Effect of the technological history on the heat denaturation kinetics of whey proteins

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EFFECT OF THE TECHNOLOGICAL HISTORY ON THE HEAT DENATURATION KINETICS OF WHEY PROTEINS

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I. Introduction

Fouling of industrial equipment is a recurring issue in food industries that impact their performance and the quality of the food products. When this phenomenon occurs, the hygienic and organoleptic qualities of the final products can be affected, downtime dedicated to cleaning chops the production and finally cleaning constitutes a high cost for companies due to the consumption of chemicals, water, the waste management and the non-productive time. The mechanisms of fouling of heat plate exchangers is particularly studied in the dairy industries because heating occurs in the manufacture of almost all industrial dairy products. It is essential to understand the mechanism of the fouling layer build up in order to limit its occurrence during the heating process and to optimize the cleaning sequence to remove it.

Depending on the composition of the heated dairy products, the structure of the fouling layer differs, but whey proteins are recognized to be the main agents participating to the fouling build-up. Industrial observations indicate that the fouling aptitude of the whey proteins is also different if they have been concentrated by under vacuum evaporation/spray dried/rehydrated or not. Several hypothesis for explaining this difference of fouling aptitude can be put forward. This difference can be explained by an insufficient rehydration of the whey protein powder when the dispersion is heated, the loss of minor compounds, such as minerals, during the under vacuum evaporation/spray drying that affects either to the heat-denaturation/aggregation of the whey proteins and/or the fouling build up. During the concentration by under vacuum evaporation/spray drying operations, the whey protein structure could also be modified and be responsible of a different susceptibility of the whey proteins to heat-denaturation/aggregation.

The present study aims to check if the under vacuum evaporation/spray drying/rehydration operations affect the kinetics of heat-denaturation of the whey proteins and could be a possible explanation for the differential fouling behavior observed on heat exchangers during whey protein heating. Therefore, our research question is whether the heat-denaturation kinetics of whey proteins can be affected by under-vacuum evaporation/spray drying/rehydration operations.

II. Bibliography

II.1. Whey proteins

Whey is the soluble fraction of milk that is separated from the casein curd during the manufacture of cheese or acid casein or that is recovered in the permeate of milk microfiltration (Singh and Havea, 2003). Whey is rich in proteins, minerals and lactose. Whey proteins are the protein fraction, which stay soluble when the pH is dropped to 4.6 or when rennet is added. They constitute about 20% of the proteins in milk (Brodkorb et al., 2016). The functional properties of the whey proteins are diverse and depend on processing applied during the production of food products. Whey proteins are often used for the manufacture of reconstituted fish or meat products, bakery products, baby food, fortified food and drinks for the elderly or sportspeople. In mass, the most important commercial whey protein ingredients are WPC (whey protein concentrate) and WPI (whey protein isolate) which is distinguished by the total protein content (Singh and Havea, 2003).

The main whey proteins are β-lactoglobulin, α-lactalbumin, immunoglobulins and bovine serum albumin. Whey protein ingredients also contains other components in various amount like lipids, lactose or minerals (Singh and Havea, 2003).

II.1.1. β-lactoglobulin

β-lactoglobulin (β-LG) is the major whey protein in bovine milk that account for 50% of the total whey proteins (Mulvihill and Donovan, 1987). It is a globular protein mainly composed of β-sheets. Its concentration in milk is between 2 and 4 g/L. Its molecular weight is 18.3kDA and it is constituted of 162 amino acid residues (Cayot and Lorient, 1998). The amino acid sequence depends on the genetic variant of the protein. There are 13 genetic variants but two variants, β-LG B and β-LG A, are the most commons (Tolkach and Kulozik, 2007).

β-LG protein stability depends on the solvent conditions. At room temperature and under physiological pH condition, β-LG exists in an equilibrium between non-covalent dimeric and monomeric forms. The equilibrium is shifted to the monomeric form at higher temperature and higher pH values (Wijayanti et al., 2014). β-LG is absent from human milk and the milk of some other monogastrics (Mulvihill and Donovan, 1987).

II.1.2. α-lactalbumin

α-lactalbumin (α-LA) is the second major bovine whey protein. It contains 20% α-helix, 14% β-sheet and 60% unordered structure (Mulvihill and Donovan, 1987). It is a small protein with 123 amino acid

residues and a molecular weight of 14.2 kDa. Its concentration in milk is between 1 and 1.5 g/L (Cayot and Lorient, 1998).

II.2. Denaturation of whey proteins

Heat treatment is one of the main processing operation to ensure the safety of food products but it causes protein denaturation (Singh and Havea, 2003). The thermal denaturation of whey proteins plays an important role in determining the functional properties of some milk products (Anema, 2017). Protein denaturation is linked to the structural stability of the native protein. The term denaturation means the breakdown of low-energy interactions that stabilize the secondary and tertiary structures of proteins. These breakdowns are followed by random rearrangements of protein regions between them during cooling (Cayot and Lorient, 1998). Hence, protein denaturation involves two steps: an unfolding step, i.e the modification of the native state of the protein without change of its molecular weight (Loveday, 2016) to an activated state called molten globule and a subsequent (irreversible) aggregation of unfolded molecules (Singh and Havea, 2003).

Whey proteins are globular proteins with high levels of secondary or tertiary structures, which make them susceptible to denaturation during heat treatment (Poon et al., 2001). Exposure of whey proteins to high temperatures (>70°C) leads to its unfolding with the exposition of reactive groups (hydrophobic amino acids, thiol groups) initially hidden in the inner core of the proteins (Anema, 2017). Protein unfolding is rapid, extensive and is followed by rapid aggregation (Loveday, 2016). The mechanism of unfolding and aggregation of β-Lg is given in Figure 1.

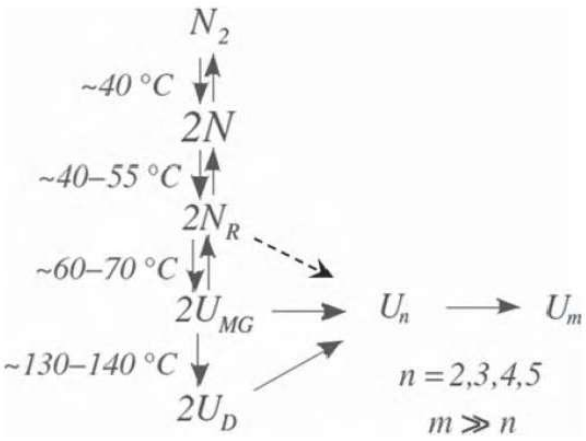

Figure 1 : Multistage process characterizing β-LG thermal denaturation (Tolkach and Kulozik, 2007).

N_2 is the native dimer state, N the native monomer state, N_R the R state, U_{MG} the molten globule state, $U_{n,m}$ the polymer and aggregate states and U_D the irreversible denatured states (Tolkach and Kulozik, 2007).

The susceptibility to denaturation of the individual whey proteins follow the order: immunoglobulin > bovine serum > β-Lg > α-LA but the denaturation depends on the medium conditions (pH, ionic strength, nature of salts, etc.) and the way it is appreciated (Cayot and Lorient, 1998).

II.3. Denaturation kinetics of whey proteins

Protein denaturation is measured in different ways: loss of solubility at pH 4.6, shift of protein elution time in reverse phase chromatography or size exclusion chromatography, decrease of band intensity in native polyacrylamide gel electrophoresis or decrease of the endotherm peak in differential scanning calorimetry (Loveday, 2016). The main kinetics parameters to modelized the heat-denaturation of proteins are n, the order of the reaction, and Ea, the activation energy. These parameters are specific to each protein and depend on the medium conditions. They are determined by following the loss of native proteins with the time of heating at defined temperatures (Anema, 2017).

The loss of native proteins due to denaturation during heat treatment is often kinetically modelled by the equation (1) (Loveday, 2016).

$$-\frac{dC}{dt} = k_n . C_t^n \qquad (1)$$

With the protein concentration C ($g.L^{-1}$), time t (min), the reaction rate constant k_n ($g^{(1-n)}.L^{(n-1)}.min^{-1}$) and the reaction order n (dimensionless) (Loveday, 2016). After integration and for n≠1 (2):

$$\frac{C_t}{C_0}^{(1-n)} = 1 + (n-1).k_n.C_0^{(n-1)}.t \qquad (2)$$

With C_t the native protein concentration ($g.L^{-1}$) at time t (min) and C_0 the initial protein concentration ($g.L^{-1}$).

Then, the relationship between the reaction rate constant and heating temperature can be expressed by the Arrhenius equation (3) (Petit et al., 2011).

$$\ln(k_n) = \ln(k_{n0}) - \frac{Ea}{R}.\frac{1}{T} \qquad (3)$$

With Ea the activation energy ($J.mol^{-1}$), k_{n0} the pre-exponential constant ($g^{(1-n)}.L^{(n-1)}.min^{-1}$) and R the ideal gas constant in $J.mol^{-1}.K^{-1}$ ($R= 8.314\ J.mol^{-1}.K^{-1}$) (Petit et al., 2011).

II.4. Fouling mechanism of plate heat exchangers

Fouling of heat plate exchangers is a problem in the dairy industry and costs billions of dollars every year. It reduces heat transfer efficiency and increases pressure drop and hence affects the economy

of a processing plant (Bansal and Chen, 2006). Fouling is a consequence of heating solutions containing heat-sensitive components. During milk heating, a layer of milk components is progressively deposited on the hot surfaces of the heat exchangers that are in contact with the milk (Truong et al., 2017).

The denaturation of β-LG and the formation of deposits on the hot plates occur simultaneously as the milk flow through the heat exchangers (de Jong, 1997). The fouling rate was correlated with the level of native β-LG in contact with the hot surface and the temperature of the milk in the heat exchangers. It is proposed that the fouling is due to reactive β-LG intermediates that interacted with the deposits on the surface of the heat exchangers (Truong et al., 2017).

III. Materials and methods

III.1. Preparation of the solutions

A permeate of milk microfiltration (852FB, Ingredia, Arras, France) was used in this study. It was received under two conditioning: a liquid form corresponding to the permeate of a microfiltrated skimmed milk concentrated by ultrafiltration/diafiltration and a powder form obtained after under vacuum evaporation/drying of the same concentrate. Three independent batches of liquid conditioning and three independent batches of powder conditioning were used in this study. The powder was composed of 81.97-84.64% of proteins, 2.61% of ashes, 7.3-7.9% lactose and 4.18-6.16% of humidity. The liquid was composed of 25.62-27.11% of dry matter of which 21.89-22.89 % of proteins, 0.66-0.68% of ashes and 0.68-3.02% lactose. The 852 FB liquid was diluted with distilled water to have a protein concentration of 10g/L and the 852FB powder was reconstituted with distilled water to have a protein concentration of 10g/L. In the following, they were called liquid and powder solutions, respectively. The liquid and powder solutions were adjusted at pH 6.7±0.03 with 1M NaOH and stored overnight at 4°C.

III.2. Heat treatment and denaturation of whey proteins

The liquid and powder solutions placed in series of glass tubes (1mL) were heated in a water bath (Fisherbrand Isotemp water bath, Thermo Fisher Scientific) set at different temperatures ranging from 72°C to 90°C by step intervals of 2°C. At each temperature, the glass tubes were removed from the water bath at different heating times. At least 10 different heating times were selected at each heating temperature. The maximum heating time at each temperature corresponds to 1.5 times the heating time required to reach 80% of protein denaturation or 20% of remaining native proteins. For each solution and temperature, the equivalent time $t_{20\%\Theta}$ to obtain 20% of remaining native proteins, was calculated with Θref (°C), $t_{20\%\Theta ref}$ (min) and z=10 (4). (Halabi et al., 2020).

$$t_{20\%\theta} = t_{20\%\theta ref}.10^{\frac{\theta ref-\theta}{z}} \quad (4)$$

One glass tube with 1mL of solution represents one heating time at one temperature. The real temperature into glass tubes was controlled with a thermometer inserted into a control glass tube filled with distilled water. Immediately after heating, the glass tubes were put into ice to rapidly cool down the solutions and to stop whey protein denaturation. Unheated solutions were used as reference.

Denatured whey proteins were precipitated at pH 4.6 with an acetic/acetate buffer solution and placed in water bath at 30°C for 5 min. Native whey proteins were separated from denatured proteins by centrifugation at 13 000 g, for 10 min at 21°C. The supernatant containing the native whey proteins was recovered and diluted with a solution of 0.106% of Trifluoroacetic acid (TFA) in distilled water.

III.3. Method for the analysis of denaturation

Samples were analyzed by reverse phase-HPLC (Dionex UltiMate 3000 HPLC System, Thermo Fisher, Germany) using a PLRP-S 300A 8µm 150*2.1mm column (Agilent Technologies, UK). Proteins were eluted from the column at a flow rate of 0.2 mL/min using a gradient of acetonitrile obtained by mixing mobile phases A and B (mobile phase A was 0.106% of TFA in distilled water and the mobile phase B was 0.1% of TFA in acetonitrile). The acetonitrile gradient started with 16% of mobile phase B for 5 min, then it increases to 32% in 15 min, 48% in 1 min, 80% in 4 min and finally 16% in 5 min. The proteins were detected at 214 and 280 nm at the exit of the column using a UV/visible detector.

The chromatographs were analyzed by the software Chromeleon, which calculated the area of protein peaks. Native whey protein concentration in each sample was quantified using a calibration curve established by injection of standards of α-LA and β-LG at a concentration of 0.5g/L.

III.4. Kinetics of protein denaturation

The kinetics of milk protein denaturation were described by equation (5) (van Boekel, 2020).

$$-\frac{dC}{dt} = k_n.C_t^n \quad (5)$$

This equation was integrated with n≠1 and gave equation (6)

$$\frac{C_t}{C_0} = \sqrt[1-n]{1+(n-1).k_n.C_0^{(n-1)}.t} \quad (6)$$

C_t is the native protein concentration at time t, C_0 is the initial concentration of native protein, k_n is the reaction rate constant at a defined temperature T_n and n is the reaction order. Concentration was given in g/L, time in min and the reaction rate constant in $g^{(1-n)}.L^{(n-1)}.min^{-1}$ (Leite et al., 2021).

The correlation between the reaction rate constant k_n and the temperature T was given by the relationship of Arrhenius. This relationship was described by the equation (7) (Leite et al., 2021).

$$k_n = k_{n0}.e^{-\frac{Ea}{R}.\frac{1}{T}} \qquad (7)$$

In this equation, Ea was the activation energy in $J.mol^{-1}$, k_{n0} was the pre-exponential constant in $g^{(1-n)}.L^{(n-1)}.min^{-1}$, R was the ideal gas constant in $J.mol^{-1}.K^{-1}$ (R= 8.314 $J.mol^{-1}.K^{-1}$) and T was the temperature in K.

Substituting k_n in equation (6) by equation (7) gave equation (8). Equation (8) may be rearranged by substituting k_{n0} by k_{n1} with an arbitrary temperature T_1 (9) (Leite et al., 2021).

$$\frac{C_t}{C_0} = \sqrt[1-n]{1 + (n-1).k_{n0}.e^{-\frac{Ea}{R}.\frac{1}{T}}.C_0^{(n-1)}.t} \qquad (8)$$

$$\frac{C_t}{C_0} = \sqrt[1-n]{1 + (n-1).k_{n1}.e^{\frac{Ea}{R}.(\frac{1}{T1}-\frac{1}{T})}.C_0^{(n-1)}.t} \qquad (9)$$

III.5. Estimation of the kinetics parameters

III.5.1. Two-step method

The “two-step method” consists of the estimation of the kinetic parameter in two steps. The first step is for estimating kinetic constants for each temperature and the second step is for estimating the activation energy and pre exponential constant (Dannenberg and Kessler, 1988).

In the literature, the reaction order was sometimes fixed arbitrary to 1.5 (Dannenberg and Kessler, 1988; Anema, 2000) to determinate the reaction rate constant. With n=1.5, the reaction rate constant for each temperature was determined by the linear regression slope of $(C_t/C_o)^{\wedge(-0.5)}$ versus time (10)

$$\frac{C_t}{C_0}^{(-0.5)} = 1 + 0.5.k_n.C_0^{0.5}.t \qquad (10)$$

The second step of the “two-step method” is to determinate the activation energy from the slope of the plot of the linear regression for the logarithm of the kinetic constant k_n, which was estimated at each temperature, versus the inverse of the heating temperature, according to equation (11). The y-intercept gives $\ln(k_{n0})$.

$$\ln(k_n) = \ln(k_{n0}) - \frac{Ea}{R}.\frac{1}{T} \qquad (11)$$

III.5.2. Analysis method with the nlsLM function

The kinetics of denaturation was analyzed with Rstudio v4.0.0 and the nlsLM function in the minpack.lm package. The nlsLM function is a non-linear adjustment according to the Levenberg-Marquard algorithm. The reaction order n, the pre-exponential constant k_{n1} and the activation energy Ea for all temperatures was estimated with equation (12). T_1 was a reference temperature and it was defined at an arbitrary temperature of 345K, corresponding approximately to the denaturation temperature of β-LG. This model took simultaneously into account the experimental points at all temperatures.

$$\frac{C_t}{C_0} = (1 + (n-1).k_{n1}.e^{\frac{Ea}{R}.\left(\frac{1}{T1}-\frac{1}{T}\right)}.C_0^{(n-1)}.t)^{\frac{1}{1-n}} \qquad (12)$$

The rate constant of the reaction k_n was calculated at each temperature thanks to the pre-exponential constant k_{n1}, the activation energy Ea of the denaturation reaction, the same arbitrary temperature T_1= 345 K according to equation (13).

$$k_n = k_{n1} \times exp\left[-\frac{Ea}{R}.\left(\frac{1}{T_1}-\frac{1}{T}\right)\right] \qquad (13)$$

IV. Results and discussion

IV.1. Characterization of liquid and powder solution of whey proteins

The reverse phase profiles of the liquid and powder solutions before heating are displayed on Figure 2.

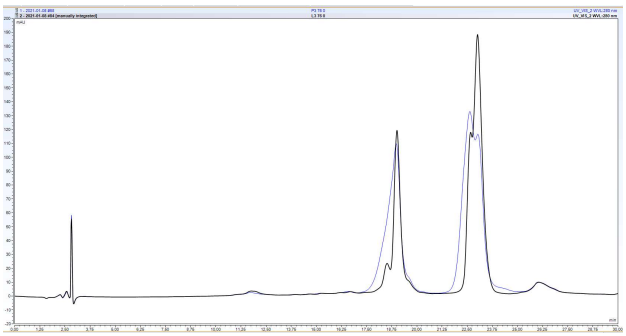

Figure 2 : HPLC Profile of unheated liquid (black peak) and powder (blue peak) solution. Proteins are detected at 280nm

The first peak eluted at retention time around 19 min corresponds to α-LA and the second double peak at retention time around 23 min represents the two variants of β-LG. (Fig. 2). The first part of the double peak represents the B variant of β-LG and the second part represents the A variant of β-LG. One small peak just ahead of α-LA peak on the liquid solution profile could correspond to an isoform of α-LA, such as a glycosylated α-LA. It was observed that the amount of glyco-α-lac represents between 3 (Hopper and McKenzie, 1973) and 10% (Slangen and Visser, 1999) of the α-LA

fraction. It may be interesting to carry out a mass spectrometry analysis to identify this entity without ambiguity.

When the two profiles are superimposed, the peaks of α-LA and β-LG in powder solution are larger than in the liquid solution, especially at lower retention time. This can be explained by protein modifications during the under vacuum evaporation, spray drying operations leading to more hydrophilic protein forms in the powder solution. These operations could be responsible of the binding of lactose molecules to the whey proteins (Norwood et al., 2017). The modification of the protein surface properties by binding of hydrophilic lactose molecules shift protein peaks to lower elution times on reverse phase chromatography.

IV.2. Denaturation kinetic of whey proteins

α-LA and β-LG denaturation in the liquid solution and the powder solution were selectively quantified by RP-HPLC from the soluble protein fraction at pH 4.6 at different heating time in the temperature range from 72°C to 90°C. One RP-HPLC profile corresponds to the heating of one sample solution (liquid or powder) at one selected temperature for one definite time.

Figures 3 and 4 represent the concentrations of native proteins according to heating time (represented by different colors) at one temperature for liquid (Fig.3) and powder (Fig.4). As expected the concentration of native proteins decreases with the increase of heating time.

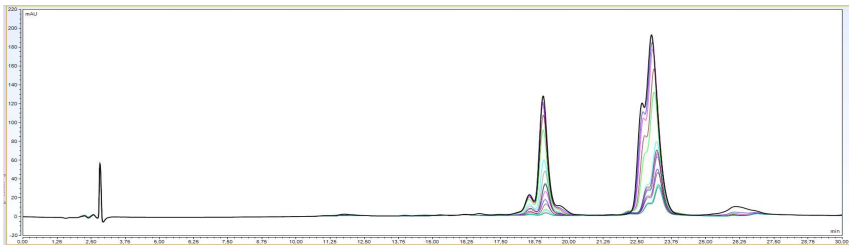

Figure 3 : HPLC profile of liquid solution after different heating time at 86°C. Proteins are detected at 280nm.

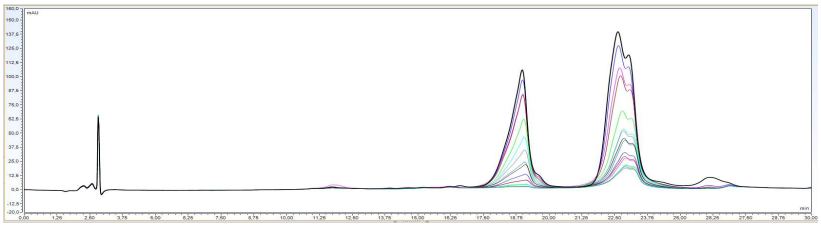

Figure 4 : HPLC profile of powder solution after different heating time at 86°C. Proteins are detected at 280nm.

IV.2.1. Heat denaturation of β-LG

Figure 5 shows the impact of the heating time on β-LG denaturation in powder solution at different temperatures. The kinetics of denaturation of β-LG at different temperatures are represented by different colors. Temperature and heating time, both contribute to an increase of the denaturation

of whey proteins. Indeed, when temperature increase the proteins denature faster. Heat treatment at temperature higher than 80°C induced a fast denaturation of β-LG (Fig.5). This was observed in the Arrhenius plot by a modification of the activation energy of denaturation of β-LG around 80°C (Fig 7). This change comes from a modification of the mechanism of denaturation of β-LG and of its kinetics parameters (activation energy, reaction order)(Tolkach and Kulozik, 2007). The same trend is observed for the denaturation of β-LG in liquid and powder solutions.

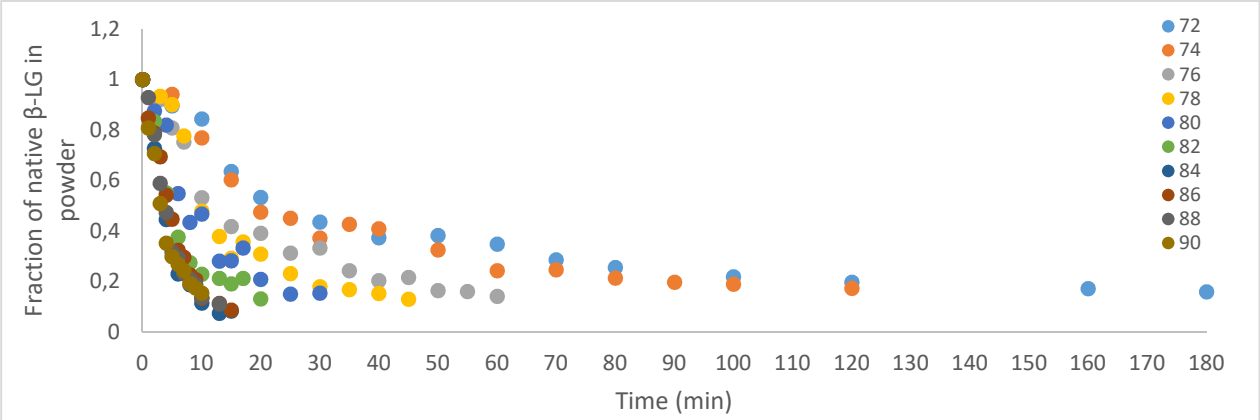

Figure 5 : β-LG denaturation of 852FB powder batch 1 according to time at each temperature.

IV.2.2. Heat denaturation of α-LA

Figure 6 show the impact of the heating time on α-LA denaturation at different temperatures. The kinetics of denaturation of α-LA at different temperature are represented by different colors. Unlike β-LG protein, α-LA denaturation kinetics increase progressively with increasing temperatures and no bend in the Arrhenius plot is observed at around 80°C (Fig.7). The Arrhenius plot of α-LA (Fig.7) shows that there is no change of its activation energy so the mechanism of denaturation of α-LA do not change in the temperature range studied.

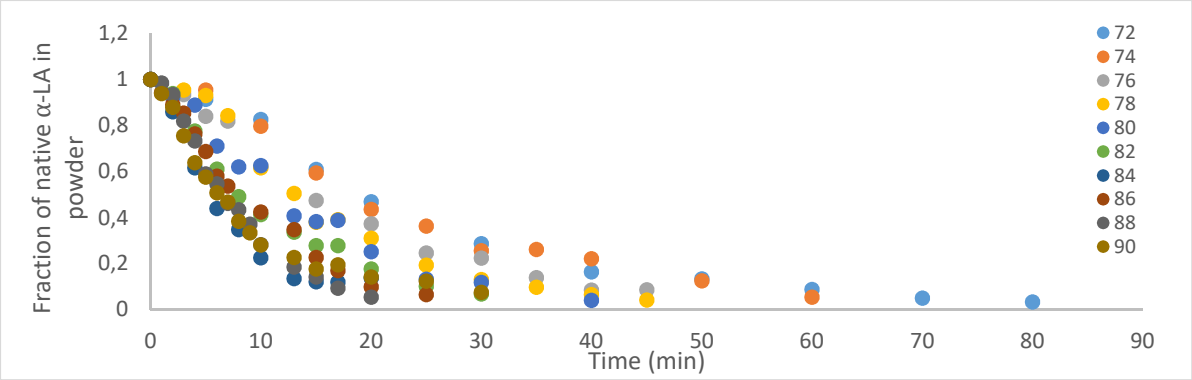

Figure 6 : α-LA denaturation of 852FB powder batch 1 according to time at each temperature.

IV.2.3. Comparison of whey proteins denaturation

Figure 7 represent Arrhenius plot of α-LA and β-LG calculated using the kinetics parameters determined with the nlsLM method. On the Arrhenius plot of β-LG, we can observe a modification of

the slope of ln(k) versus 1/T between 82 and 84°C, which represent the critical temperature where the mechanism of denaturation of β-LG changes. This critical temperature was often observed around 80 °C (Petit et al., 2011) and it slightly shifts according to the experimental conditions. This critical temperature delimits a below temperature range where β-LG unfolding was the limiting reaction of β-LG denaturation whereas above this critical temperature, the aggregation reaction becomes the limiting reaction (Petit et al., 2011). Below 82°C, α-LA denatures faster than β-LG and above 82°C, this is the opposite (Fig.7).

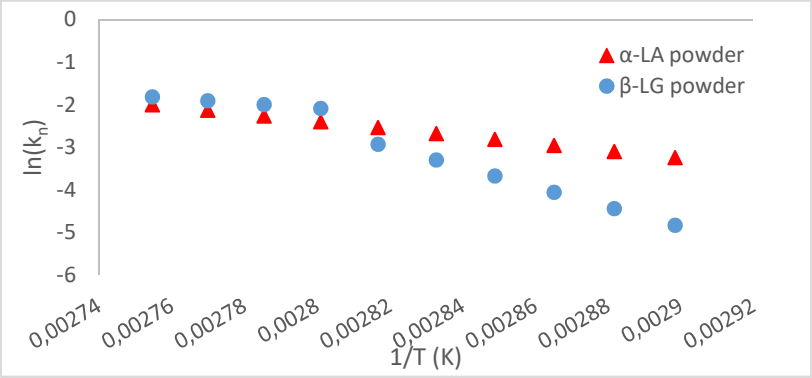

Figure 7 : Arrhenius relationship of α-LA and β-LG powder batch 1 with nlsLM method.

IV.3. Comparison of the two methods for kinetics parameter estimation

Estimation of kinetics parameters was performed using two different fitting methods, the two-step method and the nlsLM method. These two methods were used independently in other studies for estimating the kinetics parameters of β-LG denaturation. In contrary to nlsLM method, the two-step method used a linearized regression to estimate the rate constant and the activation energy of the protein denaturation kinetics. This requires that the reaction order of the reaction is known or arbitrarily fixed and consequently can affect the value of the other fitting parameters of the model. For this reason, it is interesting to compare the two methods.

In this section, the results obtained for one of the three batches of liquid and powder solutions are presented. Table 1 displays the kinetics parameters of β-LG denaturation in powder and liquid solutions using the nlsLM method. Table 2 displays the kinetics parameters of β-LG denaturation in powder and liquid solution using the "two-step" method. In the "two-step" method, the reaction order was fixed at n=1.5 for all temperatures. In our study, the critical temperature indicating a change of the mechanism of denaturation of β-LG was estimated at 82°C. Therefore, there are kinetics parameters for the denaturation of β-LG below 82°C and above 82°C.

Table 1 : Estimation of β-LG denaturation kinetics parameters with the nlsLM method (Batch 1).

	Ea ($kJ.mol^{-1}$)	Error	n	Error	k_{n1} ($g^{(1-n)}.L^{(n-1)}.min^{-1}$)	Error

ß-LG powder before 82°C	193	9	1,52	0,1	0,008	0,0014
ß-LG liquid before 82°C	202	9	1,51	0,09	0,0096	0,0016
ß-LG powder after 82°C	49	12	1,1	0,09	0,07	0,01
ß-LG liquid after 82°C	32	8	1,08	0,06	0,1	0,019

Table 2 : Estimation of ß-LG denaturation kinetic parameters with the "two-step" method (batch 1)

	Ea ($kJ.mol^{-1}$)	n	$\ln(k_{n0})$ ($g^{(1-n)}.L^{(n-1)}.min^{-1}$)
ß-LG powder before 82°C	237	1,5	77,628
ß-LG liquid before 82°C	269	1,5	88,748
ß-LG powder after 82°C	60,6	1,5	17,973
ß-LG liquid after 82°C	61,7	1,5	18,616

Activation energy values of β-LG denaturation determined with the "two-step" method were closer to the values reported in the literature than the values determined with the nlsLM method. Indeed, in the literature, activation energy was close to 300 $kJ.mol^{-1}$ below 82°C and 100 $kJ.mol^{-1}$ above 82°C (Tolkach and Kulozik, 2007). These values are higher than the values determined in this study using both methods. However, it is important to know that the value of activation energy change due to the medium conditions (pH, ionic strength, nature of salts …)(Singh and Havea, 2003).

The activation energies determined for β-LG denaturation with the nlsLM method are lower than with the "two-step" method. Interestingly, this is observed in the temperature range below than 82°C whereas the reaction order used for the fit was similar and equal to 1.5. This suggests that the mathematical operations used to rearrange non-linear data to obtained linear relationships may modify the weight of the data according to the heating time. Indeed, with the two step method, more weight on the regression are given to the data collected at longer heating time that are farther from the y-intercept.

Concerning kinetics parameters above 82°C, the reaction order determined with the two methods were significantly different. In the "two-step" method, the reaction order was assumed at 1.5 whereas it was determined near 1.1 in the nlsLM method. With this in mind, it is not possible to compare the activation energy for β-LG denaturation with the two methods because the kinetics parameters (activation energy and order of reaction) are dependent on each other for the fitting. For comparing the activation energy of β-LG denaturation above 82°C a reaction order of 1.1 should have been assumed when using the "two-step" method.

The goodness of fit of the heat-denaturation of β-LG versus heating time with the two fitting methods, the two-step method and the nlsLM method, was checked at all temperatures. Figure 8 shows the experimental points at 74°C and the fits using the two fitting methods. The red curve

represents the fit with the nlsLM function and the blue curve represents the fit with the “two-step” method. In spite of the difference of activation energy determined with the two methods, there are only small differences in the fit of the experimental data.

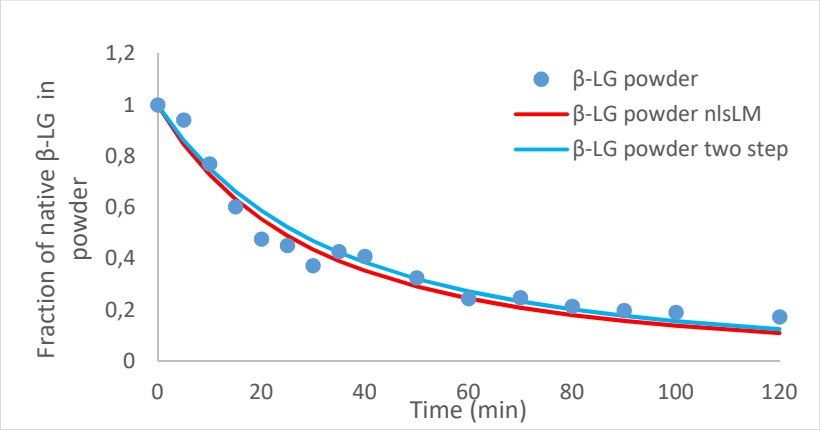

Figure 8 : Comparison of the goodness of fit of the β-LG denaturation in powder solution at 74°C using the two fitting methods (two step method and nlsLM method).

The Arrhenius plot of β-LG denaturation in powder solution using the kinetics parameters determined by the nlsLM method and the “two-step” method is displayed on Fig.9. One more time results are close to each others especially below 82°C.

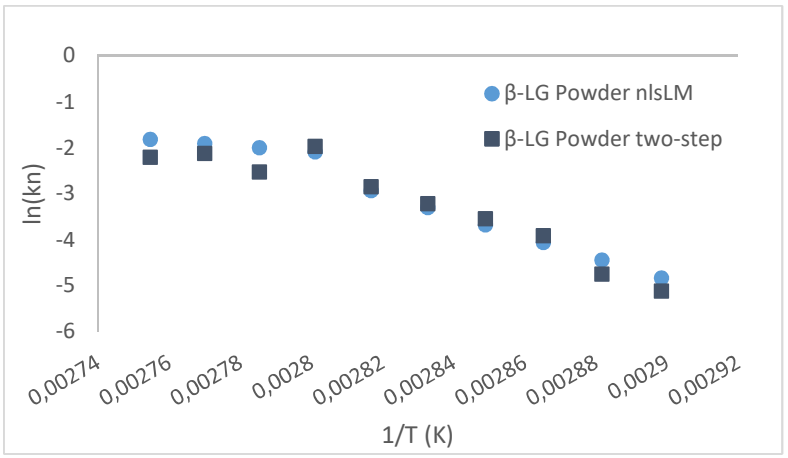

Figure 9 : Arrhenius plot of β-LG in powder solution (batch 1) calculated using the nlsLM and two-step method.

The nlsLM method which used experimental data all together to estimate the fitting parameters without consideration of a predefined reaction order was more convenient and accurate than the “two-step” method. We selected this method to compare the results of whey protein denaturation between powder and liquid solutions.

IV.4. Comparison of the kinetic parameter of powder and liquid

To compare whey protein denaturation in powder and liquid solutions, proteins at different heating time were quantified by RP-HPLC and kinetics parameters were determined with nlsLM method.

Figure 10 compares the β-LG denaturation in the powder and liquid solutions (batch 1) at 72°C according to heating time. Full orange circles represent native β-LG in powder solution and full blue circles represent native β-LG in liquid solution. Results show that β-LG denatures in the same way in the liquid and the powder solutions.

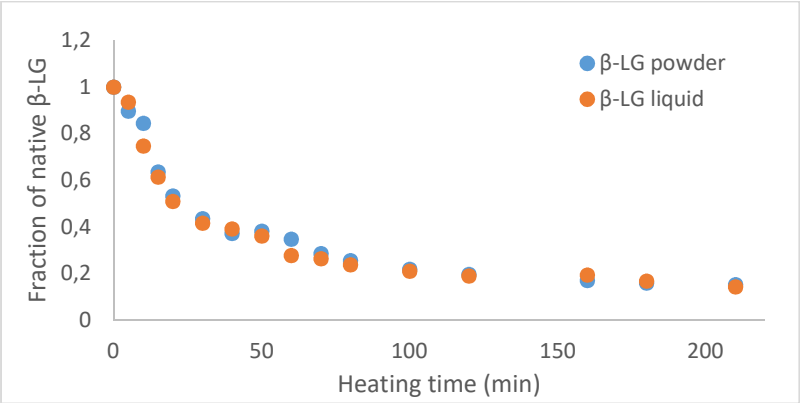

Figure 10 : Kinetics of β-LG denaturation in liquid and powder solutions (batch 1) at 72°C.

Table 3 summarizes the β-LG denaturation kinetics parameters determined for the 3 batches of liquid and powder solutions with the nlsLM method. The averaged reaction order of β-LG denaturation kinetics in powder solutions below 82°C was equal to 1.53±0.03 and it was equal to 1.60±0.06 in liquid solutions. It was equal to 1.14±0.03 and 1.20±0.08 at temperature above 82°C in the powder and liquid solutions respectively.

Table 3 : Estimation of the β-LG denaturation kinetic parameters using the nlsLM method for all batches of liquid (3) and powder (3) solutions.

		Ea ($kJ.mol^{-1}$)	Error	Moyenne	Standard error	n	Error	Moyenne	Standard error	k_{n1} ($g^{(1-n)}.L^{(n-1)}.min^{-1}$)	Error
β-LG powder before 82°C	Lot 1	194	9	218	16	1,52	0,1	1,53	0,03	0,008	0,0014
	Lot 2	232	9			1,49	0,09			0,0074	0,001
	Lot 3	228	9			1,58	0,1			0,0062	0,001
β-LG liquide before 82°C	Lot 1	202	9	231	19	1,51	0,09	1,60	0,06	0,0096	0,0016
	Lot 2	251	8			1,63	0,07			0,007	0,0009
	Lot 3	240	13			1,67	0,1			0,0075	0,0015
β-LG powder after 82°C	Lot 1	49	12	58	20	1,1	0,09	1,14	0,03	0,07	0,01
	Lot 2	37	15			1,17	0,14			0,087	0,025
	Lot 3	89	13			1,15	0,1			0,04	0,011
β-LG liquide after 82°C	Lot 1	32	8	24	7	1,08	0,06	1,20	0,08	0,1	0,019
	Lot 2	27	15			1,33	0,1			0,12	0,031
	Lot 3	14	14			1,2	0,08			0,18	0,039

On average, the reaction order is slightly higher in the liquid solutions than in the powder solutions but they are too close (regarding the standard deviation) to be statistically different. A higher number of batches is required to validate or not the statistical difference of the reaction order of β-LG denaturation between liquid and powder solutions.

The averaged activation energy of β-LG denaturation in powder solutions was equal to 218±16 ($kJ.mol^{-1}$) and it was equal in 231±19 ($kJ.mol^{-1}$) for the liquid solutions below 82°C. The averaged activation energy of β-LG denaturation was not statistically different between liquid and powder solutions. A higher averaged activation reaction compensates a higher reaction order to explain that no difference of β-LG denaturation can be observed experimentally (Fig 10). However, the averaged activation energy of β-LG denaturation above 82°C is statistically different between the liquid and powder solutions. The averages Ea of β-LG denaturation in the powder solution was equal at 58±20 ($kJ.mol^{-1}$) and it was equal at 24±7 ($kJ.mol^{-1}$) in liquid solutions.

The Arrhenius relationships for β-LG denaturation in powder and liquid solutions were plotted with the kinetics parameters estimated by the nlsLM method (Fig.11). The two plots are not statistically different.

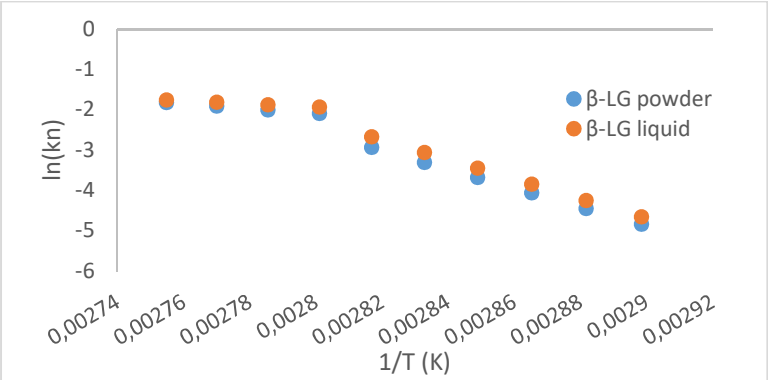

Figure 11 : Comparison of Arrhenius plot for β-LG denaturation in liquid and powder solutions (Batch 1).

Thanks to the results, we can assume that the difference of fouling of the liquid and powder solutions during heating on a heat plate exchanger can not be predicted by the denaturation kinetics of the whey proteins in the same liquid and powder solutions heated at lab scale.

V. Conclusion

The kinetics parameters of the whey protein denaturation were determined using two methods, the "two step" method and the nlsLM method. Results suggest that the nlsLM method which estimates simultaneously all kinetics parameters, is more accurate than the "two-step" method. This difference in accuracy comes from the fact that the reaction order of the denaturation kinetics of the whey proteins has to be fixed before running the "two-step" method. However, the mechanism of denaturation of the whey proteins is complex and slight change of the reaction order impacting the global fit of the experimental data is expected when the medium conditions change. For this reason, we investigate the denaturation of the whey proteins in liquid and powder solutions using the nlsLM method.

The RP-HPLC profiles of the powder and liquid samples of the whey protein ingredient 852FB exhibit some differences. They can be explained by some protein modifications during the under vacuum evaporation, spray drying operations leading to more hydrophilic protein forms in the powder solution. These modifications are not responsible of changes of the heat denaturation kinetics of the whey proteins at lab scale, especially below 82°C. This result suggests that the heat denaturation kinetics of the whey proteins is not affected by under-vacuum evaporation/spray drying/rehydration operations. In addition, the different aptitude of the liquid and powder solutions to form deposits on the surface of the plates of the heat exchangers is probably not link to the rate at which heat-denatured whey proteins are formed on heating. To progress on the understanding of the fouling mechanism, it would be necessary to focus on other hypotheses such as an insufficient rehydration of the whey protein powder, the loss of minor compounds such as calcium that are known to contribute to the build up of the fouling layer, during under vacuum evaporation and spray drying operations, etc.

Although it was not possible to distinguish liquid and powder solutions of 852FB based on the kinetics of denaturation of the whey proteins nothing could be said on other whey proteins ingredients. The whey protein ingredient 852FB is defined as an ideal whey protein ingredient because it comes from the microfiltration of a skimmed milk. Whey protein ingredients coming from cheese whey contains other protein fragments such as the caseinomacropeptide (CMP) and a higher fraction of proteose peptone that are identified on RP-HPLC at retention time around 13 min (Fig.12). These protein fragments are known to modify the heat denaturation of β-LG but no studies comparing the effect of under vacuum evaporation/spray drying and rehydratation has been done yet. It could be interesting to complete the present study by using a whey protein ingredient coming from cheese whey to know if CMP and other protein fragments recovered in cheese whey can affect the whey protein denaturation kinetics differently if the ingredient in concentrated under vacuum and spray dried, and to compare obtained results with the results on liquid and powder solutions of 852FB.

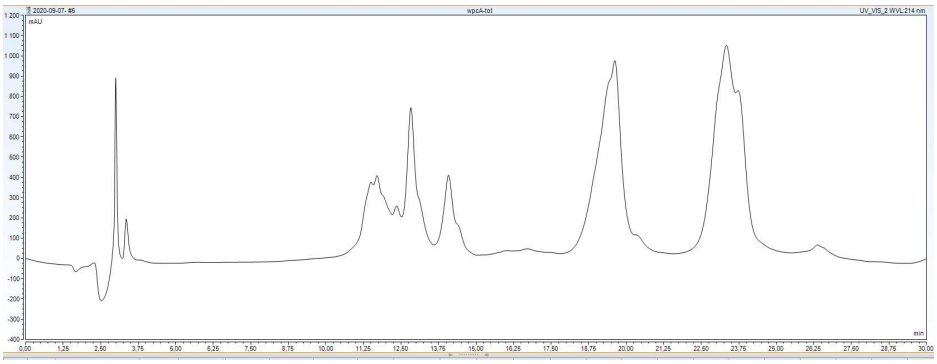

Figure 12 : RP-HPLC profile of a WPC before heating. Proteins are detected at 280nm.

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Abstract

In the dairy industry, fouling of heat-plate exchangers is a recurring phenomenon that seriously challenges production costs. Therefore, it is interesting to understand the fouling phenomenon in order to limit it. Several hypotheses have been put forward to explain protein fouling. Industrial observations indicate that fouling is impacted by under vacuum evaporation/spray drying/rehydration operations. This study is based on the hypothesis that a modification of the protein structure generated by these operations could further impact the denaturation kinetics of the whey proteins and the fouling of heat-plate exchangers. To verify this hypothesis, a permeate of microfiltration (852FB) was used in liquid form before under vacuum evaporation/spray drying/rehydration operations and in powder form after these operations. The aim of the study was to compare the kinetic parameters of protein denaturation of the liquid and reconstituted powder solutions. Heat treatments were carried out on protein solutions at several temperatures and different heating times and residual native proteins were quantified by RP-HPLC. The rate constant, k, the reaction order, n and the activation energy, Ea, of the denaturation of whey proteins were estimated using two fitting methods: the “two-step” method, which estimates the parameters in two steps and the NLSLM method, which estimates all kinetic parameters simultaneously. The results obtained showed that the NLSLM method is more accurate than the two-step method for fitting experimental results. On the other hand, the kinetic parameters of the powder solution and those of the liquid solution were not statistically different. Consequently, the fouling of heat plate-exchangers cannot be explain by the protein denaturation induced by under vacuum evaporation/spray drying/rehydration operations. Further studies are required to perfectly understand the parameters that influence whey protein fouling during industrial operations.

Résumé

En industrie laitière, l'encrassement des échangeurs à plaques est un phénomène récurrent qui remet sérieusement en cause les coûts de production. Il est donc intéressant de comprendre ce phénomène afin de le limiter. Plusieurs hypothèses ont été avancées pour expliquer l'encrassement des protéines. Les observations industrielles indiquent que l'encrassement est influencé par les opérations d'évaporation sous vide, de séchage par pulvérisation et de réhydratation. Cette étude est basée sur l'hypothèse qu'une modification de la structure des protéines générée par ces opérations pourrait avoir un impact supplémentaire sur la cinétique de dénaturation des protéines du lactosérum et sur l'encrassement des échangeurs à plaques. Pour vérifier cette hypothèse, un perméat de microfiltration (852FB) a été utilisé sous forme liquide avant les opérations d'évaporation sous vide, de séchage par atomisation et de réhydratation, et sous forme de poudre après ces opérations. Le but de l'étude était de comparer les paramètres cinétiques de dénaturation des protéines des solutions liquides et en poudre reconstituées. Des traitements thermiques ont été effectués sur les solutions de protéines à plusieurs températures et différents temps de chauffe et les protéines natives résiduelles ont été quantifiées par RP-HPLC. La constante de vitesse, k, l'ordre de réaction, n et l'énergie d'activation, Ea, de la dénaturation des protéines du lactosérum ont été estimés à l'aide de deux méthodes d'ajustement : la méthode « two-step » qui estime les paramètres en deux étapes et la méthode NLSLM, qui estime tous les paramètres cinétiques simultanément. Les résultats obtenus ont montré que la méthode NLSLM est plus précise que la méthode « two-step » pour l'ajustement des résultats expérimentaux. En revanche, les paramètres cinétiques de la solution en poudre et ceux de la solution liquide n'étaient pas statistiquement différents. Par conséquent, l'encrassement des échangeurs à plaques ne peut pas être expliqué par la dénaturation des protéines induite par les opérations d'évaporation sous vide, de séchage par pulvérisation et de réhydratation. Des études supplémentaires sont nécessaires pour comprendre parfaitement les paramètres qui influencent l'encrassement des protéines du lactosérum lors des opérations industrielles.