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Modelling the changes in viscosity during thermal treatment of milk protein concentrate using kinetic data

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Abstract

This work aimed to model the effect of heat treatment on viscosity of milk protein concentrate (MPC) using kinetic data. MPC obtained after ultrafiltration was subjected to different heat treatments with time-temperature. Heat treatment at high temperature and short time (i.e., 100 or 120°C×30 s) led to a significant increase in viscosity in MPC systems. Second-order reaction kinetic models proved a better fit than zero- or first-order models when fitted for viscosity response to heat treatment. A distinct deviation in the slope of the Arrhenius plot at 77.9°C correlated to a significant increase in the rate of viscosity development at temperatures above this, confirming the transition of protein denaturation from the unfolding to the aggregation stage. This study demonstrated that heat-induced viscosity of MPC as a result of protein denaturation/aggregation can be successfully modelled in response to thermal treatment, providing useful new information in predicting the effect of thermal treatment on viscosity of MPC.

Keywords: Milk protein concentrate, reaction kinetics, viscosity, modelling, heat stability, heat treatment
1. Introduction

Milk protein concentrate (MPC) ingredients are generally obtained by ultrafiltration
of pasteurized skim milk, often followed by diafiltration with water to remove additional
minerals and lactose (Martin et al., 2010). MPC ingredients are an excellent source of protein
with good nutritional, sensory and functional properties in many food applications (Banach et
al., 2014; Huffman and Harper, 1999). MPC ingredients contain a high protein to total solids
ratio, while the ratio of caseins to whey proteins is similar to that of the original skim milk
(Bastian et al., 1991; Green et al., 1984). Following filtration, heat treatment (high
temperature - short time) of liquid MPC is frequently carried out to inactivate microbiological
organisms. However, such heat treatments result in a number of physicochemical changes in
the liquid concentrate, in particular, denaturation and aggregation of proteins leading to an
increase in viscosity and possible gelation (Murphy et al., 2013; Singh and Havea, 2003;
Walstra and Jenness, 1984). High viscosity of the concentrate also leads to adverse effects in
the manufacturing process such as a reduction in pump efficiencies, fouling on evaporation
distribution plates/tubes of calandria, and thereby effectively limiting the total solids level
achievable prior to spray drying. This in turn affects the droplet size during atomization and
hence affects properties of the final powder (Bienvenue et al., 2003; Crowley et al., 2014;
Fryer, 1989; Schuck et al., 2005; Schuck et al., 2007).

Many previous studies have shown the significant effect of heat treatment temperature
on whey protein denaturation (Anema et al., 2004; Anema and McKenna, 1996; Buggy et al.,
2017; Kehoe et al., 2011; Oldfield et al., 2005; Oldfield et al., 1998) and subsequently
viscosity of the concentrates such as skim concentrate (Anema et al., 2014) or concentrates
containing different proportions of MPC and whey protein concentrate (Souza et al., 2015).
Means of predicting and modelling the influence of heat treatment on whey protein
denaturation of dairy ingredients, particularly whey proteins in whole milk (Anema and
McKenna, 1996), whey proteins in skim milk (Oldfield et al., 1998) or skim milk with adjusted concentration of whey protein (Oldfield et al., 2005), whey proteins in high protein concentrates (Wolz and Kulozik, 2015) and heat denaturation of β-lactoglobulin (β-lg) (Loveday, 2016) has previously been investigated using reaction kinetics. In such models, measurement of residual native protein concentration, relative to its initial concentration, as a function of time at a given temperature, is commonly used to determine the kinetic parameters of protein denaturation (Anema and McKenna, 1996; Kehoe et al., 2011; Oldfield et al., 1998). The rate of heat-induced whey protein denaturation is assumed to be proportional to the denaturation rate constant at a specific temperature and the concentration of native protein (Anema and McKenna, 1996; Oldfield et al., 1998; Petit et al., 2011).

Furthermore, the Arrhenius relationship has been used to describe the dependence of denaturation rate of native whey protein, particularly β-lg and α-lactalbumin (α-la) on temperature (Anema and McKenna, 1996; Oldfield et al., 2005; Oldfield et al., 1998; Wolz and Kulozik, 2015). In these studies, the Arrhenius plots of protein denaturation rate constant were found to be linear within a certain temperature range, while there was a noticeable break in the plotted relationship at a temperature defined as the critical temperature ($T_c$) (Anema and McKenna, 1996; Oldfield et al., 1998; Tolkach and Kulozik, 2007; Wolz and Kulozik, 2015). $T_c$ has been generally found to be in the range 78-85°C (Anema and McKenna, 1996; Oldfield et al., 1998; Tolkach and Kulozik, 2007; Wolz and Kulozik, 2015). Denaturation of whey protein is, in fact, a two-step process involving unfolding of native protein, followed by aggregation of protein (Brodkorb et al., 2016; Mulvihill and Donovan, 1987; Petit et al., 2011; Tolkach and Kulozik, 2007). Below $T_c$, the rate of protein denaturation is limited by the unfolding of the proteins, whereas at temperatures > $T_c$, the rate is limited by their aggregation (Brodkorb et al., 2016; Petit et al., 2011). Although reaction kinetics have been extensively applied to model thermal denaturation of whey proteins (Anema and McKenna,
1996; Oldfield et al., 2005; Oldfield et al., 1998; Wolz and Kulozik, 2015), modelling the viscosity changes of casein/whey protein systems due to heat treatment has not been the subject of previously published work. The objective of this study was to develop a model, which would allow quantification of the effect of heat treatment on the viscosity of MPC, obtained directly after ultrafiltration of skim milk, and thus allow determination of reaction kinetics of heat-induced denaturation.

2. Materials and Methods

2.1. Preparation of milk protein concentrate

MPC was produced by ultrafiltration (UF) of pasteurized skim milk at 12°C using 10 kDa molecular weight cut-off, spiral-wound, polymeric membranes and at a volume concentration factor of 5 in a local commercial dairy processing plant. The membrane filtration plant, operating under continuous mode, had a final UF retentate total solids (TS) content of 19.8% (w/w), pH=6.7 at 20 °C. The protein, fat, ash, and lactose contents were 87.3, 1.12, 7.04 and 2.15% (w/w, dry basis), respectively. The protein composition of the liquid MPC was as follows: κ-casein 1.48%, w/w, α₂-casein 1.75%, w/w, α₁-casein 5.98%, w/w, β-casein 5.55%, w/w, α-lactalbumin 0.52%, w/w, β-lactoglobulin 2.09%, w/w. The MPC liquid concentrate obtained directly after membrane filtration was subjected to a number of heat treatment temperatures as outlined in Fig. 1. Samples in triplicate were heated at 85, 100 or 120 °C with holding times of 15, 30, 60 or 200 s, and immediately cooled to 45 °C using a pilot scale Microthemics tubular heat exchanger (MicroThermics, NC, USA).

2.2. Viscosity measurements of milk protein concentrate

Viscosity of MPC obtained directly after UF and post-heat treatment (Section 2.1) were measured at 45 °C using a controlled-stress rheometer (AR2000ex Rheometer, TA
Instruments, Crawley, UK), equipped with a concentric cylinder geometry and Peltier controlled heating system to replicate the temperature of the evaporation stage before spray drying. Measurements were performed over a shear rate ramp ranging from 10 l/s to 300 l/s over 5 min and held at 300 l/s for 5 min. Note: At a shear rate of 300 l/s, heat-induced viscosities were found to be constant for all samples over 5 min (Appendix Fig. A1). All measurements were carried out in triplicate.

Viscosity was also measured as a function of temperature in the range from 55 to 75 °C. The MPC liquid samples obtained after UF were subjected to storage under isothermal conditions in the concentric cylinder geometry of the rheometer at different temperatures of 55, 60, 65, 70 or 75 °C. To avoid water evaporation during the measurement, three drops of tetradecane were added on top of the sample immediately after loading. Samples were rapidly heated to the controlled temperature and subsequently viscosity was recorded at a constant shear rate of 300 l/s over 5 min. The rate of viscosity increase due to heat treatment was represented as the slope of the curve at the time when viscosity initially increased (i.e., the rate > zero) (see Appendix Fig. A2). At 55 and 60 °C, a slight decrease in viscosity over time indicated thinning behaviour of the MPC liquid concentrate (Appendix Fig. A2 A and B); therefore, a linear fit was applied at the time (>100 s) when viscosity over time was observed to be linear. In Appendix Fig. A2 C, D, and E, a linear fit was applied at the time when viscosity initially increased. The rate of viscosity increase due to heat treatment was represented as the slope of the fitted curve (Appendix Fig. A.2 F). All measurements were carried out in triplicate.

2.3. Polyacrylamide gel electrophoresis

Protein profiles of MPC before and after thermal treatment (as defined in Section 2.1) were determined by polyacrylamide gel electrophoresis (PAGE) (Buggy et al., 2017). The
samples were dissolved to create reducing and non-reducing conditions in a lithium dodecyl sulphate (LDS) buffer, pH 8.4 with 10 µL of the sample added to wells in a 12% Bis-Tris Nu-PAGE Gel and electrophoresis was carried out using an X-Cell Surelock electrophoresis unit (Novex Technologies). The samples were prepared to contain 1 µg protein per µL of sample buffer solution. After electrophoresis, the gels were stained overnight using 0.05% (w/v) Coomassie brilliant blue R-250 in 25 % (v/v) isopropanol and 10 % (v/v) acetic acid. After staining, the gels were de-stained using a 10 % (v/v) isopropanol and 10 % (v/v) acetic acid solution until a clear background was achieved.

2.4. Modelling viscosity increase during heat treatment by reaction kinetics

The rate of protein denaturation was calculated using the following reaction kinetics model (Kehoe et al., 2011):

\[
\frac{\partial C_p}{\partial t} = -k_T C_p^n
\]  

(1)

where \( n \) is the reaction order, \( k_T \) ((%)\(^{1-n} \)/s) is the overall rate constant of protein denaturation at temperature \( T \) (K), \( C_p \) (% w/w) is the native protein content of the concentrate prior to heat treatment, \( t \) (s) is the holding time. Models were constructed based on zero (\( n=0 \)), first (\( n=1 \)) and second (\( n=2 \)) order reaction kinetics (see Appendix A, B and C).

Heating milk proteins at high temperature causes irreversible protein denaturation leading to aggregation and increases in concentrate viscosity (Anema et al., 2014; Souza et al., 2015). In this study, the increase rate of viscosity was assumed to be a linear response to the rate of protein denaturation.

\[
\frac{\partial \eta}{\partial t} = -\alpha \frac{\partial C_p}{\partial t} = \alpha k_T C_p^n
\]  

(2)

where \( \eta \) (mPa.s) is the viscosity of the concentrate, \( \alpha \) (mPa.s/%) is the coefficient representing response of the viscosity to protein denaturation. At a constant temperature, \( T \),
viscosity increase due to heat treatment time is shown in Table 1. Further details of equation derivations in Table 1 are described in Appendix A, B and C.

2.5. Arrhenius relationship between the rate of viscosity increase and heat treatment temperature

An Arrhenius plot involving the logarithm of relative rate of viscosity increase and the inverse of heat treatment temperature \(1/T\) (1/K) was used to investigate the effect of temperature on the viscosity rate constant and its response to heat treatment. The relative rate of viscosity increase at time zero was defined as follows:

\[
v_{\eta,0} = \left. \frac{\partial \eta}{\partial \eta_0} \right|_{t_0}
\]  

(3)

where \(\eta_0\) (mPa.s) is the viscosity of the concentrate at the initial time zero.

For low temperature heat treatments (65, 70 and 75 °C), the initial rate of viscosity increase, \(\frac{\partial \eta}{\partial t}_{t_0}\), was determined from the slope of viscosity as a function of time at time \(t_0\) when viscosity initially increased. Note that the negative rates of increase in viscosity at 50 and 60 °C were due to temperature-induced thinning behaviour and were disregarded.

For high temperature heat treatments (85, 100 and 120 °C), the relative rate of viscosity increase from time zero was calculated from the second order model as follows:

\[
v_{\eta,0} = \left. \frac{\partial \eta}{\partial \eta_0} \right|_{t_0} = \left(\frac{\eta_m}{\eta_0} - 1\right) k_T C_0
\]  

(4)

where the parameters of the model are described in Table 1.

2.6. Effect of protein content on the viscosity of milk protein concentrate

The MPC liquid concentrate described in Section 2.1 (19.8% TS and 17.3%, w/w, protein) was diluted to 13.8% TS (12.1 % w/w, protein) with a dilution factor of 0.7. The diluted concentrate was then heat treated at 120 °C with holding times of 15, 30 and 60 s, and
immediately cooled to 45 °C using a pilot scale Microthemics tubular heat exchanger. Subsequently, the post-heat treatment MPC samples were concentrated back to their original TS (19.8% TS) using forward osmosis (FO) membrane system (FO Mode Micro pilot unit, evaporEOS, Ederna SAS, Toulouse, France). The FO system was equipped with e+ membranes that allowed water to permeate from the liquid MPC across the membrane to the draw solution (25 L, H2Os™, E326, H2O, Ederna SAS, Toulouse, France). Both liquid MPC and draw solution were continuously circulated until TS content of the liquid MPC reached 19.8% (w/w). The temperature during filtration was controlled at 20°C using a Huber cooling system (Pilot ONE, Offenburg, Germany).

Viscosity of MPC obtained after FO concentration were measured using a shear rate ramp ranging from 10 to 300 1/s over 5 min and held at 300 1/s for 5 min at 45 °C using a controlled stress rheometer equipped with a concentric cylinder geometry and Peltier-controlled heating system. All measurements were carried out in triplicate. Viscosity measurements of MPC heated at 12.1% (w/w) protein were then compared to those heated at 17.3% (w/w) protein. Finally, the model of heat induced viscosity described in Section 2.4 (Table 1) was used to estimate the effect of protein content on the viscosity of MPC at 12.1% (w/w, protein). Since the MPC liquid concentrate was diluted by the factor of 0.7, the rate constant of viscosity response \( k_{120°C, P=12.1%} \) in the second-order kinetic model was assumed to be equal to \( 0.7 \cdot k_{120°C, P=17.3%} \), where \( C_{0, P=12.1%} \) and \( C_{0, P=17.3%} \) are the initial levels of native milk proteins in the concentrates containing 12.1 and 17.3% (w/w) total protein, respectively.

2.7. Statistical analysis and parameter estimation

Heat induced viscosity data were analysed using one-way analysis of variance (ANOVA), with post hoc Tukey analysis using SPSS statistics software (SPSS V.18, IBM, New York, US).
The parameters of the model described in Section 2.4 and 2.5 were estimated by minimising the sum square difference between the viscosity values at different heat treatments predicted by the model (Eq. 5 or Eq. 6 in Table 1) and the measured ones using a nonlinear estimation programme written in Matlab (The Mathworks, Inc., Natick, USA). In the model, $R^2$ which is defined in Appendix D, was used to evaluate the goodness of fit of the model.

3. Results and discussion

3.1. Effect of heat treatment on the protein profile of milk protein concentrate

SDS-PAGE protein profiles of MPC samples under non-reducing and reducing conditions before and after heat treatment are shown in Fig. 2. Whey protein bands can be observed for the control (unheated) and are also present, although more faint, for the samples subjected to heat treatment regimes of 85 and 100 °C for 30 s under non-reducing conditions. However, higher heat treatment temperatures/times resulted in complete loss of native β-lg and α-la bands in the non-reducing SDS-PAGE gel (Fig. 2; lanes 4-9). Furthermore, the presence of aggregated protein material in the stacking gel of the non-reducing SDS-PAGE gel indicated that temperatures $\geq$ 85 °C resulted in the formation of large disulphide-linked protein aggregates. Many previous studies (Anema and McKenna, 1996; Oldfield et al., 2005; Oldfield et al., 1998; Petit et al., 2011) have reported the rapid denaturation of whey proteins at temperatures greater than 78 °C. Loss of native whey protein indicated by SDS-PAGE analysis (non-reducing lanes, Fig. 2) revealed the large extent of irreversible protein denaturation at high heat treatment temperatures (i.e., 80–120 °C). These high heat treatment temperatures are typical of those used in the manufacture of MPC ingredients, which are used not only to comply with microbiological specifications but also to impart certain functionality requirements for the end-user.
3.2. Effect of heat treatment on the viscosity of milk protein concentrate

The apparent viscosity of MPC samples, taken after indirect tubular heating decreased with increasing shear rate (Appendix Fig. A3 A). However, the ratio of heat-induced viscosities to that of the unheated control sample were shown to be relatively constant over different shear rates (Appendix Fig. A3 B), indicating that the effect of heat-induced viscosity was independent of shear rate. Furthermore, the viscosity was shown to increase with increasing heat treatment temperature and holding time (Fig. 3 and Appendix Table A1). The viscosity values of all heat treated MPC samples were significantly ($P < 0.05$) greater than that of the control sample (Fig. 3). MPC heat treated at 120 °C for 15 to 30 s had significantly ($P < 0.05$) higher viscosity than those heated at 85 °C and 100 °C over the same time period. Interestingly, heat-induced viscosity levelled off in MPC samples heated at 120 °C for extended holding times (i.e., 30 to 200 s; Fig. 3). The results indicated that once protein denaturation and aggregation occurred due to the high temperature (i.e., 120 °C), holding the product for longer did not significantly ($P > 0.05$) increase the viscosity.

3.3. Modelling the viscosity increase due to protein denaturation

The heat treatment temperatures described in Section 3.1 were all $\geq 85$ °C, where the protein denaturation rate was considered to be limited by their aggregation rate (Brodkorb et al., 2016; Petit et al., 2011). The viscosity of the MPC at time zero ($\eta_0 = 8.45$ mPa.s) was measured prior to heat treatment. The parameters and their 95% confidence intervals estimated by first- and second-order reaction kinetic models are shown in Table 2. The second-order model had a better fit compared to the first-order model, with an $R^2$ value of 0.91 and 0.87, respectively. Estimated rate constants ($k_T C_0$) at 100 and 120 °C were significantly higher (2.65 and 18.9 fold, respectively), compared to at 85 °C. The zero–order
model indicated a linear response between viscosity and time at a constant temperature $T$
(Appendix Fig. A4). However, since the experimental viscosity data showed a non-linear
response to heat treatment time (Fig. 3), the zero–order model was not suitable ($R^2=0.55$) and
therefore disregarded. A number of previous studies (Anema, 2016; Anema and McKenna,
1996; Oldfield et al., 2005; Oldfield et al., 1998; Wolz and Kulozik, 2015) have examined the
reaction kinetics involved in whey protein denaturation and aggregation based on the level of
residual native whey protein using first- and second-order kinetics. However, the present
study has shown that by measuring viscosity and its response to thermal treatment a second-
order model can be applied to predict increases in product viscosity during high temperature-
short time thermal processing. In addition, the response of MPC viscosity to heat treatment
was also carried out at shear rates of 100 and 200 1/s (Appendix Fig. A5) and indicated
similar trends in the response of the measured and predicted heat-induced viscosities at the
two different shear rates. This shows the robustness of the model, indicating that the shear
rate used during viscosity measurement is not a factor in predicting heat-induced viscosity.

Viscosity of MPC heat treated (120 °C for 15, 30 or 60 s) at two different protein
concentrations (i.e., 12.1 or 17.3%, w/w) are shown in Fig. 4. Thermal treatment of the MPC
sample with a protein content of 12.1% (w/w) at 120 °C and subsequently concentrated to a
protein content of 17.3% (w/w) had a significantly lower viscosity, compared to MPC heated
at 120 °C at 17.3% (w/w) protein across all holding times (Fig. 4). In fact, protein
denaturation rate was found to increase with increasing total protein concentration (Law and
Leaver, 1997; Wolz and Kulozik, 2015). Values of denaturation rate of $\alpha$-la and $\beta$-lg were
found to increase by 84 and 92% when doubling concentration of total protein in skim milk
heated at 80 °C (Law and Leaver, 1997). Wolz and Kulozik (2015) proposed that high protein
content of concentrates induced a faster thermal denaturation, most likely due to the increased
probability of collisions between whey protein molecules. Our results confirmed promotion
of protein aggregation at high protein content resulting in large heat-induced viscosity of MPC. Overall, the model developed in this study showed it was possible to predict heat-induced viscosity in relation to the initial protein content of the MPC.

3.4. *Heat-induced viscosity changes in the protein unfolding temperature range* (55-75 °C)

Viscosity measurements of MPC liquid concentrate measured at 55, 60, 65, 70 and 75 °C are shown in Fig. 5. Over this temperature range, the rate of protein denaturation was relatively low and limited by the rate of unfolding (Brodkorb et al., 2016; Petit et al., 2011). A decrease in viscosity was observed with increasing temperature from 55 to 70 °C, confirming a negative correlation between viscosity and temperature (Fig. 5A). A slight decrease in viscosity (by < 4.5% of their initial values) was also found when MPC was measured at 55 and 60 °C as a function of time, as shown by the negative values in the slope of the curve (Appendix Fig. A2 A and B) and in the rate of viscosity increase (Fig. 5B). Non-Newtonian shear thinning behaviour of micellar casein concentrate was previously reported to be affected by both concentration and temperature, but less pronounced at temperatures above 60 °C (Appendix Fig. A2) (Sauer et al., 2012). Therefore, increase in viscosity observed in this study for the viscosity-time profiles at 65, 70 and 75 °C was due to protein denaturation/aggregation (Fig. 5). The rate of increase in viscosity by protein denaturation/aggregation was represented as the slope of the curve at the time when viscosity began to increase (Appendix Fig. A2 C, D and E). This rate of increase in viscosity, as a function of temperature is calculated and shown in Fig. 5B and Appendix Table A2. The negative rates of increase in viscosity at 50 and 60 °C due to shear thinning behaviour were disregarded and only the positive rates at 65, 70 and 75 °C were further considered for the Arrhenius plot in the unfolding-limited temperature range.
3.5. Temperature dependence of viscosity increase due to protein denaturation

The Arrhenius relationship is commonly used to describe temperature dependence on protein denaturation rate (Anema and McKenna, 1996; Oldfield et al., 2005; Oldfield et al., 1998; Wolz and Kulozik, 2015). In this study, the Arrhenius relationship was extended further to describe the relationship between temperature and the rate of increase in viscosity (Fig. 6). The slope of $\ln(\eta_0)$ as a function of $1/T$ represents the activation energy, $E_a$, indicating the changing rate of increase in viscosity as a response to temperature. Interestingly, the Arrhenius plot showed two regions in which the $E_a$ differed. Estimated $E_a$ was 99.0 kJ/mol and 442 kJ/mol in the high and low temperature regions, respectively. The critical temperature ($T_c$) was determined as the intersection point of the two fitted linear plots of $\ln(\eta_0)$, as a function of $1/T$.

The sharp deviation in the Arrhenius plot was found at $T_c$ equal to 77.9 °C (Fig. 6) and was similar to that of $\beta$-lg denaturation (78 °C), described by Tolkach and Kulozik (2007) and Blanpain-Avet et al. (2016). The deviation from the linear model in the Arrhenius plot is due to a shift from the protein unfolding-limited rate to the aggregation-limited rate at $T_c$ (Petit et al., 2011). In fact, Anema and McKenna (1996) showed a significant change in the Arrhenius plot at a $T_c$ of ~80 °C for denaturation of $\alpha$-lac and 85 °C for the denaturation of different variants of $\beta$-lg. Similarly, Oldfield et al. (1998) indicated that whey protein ($\beta$-lg and $\alpha$-lac) denaturation showed a break in the Arrhenius plot at approximately 80 to 90 °C.

The relationship between the protein denaturation rate and temperature is also presented by the activation energy ($E_a$) (Anema and McKenna, 1996; Oldfield et al., 1998; Petit et al., 2011). $E_a$ values obtained from this study compared to previous literature are shown in Appendix Table A3. Previous studies used powder ingredients such as, rehydrated whole milk, skim milk and $\beta$-lg dispersions by described by Anema and McKenna (1996),
Oldfield et al. (1998) and Tolkach and Kulozik (2007), respectively. Differences in $E_a$ values may be explained by differences in protein ingredients and their thermal history during manufacture.

Future work may examine the correlation between protein aggregation and heat-induced viscosity based on the level of protein-protein interactions, particularly between $\beta$-lg and $\kappa$-casein. In addition, an extension of the model taking into account pH, minerals and calcium ion activity would be useful in understanding the mechanisms responsible for heat-induced changes in viscosity of MPC systems.

5. Conclusion

Modelling the reaction kinetics of milk protein denaturation using viscosity data from the thermal treatment of MPC proved successful. Therefore, the effect of thermal heat treatment regimes on milk protein viscosity can be predicted and used to set limits both in terms of heat treatment temperature and holding times. In order to effectively model this data the viscosity changes due to protein denaturation a second-order reaction model proved superior over zero- or first-order reaction models. Furthermore, the use of an Arrhenius plot to profile the rate of viscosity increase in response to temperature confirmed the transition of protein denaturation behaviour from the unfolding to the aggregation state. This study could be used to simulate and/or optimise the effect of heat treatment in order to minimise protein denaturation and to avoid increases in product viscosity during evaporation in MPC manufacture, helping to improve process efficiency and product quality.
Appendices

Appendix A

Zero order reaction kinetics

From Eq. 1, the rate of protein denaturation follow zero order reaction \((n = 0)\) could be written as

\[
\frac{\partial c_p}{\partial t} = -k_T \tag{A1}
\]

At a constant temperature \(T\), Eq. (A1) could be rewritten as

\[
c_p = -C_0 k_T \tag{A2}
\]

where \(C_0\) (\% \(w/w\)) is the initial native protein level (at \(t = 0\)) of the concentrate subjected to heat treatment.

At the constant temperature \(T\), Eq. (2) could be integrated as follows

\[
\int_0^t \partial \eta = \int_0^t \alpha_k T \partial t = \int_0^t \alpha k_T \partial t \tag{A3}
\]

The viscosity \(\eta\) of the concentrate at the time \(t\) in Eq. (A3) could be solved as:

\[
\eta = \eta_0 + \int_0^t \alpha k_T \partial t = \eta_0 + \alpha k_T t \tag{A4}
\]

where \(\eta_0\) (mPa.s) is the viscosity of the concentrate at the initial time.

Appendix B

First order reaction kinetics

From Eq. 1, the rate of protein denaturation assumed to follow first order reaction \((n = 1)\) could be written as

\[
\frac{\partial c_p}{\partial t} = -k_T c_p \tag{B1}
\]

At a constant temperature \(T\), Eq. (B2) could be rewritten as

\[
c_p = C_0 \exp(-k_T t) \tag{B3}
\]
where $C_0$ (%, w/w) is the initial native protein level (at $t = 0$) of the concentrate subjected to heat treatment.

At the constant temperature $T$, Eq. (2) could be integrated as follows

$$\int_0^t \partial \eta = \int_0^t \alpha k_T C_P \partial t = \int_0^t \alpha k_T C_0 \exp(-k_T t) \partial t$$  \hspace{1cm} \text{(B3)}$$

The viscosity $\eta$ of the concentrate at the time $t$ in Eq. (B4) could be solved as:

$$\eta = \eta_0 + \int_0^t \alpha k_T C_0 \exp(-k_T t) \partial t = \eta_0 + \alpha C_0 (1 - \exp(-k_T t))$$  \hspace{1cm} \text{(B5)}$$

where $\eta_0$ (mPa.s) is the viscosity of the concentrate at the initial time.

Eq. (B5) could be rewritten as follows

$$\eta = \eta_0 + (\eta_m - \eta_0) (1 - \exp(-k_T t))$$  \hspace{1cm} \text{(B6)}$$

and $\eta_m = \eta_0 + \alpha C_0$  \hspace{1cm} \text{(B7)}$$

where $\eta_m$ (mPa.s) is the maximal viscosity at temperature $T$ due to heat treatment (the asymptotic viscosity as $t$ approaches infinity).

**Appendix C**

*Second order reaction kinetics*

The rate of protein denaturation assumed to follow second order reaction ($n = 2$) could be written as

$$\frac{\partial C_P}{\partial t} = -k_T C_P^2$$  \hspace{1cm} \text{(C1)}$$

At a constant temperature $T$, the concentration of native protein $C_P$ at time $t$ could be rewritten as

$$C_P = \frac{C_0}{1 + k_T C_0 t}$$  \hspace{1cm} \text{(C2)}$$

where $C_0$ (%, w/w) is the initial native protein level (at $t = 0$) of the concentrate subjected to heat treatment.

At the constant temperature $T$, Eq. (2) could be integrated as follows
\[ \int_0^t \partial \eta = \int_0^t \alpha k_T C_\rho \partial t = \int_0^t \alpha k_T \frac{C_0}{1 + k_T C_0 t} \partial t \]  \hspace{1cm} (C3)

The viscosity \( \eta \) of the concentrate at the time \( t \) in Eq. (C3) could be expressed as:

\[ \eta = \eta_0 + \int_0^t \alpha k_T \frac{C_0}{1 + k_T C_0 t} \partial t = \eta_0 + \alpha C_0 \left( 1 - \frac{1}{1 + k_T C_0 t} \right) \]  \hspace{1cm} (C4)

where \( \eta_0 \) (mPa.s) is the viscosity of the concentrate at the initial time. The viscosity \( \eta \) in Eq. (C4) could be rewritten as follows

\[ \eta = \eta_0 + \alpha C_0 \left( 1 - \frac{1}{1 + k_T C_0 t} \right) = \eta_0 + (\eta_m - \eta_0) \left( 1 - \frac{1}{1 + k_T C_0 t} \right) \]  \hspace{1cm} (C5)

and \( \eta_m = \eta_0 + \alpha C_0 \)  \hspace{1cm} (C6)

where \( \eta_0 \) (mPa.s) is the viscosity of the concentration at the initial time, \( \eta_m \) (mPa.s) is the maximal viscosity at temperature \( T \) due to heat treatment (the asymptotic viscosity as \( t \) approaches infinity).

### Appendix D

**Criterion for goodness of fit of the model**

\[ R^2 \] is a statistical analysis of how close the fitted model to data is. The \( R^2 \) is defined as:

\[ R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \]  \hspace{1cm} (D1)

where \( SS_{res} \) and \( SS_{tot} \) are the regression sum of squares and the total sum of squares of the data, respectively. \( SS_{res} \) and \( SS_{tot} \) are defined as:

\[ SS_{res} = \Sigma (y_i - f_i) \]  \hspace{1cm} (D2)

and \( SS_{tot} = \Sigma (y_i - \bar{y}) \)  \hspace{1cm} (D3)

where \( y_i \), \( \bar{y} \) and \( f_i \) are the measured data, the mean of the measured data and the predicted value by the model, respectively.
Appendix Table A1. Apparent viscosity (shear rate 300 1/s; 45 °C) of MPC subjected to different heat treatments.

| Sample ID | Heat treatment  | Viscosity (mPa.s) |
|-----------|----------------|-------------------|
| 1(*)      | -              | 8.45±0.89<sup>a</sup> |
| 2         | 85°C×30s       | 11.53±1.78<sup>b</sup> |
| 3         | 85°C×60s       | 13.63±1.1<sup>b</sup> |
| 4         | 85°C×200s      | 15.47±0.02<sup>c</sup> |
| 5         | 100°C×30s      | 14.09±0.13<sup>c</sup> |
| 6         | 100°C×60s      | 16.54±0.95<sup>d</sup> |
| 7         | 100°C×200s     | 17.35±0.33<sup>de</sup> |
| 8         | 120°C×15s      | 16.67±0.43<sup>d</sup> |
| 9         | 120°C×30s      | 20.11±0.44<sup>e</sup> |
| 10        | 120°C×60s      | 19.51±0.66<sup>de</sup> |
| 11        | 120°C×200s     | 20.06±0.17<sup>e</sup> |

<sup>a</sup>Values presented are the means of data ± standard deviations of triplicate measurements; values within a column not sharing a common superscript differ significantly (P< 0.05).
Appendix Table A2  Relative rate of viscosity increase ($v_{\eta,0}$) of MPC as a function of temperature.

| T (°C) | $v_{\eta,0}$ (relative unit) |
|--------|-----------------------------|
| 55     | (-7.02±0.89)×10^{-5}a       |
| 60     | (-4.37±0.33)×10^{-5}a       |
| 65     | (1.71±0.46)×10^{-5}a        |
| 70     | (2.70±0.03)×10^{-4}a        |
| 75     | (1.57±0.51)×10^{-3}b        |

aValues presented are the means of data ± standard deviations of triplicate measurements; values within a column not sharing a common superscript differ significantly (P< 0.05).
Appendix Table A3. Activation energy of whey protein denaturation (taken from Anema and McKenna (1996); Anema (2016), Oldfield et al. (1998); Tolkach and Kulozik (2007)),

| Model system        | Protein     | $T_c$  | Activation energy ((kJ/mol)) | Source              |
|---------------------|-------------|--------|------------------------------|---------------------|
|                     |             | (°C)   | Unfolding                    | Aggregation         |
| Reconstituted       | β-lg A      | 78     | 313.9                        | 80.8                | Tolkach and Kulozik (2007) |
| whole milk          |             |        |                              |                     |
|                     | β-lg B      | 70-85  | 263.49                       | 51.4                | Anema and McKenna            |
|                     |             | °C     | (70-85 °C)                   | (100-115 °C)        |
|                     | α-lac       | 70-80  | 195.11                       | 57.51               |                                |
|                     |             | °C     | (70-80 °C)                   | (85-115 °C)         |
| Skim milk           | β-lg A      | 70-90  | 285.5                        | 58.5                | Oldfield et al. (1998)        |
|                     |             | °C     | (70-90 °C)                   | (95-130 °C)         |
|                     | β-lg B      | 70-90  | 296.7                        | 44.0                |                                |
|                     |             | °C     | (70-90 °C)                   | (95-130 °C)         |
|                     | α-lac       | 70-80  | 203.3                        | 52.9                |                                |
|                     |             | °C     | (70-80 °C)                   | (85-130 °C)         |
| Reconstituted       | β-lg        | 85     | 287.14                       | 61.39               | Anema (2016)                  |
| β-lg powder         |             |        |                              |                     |
| Reconstituted       | Native whey | 85     | 287.14                       | 61.39               | Anema (2016)                  |
| whole milk          |             |        |                              |                     |
| MPC liquid          | concentrate | 78.7   | 477.0                        | 51.8                | Current study                 |
|                     |             |        | (65-75 °C)                   | (85-120 °C)         |

$T_c$ is the critical temperature in the Arrhenius plot, defining the aggregation-limited and the unfolding-limited range.
Appendix Fig. A1 Typical apparent viscosity and shear rate as a function of time at 45 °C of liquid MPCs subjected to different heat treatments. Measurements were performed over a shear rate ramp ranging from 10 1/s to 300 1/s over 300 s and held at 300 1/s from 300 to 600s. At a shear rate of 300 1/s, viscosity was found to be constant over 300 s.
Appendix Fig. A2. Viscosity of liquid MPC as a function of time (A - E) and its relative rate of viscosity increase ($\dot{\eta}$) (F). The samples were measured at 55 (A), 60 (B), 65 (C), 70 (D) or 75 °C (E) for 5 min at a constant shear rate of 300 1/s. Symbols indicate experimental data points while lines correspond to the linear fit of the data.
Appendix Fig. A.3. Heat-induced viscosity of liquid MPC as a function of shear rate (A) and its relative value to the control sample ($\eta/\eta_0$) (B), where $\eta$ and $\eta_0$ are the viscosity of the sample subjected to the heat treatment and that of the unheated sample, respectively.
Appendix Fig. A.4. Fitted model viscosity of liquid MPC to heat treatment under zero-order reaction kinetics. Symbols (□), (○) and (×) indicate experimental data points while dashed dotted (− ·), dashed (− −) and solid (− −) lines represent the fitted model at 85, 100 and 120 °C, respectively. Bars present standard errors of the triplicate measurements.
Appendix Fig. A5 Modelled viscosity of liquid MPC to heat treatment at the shear rate of 100 1/s (A) and 200 1/s. Symbols indicate experimental data points while solid lines represent the second-order models. Bars present standard errors of the triplicate measurements. In the model, the ratio $\eta_m/\eta_0$ at a specific shear rate was considered to be constant (2.44). Values of $\eta_0$ at different shear rates were obtained from the measurement while the rate constants of viscosity response to temperature were obtained from Appendix Table A3.
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Figure legends

**Fig. 1.** Schematic diagram describing heat treatment of liquid milk protein concentrates

**Fig. 2.** Non-reducing and reducing sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein analysis of liquid MPC before and after thermal heat treatment (AP: aggregated protein; β-lg: β-lactoglobulin).

**Fig. 3.** Viscosity of liquid milk protein concentrates before and after thermal heat treatment using an indirect tubular heat exchanger. Viscosity measurements were performed at a shear rate of 300 1/s at 45 °C. Error bars were obtained from triplicate trials. Modelled viscosity of liquid MPC as a function of heat treatment temperature and holding times under (A) first-order reaction kinetics and (B) second-order reaction kinetics. Symbols indicate experimental data points and solid lines represent the fitted model.

**Fig. 4.** Viscosity of milk protein concentrate heat treated at 120 °C for 15, 30 or 60 s at 17.3%, w/w, protein (×) or 12.1%, w/w, protein (○). The sample diluted to 12.1%, w/w, protein was concentrated back to 17.1%, w/w, protein prior to viscosity analysis. Symbols and the solid line (—) represent the experimentally measured data points and the second-order reaction kinetics model, respectively. Modelled parameters are shown in Table 1. Error bars represent standard deviations of triplicate measurements.

**Fig. 5.** Viscosity of liquid MPC as a function of time (A) and its relative rate of viscosity increase ($v_{\eta,0}$) as a function of temperature (B). Liquid MPC was obtained after ultrafiltration and heated at 55 (o), 60 (□), 65 (+), 70 (Δ) or 75 °C (×) for 5 min at a constant shear rate of 300 1/s. Bars represent standard errors of triplicate values.

**Fig. 6.** Arrhenius plot for the logarithm of relative rate of viscosity increase ln($v_{\eta,0}$) as a function of 1/T. $v_{\eta,0}$ is defined in Eq. (3). Symbols indicate experimentally determined data points while lines correspond to the linear regression fit of the data. In the aggregation-limited temperature area, $v_{\eta,0}$ was calculated from Eq. (4) while in the unfolding-limited
temperature area, $v_{\eta,0}$ was determined from the slope of viscosity as a function of time (see Fig. 5B). $T_c$ is the critical temperature in the Arrhenius plot, defining the aggregation-limited and the unfolding-limited regions.
### Tables

**Table 1.** First- and second-order reaction kinetics as a function of the modelled viscosity response to heat treatment

|                       | First order                                      | Second order                                     |
|-----------------------|--------------------------------------------------|-------------------------------------------------|
| Rate of protein denaturation | $\frac{\partial C_p}{\partial t} = -k_T C_p$ | $\frac{\partial C_p}{\partial t} = -k_T C_p^2$ |
| Rate of viscosity change | $\frac{\partial \eta}{\partial t} = -\alpha \frac{\partial C_p}{\partial t}$ | $\frac{\partial \eta}{\partial t} = -\alpha \frac{\partial C_p}{\partial t}$ |
| Heat induced viscosity as a function of time ($t$) at temperature ($T$) | $\eta = \eta_0 + (\eta_m - \eta_0)(1 - \exp(-k_T t))$ (Eq. 5) | $\eta = \eta_0 + (\eta_m - \eta_0)\left(1 - \frac{1}{1+k_T C_0 t}\right)$ (Eq. 6) |

$k_T$ is the overall rate constant for protein denaturation at temperature $T$ (K); $C_p$ (%, w/w) is the native protein concentration of the concentrate; $t$ (s) is the time; $C_0$ (%, w/w) is the initial native protein concentration; $\eta$ (mPa.s) is the viscosity of the concentrate; $\eta_0$ (mPa.s) is the viscosity of the concentrate at time zero prior to heat treatment; $\eta_m$ (mPa.s) is the maximum viscosity due to heat treatment; $\alpha$ (mPa.s / %) is the coefficient representing response of viscosity to protein denaturation. Details of equation derivations in Table 1 are described in Appendices B and C.
Table 2. Parameters of viscosity response to heat treatment estimated using first- and second-order reaction kinetics

| Model parameters                                      | First order                        | Second order                       |
|--------------------------------------------------------|------------------------------------|------------------------------------|
| Maximum viscosity due to heat treatment                | $\eta_m$ (mPa.s) = 19.77±0.60       | $\eta_m$ (mPa.s) = 20.6±0.84       |
| Rate constant of viscosity response at 120 °C          | $k_{120}$ (1/s) = (9.65±2.4)×10$^{-2a}$ | $k_{120} C_0$ (1/s) = (19.1±0.90) ×10$^{-2a}$ |
| Rate constant of viscosity response at 100 °C          | $k_{100}$ (1/s) = (2.03±0.67) ×10$^{-2b}$ | $k_{100} C_0$ (1/s) = (2.68±1.16) ×10$^{-2b}$ |
| Rate constant of viscosity response at 85 °C           | $k_{85}$ (1/s) = (7.52±2.55) ×10$^{-3c}$ | $k_{85} C_0$ (1/s) = (1.01±0.4) ×10$^{-2c}$ |

$\pm$ 95% confidence interval.

$\eta_m$ (mPa.s) is the maximum viscosity due to heat treatment.

$k_T$ is the overall rate constant of protein denaturation at temperature $T$ (K)

$C_o$ (%, w/w) is the initial native protein concentration

Values of the rate constant within a column not sharing a common superscript differ significantly (P< 0.05).
Fig. 1.
Fig. 2.

1= Control
2=85°C×30 s
3=100°C×30 s
4=120°C×15 s
5=120°C×30 s
6=85°C×60 s
7=100°C×60 s
8=120°C×60 s
9=100°C×200 s
Fig. 3.

A

B
Fig. 4.
Fig. 5.
Fig. 6.
Highlights

- Heat treatment (≥75°C) caused a significant increase in viscosity of MPC
- A model was developed to describe the effect of heat treatment on MPC viscosity
- Second-order kinetics proved a good fit for viscosity response to heat treatment
- The Arrhenius plot showed the transition from protein unfolding to aggregation