Influence of whey protein aggregation on the residence time distribution in a helically holding tube during heat treatment process

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ABSTRACT

The residence time distribution (RTD) of a flowing whey protein (WP) suspension through a helically tubular system was studied. The experimental system is the helical holding section of a thermal process pilot. RTD was measured in isothermal conditions at 60°C, a temperature involving any WP aggregation, and at 87°C, temperature at which WP aggregates. Two flow rates (20 L/h and 49 L/h) were tested with two different lengths of holding tube in order to maintain the same order of magnitude of the holding time. Methylene blue was used as tracer and spectrophotometer analysis were performed to determine the outlet concentration. These investigations yielded to great differences between the RTD in aggregation and no aggregation conditions. The minimum residence time is shorter when WP aggregation occurs due to the modification of the velocity field inside the holding tube with the enhancement of the viscosity. The mean residence time remains the same whatever the holding temperature at the low flow rate. However, it is slightly shorter when the flow rate increases probably due to the increasing of secondary flow circulation which narrows the RTD in curved tubes. Fitting the experimental results with the generalized laminar model confirms the shorter minimum residence time when WP aggregate. These results help to understand the WP aggregates size dispersion.

Keywords: Residence time distribution; Whey protein; Aggregation; Helical holding tube

INTRODUCTION

Elements of fluid flowing through a processing unit usually take different path and length of time. Knowing the distribution of these times is one of the essential concepts for the design and optimization of processes. This concept, called residence time distribution (RTD) study, is widely used in heat treatment processes to adequately evaluate the thermal processing and/or to understand the fluid flow behaviour. RTD in heat treatment processes, particularly in holding tube, is nowadays well known, but it concerns only processes where the product is not under transformation [1]. There is less knowledge of flow fields and RTD inside equipment in thermal processes where the product is under structural transformations during heating or cooling processes. In such processes, the rheological properties change significantly and consequently modify the flow behaviour and the velocity profile inside the equipment. This is the case for whey protein (WP) heat treatment processes where WP molecules, particularly the β-lactoglobulin (β-lg), unfold and aggregate depending on the time-temperature-shear rate history. The aggregation phenomena induce an increase of the protein volume fraction and of the fluid apparent viscosity, which can modify the fluid age dispersion.

The purpose of this paper is the experimental determination of the RTD in holding tubes of a heat induced whey protein aggregation process. We aimed to compare the fluid age dispersion inside the pipe under aggregation conditions with the one obtained without aggregation.

MATERIALS & METHODS

The continuous thermal processing system

The experimental setup is an ultra high temperature (UHT) pilot (Armfield FT174X, Ringwood, UK). This system includes a feed tank, a pre-heater unit made of helical tubes in a water bath, a heating section with a heat exchanger made of a succession of eight concentric double tubes, a helical holding tube with variable length and a cooling section similar to the heating one. The pilot plant has a nominal capacity of 20 – 50 L/h where the product is fed by a volumetric pump. Residence time distributions were investigated in the holding
tube which has an internal diameter of 7.08 mm. Three volumes (259 ml, 342 ml and 756 ml) of the holding tube can be used according to the chosen configuration (three different lengths).

The working fluid
A WP solution was used to carry out the RTD experiments. A 6% β-lg solution was prepared using deionised water at 40°C and a WP powder containing 88.9% of β-lg. A 1M solution of CaCl₂ was added to the β-lg solution to make WP solution with 5 mM CaCl₂. This solution was maintained at 40°C for 2 h for complete rehydration of the β-lg powder. Measurement of the pH of the solution gives a value of 6.8.

The RTD determination
RTD experiments were conducted using a colorimetric method. Preliminary study of solubility in β-lg solutions and of thermal stability was performed with several colouring agents in order to select a tracer. Methylene blue (MB) (Rhône Poulenc, 3.10957, Villers St Paul, France) is the more soluble among the tested tracer. The measurements of colour parameters with a colorimeter (Minolta CR200) showed that heating temperatures and times have no effect on the stability of MB. Thus MB has been chosen as tracer for the experimental RTD study.

A tracer injection system constituted by an independent piece of stainless tube (internal diameter of 8.1 mm and length of 150 mm) fitted with a syringe has been designed and constructed. A static mixer was inserted immediately after the injection point, in order to improve the tracer distribution. The tube was added in the entrance of the holding tube corresponding to the exit of the heating section. Preliminary experiments showed that a pulse injection in a short time with a good input signal could be achieved with 10 ml samples. Isothermal conditions allowing any WP aggregation (T = 60°C) and allowing aggregation (T = 87°C) were explored at a holding time of about 60 sec and at two different flow rates (20 L/h and 49 L/h). We worked with a volume of 342 ml for the low flow rate (20 L/h) and of 756 ml for the higher one (49 L/h) in order to maintain a holding time in a same order of magnitude (~ 60 sec) for the two flow rates. Continuous cup sampling was made at the outlet of the holding tube and samples analysed with a spectrophotometer (Beckman Coulter DU 730, Fullerton, USA). Tracer concentration has been selected after preliminary absorbance measurements with β-lg solutions and β-lg aggregates solutions so that to give an absorbance in the range where the Lambert-Beer’s law is valid. So we used a MB solution of 0.01 g/L for no aggregation conditions (T = 60°C) and 0.02 g/L for aggregation conditions (T = 87°C). Samples collected were diluted (dilution rate of 1/20) before the spectrophotometry analyses in order to guarantee the detection of the MB. Indeed, because of their high absorbance at the studied wavelength (662 nm), the protein aggregates tend to mask the presence of MB.

Experimental runs were conducted in triplicate for each condition of temperature and flow rate to obtain the RTD of the holding tube.

Data treatment
The RTD of a process is quantitatively characterized by the age distribution function E(t) also called E-curve. E(t) represents the fraction of fluid at the outlet stream that has been in the system for times between t and t + dt. For a pulse injection, where a small amount of a tracer is instantaneously injected at the unit inlet and its outlet concentration C(t) is continuously recorded, the mathematical expression for the E-curve is given by Eq.(1) [2].

\[
E(t) = \frac{C(t)}{\int_0^\infty C(t) \, dt}
\]

The normalization of the RTD allows to represent E(t) in such a way that the area under the curve is unity as shown in Eq. (2).

\[
\int_0^\infty E(t) \, dt = 1
\]

The mean residence time is the first moment of the distribution and is calculated from Eq. (3).

\[
\bar{t} = \int_0^\infty t \, E(t) \, dt
\]

For ideal flow, \( \bar{t} \) is equal to the geometrical residence time defined by Eq. (4) with Q being the flow rate of the fluid and V the system volume.
The dimensionless E-curve is obtained from Eq. (5) where $\theta = t/\tau$ is the dimensionless time.

$$E = \tau E(t)$$  \hspace{1cm} (5)

The integrals in Eq.(1) and (3) are calculated by the rectangle method.

RESULTS & DISCUSSION

Table 1 shows the different investigated operating conditions, the geometrical residence time, the experimental minimum and mean residence times. The reproducibility of experiments has been tested by realizing each one three times. The standard deviation on the ratio of the mean residence time to the geometrical residence time ($t/\tau$) varied from 0.005 to 0.009. Table 1 indicates the average values of the replicates. The Reynolds numbers (Re) have been calculated at the entrance of the helical holding tube. For the lowest flow rate (20L/h), Re is lower than 2000, indicating a laminar flow. For the highest flow rate (49L/h), Re is in the range of transition between laminar and turbulent flow for straight tubes. But it is known that for helically coiled system, the laminar turbulent transition is retarded by flow structures generated by the curvature effects and that the flow becomes fully turbulent at Reynolds number between 8000 and 10 000 [3].

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>60 (No aggregation)</th>
<th>87 (Aggregation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (L/h)</td>
<td>20</td>
<td>49</td>
</tr>
<tr>
<td>Re</td>
<td>1265</td>
<td>3100</td>
</tr>
<tr>
<td>Geometrical residence time $\tau$ (s)</td>
<td>61.6</td>
<td>55.5</td>
</tr>
<tr>
<td>Minimum residence time $t_{\min}$ (s)</td>
<td>38.6</td>
<td>44.6</td>
</tr>
<tr>
<td>Mean residence time $\bar{t}$ (s)</td>
<td>49.2</td>
<td>51.1</td>
</tr>
<tr>
<td>$t_{\min}/\tau$</td>
<td>0.63</td>
<td>0.80</td>
</tr>
<tr>
<td>$\bar{t}/\tau$</td>
<td>0.80</td>
<td>0.92</td>
</tr>
</tbody>
</table>

The age distributions with and without aggregation inside the holding tube at 20 l/h and at 49 L/h are respectively shown on figure 1(a) and on figure 1(b) for one of the replicates. It is obvious that the measured RTD show significant differences depending on whether there is aggregation or not for any flow rate. Table 1 shows that the minimum residence time is about 11% shorter when WP aggregation takes place for both flow rates for 87°C treatment. However, no difference was found between the mean residence time at 20 L/h under aggregation and under no aggregation conditions, and it is slightly shorter at 49 L/h in aggregation conditions. Such behaviours can be explained by the modification in flow inside the holding tube when aggregation occurs.

Figure 2 shows the evolution of the fluid velocity profile in pipes for fully developed laminar flow, and under aggregation conditions (T = 87°C). It represents the velocity parabolic profile at the entrance of the duct and how this profile is modified further when aggregation phenomena occur. The relatively high shear rate located near the wall implicates that aggregation is more important at the wall and both volume fraction and viscosity locally increase more rapidly at this point. The local viscosity enhancement leads to a decrease of the velocity close to the tube wall and its increase near the axis. As a result, the velocity distribution is gradually modified in comparison to the parabolic profile at the entrance of the duct. Thereby, WP aggregation reduces the minimum residence time and slows down the flow close to the pipe wall as indicated by the tailing effect on the RTD curves of figure 1.

This tailing effect is more important on figure 1(a) where the flow rate is low (laminar regime). However, increasing flow rate reduces the dispersion and the tailing effect as observed in figure 1(b). This latter result can be explained by a flow pattern modification due to the helical configuration of the holding tube. Indeed,
for helical system, when flow rate increase, the centrifugal forces exerted by the curvature of the tube results on secondary flow materialized by two contra-rotative vortices called Dean cells [4]. As a result of the secondary flow, the axial velocity profile becomes asymmetric and radial mixing is induced. The resulting RTD is narrower compared with that obtained in a straight tube. The age dispersion is also as narrow as the number of helices in the holding tube is high. Chagny et al. [3] reported that secondary flow instability occurs at Reynolds number around 1900 as it is the case for the high flow rate (49 L/h).

![Figure 1. Effect of WP aggregation on the RTD inside the holding tube: (a) 20 L/hr, (b) 49 L/hr](image)

The secondary flow circulation can also explain the delay on the minimum residence time obtained in this work. Indeed, the theoretical value of the ratio of the minimum residence time to the geometrical residence time (breakthrough time) is 0.5, for Newtonian fluids flowing in laminar regime in a straight tube without radial diffusion. But, it is shown in figure 1 and table 1 that the ratios are above 0.5 for all conditions explored. This result denotes a flatter axial velocity profile than the parabolic one and/or radial mixing.

The ratio of the mean residence time to the geometrical residence time, when lower to 1 as it is shown in table 1, is characteristic of the presence of dead volume. Calculations by using Eq. (6) indicate dead volumes $V_d$ of 68 ml (20%) and 83 ml (11%) respectively for the lowest flow rate (20 L/h) with short holding tube (342 ml) and the highest flow rate (49 L/h) with long holding tube (756 ml). The reduction of the dead zone can be explained by the increasing radial mixing due to the enhancement of the strength of the secondary flow with the Reynolds number.
Figure 2. Evolution of velocity profile in laminar regime inside a cylindrical tube during flowing of WP fluid subjected to aggregation

We tried to fit the experimental RTD curves obtained with the generalized laminar model shown in Eq. (7). The objective was to reinforce the results about the difference in the breakthrough time depending on whether there is WP aggregation or not. The sum of squared errors $e$ (Eq. (8)) between the model curve and the experimental points was minimized to adjust the breakthrough time $\theta_0$.

$$E = \frac{1}{1-\theta_0} \left( \frac{\theta}{\theta_0} \right)^{1-n} \quad \theta \geq \theta_0$$

$$e = \sum_{i=0}^{m} \left( E_{\text{exp},i} - E_{\text{low},i} \right)^2$$

In order to take into account the dead volume, a corrected geometrical residence time based on the active volume was used: $\tau' = (V-V_d)/Q$. Consequently, the dimensionless time and the dimensionless E-curve are corrected respectively as $\theta' = \tau' / \tau$ and $E' = \tau' E(t)$. Figure 3 brings the results of the generalized laminar model adjustments with the experimental RTD at 20 L/h, at no aggregation (figure 3(a)) and at aggregation conditions (figure 3(b)). The adjustments give a breakthrough time of 0.83 for conditions of no aggregation (figure 3(a)) and 0.73 for conditions involving WP aggregation (figure 3(b)). These values are respectively 0.92 and 0.86 at a flow rate of 49 L/h (figures not shown). As expected, the model corroborates the experimental result and show that the minimum residence time is shorter when WP aggregate during their flowing inside a tubular system. It can be seen in figure 3 that the generalized laminar model failed to represent the peak obtained in experimental RTD curves while the tail is better fitted. The radial mixing induced by the secondary flow can explain the dispersion observed around the peak of the E-curves by comparison with the sharp peak of the laminar E-curve, which denotes an absence of any radial dispersion. However a more appropriate approach with a model describing the whole flow pattern with accuracy is currently developed.

CONCLUSION

The RTD study in the helically holding tube of a heat treatment pilot plant has shown high differences in the fluid age dispersion when transformation occurs during the thermal process. The present work demonstrates that isothermal WP aggregation modify the velocity field inside the holding tube inducing a different RTD. The principal result is a reduction of the minimum residence time with the aggregation phenomena due to the increase of the maximum axial velocity. The generalized laminar model provided a good fit for the breakthrough time and for the tail on the E-curve, but don’t give a good representation of the radial mixing peculiar to the curved tubes. A general model able to describe all the flow pattern in no aggregation and
aggregation conditions is at the moment in development. Results of this work bring valuable tools to help the understanding of the aggregate size dispersion which is closely linked to the RTD.

![Graph](a) Experimental data vs. Generalized laminar model (20 L/h, (a) No aggregation conditions, (b) Aggregation conditions)

**Figure 3.** Example of adjustment of the generalized laminar model – 20 L/h, (a) No aggregation conditions, (b) Aggregation conditions

**ACKNOWLEDGEMENTS**
The authors would like to acknowledge financial support from ANR (Agence Nationale de la Recherche) within the framework of the GLOBULE project.

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