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Estimation of equivalent egg age through furosine analysis

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Abstract

The aim of this research was to study furosine as a reference index for expressing the age of commercial shell eggs in terms of equivalent egg age. Seventy five commercial samples of grade A eggs purchased on the Italian market were analysed for air cell height, Haugh Units, 3,3′,5,5′ tetramethylbenzidine test, and furosine level. The values obtained were reported as a function of egg age calculated from the minimum durability dates printed on the packages. At equal egg age, a wide data variation for all indices was observed, thus indicating the great influence of commercialization condition on the assessed indices. An average kinetics of furosine development in albumen at 20 °C was also computed from nine storage experiments of shell eggs laid by hens of different ages and breeds. The equivalent egg age of the commercial samples, expressed as days at 20 °C, was computed by substituting the furosine value of each sample in the average kinetics equation.

Keywords: Air cell height; Egg freshness; Equivalent egg age; Furosine; Haugh Units; Shell eggs; TMB test

1. Introduction

Most shell eggs marketed in Europe are fresh eggs, with no treatments (washing, oiling, etc.) allowed. European Union (EU) regulations do not require shell egg refrigeration, except in European countries where local regulations are in force, but recommend it for domestic storage. Furthermore, storage at temperatures lower than 5 °C implies downgrading of commercial fresh eggs (grade A and A-extra eggs) to the B category (EU, 2003). For this reason, marketing times are short and well defined: grade A-extra eggs are downgraded to grade A 7 days after packaging or 9 days after laying and must have an air cell height lower than 4 mm; grade A eggs must be sold and used by 21 and 28 days after laying, respectively; moreover, an air cell height lower than 6 mm must be guaranteed till the "use by" date, also known as the minimum durability date (EU, 2003).

Freshness, the characteristic most commonly related to egg quality, declines after laying mainly in dependence of time and temperature. This quality decay is associated with chemical, nutritional, functional, and hygienic changes. Since egg quality varies with time as a function of storage temperature, freshness can not be defined only by egg age.

The most common indices used to evaluate egg freshness are air cell height, affected by egg weight and storage relative humidity (Kessler, Sinell, & Wiegner, 1990; Rossi, Hidalgo, & Pompei, 1995; Sauveur & de Reviers, 1988), and Haugh Units (HU), influenced by hen age (Eisen, Bohren, & McKean, 1962; Sauveur & de Reviers, 1988; Silversides & Villeneuve, 1994; Silversides, Twizeyimana, & Villeneuve, 1993).

Furosine [ε-*N*-(2-furoylmethyl-L-lysine)], produced by acid hydrolysis of the Amadori compounds, is a promising shell egg freshness index when determined

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in albumen (Hidalgo, Rossi, & Pompei, 1995; Lucisano, Hidalgo, Comelli, & Rossi, 1996). This index shows high repeatability and low natural variability in fresh eggs (Hidalgo et al., 1995) and, moreover, is independent from egg weight, hen age and storage relative humidity. Because of its rapid increase at medium–high (20–38 °C) storage temperatures, furosine evaluation might be particularly useful for markets in which egg commercialisation at room temperature is common place.

The aim of this research was therefore to study furosine as a reference index for the evaluation of the true freshness of commercial shell eggs in terms of equivalent egg age. For comparison, the classical egg freshness/quality indices and an innovative rapid test (Rossi, Hidalgo, & Pompei, 2001) were also assessed.

2. Materials and methods

2.1. Eggs

Seventy five commercial samples, including at least 12 grade A eggs (EU, 2003), of 40 different brands were purchased on the market in different seasons and in various Italian areas and were analysed for furosine content in albumen, reaction between 3,3′,5,5′ tetramethylbenzidine and albumen (TMB test), air cell height, and Haugh Units (HU).

To study the kinetics of furosine formation in shell eggs, seven lots of newly laid eggs, laid by 40-, and 56-weeks-old Hy-Line Brown Plus hens and by 39-, 41-, 53-, 64-, and 66-weeks-old Isa-Brown Warren hens, obtained at different times directly from two producers and available in the laboratory within 24 h of laying, were stored at 20 °C up to 59–71 days.

2.2. Analytical methods

Furosine and TMB tests were performed on bulked albumen from six eggs per sample. Shelling and accurate separation of albumen from yolk were done manually. The albumens were homogenised using a Sörvall Omni-Mixer (model 17106, Dupont de Nemours & Co, Newtown, CT) at 3000 rpm for 30 s.

Furosine (mg/100 g of protein) was estimated as the average of three replicated analyses, following the HPLC method proposed for milk by Resmini, Pellegrino, and Battelli (1990), slightly modified for albumen as described by Hidalgo et al. (1995). A calibration curve was prepared in the concentration range of 0–9.9 μmol L^{-1} with 25 different levels of hydrated furosine · 2HCl (Neosystem Laboratoire, Strasbourg, France) in 3 N HCl, obtaining a linear relationship with $r = 1$ ($p \leqslant 0.001$). Based on the calibration curve, the detection limit (Miller & Miller, 1988) in the injected standard solution was 0.2 μmol L^{-1}. Protein content was calculated as total nitrogen multiplied by the factor 6.25. Total nitrogen analysis was performed using the Kjeldahl method *n*. 925.31 (AOAC, 1995, chap. 34).

TMB test was performed on albumen following the method proposed by Rossi et al. (2001). The results are the average of three measurements and are expressed as absorbance at 370 nm.

Air cell height (mm) was determined using a home-made graduated measuring card as described by Sauveur and de Reviers (1988). The results are the average of measurements performed on 12 eggs.

Albumen height for Haugh Units evaluation was measured at 12 °C using the QCD System (Technical Services and Supplies, York, England). HU were estimated following the equation proposed by Haugh (Stadelman, 1995). Results are the average of three eggs measurements.

2.3. Statistical analysis

Correlation analysis was computed with Systat software following the Pearson approach. The equation, confidence and prediction limits of the consensus furosine formation kinetics were calculated using Table Curve 2D (Jandel Scientific, San Rafael, CA) software.

3. Results and discussion

Fig. 1 presents furosine content, TMB-albumen reaction product absorbance, air cell height and HU of the 75 commercial samples analysed, as a function of the egg age calculated on the basis of the ‘‘use by’’ date reported on packages. At each egg age, a wide variation for all quality parameters is observed, suggesting that egg age, expressed as days from laying, is not sufficient to express real egg freshness. All samples complied with air cell height limit for grade A eggs (6 mm), although some samples presented border-line values several days before the expiration date. Compliance with this limit is hardly achieved without temperature and relative humidity control during egg storage: in fact, for grade A eggs stored at 20 °C the limit is actually exceeded well before reaching the 28 days set by law, and water evaporation through the shell is five times faster at 23% than 79% relative humidity (Rossi et al., 1995).

The results of the correlation analysis reported on Table 1 evidence a highly significant ($p \leqslant 0.001$) correlation between declared egg age and the four freshness parameters, with the highest correlation coefficient being with air cell height. Since Pearson's correlation indicates the prediction capacity of one variable from the other by using a linear equation (Wilkinson, Hill, Welna, & Birkenbeuel, 1992), the best correlation coefficient observed for air cell height may be a consequence of the linear kinetics pattern of this variable (Rossi et al., 1995).

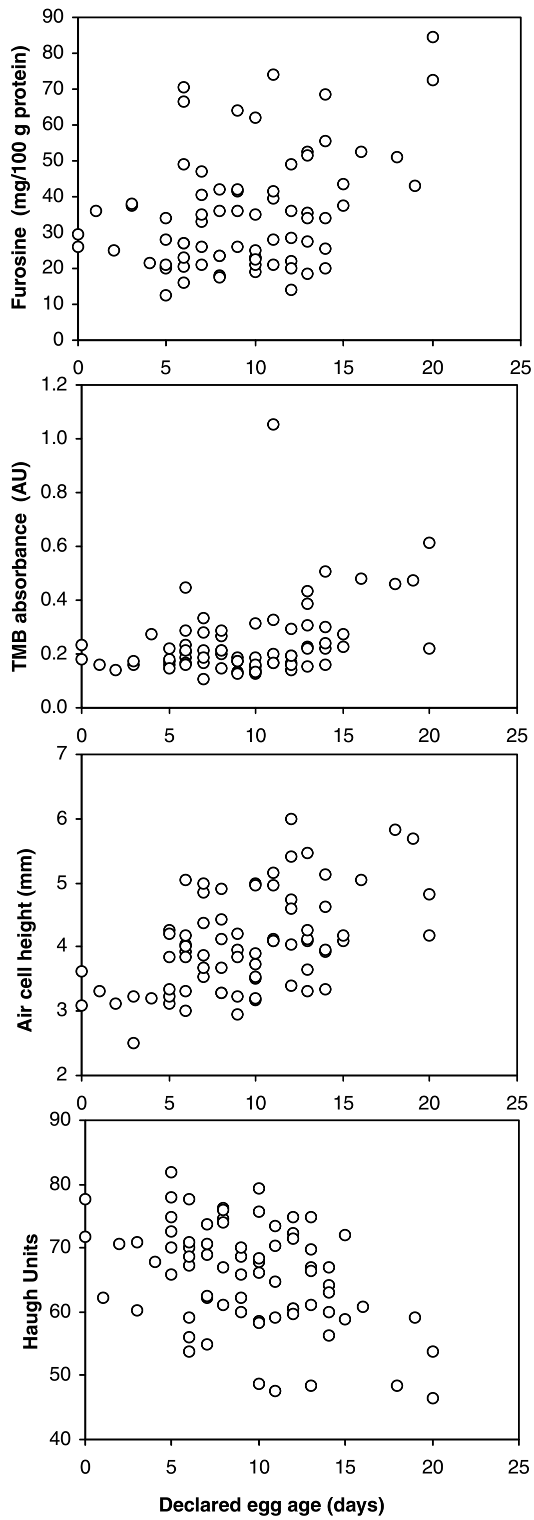

Fig. 1. Furosine, TMB-albumen reaction product absorbance, air cell height and Haugh Unit of 75 commercial shell egg samples as a function of egg age estimated from the "use by" date reported on packages.

All the other freshness parameters (furosine, TMB test, and HU) have curvilinear kinetics (Hidalgo et al., 1995; Rossi et al., 1995, 2001) and are highly correlated to each other ($p \leqslant 0.001$), while. air cell height correlation vs. furosine is non significant and vs. TMB test is significant only at $p \leqslant 0.05$.

Table 1
Correlation coefficients between egg freshness parameters measured on the 75 commercial shell egg samples

	TMB	Air cell height	HU	Declared egg age (days)
Furosine	0.605^{***}	0.154	-0.600^{***}	0.378^{***}
TMB		0.243^{*}	-0.452^{***}	0.376^{***}
Air cell height			-0.371^{***}	0.512^{***}
HU				-0.429^{***}

* Significant at $p \leqslant 0.05$.
*** Significant at $p \leqslant 0.001$.

Since furosine presents a natural variation (CV = 9.7%) (Hidalgo et al., 1995) lower than TMB test (CV = 10.3%) (Rossi et al., 2001), air cell height (CV = 15%) (Rossi et al., 1995), and HU (CV = 12%) (Rossi et al., 1995), it was chosen as the reference index to express the equivalent age of the commercial shell eggs. To this aim, a consensus furosine formation kinetics at 20 °C was computed by interpolation of the furosine levels obtained in nine different storage assays, performed using nine lots of shell eggs laid by hens of different ages and breeds (Fig. 2). The data set also included the results of the two storage assays reported by Hidalgo et al. (1995) and by Lucisano et al. (1996). Hen age does not seem to contribute to data variability,

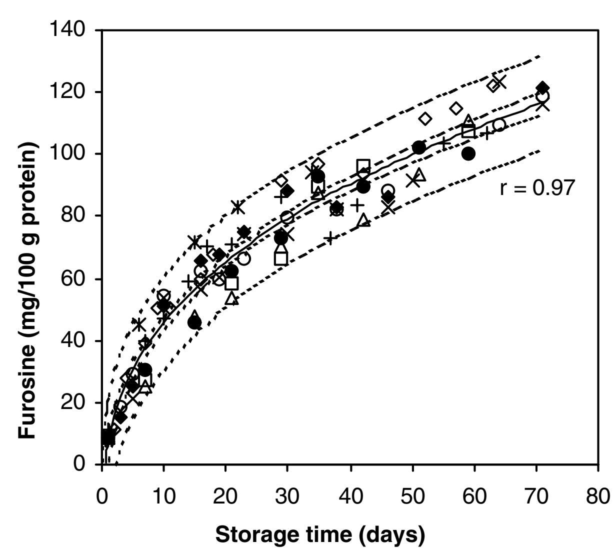

Fig. 2. Consensus kinetics of furosine development in albumen during storage at 20 °C of nine lots of shell eggs laid by 40- (○) and 56- (◆) weeks-old Hy-Line Brown Plus hens and by 39- (●), 41- (△), 53- (+), 64- (□), 66- (×), 64- (✱), and 69- (◆) weeks-old Isa-Brown Warren hens. Data relative to the last two lots are from Hidalgo et al. (1995) and Lucisano et al. (1996), respectively. Dotted lines represent the confidence and prediction limits at $p \leqslant 0.05$.

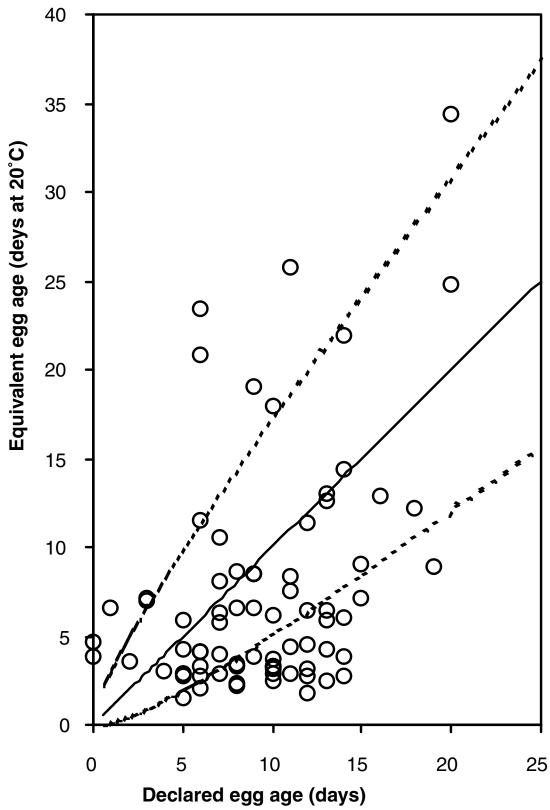

Fig. 3. Equivalent egg age of 75 commercial shell egg samples, as a function of the egg age estimated from the "use by" date reported on packages. Solid line represents the bisector while dotted lines are the prediction limits.

since single kinetics obtained with different egg lots overlap each other.

From the furosine analytical value (F) of each commercial sample and with the consensus kinetics equation of furosine formation at 20 °C ($F = 29.48\mathrm{EA}_{20\,°\mathrm{C}}^{0.36} - 22.32$), it is possible to calculate the $\mathrm{EA}_{20\,°\mathrm{C}}$ value corresponding to the equivalent egg age (days at 20 °C) of the sample. Fig. 3 reports the calculated $\mathrm{EA}_{20\,°\mathrm{C}}$ of the 75 commercial samples as a function of their declared egg ages. The solid line represents the bisector and the dotted lines are the prediction limits computed from the equation of both superior and inferior prediction curves ($p \leqslant 0.05$) reported in Fig. 2 ($F = 27.74\mathrm{EA}_{20\,°\mathrm{C}}^{0.37} - 4.91$, $F = 31.34\mathrm{EA}_{20\,°\mathrm{C}}^{0.35} - 39.85$, respectively). Most of the samples (45%) lie in the area defined by the prediction limits, indicating declared egg ages consistent with their $\mathrm{EA}_{20\,°\mathrm{C}}$. The samples lying above the superior prediction line (16% of the total), on the other hand, were not properly stored during their commercial life, since their freshness was lower than expected from the age stated on their packages. Interestingly, 75% of these poor quality samples were purchased during the warm season; in some cases, false date indication on the packages could also be hypothesized. However, a high percentage (39%) of the eggs analysed lie below the inferior prediction line: this means that, although refrigeration is not mandated by EU law, in some cases, the temperatures were kept below 20 °C during commercialisation. It is even possible that some samples were stored at temperatures inferior than 5 °C.

Although the population sample analysed is relatively small, the results depict a positive image of shell eggs freshness in the Italian market, since only three samples out of 75 presented an $\mathrm{EA}_{20\,°\mathrm{C}}$ higher than 21 days.

4. Conclusions

This research confirmed the possibility of expressing shell egg freshness as equivalent egg age, using furosine as a reference index. The same approach may be followed with other shell egg freshness parameters that are independent from hen age, egg weight and relative humidity of storage environment, i.e. TMB test.

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