Rheological measurements of yolk to characterise origin and processing properties of eggs

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Abstract

Egg proteins and lipoproteins are responsible of a wide range of interesting properties of many emulsions, foams, gels and other foods processed by industry. The modifications of functional properties of these proteins during food processing determine a complex behaviour that can result in structural changes of difficult prediction, and rheological measurements are a useful tool to study them. In this work, egg yolk samples coming from different species of fowls, different breeds of laying hens, and differently fed hens, have been analysed using steady state and dynamic tests in a rotational rheometer. Thermal transitions in egg yolk have been established by differential scanning calorimetry (DSC). The results have given valuable information about differences in egg yolk functional and structural properties that could be very useful to improve storage and processing conditions, as well as a tool to quality and fraudulence control in egg industry.

Keywords: rheological properties, egg yolk, proteins, rheology.

1. Introduction

From a nutritional and also from a technological point of view, egg white and yolk are interesting raw materials, being both of them pseudoplastic liquids (Gosset et al., 1983; Steffe, 1996). Fresh egg white and yolk contain around 10% and 16% proteins, respectively (Stadelman and Cotterill, 1986), which have important technical properties. Egg yolk is a complex association of lipids and proteins in water in which several types of particles are suspended in a protein solution or plasma. These granules contain basically phosvitin and high and low density lipoproteins (Burley and Cook, 1961), while plasma is constituted by livetins and low density lipoproteins (Martin et al., 1964).

Egg composition and physical structure is highly dependent on several factors; fowl diet has a great influence on yolk chemical composition, mainly on its fatty acids spectra (González-Esquerra and Leeson, 2000; Cherian et al., 2000; Bean and Leeson, 2003), and there are also differences due to the specie of laying fowl. Additionally, eggs coming from countries with different sanitary and environmental requirements are entering in Europe at very low prices, pressuring on industry costs and changing notably the market in different ways: development of new products with greater added value, such as new functional products (enriched Ω-3 hen eggs, Brudy…), use of eggs from species different from hen...

All these facts make necessary not only new regulations, but also the development of new controls on processes, quality and fraudulence. The development of more sensitive rheometers and more powerful software allows to calculate different characteristic parameters in an easy, precise and quick way, and that is why rheometry is recently getting great importance in food industry for process control, equipment design, and quality control (Menéndez et al., 2006). Therefore, rheometry of high resolution could be a useful technique to achieve new quality and other technical controls, so the aim of this work is to study the possibilities of some rheological measurements on egg yolk in order to control these topics in egg production and transformation industry. These studies will mainly concern the detection of mixtures of yolk from different fowl species, influence of storage time and conditions on structural changes and quality and influence of feeding on composition and hence on some technical processing properties like coagulation.

2. Materials and methods

Sample preparation. Egg yolks were prepared from fresh eggs coming from different species of fowls with a controlled feeding. The shelling of the eggs and the separation of the yolk from the albumen were performed manually. The albumen residuals were eliminated from the yolk using a blotting paper, and the removal of the vitelline membrane was achieved using tweezers. For the different analyses developed, the yolks were manually mixed with a spatula.

Analyses. The pH was measured at 20ºC using a Crisom micro 2002 pH meter. The dry matter was assessed with a HR73 Halogen Moisture Analyzer, the amount of sample was about 0.25 g. The rheological tests were developed with a Haake RS50 RheoStress rotational rheometer with a plate/plate measuring system (PP60), a gap of 1 mm and the sample amount was about 2.9 g. In steady state, flow curves were carried out in CS mode at 5±1ºC from 0 to 500 Pa of shear stress during 300s. The dynamic test ("cure test") were developed in the linear vicoelasticity range that was previously established, the frequency was 1Hz, the temperature ramp was from 5ºC to 80ºC at a heating rate of 0.6 ºC/min and they were carried out in CS mode at a constant shear stress of 10 Pa.

The differential scanning calorimetry tests were carried out in aluminium pans hermetically sealed, in a DSC 822e, developing temperature ramps from 5ºC to 80ºC at a heating rate of 0.6 ºC/min.

3. Results and discussion

Three lines of investigation were followed. Firstly, the evolution of egg yolk rheologic characteristics with storing time at its typical temperature of storage, 4ºC, was studied: the corresponding flow curves were determined and the values of their "n" (behaviour index) and "K" (consistency index) parameters were calculated fitting to the Power Law equation (1) the data obtained from these curves.

$$\tau = K.\dot{\gamma}^{n} \qquad (1)$$

A total number of 24 samples coming from caged Leghorn hens fed with laying hen fodder were analysed. An example of the flow curves obtained is shown in figure 1.

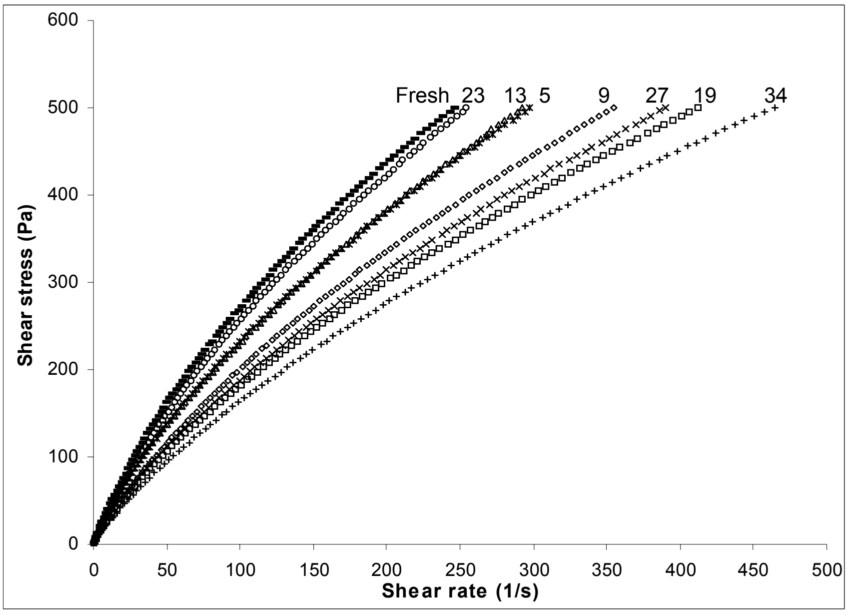

Figure 1: Flow curves corresponding to yolks from eggs of different days of age coming from caged laying Leghorn hens

Egg age (days)	"n" (dimensionless)	"K" ($Pa.s^n$)	% Dry Extract	pH
1	0.679	12.9	54.3	5.93
5	0.675	12.1	53.9	5.97
9	0.696	10.2	52.6	6.01
13	0.702	9.8	53.9	6.00
19	0.685	10.1	52.5	6.06
23	0.692	11.2	52.8	6.03
27	0.695	8.9	53.0	6.07
34	0.694	8.2	51.8	6.15

Table 1: Values of different yolk parameters obtained from eggs of different age coming from caged laying Leghorn hens

As can be seen in table 1, the results of the assays proved that, under controlled feeding conditions, certain modifications in egg yolk can be observed between 9 and 13 days of age. The evolution of the rheological parameters, pH and wet content show the physicochemical changes of egg yolk with storage time. The study of chemical and physical yolk characteristic evolution during the storage of shell eggs was also carried out in foregoing works (Hidalgo et al., 1996), but these modifications weren't described previously. A detailed study of the reasons for these modifications and their physicochemical implications would be useful in the egg quality control, mainly in order to extend the egg conservation time.

Secondly, different hen breeds (Leghorn, Bantam and Asturian Regional hen) were characterised. The parameters "n" and "K" were calculated in the same way explained before. Despite of their important morphological differences, all the breeds studied belong to the same specie *Gallus domesticus*. However, as can be seen in table 2, the values of "n" and "K" are different enough to differentiate them, so it could be possible to determinate egg yolk origin based on the value of these parameters.

		"n" (dimensionless)	"K" ($Pa.s^n$)
Leghorn	Average	0.688	9.7
	SD	0.022	3.2
Asturian Regional hen	Average	0.677	12.6
	SD	0.015	3.6
Bantam	Average	0.668	7.8
	SD	0.029	3.2

Table 2: Values of "n" (dimensionless) and "K" ($Pa.s^n$) of egg yolks obtained from different hen breeds

According to these results, and as it was to be expected, the values of "n" and "K" could be quite different with the type of fowl. Thus, and following the same procedure, three different fowl species (guinea fowl, common pheasant and domestic duck) were characterised. The values obtained are shown in table 3.

		"n" (dimensionless)	"K" ($Pa.s^n$)
Guinea fowl *(Numida meleagris)*	Average	0.596	39.6
	SD	0.040	13.8
Common pheasant *(Phasianus colchicus)*	Average	0.680	5.5
	SD	0.036	1.6
Domestic duck *(Anas domesticus)*	Average	0.705	15.1
	SD	0.020	2.4

Table 3: Values of "n" (dimensionless) and "K" ($Pa.s^n$) of egg yolks obtained from different fowl species

The consistency index seems to show better than the behaviour index the genetic differences between the fowls studied. These could be very useful at the time of detecting adulterations of hen egg yolk used as raw material, in pastry industry, for instance. With such aim, an experiment with mixed yolks was also performed. Mixtures of yolks in different percentage of domestic hen and guinea fowl were measured and their "n" and "K" indexes were calculated.

In table 4 the "K" index values are shown. The values of "K" are hence a good index of the genetic differences and were fitted to a straight line giving the following equation: K = 0.2911(guinea fowl yolk %) + 0.5804. As can be observed in figure 2 the fitting was quiet good (R^2 = 0.9804) and can allow to determine the proportion of different yolks coming from of a mixture of two different origins in a given sample of mixed yolks.

Yolk percentage *Numida meleagris*	Yolk percentage *Gallus domesticus*	"K" ($Pa.s^n$)	
		Average	SD
10%	90%	3.7	2.8
25%	75%	7.5	4.9
50%	50%	14.3	2.8
75%	25%	24.6	3.4
90%	10%	25.5	6.1

Table 4: Parameter "K" values for different percentage (p/p) of mixtures of yolks coming from domestic hen *(Gallus domesticus)* and guinea fowl *(Numida meleagris)*

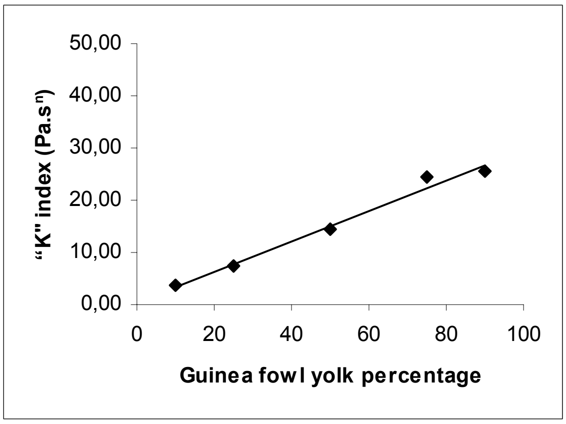

Figure 2: Values of "K" constant obtained for different percentage (p/p) of mixtures of yolks coming from domestic hen *(Gallus domesticus)* and guinea fowl *(Numida meleagris)* fitted to a straight line

Thirdly, yolk coagulation kinetics was analysed using dynamic tests, and a DSC analysis was also required. During gelation, yolk undergoes a phase transition from a liquid to a gel. The sol-gel transition is a critical point where the transition variable will be the connectivity of the physical and chemical bonds linking the basic structural units of the yolk. Experimental detection of the gel point is not easy, however different studies (Rhao, 1999) suggested that the gel point occurs at the time at which G' (storage modulus) and G'' (loss modulus) cross each other at given frequency. In this work the gel point was detected in this way.

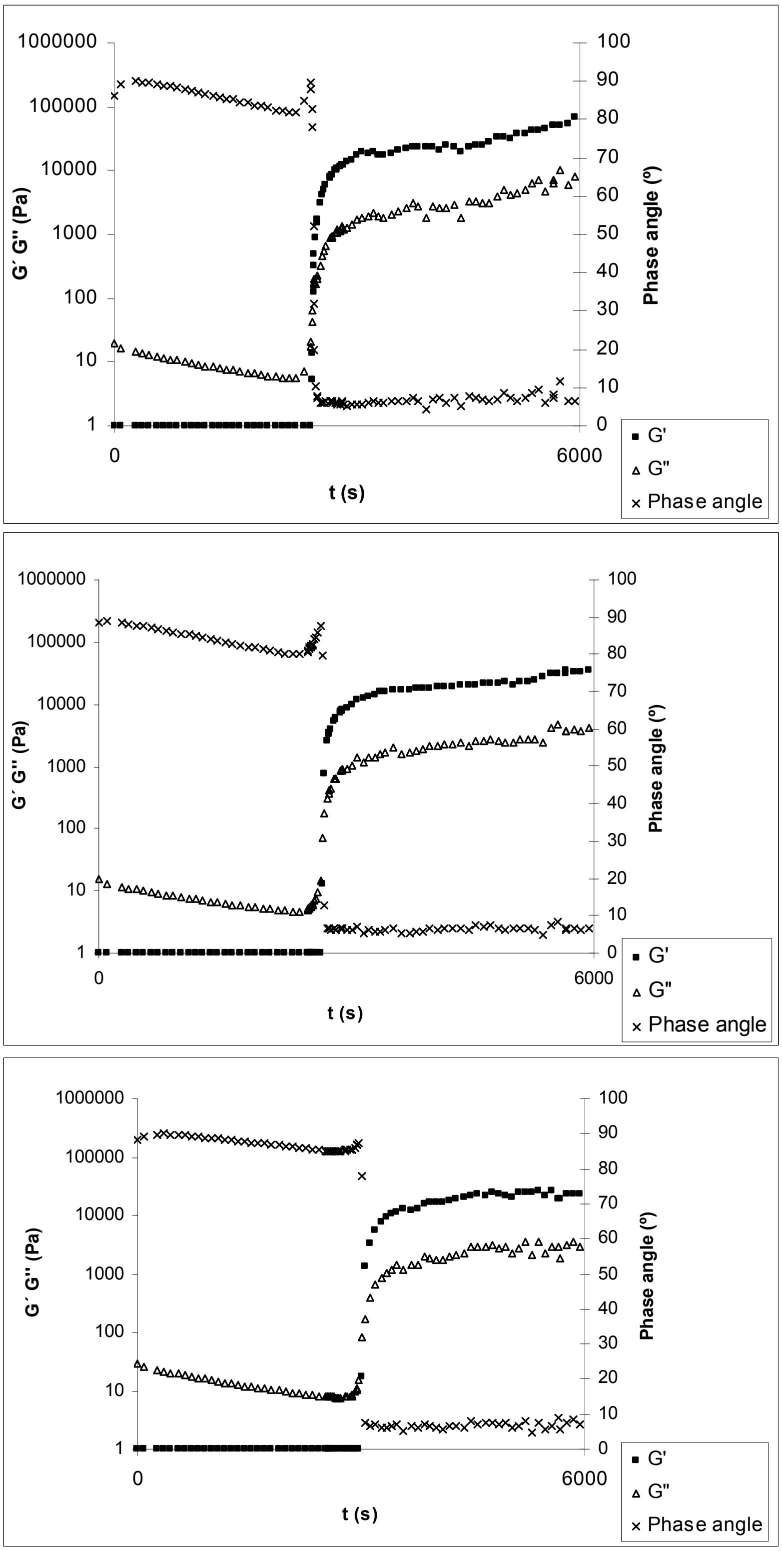

Figure 3: Cure tests for yolk eggs coming from hens with an omega-3, traditional and ecological diet (from top to down)

Three different ways of feeding the hens were developed: a "traditional diet" refers to a diet of specific laying hen fodder, an "omega-3 diet" refers to a specifically feeding with a high unsaturated acids content (the percentage of unsaturated acids in these eggs in relation to the total lipids is 5.5%) and an "ecological diet" refers to a diet of corn supplemented with vegetables and little invertebrates.

The cure test realized on yolks samples from eggs of differently fed hens are shown in figure 3. As can be seen in table 5 the values of the modules G' and G'' at the gelation point are different for hens differently fed. These different values are due to the different composition of egg yolk and therefore to its proportion of proteins, as proteins are the main responsible of coagulation kinetics (Kiosseoglou, 2003; Cordobés et al., 2004).

	G' (Pa)	G'' (Pa)
Omega-3 Diet	196.8	171.9
Traditional Diet	225.7	188.2
Ecological Diet	307.4	207.6

Table 5: Values of the parameters G' (storage modulus) (Pa) and G'' (loss modulus) (Pa) at the gelation point of egg yolks obtained from hens differently fed

The proportion of lipids is greater in yolks coming from hens traditionally fed than in those from hens ecologically fed, so its proportion of proteins is smaller. Due to these larger content of lipids, its gel point happens at smaller values of the storage (G') and loss (G'') modules. The highest fraction of lipids happens in "omega-3" yolks, these yolks come from hens specifically fed with a high unsaturated acids diet, so the protein content is lower than in the other yolks studies; that is why its gel point happens at values of G' and G'' even shorter than in the case of hens with a traditional diet.

Therefore, the point of gelation occurs at different conditions depending on the way of feeding the laying hen. After the different values of G' and G'' modules were determined for the different way of feeding the hen by the cure tests, DSC analyses were performed to assess the characteristic temperature of coagulation for the different feedings. These analyses for the three different ways of hen feeding are shown at figure 4. A well pronounced endothermic peak defines the proteins denaturation temperature (Cordobés et al., 2004); this denaturation coincides with the moment of coagulation and is different for hens differently fed: it occurs at 70ºC for the ecological diet, at 78ºC for the traditional diet and at 80ºC for the "omega-3" diet. These different temperatures of coagulation are too determined by the proportion of proteins contained in the yolk: if the proteins amount is higher, the gelification network begins to turn up at lower temperatures.

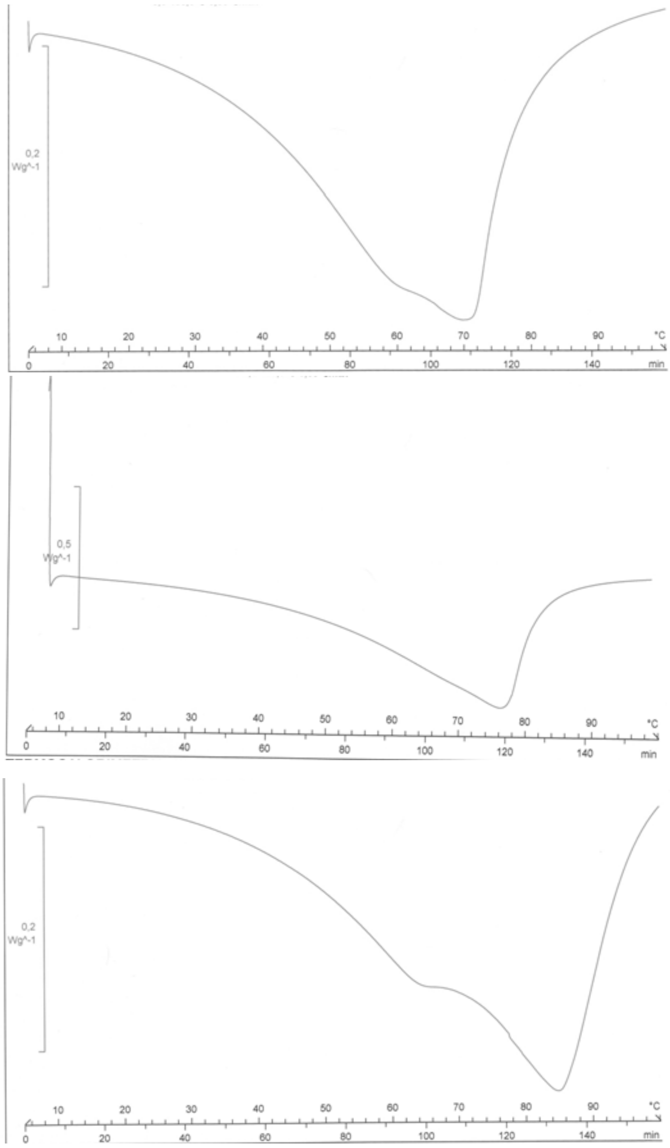

Figure 4: DSC analysis for yolk eggs coming from hens with an ecological, traditional and omega-3 diet (from top to down)

4. Conclusions

Rheological measurements can have important applications in egg industry. The egg yolks between 9 and 13 days old have shown interesting modifications. These modifications were shown not only by steady tests, but also by physicochemical analyses. A detailed study of these modifications could be of interest in the design of egg conservation conditions and therefore its quality control. Steady test also allow to determine differences between different fowl species according to their values of "n" and "K", so these indexes could be used to determine the origin of different yolk samples. The consistency index, "K", reflects better the genetic differences between fowls. As it was showed, this fact can be useful for detecting mixtures or adulterated yolk samples.

Changes in the gel structure with temperature depend on the relative protein proportion of the yolk, and hence of the way of feeding the hens, as it has been determined by DSC analysis and by cure test, giving the values of G' and G'' modules at the gelation point. In conclusion, dynamic rheological tests, particularly "cure tests", and DSC analysis, make possible to improve the knowledge of yolk coagulation kinetics which is a key parameter in many egg yolk industrial applications.

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