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Reactive extrusion of proteins: Sodium caseinate films enhanced by crystallization

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ABSTRACT

The study focuses on the reactive extrusion of sodium caseinate by an enzymatic cross-linking of proteins in the extruder barrel. A co-rotating parallel intermeshing twin-screw extruder was used for the extrusion process of proteins without prior pelletizing. Feasible process conditions were identified and transferred to a process window which allow a covalent cross-linking of sodium caseinate via the enzyme microbial transglutaminase (MTG) and an incorporation of crystalline materials in the protein matrix. Transparent, homogenous and smooth structured sodium caseinate films were produced and characterized. The mechanical properties of sodium caseinate films (non-cross-linked, cross-linked and crystallized) were determined and correlated to important process conditions. An enzymatic cross-linking leads to a significant increase of the tensile strength of the films (up to 85%) while the elongation did not deteriorate. Furthermore, the relationship between the residence time and the mechanical properties shows that residence times between 100 and 120 s offer maximum mechanical performance due to longer reaction times leading to higher cross-linking densities. It was found additionally that inner-film crystallized sodium caseinate films show significant higher elongation values. In this regard, crystalline structures (size, shape and distribution of potassium nitrate crystals) were identified and correlated to the tensile properties of the films. The synergistic effect of crystalline additives and cross-linking enhances physical and functional properties of protein films and offer additional product benefits. By combining application-oriented bio-based product design with an efficient large-scale production process, new advanced materials and products for many fields of applications could be developed and produced.

KEYWORDS: reactive extrusion, proteins, biopolymer, sodium caseinate, crystallization

1. INTRODUCTION

From a historical point of view, twin-screw extruders have been used for compounding, profile extrusion, devolatilization and reactive extrusion. In particular, co-rotating twin-screw extruders are being applied in sheet and film extrusion, where different formulation ingredients are compounded and formed in one process step. This avoids a prior pelletizing, and re-shaping in a subsequent extrusion process [1].

Extrusion is a highly efficient and continuous mixing, kneading and shaping process for a large-scale production of biodegradable films [2]. The thermoplastic processing of proteins by extrusion is a new field of interest for research and industry since the demand for environment-friendly solutions is increasing and, associated therewith, the necessity for higher production capacities.

Proteins offer a large range of possible interactions and chemical reactions. These include covalent linkages (peptide and disulfide), non-covalent

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interactions through ionic, hydrogen and van-der-Waals bonding as well as hydrophobic interactions between non-polar groups of amino acid chains [3-5]. From this results the ability of proteins to form and stabilize three-dimensional macromolecular networks such as films and coatings. Proteins, including wheat gluten, corn zein, soy, whey, gelatin and casein as well as proteins from mung bean, pea and peanut have been investigated for their film forming abilities and properties [4, 6]. Among them is casein, the major protein component of milk, which is intensively studied and characterized in relation to its ability to easily form films from aqueous solutions without further treatment [7, 8]. Caseins are random coil polypeptides with a high degree of molecular flexibility due to their low secondary structures [7]. This in turn leads to the ability to form extensive intermolecular hydrogen, electrostatic and hydrophobic bonds, resulting in an increase of the inter-chain cohesion [8, 9]. For this study, sodium caseinate was used, which is produced by adjusting acid-coagulated casein to pH 6.7 using sodium hydroxide [10, 7].

Until recently, research on sodium caseinate was mainly focused on films produced by the solution casting method, a small-scale process consisting of casting or spreading an aqueous protein solution on a hydrophobic surface followed by a subsequent drying step [11 - 14]. It was also found that cross-linking of proteins enhance certain physical properties of protein-based films. These include mechanical properties, water sensitivity, solubility and thermal resistance [15 - 17]. Cross-linking of proteins relate to inter- and intramolecular covalent bond formations including disulfide and isopeptide bonds and can be distinguished as follows: physical-induced cross-linking (by heat or irradiation), chemical cross-linking and enzymatic (bio-catalytic) cross-linking [18, 19].

A bio-catalytic cross-linking of proteins can be induced by various enzymes like transglutaminase, laccase, tyrosinase or peroxidase [20, 21]. Casein is an effective substrate for transglutaminases mainly because of its open tertiary structure. The enzymatic cross-linking is characterized by fast reaction rates resulting in high degrees of cross-linking which is an important attribute for a successful integration in an extrusion process [22].

Transglutaminase (EC 2.3.2.13, protein-glutamine γ-glutamyltransferase) catalyzes an acyl group transfer between the γ-carboxyamide groups of peptide-bound glutamine residues and primary amines, e.g. the ε-amine group of a protein-bound lysine. The reaction leads to a ε-(γ-glutamyl)-lysyl isopeptide bond, typical for enzymatic cross-linking of proteins [22, 23].

Another approach to enhance the mechanical properties of protein films is the controlled crystallization of additives in the protein matrix. Frohberg *et al.* [11] investigated the influence of crystalline additives on the tensile properties and the morphology of sodium caseinate and gelatin films made by solution casting. It was found that the synergistic effect of crystallization and enzymatic cross-linking offers the highest potential in terms of enhancing the overall mechanical performance [11, 24]. Implementation of this method in the thermoplastic processing of proteins by extrusion appears to be a promising way to incorporate crystallizing additives in protein films at a large production scale.

In the classical sense, crystallization is a process for separation, purification as well as product design and, is therefore a key technology in the production of high-value products with specific application-oriented characteristics [25]. Crystals in protein-based materials and products are able to provide completely new product benefits (e.g. additional values in terms of carrier and release functionalities) and are therefore part of the product design [11]. Furthermore, tailor-made properties can be generated and specifically optimized for the respective industrial application [11, 24].

To date, some research has been done on the thermoplastic processing of proteins to films. A successful production and characterization of protein-based films based on different raw materials is described in literature [7, 26-29, 5, 30-32]. Therefore, structure-processing relationships and their influences on the material properties are well investigated. Current literature focusing on the extrusion of proteins report a small window of operating conditions mainly due to the excessive formation of physical cross-links and/or degradation of polymer chains at high temperatures and high specific mechanical energy (SME) inputs. The early formation of covalent cross-links in the

extruder barrel decreases chain mobility, increases viscosity and leads to raptures in the extrudate. This results in inhomogeneous films with insufficient mechanical properties [27, 1]. However, the avoidance of cross-links in the extruder by lowering the temperature and the SME input restrict the possibilities of tailoring the final product properties and also hinder the production of application-oriented, high-value products in one process step. Therefore, a reactive extrusion of sodium caseinate is a novelty in the field of large-scale processing of proteins. It opens up unique opportunities for large-scale production of new, advanced bio-based products.

The objectives of this study are the production and characterization of sodium caseinate films by twin-screw extrusion and the optimization of their physical properties by enzymatic cross-linking and an incorporation of crystalline potassium nitrate in the protein matrix. In this context, the identification of a window of operating conditions and their influence on the physical properties of the product are in the center of interest.

2. MATERIALS AND METHODS

2.1. Materials

Sodium caseinate (protein 88%, fat 1.5%, lactose 0.2%, ash 4.5% and water 6%) was provided by BMI e.G. (Landshut, Germany). Microbial transglutaminase (MTG) (ACTIVA® WM, nominal activity 100 U/g) was supplied by Ajinomoto Europe Sales GmbH (Hamburg, Germany). Glycerol (99.5%) was purchased from Caldic Deutschland Chemie B.V. (Duesseldorf, Germany) and potassium nitrate was purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany).

2.2. Processing and characterization

2.2.1. Extrusion

A co-rotating parallel intermeshing twin-screw extruder (ZE25A-UTXx48D-UG, Krauss Maffei Berstoff) was used for the extrusion process of proteins. Barrel units and screw elements were designed according to the modular principle and are characterized by the following parameters: screw diameter of 25 mm with a length-diameter ratio L/D = 48, 10 independent electrically heated and water cooled barrel zones and a sheet die of 2 mm diameter and 400 mm length. The temperature profile of the barrel section was set to 30-50-50-50-70-70-70-70-70-75-75 °C (from barrel zone 1 to 10 and 11 for die temperature) and 30-50-50-50-50-50-50-50-50-50-50 °C. The rotational speed of the extruder screw was varied between 90 and 285 rpm.

The protein powder was starve-fed in barrel zone 1 by a gravimetric feeder (K-TRON Deutschland GmbH, Gelnhausen) followed by the downstream addition of the liquid materials in barrel zone 2. The liquids (water and glycerol) were fed volumetrically through a liquid injection nozzle using a positive displacement pump. Prior to the liquid feeding, glycerol as a plasticizer was added to the water in a solvent/plasticizer ratio of 2:1.

To prevent a backward flow of the feed material, low-pitch short elements were used in the feed zone followed by a high-pitch element to provide space for the powdered protein. The material was conveyed forward to the mixing section (containing kneading blocks) which provided dispersive and distributive mixing and a progressive plasticization of the protein. To avoid imperfections of the extruded product, a devolatilization (two venting ports) was applied by using vacuum pumps which removed volatiles prior to the die. Small-pitch elements in the metering section provided a sufficient conveying of the plasticized protein. In order to provide a consistent polymer flow to the die and, consequently, achieve more consistent product dimensions a gear pump was installed between the extruder and the die.

A temperature-controlled sheet die based on the coat hanger design assured a uniform thickness and flow of the material to the three-roll stack as part of sheet extrusion line. The rolls controlled the cooling rate, film thickness and surface and molecular orientation. Elastomeric coated puller rolls (synchronized with the roll stack speed) ensured the onward movement to the windup where the extruded films were wound in continuous rolls.

2.2.2. Reactive extrusion

Reactive extrusion was accomplished on a co-rotating parallel intermeshing twin-screw extruder as described in section 2.2.1. To establish the cross-linking reaction, MTG with activities of 5, 10 and 15 $U/g_{protein}$ was additionally dissolved in

the liquid feed and inserted in conjunction with the solvent and the plasticizer in barrel zone 2. The temperature profile of the barrel section was set to 30-50-50-50-50-50-50-50-50-50-50 °C to avoid the immobilization of the enzyme.

2.2.3. Crystallization

Potassium nitrate as model crystallization additive in different concentrations (KNO_3/protein ratios of 1:12 to 1:4) was dissolved additionally in the liquid feed and inserted through a liquid injection nozzle in barrel zone 2. Optical and mechanical properties were determined as described in sections 2.2.6 and 2.2.4, respectively. Furthermore, the combined effect of an enzymatic cross-linking of sodium caseinate and the controlled additive crystallization was investigated.

2.2.4. Tensile properties

Mechanical properties of preconditioned extruded films were measured according to the standard procedure DIN EN ISO 527-3 using a material testing device (BDO-FB0.5TH, Zwick/Roell, Germany). Prior to the testing the films were cut into test stripes with dimensions of 15 mm in width and 100 mm in length, considering both longitudinal and transverse direction alignment. Film thickness was determined from the mean of three measurements across each specimen using a micrometer screw. Subsequently, the sample films were tested with a grip separation of 100 mm and a crosshead speed of 50 mm/min.

2.2.5. Electrophoresis

Using SDS polyacrylamide gel electrophoresis (SDS-PAGE), protein samples can be distinguished by their molecular weight distribution. SDS-page was carried out under reduction conditions using an SDS-Tris-glycine discontinuous buffered system described by Laemmli [33, 34].

The polyacrylamide gels were prepared in a two-step process by first composing the resolving gel followed by the casting of the stacking gel on top. The staining was carried out with Coomassie blue. 10 µL of the respective protein sample (with an approx. concentration of 1 mg protein per mL) and 5 µL molecular weight marker were added to the gel. After destaining, the gels were dried between cellophane foils fixed in a frame.

2.2.6. Morphology

Optical properties (surface, opacity, coloring, crystal size, shape and distribution) were investigated using digital photography and light microscopy. Product samples were stored in a desiccator for at least 48 h to ensure constant conditions prior to the visual evaluation.

2.2.7. Statistical analysis

Analysis of variance (ANOVA; Statistica® 8, StatSoft, USA) was used to indicate a significant difference ($P < 0.05$) amongst the means. Unless specified differently, five replications were employed for each sample.

3. RESULTS AND DISCUSSION

3.1. Extrusion and reactive extrusion of proteins

By combining the effects of temperature, pressure and shearing during the extrusion process the formation of a homogenous protein melt was achieved. The array of chain interactions due to the large amount of different functional groups of these heteropolymers have to be overcome to process the protein in the extruder [7, 27]. In this respect, the relatively high glass transition temperatures of proteins, which are often above their decomposition temperatures [35-37] necessitate the usage of plasticizers to avoid degradation and to ensure an appropriate chain mobility for the thermoplastic processing of proteins [38, 28]. Moreover, a certain amount of plasticizer guarantees the required application-specific flexibility and has a profound influence on the physical properties of the final product [29, 5].

The operating window for feasible process conditions was found to be narrow. A high temperature profile leads to excessive aggregation and will result in physical cross-linking, thus reducing the melt flow. Consequently, two different temperature profiles were found suitable for the processing of sodium caseinate, which guaranteed a late melt formation and avoided extrudate disruption upon exiting: 30-50-50-50-70-70-70-70-70-75-75 °C and 30-50-50-50-50-50-50-50-50-50-50 °C. Table 1 provides an overview of the feed composition and the extrusion conditions for the thermoplastic processing of sodium caseinate.

Reactive extrusion experiments were carried out by adding the cross-linking agent microbial transglutaminase pre-dissolved along with the liquid feed. The low temperature profile was used to avoid the inactivation of the enzyme which occurs at temperatures above approx. 60 °C [39, 40]. The extrudate compositions were selected on the basis of preliminary experiments with regard to suitable extrusion conditions.

As an example, Fig. 1 shows aggregated protein in barrel zone 5 and 6 (from right to left) achieved with a temperature profile of 30-50-50-50-70-70-70-70-70-75-75 °C. Higher temperatures entail lower viscosities and earlier protein softening. As a consequence, protein aggregation increases considerably and results in physical cross-linking which may lead to disruption of the extrudate upon exiting [27, 30]. This known phenomenon was observed with barrel temperatures above 80 °C in the case of sodium caseinate. Therefore, a softening of the protein just before exiting the die in order to avoid extensive aggregation was aimed, which leads to an improved processibility and a constant extrudate flow in the barrel and through the die.

After the transformation of the protein-plasticizer to a thermoplastic melt the protein film is then formed upon cooling through the formation of new hydrogen, ionic and covalent bonds and hydrophobic interactions [5].

Sodium caseinate films with a width between 300 and 350 mm and a thickness between 150 and 300 µm were successfully produced. The product dimension differed from the lip dimension of the die due to extrudate swell (increased viscosity through temperature drop), draw down and pressure variations across the die. The relatively low temperature profile during the extrusion led to an additional increase of the extrudate swell. Pulling away the protein extrudate from the die compensated the effect of swelling that is evident when the protein extrudate is drooling out of the die lip (see Fig. 2).

Additionally, the draw led to a molecular orientation in the machine (longitudinal) direction which is reflected in the improvement of the mechanical

Fig. 1. Aggregated sodium caseinate in extruder barrel zone 5 and 6.

Table 1. Extrudate composition and extrusion conditions for the extrusion (No. 1 and 2) and the reactive extrusion (No. 3 - 6; with MTG).

No.	Solid Feed					Liquid Feed				Throughput	Temperature profile	Rpm
	Protein		MTG			Water		Glycerol				
[-]	[wt.%]	[kg/h]	[wt.%]	[U/g]	[kg/h]	[wt.%]	[kg/h]	[wt.%]	[kg/h]	[kg/h]	[°C]	[1/min]
1	50.00	5	0	0	0	33.33	3.33	16.67	1.67	10	high[a]	285
2	50.00	5	0	0	0	33.33	3.33	16.67	1.67	10	low[b]	285
3	48.78	5	2.44	5	0.25	32.52	3.33	16.26	1.67	10	low[b]	285
4	47.62	5	4.76	10	0.5	31.75	3.33	15.87	1.67	10	low[b]	285
5	46.51	5	6.98	15	0.75	31.01	3.33	15.50	1.67	10	low[b]	285
6	47.62	5	4.76	10	0.5	31.75	3.33	15.87	1.67	10	low[b]	160-285[c]

[a]30-50-50-50-70-70-70-70-70-75-75 °C; [b]30-50-50-50-50-50-50-50-50-50-50 °C; [c]rotational speed variation from 160 to 285 min^{-1}

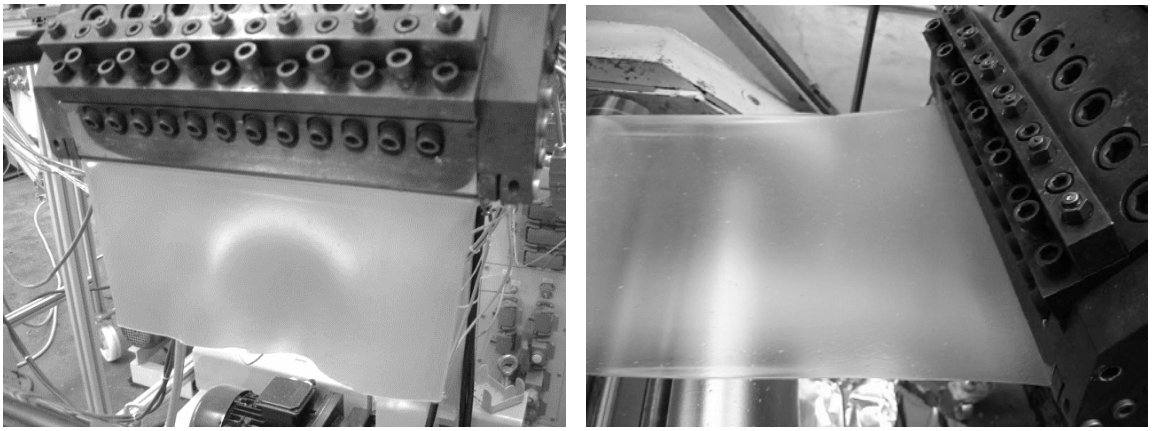

Fig. 2. Extruded sodium caseinate films; film drooling down (left) and draw down by the roll stack of the sheet extrusion line (right).

properties in comparison to properties achieved with samples measured in cross-machine (transverse) direction [1].

Occasionally, hard polymer specks protruding from the film surface, the so called “fish eyes”, were observed. This is due to high-molecular weight particles which, on the one hand, originate from dead zones leading to long residence times, allowing an additional cross-linking. On the other hand, the fusion of materials inside the die and quality fluctuation of the raw protein are other possible reasons for these defects [1].

The operating window for the formation of covalent cross-links in the protein network during the extrusion process is extremely narrow. An extensive cross-linking leads to the formation of thermosets (an irreversible hardening of the polymer after the curing) that result in an increase in torque and pressure and may cause a complete failure of the extruder. To control the cross-linking reaction, the enzymatic activity (units per gram protein), the temperature and the residence time were varied. The residence time was controlled by the rotational speed of the extruder, which in turn influences the temperature of the extrudate (higher screw speed leads to a higher melt temperature and a shorter residence time) [1]. The relationship between these important process parameters is presented in Fig. 3. The residence times were measured by adding a dye to the feed throat and monitoring the time until this coloring appeared at the die exit.

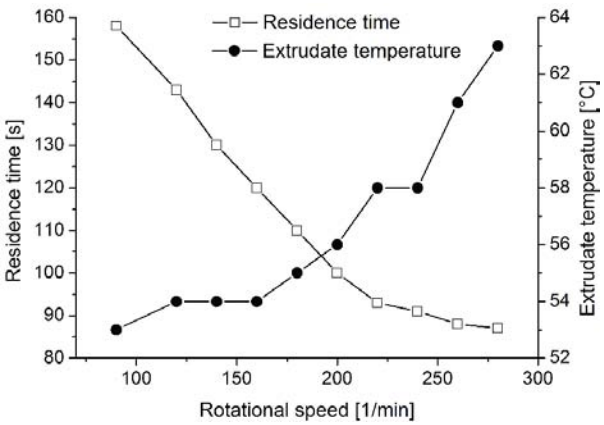

Fig. 3. Residence time and extrudate temperature at different screw speeds during a reactive extrusion with an enzymatic activity of 10 $U/g_{protein}$ and a temperature profile of 30-50-50-50-50-50-50-50-50-50-50 °C, experiment no. 6, Table 1.

The residence time shows a negative correlation to the increase of the screw speed and, consequently, a direct correlation to the extrudate temperature due to a viscous heat dissipation generated by the extruder motor turning the screw.

Regarding the implementation of a cross-linking reaction which is catalyzed by the enzyme MTG, a certain reaction time and temperature profile are required to achieve a high cross-linking density which then leads to products with enhanced properties.

SDS polyacrylamide gel electrophoresis was carried out according to the method of Laemmli [33] to

verify possible structural modifications of sodium caseinate during the reactive extrusion process. The pattern in Fig. 4 shows three protein bands (30–35 kDa) representing α-, β- and κ-casein, respectively [10]. By increasing residence time of particles in the extruder, the non-cross-linked sodium caseinate was converted to protein polymers resulting in protein bands between the stacking and the resolving gel and even on the top of the stacking gel. The ongoing cross-linking reaction was therefore visually confirmed by the residence time-dependent disappearance of the sodium caseinate substrate and the simultaneous formation of high-molecular weight protein aggregates.

In summary, the results shown in Fig. 4 reveal that a screw speed of 180 min^{-1} or less is crucial for the formation of high-molecular weight protein compounds. This in turn implies that a minimum residence time of approx. 100 s is required for a sufficient cross-linking of the proteins.

3.2. Tensile properties

The mechanical properties of the extruded sodium caseinate films, presented in Fig. 5, showed in the first instance a distinct dependence of the temperature profile used in the extruder barrel and the die. The low temperature profile (30-50-50-50-50-50-50-50-50-50-50-50 °C) which was also applied in the reactive extrusion experiments led to a significant increase in the film tensile strength ($P < 0.5$) in comparison to films extruded at a high temperature profile of 30-50-50-50-70-70-70-70-70-75-75 °C. This is probably due to an increased physical cross-linking of sodium caseinate as described in section 3.1. This leads to an inhibition of the elastic recovery of extrudates and, thereby, to network raptures lowering the tensile properties of the films.

Regarding the elongation at break no significant influence of the processing temperature could be observed. Particularly noticeable is the strong decrease in the elongation of samples measured in cross-machine (transverse) direction due to the relaxation of the extrudate at the die exit and the following molecular orientation in machine direction caused by the drawdown of the puller rolls. In general, a machine-directed molecular orientation resulted in higher tensile performance of the extruded protein films [1].

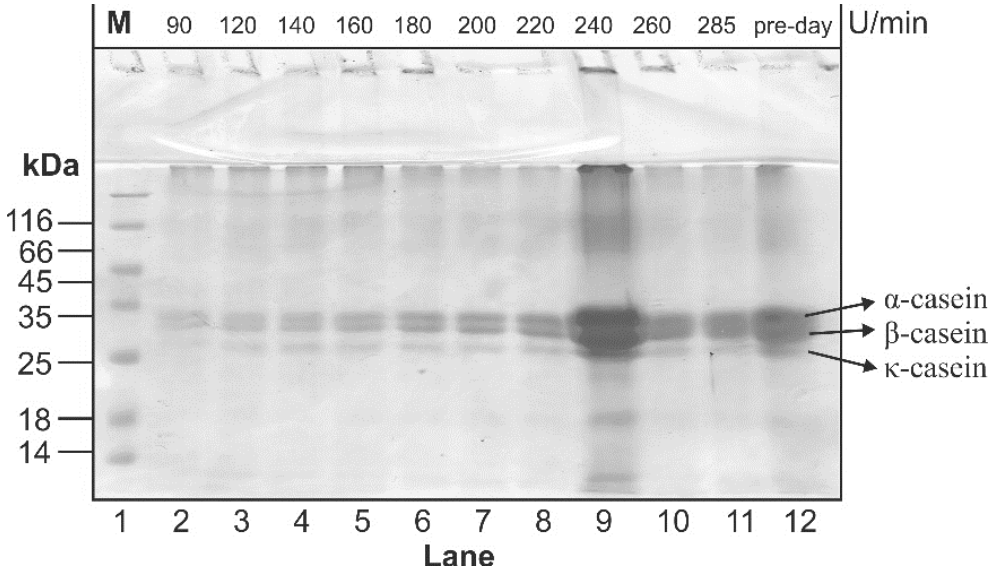

Fig. 4. SDS-PAGE pattern of extruded sodium caseinate (M: molecular weight marker (lane 1), lane 2 – 11: samples resulting from different screw speeds (90 - 285 min^{-1}), pre-day: dissolved MTG in liquid feed and stored for 24 h before fed and extruded at 240 min^{-1}); extrusion temperature profile: 30-50-50-50-50-50-50-50-50-50-50-50 °C, experiment no. 6, Table 1.

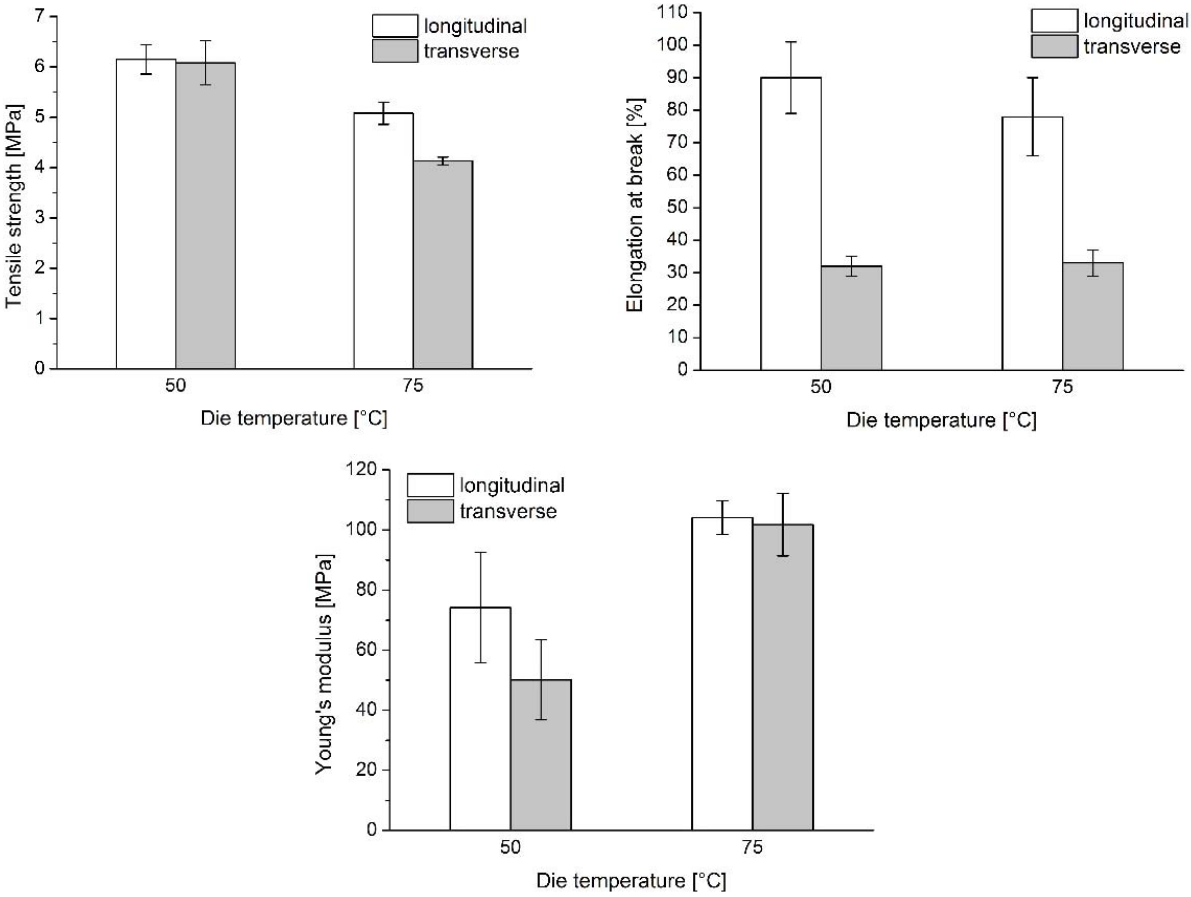

Fig. 5. Tensile strength, elongation at break and Young's modulus of extruded sodium caseinate films at different temperature profiles, experiments no. 1 and 2, Table 1; $\overline{X}$ ± SD, n = 5.

The general trend towards the influence of the preferential direction on the mechanical properties could be verified by the evaluation of the tensile properties of sodium caseinate films obtained by reactive extrusion. As shown in Fig. 6, an enzymatic cross-linking of proteins leads to significant higher tensile strength. Even with a very low enzymatic activity of 5 $U/g_{protein}$ (corresponding to 2.44 wt.% of the extrudate) an increase in the tensile strength of films from 6.15 to 11.35 MPa (longitudinal direction) was achieved - an increase of approx. 85%.

Of special relevance here is that despite the strong increase in the tensile strength no significant decrease in the elongation at break was detected. Young's modulus reaches its maximum at an enzymatic activity of 10 $U/g_{protein}$ in films measured in transverse direction.

On the basis of the correlations between the extruder screw speed and cross-linking density of the protein network, shown in Figs. 3 and 4, the relationship between the residence time (reaction time in a broader sense) and the mechanical properties is presented in Fig. 7. Here, a general trend towards the enhancement of the mechanical properties due to the increase of the residence time is observed.

In particular, residence times exceeding 95 s due to a decrease in the rotational speed of the extruder contribute to the increase in both tensile strength and elongation at break. This is due to the increase of the reaction time leading to a higher covalent cross-linking density. Altogether, a screw speed between 180 and 220 min^{-1}, which corresponds to residence times between 100 and 120 s leads to a maximal mechanical performance of the extruded sodium caseinate films.

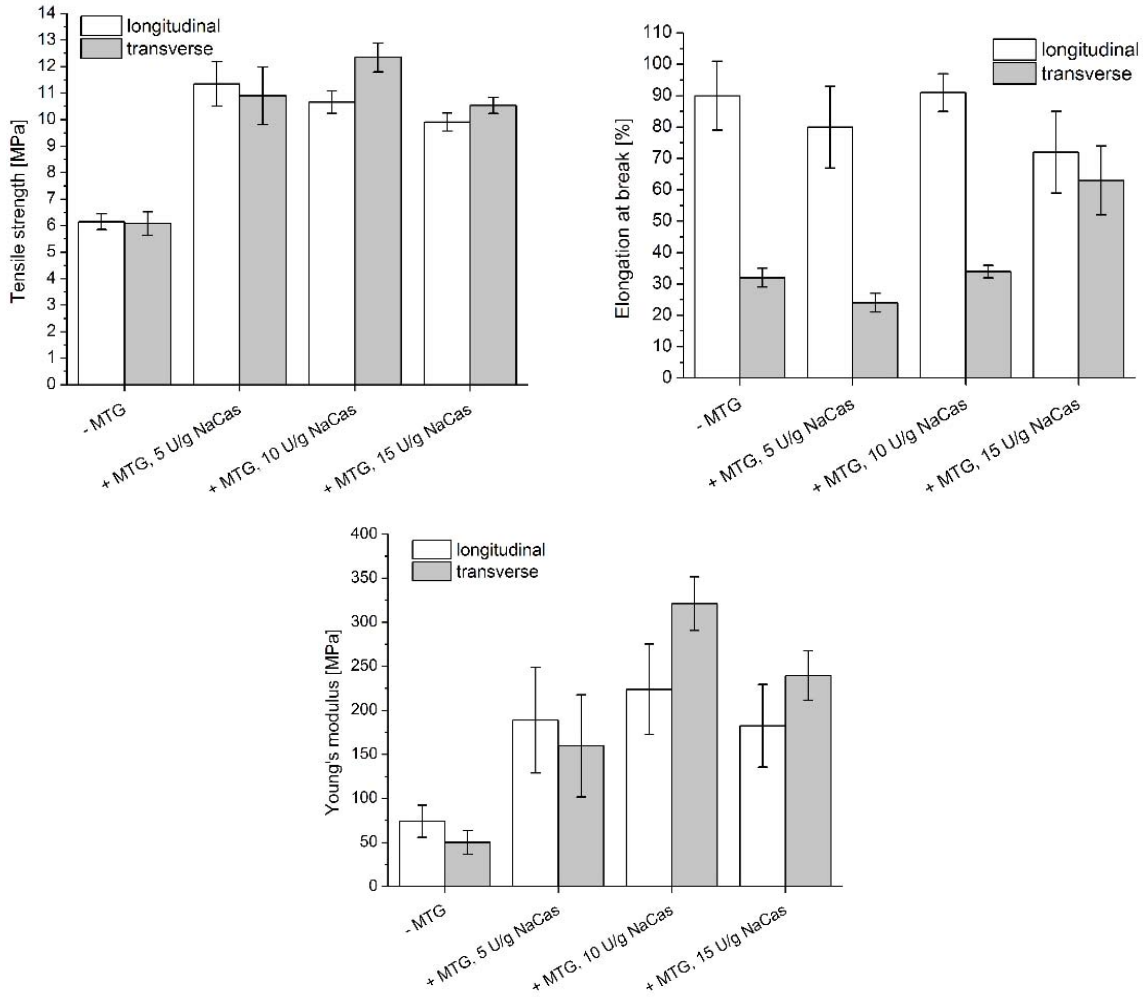

Fig. 6. Tensile strength, elongation at break and Young's Modulus of extruded sodium caseinate films at different enzymatic activities, experiments no. 2 - 5, Table 1; $\overline{X}$ ± SD, n = 5.

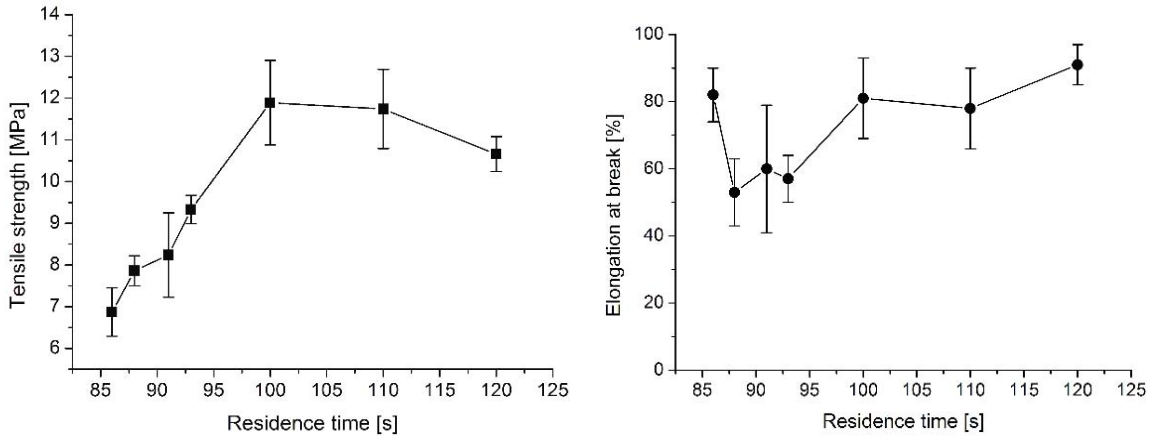

Fig. 7. Tensile strength and elongation at break of extruded sodium caseinate films at different residence times, experiment no. 6, Table 1; $\overline{X}$ ± SD, n = 5.

3.3. Crystallization

Homogenous, transparent to slightly yellowish and smooth structured sodium caseinate films were successfully produced by both the extrusion and the reactive extrusion. Visually similar protein films with inner-film crystallized potassium nitrate were the result of adding the crystalline additive in different concentrations (KNO_3/protein ratios of 1:12 to 1:4). The films showed no macroscopic visible crystalline structures wherefore micrographs were examined and presented in Fig. 8.

Independent of an enzymatic cross-linking the typical orthorhombic (aragonite-type) structures, uniformly distributed, with average crystal sizes between 30 and 100 µm were observed with KNO_3/sodium caseinate ratios of 1:6 and 1:4 at room temperature. Fig. 8 exemplarily shows incorporated potassium nitrate crystals with a KNO_3/sodium caseinate ratio of 1:6. Differences with respect to crystal sizes and structures in enzymatically treated and non-treated sodium caseinate films could not be ascertained. Stolte *et al.* [24] reported similar crystal morphologies for casted sodium caseinate films and, furthermore, investigated the influence of the drying conditions on the crystal structure.

Furthermore, tensile strength and elongation at break of inner-film crystallized sodium caseinate films dependent on the additive concentration and enzymatic cross-linking are discussed below. A distinct dependence of the potassium nitrate concentration on the mechanical properties is shown in Fig. 9.

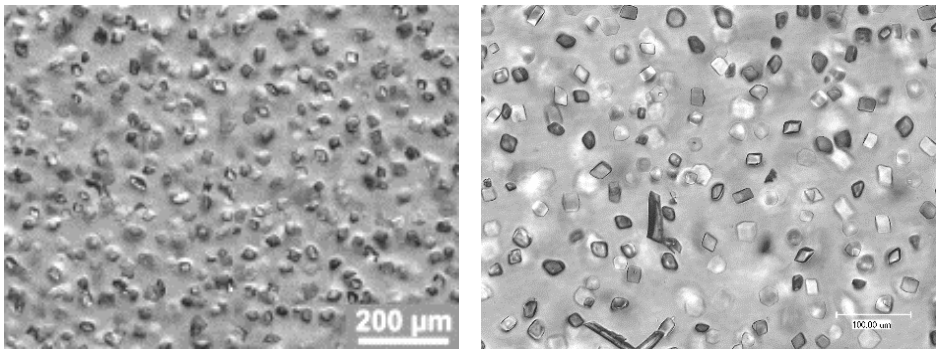

Fig. 8. Light microscopy exposures of sodium caseinate films with incorporated crystalline additive KNO_3 with a KNO_3/sodium caseinate ratio of 1:6.

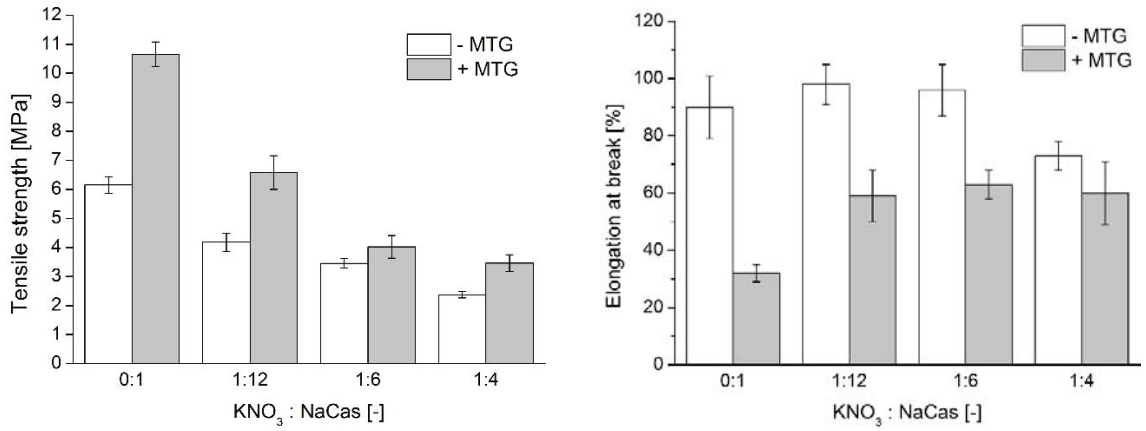

Fig. 9. Tensile strength and elongation at break of extruded sodium caseinate films at different KNO_3 concentrations; $\bar{x}$ ± SD, n = 5.

With increased additive concentration the tensile strength of the films was decreased independent of a cross-linking via MTG, whereas the elongation was enhanced, particularly in the case of cross-linked films. Even low additive concentrations in cross-linked films lead to a significant increase of the elongation ($P < 0.05$). In the case of a KNO_3/sodium caseinate ratio of 1:12 the additive yields an elongation increase of 84% compared to the films without KNO_3, but, at the expense of a significant tensile strength decrease. The results show good agreement with the influence of crystalline additives on the tensile properties of films obtained with the solution casting method reported by Frohberg *et al*. [11].

The alteration of the mechanical performance of films due to incorporated crystals appears to be related to a weakening of the intermolecular bonds which is partly compensated by covalent bondings as a result of the cross-linking reaction [13, 11].

4. CONCLUSION

In this study, the thermoplastic processing of sodium caseinate by a conventional twin-screw extrusion was successfully carried out. In a limited window of operating conditions, transparent, homogenous and smooth structured sodium caseinate films were produced without prior pelletizing of the raw protein powder. The processibility is highly dependent on the thermal transition which is related to the chain mobility. To avoid thermal degradation the use of glycerol as a plasticizer is essential to increase chain mobility by lowering the glass transition temperature of sodium caseinate below the decomposition temperature. Furthermore, structural changes and the formation of new intermolecular interactions due to chain alignment upon cooling and drawing-down the extrudate determine the physical properties of the extruded protein films [27].

Controllable process variables allow an *in-situ* cross-linking of sodium caseinate in the extruder barrel to improve the techno-functional properties of the extruded films. Therefore, the early formation of covalent cross-links needs to be avoided to ensure an elastic recovery without rapture after exiting the die. This was achieved by controlling the enzymatic activity and the reaction time. Considering the physicochemical nature of proteins an appropriate additive (KNO_3) and plasticizer (glycerol) were selected which, in combination with controlled process conditions, led to protein-based biopolymers with adequate physical properties.

The combination of the biochemical approach of an enzymatic-catalyzed cross-linking with inner-material crystallized additives offers a multitude of possibilities not only for the optimization of the material properties but also for new and advanced product benefits, e.g. carrier and release systems with time-controlled additive release and material decomposition for applications in agriculture (mulching films and seed tapes) as well as in pharmacy/medicine in the form of drug delivery systems and scaffolds. By further addition of dyes, antimicrobials, antioxidants and flavors the area of application can be widened (e.g. edible food packaging).

This study reveals the potential of covalent cross-linked and crystallized protein films produced by the extrusion technique, and thus opens a completely new field of interdisciplinary research by combining biopolymer engineering, industrial crystallization and product design through process design.

CONFLICT OF INTEREST STATEMENT

The author declares no competing financial interest.

ABBREVIATIONS AND SYMBOLS

Abbreviations

DIN	:	Deutsches Institut für Normung e.V.
EC	:	Enzyme Commission
EN	:	European Organization for Standardization
ISO	:	International Organization for Standardization
MTG	:	Microbial transglutaminase
SD	:	Standard deviation
SDS	:	Sodium dodecylsulfate
SDS-PAGE	:	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SME	:	Specific mechanical energy

Symbols

m	:	[g; kg] Mass
M	:	[Da; g/mol] Molecular weight
n	:	[-] Sample number
P	:	[-] Probability
rpm	:	[min^{-1}] Rotations per minute
T	:	[°C] Temperature
U	:	[µmol/min] Enzyme units
$\bar{x}$	:	[-] Mean value

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