Effects of shear rates on rheology, foaming properties and protein structure of egg white: structure-function relationships

V. LECHEVALIER1*, A. ARHALIASS2, J. LEGRAND2 and F. NAU1

1UMR Agrocampus Rennes-INRA Science et technologie du lait et de l’œuf, 65 rue de Saint Brieuc, CS 84215, 35042 Rennes cedex, France
2UMR CNRS 6144 Laboratoire de Génie des Procédés, Environnement, Agroalimentaire (GEPEA), CRTT, BP 406, Boulevard de l’université, 44602 Saint Nazaire, France
*lecheval@agrocampus-rennes.fr

Keywords: egg white; shear rates; protein structure; foaming properties; rheology

The transformation of shell eggs into safe liquid, frozen or spray-dried egg white with extended shelf life requires many technological operations that result in modifications to the egg white’s functional properties. During these processes, shear rates underwent by egg white may be responsible for part of the loss of its functional properties. The present study was aimed at measuring the effects of a wide range of shear rates (from 0.1s\(^{-1}\) to 36000s\(^{-1}\) during 10 minutes) on egg white’s rheology, foaming properties and protein structure. Results highlighted three ranges of shear rates leading to different behaviour of egg white: very low shear rates (0.1s\(^{-1}\)), low to medium shear rates (from 1 to 1000s\(^{-1}\)) and high shear rates (over 1000s\(^{-1}\)). At very low shear rates, egg white viscosity increased during the first few seconds. This led to a better foam stability, which was put down to protein unfolding. Medium-sheared egg white did not show any significant variation of its rheological properties and foaming capacity, compared to non-sheared egg white. Nevertheless, compared to low-sheared egg white, its viscosity, consistency and thixotropic behaviour decreased as well as protein surface hydrophobicity and foaming capacity whereas foam stability increased. These results were put down to the disruption of ovomucin-lysozyme complex as well as protein aggregation. High-sheared egg white (over 1000s\(^{-1}\)) tended to a Newtonian behaviour. It showed a strong increase of its foaming capacity and of its protein surface hydrophobicity. A multivariate factorial analysis highlighted correlations between rheological measurements, foaming properties and protein structure.

Introduction

The transformation of shell eggs into safe liquid, frozen or spray-dried egg white with extended shelf life requires many technological operations that result in modifications to the egg white’s functional properties. During these processes, shear rates underwent by egg white may be responsible for part of the loss of its functional properties (Lechevalier et al., in press). Few literatures are dedicated to shear rates effects on foodstuff liquids and especially egg white. However, Forsythe and Bergquist, 1951, suggested that high shear rates broke ovomucin chain and Thapon, 1981, explained foam stability variations by ovomucin structure changes. More recently, Hagolle, 1997, followed the viscosity of ovalbumin and lysozyme solutions at low shear rates and highlighted orthokinetic aggregation phenomena. But shear rates effects on egg white mainly stay unexplained. This is probably due to the complexity of egg white but also to the difficulty to quantify and qualify shear rates that occur in industrial processes. The present study aimed at measuring the effects of a wide range of shear rates (from 0.1s\(^{-1}\) to 36000s\(^{-1}\) during 10 minutes) on egg white’s rheology, foaming properties and protein structure.

Material and methods

Material

Shell eggs were collected from a local factory “l’œuf du Breil” (Melesse, France) and stored at 10°C during 8 days. The egg whites were manually separated from the yolks and filtered on a grille (holes
diameter: 4 mm) to separate thick egg white from thin one. Egg white was then reconstituted up to 44% of thin egg white and 56% of thick egg white.

**SHEARING**

25 ml of egg white were filled into the beaker of thermostated coaxial cylinders and sheared at 20°C during 10 minutes. For shear rates between 0.1 and 1000s\(^{-1}\), simple air gap MV DIN B cylinder geometry was used on a Haake VT 550 viscosimeter (Thermo Electron Corporation, Cergy Pontoise, France). For higher shear rates, simple air gap CC 28.7 cylinder geometry was used on a Physica MCR 500 rheometer (Anton Paar, Courtaboeuf, France). Sample viscosity was measured during shearing.

**RHEOLOGICAL MEASUREMENTS**

They were carried out using a Haake VT 550 viscosimeter operated in the controlled shear rate rotation mode. PK 5 cone plate geometry was used (cone angle: 2°). The sample was allowed to adjust to 20°C for 2 min. Shear rates increased from 0 to 1000s\(^{-1}\) within 60s in a linear ramp. It was then kept constant at 1000s\(^{-1}\) for 30s before being reduced to 0 within 60s in a linear ramp. Viscosity and shear stress were recorded as a function of shear rates. Viscosity curves obtained during rising linear ramp were modelled with Ostwald – de Waele equation to calculate egg white consistency factor (k) and flow behaviour index (n):

\[
\eta = k\dot{\gamma}^{n-1}
\]

where \(\eta\) is the apparent viscosity and \(\dot{\gamma}\) is the shear rate.

The area delimited by shear stress as function of shear rates (h) was considered as the hysteresis area which evaluate the thixotropic behaviour of egg white.

**FOAMING PROPERTY MEASUREMENTS**

Foaming properties were measured by the bubbling method described by Baniel et al., 1997.

**PROTEIN STRUCTURE MEASUREMENTS**

Fluorescence measurements were performed to check out protein structure modifications. Intrinsic fluorescence measurements were carried out at pH 7.0 after excitation at 280 nm using a spectrofluorimeter LS50B (Perkin Elmer, Norwalk, USA). Emission spectra were registered between 305 and 415 nm. The slope of the relative fluorescence intensity versus protein concentration (3 concentrations tested) and the maximal emission wavelength were then used as indexes of the protein intrinsic fluorescence.

Measurement of surface hydrophobicity was carried out using the fluorescence probe ANS as suggested by Kato and Nakai, 1980. The slope of the fluorescence intensity versus protein concentration was used as an index of the protein surface hydrophobicity.

**STATISTICAL ANALYSIS**

Response variables were analyzed using multiple factorial analysis (MFA). MFA was carried out using SPAD® (Decisia, Pantin, France).

**Results**

**EFFECTS OF SHEAR RATES ON EGG WHITE RHEOLOGY**

Figure 1 shows the evolution of egg white viscosity as a function of time for the different shear rates applied. Three different behaviours can be distinguished: at low shear rates (0.1s\(^{-1}\)), egg white’s viscosity increased during the first 45s, at medium shear rates (between 1 and 1000s\(^{-1}\)), egg white’s viscosity decreased during the first 20s, and at high shear rates (over 1000s\(^{-1}\)), egg white’s viscosity was constant with time. It can be noticed that after 10 minutes, the higher the shear rate was, the smaller the viscosity was. This result was confirmed by the viscosity curves analysis (Figure 2).
Egg white showed a shear thinning behaviour. Consistency factors and flow behaviour indexes obtained by modelling these curves using Ostwald – de Waele model as well as hysteresis areas are given in Table 1.

**Table 1** Consistency factors (k), flow behaviour indexes (n) and hysteresis area (h) of sheared egg white according to shear rates applied. Results with different letters are significantly different (p<0.05: *, p<0.01: **).

<table>
<thead>
<tr>
<th>Shear Rate (s⁻¹)</th>
<th>0s⁻¹</th>
<th>0.1s⁻¹</th>
<th>1s⁻¹</th>
<th>10s⁻¹</th>
<th>100s⁻¹</th>
<th>1000s⁻¹</th>
<th>36000s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>0.68±0.08</td>
<td>0.40±0.05</td>
<td>0.57±0.05</td>
<td>0.68±0.17</td>
<td>0.63±0.10</td>
<td>0.74±0.11</td>
<td>0.87±0.41</td>
</tr>
<tr>
<td>k</td>
<td>0.14±0.04</td>
<td>0.92±0.05</td>
<td>0.29±0.05</td>
<td>0.22±0.05</td>
<td>0.22±0.05</td>
<td>0.03±0.05</td>
<td>0.05±0.05</td>
</tr>
<tr>
<td>h</td>
<td>±482±0.04</td>
<td>±370±0.05</td>
<td>±1007±0.05</td>
<td>±2169±0.05</td>
<td>±1303±0.05</td>
<td>±259±0.05</td>
<td>±43±0.05</td>
</tr>
</tbody>
</table>

Egg white sheared at 0.1s⁻¹ showed significantly higher consistency factor and hysteresis area assuming a stronger thixotropic behaviour than before shearing. The rheological behaviour of egg whites sheared between 1 and 100s⁻¹ was not significantly different from the one of non-sheared egg white. Egg whites sheared at 1000 and 36000s⁻¹ showed significantly weaker consistency factors and hysteresis area assuming a weaker thixotropic behaviour than non-sheared egg white.

**EFFECTS OF SHEAR RATES ON EGG WHITE FOAMING PROPERTIES**

The results obtained for foaming properties are given in Table 2.

**Table 2** Foam air volume (fav), foam density (fd), foam drainage rate (fdr) and foam stability (fs) measured for the different sheared egg whites. Results with different letters are significantly different (p<0.05: *, p<0.01: **, p<0.001: ***).

<table>
<thead>
<tr>
<th>Shear Rate (s⁻¹)</th>
<th>0s⁻¹</th>
<th>0.1s⁻¹</th>
<th>1s⁻¹</th>
<th>10s⁻¹</th>
<th>100s⁻¹</th>
<th>1000s⁻¹</th>
<th>36000s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>fav (ml)</td>
<td>40.9±1.7</td>
<td>43.7±0.4</td>
<td>43.7±0.4</td>
<td>42.3±0.3</td>
<td>41.5±0.2</td>
<td>38.7±0.2</td>
<td>55.9±2.3</td>
</tr>
<tr>
<td>fd (g.l⁻¹)</td>
<td>138.6±2.2</td>
<td>138.3±3.4</td>
<td>147.9±3.4</td>
<td>138.4±4.7</td>
<td>141.1±0.3</td>
<td>133.1±0.3</td>
<td>119.4±7.5</td>
</tr>
<tr>
<td>fdr (10⁻³.s⁻¹)</td>
<td>3.9±0.2</td>
<td>3.6±0.3</td>
<td>2.8±0.4</td>
<td>4.1±0.6</td>
<td>3.4±0.4</td>
<td>4.4±0.4</td>
<td>3.8±0.4</td>
</tr>
<tr>
<td>fs (%)</td>
<td>32.7±0.1</td>
<td>53.8±0.3</td>
<td>67.0±0.2</td>
<td>57.0±0.2</td>
<td>57.0±0.3</td>
<td>63.2±0.4</td>
<td>46.8±0.2</td>
</tr>
</tbody>
</table>

Egg white sheared at 0.1s⁻¹ showed significantly higher consistency factor and hysteresis area assuming a stronger thixotropic behaviour than before shearing. The rheological behaviour of egg whites sheared between 1 and 100s⁻¹ was not significantly different from the one of non-sheared egg white. Egg whites sheared at 1000 and 36000s⁻¹ showed significantly weaker consistency factors and hysteresis area assuming a weaker thixotropic behaviour than non-sheared egg white.
Foam air volume and foam density were 2 indexes of foaming capacity. Egg white’s foaming capacity increased after low shear rates (0.1 and 1 s⁻¹). Medium-sheared egg white showed the same foaming capacity as non-sheared egg white. However, it can be noticed than foam air volume progressively decreased between egg white sheared at 1 s⁻¹ and the one sheared at 10, 100 and 1000 s⁻¹. High shear rates (36000 s⁻¹) also improved foaming capacity.

Foam drainage rate and foam stability were 2 indexes of foam stability. This one increased significantly for egg white sheared at 0.1 and 1 s⁻¹. Then it was stable for egg white sheared at 10 and 100 s⁻¹ before decreasing for egg white sheared at 1000 and 36000 s⁻¹.

Low shear rates improved foam stability and foaming capacity. High shear rates (over 1000 s⁻¹) also improved foaming capacity but rather damaged foam stability.

**EFFECTS OF SHEAR RATES ON EGG WHITE PROTEIN STRUCTURE**

Fluorescence measurements summed up in Table 3 were the indexes of protein structure changes.

**Table 3** Intrinsic fluorescence intensity (Int) and maximal emission wavelength (λ) as well as protein surface hydrophobicity (Φ) measured for the different sheared egg whites. Results with different letters are significantly different (p<0.05: *, p<0.01: **).

<table>
<thead>
<tr>
<th></th>
<th>0 s⁻¹</th>
<th>0.1 s⁻¹</th>
<th>1 s⁻¹</th>
<th>10 s⁻¹</th>
<th>100 s⁻¹</th>
<th>1000 s⁻¹</th>
<th>36000 s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int</td>
<td>2703 ±65</td>
<td>2640 ±131</td>
<td>2502 ±925</td>
<td>2541 ±26</td>
<td>2590 ±210</td>
<td>2629 ±26</td>
<td>2588 ±91</td>
</tr>
<tr>
<td></td>
<td>2640 ±131</td>
<td>2502 ±925</td>
<td>2541 ±26</td>
<td>2590 ±210</td>
<td>2629 ±26</td>
<td>2588 ±91</td>
<td></td>
</tr>
<tr>
<td>λ</td>
<td>341.5 ±0.2</td>
<td>341.9 ±0.2</td>
<td>341.8 ±0.2</td>
<td>341.8 ±0.2</td>
<td>341.7 ±0.2</td>
<td>341.7 ±0.2</td>
<td>341.6 ±0.2</td>
</tr>
<tr>
<td></td>
<td>341.9 ±0.2</td>
<td>341.8 ±0.2</td>
<td>341.8 ±0.2</td>
<td>341.8 ±0.2</td>
<td>341.7 ±0.2</td>
<td>341.7 ±0.2</td>
<td>341.6 ±0.2</td>
</tr>
<tr>
<td>Φ</td>
<td>1298 ±87</td>
<td>1402 ±96</td>
<td>1314 ±269</td>
<td>1267 ±68</td>
<td>1260 ±863</td>
<td>1124 ±120</td>
<td>1601 ±148</td>
</tr>
<tr>
<td></td>
<td>1402 ±96</td>
<td>1314 ±269</td>
<td>1267 ±68</td>
<td>1260 ±863</td>
<td>1124 ±120</td>
<td>1601 ±148</td>
<td></td>
</tr>
</tbody>
</table>

Intrinsic fluorescence intensity’s variations were gentle. Only egg white sheared at 1 s⁻¹ showed significantly lower intrinsic fluorescence intensity than non-sheared egg white. In the same way, maximal emission wavelength did not undergo large variations. Although they were significant, they were too small to be considered.

Protein’s surface hydrophobicity increased significantly after shearing at 0.1 s⁻¹. Then it decreased progressively with shear rates until 1000 s⁻¹, before increasing significantly for egg white sheared at 36000 s⁻¹.

**CORRELATIONS BETWEEN SHEAR RATES AND EGG WHITE’S RHEOLOGY, FOAMING PROPERTIES AND PROTEIN STRUCTURE**

To understand the effects of shear rates on egg white’s protein structure and their consequences on egg white’s foaming properties and rheological behaviour, a multiple factorial analysis was achieved. The results are shown on Figure 3.
Figure 3  A- Correlation circle of continuous variables (→foaming properties, →rheology, → protein structure, ← shear rates) for PCs 1 and 2. B- similarity map determined by PCs 1 and 2:  ● mean individuals, □ partial individuals for rheology, △ partial individuals for foaming properties, ◇ partial individuals for protein structure.
Principal component (PC) 1, explaining 44% of the variability was strongly correlated with rheology measurements \((r > 0.8)\), foam density and stability \((r > 0.7)\) as well as fluorescence maximal emission wavelength \((r > 0.8)\) (Figure 3A). It set egg whites with a strong thixotropic behaviour, high fluorescence maximal emission wavelength and producing stable but dense foams against egg whites with a weak thixotropic behaviour, low fluorescence maximal emission wavelength and producing unstable but expanded foams. According to Figure 3B, this corresponded to set low-sheared egg whites \((0.1 \text{ and } 1 \text{s}^{-1})\) against non-sheared and high-sheared egg white \((1000 \text{ and } 36000 \text{s}^{-1})\).

PC2, which accounted for 29% of the variability was strongly correlated with foaming capacity \((r > 0.9)\), protein’s surface hydrophobicity \((r > 0.8)\) and shear rates \((r > 0.8)\). It sets high-sheared egg whites producing expanded foams and which proteins showed a high surface hydrophobicity against low-sheared egg whites producing dense foams and which proteins showed a low surface hydrophobicity (Figure 3A).

It can be noticed that on both PCs, the nearness of non-sheared egg white and egg white sheared at 1000s\(^{-1}\) was unexpected.

This analysis showed positive correlations between shear rates and egg white’s flow behaviour index, foaming capacity and protein’s surface hydrophobicity. Thus, increasing shear rates, egg white’s protein unfolded, which gave it a nearly Newtonian behaviour and improved its foaming capacity. All correlation coefficients are given in Table 4.

Table 4  correlation coefficients between MFA variables. Significant coefficient are written in bold characters.

<table>
<thead>
<tr>
<th></th>
<th>sr</th>
<th>h</th>
<th>k</th>
<th>n</th>
<th>fav</th>
<th>fd</th>
<th>fdr</th>
<th>fs</th>
<th>Int</th>
<th>(\lambda)</th>
<th>(\phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>shear rates ((sr))</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hysteresis area ((h))</td>
<td>-0.62</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consistency factor ((k))</td>
<td>-0.32</td>
<td>0.76</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow behaviour index ((n))</td>
<td>0.68</td>
<td>-0.89</td>
<td>-0.90</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam air volume ((fav))</td>
<td>0.94</td>
<td>-0.35</td>
<td>-0.09</td>
<td>0.45</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam density ((fd))</td>
<td>-0.87</td>
<td>0.76</td>
<td>0.33</td>
<td>-0.71</td>
<td>-0.71</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam drainage rate ((fdr))</td>
<td>0.10</td>
<td>-0.56</td>
<td>-0.30</td>
<td>0.43</td>
<td>-0.15</td>
<td>-0.55</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam stability ((fs))</td>
<td>0.02</td>
<td>0.38</td>
<td>0.19</td>
<td>-0.23</td>
<td>0.19</td>
<td>0.29</td>
<td>-0.62</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence intensity ((int))</td>
<td>-0.07</td>
<td>-0.17</td>
<td>0.09</td>
<td>-0.02</td>
<td>-0.21</td>
<td>-0.25</td>
<td>0.50</td>
<td>-0.88</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. emission wavelength ((\lambda))</td>
<td>-0.36</td>
<td>0.60</td>
<td>0.66</td>
<td>-0.69</td>
<td>-0.16</td>
<td>0.47</td>
<td>-0.40</td>
<td>0.71</td>
<td>-0.59</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Surface hydrophobicity ((\phi))</td>
<td>0.81</td>
<td>-0.09</td>
<td>0.18</td>
<td>0.19</td>
<td>0.94</td>
<td>-0.57</td>
<td>-0.25</td>
<td>0.11</td>
<td>-0.07</td>
<td>-0.11</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Discussion

Shear rates had effects on egg white’s rheological behaviour, its foaming properties and its protein structure.

Very low shear rates \((0.1 \text{s}^{-1})\) unfolded egg white’s proteins as suggested by the increase of protein’s surface hydrophobicity. This led to an improvement of foam stability since it eased molecular interactions, stabilizing the interfacial film (Kim and Kinsella, 1985). Foaming capacity also tended to
increase, hydrophobic protein diffusing faster (Kato and Nakai, 1980; Kato et al., 1981; Damodaran, 1997). In addition, egg white’s apparent viscosity, consistency and thixotropic behaviour also increased. This could also be due to protein unfolding. Actually, globular proteins only have small effects on the viscosity of water except at high concentrations. Their unfolding and the hydration of exposed hydrophobic residues increased protein’s hydrodynamic volume and thus egg white’s viscosity (Rha and Pradipasena, 1986). Moreover, the increase of viscosity with time was characteristic of orthokinetic aggregation, which usually involves weak and reversible bonds such as hydrophobic interactions (Hagolle, 1997).

Medium shear rates (from 1 to 1000s\(^{-1}\)) decreased egg white’s viscosity with time. This was probably due to the disruption of the high molecular lysozyme-ovomucin complexes present in the thick egg white (Lang and Rha, 1982). However, medium-sheared egg white did not show a significantly different rheological behaviour from the one of non-sheared egg white. This result may have been due to aggregation that made up for complexes disruption. Protein’s surface hydrophobicity of medium-sheared egg white was not significantly different from non-sheared egg white but lower than low-sheared egg white, reinforcing aggregation hypothesis. Foaming capacity decrease noticed between low-sheared and medium-sheared egg whites was thus the consequence of this aggregation. However, foam stability should also have decreased with aggregation and thick egg white liquefaction (Kinsella, 1976; Trziszka, 1993; Nau et al., 1996). Phenomena involved at medium shear rates seemed then quite complicated and fluorescence measurements, carried out on egg white, did not give information on individual protein structure that could have explain foaming properties’ variations.

High-sheared egg white (over 1000s\(^{-1}\)) had a nearly Newtonian behaviour. Its apparent viscosity was 3 times lower than non- or low-sheared egg white. Protein’s surface hydrophobicity as well as foaming capacity increased whereas foam stability decreased. Such results may be explained by aggregates dissociation and suggested that protein denaturation-aggregation phenomena, such as those described by Oliva et al., 2003, may have occurred. However, Forsythe and Bergquist, 1951, put down such a loss of viscosity under high shear rates to ovomucin fibre break. Moreover, foam stability variations observed in this study were in agreement with those noticed by Thapon, 1981. He showed that foam stability increased with homogenisation until egg white’s viscosity reached 3mPa.s, under which foam stability decreased. He put foam stability increase down to a more homogeneous distribution of ovomucin in egg albumen and foam stability decrease down to changes in ovomucin tertiary structure. Nevertheless, the results obtained in this study also suggested protein interactions phenomena and ovomucin’s structure was probably not the only explanation.

Although this study did not enable to explain completely shear rates effects on egg white’s properties, some interactions between shear rates and egg white’s flow behaviour, foaming capacity and protein surface hydrophobicity were found. Further studies, especially on protein model solutions, are thus planned to better understand protein interactions and structure modifications.

References


