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The effect of temperature and shear upon technological properties of whey protein concentrate: Aggregation in a tubular heat exchanger

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ABSTRACT

Microparticulation of whey proteins at low concentration (2%, w/v), was examined in a pilot plant tubular heat exchanger (THE). Turbulent flow in combination with moderate temperatures (≤85 °C) was used in the heating section to prevent fouling, whereas the flow was varied from laminar to turbulent in the holding section of the THE. The logarithm of the formal rate of denaturation of β-lactoglobulin (β-Lg) k_f was −5.4 to −2.5 depending on the temperature. Variation of flow velocity in the holding section had a negligible impact on denaturation degree of β-Lg and particle size of agglomerates. A high increase of elastic modulus, G′, of agglomerates was combined with only bisection of water holding capacity. Advanced modifications of particle structure and properties are supposed to be achievable by more freedom in control of flow character at a heating section of a THE for example through application of direct heat transfer principles.

1. Introduction

Whey protein (WP) powders are widely used as ingredients to influence food quality, especially food texture, water holding capacity or emulsion stability. Advanced separation technologies (such as cross flow filtration) enable fractionation, concentration and, in combination with heat treatment, functionalisation of whey components.

β-Lactoglobulin (β-Lg) represents about 60% of whey proteins (Edwards & Jameson, 2014). Heat and, more specifically, the time-temperature history of WP concentrate or isolate has the potential to induce agglomeration and microparticulation of β-Lg (Spiegel, 1999; Tolkach & Kulozik, 2007), and is coupled to additional factors of the physicochemical environment such as pH (Dissanayake, Ramchandran, Donkor, & Vasiljevic, 2013; Giroux, Houde, & Britten, 2010; Mehalebi, Nicolai, & Durand, 2008; Spiegel & Huss, 2002), calcium concentration (Erabit, Flick, & Alvarez, 2014; Erabit, Ndoye, Alvarez, & Flick, 2015; Giroux et al., 2010; Spiegel & Huss, 2002), ionic strength (Nicorescu et al., 2008a,b) and whey protein concentration (Dissanayake et al., 2013; Erabit et al., 2014; Mehalebi et al., 2008; Wolz & Kulozik, 2015).

Most kinetic studies on microparticulation of whey proteins have been performed at the laboratory scale by indirectly heating a solution in a water bath (Croguennec, O'Kennedy, & Mehra, 2004; Tolkach & Kulozik, 2007) or Couette cell (Erabit et al., 2014; Simmons, Jayaraman, & Fryer, 2007; Steventon, Donald, & Gladen, 1994). A cylindrical, coaxial Couette cell generates a laminar flow in incompressible, viscous liquids and is used in this context to elucidate the impact in the shear rate upon agglomeration kinetics and tailoring of WP. Simmons et al. (2007) observed an increase of size of agglomerates due to a decrease of shear rate from 624 down to 111 s^{-1} and more specifically a local maximum at about 300 s^{-1}, when agglomeration was performed at 80 °C for 20 min. Erabit et al. (2014) found a positive correlation of shear rate and particle size if agglomeration of β-Lg was carried out at a shear rate from 0 to 400 s^{-1}. The impact of higher shear on faster heat transfer and thus temperature evolution in the solution was corrected in this study by modelling the temperature in the gap of the Couette cell. Such observations are explained by an increase in collision rate at increasing shear rate, followed by a break-up of agglomerates at shear rates higher than the critical shear rate. However, a shear rate of about 300–400 s^{-1} might be advantageous in formation of agglomerates, but the concept has not yet been screened for heat exchanger geometries as applied in dairy industry.

A specific challenge during microparticulation of WP in an industrial operation unit is to prevent fouling during heating (Guérin, Ronse, Bouvier, Debreyne, & Delaplace, 2007) and enhancing the heat transfer coefficient. Both objectives can be achieved by high shear rates and/or continuing mechanical cleaning of heat exchanger surfaces by blades. Spiegel (1999) reported on

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aggregation of a 10% (w/w) WP dispersion in a scraped surface heat exchanger (SSHE). In such a case the beneficial process stability is in opposition to a wide residence time distribution (RTD) and undefined shear rate. A further possibility to control fouling during heating und agglomeration of concentrated (20–25%, w/w) WP dispersions is implementation of twin screw extruders (Mustapha, Ruttarattanamongkol, & Rizvi, 2012; Nor Afizah & Rizvi, 2014). Shear rates vary strongly in different zones of extrusion channel and thus it is sophisticated to elucidate the impact of this parameter upon microparticulation.

Heating of WP dispersions can be carried out under laminar flow without mechanical surface cleaning in laboratory scale equipment, but rather not in pilot plant or industrial equipment due to fouling and consequently process instability. High turbulence at the heating section of plate or tubular heat exchangers (THE) counteracts heat transfer efficiency reducing fouling.

The aim of this work was to define the heating section of a THE in such a way that the process is stable, which is supposed to be the case at high turbulence and moderate denaturation temperature and the impact of shear rate on agglomeration of β-Lg at the holding section of a THE can be elucidated. The shear rate, $\dot{\gamma}$, is calculated by a one-dimensional difference in velocity only and is not in all cases satisfying for comparisons from experiment to experiment since parameters, except flow rate and hydraulic diameter, influencing flow dynamics are not integrated. For this reason $\dot{\gamma}$ is supplemented with more general, dimensionless quantities for characterisation of flow, namely the Reynolds number, Re, and the friction factor, f. As the technological properties as water holding capacity of microparticulated WP are supposed to depend strongly on denaturation degree and particle size of WP, the question should be answered weather these properties can be efficiently influenced by the character of flow at holding tubes or not.

2. Materials and methods

2.1. Preparation of reconstituted whey

A 2% (w/w) whey proteins (WP) solution was prepared using powdered whey protein concentrate (WPC) containing for a minimum 80% of WP (LEDOR MO80T, Hochdorf Swiss Nutrition Ltd., Hochdorf, Switzerland) and demineralised water. The solution contained 8.5 ± 0.2 mg g^{-1} of native β-Lg, which equates to approximately 77% of total β-Lg, and 1.9 ± 0.1 mg g^{-1} of native α-Lactalbumin (analysis done according to 2.3). To equilibrate the loss of calcium, occurring in concentration before spray drying of whey, 2 mM $CaCl_2$ (calcium chloride dihydrate, Acros Organics, NJ, USA) was added. The solution was kept under mechanical stirring at room temperature for 30 min and then without stirring at 4 °C for at least 12 h. The final pH of the solution was 6.2. Composition and concentration of whey used in industrial powder fabrication may differ. The impact of higher protein concentration was tested and will be discussed in the results section.

2.2. Tubular heat exchanger

Texturisation was performed experimentally using a double pipe tubular heat exchanger from corrugated and smooth tubes (steel alloy, X2CrNiMo17-12-2) with a thermal conductivity of 15 W m^{-1} K^{-1} (SPE Tech AG, Frauenkappelen, Switzerland). Inner tubes had a diameter of 10 mm and outer tubes a diameter of 20 mm, both with 2 mm of wall thickness and 3 m of length per tube. The product was inside the inner tube, the energy side was between the inner and the outer tubes.

The heating section was created with helically corrugated tubes. Corrugations were made of 3 parallel spirals, on the inner tube, with an inclination angle of 26° and 1.5 mm depth. This geometry was selected in pre-tests out of 7 geometries due to the highest overall heat transfer coefficient. The holding section was composed of smooth tubes. In a first set of experiments, six flow rates were tested ranging from 40 L h^{-1} to 120 L h^{-1} (±1 L h^{-1}) without modifying the residence time (44 ± 1 s), but only the total length of serially arranged tubes in the heating and holding section. The set-up is illustrated in Fig. 1a. In a second set of experiments, the flow rate was constant with 60 L h^{-1}. The heating section was composed of 3 corrugated tubes. The holding section comprised 6 smooth tubes connected in series and/or in parallel (Fig. 1b) to split the flow and create laminar up to turbulent flow, and more specifically to vary the shear rate from 28 to 168 s^{-1}.

To provide a pressure difference, a progressive cavity pump (Mono NL 15A, Socsil-Inter SA, Lausanne, Switzerland) was used. The flow rate was determined by a magnetic-inductive flow meter (Optiflux 6100, Krohne, Dordrecht, Netherlands). Pt-100 temperature sensors (TMR31 – PT100/4wD, Endress + Hauser, Nesselwang, Germany) were installed at the inlet and outlet of each section of the THE (see Fig. 1a): at the entrance and exit of product heat section, at the entrance and exit of product at the holding section and finally at entrance and exit of hot fluid at heating section. Pressure sensors (Pt-3544, Ifm electronic, Essen, Germany) were placed next to the temperature sensors: at the inlet and outlet of product at the heating and holding section. A membrane valve was placed at the end of holding section to produce a back pressure of at least 0.5 bar. Hot water was used as heat transfer fluid with a flow ≤1200 L h^{-1} and in counter-current flow. A second tubular heat exchanger (Tubotherm, APV Rosita AG, Worb, Switzerland) equipped with smooth tubes of a 4 times larger circular cross section than applied at the holding section was used for cooling of the product. Tap water of about 10 °C was used for cooling at a flow rate of 500 ± 50 L h^{-1} and in counter-current flow. The flow rate was measured with a magnetic-inductive flow meter. The product temperature after cooling varied between 15 and 20 °C depending on heating temperature.

Flow, temperature and pressure were recorded each second and the arithmetic mean of 5 min was calculated once the temperature of product was stable (ΔT ≤ 0.5 °C). Samples (3 L) were collected over 1.5–3.0 min after the process was stable, depending on the flow rate. Before subsequent analysis (see 2.5–2.9) samples were stored at 4 °C.

2.3. Moody chart

The effect of flow rate on turbulence and the type of flow in the heating and holding section of the THE was determined in a preliminary test with a 2% starch solution (Clearam® CH 20 20, Roquette, Cassano Spinola, Italy) that was injected with a flow rate that varied from 16 to 400 L h^{-1} at 40 °C and 70 °C for smooth tubes and 50 °C, 70 °C and 90 °C for corrugated tubes. The transition from laminar to turbulent flow is recognisable by the shape of the curve in a Reynolds number – Friction factor – plot (Moody-chart).

The Reynolds number, Re, is a ratio of inertial force and viscous force:

$$Re = \frac{\bar{v}_z * d * \rho}{\mu} \quad (1)$$

in which $\bar{v}_z$ (m s^{-1}) is the average flow velocity in the axial direction, d (m) is the inner diameter of the tube, ρ (kg m^{-3}) is the density of the liquid and μ (mPa s) the dynamic viscosity. The friction factor in tube flow is given by the Darcy-Weissbach-equation:

$$f = \frac{2d}{\rho * \bar{v}_z^2 * l} * \Delta p \quad (2)$$

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in which l (m) is the length of the tube and Δp (bar) is the pressure drop (Kast, 2010). For fully developed laminar flow the friction factor depends linearly on the Reynolds number ($f = 64/Re$). For turbulent flow and at the transition zone between laminar and turbulent flow the friction factor is determined by the Reynolds number and the "roughness" of the surface.

The measurements of temperature, pressure-drop and flux were made at the inlet and outlet of a series of three tubes of 3 m of length per tube. The result is an average of the flow behaviour in the entire system including bend tubes and inlet irregularities, but due to the length-diameter ratio of the tubes the contribution of fully developed flow is considered dominating. The type of flow, laminar or turbulent, for WP solutions was estimated by determining the dynamic viscosity, density and flow rate, calculation of Re and reading the corresponding position out of before developed Moody charts which are specific for a certain tube geometry.

2.4. Dynamic viscosity

The temperature dependent viscosity of a 2% (w/w) WP solution was measured with concentric cylinder geometry (Couette cell) and in rotary mode (MCR302; PTD200, CC27, Anton Paar, Graz, Austria). Temperature was linearly increased from 10 to 95 °C with a heating rate of 0.05 °C s^{-1}. The shear rate was kept constant at 40 s^{-1}. The solution behaves slightly pseudo-plastic, but viscosity is almost stable at a shear rate of 40 s^{-1} and higher. To prevent turbulent flow a low shear rate on the plateau of the viscosity curve was chosen for performing the temperature sweep. Viscosity of WP solutions in the THE trials was calculated for the average of the measured temperature at the holding section on the basis of the laboratory scale measurement of dynamic viscosity and used in calculation of Reynolds numbers. The density of 2% WP solution was assumed to equal to this of water at corresponding temperatures.

2.5. Determination of the degree of whey protein denaturation

The samples were filtered (0.45 μm; Exapure™, Switzerland) into 1.5 mL High Performance Liquid Chromatography (HPLC) vials (BGB, Rheinfelden, Germany) and sealed with aluminium crimp caps (BGB, Rheinfelden, Germany). Analysis was performed on a 1220 Infinity LC HPLC system equipped with 1220 Infinity DAD LC Detector (Agilent Technologies, Waldbronn, Germany) and a reversed-phase C18 column (Luna, 250 × 4.6 mm, 5 μm particle size; Phenomenex, Torrance, CA, USA) as well as a RP C18 guard cartridge (4 × 3 mm ID; Phenomenex). The elution method was adapted from Mayer, Raba, Meierand, and Schmid (2010). Initial conditions were 64% solvent A [0.1%, w/v, trifluoracetic acid 99% extra pure (TFA; Acros Organics, Geel, Belgium) in Milli-Q water] and 36% solvent B [0.1%, w/v, TFA in acetonitrile (Macron Fine Chemicals, origin USA, Avantor performance Materials Poland, Gliwice, Poland)]. Elution was done with the following conditions: 0–14 min, linear gradient from 64% to 50% solvent A; 14–15 min, linear gradient from 50 to 10% solvent A; 15–17 min, holding 10% solvent A and 90% solvent B. The column was brought back to the starting conditions within 3 min. UV detection was done at 205 nm. Flow rate was set to 1.2 mL min^{-1}, oven temperature was 40 °C, and injection volume was 10 μL.

Lyophilised powders of α-lactalbumin and β-lactoglobulin from bovine milk (purity ≥85% and ≥90%, respectively) were used as standards and obtained from Sigma–Aldrich (St. Louis, USA). The powders were solubilised in a 2 mM $CaCl_2$ solution at concentrations ranging from 0.05% to 1.8% (w/w). The denaturation level L_d is represented by the ratio of HPLC accessible (native) proteins after ($c_{HPLC,t}$) and before ($c_{HPLC,0}$) heat treatment of WP solution:

$$L_d = \frac{c_{HPLC,t}}{c_{HPLC,0}} \quad (3)$$

2.6. Particle size

The volume-size distribution of aggregates was measured by a static light scattering method and within a measurement range from 0.02 μm to 2000 μm (Mastersizer 2000, Malvern Instruments, Malvern, UK). The samples were diluted in a ratio of 1:50 with 2 mM $CaCl_2$ solution, used as carrier fluid. The aim of using a 2 mM $CaCl_2$ solution was to keep the ionic strength of reconstituted whey during dilution of the sample. Optical and analysis models which were applied corresponded to Fraunhofer and general purpose, respectively.

2.7. Freeze drying of reconstituted whey

Prior to analysis of water holding capacity (WHC) and rheological properties of microparticulated whey proteins, the heat treated, microparticulated reconstituted whey was concentrated with a rotor evaporator (Laborota 20 control, Heidolph Instruments, Schwabach, Germany) to about 15% dry matter at an operating temperature of 40 °C, a pressure of 30 mbar and a rotation speed of 35 rpm. Subsequently the sample was frozen in a freezer chamber at −20 °C on stainless steel plates and freeze dried (Lyolab BII − LSL, Secfroid, Aclens, Switzerland) at −55 °C. Freeze drying was carried out for at least 20 h at a pressure of 0.037 mbar. The dried sample was gently crushed using a mortar and pestle to obtain a powder and stored prior to analysis in sealed plastic bags. To compensate modifications arising possibly from the drying procedure WHC and storage modulus (G′) of microparticulated WP was related to WHC and G′ of a reference (not microparticulated, but freeze dried) for each batch.

2.8. Water holding capacity

The WHC measurements were adapted from Peters, Luyten, Alting, Boom, and van der Goot (2015). A 10% (w/w) dispersion was prepared from the freeze dried whey on a dry matter basis and demineralised water. This concentration was chosen to obtain a supernatant and a pellet. The preparation was kept at room temperature for 2 h before centrifugation (Universal 32R, Hettich Zentrifugen, Tuttingen, Germany) at 2000 × g for 20 min at 25 °C. The supernatant was discarded and the wet pellet weighed. The wet pellet was dried until a constant weight was reached (60 °C; 15 h). The WHC was calculated in g of water per g of dry matter as shown in equation (4). Analyses were made in triplicate; subsequently the arithmetic mean and standard deviation were calculated.

$$\mathrm{WHC} = \frac{\text{mass of wet pellet} - \text{mass of dry pellet}}{\text{mass of dry pellet}} \quad (4)$$

2.9. Elastic modulus

A 30% (w/w) dispersion was prepared from the freeze dried whey on a dry matter basis and demineralised water. The dispersion was kept under stirring for 2 h at 20 °C and stored at 4 °C overnight (about 14 h). G′ of this preparation was determined by an oscillatory test performed on a rheometer equipped with cone-plate geometry (MCR 302, P-PDT 200, C 25-2; Anton Paar, Graz, Austria). The amplitude of deformation γ ranged from 0.01 to 100% (5 measuring points per decade) at a constant angular frequency of 10 rad s^{-1}. The indicated G′ is the value at which G′ drops by 10% of the average (limit of linear visco-elastic range). The measurement was performed at 20 ± 0.1 °C. Analysis was made in triplicate. On

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this basis the arithmetic mean and standard deviation were calculated.

3. Results and discussion

3.1. Friction and character of flow in smooth and corrugated tubes

The friction factor is represented as a function of Reynolds number in Fig. 2. The dotted line on the Moody-Chart represents the theoretical line of laminar flow, which is given by f = 64/Re. A deviation from this line indicates the flow character change, from laminar to transient and turbulent. For the corrugated tubes this deviation occurs at a lower Reynolds number (critical Re ≈ 400) than for smooth tubes (critical Re ≈ 3000), and from this point the friction factor is larger for the corrugated tube than for the smooth tube. A flow rate of 80 L h^{-1} in a smooth tube here equals a shear rate around 100 s^{-1} ranging from zero in the centre to 226 s^{-1} at the edge of the tube (see also Fig. 6). Such a degree of shear is supposed to be related to laminar flow in a lab scale Couette cell, but might be related to a turbulent flow in a pilot plant or industrial scale THE. This should be taken in account at scaling steps or comparison of shear rate effects upon proteins agglomeration determined with different type of equipment. The corrugated tubes largely assured a turbulent flow in the heating section, needed to obtain high heat transfer coefficients, negligible fouling effects, and to increase process stability, respectively.

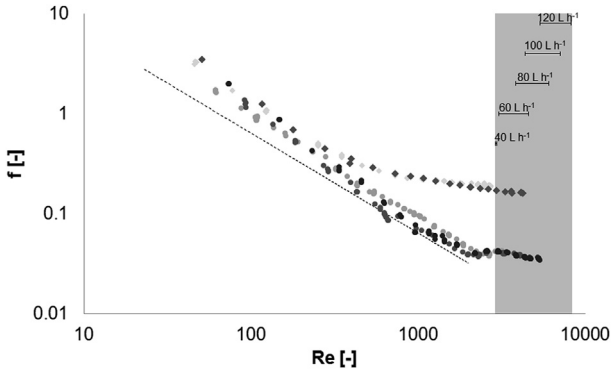

Fig. 2. Friction factors (f) for flow in smooth (●, dots) and corrugated (◆, diamonds) tubes as a function of Reynolds numbers (Re). Grey scale of symbols signify the process temperature applied during analysis of friction factors, which has an impact on viscosity und thus Re: 50 °C (●), 70 °C (●), 90 °C (●), 40 °C (●) and 70 °C (◆). The grey bar indicates the global operating area and numbers above the curve the specific shear rate at microparticulation of whey proteins. Width of error bars depends on flow rate fluctuation during experiments. The friction factor for fully developed laminar flow (f = 64/Re) is represented by the dotted line.

3.2. Influence of temperature on denaturation of β-Lg

A kinetic model for description of heat induced denaturation of β-Lg was proposed by Tolkach and Kulozik (2007). The model considers three states of β-Lg: native (N), reversible unfolded (U) and irreversible denatured state (D). States N and U can be detected together by HPLC analysis, as U refolds at temperatures below 60 °C. Denatured proteins (D) are the amount of native proteins before heat treatment (initial concentration) minus the amount of proteins in N + U after heat treatment (final concentration). At a given temperature the formal rate of denaturation k_f is described by

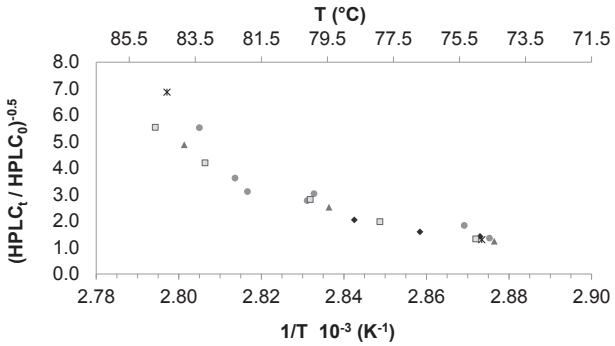

Fig. 3. Denaturation degree of β-lactoglobulin as a function of holding temperature and flow rate (L h^{-1}): ◆, 40; ●, 60; ▲, 80; ■, 100; ✳, 120. The holding time was constant and equals to 44 ± 1 s.

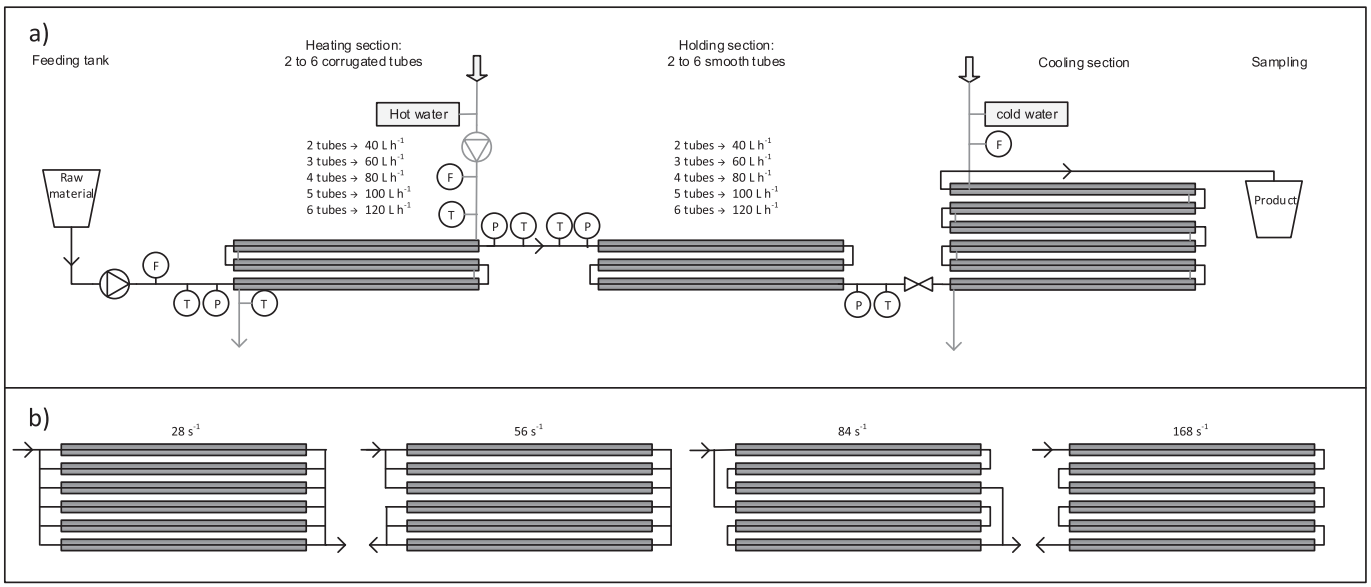

Fig. 1. Schematic drawing of configuration of tubular heat exchanger: a) A variable number of 2–6 serial tubes per section used to create the heating and holding sections in order to keep the residence time constant at 44 ± 1 s per section at a variable flow rate of 40–120 L h^{-1}, b) Parallel-serial set up of 6 tubes at the holding section, combined with 6 serial tubes at the heating section to vary the shear rate stepwise from 28 to 168 s^{-1} at a constant flow rate of 60 L h^{-1}. Further configuration was identical to a).

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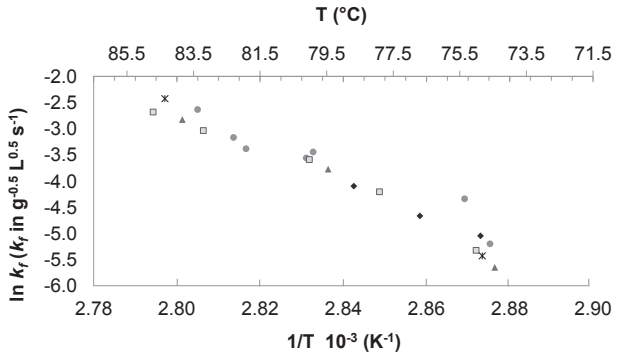

Fig. 4. Formal denaturation rate k_f as a function of holding temperature and flow rate (L h^{-1}: ◆, 40; ●, 60; ▲, 80; ■, 100; ⁎, 120) at the holding section of tubular heat exchanger.

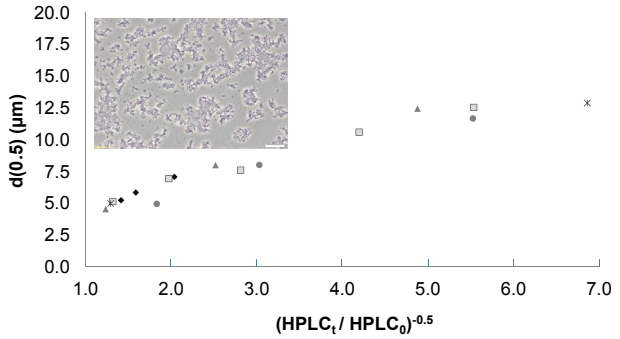

Fig. 5. Influence of denaturation degree on median volume diameter d(0.5) of agglomerates (flow rates in L h^{-1}: ◆, 40; ●, 60; ▲, 80; ■, 100; ⁎, 120). Insert: microparticulated whey protein, light microscopy, magnification × 40.

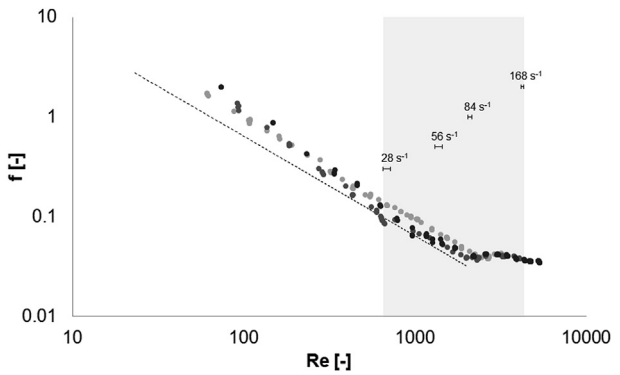

Fig. 6. Moody chart for flow in smooth tubes (●, dots). The grey bar indicates the global operating area and numbers above the curve the specific shear rate. Width of error bars depends on flow rate fluctuation during experiments. The friction factor (f) of fully developed laminar flow (f = 64/Re) is represented by the dotted line.

equation (5) where the order of reaction *n* is 1.5 (Tolkach & Kulozik, 2007). The concentration of HPLC accessible or native β-Lg before and after heat treatment is $c_{HPLC,0}$ and $c_{HPLC,t}$ respectively.

$$k_f = \left(\left(\frac{c_{HPLC,t}}{c_{HPLC,0}}\right)^{(1-n)} - 1\right) \Big/ \left((n-1)*c_{HPLC,0}^{(n-1)}*t\right) \quad (5)$$

The level of β-Lg denaturation is about to 98% at a holding temperature of 84.4 °C and is as expected a function of holding temperature (Fig. 3). For example, HPLC-accessible β-Lg was determined to be 0.19 mg g^{-1} after the heat treatment, which equals to 2 residual native β-Lg, and 8.83 mg g^{-1} before the heat treatment. Admitting that the reaction order n equals to 1.5, the denaturation level ($HPLC_t/HPLC_0$) to the power of (1−n) is 6.9 (equations (3) and (5)). The holding temperature was determined as the average of entry and exit temperatures at the holding tube.

Apart from the holding temperature, the flow rate was varied in this experimental set up. An impact on degree of β-Lg denaturation cannot be deviated from observations (Fig. 3). In addition, at low flow rates, measurements are limited to a smaller range of temperatures because of process instability due to fouling in the heating section under such conditions. For this reason at a flow rate of 40 L h^{-1} the highest holding temperature represented is about 78 °C.

Denaturation of β-Lg by thermal treatment is a combination of unfolding and aggregation events. A sharp bend at about 90 °C in the Arrhenius plot marks the transition of aggregation of β-Lg that is limited by the unfolding rate to aggregation that is limited by the aggregation rate of unfolded β-Lg. Such a behaviour was found at agglomeration of a β-Lg preparation made from freeze–dried proteins which was dissolved in ultrafiltration permeate of whey containing lactose (Tolkach & Kulozik, 2007) as well as for a preparation of a whey protein isolate dissolved in a 1 M $CaCl_2$-solution without presence of lactose (Erabit et al., 2014). At holding temperatures lower than 85 °C as applied in the herein presented experimental set up such a sharp bend in the Arrhenius plot cannot be observed (Fig. 4). The formal denaturation rate is supposed to be controlled by the rate of unfolding of β-Lg within the investigated temperature range.

The logarithm of the formal denaturation rate ln k_f was −5.4 to −2.5 in the temperature range between 76 and 85 °C (see Fig. 4), and thus higher in comparison with observations published by Tolkach and Kulozik (2007), where the ln k_f was about −10 to −7 for the same temperature range. Higher formal denaturation rates observed in this study are supposed to be related to higher heat sensibility of spray dried than freeze dried WP, used for reconstitution of whey, and other factors as a low lactose concentration or different Ca^{2+} concentration in the reconstituted whey. It should be mentioned, that an increasing lactose concentration (1.5–20%) decelerates the agglomeration of β-Lg at 80 °C (Spiegel, 1999). Tolkach and Kulozik (2007) performed kinetic studies at about 4.5% lactose concentration. Nevertheless, the slopes of k_f curves determined by Tolkach and Kulozik (2007) and in this study are similar, which can be interpreted as a similarity of the order of reaction even if the mechanism of reaction is rather complex.

The particle diameter (d(0.5)) was found to be in the range 5–13 µm. Heat treatment temperature, degree of denaturation and particle size are positively correlated (Fig. 5). Additional parameters as the calcium ion concentration or residence time have an impact on the particle size (Erabit et al., 2014), but where not studied here. Agglomerates were characterised by a loose, porous structure of unknown mechanical stability (insert in Fig. 5). Nevertheless, small particles (about 5 µm and smaller) resist to harsh shear conditions as applied in corundum stone mills or homogenisers for example (data not shown).

3.3. Influence of flow rate in holding tube on denaturation of β-Lg

To investigate the impact of laminar versus turbulent flow on agglomeration and physical properties of agglomerates, the before presented set up, which generates even in smooth tubes a turbulent flow as concluded from the Moody chart, was modified and a serially-parallel configuration of tubes at the holding section was applied (see Fig. 1b). A laminar flow was observed at shear rates of

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28 and 56 s^{-1}. The friction factor, for shear rates at this zone, follows perfectly the function f = 64/Re (Fig. 6). At a shear rate of 84 s^{-1} the flow was within the transient region of flow, and at a shear rate of 168 s^{-1} the flow was found to be turbulent.

The formal denaturation rate k_f of β-Lg is represented by a straight line in the Arrhenius plot in the temperature range 72.5–80.9 °C (Fig. 7). The variation of the denaturation rate as a function of flow rate is much smaller than the variation as a function of temperature and there is no clear difference between different flow rates. A very similar statement as for the degree of denaturation or the formal denaturation rate is indicated for the particle size. The particle size is controlled first of all by the process temperature and thus degree of denaturation at a constant flow rate and residence time (Fig. 8). Variance of particle size becomes more important at a higher degree of denaturation, but a hypothesis on a systematic impact of shear rate ranging from 28 to 168 s^{-1} cannot be deviated.

Simmons et al. (2007) observed an increase of size of agglomerates due to a decrease of shear rate from 624 down to 111 s^{-1} and a local maximum at about 300 s^{-1}. Agglomeration was performed at 80 °C for 20 min. Erabit et al. (2014) found a positive correlation of shear rate and particle size if agglomeration of β-Lg was carried out at a shear rate from 0 to 400 s^{-1}. In both studies a Couette cell was applied at heating, holding and cooling of the substrate. Such observations are in line with the hypothesis, that an increasing shear rate up to a critical value of between 300 and 400 s^{-1} increase the probability of collisions of molecules and thus aggregation resulting in larger particles size. This observation cannot be confirmed for our THE configuration, where the particle sizes do not depend on the shear rate or flow character in the holding tube. This limitation of particle size increase might be related to the required turbulent flow at the heating section. It should be remembered to the fact that the heating section was created by corrugated tubes and the flow rate as well as the friction factor was not modified during this experimental set up. A flow rate of 60 L h^{-1} corresponds to a fully turbulent flow at the heating section (Fig. 2). Denaturation of whey proteins was interrupted by cooling in a tubular heat exchanger composed of smooth tubes of a 4 times larger circular cross section as applied at the holding section. The aim was to prevent an uncontrolled shear related particle size reduction at cooling section.

In conclusion the variation of the shear rate at the holding section of this THE is supposed to be a sub-ordered or hidden factor in microparticulation of whey proteins. The impact of collision induced particle size growth and shear induced break-up was not efficiently controlled by modifications of flow character at the holding section only, but would request switching to a direct heating principle at low shear at the heating section for example.

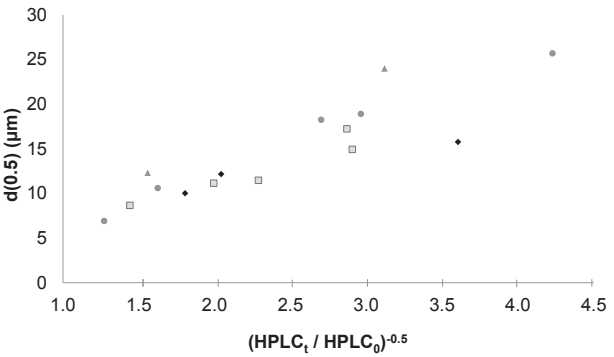

Fig. 8. Impact of HPLC accessible β-lactoglobulin fraction and shear rate (in s^{-1}: ◆, 28; ●, 56; ▲, 84; ■, 168) on median volume diameter d(0.5) of agglomerates. Flow rate was 60 L h^{-1} and average residence time 44 s at heating and holding section respectively.

3.4. Water holding capacity and elastic modulus of micro-structured β-Lg

Several physical and chemical factors impact the water holding capacity of proteins. Among them the exposition of hydrophobic-hydrophilic groups (folding), pH, intra-molecular bounds, porosity and pore size of particles are of importance. For example, binding of β-Lg molecules in a particle structure goes along with an increase in hydrophobic interactions and disulphide bridging (Tolkach & Kulozik, 2007). In conclusion the water holding capacity (WHC) reduces for more denatured proteins.

Up to a denaturation degree of 90%, which equals to the ratio $(HPLC_t/HPLC_0)^{-0.5}$ of 3.5, a decrease of WHC was observed (Fig. 9). An advanced denaturation ratio does not further reduce the WHC of the denatured part. WHC was analysed on a dry matter basis of denatured, sediment protein. The total amount of denatured protein increases with an increasing denaturation degree. But the density and electrostatic character of agglomerates seems to be unmodified at a denaturation degree of 90% and higher. In conclusion, a high yield of process is not necessarily linked to a negative impact on WHC of the product.

In contrast, the elastic modulus G′ depends only on the denaturation degree and thus on the particle size. At 30% dry matter a gel type material will be formed, where the continuous phase is composed of proteins, which is the reason for the choice of this

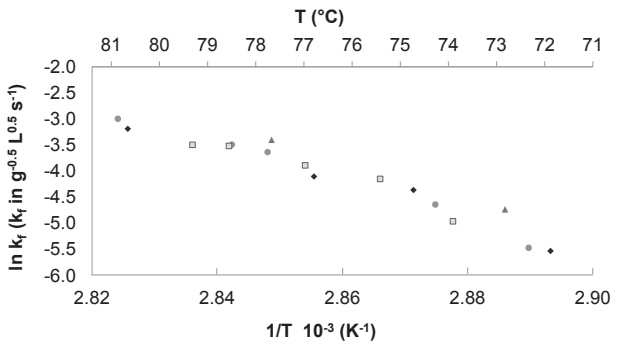

Fig. 7. Formal denaturation rate k_f as a function of holding temperature and shear rate (in s^{-1}: ◆, 28; ●, 56; ▲, 84; ■, 168) at the holding section of tubular heat exchanger at a flow rate of 60 L h^{-1} and an average residence time of 88 s at the heating and holding section respectively.

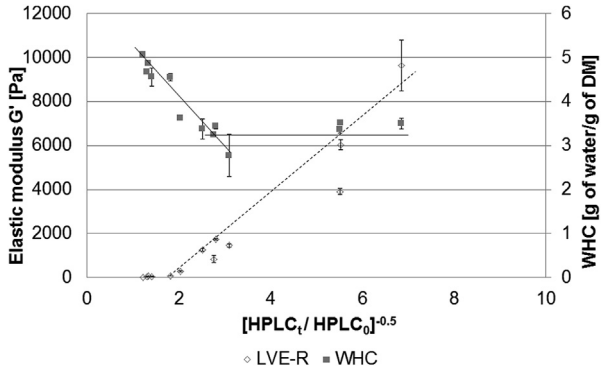

Fig. 9. Elastic modulus G′ at the limit of linear viscoelastic range (LVE-R) of micro-particulated whey protein (WP) solution (30%, w/w) and water holding capacity (WHC) of precipitated WP as a function of degree of denaturation. Flow rate was 60 L h^{-1} and average residence time 44 s at heating and holding section, respectively.

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concentration in material testing. Preparations of denatured WP were not too liquid and not too stiff over a wide range of denaturation degrees at performed G′ measurements. Native whey proteins at the same concentration represent a true liquid and an elastic modulus cannot be determined for comparison. However, the storage modulus of microparticulated whey proteins increases up to 10,000 Pa and is for comparison too small for measuring for a 30% (w/w) preparation of initial WP. The denatured WP dispersion was pre-concentrated at 40 °C and subsequently freeze–dried. Nevertheless, the amplification factor concerning rheological behaviour and modification of texture of food is supposed to be similar for spray dried microparticulated whey proteins in comparison to native, spray dried whey proteins.

4. Conclusions

Heat induced denaturation of whey proteins, validated by HPLC accessible β-Lg concentration, is a complex reaction including multiple chemical and physical factors of substrate and environment but is first guided by the temperature-time history of process. Variation of flow regime in holding section of a scalable, for industrial application relevant tubular heat exchanger has not the same dominant effect on physical and technological properties of whey proteins agglomerates as in a batch process which was performed in a Couette cell at laboratory scale for example. For scaling issues it is recommended replacing description of flow dynamics by shear rates, with a more complex description including temperature and shear dependent dynamic viscosity of liquids and shape of tubes. This leads to flow dynamics based on Reynolds numbers and friction factors. Direct as an alternative to indirect heat transfer in a steady state processes could enable studying heating and aggregation of β-Lg under laminar flow and elucidating the relation of shear induced amplification via molecule collision versus shear induced break-up of agglomerates.

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References

Croguennec, T., O'Kennedy, B. T., & Mehra, R. (2004). Heat-induced denaturation/aggregation of β-lactoglobulin A and B: kinetics of the first intermediates formed. *International Dairy Journal, 14*, 399–409.

Dissanayake, M., Ramchandran, L., Donkor, O. N., & Vasiljevic, T. (2013). Denaturation of whey proteins as a function of heat, pH and protein concentration. *International Dairy Journal, 31*, 93–99.

Edwards, P. J. B., & Jameson, G. B. (2014). *Structure and stability of whey proteins* (2nd ed., pp. 201–242). London, UK: Elsevier.

Erabit, N., Flick, D., & Alvarez, G. (2014). Formation of β-lactoglobulin aggregates during thermomechanical treatments under controlled shear and temperature conditions. *Journal of Food Engineering, 120*, 57–68.

Erabit, N., Ndoye, F. T., Alvarez, G., & Flick, D. (2015). A population balance model integrating some specificities of the β-lactoglobulin thermally-induced aggregation. *Journal of Food Engineering, 144*, 66–76.

Giroux, H. J., Houde, J., & Britten, M. (2010). Preparation of nanoparticles from denatured whey protein by pH-cycling treatment. *Food Hydrocolloids, 24*, 341–346.

Guérin, R., Ronse, G., Bouvier, L., Debreyne, P., & Delaplace, G. (2007). Structure and rate of growth of whey protein deposit from in situ electrical conductivity during fouling in a plate heat exchanger. *Chemical Engineering Science, 62*, 1948–1957.

Kast, W. (2010). Pressure drop in flow through pipes. In *VDI-Gesellschaft Verfahrenstechnik und Chemieingenieurwesen: VDI heat atlas* (pp. 1057–1064). Berlin, Germany: Springer.

Mayer, H. K., Raba, B., Meier, J., & Schmid, A. (2010). RP-HPLC analysis of furosine and acid-soluble β-lactoglobulin to assess the heat load of extended shelf life milk samples in Austria. *Dairy Science and Technology, 90*, 413–428.

Mehalebi, S., Nicolai, T., & Durand, D. (2008). Light scattering study of heat-denatured globular protein aggregates. *International Journal of Biological Macromolecules, 43*, 129–135.

Mustapha, N. A., Ruttarattanamongkol, K., & Rizvi, S. S. H. (2012). The effects of supercritical fluid extrusion process on surface hydrophobicity of whey protein concentrate and its relation to storage and heat stability of concentrated emulsions. *Food Research International, 48*, 470–477.

Nicorescu, I., Loisel, C., Vial, C., Riaublanc, A., Djelveh, G., Cuvelier, G., et al. (2008a). Combined effect of dynamic heat treatment and ionic strength on denaturation and aggregation of whey proteins – part I. *Food Research International, 41*, 707–713.

Nicorescu, I., Loisel, C., Vial, C., Riaublanc, A., Djelveh, G., Cuvelier, G., et al. (2008b). Combined effect of dynamic heat treatment and ionic strength on the properties of whey protein foams – part II. *Food Research International, 41*, 980–988.

Nor Afizah, M., & Rizvi, S. S. H. (2014). Functional properties of whey protein concentrate texturized at acidic pH: effect of extrusion temperature. *Food Research International, 57*, 290–298.

Peters, J. P. C. M., Luyten, H., Alting, A. C., Boom, R. M., & van der Goot, A. J. (2015). Effect of crosslink density on the water-binding capacity of whey protein microparticles. *Food Hydrocolloids, 44*, 277–284.

Simmons, M. J. H., Jayaraman, P., & Fryer, P. J. (2007). The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. *Journal of Food Engineering, 79*, 517–528.

Spiegel, T. (1999). Whey protein aggregation under shear conditions - effects of lactose and heating temperature on aggregate size and structure. *International Journal of Food Science and Technology, 34*, 523–531.

Spiegel, T., & Huss, M. (2002). Whey protein aggregation under shear conditions – effects of pH value and removal of calcium. *International Journal of Food Science and Technology, 37*, 559–568.

Steventon, A. J., Donald, A. M., & Gladen, L. F. (1994). Thermal aggregation of whey proteins under fluid shear conditions. In A. T. Andrews, & J. Varley (Eds.), *Biochemistry of milk products* (pp. 133–142). Cambridge, UK: Royal Society of Chemistry.

Tolkach, A., & Kulozik, U. (2007). Reaction kinetic pathway of reversible and irreversible thermal denaturation of β-lactoglobulin. *Lait, 87*, 301–315.

Wolz, M., & Kulozik, U. (2015). Thermal denaturation kinetics of whey protein at high protein concentrations. *International Dairy Journal, 49*, 95–101.

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