

Polymerization Model for Prediction of Heat-Induced Protein Denaturation and Viscosity Changes in Milk

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A bicomponent polymerization model for heat-induced protein denaturation and viscosity changes in milk is presented. The model recognizes denaturation kinetics of β -lactoglobulin (β -lg) and the association between denatured β -lg and casein micelles. The aim of the study was to develop a mathematical model based on physical and chemical fundamentals for prediction of the rheology of milk products during thermal processes. The model reproduces both the degrees of denaturation of β -lg and the viscosity change of skim milk heated at temperatures between 80 and 120 °C very well. In contrast to the volume fraction model of Snoeren, the polymerization model accounts for higher viscosities of milk heated at a higher temperature at the same denaturation degree of β -lg. This confirms that, in addition to heat load, heating rate and temperature contribute to the rheological properties of dairy products. The model developed will be a basis for future research in describing the relation between process variables and the functional properties of milk products.

Keywords: Denaturation; viscosity; rheological properties; polymerization model

INTRODUCTION

With increased competition in the dairy industry, there is a growing emphasis on production efficiency and product quality. To control the production process, it is important to predict or know the relation between the state of the process and the consequent measured quality. This relation is a basis for adjustment of control variables such as temperature, time, and component concentration in optimizing both the process and product (de Jong, 1996). Many thermal processes in the dairy industry involve fluid and semifluid materials, e.g. evaporated milk, yogurt, and custards. The viscosity of these products is an important property that often characterizes the structure of the product quite well.

In the case of milk concentration by water evaporation, lowering the viscosity of the concentrated milk by adjusting of the preheat treatment leads to a considerable reduction of the operating costs (Bouman et al., 1993). Also, for the manufacturing of stirred yogurt, the viscosity is an important fluid property. To adjust the viscosity of nonfat stirred yogurt, either nonfat dry milk solids or a skim milk concentrate is added to the yogurt milk (Tamine and Robinson, 1985; Wong et al., 1988).

It is known that the viscosity change resulting from heat treatment is related to heat-induced denaturation and subsequent aggregation of proteins (Journink and de Kruif, 1993). Snoeren et al. (1982) reported that the viscosity of skim milk concentrate depends on the protein volume fraction. They assumed that the protein volume fraction, ϕ , increases with the thermal load of

the milk and concentration, in agreement with Langley and Temple (1985). Also, the firmness of the yogurt gel is strongly related to the degree of denaturation of β -lactoglobulin (β -lg) (Dannenberg and Kessler, 1988a). Savello and Dargan (1995) found a high correlation between the gel strength and viscosity of yogurt. The authors proposed that interactions between β -lg and κ -casein initiate the formation of a branched network.

To determine the effect of heat treatment on the viscosity of the product, it is necessary to have a model which relates heat treatment to protein denaturation and the protein volume fraction. Many efforts have been made to describe the denaturation kinetics of the whey proteins, especially β -lg. However, most models consider the denaturation one overall reaction (Mulvihill and Donovan, 1987; Dannenberg and Kessler, 1988b; de Wit, 1990) or a two-stage reaction model (de Jong et al., 1992; de Jong, 1996) and provide no information about development of the dimensions or volume fractions of protein aggregates. A first attempt to develop a more mechanistic model was made by Roefs and de Kruif (1994), who described the heat-induced denaturation and aggregation of β -lg (65 °C) in water in terms of an analogy with radical polymerization. They assumed that the free reactive sulfhydryl groups in the β -lg molecule react like radicals. However, to model the heat-induced viscosity changes in milk, at least the contribution of the casein micelles has to be taken into account. Several authors reported interaction between β -lg and κ -casein on casein micelles resulting in formation of filament-like structures (Mottar et al., 1989; Guillermo et al., 1992). It has also been stated that another whey protein, α -lactalbumin, is involved in the formation of aggregates (Mottar et al., 1989; Corredig and Dalgleish, 1996).

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In this article, a bicomponent polymerization model for heat-induced protein denaturation and viscosity changes in milk is presented. The model recognizes the denaturation/aggregation kinetics of β -lg and the reaction of aggregated β -lg with casein micelles. The aim of the study is to develop a mathematical model using fundamentals of polymer chemistry for prediction of the rheology of milk products during thermal processes. The model is validated by data of denaturation and viscosity measurements on skim milk, some of which are published.

MATERIALS AND METHODS

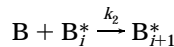
Theory of the Polymerization of β -Lactoglobulin in the Presence of κ -Casein. Bovine β -lactoglobulin (β -lg) exists in milk mainly as a dimer of two monomeric subunits noncovalently linked; it is the main constituent (more than 50%) of the whey proteins (Walstra and Jenness, 1984). Each monomer contains two disulfide bridges and one sulfhydryl group which in the native state is buried in the interior of the molecule.

In the literature, the heat-induced denaturation of β -lg is generally assumed to be a process consisting of at least two steps: a transformation of the native to an unfolded state and subsequent aggregation of the unfolded molecules (Mulvihill and Donovan, 1987). At a temperature of 55 °C and at low concentrations, β -lg is completely dissociated into two monomers (Georges et al., 1962). A further increase in the temperature (>65 °C) results in an unfolding of the molecule; breakdown of hydrogen and hydrophobic bonds and exposure of the sulfhydryl group, which is able to react with other proteins, notably, β -lg and κ -casein.

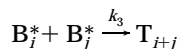
The denaturation/aggregation process of β -lg in milk can be described in terms of radical polymerization. The initiation reaction of the β -lg monomer is given by Roefs and de Kruijff (1994):



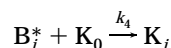
where B is the native β -lg monomer and B_1^* the activated monomer with the "radical" (sulfhydryl) group. The addition of each monomer molecule requires that a certain amount of energy be acquired since the monomer is postulated to be an active reactant for each step (Burnett, 1954). This is called the propagation step:



The polymerization reaction is terminated by the formation of disulfide bridges and thus the inactivation of the growing radicals:



where T_{i+j} is an aggregate consisting of $i + j$ β -lg monomers, assuming that the size of the β -lg radical has no effect on the reactivity (Burnett, 1954). Another termination reaction is the aggregation of growing radicals with κ -casein at the surface of the casein micelles:



where K_i is κ -casein which has reacted with a chain consisting of i β -lg monomers. The rates of disappearance and formation are given by the reaction rate equations:

$$\frac{d[B]}{dt} = -k_1[B] - k_2[B][B^*] \tag{1}$$

$$\frac{d[K_0]}{dt} = -k_4[B^*][K_0] \tag{2}$$

$$\frac{d[B_1^*]}{dt} = k_1[B] - k_2[B][B_1^*] - k_3[B_1^*][B^*] - k_4[B_1^*][K_0] \tag{3}$$

$$\frac{d[B_i^*]}{dt} = k_2[B][B_{i-1}^*] - k_2[B][B_i^*] - k_3[B_i^*][B^*] - k_4[B_i^*][K_0] \tag{4}$$

$$\frac{d[T_i]}{dt} = k_3 \sum_{j=1}^{i-1} [B_j^*][B_{i-j}^*] \tag{5}$$

$$\frac{d[K_i]}{dt} = k_4[B_i^*][K_0] \tag{6}$$

where [B], [B*], [T], and [K] are the total concentrations of the corresponding components.

It is very likely that other milk components such as α -lactalbumin are also involved in the formation of complexes (Mottar et al., 1989). Especially at relatively high heat loads, this may affect the reaction rates. However, concerning the low concentration and denaturation rates of α -lactalbumin with respect to β -lg for temperatures up to about 120 °C (Dannenberg and Kessler, 1988b), the overall effect on the outcomes of the model is assumed to be small.

The way in which the reaction rate constants are affected by temperature is assumed to be accurately described by the Arrhenius relationship.

$$k = k_0 \exp(-E_a/RT) \tag{7}$$

Given the preexponential factor (k_0) and the activation energy (E_a) of the four reaction rate constants, the conversion of the reactants can be calculated for suitable combinations of temperature and time or heating and cooling rates.

Theory of the Viscosity of Skim Milk. In 1906, Einstein derived his famous equation which describes the viscosity of highly dilute dispersions as a function of the volume fraction of spheres:

$$\eta = \eta_c(1 + 2.5\phi) \tag{8}$$

where η_c is the viscosity of the continuous phase. Batchelor (1977) derived the next higher order in the virial equation for the relative viscosity ($\phi < 0.2$):

$$\eta_r = \frac{\eta}{\eta_c} = 1 + 2.5\phi + k_H\phi^2 \tag{9}$$

where k_H is the Huggins coefficient which accounts for the hydrodynamic interactions between the spheres. The Huggins coefficient is calculated by Cichocki and Felderhof (1988)

$$k_H = 5.913 + \frac{1.9}{\tau_B} \tag{10}$$

where τ_B is the Baxter interaction parameter and a measure of adhesive interactions between the particles. For hard spheres, τ_B is infinite. For attractive spheres, τ_B is finite, resulting in a second term for eq 10. In light scattering and osmotic pressure measurements, the second virial coefficient B_2 is used, which is related to τ_B with

$$\frac{1}{\tau_B} = 4 - \frac{B_2}{V_{HS}} \tag{11}$$

where V_{HS} ($1/6\pi d^3$) is the volume of a hard sphere of diameter d .

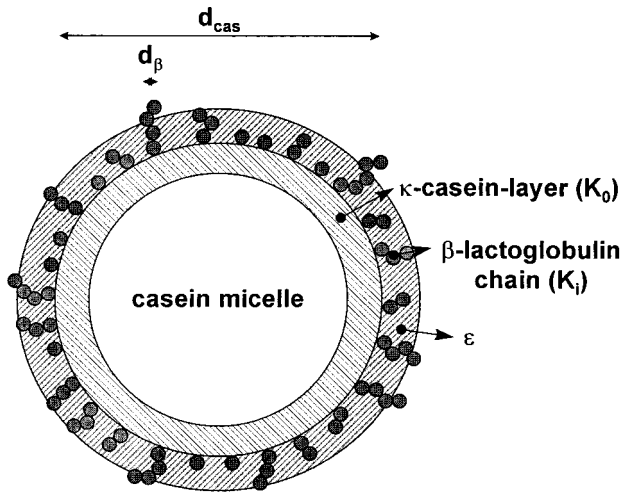


Figure 1. Highly schematic presentation of the increasing apparent volume of casein micelles by aggregation with β -lactoglobulin. Additional volume due to aggregation is indicated by the outer crosshatched ring.

Jeurnink and de Kruif (1993) considered skim milk to be a dispersion of casein micelles. They found with unheated skim milk a k_H somewhat smaller than 5.9. After the milk was heated at 85 °C for 15 min, k_H increased to 7.1. According to their theory, this indicates that during heating the micelles' self-attraction is increased from zero to a certain value. In addition to an increase of k_H , an increase of the (apparent) volume fraction of the casein micelles was determined. Thus, the total increase of the relative viscosity during heating is a result of an increase in both k_H and ϕ .

Theory of the Volume Fraction of the Protein Particles. The polymerization model described recognizes the formation of aggregates of proteins: (i) β -lg chains and (ii) complexes of β -lg chains and casein micelles. Figure 1 shows schematically the proposed model for an increase of the apparent volume of the casein micelles due to β -lg- κ -casein aggregation. The total volume fraction of the complex is related to the apparent volume of the layer of aggregated β -lg around the micelle and the apparent volume of the aggregated β -lg chains itself. Apparent volume means volume of the protein molecules plus entrapped water. According to Figure 1, the volume fraction of the complex is described by

$$\left. \begin{aligned} \phi_{\text{complex}} &= \phi_{\text{micelle}} + \phi_{\text{surface layer}} \\ \phi_{\text{micelle}} &= \frac{1}{6}\pi d_{\text{cas}}^3 N_A [\text{Cas}] \\ \phi_{\text{surface layer}} &= \frac{1}{6}\pi d_{\beta}^3 N_A \frac{1}{\epsilon} \sum_{i=1}^{N_{\text{max}}} [K_i] i \end{aligned} \right\}$$

$$\phi_{\text{complex}} = \frac{1}{6}\pi N_A \left(d_{\text{cas}}^3 [\text{Cas}] + d_{\beta}^3 \frac{1}{\epsilon} \sum_{i=1}^{N_{\text{max}}} [K_i] i \right) \quad (12)$$

where N_{max} is the maximum number of monomers per aggregated β -lg chain, $[\text{Cas}]$ the molar concentration of casein micelles, and ϵ the measure of the mean openness of the hairy layer of β -lg chains. The mean volume of the hard spheres is then derived from

$$V_{\text{HS}} = \frac{\phi_{\text{complex}}}{[\text{Cas}]N_A} \quad (13)$$

This value of V_{HS} is used to calculate the Baxter interaction parameter and the Huggins coefficient of eqs 11 and 10, respectively.

β -Lactoglobulin Denaturation Measurements. To determine the kinetic constants of the polymerization model for protein denaturation, experimental data of Dannenberg (1986) were used. He used skim milk as the medium. Dannenberg

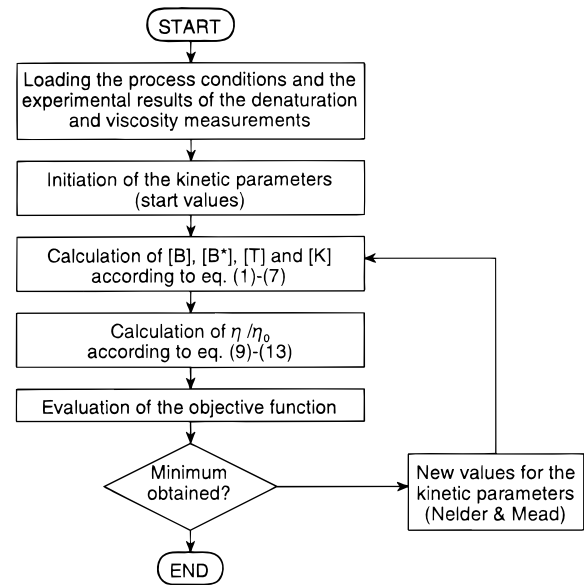


Figure 2. Method for the determination of the optimal kinetic parameters.

measured only conversions; however, the skim milk was made from normal raw milk with a total β -lg concentration of approximately 3.2 g/L. The samples were heated between 70 and 150 °C. Dannenberg acidified the heated samples to pH 4.6 to precipitate the denatured (i.e. aggregated) whey proteins. He analyzed the supernatant by ultra-thin-layer isoelectric focusing Radola (1980) and the precipitate by laser densitometry (Dannenberg, 1986).

Viscosity Measurements. To evaluate the polymerization model for the heat-induced viscosity change of skim milk according to the Batchelor equation, experimental data of Langley and Temple (1985) were used. Samples of skim milk were heated for 15 min at temperatures between 80 and 140 °C. The heating rate of the oil bath was about 1 K/s. The viscosity was measured using a "U" tube viscometer (type M2) at 20 °C.

Leguil and Roefs (1991) heated skim milk in an oil bath at 115 °C at holding times between 30 and 270 s. Heating the sample from room temperature to 115 °C took about 160 s. After heating, the bottles were cooled to 25 °C, and at least 20 min later, viscosity measurements with an Ubbelohde capillary viscometer were performed.

Numerical Methods. To obtain the optimal values of the kinetic parameters of the polymerization model, a computer program was written to fit the model with the experimental results of the denaturation (Dannenberg, 1986) and viscosity measurements (Langley and Temple, 1985). The optimization method used was the simplex method according to Nelder and Mead (Nelder and Mead, 1965; Bunday and Garside, 1987). Figure 2 is a flow chart of the computer program.

First, the experimental conditions and results of the denaturation and viscosity measurements are loaded into the program. These data will be used for comparison with the calculated values according to the model. After initiation of the kinetic parameters (first estimation), the optimization starts. For each experimental point, the concentration of the reactants and the viscosity change are calculated. The total number of experimental points was 67.

To verify the results of the polymerization model with the degrees of denaturation of β -lg determined by Dannenberg (1986), it was necessary to know which products of the polymerization are involved in the determined amount of denatured proteins. In other words, it was necessary to know which molecules remain in the supernatant after acidifying the milk sample to pH 4.6. Although there is no evidence in the literature about which molecules precipitate, it was assumed that all the molecules of β -lg which did not react with other molecules remain in the supernatant as nondenatured

Table 1. Characteristics of β -Lactoglobulin and Casein Micelles

protein	concentration in milk (mmol/L) ^a	<i>M</i> (g/mmol)	<i>d</i> (nm)
β -lactoglobulin	0.180	18.283 ^b	2 ^b
κ -casein	0.180 ^b	19.550 ^b	—
casein micelle	3.3×10^{-5} ^c	7.94×10^5 ^d	220 ^e

^a Actual concentration depends on the milk used. ^b Walstra and Jennes (1984). ^c $\phi_{\text{cas}} = C_{\text{cas}} N_A^{1/6} \pi r^2$, where $\phi_{\text{cas}} = 0.11$ (Walstra and Jennes, 1984) and $d = 220$ nm. ^d Twenty-six grams of micelles per liter (Walstra and Jennes, 1984); $M = 26 / (3.28 \times 10^{-5}) = 7.94 \times 10^5$ g/mmol. ^e Hansen et al. (1996).

protein. So the concentration of denatured β -lg was defined with

$$C_d = ([\beta\text{-lg}] - [B] - [B_1^*]) \times M_\beta \quad (14)$$

where $[\beta\text{-lg}]$ is the total concentration of β -lg in moles per liter and C_d is the concentration of denatured β -lg in grams per liter.

The next step is the evaluation of the objective function which has to be minimized. The function was defined as the sum of the correlation coefficients for the denaturation and viscosity measurements. Since the two correlation coefficients have to be maximized, the function was multiplied by -1 .

$$f_{\text{obj}} = -1(\alpha R_{\text{den}}^2 + R_{\text{visc}}^2) \quad (15)$$

with

$$\alpha = \frac{N_{\text{den}}}{N_{\text{visc}}} \quad (16)$$

where N_{den} and N_{visc} are the number of experimental points related to the denaturation and viscosity measurements, respectively.

If no minimum is obtained, the procedure is repeated with new values of the kinetic parameters determined by the simplex method of Nelder and Mead (1965).

Assumptions. Table 1 gives the characteristic properties of β -lg and the casein micelles that were used. The model evaluations are based on an average size distribution of relatively large micelles (>50 nm). Since the molar concentrations of β -lg and κ -casein are similar, the concentration of nonaggregated κ -casein was assumed to not be limiting the aggregation rate.

In addition to the data of Table 1, it was assumed that the adhesiveness of the casein micelles of unheated milk can be neglected. In other words, B_2/V_{HS} is initially equal to 4. Concerning the work of Jeurink and DeKruif (1993), it is reasonable to assume that for diluted systems ($\phi < 0.2$) B_2 remains nearly constant during (mild) heating.

The dissociation of κ -casein from the micelle under the conditions considered ($\text{pH} \leq 6.7$, $T \leq 120$ °C) is neglected. This assumption agrees with the results of Singh (1988), who did not find significant dissociation of κ -casein under these conditions.

For the initiation reaction, the kinetics of de Wit (1990) were used. The temperature dependence of the reaction rate constant k_1 is then defined by $E_a = 261.4$ kJ/mol and $\ln k_0 = 86.41$. In the literature, these data were applied for describing the unfolding of the β -lg molecules, i.e., the exposure of the radical group (de Wit, 1990; de Jong et al., 1992; de Jong, 1996).

RESULTS AND DISCUSSION

In Table 2, the results of the model parameter optimization are presented. Given are the kinetic constants of the reaction rate equations of eqs 1–6. As proposed by Hoffmann et al. (1996), the activation energies of the radical termination reactions are rela-

Table 2. Results of the Model Parameter Optimization

reaction step	$\ln k_0$	E_a (kJ/mol)
initiation ^a (k_1)	86.41	261.40
propagation (k_2)	86.44	263.88
termination of β -lg chains (k_3)	24.51	81.52
termination of β -lg/ κ -casein (k_4)	40.63	139.08

^a According to de Wit (1990).

tively low. The openness of the hairy layer (ϵ) was fitted as 0.01. This value is on the same order of magnitude as the openness of the hairy layer of the unheated micelles consisting of κ -casein. According to the data given by Walstra and Jennes (1984), the openness of the hairy layer consisting of κ -casein was estimated as 0.02.

The model presented predicts both the viscosity change and the concentration of denatured β -lg as a function of temperature and time. The total number of fitted parameters is seven (three pre-exponential factors, three activation energies, and one openness factor). Dannenberg (1986) previously used six parameters to describe the denaturation kinetics (two pre-exponential factors, two activation energies, one reaction order, and one transition temperature). Thus, the polymerization model described uses only one more parameter to describe the viscosity change as a function of heating temperature and time. Figure 3 shows a comparison between the reported percentage of denaturation (Dannenberg, 1986) and those calculated according to the model. At low temperatures, the amount of denatured β -lg is mainly in the form of terminated β -lg chains (T). With increasing temperatures, more β -lg is aggregated with κ -casein on the micelle surface. However, even at 120 °C, less than 20% of β -lg is aggregated with κ -casein.

It should be noted that in contrast with the findings of many authors (Manij and Kakuda, 1986; Luf, 1988; Dannenberg and Kessler, 1988b; de Jong, 1996) there is no shift in the kinetic constants around 90 °C. Taking this and the scattering of the data points of Dannenberg ($R_{\text{den}}^2 = 0.92$) into account, the measured degrees of denaturation and aggregation agree very well with the polymerization model ($R_{\text{den}}^2 = 0.95$). Obviously, the curious break in the Arrhenius plot is partly explained by the model. According to the model, there are different rate-determining reaction steps, for example, the propagation and the termination reaction.

At temperatures higher than 120 °C, the measured degrees of denaturation could no longer be reproduced by the model ($R^2 < 0.5$). This may be caused by a different polymerization mechanism. In the literature, there are also indications that high temperatures change the protein interactions. Langley and Temple (1985) found with skim milk heated at temperatures above 120 °C a sudden increase of the viscosity at comparable degrees of denaturation of β -lg. Others reported that heating milk at high temperatures causes disaggregation of casein micelles (Dannenberg and Kessler, 1988a; Aoki and Kako, 1988).

In Figure 4, the measured viscosity changes of skim milk heated at temperatures between 80 and 120 °C are compared with the predictions of the model. The coefficient of determination was rather high (0.97), indicating that the model was able to reproduce the measured values very well. Apparently, the polymerization model explains the temperature dependency of the heat-induced viscosity change and supports the physical background of the viscosity model for diluted

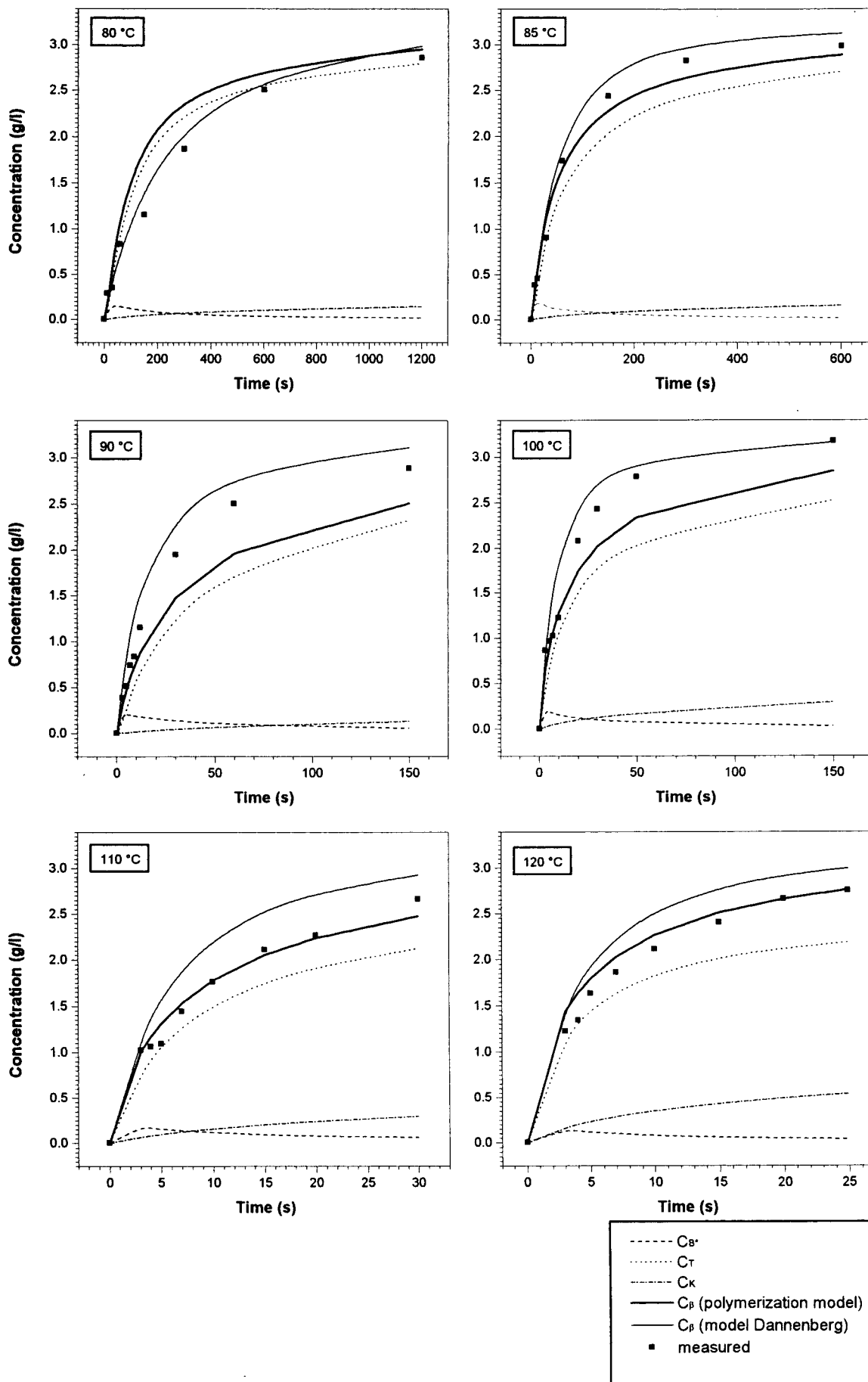


Figure 3. Comparison between the measured and the predicted denaturation degrees of β -lactoglobulin in skim milk heated at different heating temperatures.

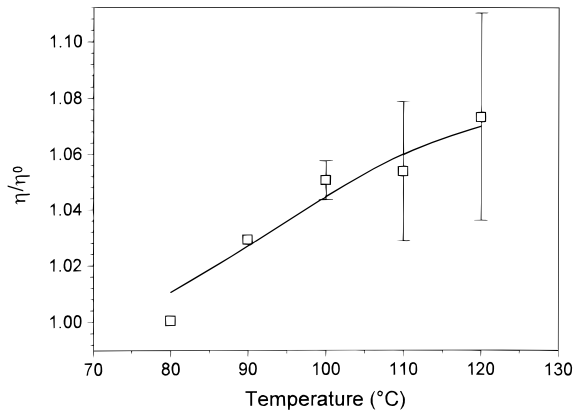


Figure 4. Comparison between the measured and the predicted viscosity change of skim milk heated for 15 min at temperatures between 80 and 120 °C (only one data point at 80 and 90 °C).

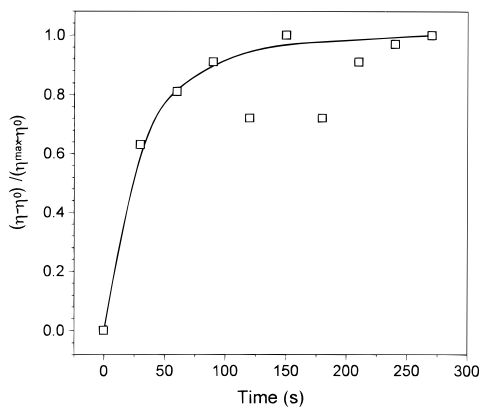


Figure 5. Comparison between the measured and predicted viscosity change of skim milk heated at 115 °C.

dispersions ($\phi < 0.2$). Also, realistic values for the interaction parameter τ_B were obtained with the viscosity model. At 120 °C, the volume fraction of the casein micelles has increased from 0.11 to 0.135 and τ_B was calculated as 1.4 which is much higher than the percolation threshold (de Kruif, 1993). According to percolation theory, the milk would flocculate at a τ_B of < 0.1 which is obviously not the case here.

Figure 5 shows a comparison between the viscosities measured by Leguil and Roefs (1991) and those predicted by the model. Since the experimental setup differed from that of Langley and Temple (1985) and the concentration of β -lg was unknown, the results were not used for estimation of the model parameters. The measured and calculated viscosities are scaled to the maximum value obtained after heating for 270 s. If the data variation between 100 and 200 s is assumed to be scatter, the course of the calculated viscosity changes fits the experimental data very well.

It is generally stated that a higher denaturation degree of whey proteins in milk results in higher viscosities (Snoeren et al., 1982; Jeurnink and de Kruif, 1993). Up to now, the effects of the heating rate and the temperature level on the viscosity change have not been established and are usually neglected. Using the model developed here, the effect of the heating rate can be estimated. Figure 6 shows some results of computer calculations with the model; the viscosity of skim milk related to the denaturation degree of β -lg as a result of holding the milk at different temperatures is presented. The holding time was adjusted for the given denatur-

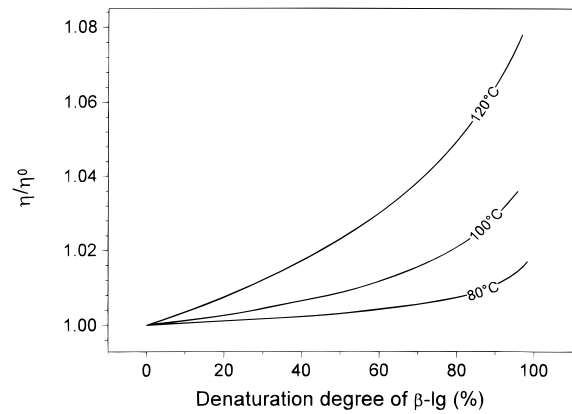


Figure 6. Viscosity change of skim milk as a function of the heating temperature and the degree of β -lactoglobulin denaturation (model simulations). The heating time was adjusted for the given denaturation degree and heating temperature.

ation degree at a certain heating temperature. For example, with 80% denatured β -lg and a heating temperature of 80 °C, the used holding time is ca. 600 s, and with 80% denatured β -lg and heating temperature of 100 °C, the used holding time is ca. 50 s. It turns out that holding at a higher temperature gives a higher viscosity of the milk at the same denaturation degree. Since a high heating rate means that a high temperature is reached relatively fast, the effect of a high heating temperature is similar to the effect of a high heating rate. The phenomenon shown in Figure 6 can be explained by the relatively high activation energy of the propagation reaction and the termination reaction between β -lg and casein micelles. At higher temperatures, larger and more β -lg chains aggregate with the casein micelles, resulting in a larger volume. This agrees with electron micrographs of Mottar et al. (1989). The micrographs show more superficial filaments at the casein micelle surface after the milk is heated at a higher temperature and with a similar degree of whey protein denaturation.

ABBREVIATIONS USED

a_s , specific reaction surface (m^2/m^3); B_2 , second virial coefficient; C , concentration (g/L); d , diameter (m); D , diffusion coefficient (m^2/s); E_a , activation energy (J/mol); f_{obj} , objective function; k , reaction rate constant ($\text{L}^{(n-1)}/\text{g}^{(n-1)} \text{ s}$); k_0 , pre-exponential factor ($\text{L}^{(n-1)}/\text{g}^{(n-1)} \text{ s}$); K , mass transfer coefficient (m/s); k_B , Boltzmann constant ($1.38 \times 10^{-23} \text{ J/K}$); k_H , Huggins coefficient; M , molecular mass (g/mol); $[X]$, concentration of X (mol/L); n , reaction order; N , number; N_A , Avogadro's constant ($6.022 \times 10^{23} \text{ mol}^{-1}$); R , gas constant (J/mol K); R^2 , coefficient of determination [$(\text{SST} - \text{SSE})/\text{SST}$ with $\text{SST} = \sum y_i^2 - \sum^2 y_i/N$ and $\text{SSE} = \sum (y_{\text{model}} - y_i)^2$]; t , time (s); T , temperature (K); V , volume (m^3); α , ratio; ϵ , openness (V/V_a) (m^3/m^3); η , dynamic viscosity (Pa s); τ_B , Baxter interaction parameter; ϕ , volume fraction; (subscripts and other abbreviations) 0, initial; a, apparent; agg, aggregated; B, monomer of β -lg; B_i^* , reactive chain of β -lg consisting of i monomers; c, continuous phase (permeate); den, denaturation; i and j , indexes; Cas, casein micelles; HS, hard sphere; K_i , terminated chain of β -lg consisting of i monomers associated with κ -casein; max, maximum; r, relative; T_i , terminated chain of β -lg consisting of i monomers; β , β -lactoglobulin.

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