishes, while the marbling within the strands increasingly adopts a parabolic shape spanning the entire width of the extrudate. At 140°C , the macroscopic strands are no longer discernible, and a pronounced parabolic microstructure dominates. In contrast, samples extruded with a gelatin-based binder-stream exhibit an irregularly marbled microstructure across all protein-mass temperatures. Macroscopic strands remain visible up to 130°C and are characterized by rough surfaces that appear entangled with adjacent strands. At 140°C , the structure becomes highly heterogeneous, indicative of extrusion instabilities.

The formation of macroscopic parallel strands in meat analogues during high moisture extrusion with breaker plates has been recently investigated by Zheng et al. (Zheng et al., 2024) and Pernice et al. (Pernice et al., 2025). This phenomenon arises from the division of the protein-mass into individual streams within the breaker plate, which form distinct strands upon exiting the plate and reconnect only partially during subsequent cooling and solidification. In the control samples, the overall structural morphology progressively transitions from parallel macroscopic strands at lower protein-mass temperatures to a lamellar parabolic structure at elevated temperatures. This parabolic morphology, in contrast, represents the typical outcome in conventional high moisture extrusion of concentrated protein systems. In water-SPI systems, comparable parabolic microstructures have been described by Wittek et al. as consisting of water-rich and water-poor domains within the protein matrix that arise from phase separation (Wittek et al., 2021). Across different protein sources, several mechanisms have been proposed to contribute to such structural formation, including protein unfolding and aggregation as well as phase separation. Moreover, the dynamic fracture and healing of intermolecular links under shear and elongational stresses is thought to play an important role, with the instantaneous network state becoming progressively permanent upon cooling (van der Sman & van der Goot, 2023). Accordingly, the increasing dominance of the parabolic microstructure observed at elevated protein-mass temperatures for the control in Fig. 3 is likely related to enhanced reconnection of adjacent protein strands due to longer residence times at temperatures above a critical gel-healing threshold. In line with this interpretation, Thornell et al. demonstrated

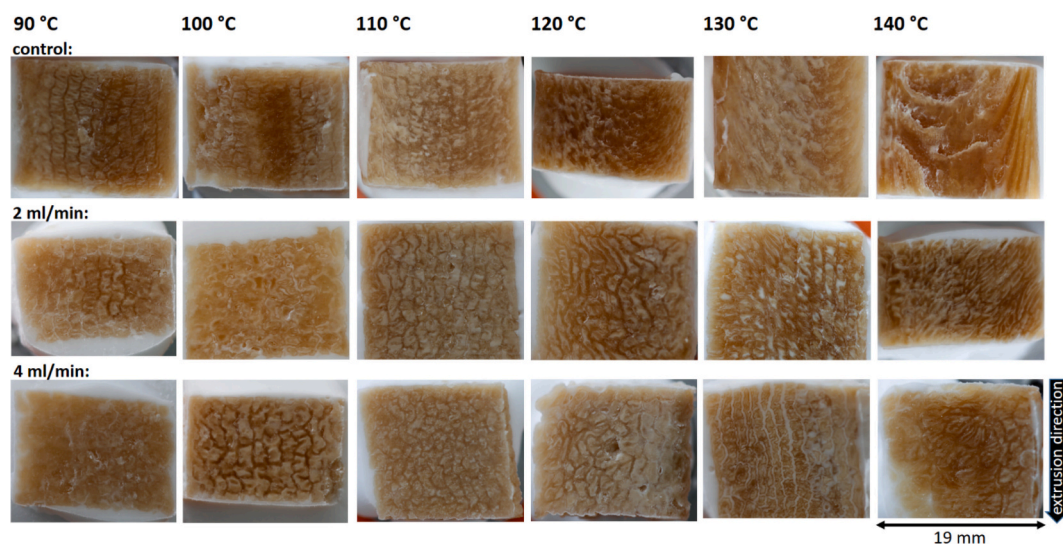


Fig. 3. Cryo-imaging top views of extrudates produced without a binder-stream (top row, control) and with binder-stream flow rates of 2 ml/min (middle row) and 4 ml/min (bottom row). Protein-mass temperature was varied from 90°C to 140°C in increments of 10°C . The extrusion direction is from top to bottom.

that network healing in thermo-reversible physical gels is promoted by higher temperatures and longer residence times (Thornell et al., 2014). For water-protein systems, this concept is further supported by the findings of Zheng et al., who reported a transition from a parabolic structure to distinct parallel strands when the cooling die temperature was reduced, thereby shortening the time available for inter-strand reconnection (Zheng et al., 2024). Similarly, Pernice et al. observed that increasing the protein-mass flow rate promoted the formation of reconnected strands and a dominant parabolic structure, which was attributed to prolonged residence times above the critical gel-healing temperature (Pernice et al., 2025). Nevertheless, it should be noted that gel fracture and network healing are not governed solely by temperature and residence time. Mechanical stresses acting on the protein-mass also play an important role (Ellwanger et al., 2025; Wittek et al., 2020).

The experimental observations obtained in this study are consistent with the hypothesis that the introduction of a gelatin-based binder-stream downstream of the bi-stream breaker plate suppresses the recombination of adjacent protein strands and thereby promotes the formation of a more pronounced anisotropic strand structure.

A plausible explanation for the persistence of macroscopic strands at elevated temperatures when a binder-stream was applied is the continuous distribution of gelatin between adjacent protein-mass streams. This gelatin layer may act as a physical barrier that inhibits interfacial healing and thus prevents the reconnection of neighboring protein strands, largely independent of the protein-mass temperature. In addition, the binder-stream enters the system at a considerably lower temperature (50 °C) than the protein-mass, which likely induces a local cooling effect on the protein strands. This temperature difference may further reduce recombination of adjacent strands. A further observation is that, the parabolic orientation of the microstructure was considerably less pronounced in samples processed with a binder-stream compared to those without binder addition across the entire investigated temperature range. One possible explanation for this observation is a lubricating effect of the gelatin phase between cooling die wall and protein strands, which may reduce shear stresses acting on the protein-mass. This interpretation is supported by the die pressure as a function of protein-mass temperature shown in Fig. A10, where pressures were consistently at least 5 bar lower when a binder-stream was applied. However, the observed structural differences may be related to flow instabilities that disrupt the formation of a coherent parabolic microstructure. Interestingly, such instabilities were not reflected in the pressure signal, where the largest standard deviations were observed for the control experiments and were considerably higher than those obtained with a binder-stream. Due to the pronounced structural inhomogeneity observed at 140 °C, this condition was excluded from further analysis.

4.2. Compressive liquid release

Fig. 4 shows the liquid release (LR) as a function of protein-mass temperature from 90 °C to 130 °C for extrusion trials with binder-stream flow rates of 0, 2, and 4 ml/min.

Across all temperatures, the incorporation of a binder-stream increased LR relative to the control, highlighting the effect of the binder-stream on liquid release. For a flow rate of 2 ml/min, LR ranged from approximately 7 to 9 wt%, whereas for 4 ml/min LR ranged from about 9 to 13 wt%. The control samples without binder-stream exhibited an LR of around 4 wt%. No clear temperature-dependent trend in LR was observed for the control or for the 2 ml/min binder-stream. For the 4 ml/min flow rate, a moderate increase in LR with protein-mass temperature was observed, from approximately 9 wt% at 90 °C to 13 wt% at 130 °C.

These results support our hypothesis that the incorporation of a gelatin-based binder-stream via the bi-stream breaker plate increases compressive liquid release compared to a control without binder-stream, with the magnitude of liquid release rising as the binder-to-protein ratio increases. It is important to note that the applied instrumental

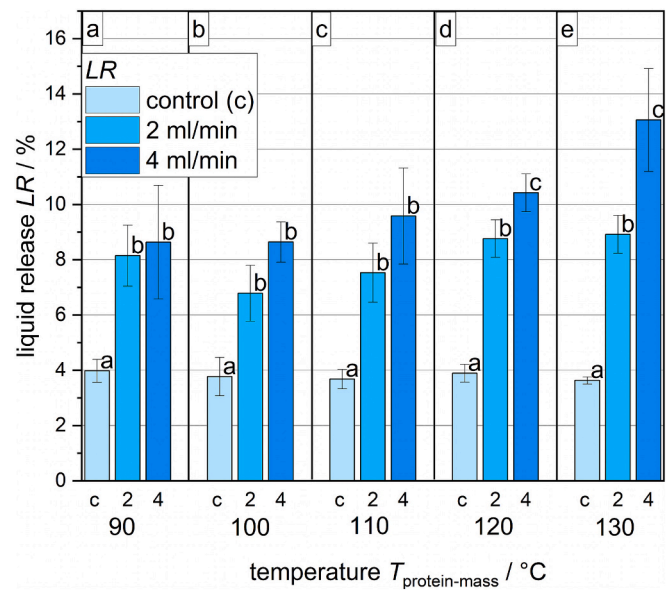


Fig. 4. a-e: Demonstration of liquid release (LR) as a function of protein-mass temperature. Subfigures a-e correspond to temperatures of 90, 100, 110, 120, and 130 °C, respectively. Within each subfigure, data is shown for the control and for binder-stream flow rates of 2 ml/min and 4 ml/min. Small letters indicate statistically homogeneous groups within each subfigure.

measurement method cannot replace or quantitatively reflect the sensory perception of juiciness. However, the observed behavior provides a qualitative indication of the potential of HEP technology to enhance juiciness in whole-cut meat analogues. This is particularly relevant given that insufficient juiciness is a well-known limitation of high-moisture meat analogues and a key factor contributing to their failure to meet consumer expectations (Xu & Falsafi, 2023).

Control samples exhibited liquid release (LR) values of approximately 4 wt% independent of protein-mass temperature, which is significantly lower than the values observed when a binder-stream was incorporated. The positive LR observed in the absence of a binder-stream can primarily be attributed to the expulsion of unbound water under compression. Nevertheless, potential artefacts arising from the sample preparation and measurement protocol cannot be excluded, as specimens were exposed to 80 °C in a saturated water-vapor environment. Additionally, water evaporation during measurement may lead to a slight overestimation of LR when determined gravimetrically.

Incorporation of a binder-stream during extrusion led to an increase in LR of 3 wt% or more, corresponding to up to 75% of the LR measured in the control. The general tendency for slightly higher LR at elevated protein-mass temperatures can be attributed to an altered distribution of gelatin within the samples. The obtained LR values exceeded those reported by Godschalk-Broers et al., who found expressible liquid values of 3.7 wt% or lower, despite applying a compressive deformation of 60%, whereas only 25% deformation was applied in the present study (Godschalk-Broers et al., 2022).

4.3. Tensile testing

To assess mechanical anisotropy, tearing tests were conducted to complement qualitative cryo-imaging. Fig. 5 presents the tensile strength of the extrudates measured parallel (longitudinal tension) and perpendicular (transversal tension) to the extrusion direction. Moreover, the corresponding anisotropy index (AI) is given as the ratio of the tensile strength in both directions according to Eq. (8). The data are shown for protein-mass temperatures ranging from 90 °C to 130 °C. Fig. 5a shows the results for the control samples without a binder-stream, while Fig. 5b and c correspond to samples processed with

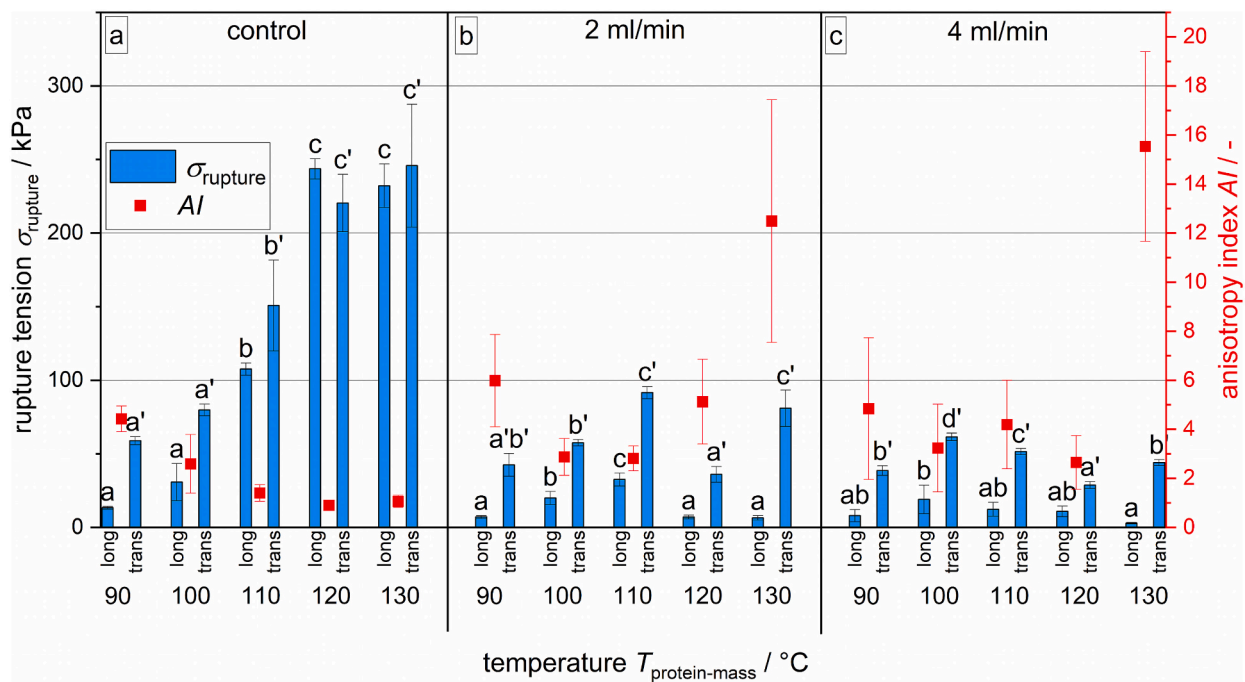


Fig. 5. a–c: Longitudinal and transversal rupture tension (σ_{rupture}) are shown as blue columns, with the resulting anisotropy index (AI) as red points, plotted over protein-mass temperature for the control (5a), 2 ml/min (5b), and 4 ml/min (5c). Within each subfigure, statistically homogeneous groups are indicated by small letters for transversal measurements and by small letters with a prime (') for longitudinal measurements. Letters are assigned separately for each subfigure.

binder-stream flow rates of 2 ml/min and 4 ml/min, respectively.

Across all temperatures, samples extruded with a binder-stream exhibited lower tensile strength in both directions compared to the control. In contrast, AI values of samples extruded with a binder-stream were consistently higher than those of the control samples except at 90 °C. AI values of samples with a binder-stream always exceeded 2.5 with the highest AI observed at 130 °C. For the control samples, increasing protein-mass temperature led to a substantial increase in both longitudinal and transversal tensile strength, with an overall rise of approximately 220% (Fig. 5a). However, AI decreased from 4.4 at 90 °C to around 1 at 120 °C and 130 °C, indicating a loss of mechanical anisotropy at elevated temperatures. For samples processed with a binder-stream (Fig. 5b and c), no clear temperature-dependent trend in tensile strength or AI was observed.

The observed decrease in AI at elevated protein-mass temperatures for the control supports the explanation proposed in connection with the top row of Fig. 3, namely that higher protein-mass temperatures at the exit of plate 2 facilitate the recombination of individual protein strands. Rheological measurements using a capillary rheometer and a closed cavity rheometer suggested that a water-SPI mass with 56 wt% moisture exhibits gel fracture behavior at lower temperatures, starting around 95 °C, whereas sharkskin and spurt phenomena preferentially occur at higher temperatures up to 140 °C (Ellwanger et al., 2025). Such effects likely contribute to the increased transversal tensile strength observed at elevated protein-mass temperatures. Higher temperatures also positively influence the healing kinetics of fractured gel domains (Thornell et al., 2014). This healing mechanism likely applies both to gel fractures occurring within individual flow streams, as described by Ellwanger et al. (Ellwanger et al., 2025), and to the reconnection of interfaces between adjacent protein strands, providing a plausible explanation for the increase in tensile strength with rising protein-mass temperature. In contrast, extrusion with a binder-stream resulted in consistently lower tensile strength than the control. This reduction can be attributed to the presence of the gelatin phase, which hinders network healing between protein strands. Consequently, the recombination of adjacent protein strands is reduced, which explains the lower

transversal tensile strength. Additionally, the gelatin may interfere with gel healing within individual protein strands, further contributing to the observed decrease in longitudinal tensile strength. The ratio of transversal to longitudinal tensile strength directly determines the AI, which was consistently higher for extrusion with the binder-stream than for the control, except at 90 °C.

4.4. Tearing pictures

Fig. 6 presents the visual appearance of the extrudates in a teared-open top view, together with isolated single protein strands, for samples processed without (top row) and with a binder-stream (middle and bottom row) at protein-mass temperatures ranging from 90 °C to 130 °C.

As already observed in the cryo-images (Fig. 3), increasing protein-mass temperature for the control promotes recombination of the protein-mass strands, resulting in a dominant parabolic structure. For samples without a binder-stream, individual strands could no longer be separated from the extrudate structure at temperatures above 100 °C.

In contrast, when a binder-stream of 2 ml/min or 4 ml/min was applied, individual strands could be isolated up to 130 °C. This observation is consistent with the AI results presented in Fig. 5, where values of approximately 3 or higher were maintained for extrudates containing a binder-stream (Fig. 5b and 5c), whereas for the control, an $AI \geq 3$ was observed only at 90 °C and 100 °C. Furthermore, for most samples with binder-stream, a parabolic shape was superimposed onto the macroscopic strands, reflecting the formation of crimped protein strands.

It could be argued that the crimping of protein strands observed in samples extruded with a binder-stream, and the resulting parabolic shape across the extrudate width, arise from wall friction. However, the lubricating effect of the gelatin solution is expected to significantly reduce such wall-friction effects. This interpretation is supported by the die pressure shown in Fig. A10, which was substantially lower when a binder-stream was applied than in the control without binder addition. Another possible explanation for the observed crimping lies in flow instabilities, which are commonly encountered in co-extrusion processes. In addition to flow-rate ratios (Tzoganakis & Perdikoulis, 2000) and

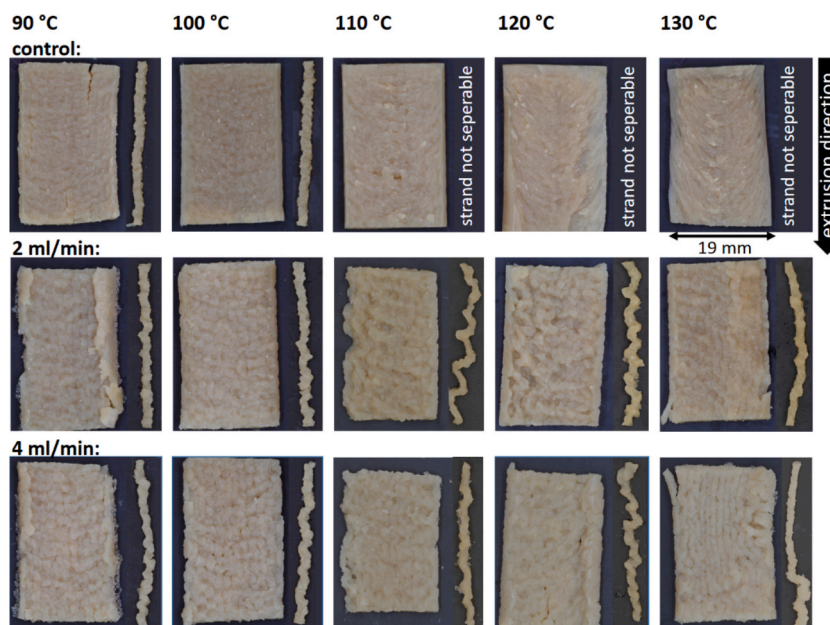


Fig. 6. Top-view images of extrudates subjected to tearing, showing samples processed without binder-stream (top row) and with binder-stream at flow rates of 2 ml/min (middle row) and 4 ml/min (bottom row). Protein-mass temperatures ranged from 90 °C to 130 °C, in 10 K increments, with the extrusion direction from top to bottom. Each panel displays both the teared-open extrudate and an isolated single protein strand in top view.

viscosity ratios (Borzacchiello et al., 2014; Vynckier et al., 2014), elasticity has been identified as a major factor governing flow stability (K. Chen & Joseph, 1992; Hinch et al., 1992; Martyn et al., 2009). While no clear effect of varying flow rate ratios on the crimping of individual strands was observed, the roles of elasticity and viscosity ratios are discussed in more detail in Section 4.5.

4.5. Rheological analysis

To investigate the role of elasticity in the observed flow instabilities, experiments were conducted to determine the relaxation time of the protein-mass and to contextualize this value with the residence time of the extruded protein-mass prior to initial contact with the gelatin binder-stream. The results are presented in Fig. 7. Decreasing the protein-mass temperature led to an exponential increase in relaxation time from approximately 0.1 s at 160 °C to about 4 s at 140 °C. Measurements below 140 °C were not possible because the low frequencies required to observe a cross-over point would have resulted in excessively long measurement times. However, fitting the available data enabled extrapolation to lower temperatures. The WLF equation provided an excellent fit to the experimental data, with a coefficient of determination (R^2) of 0.9996 when using parameters $c_1 = 3.47$ and $c_2 = 22.77$.

Considering a protein-mass flow rate of 0.76 kg/h, the combined residence time within plates 1 and 2 is approximately 0.7 s. With this information, the Deborah number (De) of the protein-mass at 140 °C can be calculated (Eq. (6)) giving a value of around 6 at 140 °C. Extrapolation of the relaxation time data via the WLF equation indicates that at 130 °C, De increases to about 84. Both values are well above 1, indicating a primarily solid behavior of the protein-mass at the mentioned temperatures. Moreover, the De values demonstrate that the protein-mass exhibits progressively slower relaxation with decreasing temperature, suggesting stronger flow instabilities due to enhanced elastic restoring forces at the outflow of the breaker plate.

It should be noted that the measured relaxation times were obtained in the linear viscoelastic regime. Beyond this regime, a nonlinear decrease in relaxation time is expected with increasing shear rate, as described by White and Metzner (White & Metzner, 1963). Assuming a Hagen-Poiseuille flow profile, the wall shear rate is calculated to be 44

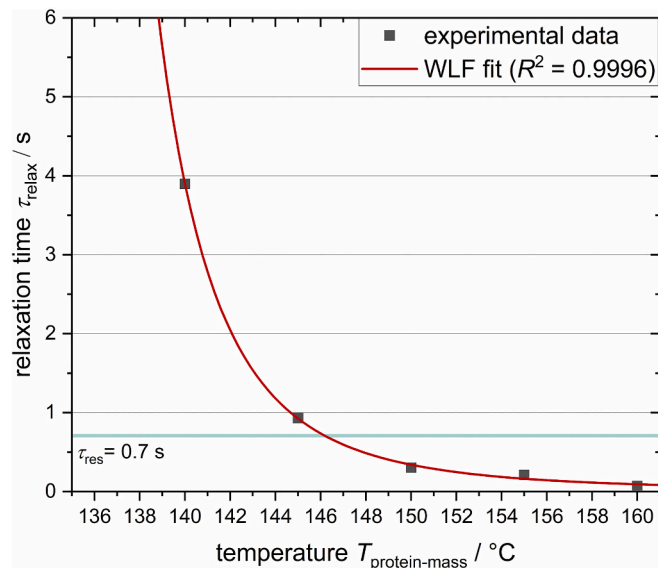


Fig. 7. Relaxation time for varied protein-mass temperature from 140 to 160 °C as black dots. The parameters c_1 and c_2 were adapted to fit the data according to the WLF equation. The resulting fit is plotted as red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

s^{-1} (Eq. (3)), exceeding the linear viscoelastic regime. Consequently, the actual relaxation times at the breaker plate may be overestimated. Partial wall slip, which is likely under these conditions, would reduce the effective maximum shear rate below this value. However, at the same time, partial wall slip would as well affect De as a consequence of a lower effective wall shear rate. For future investigations aimed at a more detailed description of the relaxation behavior, specialized experimental setups using a capillary rheometer will be considered to enable the correlation of rheological behavior with strand morphology in material systems that closely resemble HEP conditions.

For the binder-stream, the gelatin solution at 50 °C behaves

essentially as a Newtonian fluid with negligible relaxation time and is therefore not expected to contribute significant elastic effects to the flow. Detailed rheological measurements supporting this observation are presented in Fig. 8. This figure shows the shear viscosity over shear rate, and the complex viscosity over the angular frequency.

The results demonstrate that the viscosity of the protein-mass at its inlet temperature to the bi-stream breaker plate (90 °C and 140 °C, respectively) greatly exceeds that of the gelatin solution (50 °C), which exhibits constant Newtonian behavior at around 0.02 Pa·s. While the protein-mass viscosities range from approximately 20 to 300 Pa·s at 140 °C, they increase substantially at lower temperatures, reaching up to 20,000 Pa·s at 90 °C, depending on frequency. According to the Cox-Merz rule (Cox & Merz, 1958), an angular frequency of 44 Hz corresponds to a shear rate of 44 s^{-1} . This shear rate represents the Newtonian wall shear rate in the protein-mass channels at the applied mass flow rate. However, although the applicability of the Cox-Merz rule has been shown for concentrated pea protein systems at extrusion relevant conditions (Van Der Sman et al., 2023), no data has been published for soy protein. Due to the pronounced differences in viscosity, with the protein-mass remaining more than three orders of magnitude more viscous than the gelatin solution, potential deviations resulting from this approximation are not expected to alter the qualitative interpretation of the markedly different flow behavior of the two systems.

Based on a minimum viscosity ratio (protein-mass to gelatin) of over 1000, the flow stability is likely influenced by the viscosity ratios present downstream of the bi-stream breaker plate. This supports the observation that the less viscous gelatin solution tends to encapsulate the protein-mass streams. Moreover, this mechanism provides a plausible explanation for the zigzag and wave instabilities observed in Fig. 6.

5. Conclusion

This study presents a novel extrusion-based approach for the complex structuring of protein-rich matrices while simultaneously incorporating a binder component to enhance liquid release upon compression. The method is intended for applications in the field of meat analogues to approximate whole-cut meat products such as goulash pieces, roast slices, medallions, or steaks. By basing the technology on the extrusion process, medium-term integration into existing industrial production lines appears feasible. Thus, the approach may contribute to increasing consumer acceptance of plant-based meat alternatives.

In the experiments, protein-mass temperature and binder-stream

flow rate were varied. The products were examined using optical methods (tearing pictures and cryo-imaging) as well as mechanical methods (tensile and compression analysis).

Increasing the protein-mass temperature led to a progressive dominance of a parabolic microstructure over the macroscopic strands, accompanied by increased tensile strength (at least 200%) and a reduced AI (4.4 to 1) in the absence of a binder-stream. The addition of a binder-stream decoupled the mechanical properties from the protein-mass temperature and generally resulted in lower tensile strength and higher AI compared to the control. Moreover, the results indicate that increasing the binder-stream flow rate tended to enhance compressive liquid release (LR up to 13 wt%) across all protein-mass temperatures. This increase in LR was significant when comparing the control with samples produced at 2 ml/min, corresponding to a three-fold relative increase and indicating improved juiciness.

The cause of the observed flow instabilities was investigated using rheological measurements. A viscosity ratio of approximately 1000 (protein-mass to gelatin solution) was determined. Since gelatin exhibited Newtonian behavior, a relaxation time ratio could not be determined. In contrast, the protein-mass showed pronounced viscoelasticity, with $De > 6$ at temperatures below 140 °C. The results from relaxation time and viscosity measurements indicate conditions that promote encapsulation and flow instabilities, allowing interlocking (key-lock-type) protein strands to form and thereby enhancing inter-strand adhesion.

While the process shows strong potential for generating palatable whole-cut meat analogues, challenges remain in understanding the underlying flow instabilities and improving overall process stability. Further research is required to identify the governing parameters. Multiphase flow simulations could help to gain deeper insights into the flow behavior within the system. In addition, the influence of different binder-phase material systems and geometrical parameters downstream of the breaker plate should be investigated. Future studies could explore the use of ionically gelling binder-stream solutions and the incorporation of dispersed lipid phases, potentially enabling greater control over juiciness, mouthfeel, flavor release, and overall texture of the extrudates.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used ChatGPT, OpenAI in order to improve grammar, readability and style. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Laurids Pernice: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Victoria Fuhl:** Validation, Investigation, Formal analysis. **Ulrike S. van der Schaaf:** Writing – review & editing, Supervision, Resources. **Nico Leister:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Laurids Pernice reports financial support was provided by German Federal Environmental Foundation. If there are other authors, they

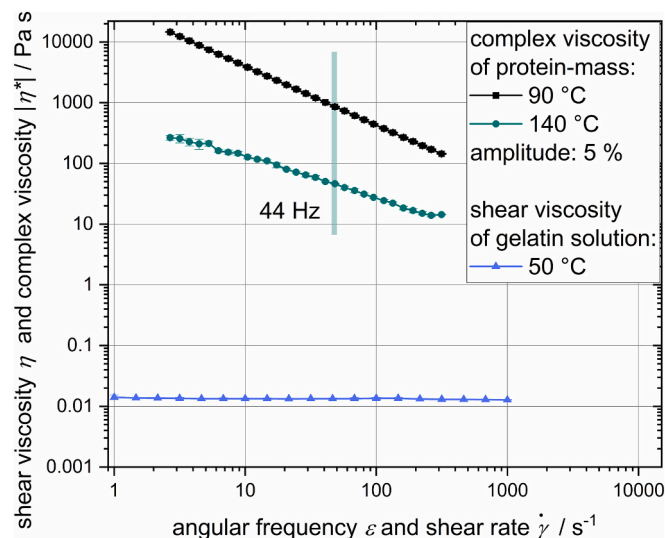


Fig. 8. Apparent complex viscosity of the 39 wt% protein-mass at 90 °C and 140 °C as a function of angular frequency (3–314 Hz) and shear viscosity of a 10 wt% gelatin solution at 50 °C as a function of shear rate (1–1000 s^{-1}).

declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work

reported in this paper.

Appendix

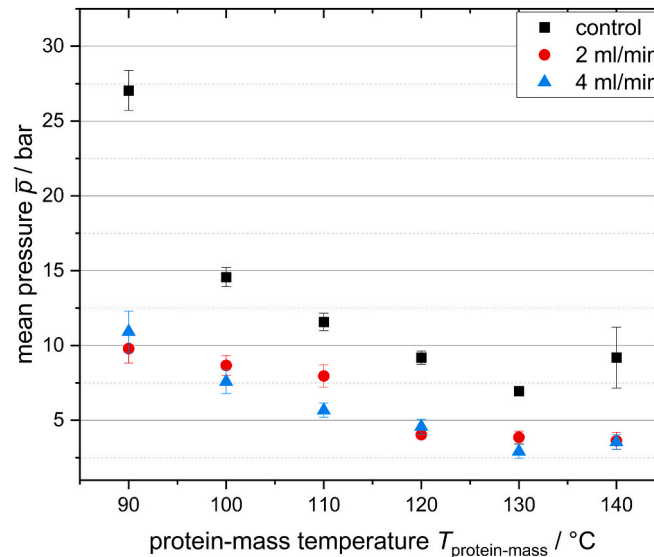


Fig. A10. Die pressure measured in the adapter for the control as well as for the extrudates with binder-stream with the mass flow rates of 2 ml/min and 4 ml/min. Data is shown for protein-mass temperatures of 90 °C to 140 °C, varied in steps of 10 K.

Data availability

Data will be made available on request.

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