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Structural, rheological and functional properties of extruded mozzarella cheese influenced by the properties of the renneted casein gels

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ABSTRACT

Renneted casein gels, often named cheese curds, are traditionally pre-heated by immersion in water before stretching, which influences cheese functional properties. This work aims to understand the effect of pre-heating (cooking) on extrusion process of cheese curds at 80 and 90 °C in a lab twin-screw, co-rotating extruder, and consequences for composition, microstructure and functional properties of the extrudates. The cooking significantly enhanced curd meltability and oiling-off, resulting in faster extrusion, higher exit temperature and lower specific mechanical energy. Microscopic observations at different length scales, combining confocal laser scanning microscopy and X-ray micro-tomography, showed that cooking caused extensive fat coalescence and cracks in protein matrix, resulting in extrudates with less fibrous structure, lower elasticity and tensile strength. The extrusion process induced significantly increased of calcium bridges in extrudates, which may replace weak water-protein interactions and increase relaxation time of the more mobile water fraction ($T_{2,2}$). The specific mechanical energy was less influenced by the extrusion temperature in cooked than uncooked curds, likely due to a more extensive oiling-off during extrusion of the cooked curd. In agreement with the microscopic observations, extrudates obtained at 80 °C differ from 90 °C specially regarding fat content respectively 23.8 – 24.8% and 29.5% (w/w) and fat globule size and distribution, influencing fiber formation. In general curds extruded at 90 °C had higher elasticity and tensile strength, which was related to the well-elongated fat domains. This study provided knowledge about extrusion process of curds generating new ideas to improve cheese processing and design customized cheese products.

1. Introduction

Mozzarella cheese is one of the most consumed cheeses worldwide (Francolino, Locci, Ghiglietti, Iezzi, & Mucchetti, 2010). As a member of the pasta-filata cheese family, mozzarella has a unique characteristic
fibrous texture that is created during the cooking-stretching process of cheese curds. In this production step the cheese curd, a renneted casein gel, is initially pre-heated (cooked), in hot water or by steam injection, and then stretched in rotating screws of variable configurations, promoting heat transfer, phase transition, molecular redistribution and structure formation. The purpose of the cooking step is to reach the gel-sol transition temperature, which is necessary to plasticize the cheese curd in order to obtain a fibrous protein structure during the subsequent stretching step (Bähler, Ruf, Samudrala, Schenk, & Hinrichs, 2016). During water cooking the protein matrix contracts, influencing the water distribution in the curd (Smith, Vogt, Seymour, Carr, & Codd, 2017), and causing coalescence of fat globules (Rowney, Roupas, Hickey, & Everett, 2003). Furthermore, fat was considerably lost during cooking, accompanied with increased protein content and changes in crossover temperature (T_c) (Banville, Chabot, Power, Pouliot, & Britten, 2016, Feng, Lillevang, & Ahnér, 2021). Thus, the cooking step determines the curd composition, structure and rheological properties which consequently will influence its behavior during the following stretching process.

Mozzarella manufacturers use a wide range of industrial equipment for processing pasta-filata cheese, and the thermomechanical treatments used for stretching the curds may differ considerably depending on the equipment used (Banville et al., 2016). Although the cooker-stretcher is the most used device for mozzarella, twin-screw extruders are of increasing interest as they are more flexible, allowing extensive variation in thermal and mechanical energy inputs and mixing efficiencies (Bouvier & Campanella, 2014). An extruder typically consists of two distinct functional sections: the screw section and the cooling die section. Thermomechanical stresses generated by the screws, induce structural changes on the material, which is subsequently forced to flow through the cooling die where the final structure and shape of the products are defined (Emin & Schuchmann, 2017). In the cooling zone, the decrease in temperature determines the rearrangement and the cross-linking of protein molecules, forming a fibrous structure. These interactions and molecular changes during extrusion have a noticeable effect on the functional properties of the final extruded products (Zhang et al., 2017).

Studies regarding high moisture extrusion of dairy proteins are limited. Whey protein isolate has been extruded and the effect of moisture and temperature on protein solubility, molecular structure, and protein quality of the extrudates have been reported (Onwulata, Konstance, Cooke, & Farrell, 2003; Afizah & Rizvi, 2014; Qi & Onwulata, 2011a; 2011b). Cheese curds have been recently extruded in a single screw extruder showing the possibility to produce homogeneous pasta-filata cheese with reduced water usage and milk solids loss, compared to conventional cooker-stretchers (Kern, Bähler, Hinrichs, & Nobel, 2019). Further studies, by Kern, Scharfe, and Hinrichs (2020), showed the possibility to develop innovative products and proposed a mechanism to explain the creation of anisotropic casein-based extrudates by sheet die extrusion. Suitable temperature for extrusion was observed at 50–70 °C and maximum shear stress <20 kPa, and products with mechanical properties similar to plant-based extruded products could be obtained (Kern et al., 2020). Our previous study, using a lab twin-screw extruder, cheese curds were extruded at specific mechanical energy (SME) ranging from 18 to 390 kJ kg⁻¹ and a higher extrusion temperature has shown to enhance curd elasticity and reduce melt strength while a higher cooling temperature led to extrudates with lower water retention and melt strength (Feng, van den Berg, Lillevang, & Ahnén, 2022). These studies provided better understanding about the extrusion process and its potential application in cheese manufacturing. To the best of our knowledge no study exist regarding the effect of the composition and structure of cheese curds on the extrusion process, and consequences for the properties of the extruded cheese.

Since water cooking of the curd is typically applied before stretching, during the manufacture of mozzarella cheese, the objective of this study is to understand extrusion of cooked curd, in comparison with uncooked curd, at temperatures 80 and 90 °C using a lab-scale twin-screw extruder. To achieve this objective, the curd composition and structure, at different length scales, of the uncooked and cooked curds were assessed and related with the compositional, structural and functional properties of extruded curds.

2. Materials and methods

2.1. Curd preparation

Frozen renneted and cultured (by lactic acid bacteria) ‘Cagliata’ mozzarella curd was provided by Arla Foods (Denmark). The curd block was defrosted at 4 °C overnight before being cooked. Cooked curd was obtained by cutting the curd into 1 cm³ cubes using a wire cutter, and cooking with 2.5% (w/w) NaCl solution at 80 °C for 4 min in a water bath shaker (Grant Instruments, Cambridge, UK) at 125 rpm. The curd-to-solution ratio was 1:1 (w/w). After cooking the curd was immediately cooled in ice water. Prior to analysis and extrusion process, both the uncooked and cooked curds stored at 4 °C in a cold storage room overnight, were kept at room temperature for at least 2 h.

2.2. Shearing of uncooked and cooked curds in a twin-screw extruder

A lab co-rotating twin-screw extruder Process 11 Hygienic (ThermoScientific, Germany) was used for the shearing process. The extruder barrel is divided into seven zones that can be heated separately. Grinded curd and cooked curd were fed through a custom build feeding tube located at the first zone (at the very beginning of the screws). The heating temperature, 80 or 90 °C, was the same for all the zones. Screw speed and cooling temperature were respectively 150 rpm and 30 °C. Torque and pressure were recorded during the process, as was the exit temperature (T_exit) of extrudates. Each production was done in duplicate, and the collected extrudates were vacuum-packaged and aged at 4 °C for 2 weeks before analysis. Mass flow, residence time (RT), net torque (Torque_net) and SME were calculated as reported in Feng et al. (2022). Images of the extruded curds were taken by the Videometer system (Videometer A/S, Harsholm, Denmark).

2.3. Compositional analysis

Moisture content was determined by the oven-drying method at 105 °C by mixing 2 g sample with 20 g sand (Horwitz, 2000). Nitrogen content was quantified using a Dumas protein analyzer (rapid MAX N exceed, Elementar, Germany). A conversion factor of 6.38 was used to obtain the protein content from the nitrogen content. Fat content was determined by Gerber method (IDF, 1981). Calcium content was measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Agilent Technologies, Santa Clara, CA, USA). Before analysis, 1 g of sample was digested with 8 ml HNO₃ (65%) and 2 ml HCl (37%) using a Multiwave GO microwave (Anton Paar, Graz, Austria). Standards in a range of 0.04–20 mg L⁻¹ were prepared from multi-element (Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Ti, Zn) standard solution IV from Merck KGaA. Standard curves were determined at the wavelength of 396.847 nm. All analyses were performed in duplicate for each of the duplicated extrusion process.

2.4. Rheology of uncooked and cooked curds

Crossover temperature T_c of the curds was determined on a rheometer (Discovery HR-2, TA Instruments) with 25 mm diameter serrated parallel plates. Disc-shaped samples of 25 mm diameter and ~2 mm thickness were prepared and equilibrated for 2 min at 20 °C. A 1N normal force was used to define the measurement gap. A ring of paraffin oil was placed around the sample periphery to avoid moisture loss during rheological measurements. During the temperature sweep test, strain and frequency were 0.1% and 1 Hz respectively and the
temperature was increased from 20 to 100 °C. The rate of the Peltier heating system was set at 3 °C-min⁻¹. The measurement was conducted at least in duplicate for uncooked and cooked curds.

2.5. Meltability and oiling-off

A test modified from the Schreiber test was used (Kindstedt, Duthie, & Rippe, 1988) with conditions based on Feng, Barjon, van den Berg, Lillevang, & Ahnér (2021b) to evaluate the curd meltability, in which a curd cylinder (15 mm thick, 25 mm in diameter for uncooked and cooked curds; 3 mm thick, 5 mm in diameter for extrudates) was placed in a glass Petri dish in a 200 °C oven, kept for 2 min, and cooled to room temperature. Images of the samples were taken by the Videometer system. The area of melted curd was measured using image analysis software Fiji (Fiji 1.53c, USA). Meltability was evaluated as dividing the area of melted curds by the original area of the curd cylinders. Measurements were done in duplicate for uncooked, cooked and extruded curds.

Oiling-off was determined by the methods of Breene, Price, and Ernstström (1964) and Feng, Barjon, van den Berg, Lillevang, & Ahnér (2021). Whatman No.1 filter paper was placed on top of an aluminum pan. A curd cylinder (5 mm thick, 25 mm in diameter for uncooked and cooked curds; 1 mm thick, 5 mm in diameter for extrudates) was placed on the filter paper, put in an 80 °C oven for 10 min, and cooled at room temperature for 30 min. Images with the area of each oil ring formed on the filter paper were measured by Fiji. Oiling-off was evaluated by dividing the oily area of melted curds by the original area of curd cylinders. Measurements were done in duplicate for uncooked, cooked and extruded curds. One extruded sample was taken from each of the duplicated extrusion productions for the duplicated analyses.

2.6. Tensile test of extruded curds

The tensile test was carried out with a TA.XTplus texture analyzer (Stable Micro Systems, Godalming, UK) to describe the tensile behavior of the extruded curds, as previously described in Feng et al. (2022). More specifically, 20 cm of extruded curd was assembled to the Spaghetti tensile grips (A/SPR) with an initial distance of 2 cm between the grips. Pre-test speed was 0.1 cm s⁻¹, test speed 0.3 cm s⁻¹ and post-test 1.0 cm s⁻¹. The tensile force was recorded until breaking of the curd strips. The distance at the breaking point relative to 2 cm was used to evaluate tensile behavior, expressed as elasticity (%), and the force at the breaking point was expressed as tensile strength (g). Measurements were done in at least triplicate at room temperature. Three extruded samples were taken from each of the duplicated extrusion productions for the duplicated analyses.

2.7. Water mobility

Low field nuclear magnetic resonance (LF-NMR) experiment was performed at 20 °C using an Oxford Instruments spectrometer (UK) with a receiver gain of 5.0%, tau value of 100 μs and 8 scans with 8000 echoes. Samples were placed into 1.8 cm diameter NMR tubes and the T2 relaxation times and the corresponding area fractions were obtained using the Carr, Purcell, Meiboom and Gill (CPMG) pulse sequence. A two-component model was used to fit the exponential decay. More details can be found in Feng et al. (2022). Measurements were done in triplicate for uncooked, cooked and extruded curds. Three extruded samples were taken from each of the duplicated extrusion productions for the triplicated analyses.

2.8. Protein secondary structure

Freeze-dried (24h) curd samples were grinded to powder for FTIR analysis according to (Carbonaro, Maselli, & Nucara, 2012) using an ABB Bomem FTIR spectrometer (Canada). Measurements were performed in triplicate at room temperature, in the mid-infrared region (4000-500 cm⁻¹) with a resolution of 4 cm⁻¹, 64 scans. The relative weights obtained by multi-peak fitting were considered as estimates of the secondary structure percentages of the protein amide range. The attribution of secondary structure was performed according to frequency in the region of amide I band (~1600-1700 cm⁻¹) as defined in Table 1, and the quantification was done as percentages from the integrals of the deconvoluted bands (Hu, Zheng, Liu, Deng, & Zhao, 2016). Measurements were done in at least triplicate for uncooked, cooked and extruded curds. Three samples were taken from each of the duplicated extrusion productions for the triplicated analyses.

2.9. Protein interactions

Different types of bonds (covalent and non-covalent) responsible for stabilizing the protein structure of the curd samples were semi-quantitatively estimated. The analysis was performed according to the procedure described in Gonçalves & Cardarelli, 2019 with some changes. 2 g of grinded sample was transferred to 50 mL plastic tubes with 20 g of the different buffer solutions. Buffering solution B1 contained 12.12 g L⁻¹ Tris (Merk, Germany) and 1 g L⁻¹ SDS (Merk, Germany). The pH value was adjusted to 7.2 with 1M HCl (Merk, Germany). Buffer B2 was prepared directly before application by adding DTT (Sigma, Germany) to B1 (5g L⁻¹). Buffer B3 contained 9 g L⁻¹ sodium chloride (Merk, Germany) and 7.8 g L⁻¹ sodium dihydrogen phosphate dihydrate (Merk, Germany). The pH value was adjusted to 7.2 with 1M NaOH (Merk, Germany). Buffer B4 contained 14.6 g L⁻¹ EDTA (Merk, Germany) and 17.9 g L⁻¹ tri-Na-citrate (Merk, Germany). The pH value was adjusted to 8.0 with 1M NaOH (Merk, Germany). The solutions were intensively mixed using an Ultra-Turrax (T25 basic, IKA, Germany) at room temperature for 5 min. Then, the samples were placed on a multipoint stirrer (Cimarec-i Poly 15, Thermo Scientific) for 30 min and centrifuged (Sorvall RC-6 Centrifuge, LabX) at 15000 g for 20 min at 20 °C. The supernatant was then filtered with 0.2 μm membrane filters (OE66, Whatman, Germany), and the total nitrogen content (Nₗ) was determined. The nitrogen contents of the buffer solutions (Nb) and curd samples (Nc) were also determined. The nitrogen content is a measure of the amount of the bonds, which participates in the stabilization of the curd protein network. In order to obtain information about the amount of protein stabilized by the different interactions, following equations were defined (Gonçalves & Cardarelli, 2019):

\[ N_{\text{hydrophobic}} = \frac{m_B + m_C}{m_C} N_s - \frac{m_B}{m_C} N_b \]  

\[ \frac{N_{\text{hydrophobic}}}{N_c} = P(EB) + P(Hy) + P(HB) + P(ub) \]  

\[ \frac{N_{\text{hydrophobic}}}{N_c} = P(EB) + P(Hy) + P(HB) + P(SS) + P(ub) \]  

\[ \frac{N_{\text{hydrophobic}}}{N_c} = P(EB) + P(HB) + P(ub) \]  

Table 1: Assignments of secondary structure of proteins in the region of the deconvoluted band amide I.

| Secondary structure | Stretch frequency (cm⁻¹) |
|---------------------|--------------------------|
| β-sheet             | 1600-1640                |
| random coil         | 1640-1650                |
| α-helix             | 1650-1660                |
| β-turn              | 1660-1700                |
\[ \frac{N_{\text{bond,B4}}}{N_c} = P(EB) + P(HB) + P(CaB) + P(ub) \]  
\[ P(SS) = \frac{N_{\text{bond,B1}}}{N_c} - \frac{N_{\text{bond,B3}}}{N_c} \]  
\[ P(Hy) = \frac{N_{\text{bond,B1}}}{N_c} - \frac{N_{\text{bond,B3}}}{N_c} \]  
\[ P(EB) + P(HB) + P(ub) = \frac{N_{\text{bond,B3}}}{N_c} \]  
\[ P(CaB) = \frac{N_{\text{bond,B4}}}{N_c} - \frac{N_{\text{bond,B3}}}{N_c} \]

where \( N_{\text{bond,B1}}, N_{\text{bond,B2}}, N_{\text{bond,B3}}, N_{\text{bond,B4}} \) are the nitrogen contents of the supernatant obtained from the treatment of the samples with buffer solutions B1, B2, B3 and B4 (g·g\(^{-1}\)); and \( P(x) \) is the amount of protein (%) stabilized by x interactions, where x corresponds to electrostatic interactions (EB), hydrophobic interactions (Hy), hydrogen bridges (HB), disulphide bridges (SS), calcium bridges (CaB) and free proteins (ub). Measurements were done in duplicate for uncooked, cooked and extruded curds. Two extruded samples was taken from each of the duplicated extrusion productions for the duplicated analyses.

### 2.10. Microstructure

#### 2.10.1. Confocal laser scanning microscopy (CLSM)

The sample preparation and microscopy method were adapted based on previous work by Ong, Dagastine, Kentish, & Gras. (2011) and Lamichhane, Auty, Kelly, & Sheehan. (2020). A piece of curd (1 mm thick) was cut using a razor blade at room temperature (20°C) and the extrudates were cut parallel to the extrusion direction. Fast Green FCF and Nile Red of concentration 0.01% (w/v), prepared in deionized water (80°C) and 1,2-propanediol respectively, were mixed in a ratio of 1:3.50. The image pixel resolution was 1024 \( \times \) 1024. The imaging was performed with Leica LAS AF software and showed the fat in red and protein in green.

#### 2.10.2. X-ray microcomputed tomography (\( \mu \)CT)

The 3D microstructure of the curd samples was imaged with X-ray micro-tomography (Zeiss Versa 520 \( \mu \)CT). The source voltage was set to 60 kV with 5W power. The effective pixel size on the detector was adjusted to 3 \( \mu \)m using a 4 \( \times \) magnifying microscopic objective coupled to a scintillator for X-ray to visible light conversion. 1600 X-ray transmission projection images were acquired while rotating the sample over 360°. The exposure time for each angular projection was set to 3 s resulting in a total scan time of 80 min. The tomographic reconstruction resulted in 3D images with the dimensions of 1000 \( \times \) 1000 \( \times \) 1000 voxels covering a total volume of 3 \( \times \) 3 \( \times \) 3 mm\(^3\) in the interior of the larger sample. We used Avizo (Avizo 2021.1, FEI SAS, Thermo Fischer Scientific) for the 3D visualization of the structure.

### 2.11. Statistical analysis

The extrusion process was performed in duplicate, and the uncooked and cooked curds, as well as extruded curd products, were analyzed in at least in duplicate. For each analyses, at least two extruded samples were taken from each of the duplicated extrusion productions for the analyses. One-way analysis of variance (ANOVA) followed by the Duncan test was done to verify differences between means of properties of all the curd samples using IBM SPSS Statistics 28 (IBM Corporation, Somers, NY, USA). The t-test was applied when comparing only two datasets (i.e. uncooked and cooked curds) using the same software. Differences were considered significant at the probability level \( p \leq 0.05 \).

### 3. Results and discussion

#### 3.1. Effect of cooking on curd composition, microstructure and physicochemical properties

The composition, microstructure and physicochemical properties of uncooked and cooked curds – the two distinct materials to be extruded in this study - were analyzed to better understand the effect of cooking on curd behavior during the extrusion process.

The moisture, fat and calcium contents of the curd were not significantly influenced by the cooking process (Table 2), while a significant increase (10%) in protein content was observed (\( p < 0.05 \)). Banville et al. (2016) also reported increase in 1–3% of protein content after a thermomechanical processing (without water) at 60–70°C. The increase in protein content maybe due to calcium binding to caseins that is promoted at higher temperature due to the calcium phosphate having inverse solubility, i.e. less soluble at high temperature (Barone, Yazdi, Lillevang, & Ahnre, 2021). Consequently, a decreased Ca:protein ratio from 32.2 to 30.0 mg Ca g\(^{-1}\) protein was observed due to the increase of

| Table 2 | Composition, water distribution, secondary protein structure, protein interaction, rheology and functionality of uncooked and cooked curds. |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| Properties | Uncooked curd | Cooked curd |
| Composition | Moisture (%) | 45.5 ± 0.2a | 45.4 ± 0.3b |
| | Protein (%) | 23.3 ± 0.1a | 25.7 ± 0.7b |
| | Fat (%) | 28.0 ± 0.7a | 28.5 ± 0.7a |
| | Ca (%) | 0.75 ± 0.01b | 0.77 ± 0.03b |
| | Caprotein(mg g\(^{-1}\) protein) | 32.2 ± 7.8 | 30.0 ± 0.1 |
| Water distribution | T\(_{2,1}\) | 14.9 ± 0.4a | 16.2 ± 0.1a |
| | A\(_1\) (%) | 82.3 ± 0.1a | 83.1 ± 0.1b |
| | T\(_{2,2}\) | 61.3 ± 0.5a | 65.5 ± 0.1b |
| | A\(_2\) (%) | 17.7 ± 0.1b | 16.9 ± 0.1 |
| Secondary protein structure | \( \alpha \)-helix | 37.3 ± 0.2 | ND |
| | \( \beta \)-sheet | 13.1 ± 1.3 | 20.4 ± 4.6 |
| | \( \beta \)-turn | 10.8 ± 0.5b | 10.3 ± 3.4 |
| | RC | 38.8 ± 1.6 | 69.3 ± 3.2 |
| Protein interaction | SS | ND | 6.6 ± 1.4 |
| | HY | ND | ND |
| | EB + HB | 32.4 ± 6.1a | 28.6 ± 0.6a |
| | CaB | 36.1 ± 7.8a | 29.7 ± 5.8 |
| Rheology/Crossover temperature | T\(_{c}\) | 70.9 ± 2.8 | 72.7 ± 1.0 |
| Functionality | Meltability | 1.1 ± 0.0b | 1.3 ± 0.05 |
| | Oiling-off | 1.1 ± 0.0b | 3.0 ± 0.3b |

\( a, b \) Means with different superscript letters in the same row are significantly different.
protein content and insignificant loss of calcium. The Ca/protein ratio has been reported to increase the strength of the protein network (Pastorino, Hansen, & McMahon, 2003). The protein network strength is expected to influence the extrusion of the curd.

Significant differences were observed between the microstructure of the uncooked and cooked curds analyzed by confocal laser scanning microscopy (CLSM) (Fig. 1a and b) and X-ray microcomputed tomography μCT (Fig. 1c and d). Fig. 1a, shows that uncooked curd exhibited evenly distributed, small, native fat globules (red) embedded in the protein network (green). In comparison, the curd structure after cooking was characterized by bigger fat domains (Fig. 1b) due to fat coalescence. Cracks/water cavities (black) in the protein matrix of cooked curd were also observed, suggesting breakdown of the curd protein network. Fig. 1c and d shows the grey level cross-section images of the curds acquired by μCT. The contrasts in these images are caused by the variations in density and composition of the curds (Laverse, Mastromatteo, Frisullo, & Del Nobile, 2011). The brighter regions correspond to the higher density state (Frisullo, Laverse, Marino, & Del Nobile, 2009), i.e., protein matrix in this study. The dark grey areas represent fat since they have a lower absorption coefficient compared to protein (Nagdalai et al., 2021). The white dots were recognized as salt particles (Einarsdottir et al., 2014), only presented within the protein network. One advantage of μCT, compared to CLSM, is the 3D nature that provides a complementary, complete view and additional details (e.g., isotropy, salt distribution) of the curd sample at the micrometer scale. As shown in Fig. 1c, fat and salt were homogeneously distributed in the protein network of uncooked curd, while the cooked curd exhibited substantially coalesced fat or water domains, presented as large, irregularly-shaped dark areas (Fig. 1d). The μCT cannot distinguish between fat and water, provided relevant information about the anisotropy of the curds, that is not so clearly observed by CLSM.

Water mobility in the curd maybe associated with the structure density of the protein network. Water translation and the rotation of molecules, as well as, the chemical exchange process between water molecules and biopolymers or other solutes can be estimated by the relaxation time of protons (T2) (Luo, Pan, Guo, & Ren, 2013). Components with shorter (T2,1) and longer relaxation times (T2,2) are attributed to the protons in less and more mobile fractions of water, respectively. A1 and A2 are the corresponding proportions of these two fractions. Although, the microstructure of cooked curds significantly differs from the uncooked curds, in general the cooking process did not induce major changes on water mobility as shown in Table 2. The relevant values i.e. T2,1, A1, T2,2 and A2, although being significantly different (p > 0.05) due to the small uncertainty in the LF-NMR measurements, the largest difference was observed for the more mobile fraction of water (T2,2) to be approx. ~7%. The overall increase in water mobility after cooking process is in agreement with the presence of cracks in the protein network and fat coalescence, as observed in the microstructure characterization (Fig. 1). Previous studies reported that fat opens up the protein matrix allowing water to accumulate in serum pockets (McMahon, Fife, & Oberg, 1999). As also found by Smith et al. (2017), heating could cause an increase in molecular mobility in cheese as the association between the casein and water is reduced at elevated temperatures.

To investigate the structural changes on the protein matrix network caused by cooking, the protein secondary structures and the type of protein interactions in the cheese curd were analyzed. Alterations of the protein secondary structure suggest changes in the functional/reactive sites located on these structures (Tarhan & Kaya, 2021), which consequently affect the curd structure during processing. The change in protein structure in cheese have been linked to changes induced by microbial proteolytic activity during ripening (Lucia, Daniela, & Rosalba, 2001; Wang et al., 2011). The presence of β-sheet structures has been related to the increase in protein digestibility (Carbonaro et al., 2012). In this study, β-sheet slightly increased while β-turn was not significantly influenced by the cooking process (Table 2). Heat treatment was reported to alter milk protein conformation in the unfolded molecular structure, causing exposure of β-sheets and β-turns (Tarhan & Kaya, 2021). The slight changes in the two structures observed in this study might be due to a less extensive heating process. A significant increase from 38.8 ± 1.6 to 69.3 ± 3.2% was observed in the random coil (RC) conformation as well as a decrease of α-helix from 37.3 ± 0.2 to 0%. RC has been claimed to be typical of caseins, the major class of protein in milk and mozzarella cheese (Carbonaro et al., 2012). The increase of RC was due to a reduction of α-helices that transitioned to RC structures. Hence, the cooking process induced substantial changes in the protein structures, possibly related to the unfolding and aggregating of casein proteins during heat treatment, which will be further discussed in the following sections.

The main protein interactions observed in uncooked curd are electrostatic interactions (EB), hydrogen bonds (HB) and calcium bridges (CaB) while hydrophobic interactions (Hy) and disulfide bridges (SS) were not found (Table 2). This is in agreement with previous studies showing that during the renneting process, paracasein micelles in curd interact mainly by exposing their hydrophobic cores and through electrostatic and calcium-mediated ionic bonds (Ono et al., 2017; Panteli, Zoidou, & Moatsou, 2015). Contrary, Gonçalves & Cardarelli (2019) found the predominant bonds among the proteins in the renneted curd to be Hy, and the number of CaB to be minimal, as was the number of SS (Gonçalves & Cardarelli, 2019). The contradiction might be explained by the different curd compositions and processing treatments in the different studies. A key factor is the concentration of calcium ions, which act by crosslinking caseins containing serine phosphate residues or by neutralizing charged compounds/structures (Fox, O’connor, McSweeney, Guinee, & O’Brien, 1996). The protein structure always turns to the more thermodynamically stable form (Happler, 2013), and the bond reorganization process takes place following the hierarchical order CaB > EB > HB > Hy in which stronger bonds tend to replace weaker bonds as the disaggregation of the structure exposes new reactive sites (Amaro-Hernández et al., 2022). Therefore, in this study, the higher calcium content in Cagliata curd (0.75%), compared to that in the curds reported by Gonçalves & Cardarelli (2019) (0.4-0.5%) (Gonçalves & Cardarelli, 2019), could lead to a replacement of Hy by CaB during curd processing. Only a small proportion of SS (7%) appeared in the curd after cooking, while the cooking process did not significantly influence Hy or even the dominant bonds EB + HB and CaB, perhaps because of the similar calcium content in the two curds (0.75 ± 0.01 and 0.77 ± 0.01).
Cooking increased, but not significantly (p > 0.05), the crossover temperature $T_c$ (70.9 ± 2.8 to 72.7 ± 1.0 °C) indicating that the thermal energy required for the occurrence of gel-sol transition was not so different for uncooked and cooked curds. In our previous study (Feng, Lillevang, & Ahné, 2021), curd cooked at 80 °C/4 min showed a $T_c$ of 76 °C. Although, the value is in the same order of magnitude the different results are likely due to variations in the composition of the curd.

However, the cooking process significantly increased the curd meltability and oiling-off (p ≤ 0.05) (Table 2). Meltability of cooked curd is 18% higher than that of uncooked curd, and oiling-off increased by 168% after cooking. These two properties have been associated with size of fat globule domains where larger fat globules were reported to induce higher meltability and oiling-off (Rowney et al., 2003). This is a good agreement with the differences in microstructure between cooked and uncooked curds observed in Fig. 1. The uncooked curd exhibited evenly distributed, small, native fat globules (red), which could delay the melting and oiling-off because they were embedded in the protein network (green). In addition, the cracks/water cavities (black) in the protein matrix of cooked curd, further accelerate curd melting and free oil release.

### 3.2. Characterization of extrusion process of curds

The measured/calculated extrusion parameters (pressure, mass flow, RT, $T_{exit}$, torque$_{net}$ and SME) obtained with the cooked and uncooked curds as feeding materials and controllable parameter $T_h$ are presented in Table 3. In general, a higher extrusion pressure was needed to extrude the uncooked curds (4–16 bar) compared with cooked curds (0–4 bar), indicating that melted cooked curds have a lower viscosity than uncooked curds since pressure is a function of material viscosity (Feng et al., 2022). These results were in agreement with the higher meltability and oiling-off of cooked curds, as indicated in Table 2. The higher meltability indicated a softer curd protein matrix, which enabled easier flow through the taper inlet and cooling die. Besides, the higher amount of oil released from cooked curds could lubricate the extruder and die, which reduces shear stress (Fox, Guinee, Cogan, & McSweeney, 2017) and further increase the curd flow. Hence, the mass flow of cooked curds during extrusion was 48–147% higher than uncooked curds, and consequently lowered the residence time (RT) to 65 s compared with 97–156 s, respectively. Cooked extrudates showed a higher $T_{exit}$ due to its shorter stay in the cooling die. Torque$_{net}$ is similar for both types of curds, while extrusions at 90 °C displayed lower torque$_{net}$ values due to the lower curd viscosity and/or filling degree in the extruder barrel compared with extrusion at 80 °C. SME, calculated based on mass flow and torque$_{net}$, was higher for extrusion of uncooked curds than that of cooked curds at both extrusion temperatures. It was noticed that extrusion of cooked curds did not show a difference in mass flow, RT, torque$_{net}$ and SME between the extrusion temperature of 80 or 90 °C ($T_h$) while that of uncooked curds showed significant differences (p ≤ 0.05). This was attributed to the extensively released oil from the cooked curd as a lubricant that reduced the effect of processing variations.

A decrease in pressure and increase of mass flow were noticed when $T_h$ increased from 80 to 90 °C for uncooked curds. However, for extrusion of cooked curds, the pressure was lower at 80 °C. This result was confirmed by our visual observation during the extrusion trials. Differently from other extruded curds, cooked curds extruded at 80 °C quickly flowed out of the cooling die as a thin and pitted mass, during which friction or shear forces between the material and the wall of the cooling die appeared to be absent, causing the absence of pressure buildup. It is speculated that, although the cooked extrudates showed similar SME values, the majority of mechanical work for the 80 °C extrusion occurred in the screw section, while most of the SME for the 90 °C extrusion were produced in the cooling die section.

### 3.3. Appearance and microstructure of extrudates

Images of the extruded curd strips are shown in Fig. 2, for both the inner structure/cross-section after cutting and the formed protein fibers after tearing the strings. Uncooked extrudates displayed a more fibrous structure than cooked extrudates at both 80 and 90 °C extrusion temperatures, which could be related to their relatively higher SME values. To produce a fibrous curd structure, a certain extent of shearing of curd must occur in the cooling die to enable the elongation of protein and fat particles. The relatively higher SME (31–74 kJ kg$^{-1}$) with lower meltability (1.1) and oiling-off (1.1) of uncooked curd compared to that of cooked curd during extrusion promotes the creation of protein fibers. It was also noticed that curds extruded at 80 °C showed a less fibrous behavior than those extruded at 90 °C, especially for cooked products. Correspondingly, the microstructure of the 80 °C extrudates showed little anisotropic behavior (Fig. 3a, c, e and g). $\mu$CT images, Fig. 4a, d, g and j showed part of the cross-section areas perpendicular to the extrusion direction (the approximate diameter of the extruded curd strips is 5 mm), from the edge towards the center part of the cylindrical extruded curd strips. Fig. 4a–e presented unevenly distributed fat domains in the uncooked curd extruded at 80 °C, showing more coalesced fat domains in the center part of extrudate due to the higher center temperature of curd in the cooling die. The absence of anisotropy in this sample was attributed to the longer RT and lower temperature of curd in the cooling die. The rigid protein and fat molecules were hardly deformed and elongated under shearing when the curd temperature was close to or below its gel-sol transition temperature that is a precondition for curd stretching (Bähler et al., 2016), impeding the formation of long fibers.

| Material | $T_h$ (°C) | Pressure (bar) | Mass flow (g s$^{-1}$) | RT (s) | $T_{exit}$ (°C) | Torque$_{net}$ (%) | SME (kJ kg$^{-1}$) |
|----------|-----------|---------------|---------------------|--------|---------------|-------------------|-----------------|
| Uncooked | 80        | 16 ± 1        | 0.19 ± 0.00$^a$     | 156 ± 2 | 38 ± 3        | 6 ± 0$^a$         | 74 ± 0$^a$     |
|          | 90        | 4 ± 2         | 0.31 ± 0.03$^b$     | 97 ± 4  | 50 ± 2        | 4 ± 0$^b$         | 31 ± 1$^b$    |
| Cooked  | 80        | 0 ± 0$^c$     | 0.47 ± 0.00$^c$     | 65 ± 0  | 42 ± 1$^b$    | 6 ± 1$^a$         | 27 ± 3$^b$    |
|          | 90        | 4 ± 0$^c$     | 0.46 ± 0.02$^c$     | 65 ± 3  | 61 ± 1$^d$    | 5 ± 0$^a$         | 27 ± 0$^b$    |

Means with different superscript letters in the same column are significantly different.

$^a$ RT: residence time; SME: specific mechanical energy; $T_{exit}$: exit temperature; $T_h$: heating temperature; Torque$_{net}$: net torque.
protein fibers.

The numerous small fat particles shown in cooked extrudate obtained at 80 °C (Fig. 3c and g), looking like the original uncooked curd (Fig. 1a), also suggested a lack of fibrous behavior for this sample. Looking at the μCT images (Fig. 4d–f), continuous fat areas were observed in the entire object, but to a greater extent at the surface. This

| Location          | Uncooked Curds                        | Cooked Curds                        |
|-------------------|--------------------------------------|-------------------------------------|
|                   | 80 °C extrudate | 90 °C extrudate   | 80 °C extrudate | 90 °C extrudate |
| Peripheral regions| ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) |
| Center            | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) |

Fig. 3. Microstructure of uncooked curd extruded at 80 °C, cooked curd extruded at 80 °C, uncooked curd extruded at 90 °C and cooked curd extruded at 90 °C (center and edge) observed by confocal laser scanning microscopy (CLSM). The red, green and black areas respectively indicate fat, protein and serum phases. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Microstructure of uncooked curd extruded at 80 °C, cooked curd extruded at 80 °C, uncooked curd extruded at 90 °C and cooked curd extruded at 90 °C visualized by X-ray micro-tomography (X-ray μCT). The brighter regions, dark grey areas and white dots respectively correspond to the protein matrix, fat/serum and salt. The three orthogonal slices are shown in each row.
observation was in agreement with the pitted appearance of cooked curd extruded at 80 °C as shown in Fig. 2. As mentioned previously, extrusion of this sample resulted in unexpected low pressure, which manifested as a small and fragile strip with almost no fiber formation, indicating that it was not properly sheared, structured and shaped in the cooling die. It is therefore confirmed that the texturization of cheese curds is actually determined by the shear work produced in the cooling die, instead of the screw section.

In comparison, the microstructure in peripheral regions of both uncooked and cooked extrudates obtained at 90 °C clearly showed aligned proteins with elongated fat domains (Fig. 3h and d). The center part did not show a clear anisotropy (Fig. 3f and h), while in the μCT images (Fig. 3h and k) a small degree of anisotropy is observed, though less obvious than in the peripheral part (Fig. 3i and l). As mentioned, the curd strips in the cooling die had a higher temperature in the center than surface, which was related to less shearing at the center and thus, the less fiber formation, which was also previous observed (Akdogan, 1999; Feng et al., 2022).

According to the discussion above, two aspects should be considered regarding the protein fiber formation. First, it is necessary for the curd to have a proper temperature before entering and passing through the die section: at a temperature below gel-sol transition point, the rigid curd structure (fat and protein network) cannot be deformed and structured by the shear forces produced in the cooling die; if the temperature is too high, the low curd viscosity result into low shear forces that are not sufficient to induce stretching/elongation of the curd components. Second, the ‘screw section’ determine the fat distribution in the curd matrix, influencing oiling off, while the ‘die section’ flow and cooling rate play an important role in efficiently stretching the protein matrix. Hence, in future studies, it is suggested to differentiate the ‘screw section’ from the ‘die section’ to assess the different types of shearing effects separately on the extrudate properties.

### 3.4. Composition of extrudates

The composition of the extrudates is shown in Table 4. In general, moisture content, ranging from 44.4 ± 0.1 to 45.2 ± 0.2% (w/w), was not significantly or only slightly influenced by the extrusion temperature or cooking. This is in agreement with our previous work that reported that the only factor influencing the moisture content was the cooking temperature, which determined the curd viscosity in the die, thus the extent of water retention (Feng et al., 2022). After extrusion, a significant increase in protein content was observed (p ≤ 0.05) in comparison to the feeding materials (uncooked and cooked curds in Table 2). Cooked extrudates showed a higher protein content (28.1 ± 0.0 and 26.9 ± 0.3% w/w) than uncooked ones (26.4 ± 0.1 and 24.9 ± 0.2% w/w), indicating increased protein network stability and loss of other components in curds during extrusion. The protein stabilization might be linked to the stronger protein interactions induced by thermomechanical process, such as calcium-protein bonds that will be discussed in the following sections. Moreover, the 80 °C extrudates had a higher protein content than those produced at 90 °C due to their higher fat loss during extrusion.

For fat content, no statistical significant differences are observed between extruded curds from uncooked or cooked curds, but significant differences (p ≤ 0.05) were observed between the two extrusion temperatures (T_e), suggesting that fat loss requires involvement of shear forces. Fat content was significantly reduced from ~28.0% w/w in the feeding materials (Table 2), to 23.8–24.8% w/w in extrudates obtained at 80 °C, but slightly increased or did not change in extrudates obtained at 90 °C (29.5% w/w). Previous studies by Banville et al. (2016) using a torque rheometer showed that the extent of fat loss in cheese curds was impacted by the temperature, mixing speed and mixing time (shear applied). The microstructure (Fig. 3a, c, e and g) showed that the extrudates exhibited smaller fat droplets in 80 °C while large oil domains are observed in 90 °C extrudates. The differences in fat loss between 80 and 90 °C are hypothesized to be due to differences in capability of the protein network to hold the fat globules during heating and cooling in the die. The extrusion at 90 °C, allowed for both cooked and uncooked curds to keep the large fat domains entrapped and therefore a higher final fat content in the extrudates, as also observed in our previous work (Feng, Lillevang, & Ahrné, 2021).

Furthermore, compared with the results shown in Table 2, extrusion induced an increase of calcium content in curds, accompanied with the increase of protein content (up to 21%), and Ca:protein ratio increased from 30 to 32 to 32–38 mg Ca g−1 protein (up to 27%), which could be attributed to the enhanced calcium-protein interactions induced by extrusion that will be illustrated in a following section. It was found that for extrusion at 80 °C, calcium significantly reduced in the cooked compared to uncooked extrudate. This could be due to the soluble calcium in the fat/serum phase that was lost during extrusion, as suggested in the large areas throughout the extrudate shown in Fig. 3d–f. Contrast, the calcium difference between uncooked and cooked extrudates was not significant at 90 °C, which is related to the well-distributed fat droplets and serum phase dispersed between aligned proteins indicated in Figure g–l.

### 3.5. Protein secondary structure in curds

Fig. 5 shows the distribution of α-helix, β-sheet, β-turn and RC structures in uncooked, cooked and extruded curds. Uncooked curds exhibited significantly different secondary structure distribution compared with cooked and extruded curds. As previously mentioned, the main secondary structures in uncooked curd are α-helices and RGS, whereas after cooking or extrusion treatments, α-helices disappeared and transited to mainly RGS and β-sheets. Likewise, a study on extrusion of wheat gluten reported a conformational transition from α-helix into β-sheet, β-turn and RC as the extrusion temperature increased (Chen et al., 2021). α-helix was found to be the most unstable structure and would gradually transform into a more stable structure, followed by sub-stable β-sheet and stable β-turn (Zhang, Liu, Liu, Yoon, Rizvi, & Wang, 2019), which participates in formation of protein aggregates (Beck, Knoerzer, & Arcot, 2017). However, extruded curds were not significantly different from the cooked curd. Therefore, the heat treatment at temperature ≥80 °C during cooking or extrusion was likely to be the predominant factor that caused the structural transition, no matter if the curds was heated in water, or during extrusion (no additional water). It was reported that pasteurization at 85 °C markedly induced conformational alterations in the unfolded molecular structure of milk proteins, resulting in exposure of β-sheets, turns and RGS (Tarhan & Kaya, 2021). The significant loss of helical structure in the uncooked curd might be attributed to the inaccessibility of these structural

### Table 4

| Concentration (% w/w) | Extruded at 80 °C | Extruded at 90 °C |
|------------------------|------------------|------------------|
|                        | Uncooked | Cooked | Uncooked | Cooked |
| Moisture               | 45.2 ± 0.2b | 45.2 ± 0.2b | 45.1 ± 0.3b | 44.4 ± 0.2b |
| Protein                | 26.4 ± 0.1b | 28.1 ± 0.2b | 24.9 ± 0.2b | 26.9 ± 0.2b |
| Fat                    | 23.8 ± 0.4b | 24.8 ± 0.4b | 29.5 ± 0.4b | 29.5 ± 0.4b |
| Ca                     | 1.01 ± 0.00c | 0.93 ± 0.00c | 0.88 ± 0.00c | 0.87 ± 0.00c |
| Capprotein (mg g−1)    | 38.2      | 33.1      | 35.4      | 32.4      |

a The standard deviations of results of extrudates derived by putting samples from technical replicates (replicates of extrusion process) together and analyzing by duplicate. Means with different superscript letters in the same row are significantly different. Ca: calcium; Caprotein: calcium-to-protein ratio.
elements buried in aggregated milk proteins (Tarhan & Kaya, 2021) due to the intermolecular cross-linking of β-sheets (Rahaman, Vasiljevic, & Ramchandran, 2015). Hence, the α-helix structure can possibly be seen as an indicator of the extent of heat treatment.

To our knowledge, studies regarding changes in dairy proteins secondary structure induced by extrusion process are limited. Qi and Onwulata (2011a) studied the changes in whey protein paste extruded at different temperatures, finding that compared to extrusion at 50 °C, the samples processed at 75 °C exhibited significant loss of secondary structure, and the extrudates at 100 °C showed almost complete RC structures. However, study on protein structures in extruded casein-concentrated material were not found.

### 3.6. Protein interactions in curds

Fig. 6 shows that all the curds, uncooked, cooked or extruded, show a small proportion of disulfide bonds (SS) (up to 7%) and only minor differences were observed among these samples, which has been attributed to the limited number of thiols in caseins (Broyard & Gaucheron, 2015; Fox et al., 2017). Hydrophobic interactions (Hy) represented up to 9% of the protein interaction and a tendency of increase due to extrusion, not being cooked, was observed. Electrostatic interactions and hydrogen bridges i.e. EB + HB (21–32%), and calcium bridges i.e. CaB (30–59%), were the dominant interactions stabilizing the proteins in the curds, which were reported by previous work showing that these are the primary interactions influencing cheese melting behavior (Horne, 1998; Lucey, Johnson, & Horne, 2003). The extrusion process resulted in a significant increase of CaB (up to 97%), at the expense of a reduction of EB + HB. An increase of temperature from 40 to 50 °C was previously reported to reduce the contribution of EB (Giroux, Bouchard, & Britten, 2014), which to some extent was also observed here as the reduced EB + HB with the heat treatment in the extruder. Moreover, the heat treatment in the range of 70–90 °C is known to induce an increase in insoluble calcium (i.e. colloidal calcium phosphate) content (O’Mahony, McSweeney, & Lucey, 2006; Udayarajan, Lucey, & Horne, 2005), which could reinforce the protein interactions and strengthen the dense para-casein fibers through CaB (Gonçalves & Cardarelli, 2019). The increase of CaB in the curds after the extrusion process was also consistent with the composition results shown in Tables 2 and 4, as mentioned, which corresponded to the increased calcium (from 0.75-0.77% to 0.87-1.01%) and protein (from 23.3-25.7% to 24.9-28.1%) contents, as well as Caprotein ratio (from 30.0 to 32.2 to 32.4–38.2 mg Ca-g⁻¹ protein) in curd after extrusion. However, the extrudates did not differentiate among each other.

#### 3.7. Water mobility in curds

Relaxation times and the corresponding abundances for both components of signal transverse magnetization in all the curds are given in Fig. 7. T2,1 with a relaxation time of 14.9–16.3 ms was the faster relaxation component and was estimated to be 76–84% (A1) of the water/protons in the curds, and correspondingly, T2,2 of 61–81 ms was the slower relaxation component and was approximately 16–23% (A2) of the water. T2,1 did not show big differences among samples, while T2,2 clearly increased after the extrusion process, reflecting more mobile water molecules that could be linked to the increased CaB. The water-protein interactions were likely to be replaced by stronger interactions between caseins like CaB, promoting micro-syneresis, thus a higher water mobility and a denser protein matrix (Kuo, Gunasekaran, Johnson, & Chen, 2001). Correspondingly, curds extruded at 90 °C showed significantly lower A1 and higher A2, indicating the higher proportion of the more mobile fraction of water after 90 °C extrusion.

#### 3.8. Tensile behavior of extruded curds

The anisotropic structure formed by the protein matrix being deformed by shear stresses in the cooling die can be evaluated in terms of tensile behavior. The distance to break the curd strands is a measure of extensibility, or elasticity. A tensile test of curds before extrusion...
could not be carried out due to the difficulty in shaping them in a geometry similar to the extruded curds. Extruded curds produced from uncooked curds clearly showed higher elasticity (300–450%) than the ones produced from cooked extrudates (<250%) (Fig. 8). This was attributed to the observed cracks in the microstructure (Fig. 1b) and weakened the curd protein matrix. Moreover, although calcium bridges (CaB) did not differ in the extrudates, as previously mentioned, the cooked curds had a lower level of Ca:protein ratio, which might also weaken the curd protein matrix.

It was unexpected that cooked extrudates obtained at 90 °C, which exhibited a fibrous structure (Figs. 2–4), showed an elasticity as low as the extrudates obtained at 80 °C that did not show a clear fibrous structure. To further evaluate the tensile behavior, tensile strength (the force at the breaking point of curd strips) was measured and shown in Fig. 8, though it has been reported that it correlates well with elasticity (Affifah & Ratnawati, 2017). Tensile strength provided additional information about protein network strength in extrudates, presenting a higher value for the cooked products obtained at 90 °C and corresponding to the macro-behavior of the extrudates. The low tensile strength for cooked curd extruded at 80 °C suggested that the texturization in the cooling die and a certain level of ‘die section SME’ are preconditions to obtain curd products with strong protein network. It was also found that an increase of T弛 enhanced curd elasticity and tensile strength, which was consistent with our previous work (Feng et al., 2022). This was related to the microstructural observation in this study, which showed many small fat domains in 80 °C extrudates that resulted in short protein fibers.

### 3.9. Meltability and oiling-off of extrude curds

The cooked curd and extrudates showed similar meltability and oiling-off (Fig. 9), except for the extrude obtained at 80 °C without cooking that exhibited a low oiling-off level that was similar to the original curd. This was attributed to the greatly reduced fat content (Table 4) in this product. It was mentioned that EB = HB and CaB are the primary interactions influencing cheese melting behavior [5, 39], whereas the different functional properties could not only be explained by the protein interactions. Meltability and oiling-off have also been commonly related to fat content and fat globule size (Cai-Sokolińska & Pikul, 2009; Noronha, O’Riordan, & O’Sullivan, 2008; Rowney et al., 2005; Schenkel, Samudrala, & Hinrichs, 2013). Our results shows that meltability and oiling-off of cooked extrudates obtained at 80 °C were substantially higher than other curds. The curd behavior upon heating was already noticed by visualization after the measurements, showing that the aforementioned curd lost its cylindrical shape, displaying a
liquid state with a serious separation of water and fat phases. This reflected the fast breakdown of the curd upon heating and further confirmed the weak protein network of this product as indicated previously. Hence, protein structure is an important factor to be considered to obtain dairy products with desired functional properties.

4. Conclusions

This study provides new insights regarding the effect of curd properties, modified by water cooking, on extrusion behavior at temperatures of 80 and 90 °C using a lab-scale twin-screw extruder and their impact on extrudates properties.

The cooking process significantly affected the curd composition and structure by enhancing curd meltability and oiling-off, which resulted in decreased extrusion pressure, faster curd flow, higher \( T_{\text{cat}} \) and lower specific mechanical energy (SME). Cooking induced changes in the curd protein matrix, as was observed by an increase of protein content and formation of cracks in the protein matrix microstructure, which may be related with a significant increase of RC conformations (38.8 ± 1.6 to 69.3 ± 3.2%) and appearance of SS bonds (7%). These structural changes led ultimately to extrudates with a less fibrous structure and lower elasticity and tensile strength.

The extrusion process induced a significant increase of Ca:protein ratio and % of calcium bridges (CaB) in the protein network of the extrudates. The temperature of extrusion (80 or 90 °C) had less effect on measured and calculated extrusion parameters (pressure, mass flow, residence time, torque and SME) of cooked than uncooked curds, which may be due to a higher meltability of cooked curds and a lubricating effect caused by the more extensive oiling-off of cooked curds during extrusion. Microscopic observations at different length scales by combining CLSM and X-ray \( \mu \)CT techniques and composition analysis showed that extrudates obtained at 80 °C have less fiber formation, lower fat content and smaller fat globules compared with extrudates at 90 °C. Thus, in general curds extruded at 90 °C had higher elasticity and tensile strength, which was related to the well-elongated and distributed fat.

Author contributions

Ran Feng: Conceptualization, Formal analysis, Methodology, Investigation, Writing – original draft. Franciscus Winfried J van der Berg: Conceptualization, Formal analysis, Validation, Writing – review & editing. Rajmund Mokso: Methodology, Investigation, Writing – review & editing. Søren Kristian Lillevang: Conceptualization, Methodology, Writing – review & editing. Lilia Ahrné: Conceptualization, Formal analysis, Methodology, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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