Furosine as a Freshness Parameter of Shell Eggs

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Furosine, an index of the initial steps of Maillard reaction, has been used for the evaluation of shelf life in UHT milk and of heat damage in various food products. The aim of this paper was to study furosine as an index for egg freshness. Fresh eggs from different hen breeds were used to assess HPLC method repeatability, to investigate natural variability of furosine content in fresh shell eggs, and to study furosine formation during storage at 5, 20, 30, and 38 °C. Furosine, present in fresh egg albumen at a level of about 10 mg/100 g of protein, increased during storage with dependence on temperature, reaching after 40 days at 20 °C a level of about 100 mg/100 g of protein. The modification of furosine content in yolk during storage at 20 °C was minimal. Furosine, when measured in albumen, resulted in a very reliable index for egg freshness. In albumen, maximum furosine contents of 60 and 90 mg/100 g of protein, respectively, for grades A-extra and A eggs commercialized in the European Community are suggested.

Keywords: *Albumen; egg freshness; furosine; whole egg; yolk*

INTRODUCTION

Furosine, ϵ-*N*-(2-furoylmethyl-L-lysine), is an interesting index of the initial steps of Maillard reaction (Erbersdobler, 1986). Furosine is produced by acid hydrolysis of the Amadori compounds ϵ-*N*-deoxyfructosyl-L-lysine (ϵ-fructose–lysine) and ϵ-*N*-deoxylactulosyl-L-lysine (ϵ-lactulose–lysine). It is detected by different chromatographic techniques such as gas chromatography (Büser and Erbersdobler, 1985), ion exchange chromatography (Erbersdobler et al., 1987), reversed phase HPLC (Chiang, 1983), and, recently, ion-pair reversed phase HPLC (Resmini et al., 1990a,b).

Furosine detection has been used for the evaluation of heat treatment severity in UHT sterilized milk (Erbersdobler and Dehn, 1989; O'Brien and Morrisey, 1989) and pasta products (Resmini et al., 1990a) and has been proposed as an index for heat damage in other food products (Resmini and Pellegrino, 1991). Particularly, the level of furosine in milk has been utilized to evaluate the concentration of reactive and blocked lysine (Finot et al., 1981).

The development of Maillard reaction is affected by temperature, pH, water activity, and type of reducing sugars (Eskin, 1990). Although high temperatures (above 100 °C) favor the reaction, even low temperatures over a long period allow the beginning of the first steps. This explains why furosine may be utilized as a shelf-life index for food products usually stored at room temperature. For example, the furosine content of UHT milk, during 6 months of storage at 20 °C, increases from about 80 to 160 mg/100 g of protein (Parmigiani, 1993). Product water activity of 0.6–0.7 (Loncin et al., 1968) and basic pH (Eskin, 1990) also favor the reaction. Among the sugars, the aldopentoses (e.g. ribose) are the most reactive, followed by the aldohexoses (e.g. glucose) and the reducing disaccharides (e.g. lactose) (Eskin, 1990).

Since lysine and glucose are present in whole egg at levels of 0.82% (Posati and Orr, 1976) and 0.34% (Zambelli, 1992) respectively, Maillard reaction can occur during the aging of shell eggs.

The aim of this paper is to evaluate Maillard reaction through the furosine measurement in shell eggs stored at different temperatures in view of proposing furosine as an index for egg freshness.

MATERIALS AND METHODS

Eggs. Grade A and grade A-extra fresh eggs (EEC, 1991), from different commercial channels, and grade A-extra fresh eggs, directly from the producer and available in the laboratory after less than 24 h from laying, were utilized in this research. The eggs obtained directly from the producer were laid by hens of known breed and age, expressed as weeks from hatching. Different egg lots were used as follows: (a) grade A fresh commercial eggs, to assess the optimal hydrolysis time of yolk, to assess the repeatability of the method in albumen, and to determine the freeze-drying effects on furosine levels in the albumen; (b) grade A-extra fresh commercial eggs, to assess the repeatability of the method in yolk and whole egg; (c) eggs laid by hens of the breeds Brown Hy-line (30 and 53 weeks old) and Isa-Brown Warren (30 and 67 weeks old), to study the natural variability of furosine content in albumen; (d) eggs laid by hens of the breeds White Hy-line (43 weeks old), Brown Hy-line (62 weeks old), and Isa-Brown Warren (65 weeks old), to study the natural variability of furosine content in yolk and whole egg of fresh eggs; (e) eggs laid by hens of the breed Isa-Brown Warren (64 weeks old), to study furosine formation in yolk, albumen, and whole egg during egg aging.

Furosine HPLC Analysis. Shelling of eggs and accurate separation of yolk from albumen (when needed) were done manually. Samples to be analyzed were homogenized using a Sörvall Omni Mixer (Model 17106, DuPont de Nemours & Co., Newton, OH) at 3000 rpm for 30 s.

Furosine content was determined by the HPLC method proposed by Resmini et al. (1990b) for milk. This method was slightly modified as follows: 8 mL of 8 N HCl was added to about 400 mg of albumen or whole egg or 250 mg of yolk, accurately weighed in 10 mL screw-cap Pyrex vials. After nitrogen was bubbled for 1 min, the vials were sealed and kept at 110 °C for 23 h. Afterward, the sample was filtered with a 0.22 μm Millipore GS membrane (Millipore, Bedford, MA). A volume of 0.5 mL of filtrate underwent solid-phase extraction in a Sep-Pak C_{18} Millipore cartridge, prewetted with methanol and water. Furosine was eluted from the cartridge using 3 mL of 3 N HCl, and 20 μL of the eluate was injected in a liquid chromatography apparatus consisting of two 510 HPLC pumps, a 680 automated gradient controller, and a 490 programmable multiwavelength detector, all from Millipore Waters (Milford, MA). The instrument was connected to a D-2500 chromato-integrator (Merck-Hitachi, Darmstadt, Germany).

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Table 1. Variability of Furosine Content (Milligrams per 100 g of Protein) in Albumen, Yolk, and Whole Egg of Fresh Eggs Laid by Different Hen Breeds of Different Ages (in Weeks)

	White Hy-line, 43 weeks		Brown Hy-line				Isa-Brown Warren			
			30 weeks, albumen	53 weeks, albumen	62 weeks		30 weeks, albumen	67 weeks, albumen	65 weeks	
	yolk	whole egg			yolk	whole egg			yolk	whole egg
	15.8	11.7	12.0	10.7	14.8	13.1	11.6	11.0	15.9	12.4
	18.1	11.7	9.7	8.6	14.3	14.5	10.9	11.6	17.6	11.1
	16.0	12.2	10.5	9.7	16.4	10.9	9.2	11.6	13.2	12.3
	18.6	12.1	10.9	11.3	16.4	12.1	9.9	12.3	15.0	10.7
	15.2	9.2	10.4	10.3	18.3	12.6	11.1	9.4	19.1	13.9
	15.3	10.4	12.2	9.3	21.6	10.9	9.9	10.2	16.4	14.5
mean	16.50	11.22	10.95	9.98	16.97	12.35	10.43	11.02	16.20	12.48
SD[a]	1.47	1.18	0.97	0.98	2.67	1.38	0.91	1.06	2.05	1.50
CV[b] (%)	8.91	10.52	8.89	9.82	15.73	11.17	8.69	9.61	12.65	12.02

	albumen	yolk	whole egg
total mean	10.60	16.55	12.02
total SD[a]	1.01	2.02	1.40
total CV[b] (%)	9.54	12.20	11.65

[a] Standard deviation. [b] Coefficient of variation.

Operative conditions of the HPLC analysis of furosine were as follows: a C_8 furosine-dedicated column (250 × 4.6 mm, Alltech Italia S.R.L., Milan, Italy); column temperature, 35 °C; detection multiwavelength 280 nm; mobile phase (A) 0.4% acetic acid in water, (B) 0.3% potassium chloride in solvent A; flow rate, 1.2 mL/min. The elution gradient, expressed as proportion of eluent B, was as follows: initial condition, 2% for 13.5 min; from 2 to 50% in 7 min, 50% for 1 min; from 50 to 2% in 1.5 min, 2% for 10 min.

A calibration curve was built, using eight different concentrations (between 0 and 5 μmol/L) of hydrated furosine·2HCl (Neosystem Laboratoire, Strasbourg, France) in 3 N HCl. Based on the calibration curve, the limit of detection was calculated as the intercept value of the regression line plus 3 times the standard error of the estimate (Miller and Miller, 1988).

The results, expressed as milligrams of furosine/100 g of protein, are the average of triplicate measurements.

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 (AOAC, 1990).

Method Assessment. The influence of hydrolysis duration was assessed by determining furosine content on four aliquots of a yolk sample that underwent different hydrolysis times (between 22 and 29 h). One-way analysis of variance was performed to determine significant differences among the four hydrolysis times. The repeatability of the furosine analytical method in albumen, yolk, and whole egg was assessed by performing, in each case, 10 replicate measurements on the same sample made of 5 albumens or 5 yolks or 5 whole eggs homogenized together. The results were expressed in terms of standard deviation (SD) and of coefficient of variation (CV). Furosine contents of an albumen sample analyzed before and after freeze-drying at 4 and 20 °C were also compared.

Natural Variability of Furosine Content. Natural variability of furosine content in albumen, yolk, and whole egg of fresh eggs was studied by analyzing, for each material, six individual eggs laid by each group of hens. Four different groups of hens for albumen and three for yolk and whole egg were analyzed, as reported before. Within-group variability and total variability were expressed in terms of CV. One-way analysis of variance was performed to determine significant differences among the groups.

Aging of Eggs. A group of eggs laid by Isa-Brown Warren hens (64 weeks old) was divided into four lots that were maintained at 5, 20, 30, and 38 °C for up to 40 days, and furosine analysis of the albumen was performed at different times. In the eggs stored at 20 °C yolk and whole egg were also analyzed. Each analysis was performed on four eggs homogenized together.

RESULTS AND DISCUSSION

Calibration Curve and Analytical Method Assessment. The furosine calibration curve was linear in the range 0−5 μmol/L (r^2 = 0.9997), showing a detection limit of 0.11 μmol/L for the standard solution.

The original method for furosine detection in milk (Resmini et al., 1990b) suggested a 23 h acid hydrolysis time. Since yolk has a high lipidic content that could protect the proteins against acid action, the influence of hydrolysis duration on the results of the analysis was also assessed by performing analysis on the same yolk sample after four different hydrolysis times between 22 and 29 h. No significant differences were found (P > 95%); hence, as suggested in the original method, all of the samples were afterward hydrolyzed for 23 h.

The repeatability of the method, expressed in terms of mean ± SD and CV, was 23.58 ± 1.08 (CV = 4.58%) in albumen, 17.50 ± 1.95 (CV = 11.16%) in yolk, and 12.51 ± 0.84 (CV = 6.72%) in whole egg. Thus, method repeatability seemed to be negatively related to lipid content in the egg fractions ($CV_{albumen} < CV_{whole\ egg} < CV_{yolk}$), which could represent a variability factor during the hydrolysis step of the method. On the basis of the CV values, method repeatability in albumen was very good and in yolk and whole egg was acceptable (Horwitz, 1983).

Furosine contents of an albumen bulk sample before and after freeze-drying at 4 and 20 °C were also determined. This process modifies furosine content in the sample increasing from 30.7 to 58.1 and 82.1 mg/100 g of protein when freeze-drying is performed at 4 and 20 °C, respectively. This result could be explained by the fact that during freeze-drying the increasing solids concentration favors the interaction between sugars and proteins and decreases water activity in the product up to levels more favorable to Maillard reaction. It is therefore clear that freeze-drying is not a good method to preserve egg samples to be analyzed for furosine content.

Natural Variability of Furosine Content. Table 1 shows the variability of furosine content in albumen, yolk, and whole egg of fresh eggs laid by different groups of hens. For each egg fraction, no significant differences (P > 95%) have been found among the groups. The natural variability seemed to be related to the lipid content of the egg fraction ($CV_{albumen} < CV_{whole\ egg} < CV_{yolk}$). Total variability for furosine content in the

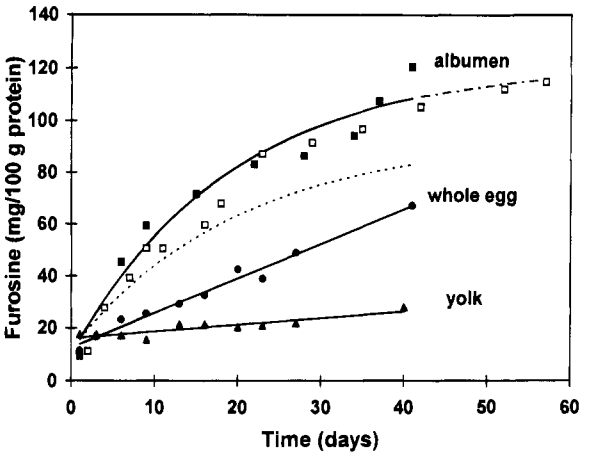

Figure 1. Furosine content in albumen, yolk, and whole egg of eggs stored at 20 °C. The open symbols represent furosine content in the albumen of another lot of eggs stored at 20 °C, as reported by Comelli (1994). The interpolation curve of albumen was calculated on the basis of our data only (solid symbols). The dotted line represents the calculated furosine content in whole egg on the basis of the weight ratio of albumen and yolk.

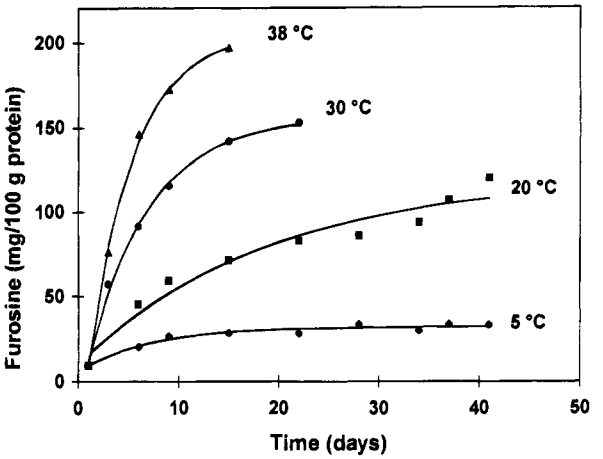

Figure 2. Formation kinetics of furosine in albumen during storage of eggs at different temperatures.

three egg fractions, when compared with other egg freshness parameters, is lower than that of uridine and higher than that of pH (Rossi and Pompei, 1995).

Furosine Content Evolution during Egg Aging. Figure 1 reports the increase in furosine content in yolk, albumen, and whole egg of eggs stored at 20 °C for 40 days. In very fresh eggs (those analyzed 1 day after laying) yolk has a higher furosine content than albumen and, as a consequence, than whole egg. During storage at 20 °C the increase of furosine content in yolk is minimal when compared with the increase in albumen.

Since Maillard reaction is favored by alkaline conditions, the different reaction rates in yolk and albumen may be explained by the different values of pH in these two fractions. Following Romanoff and Romanoff (1949) albumen and yolk pH values in newly laid eggs are 7.6 and 6, respectively. During egg storage at 20 °C, albumen pH rapidly reaches values of 9.3–9.4 in the first 10 days, remaining constant afterward, while yolk pH increases slowly and evenly, reaching at 40 days values of about 6.6 (Rossi et al., 1995).

In whole egg, the furosine increase does not reflect the weight ratio existing between yolk and albumen. In fact, measured furosine values in whole egg are quite below the expected ones on the basis of furosine content of albumen and yolk. A plausible explanation for this discrepancy cannot be supposed at the moment. Experiments are in progress to investigate the phenomenon.

In Figure 1 experimental points of furosine content in albumen of another lot of eggs stored at 20 °C (Comelli, 1994) have been added for comparison. This set of data is fairly consistent with the kinetics observed with our experimental points and with the supposed curve trend after 40 days of storage.

Figure 2 reports kinetics of furosine content in albumen during eggs storage at different temperatures. The experimental points at each temperature have been interpolated by the best fitting curve.

During the first days of storage, there is a quick increase in furosine which is clearly and strongly dependent on temperature; afterward, the increase continues but at a lower rate. As a whole, the curves show an exponential trend, as already observed by Resmini et al. (1990b) in cow milk treated at 110 and 118 °C for up to 60 min.

Chiang (1988), analyzing furosine content in powdered meal replacer products treated at 110 °C for up to 5 h, stressed that furosine level, after reaching a maximum, decreases gradually with treatment time at that high temperature. An increase followed by a decrease is also reported by Kato et al. (1982) in their study of the formation kinetics of ϵ-fructose–lysine in a model system of L-lysine and D-glucose heated at 75 °C for a maximum of 72 h. Actually, ϵ-fructose–lysine is only an intermediate in Maillard reaction. Under severe treatment conditions, Maillard reaction continues with a decrease of furosine (Erbersdobler, 1986) and the formation of other products such as ϵ-pyrrole–lysine, as shown by Chiang (1988).

Egg storage conditions used in the present work limit Maillard reaction progress at its first stages when ϵ-fructose–lysine shows increasing trends. Because albumen pH is strongly dependent on storage time and temperature, as already mentioned (Rossi et al., 1994), a proper calculation of the activation energy (E_a) for furosine formation reaction based on experimental kinetics is not possible, given the influence of pH on formation of Amadori compounds (Lee et al., 1984).

Based on the interpolation curves of the experimental values in Figure 2, the curves of identical levels of furosine formation in the albumen of eggs stored at different temperatures are presented in Figure 3. The curves are mostly parallel, except the one relative to 20 mg/100 g of protein that diverges from the others. This is because the low levels of furosine are strongly affected by Maillard reaction development during the first 24 h of egg storage, that in our case was under noncontrolled conditions.

The first point (first day) reported in Figure 2 does not belong to any of the four curves because eggs in the first 24 h, during gathering, candling, and transporting, were exposed at noncontrolled temperatures. This fact mostly influences the shape of the curve at 5 °C (Figure 2) that causes a different trend for the formation of 20 mg/100 g of protein of furosine in Figure 3.

What has been discussed before does not justify completely this phenomenon, and a different kinetics of furosine formation at 5 °C with respect to the other temperatures could be hypothesized.

Conclusions. This work demonstrates that furosine is a good index of shell egg freshness, especially when

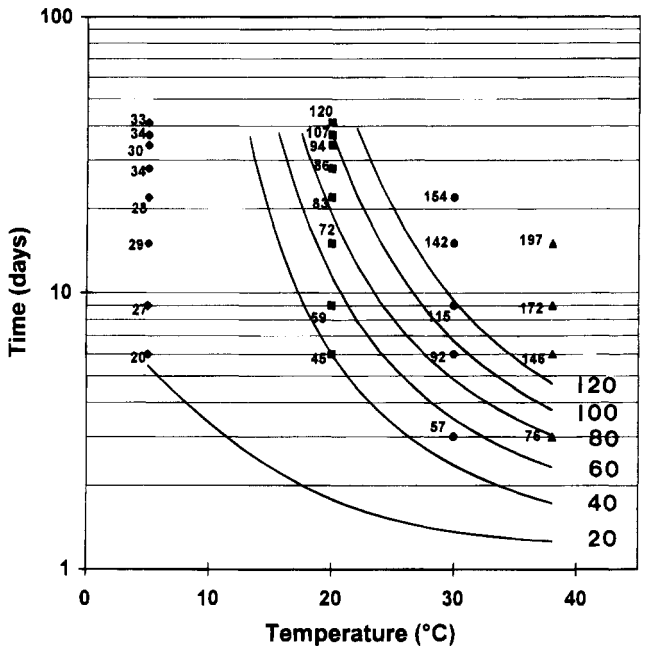

Figure 3. Levels of furosine formation in albumen of eggs stored at different temperatures. The curves from the bottom to the top correspond to a furosine formation level from 20 to 120 mg/100 g of protein. Smaller numbers represent the experimental furosine values.

determinated in the albumen, where it is formed at higher levels as a function of storage temperature. Using the curves of identical levels of furosine formation (Figure 3), it is possible to predict furosine levels at nonexperimental conditions or to ascribe different combinations of time and temperature of storage to each furosine concentration. In this way furosine determination could be interesting particularly for markets in which egg commercialization at room temperatures is diffused. European Community (EC) regulations do not require egg refrigeration but recommend it for domestic storage. When fresh eggs (grade A-extra and grade A eggs) are maintained at temperatures lower than 5 °C, they are downgraded and are not considered as *fresh eggs* anymore. Grade A-extra and grade A eggs must have air cell heights lower than 4 and 6 mm, respectively (EEC, 1991). After 7 days from packaging, grade A-extra eggs are downgraded to grade A (EEC, 1990). For grade A eggs, EC regulations do not state a limiting time for consumption but prescribe the indication "best before date" on egg packages (EC, 1993).

The kinetics of furosine formation reported in Figure 2 allow us to hypothesize limits for furosine concentration in shell eggs commercialized in the EC. We propose limits of 60 and 90 mg/100 g of protein in albumen for grade A-extra and grade A eggs, respectively. These limits were calculated on the basis of a maximum storage temperature of 20 °C for periods of 10 and 30 days after laying for grade A-extra and A eggs, respectively. For grade A-extra eggs the 10 days considered include 7 days from the date of packaging as mentioned above. For grade A eggs we considered a limiting time of 30 days after laying (as indicated by some Italian producers). We suggest that a very fresh egg, suitable even for raw consumption, must have less than 25 mg of furosine/100 g of protein, taking into account a maximum of 3 days after laying.

Furosine seems to be a more sensible indicator for measuring shell egg freshness than the criteria adopted by EC regulations (EEC, 1991) or the physical and chemical parameters formerly proposed (Kessler et al., 1990; Silversides et al., 1993; Rossi et al., 1995). Physical methods are fast and cheap but are dependent on several variables (Eisen et al., 1962; Sauver and de Reviers, 1988; Kessler et al., 1990). Chemical methods are advantageous when the estimation of raw material freshness in the egg products is needed. Hence, furosine determination in albumen and whole egg products (i.e. pasteurized and frozen) could be a promising index for egg products quality.

ABBREVIATIONS USED

CV, coefficient of variation; E_a, activation energy; EC, European Community; HPLC, high-performance liquid chromatograph; P, probability; SD, standard deviation; UHT, ultrahigh temperature.

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