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Traitement thermomécanique à l'échelle laboratoire de solution de protéines sériques en conditions proches des équipements industriels – Influence du cisaillement

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Abstract

The aim of this study was to describe a thermal aggregation process at laboratory scale by using an experimental process simulator. This device is able to impose fast variations of temperature and shear in the same range as in industrial equipments. Thermally-induced aggregation was studied on β-lactoglobulin (β-lg) solutions. We observed the influence of a moderate shear and of treatment duration, on the final product characteristics: aggregate size and concentration and residual native fraction. The first results provide information about the shear influence on β-lactoglobulin denaturation/aggregation during thermo-mechanical process: shear has no effect on the aggregated fraction but has a significant influence on the size distribution. The higher is the shear rate during heating, the higher is the concentration of large particles (20-1000 µm). These results are discussed in regards to the theoretical aspects of particles motion in suspension and the role of both perikinetic (Brownian motion) and orthokinetic (shear) collisions on β-lactoglobulin denaturation and aggregation.

Key-words: process simulator, laboratory-scale thermomechanical process, β-lactoglobulin aggregation, shear.

Résumé

L'objectif de l'étude est de décrire un processus d'agrégation à l'échelle laboratoire à l'aide d'un simulateur de procédés. L'appareil permet d'imposer des variations de température rapides et un cisaillement dans des gammes comparables à ce qui est réalisé dans les équipements industriels. Une solution de β-lactoglobuline (β-lg) est utilisée comme modèle pour étudier l'agrégation thermo-induite. Nous avons exploré l'effet d'un cisaillement modéré et de la durée de traitement thermique sur les caractéristiques de la suspension après traitement thermomécanique : concentration et taille d'agrégats et taux de dénaturation. Les premiers résultats révèlent que le cisaillement n'a pas d'effet sur le taux de dénaturation mais a un effet significatif sur la distribution granulométrique. Plus le cisaillement imposé est élevé, plus la concentration en grosses particules (20-1000 µm) sera élevée. Ces résultats sont discutés en considérant les aspects théoriques du mouvement des particules en suspension et du rôle des collisions péricinétiques (mouvement Brownien) et orthocinétiques (cisaillement) sur la dénaturation et l'agrégation de la β-lactoglobuline.

Mots-clés : simulateur de procédés, traitement thermomécanique, échelle laboratoire, agrégation de la β-lactoglobuline, cisaillement.

1. Introduction

In food industry, evaluation of new equipment or modified process parameters is necessary in many cases like for cost and energy saving requirements, higher productivity needs or new product development. However, tests at industrial scale are complex to achieve (large amount of product, difficult instrumentation…) and expensive in terms of time and money. Therefore, it is useful to develop laboratory-scale methods to characterize the influence of process parameters close to the process condition (high heating rate, shear rate, residence time) on the characteristics of the final product. Most of the works in literature at laboratory-scale are performed at constant temperature or for slow heating kinetics and neglect shear effect whereas in industrial conditions heating is higher than 1°C.s^{-1} and occurs under relatively high shear flow.

In this study, an original laboratory-scale process simulator previously developed (Alvarez, 2006; Lagarrigue & al., 2007) was used to study a food processing in industrial process-like conditions. Experimental study of aggregation was carried out on β-lactoglobulin solutions which are very sensitive to heat treatment and shear: unfolding, aggregation and aggregate growth (Cayot & Lorient,1998). The aggregation is the results of collisions between reactive particles. Smoluchowski (Elimelech & al., 1998) defines two types of collisions related to the mode of transport of particles in suspension: perikinetic (Brownian motion) and orthokinetic (shear). By studying the effect of shear rates on the denaturation rate and the size distribution, it would be possible to discuss the role of each mode of collision on the different steps of aggregate formation.

2. Materials and Methods

2.1 Solution preparation

Preparation of 6% β-lactoglobulin solutions (industrial powder) was made in warm deionized water (40°C) under mixing. $CaCl_2$ 1M was added to the protein solutions in order to obtain a concentration of 6.6 mM. After this mixing, we placed the solution in a water bath at 40°C for 2 hours to assure a complete rehydration of the β-lactoglobulin powder. The solution was kept at 4°C and used within a day to prevent any destabilization with time or temperature.

2.2 Thermomechanical treatments

Heat treatments were performed with a process simulator which consists in a reactor specially designed (Alvarez, 2006) and installed on a rheometer Physica UDS 200® equipped with a Couette geometry. The cup of the reactor inner cylinder is made of stainless steel (ρ_m = 7900 kg.m^{-3}, cp_m = 510 J.kg^{-1}.K^{-1}, λ_m = 15 W.m^{-1}.K^{-1}) of 1.25 mm thickness. Maximum heating rate applied with electrical heaters could be 5 °C s^{-1} during heating treatment and 2.5 °C s^{-1} during cooling. Temperature was measured with a calibrated Copper-Constantan thermocouple inserted at the outer cylinder wall. To control and to impose any temperature profile at the outer cylinder wall, we developed a predictive control algorithm, based on least square method.

In order to simulate a thermal process a simple thermal model was established in a Couette geometry (concentric cylinders) (Lagarrigue & al., 2007). This model takes into account the temperature gradient in the gap which is a function of heating rate and the time response of the system. The temperature in the middle of the gap (Tm) was used as the representative temperature of the sample and depends on the bob temperature T_{bob} and heating rate.

Five different steps composed experimental heat treatments. Process started with a slow preheating step from about 4°C to 60°C at 0.5°C.s^{-1} followed by 60 s of stabilization. This preheating step had no effect on protein denaturation and aggregation but helped in reducing temperature heterogeneity. Heating treatments were imposed at 5°C s^{-1} up to T_{heat} = 85°C and this temperature was then maintained for 60 or 240 seconds. This time is defined as holding time (t_{hold}). Controlled cooling was performed to the sample down to 25°C at a cooling rate of 2.0°C.s^{-1}. For this study, heat treatments were carried out with different shear rates : 0, 100 and 400 s^{-1}.

2.3 Residual native fraction determination

In order to promote agglomeration of the aggregated fraction, the sample is diluted (1/20th) in an acetate buffer at pH 4.6. The agglomerated materials is removed thanks to a syringe filter (Ø 0.2 µm). The residual native fraction of β-lg (variant A and B) is then determined by HPLC.

2.4 Granulometric characterization

2.4.1 FBRM

Post-cooling characterization of the suspension was made with Focused Beam Reflectance Measurement (Mettler Toledo). It was used to determine aggregate chord size distribution and concentration determination for particles above 1 µm. The FBRM measurement was made after a four times dilution of the sample in the unheated solution.

2.4.2 Laser granulometer

Laser granulometer Mastersizer (Malvern Instruments) was used to determine the volume size distribution of the suspension of aggregates for particles from 0.1 to 1000 µm. The measurement was made after the dilution of the sample in water. The level of dilution needed is determined by the granulometer depending on the turbidity of the suspension.

3. Results and discussion

3.1 Temperature profile

Figure 1 shows the different temperatures imposed (T_{wall}) and calculated (T_m and T_{bob}) during a heat treatment at 85°C for 240 seconds. It can be seen that the heating and cooling phases are very quick compare to the holding time.

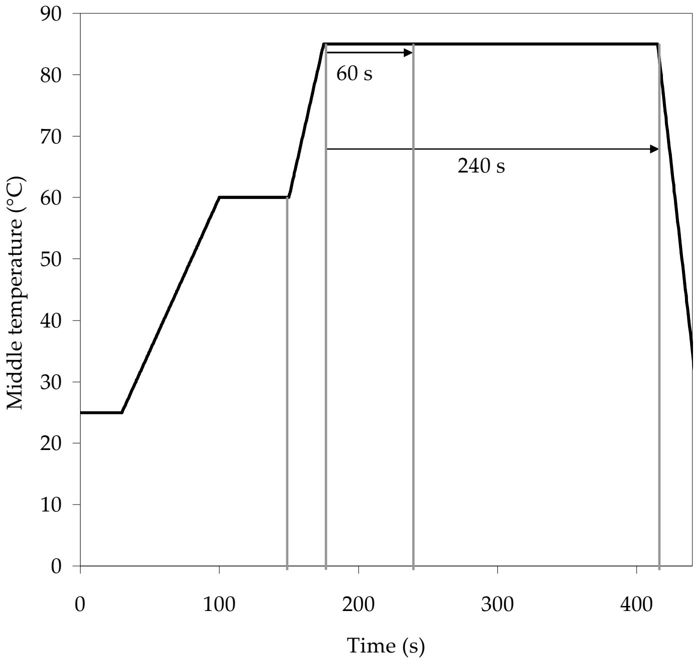


Figure 1.Temperature profile obtained with the process simulator for a heat treatment at 85°C for 240 s.

3.2 Residual native fraction determination

The Table 1 presents the results of residual native fraction determination by HPLC for the different experiments. It can be seen that the irreversible aggregation at 85°C is fast: more than 60% is aggregated after 60 seconds at 85°C and around 90% after 240 seconds.

Table 1. Residual native fraction determined by HPLC after heat treatment

Temperature (°C)	Shear rate (s^{-1})	Treatment duration (s)	Residual native fraction
85°C	0 s^{-1}	60 s	0.38
		240 s	0.07
	100 s^{-1}	60 s	0.36
		240 s	0.15

	$400\ s^{-1}$	60 s	0.33
		240 s	0.06

Analysis of variance underlines that for a heat treatment at 85°C, the imposed shear rates (from 0 to 400 s-1) has not a significant effect (P_{value} = 0.41) on the residual native fraction. The treatment duration has logically a significant effect on the residual native fraction (P_{value} = 0.011). The mechanism of β-lg thermally-induced denaturation is well described (Roefs & Kruif, 1994). It needs collisions between reactive (reversibly unfolded) monomer to give irreversible aggregation. The shear (orthokinetic collision) does not significantly affect the probability of collision between reactive monomer. This result is in agreement with to theoretical basis developed by Elimelech & al. (1998). It can be seen that for small particles like β-lg monomer (radius of around 2 nm (Aymard & al., 1996)) and moderate shear, the collisions are mainly due to Brownian motion (perikinetic collision).

3.3 Size measurements

3.3.1 Volume size distribution - Laser granulometer

The figure 2 shows the volume size distribution for the different experiments. Thermomechanical treatments of β-lg solution lead to a large diversity of aggregates size in the suspension.

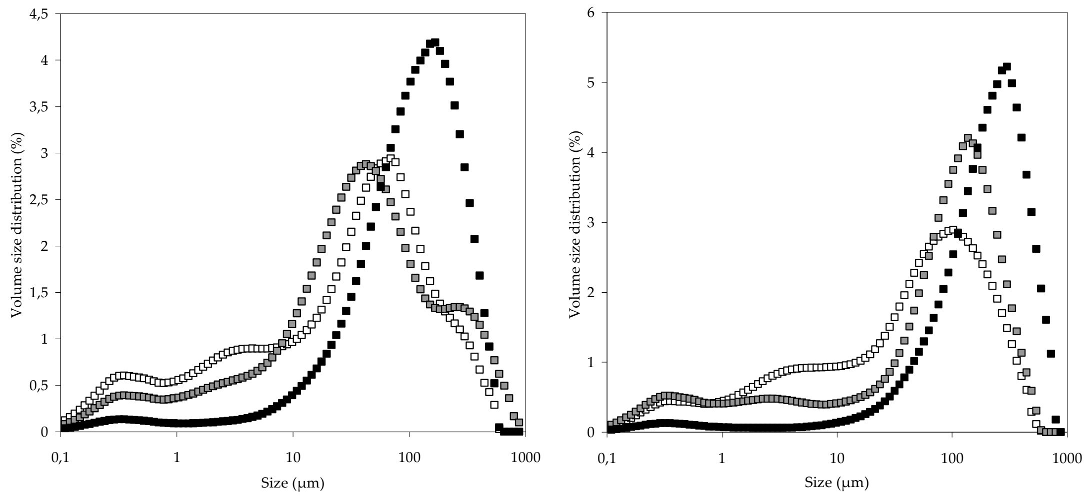
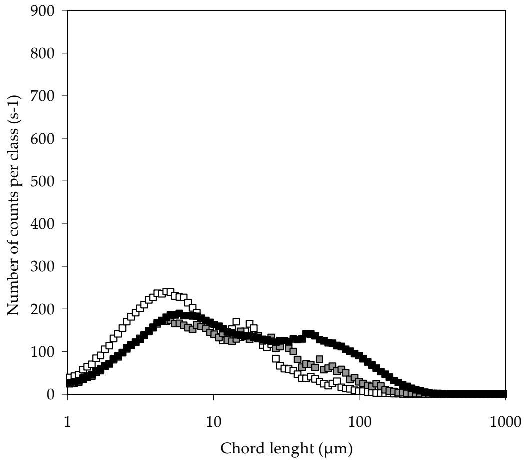


Figure 2. Volume size distribution of the β-lactoglobulin aggregates after heat treatment at 85°C for 60 (left) and 240 s (right) with different shear rates: 0 s^{-1} (white), 100 s^{-1} (grey) and 400 s^{-1} (black)

On the figure 2, it can be seen that increasing shear rates from 0 to 400 s^{-1} seems to increase the part of volume represented by the large particles (20-1000 µm) and to reduce the part of the small (0.1-5 µm) and medium particles (2-20 µm).

3.3.2 Number size distribution – Focus Beam Reflectance Measurement

The figure 3 shows the number size distribution obtained by FBRM for the six experiments. This method gives information on the chord size but also provides information about the relative concentration of aggregates. It should be taken into account that FBRM measured distribution in number compared to the volume distribution of the laser granulometer. That point explains the difference observed: by FBRM the particles in the range of 20-1000 µm are not the more numerous but represents the main part of the volume as it is observed in volume size distribution with Mastersizer.



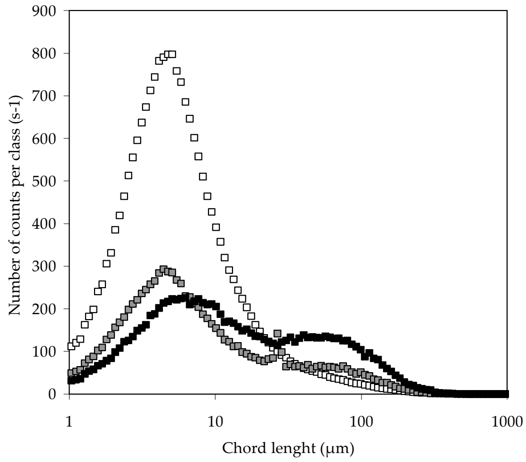


Figure 3. Number size distribution of the β-lactoglobulin aggregates after heat treatment at 85°C for 60 s (left) and 240 s (right) with different shear rates: 0 s-1 (white), 100 s-1 (grey) and 400 s-1 (black)

Characterization by FBRM confirms the tendencies observed with the laser granulometer. Imposing shear (in the range 0 to 400 s^{-1}) increase the formation of large particles. Information about the concentration of aggregates is useful to describe the mechanism of aggregate growth. When no shear is imposed (0 s^{-1}), increasing the treatment duration from 60 to 240 seconds, strongly increases the number of particles in the range 1-10 µm. When shear is applied, the number of 1 to 10 µm particles only increases slightly, maybe because these particles are used to formed larger particles.

The table 2 gives the relative concentration of aggregates (number of aggregates seen per second) classified by ranges of aggregates: small (1-5 µm), medium (5-20 µm) and large (20-1000 µm). Analysis of variance shows that imposing shear in the range (0 to 400 s-1), significantly (P_{value} = 0.019) increase the concentration of large particles (20-1000 µm). It can be seen that increasing treatment duration only leads to a small increase of the large aggregate concentration.

Table 2. Relative concentration of particles determined by FBRM after heat treatment for variable process conditions in different size ranges

Temperature (°C)	Shear rate (s^{-1})	Treatment duration (s)	Concentration of particles for different size ranges (s^{-1})			
			1-5 µm	5-20 µm	20-1000 µm	Total
85°C	0 s^{-1}	60 s	3252	3467	1214	*7934*
		240 s	10108	8824	1722	*20655*
	100 s^{-1}	60 s	2129	2939	2098	*7167*
		240 s	3882	3354	1978	*14325*
	400 s^{-1}	60 s	2173	3265	3500	*8939*
		240 s	2618	3815	3770	*10204*

Looking to the total of particles formed, it can be seen that the higher the shear rate, the less total particles are created after 240 seconds of heat treatments. The explanation could be that large particles are a combination of some smaller particles. Then, creating more large particles, logically leads to reduce the total of particles in the suspension.

Regarding to the mode of transport of particles describe before, it seems that for aggregate growth and for large aggregate formation both perikinetic and orthokinetic collisions are involved. Indeed, in the absence of orthokinetic collisions (i. e. without shear) large particles are formed but imposing shear increases the concentration of these large aggregates.

4. Conclusion

Experiments carried out on β-lg solution with different shear rates make it possible to determine the role of perikinetic (Brownian motion) and orthokinetic (shear) on the whole aggregation process. It appears that for the primary step of aggregation (leading to the denaturated state) only perikinetic collisions are involved and that for the formation of large particles both perikinetic and orthokinetic collisions occur. Controlling shear in industrial equipment could be a key for a better control of the aggregate functionalities. The device used is an effective tool for process simulation. Indeed, the simulator can be used to analyze the influence of different process parameters (holding time, heating rate, cooling rate and shear rate) and of the initial product composition (concentration, pH, ionic strength) on the final product characteristics (rheology, aggregate sizes). It is then possible to select orientations about industrial process conditions (pre-treatment, time, temperature, flow and geometry).

Referees

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