

Evolution of Chemical and Physical Albumen Characteristics during the Storage of Shell Eggs

Mara Lucisano,* Alyssa Hidalgo, Elena M. Comelli, and Margherita Rossi

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy

The aim of this paper was to study the evolution of furosine, pyroglutamic acid, and uridine and rheological characteristics of albumen from shell eggs stored over long times at 30, 20, and 5 °C. The chemical variables showed an increase in function of the storage temperature with more evident variations at room temperatures. At 5 °C, furosine increase was very low, while uridine presented a rapid increase after 150 days; pyroglutamic acid, instead, showed an even increase throughout the storage. The main decrease of viscosity happened during the first storage period; viscosity remained almost constant after 4, 6, and 36 days at 30, 20, and 5 °C, respectively. During the storage of eggs, a transition from pseudoplasticity to Newtonianity was observed, while the aggregation temperature, evaluated through viscosity measurements, remained constant at 61–62 °C. Furosine, pyroglutamic acid, and uridine were generally well-correlated ($P > 99\%$) between them, while the correlation with viscosity was lower.

Keywords: Albumen; egg freshness; furosine; pyroglutamic acid; rheological behavior; uridine

INTRODUCTION

Immediately after they are laid, aging processes begin in shell eggs, altering their chemical, physical, and functional characteristics. These modifications depend on the temperature of storage and follow different kinetics depending on the reactions involved. Well-known phenomena are the increase of albumen pH (Powrie, 1977; Burley and Vadehra, 1989), the thinning of thick albumen (Burley and Vadehra, 1989), the evaporation of water through the shell, the weakening of the vitelline membrane that surrounds the yolk, and the increase in volume of the air cell (Thapon, 1994).

According to the European Union (EU) legislation, a shell egg is fresh up to 28 days after being laid (EU, 1994); furthermore, it must not be exposed to preservation treatments (refrigeration at temperatures lower than 5 °C, oiling, etc.). Fresh eggs must present normal internal and external characteristics, an absence of off-flavors, and an air cell height lower than 6 mm (EEC, 1991). Other chemical or physical parameters such as the Haugh Unit are not taken into consideration.

Recently, new chemical indices that vary during the storage of eggs have been considered as descriptors of shell egg freshness (Rossi et al., 1995a). Among them, uridine and pyroglutamic acid increase in the albumen and in the yolk during the storage of eggs, depending on the temperature. Relying on these indices and considering also their natural variability (Rossi and Pompei, 1995), tentative limits for egg classification in markets where eggs are kept at room temperatures throughout the whole commercialization cycle (i.e. EU) were proposed by Rossi et al. (1995a). Furosine, an indicator of the initial steps of Maillard reaction, has also been proposed as a descriptor of shell egg freshness because of its reproducibility and low natural variation in fresh eggs (Hidalgo et al., 1995). The descriptors proposed by Rossi et al. (1995a) and by Hidalgo et al. (1995) are preferably evaluated in the albumen, the egg fraction that undergoes the major changes during storage. Since these parameters are not influenced by heat treatments of pasteurization (Rossi et al., 1995b), they may also be useful for evaluation of the freshness

of the raw material used for egg products (pasteurized albumen, pasteurized whole egg); hence, it is necessary to better investigate their evolution in albumen from eggs stored over long periods of times.

Besides the reaction of Maillard, the proteins of the albumen are involved in other phenomena that change their aggregation status. The thinning of the thick albumen is an example of such modifications and may depend on the pH (Burley and Vadehra, 1989), whose value influences the breakage of the ovomucin–lysozyme complex (Kato et al., 1975). This complex is stabilized by electrostatic linkages and is responsible for the superior viscosity of fresh egg albumen (Kato et al., 1971, 1979; Sauveur and de Reviers, 1988). The amount of the ovomucin–lysozyme complex in the thick albumen is almost directly proportional to its apparent viscosity (Robinson and Monsey, 1972). Similarly, Beveridge and Nakai (1975) utilized viscosity values to assess thick albumen thinning.

Many authors described the rheological behavior of fresh albumen as a function of measuring time (Tung et al., 1970; Beveridge and Nakai, 1975; Pitsilis et al., 1984), shear rate, and temperature (Payawal et al., 1946; Tung et al., 1970; Scalzo et al., 1970; Pitsilis et al., 1975, 1984; Gossett et al., 1983). On the contrary, very few studies are concerned with the modifications of the rheological behavior of albumen during shell egg storage. Tung et al. (1970) described a decrease of the albumen consistency index (K) from 1.9 to 1.6 $\text{dyn}\cdot\text{s}^n\cdot\text{cm}^{-2}$ (0.19–0.16 $\text{Pa}\cdot\text{s}^n$) for eggs stored at 20 °C for 2–4 weeks but did not find any variation in the flow behavior index (n). A decrease of the apparent viscosity of thick albumen fraction from 9.9 to 4.8 cP ($9.9\text{--}4.8 \times 10^{-3}$ Pa·s), with a shear rate of 390.9 s^{-1} , after 47 h of conservation at 37 °C, and the evolution toward a Newtonian-type behavior have been reported by Robinson and Monsey (1972).

Albumen proteins undergo structural modifications during storage; these modifications lead to a different behavior of the product when it is heated. Ovalbumin changes to a more thermostable form (Donovan and Mapes, 1976; Rossi et al., 1992) with a denaturation

temperature of 89 °C (Shitamori et al., 1984), compared to a denaturation temperature of 81.6 °C for the native protein. The sudden increase in viscosity during albumen heating was utilized by Richardson and Ross-Murphy (1981) to determine the temperature of formation of the insoluble gel network structure.

The aim of this research was to study the evolution of furosine, pyroglutamic acid, and uridine in albumen over long storage times of shell eggs at different temperatures. Furthermore, the rheological behavior of the albumen was examined with the aim of providing some useful information for the choice of pasteurization conditions. Finally, the relationships between rheological parameters and chemical characteristics were also scrutinized.

MATERIALS AND METHODS

Eggs. A lot of grade A, extra shell eggs of weight class 1 (70–75 g) (EEC, 1990, 1991), laid by Isa-Brown Warren hens (69 weeks old, 1 week after molting), directly from the producer, and available in the laboratory 24 h after being laid, was used. The egg lot was divided into three groups and stored in thermostat chambers at 30, 20, and 5 °C for 31, 98, and 204 days, respectively. Storage at 5 °C was continued up to 354 days to study the formation of furosine, pyroglutamic acid, and uridine.

Sample Preparation. The measurements were made on bulked albumen from four random eggs per group; after manual shelling of the eggs, yolk and albumen were separated and the chalazae were removed. For the rheological tests, the albumens were homogenized at 2000 rpm for 10 s with a Sorvall Omni Mixer (Model 17106, DuPont de Nemours & Co., Newton, CO). The mixed sample was then filtered twice through a 20 mesh sieve. For the chemical analyses, the samples were further mixed at 3000 rpm for 15 s.

Chemical Analysis. The pH was detected potentiometrically, using a PHM82 Standard pH Meter (Radiometer Analytical A/S, Bagvaerd, Denmark).

Dry matter (average of two measurements) was assessed following the AOAC method no. 925.30 (AOAC, 1990).

Furosine content (milligrams per 100 g of protein) was computed as the average of three replicated analyses and following the HPLC method as proposed for milk by Resmini et al. (1990), slightly modified for egg as described by Hidalgo et al. (1995a). A calibration curve was prepared in the concentration range between 0 and 5 $\mu\text{mol/L}$ hydrated furosine $\cdot 2\text{HCl}$ (Neosystem Laboratoire, Strasbourg, France) in 3 N HCl, following Hidalgo et al. (1995), which produced a linear relationship with a determination coefficient (r^2) > 0.999 and a detection limit of 0.11 $\mu\text{mol/L}$ in the injected standard solution.

Protein content was calculated as total nitrogen multiplied by the factor 6.25 and expressed as grams of protein per 100 g of product. Total nitrogen analysis was performed using the Kjeldahl method (no. 925.31; AOAC, 1990).

Pyroglutamic acid and uridine analyses were performed by HPLC following the procedure described by Rossi and Pompei (1995). The results, expressed as parts per million of each compound, are the average of duplicate analyses. The pyroglutamic acid (Fluka AG, Buchs, Switzerland) calibration curve was prepared using 8 different standard concentrations, from 0.2 to 4 ppm, while the uridine (Sigma Chemical Co., St. Louis, MO) calibration curve was prepared using 15 different standard concentrations, from 0 to 60 ppm.

The repeatability of the method for pyroglutamic acid and uridine determination was assessed by replication of the measurement ten times on the same albumen sample from commercial eggs. The results are expressed in terms of mean \pm standard deviation (SD) and coefficient of variation (CV).

Rheology. Rheological tests were made on 13 mL samples using a controlled rate Böhlin VOR Rheometer (Böhlin Reologi AB Corporate Headquarters, Lund, Sweden) with a closed concentric cylinder measuring system (C25), a torsion bar of

Table 1. Consistency Index (K), Flow Behavior Index (n), and Dry Matter in Albumen of Eggs Stored at 30, 20, and 5 °C

storage temp (°C)	time (days)	shear rate (9.2–46.0 s ⁻¹)		shear rate (23.2–146.0 s ⁻¹)		dry matter (%)	
		K ($\times 10^{-2}$)	n	K ($\times 10^{-2}$)	n		
30	fresh	0.64	6.90			11.00	
	4	0.79	2.01			11.50	
	9	0.88	1.11				
	11			1.01	0.58	12.85	
	18			0.91	0.95	13.20	
	22			0.93	0.92	14.02	
	29			0.99	0.72	14.70	
	20	7	0.80	2.18			12.25
	10	0.73	2.69			12.40	
	16			0.83	1.70	12.26	
20	24			0.91	1.04	12.75	
	28			1.07	0.46	13.00	
	35			1.04	0.49	13.25	
	42			0.99	0.70	13.55	
	98			0.91	1.20		
	5	8	0.68	5.05			11.91
	21	0.71	3.79			11.85	
	31	0.77	3.11			12.17	
	36	0.82	2.47			12.22	
	46			0.96	0.81	12.33	
5	57			0.97	0.93	12.75	
	100			1.07	0.49	12.50	
	147			0.99	0.80	13.45	
	171			1.00	0.75	13.71	
	204			0.96	1.28	13.30	

20.83 g \cdot cm, and a gap of 0.15 mm. The rheological behavior of albumen was studied with two series of trials in rotation (repeated three times), the first series to evaluate shear stress (τ) as a function of shear rate ($\dot{\gamma}$) (flow curve) at 20 °C and the second series to assess viscosity (η) as a function of temperature at a constant shear rate ($\dot{\gamma} = 14.6 \text{ s}^{-1}$).

In the first series of trials, every measurement was from two sequential cycles of assessment on the very same sample, with a shear rate increasing and then decreasing continuously, and no rest period between cycles. Experimental data of the second flow curve with an increasing shear rate were elaborated following the power law equation ($\tau = K(\dot{\gamma})^n$) using a data elaboration program that came with the rheometer (Böhlin software) to obtain the viscosity curves ($\eta = K(\dot{\gamma})^{n-1}$). The values are the average of three measurements.

To evaluate the rheological characteristics of the albumen, two different shear rate intervals (from 9.2 to 46.0 s⁻¹ and from 23.2 to 146.0 s⁻¹) were used during storage, as reported in Table 1. As albumen viscosity decreased during egg aging, in order to have measurements falling in the detection field of the instrument, it was necessary to use shear rates superior to 23.2 s⁻¹.

The very same sample, after the two cycles of increasing and decreasing shear rate, was warmed with a heating rate of 2 °C/min and with $\dot{\gamma} = 14.6 \text{ s}^{-1}$ to detect the temperature of aggregation from the sudden viscosity increase.

Data Analyses. The interpolation curves of the figures were computed using *Table curve* software. The correlation analyses (following the Pearson approach) among all measured variables were computed with *Systat* software.

RESULTS AND DISCUSSION

Chemical Analyses. Albumen pH was dependent on the temperature of storage and showed a rapid increase during the initial 5–10 days, as observed by Donovan and Mapes (1976), followed by a decrease over long storage times at 20 and 5 °C. The values were always superior to pH 9.

Dry matter content (Table 1) increased linearly as a function of storage temperature as a consequence of water evaporation through the shell and the migration

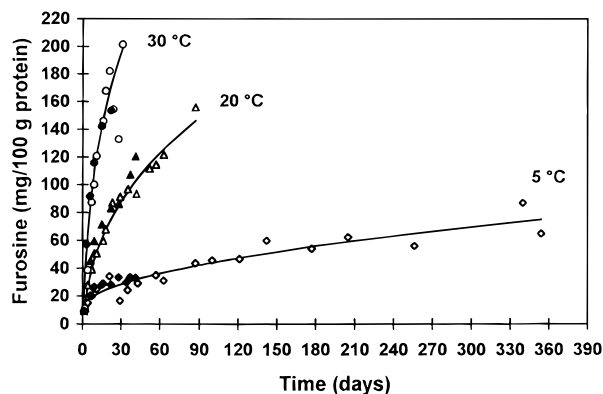


Figure 1. Albumen furosine content during storage of eggs at 30, 20, and 5 °C. Solid symbols represent furosine content in the albumen of another lot of eggs, as reported by Hidalgo et al. (1995). The interpolation curves were calculated on the basis of our data only (open symbols).

of water through the vitelline membrane from the albumen to the yolk (Sauveur and de Reviere, 1988). Considering the dry matter values of yolk reported by Hidalgo et al. (1996), a mass balance between fresh egg and eggs stored for different times was computed, showing that water evaporation through the shell is the most important phenomenon ($83.3 \pm 9.9\%$ of the water loss).

Figure 1 depicts furosine content in the albumen of shell eggs stored at the three temperatures. The results obtained by Hidalgo et al. (1995) during the storage of eggs of 64-week-old Isa-Brown hens are presented for comparison (but they have not been used to compute the regression curves). There is a perfect agreement between the two series of results obtained with the two egg lots laid by hens of different ages and breeding factors. For long storage times, a decrease of furosine due to the progress of the Maillard reaction has never been observed.

Pyroglutamic acid and uridine calibration curves were linear ($r^2 = 0.999$) in the concentration ranges studied. Detection limits in the standard solution injected resulted in 0.15 and 0.40 ppm, respectively. The repeatability of the method, expressed in terms of mean \pm SD and coefficient of variation (CV), was 4.84 ± 0.27 ppm (CV = 5.57%) for pyroglutamic acid and 13.18 ± 0.15 ppm (CV = 1.15%) for uridine. On the basis of CV values, the method repeatability in albumen for both acids was very good (Horwitz, 1983).

Pyroglutamic acid and uridine contents of albumen during the storage of eggs at the three temperatures are presented in Figures 2 and 3, respectively, along with their regression curves. The concentration of pyroglutamic acid in the albumen of fresh eggs was extremely low (about 1 ppm) and increased linearly as a function of temperature and storage time, even under refrigeration. The values are consistent with those observed by Rossi et al. (1995a) in albumen of eggs from 68-week-old Isa-Brown Warren hens but are far lower than those observed by the same authors for albumen of eggs from 40-week-old hens of the same breed. Rossi and Pompei (1995), studying pyroglutamic acid content in fresh egg yolk as influenced by the age of layers, observed a peak with higher concentration levels at about 40–47 weeks of life, while the levels in fresh egg albumen were practically nil at all hen ages. The increase of pyroglutamic acid in albumen during storage may be due, at least partially, to the transfer of this compound from the yolk (naturally rich even in fresh

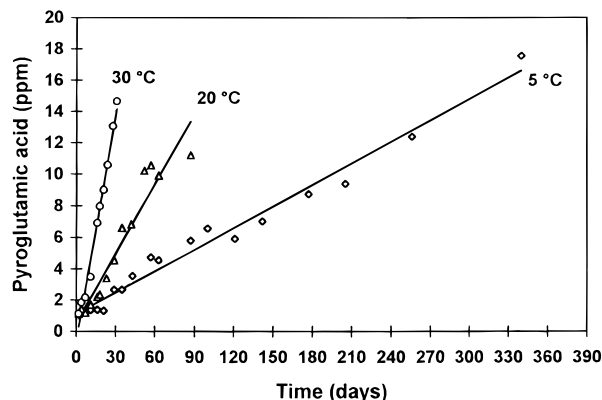


Figure 2. Pyroglutamic acid content in albumen during storage of eggs at 30, 20, and 5 °C.

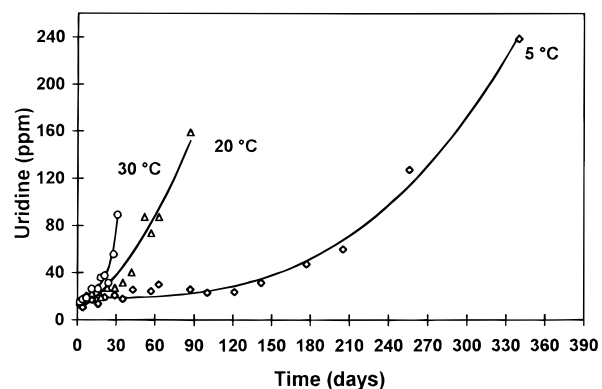


Figure 3. Uridine content in albumen during storage of eggs at 30, 20, and 5 °C.

eggs) as a consequence of the weakening of the vitelline membrane.

The uridine content of albumen increased exponentially during the storage of eggs, depending on temperature. At 5 °C, the uridine concentration started from 15.3 ppm, remained constant up to 150 days, and increased rapidly afterward, reaching a value of 240 ppm after 340 days. This late increase may be interesting for detection of eggs stored over long periods (above 5 months) at 5 °C.

Rheological Analysis. The albumen of fresh and short-stored eggs showed a slight hysteresis, since the increasing shear rate flow curves were not identical to the decreasing ones. In the albumen of short-stored eggs (10 days at 20 °C, 21 days at 5 °C), this behavior was just evident and was absent for longer storage times. This is a consequence of the thinning of the albumen during egg aging; in stored eggs, the albumen has lost its original structure and its behavior becomes time-independent. A similar result was obtained by Tung et al. (1970) with a shear rate application time of 60 s ($\dot{\gamma} = 147 \text{ s}^{-1}$). Beveridge and Nakai (1975) evaluated the changes in shear stress decay curves at a shear rate of 1370 s^{-1} and at 1 °C, which occur in thick albumen held at 37 °C up to 5 days, and showed lower viscosity values and a less evident thixotropy at increasing storage times. Pitsilis et al. (1984), studying the behavior of the albumen in eggs stored at 10–12 °C for 12 days, found a lag time to the equilibrium stress of 100–20 s, depending on the shear rate applied (between 36.7 and 345 s^{-1}); the higher the rate, the lower the time requested.

For the evaluation of viscosity variation during egg aging, the second flow curve made with an increasing shear rate was considered. Figure 4 reports the viscos-

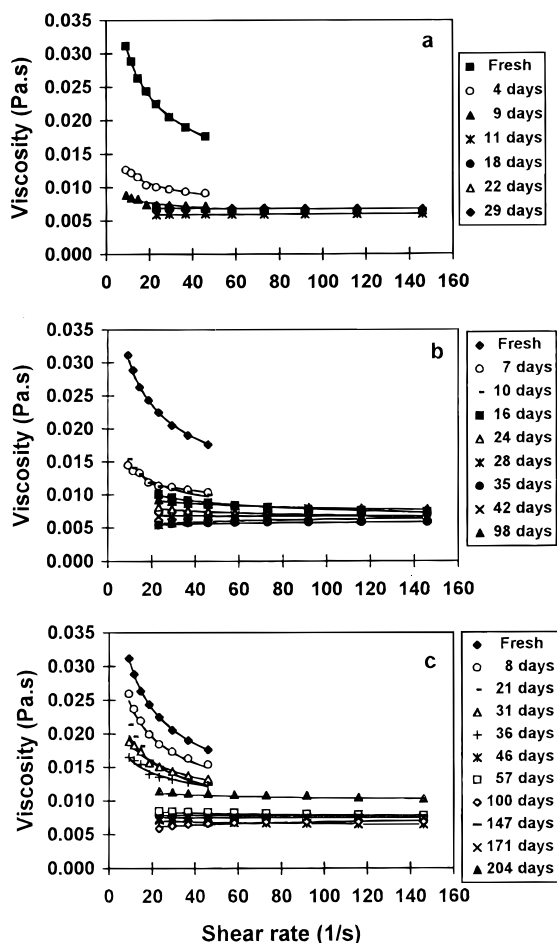


Figure 4. Viscosity as a function of increasing shear rate for albumen of eggs fresh and stored at 30 (a), 20 (b), and 5 °C (c).

ity curves of albumen from eggs stored at 30 (a), 20 (b), and 5 °C (c) for different times. Table 1 shows the values of the flow behavior index (n) and of the consistency index (K), computed with the power law. Fresh egg albumen presented an n value of 0.64, suggesting a pseudoplastic behavior for shear rates between 9.21 and 46 s^{-1} . A comparison of our viscosity values with those reported in the literature is difficult, because of the different shear rates adopted and the different sample preparation methods. An n value of 0.563, comparable with the value in our work, was obtained by Tung et al. (1970), analyzing nonmixed albumen samples from fresh eggs and using shear rates between 8.1 and 147 s^{-1} at 20 °C. On the other hand, working at superior shear rates (38.8–3140 s^{-1}) and at 20 °C, Gossett et al. (1983) reported an n value of 0.83 for albumen samples at pH 9.

Pitsilis et al. (1975), analyzing fresh egg albumen, found a viscosity of 7×10^{-3} Pa·s ($\dot{\gamma} = 36.7$ s^{-1} , at 18 °C and a pH of 9), lower than our value (19×10^{-3} Pa·s at $\dot{\gamma} = 36.7$ s^{-1}). This difference may be due to the more energetic homogenization of the sample (6000 rpm for 45 s) used by many authors. Scalzo et al. (1970) showed viscosity values of 3.5×10^{-3} Pa·s at 20 °C and values even lower for albumen samples obtained from three industries, stabilized at pH 7, hand mixed further and filtered through a sieve before analysis. The same authors found a Newtonian behavior in the shear rate utilized ($\dot{\gamma} = 10$ –5000 s^{-1}).

During egg storage, a decrease of viscosity was observed. An increase of the flow behavior index over

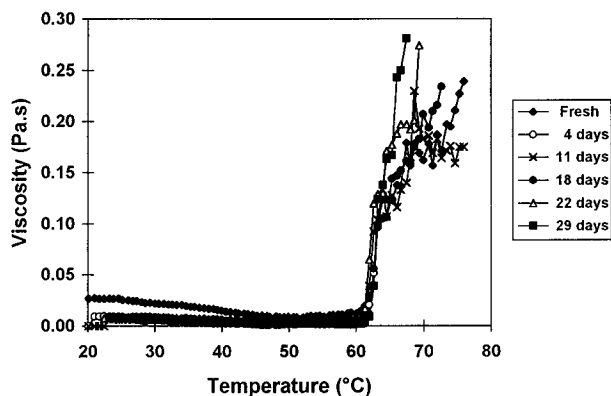


Figure 5. Viscosity as a function of temperature ($\dot{\gamma} = 14.6$ s^{-1}) for albumen of eggs fresh and stored at 30 °C.

all temperatures was also evident, indicating a transition from pseudoplasticity to Newtonity (Table 1).

The viscosity of the albumen depends on the integrity of the ovomucin–lysozyme complex, and particularly on the β -fraction of ovomucin, rich in carbohydrates (Kato et al., 1970a). The ovomucin concentration is 4 times higher in thick albumen than in thin albumen (Burley and Vadehra, 1989), and its complex with lysozyme is stronger (Kato et al., 1970a). The destabilization of the complex is due to a pH increase that, during storage, approaches the lysozyme isoelectric point (10.7). Hence, the β -fraction of ovomucin separates (Kato et al., 1981) and is released in solution (Sato et al., 1976; Kato et al., 1971), causing the thinning (Robinson and Monsey, 1972) and the decrease (Kato et al., 1970b; Rossi et al., 1995a) of thick albumen.

The strongest viscosity variations happened during the initial storage days (Figure 4), when the highest pH variation for all temperatures occurred. At 30 °C, the viscosity, measured at $\dot{\gamma} = 36.7$ s^{-1} , decreased up to 11 days, from 19×10^{-3} Pa·s in the fresh product to 5.9×10^{-3} Pa·s in the stored product; after this period, there was no further increase of pH and the viscosity curves were overlapping. For the albumen of eggs stored at 20 and 5 °C, viscosity at $\dot{\gamma} = 36.7$ s^{-1} decreased up to 35 and 46 days of storage, reaching values of 5.6×10^{-3} and 6.9×10^{-3} Pa·s, respectively. Afterward, the viscosity increased at the same time as a slight decrease of water content and pH.

Viscosity curves during albumen heating from fresh eggs and eggs stored at 30 °C are presented in Figure 5. The results at 20 and 5 °C are not reported because they are analogous. A sudden increase in viscosity happened at 61–62 °C, indicating the initial formation of egg albumen aggregates. Hsieh and Regenstein (1992), studying the concentration of soluble proteins, determined a temperature of phase transition of 60 °C. The initial temperature of albumen aggregate formation was about 61 °C by the loss of energy detected with a thermal-scanning rigidity monitor (Montejano et al., 1984) and was about 61.5–62.5 °C by the determination of gel strength through a compression test (Johnson and Zabik, 1981).

The increase of viscosity at 61–62 °C is coincident with the beginning of the endothermic peak of the DSC thermograms corresponding to the conalbumin denaturation, as shown by Donovan et al. (1975) working with albumen at pH 9, and by Rossi et al. (1992). For pure conalbumin at pH 8, Hegg et al. (1978) reported a denaturation and precipitation temperature of 60 °C.

At temperatures above 64 °C, the viscosity increases irregularly because of the aggregate mechanic breakage,

Table 2. Correlation Coefficients between the Considered Variables

		dry matter	furosine	pyroglutamic acid	uridine	apparent viscosity ^a
pH	30 °C	0.701 ^c	0.821 ^c	0.574 ^b	0.427	-0.960 ^c
	20 °C	0.250	0.329	0.035	-0.076	-0.888 ^c
	5 °C	-0.176	-0.092	-0.241	-0.202	-0.198
	overall	0.172	0.691 ^c	0.103	-0.125	-0.669 ^c
dry matter	30 °C		0.892 ^c	0.946 ^c	0.895 ^c	-0.738
	20 °C		0.966 ^c	0.939 ^c	0.856 ^c	-0.962 ^c
	5 °C		0.864 ^c	0.944 ^c	0.882 ^c	-0.755 ^c
	overall		0.645 ^c	0.943 ^c	0.800 ^c	-0.779 ^c
furosine	30 °C			0.838 ^c	0.707 ^c	-0.805 ^b
	20 °C			0.915 ^c	0.830 ^c	-0.960 ^c
	5 °C			0.920 ^c	0.711 ^c	-0.716 ^b
	overall			0.642 ^c	0.300 ^b	-0.685 ^c
pyroglutamic acid	30 °C				0.882 ^c	-0.586
	20 °C				0.878 ^c	-0.773 ^b
	5 °C				0.886 ^c	-0.680 ^b
	overall				0.797 ^c	-0.616 ^c
uridine	30 °C					-0.608
	20 °C					-0.831 ^b
	5 °C					-0.390
	overall					-0.511 ^c

^a Measured at $\dot{\gamma} = 36.7 \text{ s}^{-1}$. ^b Significant at the 5% level of probability. ^c Significant at the 1% level of probability. Degrees of freedom ($n - 2$) for the correlation between chemical variables: 30 °C, 12; 20 °C, 17; 5 °C, 27; overall = 59. Degrees of freedom ($n - 2$) for the correlation between apparent viscosity and chemical variables: 30 °C, 4; 20 °C, 6; 5 °C, 9; overall = 22.

giving nonsignificant values. Hence, it was not possible to identify the contribution of other proteins and especially of the ovalbumin, which has a starting aggregation temperature of 72 °C (Hsieh and Regenstein, 1992) and a denaturation temperature of 81.1–82 °C (Shitamori et al., 1984).

From the curves of Figure 5, it is possible to deduce that the aggregation temperature stays constant at ca. 61–62 °C; the rheological analysis only records the conalbumin contribution, which does not vary during storage, showing the same denaturation temperature (Rossi et al., 1992). Similarly, for the albumen of eggs stored at 12.8 °C for 24 days and at 35 °C for 7 days, Meehan et al. (1962) found an aggregation temperature of 61 °C.

Correlation between Variables. Table 2 contains the results of the correlation analysis between the variables for every storage temperature and for all the temperatures together (overall). The coefficient of correlation depends on the storage temperature, as well as on the correlated variables. At 36.7 s^{-1} , the apparent viscosity is negatively correlated to pH, dry matter, furosine, pyroglutamic acid, and uridine. Although dry matter increases during storage, the viscosity decreases because of the predominant phenomenon of thick albumen thinning.

The correlations between dry matter, furosine, pyroglutamic acid, and uridine are highly significant and similar at all the storage temperatures. The overall coefficients of correlation are lower than those at the individual temperatures, particularly for the correlation among furosine and uridine, because of the different formation rate of the two compounds at the three different storage temperatures.

The pH is slightly correlated with the other chemical parameters, except with furosine at 30 °C. The lack of correlation between pH and furosine at 20 and 5 °C is due to the different development of the two variables, because the slight pH decrease (0.2 unit) after 20 days of storage at these temperatures does not influence furosine formation velocity, which continues to increase.

Conclusions. The results confirm that furosine is a good indicator of shell egg freshness for markets where egg refrigeration is not common practice, because it increases even over long storage times and presents a

similar evolution in different egg lots. Uridine may be an additional parameter for detection of eggs stored over a long period at 5 °C. Pyroglutamic acid, on the other hand, is unsuitable as an egg freshness index because, even if it increases linearly during the storage of eggs, it has a high natural variability and a low range of increase.

With regard to the rheological analysis, a transition from pseudoplasticity to Newtonity and a high decrease of viscosity happen during the first storage period, depending on temperature. The aggregation temperature remains constant at 61–62 °C during the storage of eggs.

The parameters furosine, pyroglutamic acid, and uridine are generally well-correlated among them ($P > 99\%$). On the other hand, the correlation with apparent viscosity is lower, since this parameter depends on several factors (such as pH, ovomucin-lysozyme complex scission, and dry matter) that influence albumen thinning. All these factors change with different rates in relation to storage temperature.

ABBREVIATIONS USED

CV, coefficient of variation; DSC, differential scanning calorimetry; HPLC, high-performance liquid chromatography; K , consistency index; r^2 , determination coefficient; n , flow behavior index; P , probability; $\dot{\gamma}$, shear rate; τ , shear stress; SD, standard deviation; η , viscosity.

LITERATURE CITED

- AOAC. Eggs and egg products. In *Official Methods of Analysis of the Association of Official Analytical Chemists*; Helrich, K., Ed.; Association of Official Analytical Chemists: Arlington, VA, 1990; Vol. 2, pp 853–863.
- Beveridge, T.; Nakai, S. Effects of sulphhydryls blocking on the thinning of egg white. *J. Food Sci.* **1975**, *40*, 864–868.
- Burley, R. W.; Vadehra, D. V. The albumen: chemistry. In *The Avian Egg. Chemistry and Biology*; Wiley: New York, 1989; pp 65–128.
- Donovan, J. W.; Mapes, C. J. A differential scanning calorimetric study of conversion of ovalbumin to S-ovalbumin in eggs. *J. Sci. Food Agric.* **1976**, *27*, 197–204.
- Donovan, J. W.; Mapes, C. J.; Davis, J. G.; Garibaldi, J. A. A differential scanning calorimetric study of the stability of egg white to heat denaturation. *J. Sci. Food Agric.* **1975**, *26*, 73–83.

- EEC (Economic European Community). Regulation 1274/91, May 15, 1991 Council. *Gazz. Uff. Comunità Eur.* **1991**, L121, 11–24.
- EEC (Economic European Community). Regulation 1907/90, June 26, 1990 Council. *Gazz. Uff. REPUB. Ital.* **1990**, 71, 55–61.
- EU (European Union). Decision 94/371/CEE, June 20, 1994 Council. *Gazz. Uff. Comunità Eur.* **1994**, L168, 94.
- Gossett, P. W.; Rizvi, S. S.; Baker, R. C. Selected rheological properties of pH-adjusted or succinylated egg albumen. *J. Food Sci.* **1983**, 48, 1395–1399.
- Hegg, P.-O.; Martens, H.; Löfqvist, B. The protective effect of sodium dodecylsulphate on the thermal precipitation of conalbumin. A study on thermal aggregation and denaturation. *J. Sci. Food Agric.* **1978**, 30, 981–993.
- Hidalgo, A.; Rossi, M.; Pompei, C. Furosine as a freshness parameter of shell eggs. *J. Agric. Food Chem.* **1995**, 43, 1673–1677.
- Hidalgo, A.; Lucisano, M.; Comelli, E. M.; Pompei, C. Evolution of chemical and physical yolk characteristics during the storage of shell eggs. *J. Agric. Food Chem.* **1996**, in press.
- Horwitz, W. Today's chemical realities. *J. Assoc. Off. Anal. Chem.* **1983**, 66, 1295–1301.
- Hsieh, Y. L.; Regenstein, J. M. Modeling gelation of egg albumen and ovalbumin. *J. Food Sci.* **1992**, 57, 856–861.
- Johnson, T. M.; Zabik, M. E. Gelation properties of albumen proteins, singly and in combination. *Poult. Sci.* **1981**, 60, 2071–2083.
- Kato, A.; Nakamura, R.; Sato, Y. Studies on changes in stored shell eggs. Part V. The difference in the chemical and physicochemical properties of ovomucin (B) between the thick and thin white. *Agric. Biol. Chem.* **1970a**, 34, 854–859.
- Kato, A.; Nakamura, R.; Sato, Y. Studies on changes in stored shell eggs. Part VI. Changes in the chemical composition of ovomucin during storage. *Agric. Biol. Chem.* **1970b**, 34, 1009–1013.
- Kato, A.; Nakamura, R.; Sato, Y. Studies on changes in stored shell eggs. Part VII. Changes in the physicochemical properties of ovomucin solubilized by treatment with mercaptoethanol during storage. *Agric. Biol. Chem.* **1971**, 35, 351–356.
- Kato, A.; Imoto, T.; Yagishita, K. The binding groups in ovomucin-lysozyme interaction. *Agric. Biol. Chem.* **1975**, 39, 541–544.
- Kato, A.; Kiminori, O.; Kuramoto, Y.; Kobayashi, K. Degradation of the o-glycosidically linked carbohydrate units of ovomucin during egg white thinning. *J. Food Sci.* **1979**, 44, 1341–1344.
- Kato, A.; Ogata, S.; Matsudomi, N.; Kobayashi, K. A comparative study of aggregated and disaggregated ovomucin during egg white thinning. *J. Agric. Food Chem.* **1981**, 29, 821–823.
- Meehan, J. J.; Sugihara, T. F.; Kline, L. Relationship between shell egg handling factors and egg products properties. *Poult. Sci.* **1962**, 41, 892–900.
- Montejano, J. G.; Hamann, D. D.; Ball, H. R.; Lanier, T. C. Thermally induced gelation of native and modified egg white; rheological changes during processing; final strengths and microstructures. *J. Food Sci.* **1984**, 49, 1249–1256.
- Payawal, S.; Lowe, B.; Stewart, G. F. Pasteurization of liquid-egg products. II. Effect of heat treatments on appearance and viscosity. *Food Res.* **1946**, 11, 246–249.
- Pitsilis, J. G.; Walton, H. V.; Cotterill, O. J. The apparent viscosity of egg white at various temperatures and pH levels. *Trans. ASAE* **1975**, 18, 347–349.
- Pitsilis, J. G.; Brooker, D. B.; Walton, H. V.; Cotterill, O. J. Rheological properties of liquid egg white. *Trans. ASAE* **1984**, 27, 300–304.
- Powrie, W. D. Chemistry of eggs and egg products. In *Egg Science & Technology*; Stadelman, W. I., Cotterill, O. J., Eds.; AVI Publishing: Westport, CT, 1977; pp 65–91.
- Resmini, P.; Pellegrino, L.; Battelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.* **1990**, 3, 173–183.
- Richardson, R. K.; Ross-Murphy, S. B. Mechanical properties of globular protein gels: 1. Incipient gelation behavior. *Int. J. Biol. Macromol.* **1981**, 3, 315–322. Cited by: Hsieh, Y. L.; Regenstein, J. M. Modeling gelation of egg albumen and ovalbumin. *J. Food Sci.* **1992**, 57, 856–861.
- Robinson, D. S.; Monsey, J. B. Change in the composition of ovomucin during liquefaction of thick egg white. *J. Sci. Food Agric.* **1972**, 23, 29–38.
- Rossi, M.; Pompei, C. Changes in some egg components and analytical values due to hen age. *Poult. Sci.* **1995**, 74, 152–160.
- Rossi, M.; Pompei, C.; Schiraldi, A.; Riva, M. Kinetics of ovalbumin transformation. In *Applicazioni della calorimetria ai prodotti alimentari*; Riva, M., Schiraldi, A., Eds.; RAISA-CNR: Milano, Italy, 1992; pp A.33–A.36.
- Rossi, M.; Pompei, C.; Hidalgo, A. Freshness criteria based on physical and chemical modifications occurring in eggs during aging. *Ital. J. Food Sci.* **1995a**, 7, 147–156.
- Rossi, M.; Pompei, C.; Hidalgo, A. Influence of pasteurization on some yolk and whole egg characteristics. In *Proceedings of the second Italian Conference in Food Science and Technology*, Cernobbio, Italy; 1995b; in press.
- Sato, Y.; Hayakawa, S.; Nakamura, R. Studies on factors of solubilization of insoluble ovomucin during egg white thinning. *J. Agric. Food Chem.* **1976**, 24, 798–803.
- Sauveur, B.; de Reviers, M. Egg quality. In *Reproduction des volailles et production d'œufs*; INRA: Paris, 1988; pp 377–436.
- Scalzo, A. M.; Dickerson, R. W., Jr.; Peeler, J. T.; Read, R. B., Jr. The viscosity of egg and egg products. *Food Technol.* **1970**, 24, 1301–1307.
- Shitamori, S.; Kojima, E.; Nakamura, R. Changes in the heat-induced gelling properties of ovalbumin during its conversion to S-ovalbumin. *Agric. Biol. Chem.* **1984**, 48, 1539–1544.
- Thapon, J.-L. Egg modification during the storage of eggs. In *L'œuf et les ovoproduits*; Thapon, J.-L., Bourgeois, C.-M., Eds.; Technique et Documentation (Lavoisier): Paris, 1994; pp 84–94.
- Tung, M. A.; Richards, J. F.; Morrison, B. C. Rheology of fresh, aged and gamma-irradiated egg white. *J. Food Sci.* **1970**, 35, 872–874.

Received for review July 26, 1995. Revised manuscript received February 6, 1996. Accepted February 23, 1996.® Research supported by National Research Council of Italy, Special Project RAISA, Subproject 4, Paper 2623.

JF9504850

® Abstract published in *Advance ACS Abstracts*, April 1, 1996.