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Modulating the texture of pea-based meat analogs: Influence of methylcellulose on mechanical and rheological properties of high moisture extrudates

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ABSTRACT

Adding hydrocolloids to plant-based meat analogs is a useful way to modulate the extrudates texture. However, it can alter the rheological properties of the melt, hence affect the extrusion process and change the extrudate's structure in an unknown manner. This study focuses on the influence of methyl cellulose (MC) as a model hydrocolloid on the mechanical properties of pea protein extrudates produced through high-moisture extrusion cooking. MC was introduced in solutions with different molecular weights at varying concentrations during extrusion. The study aims to assess whether MC affects the anisotropic structure of pea extrudates due to protein interactions or melt viscosity effects. Observations included changes in specific mechanical energy and die pressure, mechanical and rheological characterizations, and protein solubility analyses. MC decreased the melt viscosity during extrusion by 20 % for all MC concentrations, although offline rheology measurements of protein-MC mixtures showed an increase dependent on concentration and type of MC. The meat analogs E-Moduli decreased due to MC addition by up to 75 %. Protein analysis revealed protein-MC complexation, regardless of the MC molecular structure. These results show that anisotropic structuring cannot be predicted alone through offline rheological characterization. Understanding these protein-hydrocolloid interactions can improve plant-based products.

1. Introduction

The global population is projected to reach nearly 10 billion by the year 2050, which poses significant challenges to food security, nutrition, and sustainable development [1]. To address the need for providing enough protein to a growing population while minimizing the environmental impact of the food industry, substituting animal proteins with plant proteins offers a promising

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solution [2]. Plant-based meat analogs, produced through high moisture extrusion cooking, serve as an alternative to traditional meat. Producing high-moisture meat analogs (HMMA) involves the alteration of raw plant proteins through shear and thermal energy, resulting in a fibrous protein matrix that closely resembles the structure of meat. The exact mechanisms behind the formation of these fibers are still being studied [3–5]. Shear stress leads to the rearrangement and alignment of the denatured proteins, resulting in various intra- and intermolecular interactions [6,7]. The cooling step in the cooling die is responsible for solidifying the protein network, resulting in a cross-linked protein gel with layered fibers [8–10].

Despite the growing demand for plant-based meat alternatives worldwide, consumers still have limited options in terms of meat types and protein sources. To meet consumer expectations, new products must closely replicate the taste, aroma, and texture of meat [11]. One way to enhance the textural properties of meat analogs is by incorporating hydrocolloids. Hydrocolloids improve water binding in extrudates and have the potential to enhance fiber formation [12]. The effectiveness of hydrocolloids, however, depends on factors such as concentration, interactions with the protein matrix, and molecular features [12–14]. Like in animal products, it is not novel that hydrocolloids are to be included in plant-based patties, sausages, and nuggets for structuring and texture, and in some case, to improve the nutritional values of the finished products [15,16]. Dekkers et al. (2016) [17] showed that the addition of soluble fibers, like pectin, enhances the formation of anisotropic structures in soy protein meat analogs. Other groups focused on the addition of dietary fibers in meat analogs [12–14,17]. Ramos Diaz et al. (2022) [18] included beta-glucan in high moisture meat analogs, where the addition of fibers resulted in softer extrudate structures. Wagner and Ganjyal (2024) [19] incorporated pea hull fibers, citrus fibers and oat bran to wheat protein in different concentrations. The authors demonstrated that adding dietary fibers to HMMA influenced the texture of the extrudates depending on the water-holding capacity (WHC) and water-binding abilities of the investigated fibers. Increased WHC results in a softer texture and less fibrous appearance in the final product. Interestingly, fibers with amphiphilic behavior interacted with protein's hydrophobic regions, strengthening the HMMA matrix.

Besides affecting the composition of meat analogs and thus the textural properties, hydrocolloids also affect the rheological properties of the protein melt during extrusion [13]. The rheological properties of the raw materials used in the process have a significant impact on the extrusion process as a whole. For instance, the shear stresses generated by screw rotation, which are responsible for physical and chemical changes in the material, are influenced by viscosity [19]. Moreover, the rheological properties of the melt affect the fiber formation inside of the cooling die. The cooling die, which plays a significant role in determining the final structure of the product, is essential for the formation of the characteristic anisotropic structures in high moisture meat analogs. During the cooling step, the flow behavior inside the die is determined by the rheological properties of the protein melt. Yao et al. (2004) [20] showed in dead-stop experiments that the formation of anisotropic structures occurs at the beginning or within the die section. The influence of the die section becomes evident when considering the shape or orientation of the anisotropic structures, which typically exhibit a fibers-shape resembling a laminar flow profile. This viscosity change due to the addition of fibers is more significant with ingredients that have a higher affinity for water. Ultimately, the altered melt viscosity affects the flow properties of HMMA as it enters the cooling die, contributing to the observed changes in texture when different ingredients are used [19].

All studies up to date on the effect of fibers from different sources on textural properties of HMMA, had the fiber ingredients added in the powder blend. The effects on structure are attributed to the WHC of the fibers or gelling properties. However, it remains unclear whether this effect depends on the molecular weight of the hydrocolloids, the temperature treatment and/or interactions with the matrix. This study focuses on the influence of methyl cellulose as added model hydrocolloid in solution during the extrusion process, with defined molecular weight and structure. Methyl cellulose (MC) is a semiflexible, non-ionic cellulose ether derivate that is wide used in industrial application due to its excellent thickening properties [21]. In the food industry, MC is structuring agent for meat, dairy and alternative products. MC is soluble at low temperatures and shows thermos-reversible gelling behavior at higher temperatures (around 52 °C) [22]. Moreover, it has been shown that MC can interact with proteins, such as dairy proteins [23,24] and soy in aqueous solution. It can help creating a robust gel network structure that yields denser and more uniform gels [23]. Wittek et al. (2020) [25] demonstrated the influence of MC on the viscosity of soy protein doughs and render the samples more elastic, which in turn could affect the flow profile of the protein melt in the die. However, possible molecular interactions between proteins and MC were not investigated.

This represents a growing challenge in the meat-analog production. Protein sources are hardly pure isolates, as rest hydrocolloids from their botanical origin are almost always present. This few percentages can affect the anisotropic structure dramatically [26]. The economic and ecologic pressure on the meat analog market will additionally shift the ingredients from wet fractionated protein isolate to dry fractionated protein concentrates or even protein-rich flours. With each reduction of the side-streams from the plants of origin, higher amounts of fibers will be in the process and in the product. Therefore, there is a high need for assessing interactions amongst hydrocolloids and proteins under high shear and temperature.

This study aims to elucidate the impact of methyl cellulose on the mechanical properties of pea protein extrudates produced through high-moisture extrusion cooking. Pea protein was chosen due to its low allergen potential and local sourcing in Europe. Methyl cellulose of varying molecular weights was introduced in the water stream at 0.5 and 1 wt% during the extrusion process. The addition of pre-dissolved fibers can potentially change the water distribution in the final product significantly. The main goal of the work was to assess whether the addition of methyl cellulose an influence on the anisotropic structure of pea extrudates, either due to interaction with the protein molecules or due to viscosity effects. Additionally, the effect of the methyl cellulose solutions on the extrusion process parameters was also observed. In this regard, the viscosity of protein-MC melt upon entering the cooling due was determined, as well as changes in SME and die pressure were recorded. Moreover, mechanical and rheological characterization were conducted as an approach to gain insights into formulation and process parameters. Finally, protein solubility analyses were performed to clarify possible protein MCE interactions that might affect structuring. The goal of this study is to understand the viscosity effect caused by the addition of fibres to plant-based meat analogues made of proteins with no allergen potential, such as pea protein. Understanding these interactions can facilitate formulation and process design to improve plant-based meat quality, offering

consumers better vegan alternatives.

2. Materials and methods

2.1. Materials

Commercially available pea protein isolate (PPI) Nutralys F85M (Roquette, Lestrem, France) was used for all extrusion experiments. The protein content of PPI > 83 % and max. 10 % of water content according to the manufacturers. Methyl cellulose Tylopur MCE-4000 and Tylopur MCE-100TS was kindly gifted by ShinEtsu (SE Tylose GmbH & Co. KG, Wiesbaden, Germany) were used for this work. The investigated MC had a molecular weight of $\widetilde{M}_w \sim 144$ kDa and $\widetilde{M}_w \sim 385$ kDa for MCE-4000 and MCE-100TS, respectively. The specifications of both ME provided by the manufacturer are found in the table below.

Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, sodium dodecyl sulfate, Urea and dithiotreitol Chemical for protein solubility assays were purchased from Carl Roth (Karlsruhe, Germany).

2.2. Preparation of methyl cellulose solutions

MC solutions were prepared 1 day before extrusion, according to instructions given by the manufacturer. For this purpose, 0.5 or 1 wt% MC powder was added gradually to 500 g of water with a temperature of 70 °C, while stirring with a magnetic stirrer and magnetic plate. The mixture was stirred for 10 min until the powder was homogeneously dispersed in the water. After this, additional 500 g of cold water at 4 °C were added to the mixture while continuous stirring. Subsequently, the solutions were cooled down in an ice bath while stirring for another 15 min until the solution became transparent, and no particle was detected at suspended in the water phase.

2.3. Extrusion process

Meat analog products were produced with a fully ported Process 16 Hygienic Twin-screw Extruder and a cooled slit die (Thermo Electron (Karlsruhe) GmbH, Karlsruhe, Germany). Pea protein isolate powder was fed into the extruder with a gravimetric twin-screw feeder DDRS20. One peristaltic pump was used for water or methyl cellulose solution feeding, respectively. Both water and MC solution feeding occurred in the third port of the extruder. The temperature zones of the barrel were set to the following: 40/60/80/100/120/130/130/130/130. The die section was composed of the main body of the cooled slit die and an additional extension. The dimensions of cooled slit die were W x H x L: 6 × 28 x 320 mm, and the extension was W x H x L: 28 × 6 x 160 mm. These were kept at a constant temperature of 50 °C.

An overview of all the formulations is presented in Table 1. The schematic process setup is shown in Fig. 1. The trials were performed at otherwise constant process parameter settings, where screw speed was kept at 400 rpm and total feed rate at 1.5 kg/h. Material temperature of the melt was measured at extruder exit (before entering the cooling die) and verified to be 130 °C for all PPI formulations. After extrusion trials, samples were taken and stored at 5 °C in plastic bags to prevent drying and spoilage of the samples. Samples were analyzed within the next two days after production.

2.4. Mechanical measurements

The Young's modulus, fracture stress, and fracture strain of the investigated samples were determined under compressive stress. For this purpose, extrudates were subjected to compressive stress using a stress controlled HAAKE MARS 60 rheometer (Thermo Electron (Karlsruhe) GmbH, Karlsruhe, Germany) and a stress-strain diagram was recorded. The Young's modulus was determined by analyzing the linear portion of the stress-strain diagram, using Equation 1 as described by Willats et al. in 2001 and Fraeye et al. in 2010 [27,28].

$$E = \frac{\sigma}{\varepsilon} = \frac{F}{A * \varepsilon} \tag{1}$$

Where E = Young's modulus
σ = stress
F = measured force
A = probe's cross-sectional area

Table 1
Specifications of used methyl cellulose according to the manufacturer specifications.

	MEC-400	MEC-100TS
Degree of substitution	27.5 – 31.5 %	27.5 – 31.5 %
Residual water	Max. 5 %	Max. 5 %
Apparent viscosity at 20°C, 2 % dry basis	3000–5600 mPa s	82,500 – 154,000 mPa s

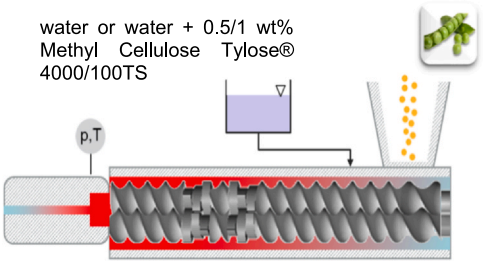


Fig. 1. Schematic extruder setup.

ε = strain

ε was calculated as shown in Equation 2.

$$\varepsilon = \frac{\Delta l}{l_0} \tag{2}$$

Where Δl = change in the height of the sample caused by the applied stress

l_0 = samples' initial height

Cylindrical shaped extrudates with a 6 mm diameter and 5 mm height were cut for the determination of the Young's modulus under compression stress. The height-to-diameter ratio of 0.83 is rather small, which can lead to slightly overestimated modulus values. Smaller diameters are however difficult due to the fibrous structure of the products. Samples were compressed using a serrated plate-plate measurement system with a plate diameter of 20 mm at a constant velocity of 1 mm*s^{-1}. The measurements were conducted 10 times for each sample to ensure statistical relevance.

For the elasticity tests at consumption relevant conditions, the extrudate was cut into cylindrical samples with a diameter of 15 mm and a height of 6 mm. The compression trials were conducted using a serrated plate-plate geometry with a diameter of 35 mm. Prior to the actual measurement, two compression-decompression cycles were performed while heating the samples from room temperature to 35 °C. These preliminary compressions reduced the plate-plate distance of the sample to 80 % of its original height at a constant velocity of 0.25 mm/s before decompression to original plate-plate distance.

The actual measurements at 35 °C were conducted to determine the Young's modulus (E) at consumption relevant conditions for reference, 1 % 100TS and 1 % MC4000 samples.

2.5. Closed cavity rheometer

To determine the die entrance viscosity of the investigated formulations, a rubber process analyzer flex, also known as a closed cavity rheometer (CCR), from Bareiss Prüfgerätebau GmbH, Oberdischingen, Germany, was used. The specific details of this rheometer and its features are thoroughly described by Emin et al. (2017) [29]. The closed bi-conical, grooved geometry with a radius of 20.625 mm and an angle of 7.16° is widely employed for investigating the rheological properties of protein-water mixtures at extrusion-like conditions [25,29,30]. The measuring geometry is sealed during the measurement process, using a closing pressure of 4.5 bar, which prevents the evaporation of water at temperatures exceeding 100 °C.

All samples were analyzed in oscillatory strain at different temperatures with a temperature ramp, ranging from 40 °C to 150 °C for PPI samples. The heating rate was maintained at a constant 5 K/min. The frequency and strain were kept constant at 1 Hz and 1 % deformation, as favorable conditions to minimize signal-to-noise ratio within the linear visco-elastic (LVE) region. Temperature sweeps were repeated three times, and the mean value and deviation were determined.

2.6. Protein solubility

To elucidate possible interaction between MC and PPI during the extrusion process, the solubility of all extruded sample in reducing and non-reducing conditions was studied, according to the method describe by Areas (1992) [31] and Liu and Hsieh (2008) [32]. For this purpose, three different buffers were prepared, whose purpose was to suppress different protein-protein interactions or bonds. Electrostatic interactions were disrupted using a sodium phosphate buffer with 0.132 M sodium dihydrogen phosphate, 0.68 M disodium hydrogen phosphate and 0.05 M sodium chloride (buffer 1). For solubilizing non-covalent protein bond, additional 17.3 mM sodium dodecyl sulfate (SDS) and 8 M urea were added to the phosphate buffer (buffer 2). Lastly, covalent bonds were suppressed in adding 10 mM of dithiotreitol (DTT) to buffer 2, resulting in buffer 3. All buffers had a resulting pH after dissolving the extrudates between 6.7 and 6.9.

All extrudates were extracted following the same procedure. The extrudates were vacuum dried at 50 °C and 5 mPa and for 24 h. All samples were then ground and sieved to a particle size $<$ 280 µm. 10 mg of the sample was mixed with 20 mL buffer, vortexed for 30 s, and dissolved at room temperature for 1 h in a rotary shaker from Edmund Bühler GmbH (Bodelshausen, Germany) at 200 rpm. Afterwards, samples were centrifuged at 4310 × g for 50 min with a lab centrifuge from Eppendorf AG (Hamburg, Germany). The absorbance of the supernatant was determined at 280 nm with an Evolution 201 spectrophotometer by Thermo Fisher Scientific Inc.

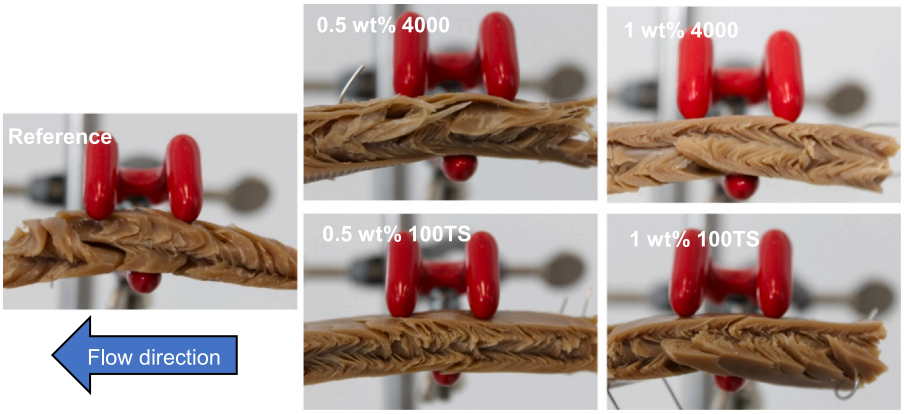


Fig. 2. Pea protein extrudates produced with water, 0.5 wt% 4000 MC, 0.5 wt% 100TS MC, 1 wt% 4000 MC and 1 wt% 100TS MC. Flow direction in the extruder die labelled with arrow.

(Waltham, MA, USA). All absorbance values were compared with a bovine serum albumin (BSA) calibration curve to determine the BSA equivalent protein content. The BSA calibration curve was obtained with measuring the absorbance of different BSA concentration (concentration range 0.005–2 mg BSA/mL buffer, $R^2 > 0.99$). Solubility and absorbance measurements were conducted in triplicate, resulting in a total of nine absorbance values per sample.

2.7. Statistical analysis

If not specified otherwise, all analyses were conducted at least three times per independent test. All data was assessed by a multifactorial analysis of variance (ANOVA) and a Tukey test as post-hoc test. Dissimilarities in samples were considered statistically relevant at a level of $p \leq 0.05$. The software OriginPro 2019 (OriginLab Corp., Northampton, MA, USA) was used for the statistical analysis, calculation of averages, and standard deviations.

3. Results and discussion

3.1. Influence of MC on visual appearance of meat analogs

The first assessment of extruded samples was optically. For this, all samples were cut in the middle and folded, so the resulting fiber structure is clearly showed. Photographs of all samples, taken directly after extrusion, are depicted in Fig. 2. As seen from the images below, in the presented work, changes in anisotropic structure were noticeable due to the addition of MC to the formulations. The fibrous structure of the reference sample is less pronounced compared to the samples containing MC. Samples with 0.5 and 1 wt% MC seem more layered, and the typical v-shape of laminar flow profiles is also more pronounced. One reason why these samples are different even though the processing conditions remained the same could be that the addition of MC influences the viscosity of the protein melt. Moreover, the effect of MC on the protein melt viscosity depends on the molecular weight of MC and its its final concentration on the extrudate.

Usually, an increase in viscosity would cause a shift from the parabolic flow profile to a kind of plug profile, whereas a decrease in viscosity leads to a more pronounced v-shape laminar flow [33,34]. Ellwanger et al. (2024) [35] demonstrated that an increase in the complex viscosity of soy protein melt prior entering the cooled slit die rendered the extrudates brittle and less structured. The authors modified the pH of the melts during extrusion, which besides increasing the melt viscosity, affected the reaction behavior of the investigated proteins. pH clearly affects the reactivity of the protein by changing the surface charge of molecules, therefore, lesser structured extrudates is expected. According to Wittek et al. [25] the rheological properties of the melt are key for structuring the layered fibers. The changes in the entrance viscosity can affect the flow profile inside the cooled slit die, potentially resulting in different fibrous structures. The obtained fibers in this study are even more defined and layered due to the addition of MC. Therefore, although counterintuitive, the addition of MC to the formulation is assumed to decrease the viscosity of the melt prior to the cooling die. For this reason, all investigated formulations were rheologically characterized using a closed cavity rheometer (CCR) in order to assess the influence of MC on the melt viscosity.

3.2. Influence of MC on the melt viscosity of protein-MC mixtures in offline measurements and in the extrusion process

The influence of methyl cellulose on the rheological properties of the protein melt was determined at temperatures comparable to extrusion conditions. Samples' complex viscosity was measured with a temperature ramp, at constant frequency and amplitude strain. Frequency and amplitude values were inside the linear visco-elastic region of the melt; hence a reversible mechanical strain can be assumed, and the focus of the measurements would lay in the assessing the temperature effect. The values are chosen for comparison

Table 2
Overview of sample formulations. In the liquid phase, parts of the water are replaced by MC.

Sample's name	Protein content	Liquid phase	MC in liquid phase	MC in extrudate
Reference	50 wt% PPI	50 wt%	0 wt%	0 wt%
0.5 % 4000	50 wt% PPI	50 wt%	0.5 wt%	0.25 wt%
1 % 4000	50 wt% PPI	50 wt%	1 wt%	0.5 wt%
0.5 % 100TS	50 wt% PPI	50 wt%	0.5 wt%	0.25 wt%
1 % 100TS	50 wt% PPI	50 wt%	1 wt%	0.5 wt%

were measured at 120 °C, which is close to the entrance temperature of the melt into the die. These are listed in the table below (Table 2).

It can be seen from the values above that the complex viscosity increases with the addition of MC, which is not surprising, but do not correspond the optical assessment. It is also noticeable that the increment depends on the concentration and the molecular weight of the MC used. The viscosity of the melt reaches two times or more the reference's viscosity value when 1 wt% MC solution was added, with the formulation containing 1 wt% 100TS MC (highest molecular weight) having the highest viscosity value. This was also expected, yet again contrary to the actual extrusion experiments. As mentioned above, changes in viscosity can affect the melt's flow profile inside the cooling die, but can also affect the heat transfer inside of the die and affect the thermo-mechanical treatment inside of the extruder. This, in turn, can also change the anisotropic structure of the extrudates. However, the data summarized in Table 2 show an increase in viscosity which should result in less structured extrudates, which is not the case. Therefore, the CCR measurements do not completely represent what is happing inside the extruder. The complex viscosity data discussed above reflects only the changes in viscosity in dependency of the temperature and the formulation of the melt. The mechanical effect is not regarded in this setup, so the values are indicative and qualitative. The values should indicate tendencies but should not be regarded as absolute, as the high shear in the barrel section could also increase molecular interaction or decrease the viscosity of the melt due to energy dissipation. Therefore, further analysis is still necessary to shed light on possible MC-protein interaction. First, the viscosity effect of MC on the protein melt under thermal and mechanical treatment should be assessed. For this reason, all investigated formulations were extruded under constant conditions and the system response values, such as pressure and torque were recorded.

To determine possible changes in viscosity due to the addition of MC during extrusion, the process parameters during the whole extrusion process were also recorded for all investigated formulation and are displayed in Table 3.

As seen from the results summarized in Table 3, both pressure at die entrance and torque – with torque values decreasing in a slight less significant manner than the pressure values– decrease with the addition of methyl cellulose solutions. Another important fact is that the decrease in torque causes a reduction in the total SME. This means that the total mechanical energy input is also reduced by the addition of MC, which could affect the reaction kinetic and consequently the optical appearance of the extrudates.

Similar to the optical assessment, these results are counter intuitive, as one would expect that the addition of a gelling agent, such as MC, to protein melts would result in higher viscosity and conversely result in higher pressures at die entrance. However, this is not the case. The decrease in pressure at die entrance show that the melt viscosity and/or the filling degree of the extruder decrease as well. However, since throughput, screw configuration, screw speed and temperatures remained constant for all formulations, most probable is the melt viscosity that changed with the MC solution. Wagner et al. (2024) [19] showed that the incorporation of dietary fiber in high moisture meat analogs affected the in-process behavior (torque and pressure) of the resulting HMMA, as well. The authors measured a decrease in dependable variables torque and pressure when introducing hydrocolloid bran, which was attributed to water absorption capacity of the fiber. It was hypothesized by the authors that the fibers took away that available water from the proteins, which conversely reduces the protein reactivity. This could also be applicable to formulations investigated in this work. MC is also known for its high-water binding capacities. Additionally, contrary to previous studies, MC was incorporated already in the liquid phase during the extrusion process. Therefore, it would be imaginable that the proteins would have less available water for hydration in the short residence time inside of the extruder, and subsequently show a diminished reaction/interaction behavior. Additionally, the decrease in pressure at die entrance of melts with 0.5 wt% and 1 wt% MC formulations are in the same range, respectively, and irrespectively of the MC's molecular weight. Change in viscosity alone cannot predict the formation of more, or less pronounced fibers, but rather the reason why the increase happens need to investigated. In this case, the interaction between MC and pea protein are still unknown and need clarification to assess the possible reason for the more pronounced fibers. This could rather

Table 3
Complex viscosity of pea protein isolate melts with and without addition of MC at different concentrations.

Sample	Complex viscosity at 120 °C, 1 Hz [Pa*s]
Reference	1619
0.5 wt% 4000	1984
1 wt% 4000	3090
0.5 wt% 100TS	2532
1 wt% 100TS	3659

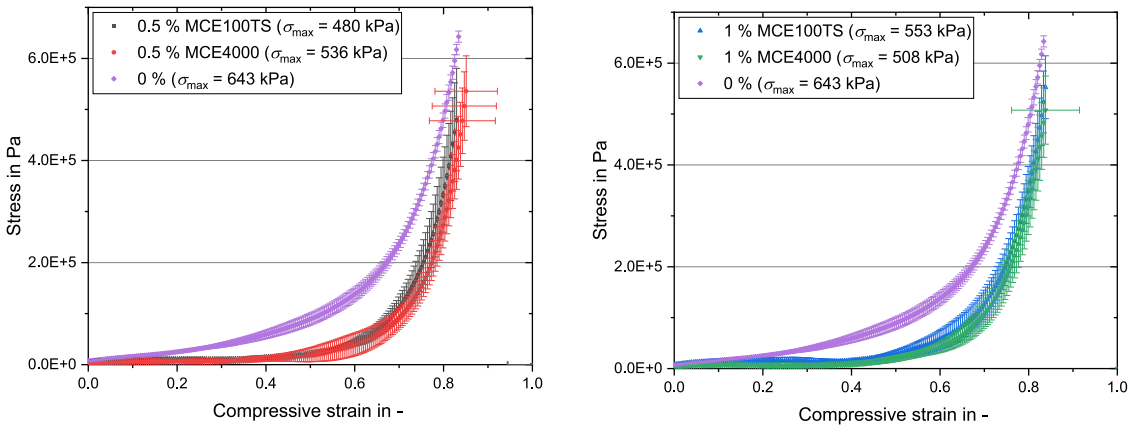


Fig. 3. Stress-strain curves under compression stress of pea protein extrudates with and without the addition of either 0.5 wt% MC (left) or 1 wt% MC (right).

support a hypothesis based on the reaction and/interaction of proteins with the components of the formulation than based solely on the gelling behavior of MC. In order to clarify possible interactions between MC and pea protein, further analyses are required.

The more pronounced anisotropic structure could be caused by the inclusion of MC as an additional component in the multiphase system of the pea protein melt. According to the multiphase theory proposed by Tolstoguzov [4], a multiphase mixture comprising both continuous and dispersed phases is essential. The dispersed phase, which can consist of various protein fractions, fat, polysaccharides, proteins, water, or air, undergoes deformation and elongation due to thermo-mechanical stress. This phase subsequently solidifies in its elongated form, resulting in the formation of fibers. Additionally, changes in the solubility of proteins due to the presence of additional phases can influence the formation of the multiphase system. It has been demonstrated in the literature that a water-poor, protein-rich phase and a water-rich, protein-poor phase can occur, which in turn result in more fibers [9].

3.3. Influence of MC on textural parameters of extrudates

One way to assess the possible interactions between pea protein and MC is by texture analysis. Particles that are not incorporated into a protein gel, referred to as inactive fillers, lead to a reduction in gel strength and a higher incidence of syneresis [36]. Conversely, gels containing bound particles, such as fibers, known as active fillers, exhibit markedly enhanced mechanical stability. For this reason, samples were analyzed under compression stress. Stress-strain diagrams were recorded and are depicted in Fig. 3 left for samples with 0.5 wt% MC and Fig. 3 right for samples with 1 wt% MC, respectively.

The extrudates become softer upon addition of methyl cellulose. The stress-strain curves shift to the left, and this effect is observed regardless of the concentration and type of methyl cellulose. The strain-stress curves from all samples prepared with MC display a flatter slope, which can be interpreted as a higher deformability of the protein matrix. Ramos Diaz et al. (2022) [18] had similar results with the addition of beta glucans to protein extrudates, therefore, these results line up with the ones reported in literature. Leonard et al.: (2020) [37] reported that the addition of fiber disrupts the internal structures of plant-based extrudates. In this work, the shift in maximum strain and less stress required for compression of the samples could be an indicator for a lesser crosslinked/compact protein gel structure.

One way to assess the influence of MC on the protein gel strength is over the Young's Modulus. Compression stress deforms the protein matrix in one direction and the capacity of a material to endure this uniaxial deformation is described by the Young's modulus, amongst others characteristic values. Many authors have shown that an increase in Young's Modulus is caused by an increase in the molecular rigidity [38,39], which for pea protein extrudates, as for other cross-linked polymer gel, could be a consequence of higher number of cross-links [40]. The Young's modulus of all investigated samples was calculated from the slot of the regression of linear section of the curves. The calculated values are found in Table 4.

As seen from results presented in Table 4 and both strain-stress-diagrams, the addition of MC to the pea protein matrix reduces the stiffness of the samples. Thus, it appears that MCE loosens the structure of the extrudates. Ramos Diaz (2024) [18] also observed

Table 4
Pressure at die, torque and calculated SME of pea protein isolate melts with and without addition of MC at different concentrations.

Name	Pressure [bar]	Torque [Nm]	SME [kJ/kg]
Reference	18.2 ± 0.5	2.5	251
0.5 wt% 4000	14.0 ± 0.2	2.1	211
1 wt% 4000	14.8 ± 0.3	2.1	211
0.5 wt% 100TS	13.6 ± 0.2	2.1	211
1 wt% 100TS	14.6 ± 0.1	2.1	211

Table 5
Young's Modulus of pea protein isolate extrudates with and without the addition of either 0.5 or 1 wt% MC solutions during extrusion, measured at room temperature.

Sample	Young's Modulus [kPa]
Reference	86
0.5 wt% 4000	23
1 wt% 4000	32
0.5 wt% 100TS	23
1 wt% 100TS	35

spherical bodies entrapped between the fibrous structure of the extrudates that were hypothesized to be traces of fibers or protein-fiber complexes. This could also be the case of this work. These complexes or traces could act as failure points and prevent protein-protein interactions, hence affect the mechanical strength of the investigated samples. However, viscosity effects could also be responsible for the decreased number of crosslinks. As mentioned before and as seen from the processing parameters, the SME is reduced by the addition of MC, which means that there is less mechanical energy is put into the protein melt for reaction. Whether MC-protein interactions or solely viscosity effects are responsible for the changes in the mechanical properties of the pea protein extrudates is only assessable with offline viscosity measurements and protein solubility studies.

3.4. Protein MC interactions

Still, it remains unclear whether the changes observed in textural properties and in the anisotropic structure are solely related to the water holding capacity of MC/gelling properties of MC and the resulting increase in viscosity of the melt, or if there are any interactions between pea protein and MC. In order to shed light on possible interactions, protein solubility tests performed on all samples. For this, extrudates were dried and milled. The dried powder was then extracted in three different buffers that solved electrostatic, reducing and non-reducing conditions. The solubility of the samples was then compared to a BSA calibration curve. The results are depicted in Fig. 4.

As observed in Fig. 4, reducing conditions are hardly affected by the addition of MC, as all samples, including the reference samples, display similar equivalent solubility. Similar results are obtained when samples are extracted under non-reducing conditions. However, the samples containing the highest amount of MC show reduced solubility when electrostatic interactions are diminished. Previous studies have reported that a moderate amount of MC interacted with soy protein isolate and enhanced the proportion of flexible structures within the SPI [23]. The authors noticed an increase in the formation of intermolecular hydrogen bond in the presence of MC: This could also apply to this study. Since the solubility of pea protein decreases due to the addition of MC, larger molecules could be formed. Additionally, these larger molecules are only significantly noticeable at a p-value > 0.05 when 1 wt% MC is added to the melt. Hence, it could be concluded that a minimal amount of MC (greater than or equal to 1 wt%) is

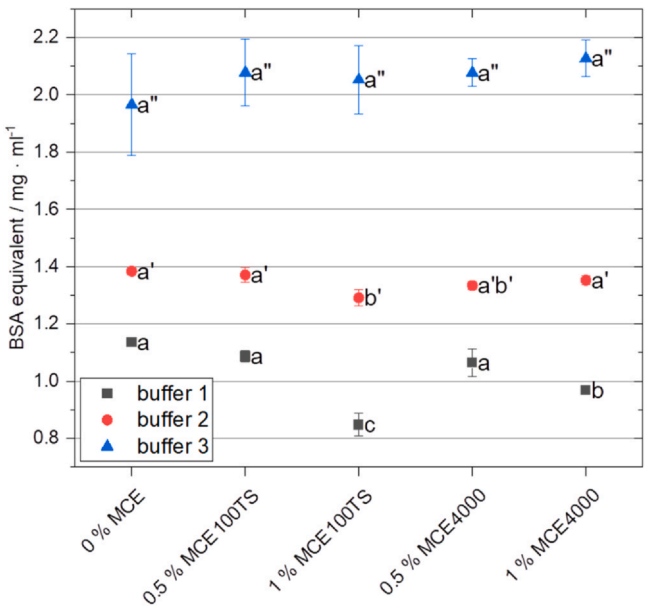


Fig. 4. Solubility of pea protein extrudates, with and without the addition of MC in reducing and non-reducing conditions.

required for complexation. Although the solubility data indicate protein-MC complexation, there is no information on the molecular size, structure, and/or bond type. Therefore, additional analytical methods should be employed to clarify these queries. Nevertheless, these results indicate that the melt viscosity increase discussed throughout this paper could be also attributed to formation of protein-MC complexes.

4. Conclusions

The addition of methyl cellulose (MC) to high-moisture pea protein extrudates significantly influenced their properties. Optical assessments revealed that MC addition resulted in more layered and pronounced anisotropic structures. The complex viscosity measurements showed increased viscosity by the addition of MC to the pea protein melts at high temperatures. This is a surprising finding, since the extrusion process parameters indicated that both pressure and torque decreased with MC addition, contrary to expectations, suggesting reduced melt viscosity or extruder filling degree. It is hypothesized that MC's high water-binding capacity affects protein hydration and reactivity, leading to a changed viscosity and more pronounced fibers.

Texture analysis showed that extrudates with MC became softer and more deformable, indicating a less crosslinked protein gel structure. Young's Modulus calculations confirmed reduced stiffness with MC addition, possibly due to MC-protein complexes or viscosity effects reducing crosslinks.

Protein solubility tests under various conditions suggested that MC interacts with pea protein, forming larger molecules, especially at higher MC concentrations (≥ 1 wt%). Although further analytical methods are needed to clarify these interactions and their impact on melt viscosity and extrudate properties, these initial results highlight the complexity of interactions between fibers and proteins in the extrusion process. Understanding these interactions could help tailor the structure of meat analogs to increase consumer acceptance and meet the demand for better meat-like structures.

CRediT authorship contribution statement

Nico Leister: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Valerie L. Stahl:** Writing – review & editing. **Valentin Glubrecht:** Methodology, Conceptualization. **Laurids Pernice:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Saavedra Isusi Gabriela I.:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests (e.g., any funding for the research project)/personal relationships (e.g., the author is an employee of a profitable company) which may be considered as potential competing interests: Gabriela Itziar Saavedra Isusi and Valerie L. Stahl are employed by the company Thermo Fisher Scientific. Valentin Glubrecht is employed by the company SE Tylose GmbH & Co. KG. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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