

Influence of a thermal treatment on the functionality of hen's egg yolk in mayonnaise

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Abstract

The viscosity and emulsifying properties of egg yolk submitted to a heat treatment at 68 °C for up to 11 min were investigated in a standard mayonnaise recipe. Heating of the egg yolk prior to emulsification was found to lead to a reduction of the average oil droplet size in mayonnaise of up to 40% compared to non-heated egg yolk. This led to an increased viscosity and greater pseudoplasticity of the mayonnaise prepared with heated egg yolk. In order to investigate the effect of egg yolk heating on the rheological properties of the mayonnaise independently from the oil droplet size, emulsions having various median oil droplet diameters were prepared with non-heated and heated egg yolk. This work shows that heating of egg yolk prior to emulsification significantly impacts the rheological properties of mayonnaise when the median oil droplet diameter exceeds 5 µm. The presence of thermally unfolded and interacting proteins in the aqueous lamella of continuous phase surrounding each oil droplet of the mayonnaise may explain the impact of heating on the rheological properties of the product. The increased thixotropy observed in mayonnaise prepared with heated egg yolk may reflect the formation of a network of oil droplets connected by weak bonds formed between proteins adsorbed at the interface and within the continuous phase. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Egg yolk; Protein denaturation; Pasteurization; Mayonnaise; Rheology; Thixotropy

1. Introduction

Hen's egg yolk (EY) is a very effective and therefore widely used food emulsifier, notably in the preparation of mayonnaise and numerous other salad dressings and sauces. The very high sensitivity of EY to microbiological spoilage makes it necessary to thermally treat this ingredient, so as to ensure its innocuity. Pasteurisation of industrially produced EY typically consists of a heating at temperatures between 60 and 68 °C for times ranging from a few seconds to about 10 min, depending on the temperature (Le Denmat, Anton, & Gandemer, 1999). This treatment is designed to inactivate pathogenic microorganisms such as salmonella without damaging EY

proteins and their functional properties. However, they do not ensure complete eradication of the microbial flora from EY, which leads to only limited shelf life and imposes storage at 4 °C (Cunningham, 1995, Chap. 12). Some egg processors and users would prefer to apply stronger heat treatments to further ensure microbial safety of EY and increase shelf life. However, such heat treatments could alter physical and functional properties of EY, because of the thermal sensitivity of some of its components. From a structural point of view, EY is a dispersion of low density lipoproteins (LDL) and insoluble egg yolk granules in an aqueous solution of soluble glycoproteins called livetins. In native egg yolk, granules consist of a complex of high density lipoproteins (HDL) and a phosphoprotein called phosvitin, which is held together by phosphocalcic bridges. On the other hand, LDL consist of a core of lipids surrounded by an interfacial layer of phospholipids and proteins called the LDL apoproteins (Burley & Vadehra,

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1989). The most heat sensitive proteins of EY are thought to be the LDL apoproteins (Tsutsui, 1988) and some of the livetins (Ternes & Werlein, 1987). These components have been shown to denature at temperatures as low as 62 to 65 °C (Dixon & Cotterill, 1981; Le Denmat et al., 1999). Mayonnaise has been used as a model system to study the emulsifying properties of EY (Harrisson & Cunningham, 1986; Paraskevopoulou & Kiosseoglou, 1995; Yang & Cotterill, 1989). However, more recent studies have mostly used rather liquid model emulsions with a dispersed phase volume fraction (φ) between 0.2 and 0.4 (Anton, Chapleau, Beaumal, Delepine, & de Lamballerie-Anton, 2001; Le Denmat, Anton, & Beaumal, 2000; Mine, 1998). The complex rheological behaviour of mayonnaise has received a lot of attention and has been studied using steady shear rotational rheology (Franco, Berjano, Guerrero, Munoz, & Gallegos, 1995; Pons, Galotto, & Subirats, 1994), small amplitude oscillatory shear (Gallegos, Berjano, Guerrero, Munoz, & Flores, 1992; Guerrero, Partal, & Gallegos, 2000), or even both techniques (Moros, Franco, & Gallegos, 2002; Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004). It is generally agreed that mayonnaise exhibits pseudoplastic behaviour with a flow threshold and presence of thixotropy (Pons et al., 1994). This is the result of the internal microstructure of mayonnaise, which is made of very tightly packed oil droplets separated by a lamella of continuous phase (Langton, Jordansson, Altskär, Sorensen, & Hermansson, 1999). The close packing of oil droplets in mayonnaise adds to the tendency of the system to destabilise through coalescence, which shows the importance of having a very resistant interface in order to avoid this phenomena. This is the role of the interfacial film that surrounds each oil droplet, and which is approximately 140 Å in width. This film is composed of coalesced low density lipoprotein of EY and microparticles of EY granules. The continuous aqueous phase contains electron dense particles, which are polymorphic in shape, average 550 Å in size, and are presumed to be fragments of EY granules. The protein particles adhere to one another forming a network between lipid droplets, as well as forming a layer or coating on the interfacial film. Not only the particle size distribution, but also the protein network undoubtedly influence the rheological properties of mayonnaise (Ford, Borwankar, Pechak, & Schwimmer, 2004, Chap. 13).

The impact of a thermal treatment on the emulsifying properties of EY has received very little attention, even though the few studies available seem to indicate that the stability of O/W emulsions is not affected by EY pasteurisation (Cotterill, Glauert, & Basset, 1976; Cotterill, Glauert, Steinhoff, & Baldwin, 1974; Varadarajulu & Cunningham, 1972). A recent study concluded that a thermal treatment of about 3 min at temperatures as high as 76 °C led to a slight decrease of emulsifying activity and no modification of emulsion stabilisation properties of EY (Le Denmat et al., 1999). Campbell, Raikos, and Euston (2005) measured that heating EY at 77 °C for

2 min did not impact its emulsifying activity in concentrated emulsions (oil volume ratio $\varphi = 0.65$), but the rheological properties of the obtained emulsions were not characterised.

There is a lack of studies where the influence of thermal denaturation of EY is systematically related to the physical properties of the prepared emulsions. This constitutes a gap regarding the understanding of the impact of the pasteurisation conditions of EY on the properties of emulsions. Differences in the pasteurisation intensity between egg product distributors leads to difficulties for the food emulsion manufacturers in controlling the properties of the final product. The objective of this study is to evaluate the impact of the intensity of a thermal treatment on the emulsifying properties of EY, using mayonnaise as a model food emulsion. The heating conditions have been chosen to cover standard pasteurisation as well as a slightly more severe treatment intensity. A situation where the EY has started to form a weak gel, which would normally be avoided by egg product manufacturers, is also covered in this study. The oil droplet size distribution and rheological properties of the mayonnaise are studied and the results are discussed with regards to potential modifications of the emulsion's internal structure.

2. Material and methods

2.1. Preparation and heating of an egg yolk suspension

Freshly laid eggs from “Lohman Tradition” hens were collected from the University's research farm (Thalhausen) and used within 48 h after collection. Whole egg yolks were manually separated from the albumen, and the vitellin membrane was carefully rinsed with a 1% NaCl (w/v) solution. Albumen-free egg yolks were then mixed and diluted with NaCl 1% (w/v) solution, so as to obtain a suspension containing 80% (w/w) pure EY and 20% (w/w) NaCl 1% (w/v). The suspension was sieved through a 1 mm mesh sieve to remove the fragments of vitellin membrane.

2.1.1. Thermal treatment of the egg yolk suspension

The EY suspension was heated-up from 25 °C to 68 °C in 3 min using a batch scraped-surface heat exchanger. The heating fluid (water) had a temperature of 68 °C in order to avoid over-heating at the heating surface. The suspension was then held at 68 °C under constant mild agitation for various times between 1 and 11 min. The product was then rapidly cooled using ice water while maintaining the agitation.

2.2. Composition and fabrication of the model mayonnaise

The composition of the model mayonnaise was kept constant throughout the study. It contains (in % w/w): 80% sunflower oil, 7.5% de-ionised water, 7.5% EY, 3.5% acetic acid 10% solution, 0.5% sugar and 1% NaCl. The sunflower oil all came from the same batch of 2001

purchased from a food ingredient supplier (Cereol, Mannheim, Germany). The 10% (w/v) acetic acid solution was prepared from glacial 99–100% acetic acid (J.T Baker, Deventer, Holland). Commercial sugar (Südzucker, Mannheim, Germany) and salt (Bad Reichenhaller, Berchtesgaden, Germany) were purchased from a local supermarket.

2.2.1. Preparation of the mayonnaise

For the preparation of the pre-emulsion, the water phase was first prepared by mixing all of the ingredients apart from the oil in a 3 l plastic beaker. The oil was then incorporated with help of a peristaltic pump at a constant rate of 200 ml/min while mixing with a flat bladed turbine rotating at 200 tr · min⁻¹. Great care was taken to minimise air incorporation during mixing. The so-obtained pre-emulsion was then further emulsified by passing it once through a colloid mill with help of a positive displacement pump at a rate of 50 l/h. The colloid mill (model Labor 2000/4, IKA, Staufen, Germany) was rotating at a constant 4656 tr · min⁻¹ with a gap width setting between rotor and stator of 830 µm.

For each heat treatment condition of the EY, both the heating and the preparation of the mayonnaise were carried out twice on two different days, in order to check the overall reproducibility of the whole preparation procedure. Each set of results obtained are presented as distinct data points, and a curve best fitting the average of the two repetitions is also presented.

For the preparation of mayonnaise with different oil droplet diameter, the energy input was varied by altering the rotation speed of the colloid mill, using the manufacturer's controller.

2.3. Rheological characterisation of egg yolk and mayonnaise

The viscosity of the EY was measured at 20 °C with a controlled stress rheometer, model Carri-Med CSL 500 (TA Instruments, Alzenau, Germany). The rheometer was equipped with a stainless steel cone having a diameter of 40 mm and an angle of 4°. A solvent trap filled with de-ionised water was used in order to prevent sample drying during measurement. The values of apparent viscosity reported in this work are taken at a shear rate of 100 s⁻¹ after 3 min of shearing.

2.3.1. Rheological characterisation of the mayonnaise

The same controlled stress rheometer was used for the characterisation of the mayonnaise flow properties at 20 °C, using a stainless steel cone having a diameter of 40 mm and an angle of 2°. A solvent trap filled up with de-ionised water was also used. A thixotropic loop measurement was carried out by first increasing the shear rate logarithmically from 0 to 150 s⁻¹ in 240 s, then maintaining it at 150 s⁻¹ for 240 s, and finally decreasing it logarithmically back to 0 s⁻¹ in 120 s. The values of apparent viscosity reported in this work were taken at a shear rate of

150 s⁻¹ at the very end of the holding time. The decreasing part of the thixotropic loop was modelled using the Herschel–Bulkley model, which is presented in Eq. (1). τ is the shear stress (Pa), τ_o the yield stress (Pa), γ the shear rate (s⁻¹), K the consistency index (Pa · sⁿ) and n the flow index.

$$\tau = \tau_o + K \cdot \gamma^n \quad (1)$$

The area comprised within the thixotropic loop is referred to as the thixotropy (Pa · s⁻¹).

2.4. Oil droplet size distribution measurement

An oil droplet size distribution was obtained for each mayonnaise sample using a Laser diffraction Spectrometer model LS 230 (Beckman Coulter, Krefeld, Germany). A sample of the mayonnaise was carefully dispersed in water while ensuring the absence of any lumps, and then suspended in a solution containing 0.5% (w/v) of sodium-dodecyl-sulphate prior to measurement. The volume based distribution is used. The variation index V_d of the droplet size distribution is defined as indicated in Eq. 2 below:

$$V_d = \frac{(d_{90.3} - d_{10.3})}{d_{50.3}} \quad (2)$$

The volume distribution is used to calculate the characteristic diameters of the oil droplets. By definition, 90%, 50% and 10% of the oil volume is dispersed in the form of droplets with a diameter below the values of $d_{90.3}$, $d_{50.3}$ and $d_{10.3}$, respectively.

3. Results and discussion

3.1. EY viscosity as a function of heating conditions

The apparent viscosity of the EY suspension increases during heating, as can be seen in Fig. 1. The viscosity increase which is relatively slow during the first 4 min at 68 °C, accelerates greatly between 4 and 8 min heating, before it starts to slow down again. As highlighted by Kiosseoglou (2003), EY gelation implies a destabilisation of LDLs resulting from the unfolding of their apoproteins

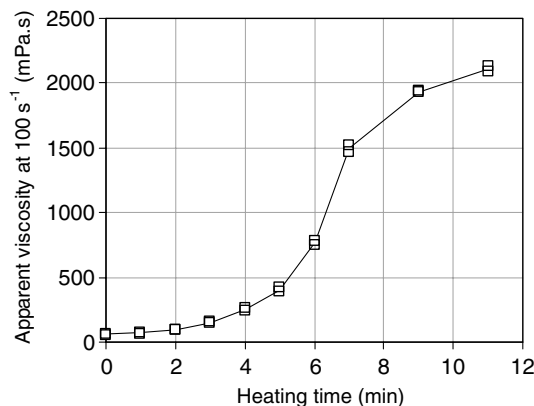


Fig. 1. Effect of heating at 68 °C on the apparent viscosity of an egg yolk suspension.

leading to attractive molecular interactions and finally to interparticle network formation. This process was shown to involve in priority the proteins of EY plasma (Anton, LeDenmat, Beaumal, & Pilet, 2001), and the network formation was recently reported as being dominated by hydrophobic interactions, while disulfide linkages appear to play a complementary role (Kiosseoglou & Paraskevopoulou, 2005). The initial phase observed in Fig. 1 (0–4 min at 68 °C) presumably corresponds to the time necessary for the EY apoproteins to unfold under the effect of heat, before they start interacting with each other, once a critical concentration of unfolded protein has been reached (after 4 min at 68 °C). For heating times above 7 min, the EY suspension formed a gel which was too thick to be evenly dispersed in an aqueous solution without forming large lumps. For this reason, mayonnaise was only prepared with EY heated up to 7 min at 68 °C.

3.2. Emulsifying activity of EY as a function of the heating conditions

Fig. 2 shows that the average diameter of oil droplets formed in mayonnaise decreases for a heating time of the EY up to 4 min. The achieved droplet size stabilises at a low value for any further heating up to 7 min. The processing parameters during the emulsification of the mayonnaise were kept constant in order to ensure a constant energy input for the disruption of oil droplets. These results suggest that the emulsifying activity of the EY has been improved by the heating treatment applied prior to mayonnaise preparation.

It is particularly noticeable that the improvement of the emulsifying activity of EY occurs during the first 4 min of heating, which is the time associated with the least viscosity increase of the EY. However, as the EY viscosity increases steeply between 4 and 7 min heating, the emulsifying activity of EY does not change anymore. These observations suggest that the improved emulsifying activity of EY during heating is due to a structural modification of its most

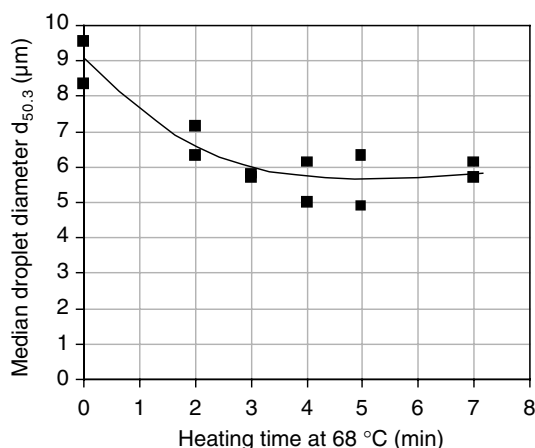


Fig. 2. Median oil droplet diameter in mayonnaise made with EY heated at 68 °C for different times.

heat sensitive components, which are thought to be the LDL apoproteins as well as some of the livetins (see chapter 1). Although the role of livetins in the emulsifying properties of EY is not well understood, the LDL apoproteins have been shown to play a key role in the stabilisation of oil/water interfaces (Kiosseoglou & Sherman, 1983; Melnikov, 2002; Mitzutani & Nakamura, 1985). The observed increased emulsifying activity of EY upon heating could therefore be a consequence of the thermal unfolding of LDL apoproteins. Structural changes of these proteins could lead to an increased surface hydrophobicity and molecular flexibility, allowing a faster and more effective adsorption of these molecules at the O/W interface. Live-tins may also play a similar role provided that they can favourably compete with other EY components for the adsorption at the interface, which is not sure at present. Moreover, the dissociation of the native LDL structure during the thermal unfolding of their apoproteins can lead to a greater availability of the constituents of LDL for the O/W interface, including the very surface-active phospholipids.

3.3. Rheological properties of mayonnaise as a function of EY heating conditions

The significant decrease of oil droplet diameter in mayonnaise prepared with heated EY leads to important modifications of the product's flow properties. A four min heating at 68 °C has been measured to lead to a three fold increase of the consistency index of mayonnaise, based on the Herschel–Bulkley model (Fig. 3). This is accompanied by an increased sensitivity of the emulsion's structure to shear intensity, as indicated by the decrease of flow index from 0.45 to 0.35 (Fig. 3). In mayonnaise, the large contact surface area between oil droplets leads to important friction forces which oppose to the free flowing of the emulsion in a shear field, hence increasing its viscosity. A decrease of oil droplet diameter leads to a greater contact surface area

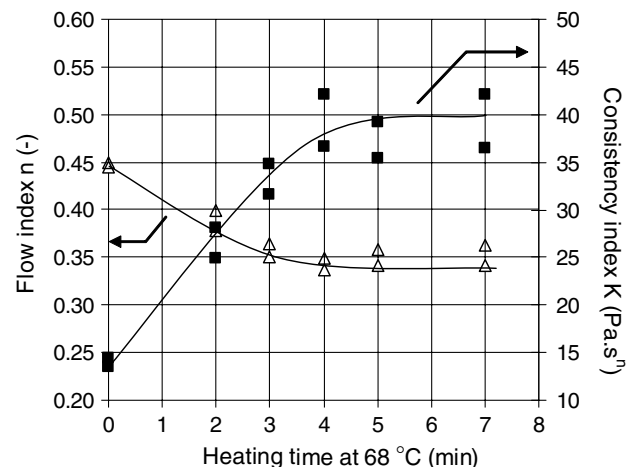


Fig. 3. Consistency and flow indexes in mayonnaise made with EY heated at 68 °C for different times.

between droplets, and therefore to an increased viscosity (Langton, Åström, & Hermansson, 1999).

But the flow properties of such concentrated emulsions can also be greatly impacted by the nature and intensity of interactions taking place between droplets, and within the lamella of continuous phase between each droplets. The formation of a protein network between oil droplets under the effect of a thermal treatment in emulsions containing EY, and its impact on the rheological properties of such emulsions was reported by Anton et al. (2001). It is however difficult to dissociate the role played by the reduction of oil droplet size from that played by increased inter-droplets interactions, since they both lead to an increased consistency of mayonnaise. In order to look at the impact of EY protein denaturation independently from the oil droplet size, at least 10 samples of mayonnaise containing either non-heated or heat treated EY (68 °C for 6 min) were produced so as to contain oil droplets with various median diameters (see Fig. 4). The difference in the achieved droplet size between non-heated and heated EY is particularly important when the rotation velocity is low, which indicates that the energy input is the factor limiting the decrease of droplet size in these conditions. At high shear rate, the limiting factor is more likely to be the velocity at which the interfacial tension is decreased and the new interface is stabilised.

As expected, we observe that the consistency index of the emulsions prepared with non-heated and heated EY decreases with increasing oil droplet diameter (Fig. 5). Moreover, it appears that when rather small oil droplets are produced (<4.5 µm), the consistency index of mayonnaise containing non-heated and heated EY is very similar. However, when larger droplets are obtained (> 4.5 µm), the presence of heated EY in the mayonnaise leads to a higher consistency index than that obtained with non-heated EY. This suggests the existence of a critical average droplet size below which the rheological behaviour of mayonnaise is driven by the total contact surface area between droplets, and therefore mainly influenced by the droplet size

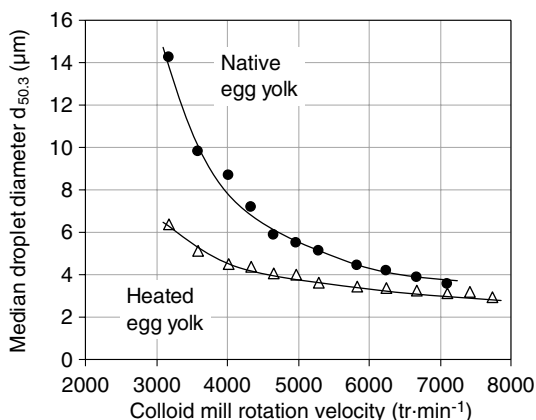


Fig. 4. Median oil droplet diameter in mayonnaise prepared with non-heated and heated EY (68 °C for 6 min) using different intensity of mechanical energy.

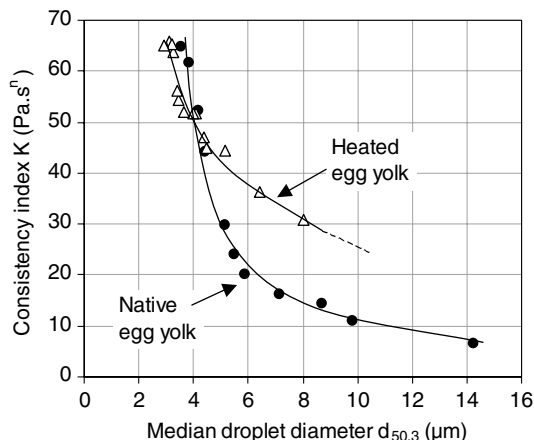


Fig. 5. Consistency index of mayonnaise prepared with non-heated and heated EY (68 °C for 6 min) for various median oil droplet diameter.

achieved at any given dispersed phase volume ratio. When the achieved average droplet size is larger than the critical value, the rheological properties of the emulsion can then be dominated by other factors such as molecular interactions between droplets.

The flow curves obtained for samples of mayonnaise having similar oil droplet size distributions (see Table 1), but containing either non-heated or heated EY, are presented on Fig. 6. An hysteresis loop was obtained for each sample by successively increasing, maintaining and decreasing the shear rate as indicated in chapter 2.3. The surface of the area comprised within the hysteresis loop,

Table 1
Parameters characterising the oil droplet size distribution of two samples of mayonnaise having similar median droplet diameter (V_d as defined in chapter 2.4)

	$d_{10.3}$ (µm)	$d_{50.3}$ (µm)	$d_{90.3}$ (µm)	V_d (-)
Non- heated EY	3.71	5.13	7.97	0.830
Heated EY	3.65	5.14	8.45	0.934

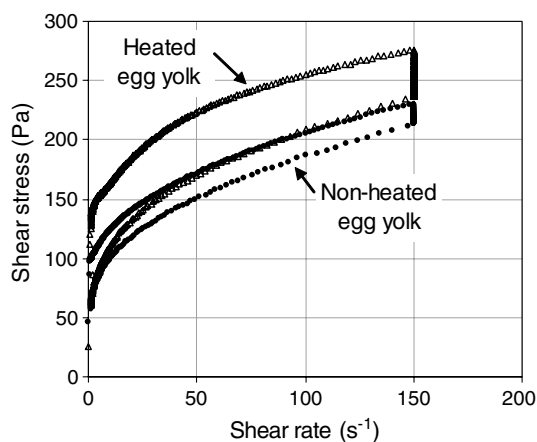


Fig. 6. Thixotropic loop for mayonnaise prepared with non-heated and heated EY (68 °C for 6 min).

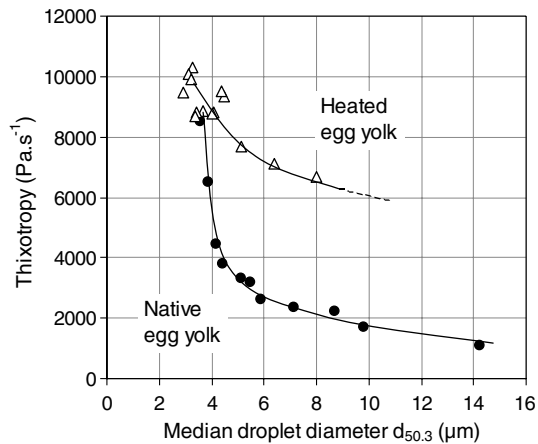


Fig. 7. Thixotropic loop area for mayonnaise prepared with non-heated and heated EY (68 °C for 6 min) for various median oil droplet diameter.

referred to as thixotropy, is larger in mayonnaise containing heat-treated EY compared to that obtained with non-heated EY. This increased level of thixotropy corresponds to a progressive breakdown of the product's structure as the time of shear is increased (Abu-Jdayil, 2003).

The effect of EY heating on the thixotropy of the mayonnaise for different oil droplet diameter is presented on Fig. 7. It shows that the thixotropy is little affected by heating in mayonnaise with a median oil droplet diameter below 4 μm . However, the thixotropy of mayonnaise drops much more rapidly with increasing oil droplet diameter if non-heated EY is used. For a median oil droplets diameter of about 6 μm , the thixotropy of mayonnaise prepared with heated EY is more than three times that of mayonnaise made with non-heated EY. These results show that the structural modifications of EY proteins taking place upon heating result in an increased level of structure formation between oil droplets in a concentrated emulsion system.

Considering that EY proteins denaturation starts at temperatures close to 65 °C (see chapter 1), it is expected that the temperature of 68 °C used to heat up the EY in this study allows a relatively slow unfolding, and affects particularly the most heat sensitive components of EY. Different hypothesis regarding the effect of having unfolded EY proteins in the emulsion can be used to explain the measured increase of time-dependant structure and consistency. Some of the EY proteins adsorb at the O/W interface and form the cohesive and elastic interfacial film which ensures long-term stability of the emulsion by preventing coalescence of the oil droplets. But a lot of the EY proteins remain in the continuous phase, and therefore participate in the properties of the lamella between oil droplets. The presence of thermally unfolded and possibly partly aggregated proteins in the lamella probably leads to an increased viscosity of the continuous phase, which participates in the increased consistency of the emulsion. Furthermore, non-adsorbed proteins from the lamella are likely to interact with adsorbed proteins, and possibly even form a continu-

ous network of interconnected droplets, as observed with transmission electronic microscopy by Anton et al. (2001). These interactions are expected to be of hydrophobic nature, as EY proteins are known to form such bonds upon heating (Kiosseoglou & Paraskevopoulou, 2005). The sum of the elementary energy associated with each of the bonds could lead to increased consistency and thixotropy, which may be related to the increased storage modulus observed when heating up egg yolk stabilised emulsions (Anton et al., 2001; Moros, Cordobes, Franco, & Gallegos, 2003). The sensitivity of this type of bonds to shear intensity and time of shearing is reflected in the measured increased levels of pseudoplasticity and thixotropy when heated EY is used, as shown in Figs. 3 and 7, respectively.

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