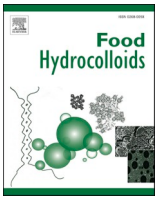
Food Hydrocolloids 144 (2023) 108932

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



# Towards creating sustainable foods from side streams: Heat-induced structure formation in blends of sunflower seed press cakes and cheese whey under moderate shear

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ARTICLE INFO

*Keywords:*
Plant protein
Whey protein
Circularity
Food waste
By-product
Heat treatment

ABSTRACT

Processing of oilseeds generates low value by-products, which still contain valuable components. Sustainable and circular food chains require valorising the entire stream or producing less refined fractions of it. One approach could be blending with other protein-containing side streams to obtain novel, nutritionally valuable and techno-functional food ingredients. In this study, sunflower press cake was co-processed with components from whey, a cheese-making by-product. Blends with constant dry matter and protein content but different press cake to whey protein ratios (0–225 g/kg press cake) were used to investigate the contributions of both side streams to structure formation during heating (80–140 °C) under moderate shear using a Rapid Visco Analyser. The denaturation of whey proteins contributed to an increased viscosity, but the highest viscosities were still achieved at high ratios of press cake, underlining the importance of the fibre fraction for structure formation. Treatments at 120 and 140 °C increased the amount of insoluble material and water holding capacity of the blends, and analyses of the serum phase and curd showed that sunflower and whey proteins formed heat-induced, insoluble aggregates. Confocal laser scanning microscopy confirmed the presence of large protein particles dispersed in the matrix rather than the presence of a continuous network, as was the case for heating without shear. Furthermore, the protein particles were more defined and showed a smoother appearance with increasing press cake concentration. This research provides fundamental insights in the colloidal interactions between biomacromolecule blends during processing (e.g., protein cross-linking, microphase separation), and demonstrates the importance of understanding the critical process parameters (e.g., heat, shear) leading to structure formation, facilitating the successful integration of complex materials such as press cakes and setting the basis for further processing of the blends and their utilisation as ingredients, for instance in functional drinks, snacks, or semi-solid spreads.

## 1. Introduction

Production and distribution of food have significant impact on our environment through, e.g., use of land, nutrient resources, energy, and freshwater (Aiking, 2011). Processing of raw materials often generates side streams, which still contain some of the macro- and micronutrients of the raw material, such as protein, fibre, lipids, minerals, and polyphenols, but that mostly end up as animal feed, substrates for biofuel production, or as landfill rather than in the human food chain (Raak, Symmank, Zahn, Aschemann-Witzel, & Rohm, 2017). Many concepts have been developed to extract valuable compounds from food processing side streams (Mirabella, Castellani, & Sala, 2014). However, in processes of purification, circularity and sustainability are not top priorities (Karefyllakis, Octaviana, van der Goot, & Nikiforidis, 2019; van der Goot et al., 2016). Albeit using purified ingredients is justified in some cases such as infant formula and nutritional beverages to reach consistency and/or predictable processing behaviour, they are recombined with other purified ingredients in many applications. Using less refined ingredients that already contain different techno-functional biomolecules (proteins, fibre, lipids) as novel components is thus a promising way to decrease the environmental impact of food production (Lie-Pang, Braconi, Boom & van der Padt, 2021), especially when produced from food processing side streams constantly generated in large quantities.

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https://doi.org/10.1016/j.foodhyd.2023.108932
Received 13 March 2023; Received in revised form 15 May 2023; Accepted 29 May 2023
Available online 5 June 2023

One prominent example for food processing side streams is whey, which is a nearly casein- and fat-free liquid generated in large volumes during the production of many cheese varieties (~3–11 kg per kg cheese). Whey still contains ~50 g/L lactose, as well as ~10 g/L of major and minor whey proteins (in particular α-lactalbumin and β-lactoglobulin) with excellent amino acid profiles, making whey one of the most valuable food processing side streams in terms of protein quality (Prandi et al., 2019). In the efficient supply chains of large dairy factories, the valorisation of cheese whey has been brought to perfection by fractionating the different compounds to produce ingredients such as whey protein isolates and concentrates as well as lactose, which are widely applied in, e.g., infant formula and sports nutrition (Smithers, 2015). However, this may not be the case for small, artisanal cheese manufacturers, where whey streams are still not further utilised for human consumption.

Another, currently underutilised side stream is the solid pressing residue from vegetable oil production, so called press cake, which is a rich source of proteins and fibre. Soybean, rapeseed, and sunflower are three of the globally most abundant crops due to the production of vegetable oil. The side streams from soybean oil production have been valorised for many years due to their economy of scale to produce soy protein isolates and concentrates. These ingredients have been successfully used in nutritional beverages as well as plant-based dairy and meat alternatives (Zhang et al., 2021). However, other oilseed press cakes have received increased attention only in the recent years (Arrutia, Binner, Williams, & Waldron, 2020; Avelar, Rodrigues, Pereira, & Vicente, 2022; Filho & Egea, 2021). Some oilseeds contain significant amounts of antinutritional compounds such as phytic acid, glucosinolates, or chlorogenic acid, which have undesired effects on digestion and uptake of nutrients (Ancuța & Sonia, 2020). However, strategies to decrease the amount of antinutritional compounds are currently under development. For example, extrusion cooking of press cakes at temperatures between 100 and 130 °C was proven powerful to degrade antinutritional compounds while resulting in only minor aggregation, denaturation, and structural changes of the proteins (Vidal et al., 2022).

As many plant proteins have deficiencies in some essential amino acids such as lysine, using blends of different protein sources is a promising way to not only enhance their biological value, but also to modulate their techno-functional behaviour (Jiménez-Munoz, Tavares, & Corredig, 2021), especially when blending plant and animal proteins (Alves & Tavares, 2019). Side stream blends such as whey and oilseed press cakes have therefore a great potential to obtain novel, sustainable food ingredients of high nutritional value and good techno-functional properties (Raak et al., 2022). Using such complex matrices that contain various types of biomacromolecules such as carbohydrates and different proteins, however, requires a complete understanding of the synergies and thermodynamic incompatibilities of the components present in the system in terms of structure formation during processing, from the molecular level up to aggregates and networks (Corredig, Young, & Dalsgaard, 2020).

The aim of this research was to investigate systematically the structure formation in blends of sunflower press cake and whey during different heat treatments under moderate shear conditions. Heat treatment is an important process to ensure microbial and enzymatic stability of food products, while shear at different intensities is often applied in food manufacturing to homogenise products and modulate their structure formation and texture. For instance, high shear is applied in extrusion to create fibrous structures of plant proteins, whereas moderate shear can be used to break yoghurt gels to obtain a smoother texture. Blends with varying amounts of press cakes but same overall dry matter and protein content were studied, thus allowing to draw conclusions on the individual contributions of press cake and whey constituents on structure formation and physical properties of the blends. For the first time, this study describes in detail the structuring mechanisms of such side stream blends to facilitate their implementation in a circular food chain.

## 2. Materials and methods

### 2.1. Gross composition of basis materials

The materials used in this study and their composition are summarised in Table 1.

The press cake from Schalk Mühle (Ilz, Austria) was obtained from cold pressing (55–65 °C) of dehulled sunflower seeds, and milled in a hammer mill. Sweet whey powder and whey protein concentrate were provided by Bayrische Milchindustrie eG (Palting, Germany) and Arla Foods Amba (Viby, Denmark). Milk ultrafiltration permeate was produced from pasteurised (72 °C, 15 s) skim milk (Arla Foods Amba) by ultrafiltration (MWCO 30 kDa, PESH) using a Vibro LE System (Sani Membranes ApS, Allerød, Denmark) as described previously (Raak & Corredig, 2022). 0.2 g/L $NaN_3$ was added to the skim milk for preservation prior to ultrafiltration, ensuring also the microbial stability of the samples prepared with the resulting permeate.

Total dry matter was determined by drying the samples at 105 °C in a heating chamber (Memmert GmbH + Co. KG, Schwabach, Germany) until constant mass was reached. Protein contents were obtained from their nitrogen contents (Gerhardt Dumatherm, C. Gerhardt GmbH&Co. KG, Königswinter, Germany) by multiplying with conversion factors of N × 5.80 (sunflower proteins) and N × 6.38 (whey proteins). The lipid contents were determined according to AOAC method 954.02 using a Hydrotherm and Soxtherm® from C. Gerhardt GmbH&Co.KG. Total carbohydrate contents were determined according the phenol-$H_2SO_4$ colorimetric method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956), which detects all classes of carbohydrates (i.e., mono-, di-, oligo-, and polysaccharides). Total starch content of the sunflower press cake was determined according to method 76–13.01 (AACC, 2015) using the Megazyme total starch assay kit (K-TSTA-100A, Wicklow, Ireland). Both, resistant starch and glucose are detected by the method. Ash contents were determined by thermo-gravimetric analysis (TGA-2 STAR, Mettler Toledo, USA). Approx. 12 mg of sample were weighed into aluminium crucibles with punctured lids (Mettler Toledo) and placed in the instrument. The samples were heated from ambient temperature to 600 °C at 10 K/min under nitrogen atmosphere and subsequently held at 600 °C for 45 min under air environment while constantly measuring the sample weight. The ash content was calculated from the ratio of final to initial sample weight. All compositional analyses were performed at least in duplicate and are reported as mean values.

### 2.2. Sample preparation

#### 2.2.1. Preparation of basis samples

Various blends with sunflower press cake contents of 0–225 g/kg

**Table 1**
Composition of the raw materials used for sample preparation.

| | Sunflower press cake | Whey protein concentrate | Sweet whey powder | Milk ultrafiltration permeate |
|---|---|---|---|---|
| Dry matter (g/kg, w.b.) | 950 | 991 | 989 | 58 |
| Protein (g/kg, w.b.) | 462 | 781 | 121 | n.d. |
| Lipids (g/kg, w.b.) | 90 | n.d. | 9 | n.d. |
| Total carbohydrates (g/kg, w.b.) | 212 | 97 | 731 | n.d. |
| Total starch (g/kg, w.b.) | 11 | n.d. | n.d. | n.d. |
| Ash (g/kg, w.b.) | 96 | 3 | 77 | n.d. |

w.b. – wet basis.
n.d. – not determined.

were prepared by mixing press cake, whey protein concentrate, sweet whey powder, and milk ultrafiltration permeate using an Ultra Turrax (Ø18 mm dispersing element, IKA Werke GmbH&Co.KG, Stauffen, Germany). The rotational speed was set to 9,500 rpm for 4 min, followed by 13,500 rpm for 4 min, and finally 20,500 rpm for 2 min, allowing a thorough homogenisation without formation of clumps. Table 2 lists the composition of the all blends prepared in this study. The blends were adjusted to both equal dry matter (259 g/kg) and equal protein content (104 g/kg) to evaluate the effects of the various components on the structure formation during heat treatment.

#### 2.2.2. Heat and shear treatment

Heat treatment of the blends were performed using a Perten rapid visco analyser (RVA 4800, PerkinElmer Inc., Waltham, MA, USA) equipped with a pressure cell. The container was filled with 30 g of sample, hermetically closed, and placed within the instrument. At the beginning of the measurement, the temperature was held at 30 °C for 4 min, followed by heating with 10 K/min, holding at a peak temperature of either 80, 120, or 140 °C for 5 min, cooling down to 30 °C with 5 K/min, and holding at 30 °C for 2 min. During the experiment, the angular velocity of the paddle was 160 rounds per minute (rpm), corresponding to an average shear rate of ~54/s (Lai, Steffe, & Ng, 2000). Reference samples without shear were prepared using the same procedure but without the paddle inserted. Additionally, to study the structure formation at various points of the process, the procedure was aborted at characteristic time points, and the samples were then rapidly cooled in ice water.

In view of the complexity of the samples and the instrument geometry, the viscosity values calculated automatically by the RVA were transformed back to the original torque $M = \eta \bullet N/k''$ (mN.m), where $\eta$ is the viscosity provided by the instrument (mPa.s), $N$ = 160/min the angular velocity of the paddle, and $k''$ = 2,000/m$^3$ is the instrument constant (Lai et al., 2000).

### 2.3. Physical analyses

#### 2.3.1. pH measurements

The pH was measured using an InoLab 7310 pH meter (WTW GmbH, Weilheim, Germany) equipped with a SenTix 82 electrode (WTW GmbH).

**Table 2**
Composition and pH of side stream blends used in this study.

| Sunflower press cake (g/kg) | Whey protein concentrate (g/kg) | Sweet whey powder (g/kg | Milk ultrafiltration permeate (g/kg) | Sunflower Protein to Whey Protein Ratio | pH (−) |
|---|---|---|---|---|---|
| 0 | 118.0 | 97.4 | 784.6 | 0:100 | 6.14 ± 0.06$^{ab}$ |
| 50 | 91.8 | 75.8 | 782.5 | 22:78 | 6.12 ± 0.00$^{a}$ |
| 100 | 65.5 | 54.1 | 780.3 | 44:56 | 6.14 ± 0.03$^{ab}$ |
| 150 | 39.3 | 32.5 | 778.2 | 67:33 | 6.25 ± 0.01$^{bc}$ |
| 175 | 26.2 | 21.7 | 777.1 | 78:22 | 6.22 ± 0.04$^{abc}$ |
| 200 | 13.1 | 10.8 | 776.1 | 89:11 | n.d. |
| 225 | 0 | 0 | 775.0 | 100:0 | 6.29 ± 0.00$^{c}$ |

n.d. – not determined.

#### 2.3.2. Viscosity measurements

The apparent viscosity of the various blends of press cake and whey proteins was measured using a stress-controlled rheometer AR-G2 (TA Instruments, New Castle, DE, USA) equipped with a 40 mm cross-hatched parallel plate geometry and a Peltier element for temperature control. Gap width and temperature were 1 mm and 25 °C, respectively. The shear rate was increased from $\dot{\gamma}$ = 0.1/s to 1,000/s measuring 10 points per decade. At each point, the sample was pre-sheared for 20 s, followed by measuring the average apparent viscosity over a duration of 10 s. Data was collected and analysed using Rheology Advantage v5.7.0 (TA Instruments).

#### 2.3.3. Colour measurements

The colour of the side stream blends before and after heat treatment was measured using a CR400 chromameter (Konica Minolta Sensing Europe B.V., Nieuwegein, The Netherlands) and is reported in the CIE-LAB colour space, resulting in the coordinates $L^*$, $a^*$, and $b^*$ for lightness, green/red, and blue/yellow, respectively. The change in colour induced by the heat treatments is expressed as the colour difference $\Delta E^*$, where the colour values of the unheated samples were taken as reference:

$$\Delta E^* = \sqrt{\left(L^* - L^*_{reference}\right)^2 + \left(a^* - a^*_{reference}\right)^2 + \left(b^* - b^*_{reference}\right)^2} \quad (1)$$

#### 2.3.4. Water expression

25 g of each sample were centrifuged at 3,000×$g$ for 30 min at 25 °C using a centrifuge (SL40R, ThermoFisher Scientific, Waltham, MA, USA). The supernatant was collected for further analyses, and the sediment was weighed out and is given as percentage of the original sample mass.

#### 2.3.5. Microstructure

A 2 mg/mL solution of fluorescein isothiocyanate (FITC) dissolved in acetone was used for protein staining, and 60 μL of the staining solution were mixed with 30 g of side stream blend prior to treatments in the RVA. Small portions of each sample were placed on a glass slide, and the microstructure was visualised using a confocal laser scanning microscope (CLSM; Nikon C2, Nikon Instrument Inc., Tokyo, Japan). A laser beam with a wave length of 488 nm was applied for excitation to induce fluorescence emission of the dyed protein particles, and pictures were taken at a magnification of 20 × . Representative images are shown.

### 2.4. Protein analysis

Total nitrogen contents of the unheated blends as well as of the centrifugal supernatants of heated and unheated blends was determined using a Gerhardt Dumatherm (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and taken as indicator for serum protein. The nitrogen contents were not further converted to protein contents due to the complexity of the sample with regard to different conversion factors and non-protein nitrogen in the milk ultrafiltration permeate.

In order to identify serum protein fractions and heat-induced cross-linking of proteins, the side stream blends and their supernatants were further analysed via denaturing gel electrophoresis using an Invitrogen™ system and the corresponding supplies (ThermoFisher Scientific, Waltham, MA, USA). All samples were diluted 1:50 (w/v); the centrifugal supernatants in demineralised water, whereas the curds were diluted in a solution of 0.1 mol/L Tris and 10 g/kg sodium dodecyl sulphate (pH 7.4) and subsequently sonicated in a bath sonicator (IKA-Werka, Staufen am Breisgau, Germany) for 30 min at room temperature (Nasrollahzadeh et al., 2022). The diluted samples were mixed 6.5:2.5:1 (v/v) with NuPAGE™ LDS buffer and NuPAGE™ reducing agent, followed by heating at 95 °C for 5 min in a thermoshaker (IKA Werke GmbH&Co.KG). Aliquots of 7 μL were injected to a NuPAGE™ precast gradient gel (4–12% polyacrylamide), and a molar mass standard (Precision Plus Protein™, Bio Rad Laboratories, Inc., Hercules, CA, USA)

was used for band identification. The experiments were run at 200 V for 35 min using an XCell SureLock™ Mini-Cell filled with NuPAGE™ MES SDS running buffer. The gels were stained overnight in SimplyBlue™ SafeStain solution, rinsed in demineralised water for 24 h, and subsequently digitalised using a ChemiDoc XRS + gel imaging system (Bio Rad Laboratories). The protein bands were analysed semi-quantitatively using Image Lab (v6.0.1; Bio Rad Laboratories).

### 2.5. Statistical analysis

All samples were prepared in duplicate. Statistically significant differences between unheated blends were identified using a one-way analysis of variance (ANOVA), and the effect of both heating temperature and press cake concentration was analysed using a two-way ANOVA. The statistical acceptance level was $P < 0.05$.

## 3. Results and discussion

### 3.1. Composition of the sunflower press cake and the side stream blends

The gross composition of the sunflower press cake (Table 1) was within the range of previously reported data (Ancuța & Sonia, 2020). However, due to the use of dehulled sunflower seeds for pressing, the protein content (462 g/kg) was higher and carbohydrate content (212 g/kg) lower compared recent studies (Bárta et al., 2021; Filho & Egea, 2021; Mangieri et al., 2022). The press cake also contained 90 g/kg unextracted lipids, probably in form of native oleosomes.

The blends (Table 2) were obtained by mixing 0–225 g/kg sunflower press cake with different amounts of sweet whey powder, whey protein concentrate, and milk ultrafiltration permeate (containing mainly lactose and minerals) necessary to reach equal dry matter (259 g/kg) and protein content (104 g/kg). As a consequence, the blends differed in their sunflower protein:whey protein ratio (0:100–100:0), lactose concentration, and polysaccharide content. The pH ranged from 6.12 to 6.29, with a significant difference only between the blends without and with the highest amount of press cake (Table 2).

It is expected that the polysaccharides of the sunflower press cake (fibre and starch) will contribute to the overall viscosity of the blends, whereas the presence of lactose will play a role in Maillard-type protein cross-linking during heating. In Maillard reactions, reducing sugars such as lactose or glucose react with amino groups of proteins, initiating a cascade of chemical reactions, which eventually lead to protein cross-linking (Lund & Ray, 2017). A small proportion of reducing sugars such as glucose and galactose in the blends is deriving from the sunflower press cake (Guo, Klinkesorn, Lorjaroenphon, Ge, & Jom, 2021), whereas the major part stems from the lactose present in the milk ultrafiltration permeate and the sweet whey powder.

### 3.2. Rheological characterisation of the unheated side stream blends

Fig. 1A shows the apparent viscosity of the different side stream blends as a function of shear rate. With increasing press cake concentration, the apparent viscosity increased and the rheological behaviour changed. Blends with 0 g/kg press cake (i.e., only whey components) had a low apparent viscosity and Newtonian flow behaviour, i.e., the apparent viscosity was independent of the shear rate. Although the press cake corresponded to less than 20% of the dry matter of the blend, the samples containing 50 g/kg press cake already showed a decreasing apparent viscosity with increasing shear rate, i.e., shear thinning behaviour, with both the low shear ($\dot{\gamma} < 0.3$/s) and high shear plateau ($\dot{\gamma} > 100$/s) being clearly visible. With increasing press cake content, these plateaus were shifted outside the measured shear rate range, implying that the shear thinning occurred over a broader range of shear rates. The protein content was 104 g/kg in all blends, lower than the concentrations at which shear thinning occurred in suspensions of whey proteins (~150 g/kg; Purwanti et al., 2011) and sodium caseinate (~140 g/kg; Pitkowski, Durand, & Nicolai, 2008; Raak et al., 2020). It is possible to conclude that the pronounced shear thinning behaviour as well as the increase in apparent viscosity with increasing press cake concentration was a consequence of the higher fibre contents, as it is known that soluble and insoluble fibres such as pectin and cellulose dramatically increase the apparent viscosity of a solution and result in shear thinning behaviour (Dikeman & Fahey, 2006).

Fig. 1B illustrates the apparent viscosity of the blends at low shear rate ($\dot{\gamma} = 0.1$/s) as a function of the press cake concentration and sunflower protein to whey protein ratio. It is evident that the apparent viscosity increased dramatically with increasing press cake content above 150 g/kg, which corresponds to 55% of the dry matter and ~50% of the carbohydrates in the blend coming from the press cake. Such a behaviour is very typical for biomacromolecules such as polysaccharides and proteins, which are jammed at high concentrations, leading to increased interactions due to entanglements and interpenetration of the polymer chains or soft particles, respectively (de Kruif, Bhatt, Anema, & Coker, 2015; Nachtigall et al., 2020; Raak et al., 2020). The protein content was equal in all blends (104 g/kg), and we consider the difference in protein composition to be negligible. Therefore, this behaviour must stem from the increased fraction of polysaccharides with increasing press cake content. These results demonstrate the contribution of biomacromolecule interactions to the bulk apparent viscosity during processing of unrefined, protein-rich food matrices.

### 3.3. Characterisation of the heated blends

#### 3.3.1. Heat-induced colour changes of side stream blends

All side stream blends were subjected to heat treatment under

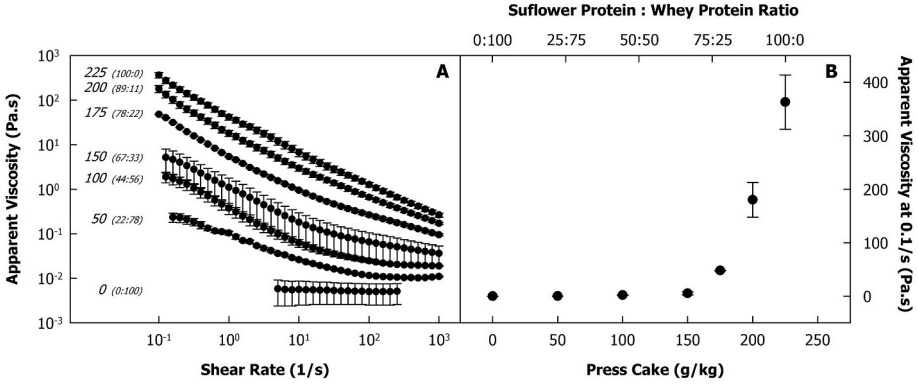


**Fig. 1.** (A) Apparent viscosity of side stream blends as a function of shear rate (numbers refer to press cake concentration in g/kg and sunflower protein to whey protein ratio). (B) Apparent viscosity of side stream blends at a shear rate of 0.1/s as a function of the press cake concentration and sunflower protein to whey protein ratio. All blends had a dry matter of 259 g/kg and a protein content of 104 g/kg.

continuous shear in the RVA, and the samples were taken for further analyses at the end of the treatment.

Fig. 2 illustrates the colour properties of the side stream blends as affected by the press cake concentration and heating temperature. The lightness ($L^*$) of the unheated side stream blends decreased significantly with increasing press cake content up to 150 g/kg and reached a plateau at higher concentrations (Fig. 2A). With increasing press cake content, $a^*$ increased from −5 to 5 (Fig. 2B), indicating a shift from slightly green to slightly red colour. As both the whey ingredients and the sunflower press cake had a yellow colour, $b^*$ had positive numbers in all cases (Fig. 2C). However, the parameter decreased slightly with increasing press cake content, indicating a slight shift towards a more neutral colour. It is worth noting that there was no more change in the colour parameters with increasing press cake content above 150 g/kg, when more than half of the solids in the blends derived from press cake.

As indicated by $\Delta E^*$ (Fig. 2D), heat treatment at 80 and 120 °C caused only a minor change in colour. At low press cake contents (≤150 g/kg), this was mainly due to the significantly increased $L^*$, whereas $\Delta E^*$ was generally lower with more press cake present. In contrast, heating of the blends at 140 °C caused more severe colour changes, which was reflected in decreased $L^*$ (Fig. 2A) and increased $a^*$ (Fig. 2B) and $b^*$ values (Fig. 2C), demonstrating the pronounced browning during heat-induced Maillard reactions (Maruta, 2021). It is important to note that the colour change induced by heating at 140 °C was significantly more pronounced at press cake contents below 150 g/kg due to the higher contents of lactose in these blends, which plays a major role in Maillard reactions. At higher contents of press cake (≥150 g/kg), however, the dark colour of the blends changed to a much lesser extent with heating.

### 3.3.2. *Heat-induced changes in the distribution of soluble and insoluble material in the blends*

The side stream blends were centrifuged before and after heat treatment to evaluate the effects of heat treatment on the proportions of soluble and insoluble material. Fig. 3 shows the relative amounts of sediment after centrifugation of the blends, providing information on the amount of insoluble material as well as its water holding capacity, and Fig. 4 depicts the nitrogen contents of the whole blends (~17 mg/g for all samples) as well as of the serum phases expelled by centrifugation.

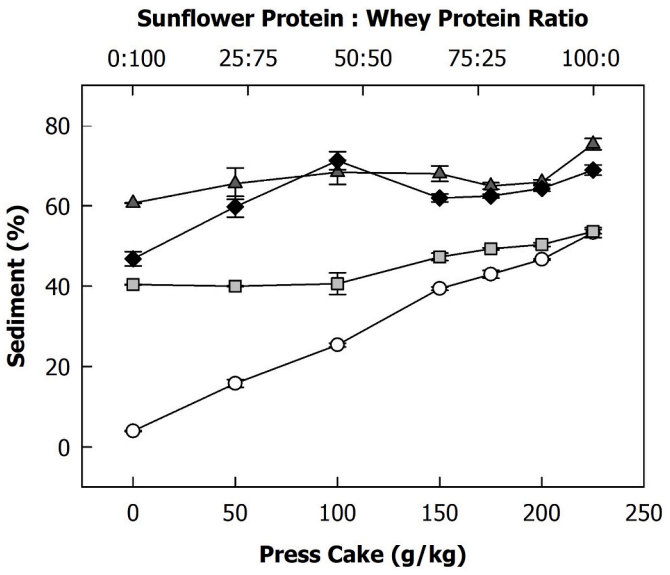


**Fig. 3.** Relative amount of sediment after centrifugation (3,000×g, 30 min, 25 °C) of side stream blends with various press cake concentrations and sunflower protein to whey protein ratios after heat treatment under moderate shear in the rapid visco analyser (white circles: untreated, light grey squares: 80 °C, dark grey triangles: 120 °C, black diamonds: 140 °C). All blends had a dry matter of 259 g/kg and a protein content of 104 g/kg.

Samples with 0 g/kg press cake showed almost no sedimentation, as these blends contained only whey constituents, which have a high solubility. Heat treatment at 80 and 120 °C significantly increased the amount of sediment due to whey protein denaturation and protein cross-linking (Fig. 3). This was also reflected in a decrease of the nitrogen content in the supernatant (Fig. 4), which served as an indicator for serum protein. Tolkach and Kulozik (2007) showed that β-lactoglobulin is almost completely denatured after heating at 80 °C for 5 min. However, this is not necessarily related to a loss in solubility, which requires

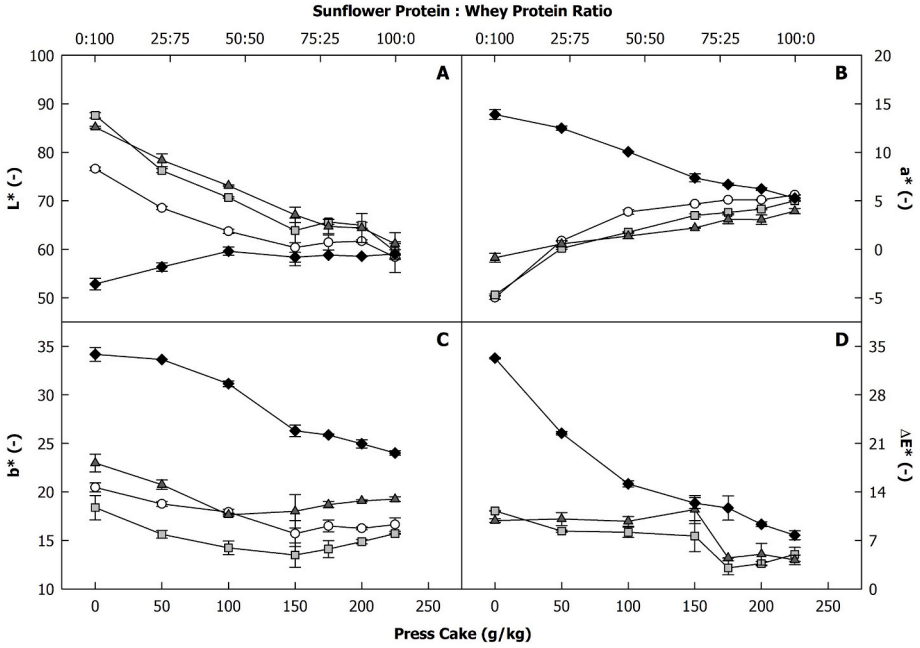


**Fig. 2.** Colour parameters $L^*$ (A), $a^*$ (B), $b^*$ (C) of side stream blends with various press cake concentrations and sunflower protein to whey protein ratios after heat treatment under moderate shear in the rapid visco analyser (white circles: untreated, light grey squares: 80 °C, dark grey triangles: 120 °C, black diamonds: 140 °C), and colour difference $\Delta E^*$ (D) of heated compared unheated blends. All blends had a dry matter of 259 g/kg and a protein content of 104 g/kg.

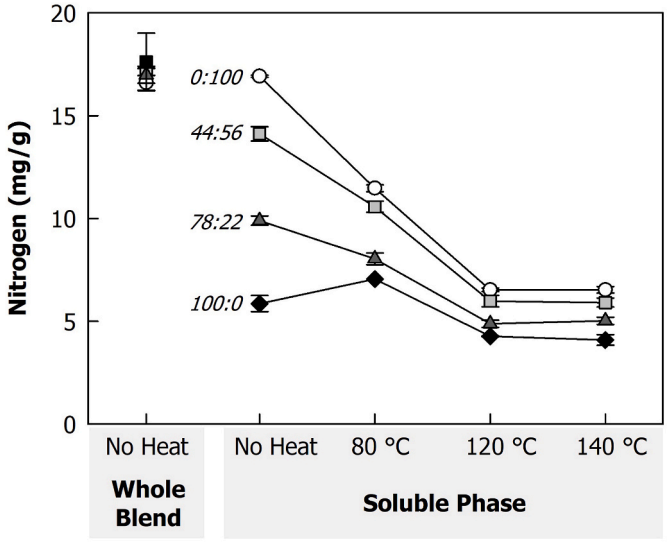


**Fig. 4.** Nitrogen content of side stream blends with 0 (white circles), 100 (light grey squares), 175 (dark grey triangles) or 225 (black diamonds) g/kg press cake and their serum phases extracted by centrifugation (3,000×*g*, 30 min, 25 °C) after different heat treatments under moderate shear in the rapid visco analyser. All blends had a dry matter of 259 g/kg and a protein content of 104 g/kg. Italic numbers refer to sunflower protein to whey protein ratio.

more severe aggregation that is favoured by heat-induced cross-linking at higher temperatures. Heat treatment at 140 °C resulted in a significantly lower amount of sediment compared to heating at 120 °C (Fig. 3), which in turn means a greater amount of serum expelled by centrifugation. This indicates a reduced water holding capacity of the whey protein aggregates due to more extensive protein cross-linking at 140 °C, as the amount of nitrogen in the serum did not change (Fig. 4).

In unheated blends, the sediment amount increased significantly with increasing press cake content (Fig. 3) due to the presence of both insoluble fibre and insoluble sunflower proteins. This was also reflected in the significant decrease in serum nitrogen with increasing press cake content, although the whole, uncentrifuged blends showed no difference (Fig. 4). After heat treatment at 80 °C, the amount of sediment was higher for blends with ≤175 g/kg press cake, whereas no significant effect was found for press cake contents of 200 and 225 g/kg, possibly due to the increased fraction of insoluble polysaccharides. The nitrogen content in the expelled serum phases also decreased upon heating at 80 °C for blends containing whey proteins (≤175 g/kg press cake), whereas a slight increase was observed for the sample containing only press cake (Fig. 4). This demonstrates that heat treatment at 80 °C affected mainly the whey proteins, which were dominant in blends with low press cake contents (Table 2).

Heat treatments at 120 and 140 °C resulted in significantly less expelled serum and, in turn, significantly higher sediment amounts (Fig. 3). High temperature treatments cause a higher degree of protein denaturation and polysaccharide hydration, thus reducing the amount of material in the serum (Fig. 4) and resulting in a greater water holding capacity of the sediment. For all blends containing press cake (>0 g/kg), there were no differences between the two heating temperatures in terms of sedimentation, and the effect of the press cake content was small. Since the amount of fibre present in the blends increased with increasing press cake content, one would expect a better water holding of the sediment of those blends. It might therefore be hypothesised that the denatured whey proteins have a high water holding capacity, and that their substitution with sunflower press cake was compensated due to the presence of fibres.

The proteins present in the blends and in the expelled centrifugal supernatants were further characterised using denaturing and reducing gel electrophoresis (Fig. 5). Samples containing sunflower press cake showed a number of bands corresponding to two major groups of sunflower storage proteins, namely sunflower albumins (SFA; 10–18 kDa) and helianthinin (Hel). The latter belongs to the protein class of globulins and is an oligomer of 300–350 kDa composed of six polypeptide chains with either acidic (30–44 kDa) or basic (20–27 kDa) isoelectric point (González-Pérez, 2015), which dissociate under reducing and denaturing conditions. Bands at ~50 kDa corresponded to the undissociated Hel trimer (7S). Due to similar molar masses, the major whey proteins α-lactalbumin and β-lactoglobulin (14 kDa and 18 kDa, respectively; Farrell Jr. et al., 2004) migrated to a similar position as SFA and could barely be distinguished in the blends. In contrast, bovine serum albumin (66 kDa; Farrell Jr. et al., 2004) was separated from all other protein bands.

Overall, the gel electrophoresis patterns confirm the findings on serum nitrogen (Fig. 4): with increasing press cake content, less protein was found in the supernatants, and heat treatment further decreased the amount of serum protein, with almost no protein bands detectable in the serum phases of blends treated at 120 and 140 °C (Fig. 5). This also confirmed that the 4–6 mg/g serum nitrogen found in the supernatants after high temperature treatments of the curds corresponded to non-protein nitrogen. Fig. 5 clearly shows that the band intensities of the major whey proteins (α-lactalbumin, β-lactoglobulin, bovine serum

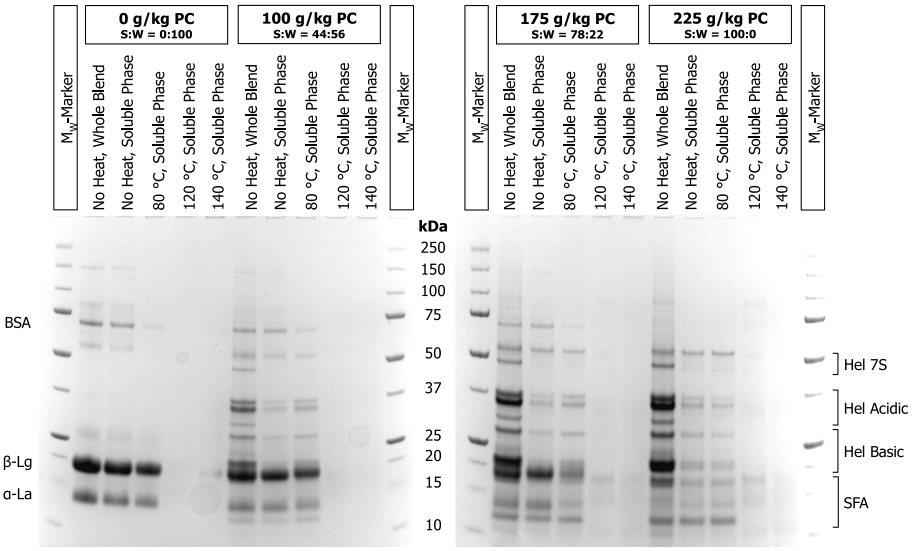


**Fig. 5.** Reducing and denaturing gel electrophoresis of side stream blends with different press cake (PC) contents and sunflower protein to whey protein ratios (S:W), and their serum phases extracted by centrifugation (3,000×*g*, 30 min, 25 °C) after different heat treatments under moderate shear in the rapid visco analyser. Different protein fractions are indicated: BSA – bovine serum albumin, β-lg – β-lactoglobulin, α-la – α-lactalbumin, Hel acidic – helianthinin acidic polypeptides, Hel basic – helianthinin basic polypeptides, SFA – sunflower albumins, Hel 7S – helianthinin trimeric subunits.

albumin) were similar for the whole blends and the corresponding supernatants, indicating a high solubility of these proteins. In contrast, all major sunflower proteins showed weaker band intensities in the supernatants compared to the whole blend, especially the Hel subunits, indicating a poor solubility in water. Heat treatment at 80 °C did not change the composition of sunflower proteins in the serum phase, whereas the intensities of the whey proteins became lower, as is consistent with the increase in sediment (Fig. 3) and decrease in serum nitrogen (Fig. 4) of blends containing more whey proteins. In contrast, almost all proteins except for some SFA disappeared from the serum phase after heating at 120 and 140 °C. Interestingly, no high molecular weight molecules appeared on the top of the gel, indicating that all proteins that were covalently cross-linked during the heat treatment were insoluble.

### 3.4. Structuring of side stream blends during heat treatment under moderate shear

#### 3.4.1. RVA torque profiles

The structural changes occurring in the blends during heating under moderate shear were characterised in more detail by following the changes in torque measured by the RVA. Fig. 6 shows the temperature profiles as well as the torque development in selected samples during heating to 80 (A), 120 (B), or 140 °C (C) and subsequent cooling. The torque is a measure for the resistance of the sample against the constant stirring of the RVA paddle at 160 rpm and can thus be seen as an indicator for viscosity changes and a mean to follow structure formation.

The initial torque values were in agreement with the bulk viscosities measured using rotational rheometry (Fig. 1B), showing higher values with increasing press cake content in the blends. For all blends with >0 g/kg press cake, the torque decreased during the initial holding at 30 °C, pointing to also thixotropic behaviour in addition to the shear thinning observed for these samples (Fig. 1A), and heating first caused a decrease in torque, reaching a minimum between 80 and 90 °C. Further heating had different effects on the blends depending on composition and temperature.

At 80 °C (Fig. 6A), the initial low torque value of the sample with 0 g/kg press cake (~0.04 mN m) increased continuously by more than a factor of 10 to ~0.45 mN m, whereas it remained nearly constant during the holding stage and increased to the original value during cooling for the sample with 225 g/kg press cake and without whey proteins. The other blends containing both whey proteins and press cake showed an intermediate behaviour between these two samples, with a slight decrease in torque during heating and an increase in torque during cooling to a value greater than the initial torque.

At 120 and 140 °C (Fig. 6B and C), there was a steep increase in torque with a first peak at a temperature of ~120 °C. Two peaks were observed in each torque profile, where the second one became more pronounced with increasing press cake content and was followed by a decrease in torque, with a larger drop for heat treatment at 140 compared to 120 °C. During the cooling stage, there was an increase in torque due to the formation of hydrogen bonds at lower temperatures. The torque profile of the sample with 225 g/kg press cake treated at 120 °C does not seem to match this general pattern. However, it has to be pointed out that the repeatability of this measurement was poorer than for the other treatments, especially after reaching the first peak, and while the second peak could be observed in some of the individual measurements, it was evened out when averaging all curves. This might have been due to the very high peak viscosity, possibly resulting in an unstable measurement signal due to an inhomogeneous flow within the sample. Remarkably, the final samples showed only small deviations with regard to colour (Fig. 2), insoluble material (Fig. 3), and serum nitrogen (Fig. 4).

The RVA has been extensively used to study the pasting and gelatinisation of starch suspensions, for which different parameters such as peak viscosity, breakdown viscosity, and setback have been defined and related to structural changes in the starch granules and interactions between the starch molecules (Balet, Guelpa, Fox, & Manley, 2019). In the current study, however, starch was a minor component, accounting for only ~11 g/kg of the original press cake material (Table 1) and thus for 0–2.5 g/kg in the different blends. Proteins, which accounted for 104 g/kg in the blends, are known to form elastic gel networks during heating due to non-covalent interactions (e.g., hydrophobic, hydrogen bonds) and covalent disulphide bonds, even at lower concentrations (Nicolai, 2019). Treatments that are more severe in terms of heating time and/or temperature can also result in covalent proteins cross-links deriving from advanced Maillard reaction products, which can additionally contribute to the gel network (Hannß, Hubbe, & Henle, 2018).

However, the RVA has also been used to evaluate protein-rich systems such as processed cheese, were changes in viscosity at 80 °C were related to hydration and oil uptake of the casein proteins (Kapoor, Lehtola, & Metzger, 2004; Kapoor & Metzger, 2005). Furthermore, heat-induced structure formation in blends of whey proteins and starch were studied using the RVA, showing a shift from starch-dominated networks to whey protein-dominated networks with increasing content of whey proteins (Sopade, Hardin, Fitzpatrick, Desmee, & Halley, 2006). The authors, however, pointed out that complementary methods are crucial to be able to relate the torque profiles obtained from the RVA to the heat-induced structure forming behaviour of biomacromolecule blends. Therefore, the blends were analysed also by gel electrophoresis and CLSM at different time points of the treatment.

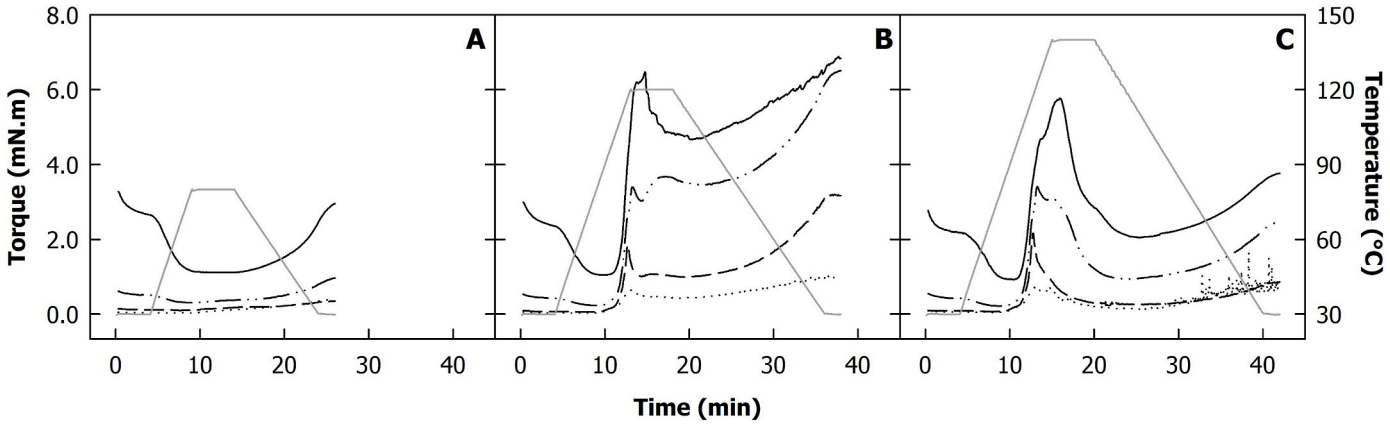


**Fig. 6.** Temperature profiles (grey curves) and torque development (black curves) of side stream blends with 0 (dotted lines), 100 (dashed lines), 175 (dotted dashed lines), and 225 g/kg press cakes (full lines) during heat treatment under moderate shear in the rapid visco analyser. Peak temperature were 80 (A), 120 (B), or 140 °C (C). All blends had a dry matter of 259 g/kg and a protein content of 104 g/kg.

*3.4.2. Protein profile of the press cake blends at different stages of heating*

To evaluate the formation of protein cross-links during heating, the blends were analysed for their protein composition at various stages of the treatments in the RVA. The selected time points corresponded to the first local minimum (10 min), the first and second peak, the second local minimum, and the end point in the RVA curves (see Fig. 6). Fig. 7 shows exemplarily the reducing gel electrophoresis patterns of the blends containing 175 g/kg press cake (78:22 sunflower protein to whey protein ratio) taken before and at different time points during heat treatment at 80, 120 or 140 °C in the RVA. Heating at 80 °C barely affected the protein profile of the blend, suggesting that interactions were mainly non-covalent ones as well as disulphide bonds, which were disrupted by the addition of the reducing agent. Samples treated at 120 and 140 °C, however, showed decreased band intensities of the monomeric proteins and increased intensities of high molar mass polymers appearing at the top of the gel, which were most likely a result of covalent protein cross-linking at high temperatures. Heat treatment at 140 °C caused a more pronounced decrease of monomeric proteins, indicating a greater extent of polymerisation due to covalent cross-linking of proteins, which is in line with the more pronounced change in colour due to Maillard reactions (Fig. 2). In both cases, 120 and 140 °C, high molar mass polymers were already noticeable in samples taken from the RVA at the first peak in the torque profile, but appeared even more clearly at the second peak in the torque profile, indicating the onset of the Maillard-type cross-linking of the proteins. Remarkably, a considerable amount of proteins in the blends heated at 120 and 140 °C was still detected in their monomeric form, although no monomeric proteins were found in the respective serum phases (Fig. 5). This indicates that the proteins in the curd were largely cross-linked by disulphide bonds, which were disrupted in gel electrophoresis by a reducing agent.

*3.4.3. Microstructure of the press cake blends at different stages of heating*

The microstructure of the side stream blend with 175 g/kg press cake (78:22 sunflower protein to whey protein ratio) before and at different time points of heat treatment at 80, 120, or 140 °C is shown in Fig. 8A. Only the proteins were stained and visualised in green. The unheated blend was a rather heterogeneous suspension of small protein particles with various sizes, which were dispersed in the aqueous, fibre-containing matrix. The treatment at 80 °C affected the microstructure only marginally, and no effect of shear was noticeable. In contrast, large protein aggregates of 10–100 μm were observed for heat treatment at 120 and 140 °C, where no effect of the heating temperature and no difference between samples taken from the RVA at the first peak and at the end could be noticed. The microstructure of blends heat treated without shear was, however, considerably different, showing a dense, homogeneous gel network. The effect of shear on the heat-induced formation of protein microparticles was also studied in the past, particularly with regard to microparticulated whey proteins (Erabit, Flick, & Alvarez, 2014; Tanger, Ramos, & Kulozik, 2021). Fig. 8B compares the microstructures of side stream blends containing 100, 175, or 225 g/kg press cake and heated at 120 °C under moderate shear. With increasing press cake content, the protein aggregates seemed more compact and showed a more defined surface structure, whereas at 100 g/kg press cake, the aggregates seemed bigger with a less homogeneous surface. This is most likely a consequence of the increased fibre content with increasing press cake concentration, as the fibres will compete with the proteins for water, leaving the protein aggregates less hydrated compared to blends with lower fibre content.

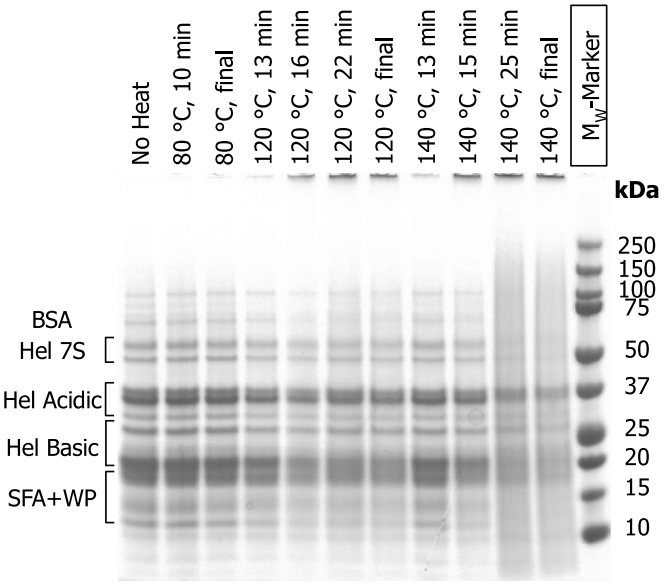


**Fig. 7.** Reducing and denaturing gel electrophoresis of side stream blends with 175 g/kg press cake (78:22 sunflower protein to whey protein) at different time points during heat treatment at 80, 120 or 140 °C under moderate shear in the rapid visco analyser. Different protein fractions are indicated: BSA – bovine serum albumin, WP – whey proteins (β-lactoglobulin and α-lactalbumin), Hel acidic – helianthinin acidic polypeptides, Hel basic – helianthinin basic polypeptides, SFA – sunflower albumins, Hel 7S – helianthinin trimeric subunits.

*3.5. Proposed mechanism for structure formation during heat treatment under moderate shear*

From these results, the following mechanisms for structure formation during heat treatment of the side stream blends under moderate shear might be proposed (Fig. 9).

Heat treatment at 80 °C mainly had an impact on the whey proteins, which were denatured and partly aggregated through cross-linking by disulphide bonds, leading to an increased sample viscosity (Fig. 6A) and higher amounts of sedimentable material of blends with higher whey protein content and less press cake (Fig. 3). On the other hand, the treatment had only minor effects on the sunflower press cake, as the starch content was too low to cause major changes in sample viscosity with heating, and the majority of the sunflower proteins were already insoluble before the treatment (Fig. 4).

The high temperature treatments, however, caused severe changes to the sample. A steep increase in torque induced at temperatures of >85 °C indicated pronounced protein denaturation and aggregation (Fig. 6B and C). The first peak in torque was reached at around 120 °C for both high temperature treatments, and was followed by a second peak, which became more pronounced with increasing press cake content, suggesting that this second peak was somehow related to the fibres in the press cake. Assuming that the initial increase in torque was solely related to protein denaturation and aggregation, the first peak might be due to a loss in the water holding capacity of the protein particles, thereby decreasing their volume fraction and reducing the serum viscosity. As shown in Fig. 7, there was a considerable increase in high molecular weight protein polymers between the first and second peak in the RVA torque profile, suggesting severe covalent protein cross-linking in this stage of the treatment. This might have resulted in a compacting of the protein particles and thus a loss of water, which could then have been taken up by the fibres, resulting in an increase in bulk viscosity and thus the second peak in the torque profiles (Fig. 6B and C). A competition for water between the protein particles and the fibres was also indicated by the micrographs, which showed more compact protein particles in blends with higher fibre content (Fig. 8B). The second peak maximum was reached even before the cooling stage, followed by a decrease in the torque signal. No differences in the microstructure of the samples taken out at different times of the treatment were found using CLSM, indicating that this drop in viscosity is related to structural changes at smaller length scales. This could, for instance, be a transition of the polysaccharide fraction from crystalline to amorphous structures. When entering the cooling stage, the torque signal increased again, indicating

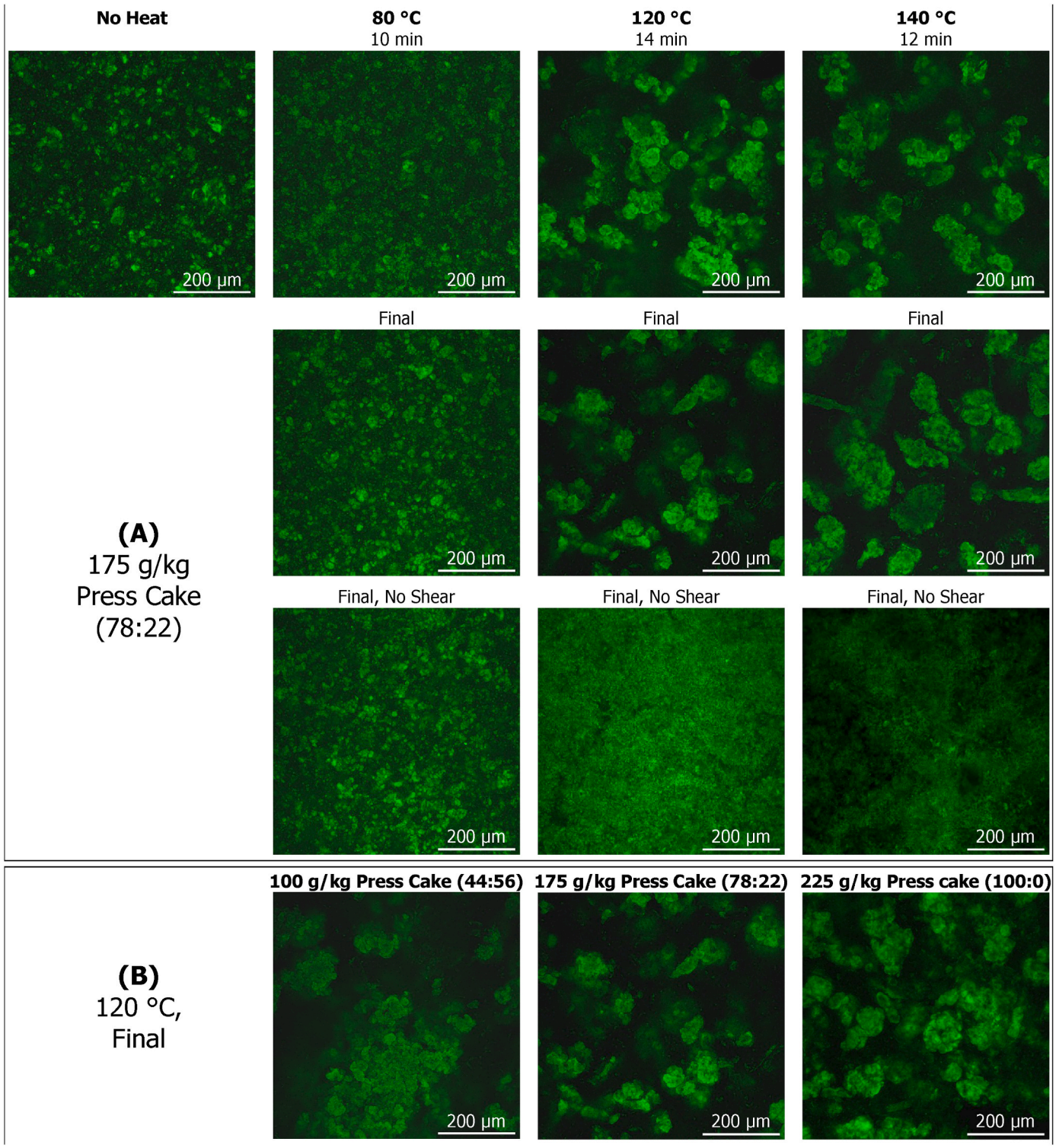


**Fig. 8.** Confocal laser scanning microscopy of (A) side streams blends with 175 g/kg press cake at different time points during heat treatment at 80, 120 or 140 °C with or without moderate shear in the rapid visco analyser, and of (B) side stream blends with different press cake concentrations at the end of heat treatment at 120 °C under moderate shear. Numbers in brackets refer to sunflower protein to whey protein ratio.

an increase in viscosity due to swelling of the protein particles and fibres (de Kruif et al., 2015) as well as the formation of hydrogen bonds.

The torque (Fig. 6) at the end of the process relative to the torque at the beginning of the heating ramp was higher at press cake contents of ≤175 g/kg (78:22 sunflower protein to whey protein ratio), meaning a greater relative viscosity increase in systems containing more whey proteins and less press cake. This could be due to the better gelling properties of whey proteins compared to both sunflower proteins and fibres.

## 4. Conclusions

This study described the structure formation in different blends of sunflower press cake and whey ingredients with equal dry matter and

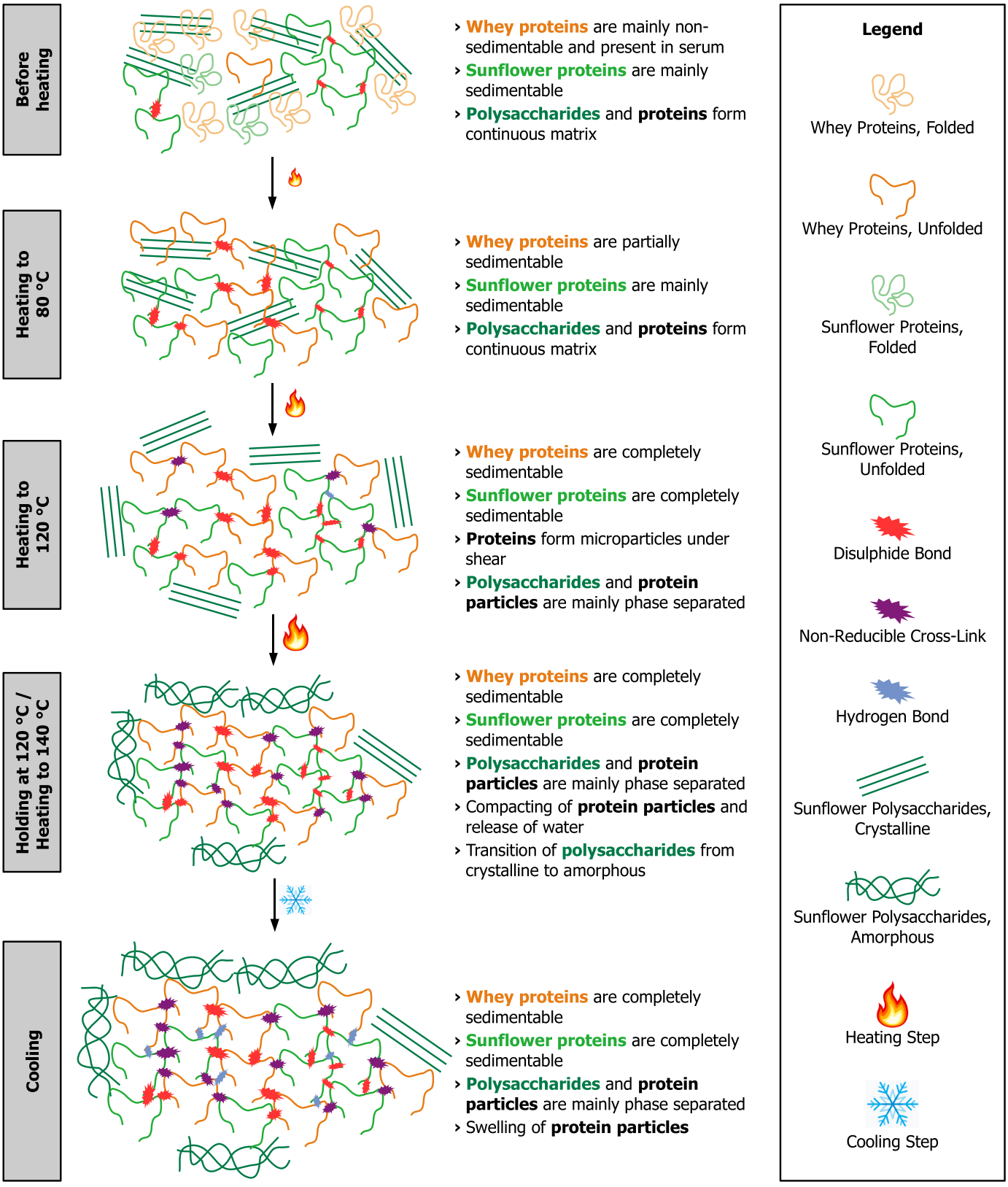


**Fig. 9.** Proposed mechanism for structure formation during heat treatment of side stream blends under moderate shear.

protein content but different press cake contents during heat and shear treatment performed in the RVA. The results suggested a complex interplay of protein denaturation and aggregation and fibre swelling and breakdown. It seemed that the whey proteins were generally more capable of increasing the apparent viscosity of the blends due to their heat-induced gelling, whereas the fibres in the press cake contributed more to the overall apparent viscosity. Heat treatment at 120 °C seemed most favourable in terms of viscosity development, phase stability, and colour stability. The results of this study will help valorising these two food processing side streams in future, sustainable food applications, as

it provides a detailed understanding of the systems. Future investigations will include the fermentation of the heated side stream blends using co-cultures of lactic acid bacteria and yeast strains (Mangieri et al., 2022), which will increase their microbial stability and enhance their sensory properties, fostering the development of sustainable food applications.

## Funding

The authors acknowledge the financial support for this project provided by translational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofunds, under the Joint SUSFOOD2/CORE Organic Call 2019. The national partner organisation was the Ministry of Food, Agriculture and Fisheries of Denmark.

Additional funding was received from Novo Nordisk Fonden (grant number NNF21OC0071375) for NR, and from Villum Fonden (grant number 37759) for MC.

## Credit authorship statement

Norbert Raak: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Data curation; Writing – original draft; Visualization. Milena Corredig: Conceptualization; Methodology; Resources; Writing – review & editing; Supervision; Project administration; Funding acquisition.

## 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.

## Acknowledgements

The authors kindly thank Schalk Mühle Gmbh & Co KG (Ilz, Austria) for donating the sunflower press cake, Bayerische Milchindustrie eG (Palting, Germany) for gifting the sweet whey powder, and Arla Foods Amba (Viby, Denmark) for providing the whey protein concentrate and skim milk. Special thanks go to Laura Roman Rivas, Kubra Tarin, and Rita Albrechtsen (Department of Food Science, Aarhus University) for support in the laboratory work.

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