

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

Alyssa Hidalgo,* Mara Lucisano, Elena M. Comelli, and Carlo Pompei

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

Yolk modifications during the storage of eggs could influence the quality of yolk products. The aim of this research was to study the rheological behavior and the evolution of some chemical parameters in yolk during the storage of shell eggs at 30, 20, and 5 °C. Water content, pH, furosine, pyroglutamic acid, and uridine increased during storage as a function of temperature. The yolk of fresh eggs showed pseudoplasticity ($n = 0.87$) with a viscosity decrease from 1.85 to 1.49 Pa·s for an increase in the shear rate from 7.36 to 46.3 s⁻¹. A progressive transition from pseudoplasticity to Newtonianity and a decrease in apparent viscosity were observed during storage. The aggregation temperature evaluated by loss modulus and storage modulus measurements rose from 72 °C (fresh eggs) to 80.5 °C (stored eggs). Highly significant correlations between the chemical parameters, especially for the storage at 30 °C, were found; viscosity was highly correlated with dry matter.

Keywords: *Egg storage; furosine; loss modulus; pyroglutamic acid; rheological behavior; storage modulus; uridine; viscosity; yolk*

INTRODUCTION

Freshness is the most important criterion to classify shell eggs. The most common quantitative parameters utilized to evaluate shell egg freshness—air cell height and Haugh unit—are related to phenomena that occur during albumen aging. Some authors (Rossi et al., 1995a; Hidalgo et al., 1995) have proposed furosine, pyroglutamic acid, and uridine as freshness indices of shell eggs when evaluated in albumen. These chemical parameters could be also useful to evaluate the freshness of the raw material used for egg products (pasteurized albumen, pasteurized whole egg) because they are not influenced by the heat treatments of pasteurization (Rossi et al., 1995b), an essential step in egg products processing.

During shell egg aging, the yolk undergoes several modifications of its chemical and physical characteristics. The passage of water from the albumen to the yolk is a well-known phenomenon that determines a decrease in both viscosity (Meyer and Woodburn, 1965; Kline et al., 1965; Colas, 1981) and yolk index (the ratio of yolk height to yolk diameter) (Sauveur and de Revers, 1988; Thapon, 1994). During egg aging there is also a weakening of the vitelline membrane and hence a higher difficulty of yolk–albumen separation (Sauveur and de Revers, 1988; Thapon, 1994); furthermore, there are increases in pH (Powrie, 1977), pyroglutamic acid, and uridine (Rossi et al., 1995a). All these changes could influence the quality of yolk products.

The aim of this research was to study the evolution of some chemical parameters in yolk during long-term storage of shell eggs at different temperatures, in order to propose them as possible indices of raw-material freshness in yolk products. The rheological behavior of yolk during egg storage was also studied with the aim of providing useful information for the choice of pasteurization conditions.

MATERIALS AND METHODS

Eggs. A lot of grade A-extra eggs of weight class 1 (70–75 g) (EEC, 1990; EEC, 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 laying, was utilized in the trials. The egg lot was divided in three groups and stored in thermostat chambers at 30, 20, and 5 °C for 29, 87, and 204 days, respectively. For chemical analyses purpose, storage at 5 °C was continued up to 354 days.

Sample Preparation. The measurements were made on bulked yolk samples of four random eggs per group. 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 a spatula. For the rheological tests, the yolks were manually mixed for 5 s with a spatula. For the chemical analyses, the samples were further mixed at 3000 rpm for 20 s with a Sorvall Omni Mixer (Model 17106, Dupont de Nemours & Co, Newton, CO).

Chemical Analyses. 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 by following the AOAC method 925.30 (1990).

Furosine content (mg of furosine/100 g of protein) was computed as the average of three replicated analyses performed by following the HPLC method as proposed for milk by Resmini et al. (1990), slightly modified for egg as described by Hidalgo et al. (1995).

Protein content (average of two measurements) was calculated as total nitrogen multiplied by the factor 6.25 and expressed as grams of protein/100 g of product. Total nitrogen analysis was performed using the Kjeldahl method 925.31 (AOAC, 1990).

Pyroglutamic acid and uridine analyses were performed by HPLC by following the procedure described by Rossi and Pompei (1995). The results, expressed as parts per million of each compound, were the average of duplicated analyses.

Rheology. The rheological behavior of yolk was studied in rotation and in oscillation using a controlled-rate Böhm VOR Rheometer (Böhm Reologi AB Corporate Headquarters, S-223 70 Lund, Sweden) with a cone/plate measuring system (CP 5/30) (5.4 rad cone angle, 3 cm diameter), a gap of 0.15 mm, and torsion bars of 88.3 g·cm (for the rotation tests) and of 20.83 g·cm (for the oscillation tests). The sample quantity was about 0.7 mL, enough to cover the plate surface.

The tests in rotation consisted of two consecutive flow curves, the first one in increasing and the second one in decreasing shear rate ($\dot{\gamma}$), performed twice on the same sample,

Table 1. Flow Behavior Index (*n*), Consistency Index (*K*), and Solids Percentage in Yolk during the Storage of Eggs at 30, 20, and 5 °C, Measured at a Shear Rate Range from 7.36 to 46.3 s⁻¹

temp (°C)	time (days)	<i>n</i>	<i>K</i> (Pa·s)	% dry matter
	fresh	0.87	2.42	52.3
30	4	0.83	2.13	50.8
	9	1.02	0.67	49.1
	11	1.04	0.49	48.7
	18	0.90	0.71	48.6
	22	0.89	0.50	47.8
20	7	0.98	1.08	50.4
	10	0.86	1.82	50.9
	16	0.86	1.57	50.2
	24	0.96	0.86	49.0
	28	0.87	0.95	50.4
	35	0.96	0.64	49.4
	42	1.02	0.52	48.5
	52	0.76	1.18	48.2
	63	1.06	0.34	49.0
	87	1.14	0.23	47.9
	5	8	0.89	1.66
21		0.96	1.09	51.0
31		0.96	1.44	50.9
36		0.91	1.58	50.3
46		0.70	2.77	50.4
57		0.95	1.00	49.9
100		0.82	1.65	50.0
122		0.74	2.21	49.6
147		0.77	2.47	48.8
204		0.91	0.84	48.8

at a constant temperature of 20 °C. To obtain the viscosity curves (viscosity vs shear rate), the experimental data for the increasing shear rate flow curve from the second trial were elaborated by following the power law equation ($\tau = K(\dot{\gamma})^n$, where τ = shear stress, K = consistency index, $\dot{\gamma}$ = shear rate, and n = flow behavior index) and using the data elaboration program of the rheometer (Böhlín software). The values are the average of three measurements.

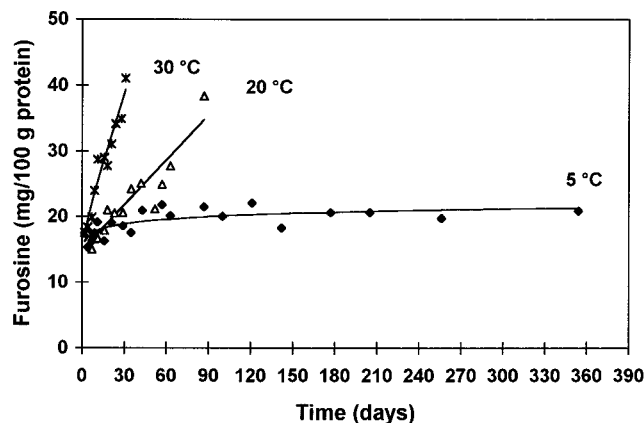
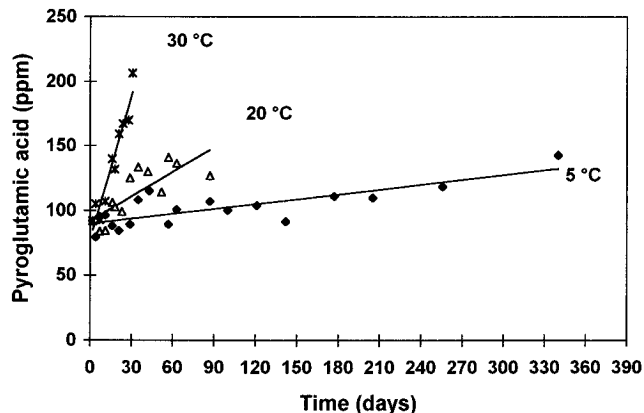
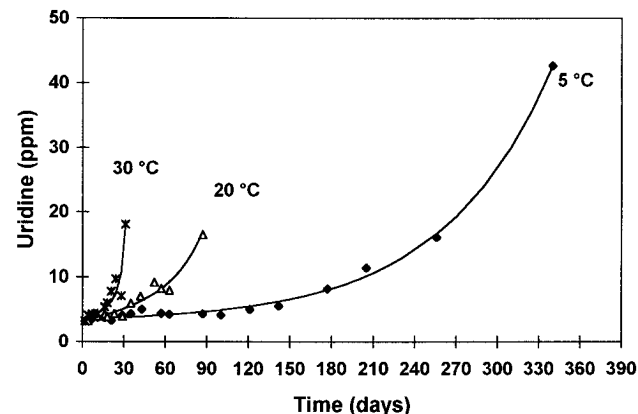
To determine the conditions of the linearity field in the oscillation tests, preliminary analyses in frequency sweep (from 0.5 to 5 Hz, at a deformation of 0.08 and at a temperature of 20 °C) and in strain sweep (from 0.02 to 0.2, at a frequency of 1 Hz and at a temperature of 20 °C) were performed. On the basis of these results, the evaluation of the storage modulus (G') and of the loss modulus (G'') as a function of temperature (from 20 to 90 °C; heating rate = 2 °C/min) was done at a frequency of 1 Hz and at a deformation of 0.08 on yolk samples previously undergone to two strain sweep. The phase angle (δ , $\tan \delta = G''/G'$) for each temperature was calculated.

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. During egg storage the yolk pH increased linearly from 6.1 to 6.6, 6.5, and 6.8 after 29 days at 30 °C, 87 days at 20 °C, and 354 days at 5 °C, respectively. Dry matter content (Table 1) decreased exponentially as a function of the storage temperature, as a consequence of water migration from albumen to the yolk (Thapon, 1994).

Figure 1 reports the furosine content of the yolk during the storage of eggs at three temperatures. Furosine level increased linearly during the storage at 30 and 20 °C. At 5 °C, on the other hand, the increase was limited to the initial 60 days of storage, up to an asymptote of about 20 mg/100 g of protein during the following days. The values found for the yolk were much lower than the ones observed for the albumen by

**Figure 1.** Furosine content in yolk during the storage of eggs at 30, 20, and 5 °C.**Figure 2.** Pyroglutamic acid content in yolk during the storage of eggs at 30, 20, and 5 °C.**Figure 3.** Uridine content in yolk during the storage of eggs at 30, 20, and 5 °C.

Hidalgo et al. (1995). The different furosine formation rate in the two egg fractions was attributed by Hidalgo et al. (1995) to the different medium alkalinity, which influences the development of the Maillard reaction.

Pyroglutamic acid and uridine content of yolk during the storage of eggs are presented in Figures 2 and 3, respectively. The pyroglutamic acid of fresh egg yolk was 92 ppm. Rossi and Pompei (1995) reported values of 71 ppm in the yolk of eggs laid by 70-week-old Warren and Isa-Brown hens (without molt). Even lower values (ca. 50 ppm) were found by Rossi et al. (1995a) in the yolk of fresh eggs laid by 60-week-old Warren hens (without molt). These discrepancies suggest a high natural variability due not only to the hen age (Rossi and Pompei, 1995) but also to other factors (e.g. the

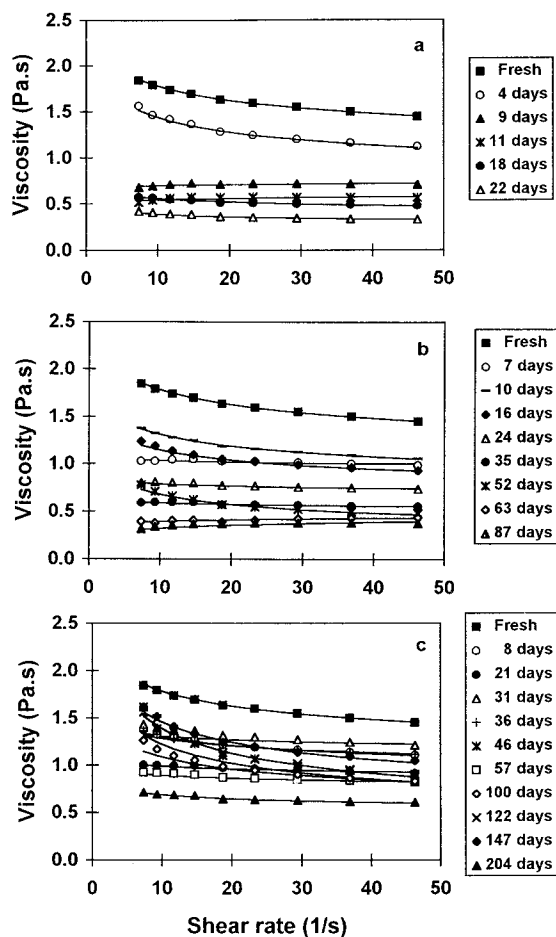


Figure 4. Viscosity in function of increasing shear rate for yolk of fresh eggs and of eggs stored at 30, 20, and 5 °C.

molt). During the storage, the content of pyroglutamic acid increased linearly as a function of the temperature, reaching (at 30 and 20 °C) values higher than the ones reported by Rossi et al. (1995a).

Uridine content in the yolk of fresh eggs was 3.2 ppm, similar to the values reported by Rossi and Pompei (1995) and by Rossi et al. (1995a). During the aging of the eggs there was an exponential increase of uridine content, but the values reached were lower than those detected by Rossi et al. (1995a). Particularly, at 5 °C there was a rapid increase of uridine after ca. 150 days of storage. High uridine values (>10 ppm) and low furosine values (ca. 20 mg/100 g of protein) may permit the identification of eggs stored under refrigeration over long periods.

Rheological Analysis. The yolk of fresh and stored eggs did not show hysteresis, because the curves of the shear stress in function of increasing and of decreasing shear rate overlapped in the two tests. Hence, the decrease in viscosity with an increasing shear rate was reversible, as already observed in samples of pasteurized yolk by Ibarz and Sintés (1989). On the contrary, Pitsilis et al. (1984) found a time-dependent behavior for shear rate vs time curve and stated that the time needed to reach the equilibrium was between 25 and 110 s, depending on the shear rate applied.

To characterize the rheological behavior of yolk during egg aging, the flow curves of the second test, obtained with an increasing shear rate, were considered. Figure 4 depicts the viscosity curves for yolk of fresh eggs and of eggs stored at 30 (a), 20 (b), and 5 °C (c). Table 1 reports the values of the flow behavior index (n) and of

the consistency index (K), computed with the power law equation; the dry matter contents of the samples are also reported. A viscosity decrease from 1.85 to 1.49 Pa·s following an increase in the shear rate from 7.36 to 46.3 s⁻¹ (Figure 4) and an n value of 0.87 was evidenced for the yolk of fresh eggs with a dry matter content of 52.3%, indicating a pseudoplastic behavior, as reported by Lorient and Matringe (1994). Chang et al. (1970) observed a pseudoplastic behavior in fresh egg yolk with a similar dry matter content (52.5%), detecting a decrease in viscosity from 2.3 to 1.8 Pa·s with the increase of the shear rate from 1.9 to 76.8 s⁻¹. An analogous dependence of the viscosity from the shear rate was highlighted by the same authors for yolk diluted with 5, 10, and 20% of albumen. Even for pasteurized yolk with a dry matter content of between 42.6 and 44.4%, Ibarz and Sintés (1989) observed a pseudoplastic behavior, obtaining n values between 0.88 and 0.85. On the other hand, Scalzo et al. (1970), working at different shear rate intervals (between 0 and 700 s⁻¹) and at different analysis temperatures (between 5 and 60 °C), showed a Newtonian behavior in yolk samples from industrial plants and with a dry matter content of 45%.

The apparent viscosity observed in fresh egg yolk (from 1.85 to 1.49 Pa·s) was lower than that reported by Moran (1935) (2.5 Pa·s) for yolk with a dry matter content of 52.5–53% and than that reported by Powrie et al. (1963) (2.0–2.9 Pa·s) analyzing manually mixed fresh egg yolk with a Brookfield viscometer. Davey et al. (1969), instead, reported a lower viscosity (1.37 Pa·s).

The viscosity of the yolk varies if a good separation of the albumen is not achieved and during the mixing operations that precede the analysis. For example, lower viscosity values for fresh egg yolk vigorously mixed were recorded: 0.26 Pa·s (Palmer et al., 1969), 0.13 Pa·s at a $\dot{\gamma}$ of 2.5 s⁻¹ and at 30 °C (Yang and Cotterill, 1989). Using a Brookfield viscosimeter, a viscosity decrease of yolk of fresh eggs (less than 48 h) was observed, from 3.42 to 1.28 and to 0.64 Pa·s for dilution with 5 and 10% of albumen, respectively (Varadarajulu and Cunningham, 1972). Chang et al. (1970) found that the addition of 5% of albumen determines a viscosity reduction of yolk (52.5% of dry matter) from 2.3 to 0.95 Pa·s ($\dot{\gamma} = 1.9$ s⁻¹) in diluted yolk (50.9% of dry matter).

During the storage of eggs, there is a viscosity decrease and a transition from pseudoplasticity to Newtonity (Figure 4). The variation rate of the parameters n and K (Table 1) depends on the storage temperature. The variations in viscosity, sharper during the initial days of storage and then gradual during time, are more evident and regular at 30 °C. Colas (1981) found a decrease in apparent viscosity and in consistency index of the yolk as a function of temperature, more rapid at 20 °C than at 4 °C.

The viscosity decrease may be partially due to the gradual dilution of the yolk during the storage of eggs, because of water passage from the albumen through the vitelline membrane (Thapon, 1994); the dry matter decreased from 52.3%, in fresh egg yolk, to ca. 48%, in eggs stored at 20 and 30 °C, and to ca. 49%, in eggs stored at 5 °C (Table 1). Similarly, Meyer and Woodburn (1965) observed a viscosity of ca. 2.7 Pa·s in fresh egg yolk with ca. 52.7% dry matter and a viscosity of 0.9 Pa·s in stored egg yolk with 50% dry matter. Kline et al. (1965) reported an average decrease of yolk viscosity from 1.63 ($\bar{X}_{\text{dry matter}} = 52.8\%$) to 0.87 Pa·s

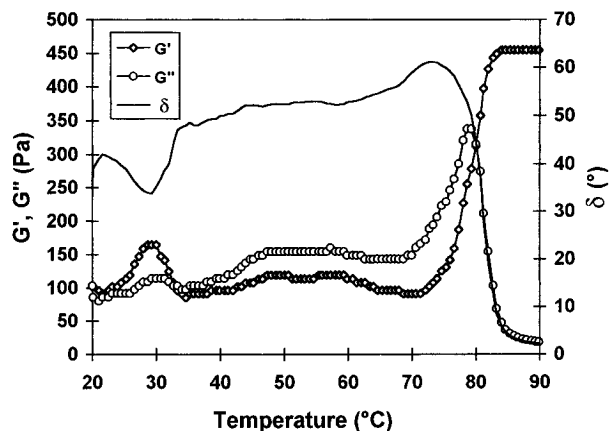


Figure 5. Variation of the storage modulus (G'), loss modulus (G''), and phase angle (δ) during heating (from 20 to 90 °C) of fresh egg yolk.

($\bar{X}_{\text{dry matter}} = 51.1\%$) after 16 days of storage at 12.8 °C of eggs laid by White Leghorn hens of different age. Payawal et al. (1946), using a capillary viscometer, observed an apparent viscosity of 0.9 Pa·s in the yolk of eggs stored at 30 °C for 7 days (50.5–51% dry matter), similar to the value found in our research under similar storage conditions.

With regard to the variation of the flow behavior index during storage, Pitsilis et al. (1984) described a pseudo-plastic behavior ($n = 0.89\text{--}0.88$) in the yolk of eggs stored at 10–12 °C for 12 days, coming from an industrial plant and with a dry matter content between 43 and 47% by dilution with albumen.

Figure 5 shows the trend of the storage modulus (G'), of the loss modulus (G''), and of the phase angle (δ) as a function of temperature for the yolk of fresh eggs. The G' and G'' values at 20 °C are comparable with those obtained by Sharma (1979) at the same frequency (1 Hz) and at a maximum strain amplitude of 0.35 rad and remain constant up to a temperature of ca. 72 °C, with a prevalence of the viscous component ($\delta > 45^\circ$) in the temperature interval between 35 and 80 °C. At 72 °C there is a rapid increase of the values of both moduli, to show that the yolk aggregation begins at such a temperature. Above 85 °C the phase transition sol-gel is over and the coagulated yolk has a G' value of 546 Pa and a G'' value of 18 Pa.

The viscosity increase of yolk at temperatures above 72 °C is surely due to structural changes of the proteins. Following Tsutsui and Obara (1980) the lipoproteins, and specifically the lipovitellins, are mainly responsible for this phenomenon. Lipovitellins are the more abundant protein fraction of the yolk (Parkinson, 1966), keep stable up to 72 °C (Dixon and Cotterill, 1981) and have a viscosity higher than lipovitelinins and livetins (Davey et al., 1969). The thermoresistance of yolk proteins was studied by Dixon and Cotterill (1981) by gel electrophoresis on yolk pasteurized for 210 s at temperatures between 54 and 84 °C (at intervals of 3 °C). No relevant changes were observed up to 60 °C, when the γ -livetins band starts to decrease and then disappears at 75 °C. The α - and β -livetins keep stable up to 69 °C: the former ones disappear at 72 °C, and the latter ones at about 81 °C.

Figure 6 depicts the variations of G' and of G'' as a function of temperature for yolk of eggs stored at 30 °C. The increase of the aggregation temperature, 72 °C in the fresh product vs 80 °C in product stored at 30 °C for 29 days, is evident. The temperature of the maxi-

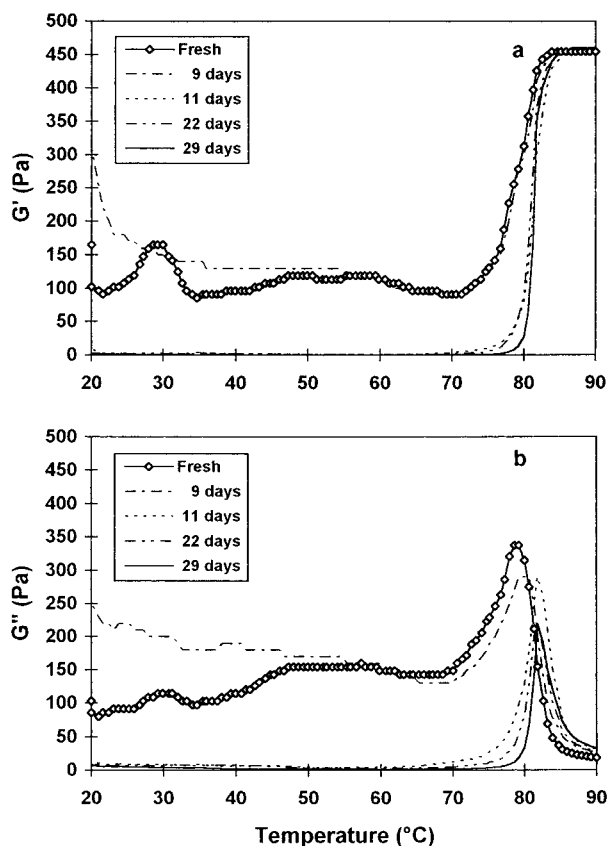


Figure 6. Variation of the storage modulus (G') (a) and the loss modulus (G'') (b) during the heating (from 20 to 90 °C) of yolk of eggs stored at 30 °C.

imum value of the storage modulus also increased from 78 °C (fresh product) up to 81–82 °C. Comparing the G' and G'' for the yolk of fresh eggs and of eggs stored 11 and 29 days at 30 °C, it is evident that, in this situation too, the rheological properties changed mainly during the initial 11 days of storage. After this period, G' and G'' reached values of about 1–2 Pa and of 4–6 Pa at 40 °C, much lower than in the fresh product (G' of 96.3 and G'' of 115 Pa), and even the aggregation temperature reached its maximum value. At temperatures inferior to the aggregation one, there was a progressive increase of δ from ca. 50 to 80°, to indicate a major decrease of G' than of G'' .

The viscoelastic parameters trend during the warming of the yolk of eggs stored at 20 and 5 °C (Figures 7 and 8) confirmed the considerations on the storage at 30 °C, with different kinetics as a function of the storage temperature: the aggregation temperature increases from 72 (fresh product) up to 80.5 and 81 °C after 87 days at 20 °C and after 100 days at 5 °C, respectively.

Correlation between Variables. Table 2 presents the correlation coefficients between the various variables studied, at every storage temperature and for the temperatures considered together (global). Very often, the viscosity seems inversely correlated to the chemical parameters, and in a more relevant way to dry matter ($P > 99.9\%$), with the exception of the correlation at 5 °C. The interdependence between these two variables was previously discussed.

The chemical variables were directly and highly correlated, with higher correlation coefficients at 30 °C, a temperature that showed a clearer evolution of the parameters.

Conclusions. During the storage of shell egg, some variations of the rheological characteristics of the yolk

Table 2. Correlation Coefficients between the Different Variables^a

		dry matter	furosine	pyroglutamic acid	uridine	apparent viscosity ^a
pH	30 °C	-0.943 ^d	0.942 ^d	0.948 ^d	0.914 ^d	-0.826 ^b
	20 °C	-0.867 ^d	0.704 ^c	0.528	0.785 ^d	-0.855 ^c
	5 °C	-0.833 ^d	0.518 ^b	0.829 ^d	0.752 ^d	-0.616 ^b
	global	-0.703 ^d	0.466 ^c	0.591 ^d	0.712 ^d	-0.698 ^d
dry matter	30 °C		-0.930 ^c	-0.821 ^c	-0.831 ^c	0.953 ^d
	20 °C		-0.729 ^c	-0.701 ^c	-0.777 ^d	0.946 ^d
	5 °C		-0.666 ^c	-0.646 ^c	-0.411	0.686 ^b
	global		-0.805 ^d	-0.778 ^d	-0.307 ^b	0.910 ^d
furosine	30 °C			0.905 ^d	0.841 ^c	-0.886 ^c
	20 °C			0.718 ^c	0.910 ^d	-0.752 ^b
	5 °C			0.554 ^b	0.274	-0.542
	global			0.814 ^d	0.207	-0.790 ^d
pyroglutamic acid	30 °C				0.919 ^d	-0.699
	20 °C				0.575 ^b	-0.808 ^c
	5 °C				0.730 ^d	-0.207
	global				0.365 ^b	-0.703 ^d
uridine	30 °C					-0.740 ^b
	20 °C					-0.726 ^b
	5 °C					-0.623 ^b
	global					-0.674 ^d

^a Measured at $\dot{\gamma}$ of 14.7 s⁻¹. ^b Significant at the 5% level of probability. ^c Significant at the 1% level of probability. ^d Significant at the 0.1% level of probability. ^e Degrees of freedom ($n - 2$) for the correlation between chemical variables: 30 °C, 8; 20 °C, 12; 5 °C, 17; global, 41. Degrees of freedom ($n - 2$) for the correlation between viscosity and chemical variables: 30 °C, 6; 20 °C, 7; 5 °C, 9; global, 26.

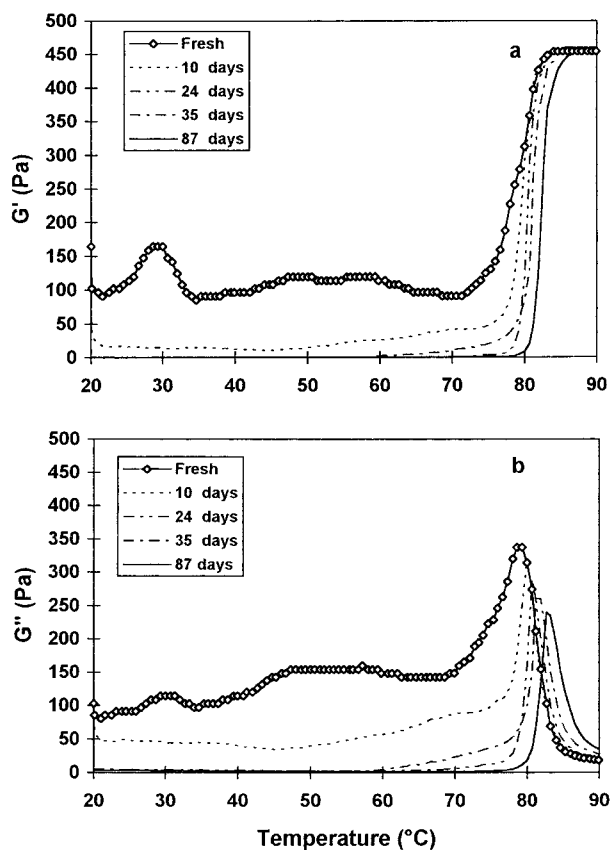


Figure 7. Variation of the storage modulus (G') (a) and the loss modulus (G'') (b) during the heating (from 20 to 90 °C) of yolk of eggs stored at 20 °C.

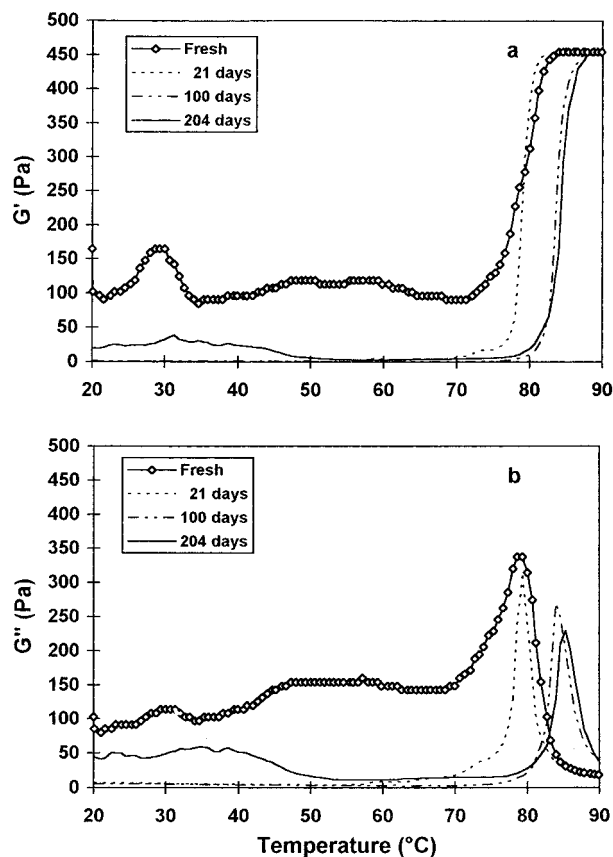


Figure 8. Variation of the storage modulus (G') (a) and the loss modulus (G'') (b) during the heating (from 20 to 90 °C) of yolk of eggs stored at 5 °C.

were evidenced. Among them, there were a progressive transition from pseudoplasticity to Newtonity, a decrease of viscosity, and an increase of the aggregation temperature. The improved thermostability of the yolk during egg storage would allow the utilization of higher temperatures during pasteurization treatments.

The low levels of furosine at refrigeration temperatures and the high natural variability of pyroglutamic acid and uridine limit the usage of these parameters as

indices to evaluate the freshness of the raw material utilized for yolk products. Furosine evaluation in yolk can be useful in the European Union where eggs are distributed at room temperatures, but not in the United States, where egg refrigeration is common practice. Only a combination of the chemical parameters (for example furosine and uridine) could evidence the presence of eggs stored over long times under refrigeration in yolk products.

ABBREVIATIONS USED

K , consistency index; n , flow behavior index; HPLC, high-performance liquid chromatograph; G' , loss modulus; δ , phase angle; P , probability; $\dot{\gamma}$, shear rate; τ , shear stress; G' , storage modulus.

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