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Analytical Methods

Changes of yolk biogenic amine concentrations during storage of shell hen eggs

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ABSTRACT

The RP-HPLC/UV method, using dabsyl derivatization, optimised for the determination of biogenic amines in egg yolk, was appropriate for quantification of putrescine, cadaverine, histamine, ethylamine, propylamine, ethanolamine, tyramine, tryptamine, spermine, spermidine, phenylethylamine. Detection limits ranged between 0.05 and 0.06 mg of biogenic amine/kg of egg yolk. Two experiments using, respectively, farm and avian eggs were conducted to evaluate yolk biogenic amine concentrations of fresh and stored eggs, and to explain the effect of temperature and time of storage in the levels of biogenic amines during egg shelf-life. Only five of the 11 biogenic amines under study were detected: putrescine, cadaverine, propylamine, ethylamine and ethanolamine. Storage time during shelf-life presented a significant effect on the levels of the five amines ($p < 0.01$). On the contrary, storage temperature did not presented a significant effect on the levels of the mentioned amines, $p > 0.01$. The significant reduction of biogenic amine concentration during the shelf-life justified the application of a multiple linear regression using stepwise method to estimate the storage time. The regression equation was applied with success to confirm the storage time of farm eggs and avian eggs that were stored at two different temperatures.

1. Introduction

An egg must be sold and used by 21 and 28 days after laying, respectively. 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 (Scott & Silversides, 2000; Silversides & Scott, 2001). Egg quality varies with time as a function of storage temperature. Most shell eggs for both retail and food processing are stored, with or without cooling, prior to use, and the storage conditions are quite essential for egg quality (Karoui et al., 2006).

The changes that occur in shell eggs during storage are many and complex and include, thinning of albumen, increase of pH, weakening and stretching of the vitelline membrane and increase in water content of the yolk, changes in protein conformation, loss of vitamins, such as vitamin B1, among others (Hammershøj, Larsen, Andersen, & Qvist, 2002; Hidalgo, Rossi, & Pompei, 2006; Hisil & Ötles, 1997; Karoui et al. 2006; Silversides & Budgell, 2004). To our knowledge, no study on the evolution of biogenic amine concentration of egg yolk during shelf-life of hen eggs has been published.

Biogenic amines are formed by the action of living organisms. Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine, and spermidine are considered to be the most important biogenic amines occurring in foods (Ruiz-Capillas & Jiménez-Colmenero, 2004; Shalaby, 1996; Smith, 1980). The simple aliphatic monoamines are widespread. The diamine putrescine and the polyamines spermidine and spermine occur universally in animals and plants and play regulatory roles in cell growth (Bardocz, Grant, Brown, Ralph, & Pusztai, 1993; Bardócz et al., 1995; Nishimura, Shiina, Kashiwagi, & Igarashi, 2006; Smith, 1980). Intake of polyamines from foods is of significant interest because as people become older it is known that the activity of one of the key polyamine biosynthetic enzymes, ornithine decarboxylase, decreases with increasing age (Nishimura et al., 2006). Amino acid decarboxylation is the most common mode of synthesis of amines in foods. The aromatic amines may render a food toxic.

The biogenic amine content of various foods, such as, cheese, fish and meat products have been widely studied (Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, & Vidal-Carou, 1997; Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997; Ruiz-Capillas & Jiménez-Colmenero, 2004; Shalaby, 1996). However, little information is available on the polyamine content and composition of eggs (Cipolla, Havouis, & Moulinoux, 2007; Eliassen, Reistad, Risøen, & Rønning, 2002), part of the published data is based only on 2–5 samples. Polyamine contents in boiled eggs were found to be very low, however, only values of five samples were reported (Kalač & Krausová, 2005; Larqué, Sabater-Molina, & Zamora, 2007). According to Nishimura et al. (2006), only traces of polyamines were found in egg albumen whereas higher amounts (between 0.58 and 26.7 mg/kg) were found in egg yolk. Thus, egg yolk seems to be the major source of egg biogenic

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amines. Hen egg yolk represents an essential ingredient, which has been used for many years by the food industry because of its excellent nutritional, organoleptic and functional properties. Thus, the knowledge of its biogenic amine concentrations is important to better understand the nutritional composition of this food ingredient.

The aim of this research was to study the evolution of yolk biogenic amine contents during shelf-life of hens shelled eggs. Two duplicate experiments were conducted to determine: (i) the effects of storage time of hens shelled eggs on biogenic amine levels of egg yolk; (ii) the effects of two storage temperatures (5 and 25 °C); (iii) find a possible interaction between storage time and temperature and (iv) select parameters to estimate egg storage time during shelf-life.

Biogenic amines were quantified by RP-HPLC-UV using dabsyl derivatization. Modifications of the method of Pinho et al. (2004) were directed to optimise extraction and chromatographic separation of biogenic amines in egg yolk without the presence of free amino acids. Several advantages of the dabsyl method over other derivatising reagents are stability of dabsyl derivatives at room temperature and detection in the visible region (λ = 436–460 nm) with high specificity and sensitivity.

2. Materials and methods

2.1. Experimental design

Two experiments were carried out on hen eggs. A total of 164 eggs weighing between 53 and 62 g for were used.

Experiment 1. Twenty-four eggs, weighing between 53 and 58 g, obtained in the same day, in the same farm, from a group of around 30 hens at age between 42 and 61 weeks were selected for this experiment. Hens were given free access to feed and water since age of 10 weeks. Feed included corn and vegetables (carrots, cabbage, fruits and others). After collection, the eggs were stored, in the laboratory, during its shelf-life at two different temperatures (e.g. 5 and 25 °C) and at different days of storage (1, 4, 8, 12, 16 and 20 d) the biogenic amine contents of yolk was evaluated after separation from albumen.

Experiment 2. One hundred and forty shelled avian eggs from five different brands were collected from the market. Eggs with the same age (±3 days) without cooling were used. After arriving to the laboratory, the eggs were stored during its shelf-life at two different temperatures (e.g. 5 and 25 °C) and at different days of storage (3, 8, 10, 13, 17, 23 and 26 d) the biogenic amine contents of yolk was evaluated after separation from albumen.

2.2. Sample treatment

Biogenic amines were extracted according to the following procedure. Egg yolk (5-g) was suspended in 5% trichloroacetic acid up to 6 ml; the mixture was homogenised in an Ultra Turrax blender (Sotel, Warsawa, Poland) for 1 min and centrifuged at 9000*g* for 10 min at 25 °C. From the resulting extract 2 ml were taken and 500 μl of phosphate buffer 0.2 M, pH 7.4 and 1.0 ml of 0.1 M 2-ethylhexyl phosphate/chloroform. After centrifugation (5000 rpm, 5 min, 25 °C) two phases were obtained, chloroform phase is separated and 1.0 ml of HCl 0.1 M were added. The mixture was homogenised in a vortex and 400 μl of upper phase were evaporated under a stream of nitrogen at 55 °C and the analytes were redissolved in 200 μl of reaction buffer (0.15 mol/l $NaHCO_3$, pH 8.6).

Derivatization was carried out with 200 μl of dabsyl chloride solution (12.4 mM in acetone) at 70 °C for 15 min. The reaction was quenched by placing the vials in an ice bath for 5 min.

2.3. HPLC analysis

The HPLC separations were performed at 50 °C, using a 150 mm × 4 mm × 3 μm Spherisorb ODS C_{18} column. An analytical HPLC unit (Jasco) equipped with two Jasco PU-2080 Plus HPLC pumps, a MD-2010 Plus Multiwavelength detector and a type 7125 Rheodyne Injector with a 20 μl loop. The Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was also used. Elution was carried out at a flow rate of 1 ml min^{-1}, using a volumetric gradient of solution A – 9 mM aqueous sodium dihydrogenophosphate, 4% (w/v) dimethylformamide and 0.1% (w/v) triethylamine (adjusted to pH 6.55 with phosphoric acid), and solution B – 80% (v/v) aqueous acetonitrile. The gradient applied comprised a step from 40% to 76% (v/v) B in A within 15 min, from 76% to 78% (v/v) B in A within 5 min, from 78% to 100% (v/v) B in A within 10 min, and return to initial conditions in 5 min. Detection was performed by measuring absorbance at 436 nm.

2.4. Quantification of biogenic amines in egg yolk

Ethylamine, propylamine, tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, and ethanolamine were from Sigma Chemical. Spermine and spermidine were obtained from Aldrich. Quantification was carried out by external standard method. A stock solution for each standard was prepared at a concentration of 4 g/l. Working standard solutions containing a mixture of the biogenic amines under study in HCl 0.1 M were prepared in the following dilutions 1.56; 3.13; 6.25; 12.5; 25.0; and 50.0 mg/l. Standard solution (20 μl) were evaporated under a stream of nitrogen at 55 °C, redissolved in 200 μl of reaction buffer (0.15 mol/l $NaHCO_3$, pH 8.6) and derivatised according to the conditions described above for samples.

The biogenic amine content in egg yolk is presented in mg of each amine/kg of egg yolk. Calculations were performed according to the egg yolk weight used in the extraction procedure and the successive volumes of extract used as described in sample treatment.

2.5. Statistical analyses

The averages and standard deviations were calculated for each biogenic amine. Descriptive statistics, analyses of variance (Two Way-ANOVA) and multiple linear regressions were all performed with SPSS for Windows, v. 15.0 (SPSS, Chicago IL, USA).

3. Results and discussion

3.1. Quantification of biogenic amines in egg yolk

Fig. 1 shows a typical chromatogram for a standard solution mixture containing 12.5 mg/l of each standard. The identification was made by comparison of the retention time with that of the corresponding standard.

The linearity of the RP-HPLC-UV method has been investigated over the ranges 1.5–50.0 mg/l for each amine. Calibration curves were calculated from the representation of the peak area of the analytes versus the concentration of each compound in standard solutions. Curves were fitted to a linear function, obtaining regression coefficients higher than 0.9991 in all cases. To evaluate the analytical performance of the HPLC-Diode array method repeatability or run-to-run precision and medium term or day-to-day precision were determined. To calculate repeatability and medium term precision, six daily replicate analysis of a standard solution of all the analytes at 10 mg/l were carried out on the same day and on different days. A study of the variance of one factor for retention

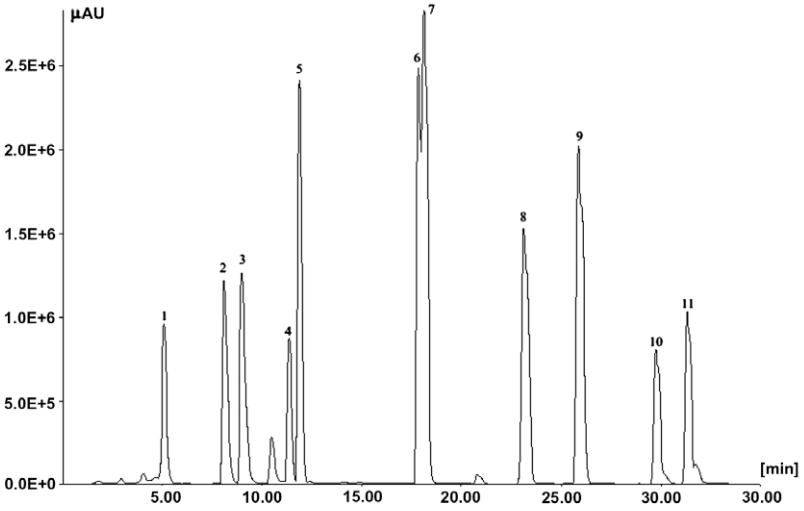

Fig. 1. Typical chromatogram for standard solution containing 12.5 mg/l of each biogenic amine (chromatographic conditions described in the text): 1 – putrescine, 2 – cadaverine, 3 – histamine, 4 – ethanolamine, 5 - tyramine, 6 – ethylamine, 7 – propylamine, 8 – tryptamine, 9 – phenylethylamine, 10 – Spermine and 11 – spermidine.

and time concentration was then performed. The RSD for retention time ranged between 0.05% to 0.26% for run-to-run precision and between 0.04% and 0.18% for day-to-day precision. For concentration, run-to-run precision, expressed as RSD, ranged between 0.02% and 5.72%, and day-to-day precision ranged between 3.07% and 6.52%. Detection limits (LODs), based on a signal-to-noise ratio of 3:1 ranged between 0.15 and 0.20 mg/l, corresponding to 0.05 and 0.06 mg of biogenic amine/kg of egg yolk. Similar detection limits were obtained by other authors using derivatization with dabsyl chloride and diode array detection (Krause, Bockhardt, Neckermann, & Klostermeyer, 1995; Pinho, Ferreira, Mendes, Oliveira, & Ferreira, 2001).

Yolk samples were purified using the extraction procedure described in Materials and Methods, which proved a suitable clean-up of the biogenic amines to permit their analysis by HPLC/diode Array. Thus, the method was appropriate for the analysis on 11 biogenic amines in egg yolk.

Only five biogenic amines were detected and quantified in egg yolks from experiment 1. Concentrations ranged between 9.32 and 1.08 mg of ethanolamine/kg of yolk, 7.79 and 0.59 mg of ethylamine/kg of yolk, 9.92 and 2.72 mg of propylamine/kg of yolk, 20.8 and 0.19 mg of putrescine/kg of yolk, 11.32 and 6.89 mg of cadaverine/kg of yolk (Fig. 2a and b).

Samples of egg yolk from experiment 2 presented the same five biogenic amines. Mean values and standard deviation are shown in Table 1. In general similar behaviour was observed for all amines during storage time, its content decreased, specially putrescine, ethanolamine and ethylamine. Apparently, similar results were obtained at 25 and 5 °C.

Some authors mentioned lower contents of biogenic amines in eggs, since maximum levels of amines were 0.4 mg/kg of putrescine, 0.5 mg/kg of cadaverine, 0.5 mg/kg of histamine and 1.4 mg/kg of spermidine (Kalač & Krausová, 2005; Okamoto et al., 1997). However, these results respect to the analysis of a small number of egg samples (whole egg) and no reference is made concerning storage time. Similar situation was observed with respect to results presented by Bardócz et al. (1995) that refer contents of 0.26–0.35, 0–0.14 and 0.20–0.60 mg/kg, respectively, for putrescine, spermidine and spermine in boiled egg. Recently, Nishimura, Shiina, Kashiwagi, and Igarashi (2006), described the contents of putrescine, spermidine and cadaverine found in egg yolk, respectively, 0.88, 0.58 and 26.66 mg/kg. Other authors identified the polyamines putrescine, spermidine and spermine in the following concentrations 0.05, 0.10 and 0.05 mg/kg of egg (Larqué et al., 2007).

In some cases the levels of biogenic amines, found in the two experiments were lower than those mentioned in literature. However, the analyses were performed in egg yolk and not in whole egg. Additionally, storage time and temperature is not referred by other authors. On the other hand, spermine and spremidine usually described by other authors, were below the detection limit of the method.

3.2. Effect of storage time and temperature during eggs shelf-life on the contents of yolk biogenic amines

Statistical test two-way-ANOVA was used, in order to verify whether the average values obtained in experiment 2, during storage time at two temperatures for each variable, (ethanolamine,

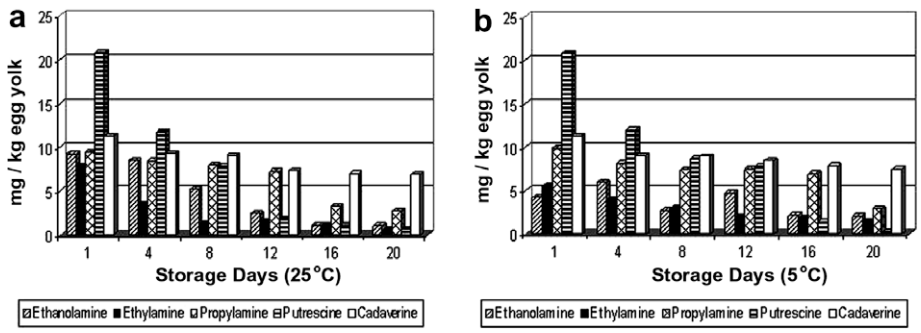

Fig. 2. Biogenic amine content of yolk from experiment 1 eggs: (a) stored at 25 °C, (b) stored at 5 °C.

Table 1
Composition of biogenic amines of yolk from experiment 2 eggs[a].

Storage days	Ethanolamine (mg/kg yolk)		Ethylamine (mg/kg yolk)		Propylamine (mg/kg yolk)		Putrescine (mg/kg yolk)		Cadaverine (mg/kg yolk)	
	25 °C	5 °C	25 °C	5 °C	25 °C	5 °C	25 °C	5 °C	25 °C	5 °C
3	14.28 ± 2.66	13.18 ± 2.57	6.86 ± 0.17	7.45 ± 0.89	10.47 ± 0.73	9.54 ± 0.93	12.04 ± 0.73	14.18 ± 0.93	8.65 ± 0.01	10.80 ± 1.84
8	8.92 ± 2.44	8.47 ± 186	4.45 ± 2.01	5.10 ± 0.32	10.13 ± 0.78	9.33 ± 0.52	10.05 ± 0.78	14.51 ± 4.09	8.62 ± 0.51	9.92 ± 0.97
10	5.39 ± 3.37	2.92 ± 0.99	2.08 ± 0.39	2.58 ± 2.47	9.87 ± 0.96	7.73 ± 3.07	6.77 ± 1.96	11.57 ± 3.07	8.69 ± 0.51	8.18 ± 0.73
13	4.82 ± 3.72	2.05 ± 2.61	1.43 ± 0.77	1.87 ± 0.72	5.61 ± 2.70	6.82 ± 2.48	6.77 ± 2.70	9.21 ± 4.67	5.77 ± 3.32	8.11 ± 1.73
17	3.80 ± 2.04	2.18 ± 0.86	1.75 ± 1.17	1.49 ± 0.23	7.30 ± 2.77	6.67 ± 0.72	3.41 ± 2.77	3.75 ± 0.72	8.14 ± 0.92	8.40 ± 1.19
23	3.46 ± 0.56	2.33 ± 0.79	1.48 ± 0.79	1.75 ± 1.15	6.59 ± 1.96	5.70 ± 2.30	1.97 ± 1.96	1.41 ± 2.30	7.46 ± 1.02	7.62 ± 0.42
26	3.30 ± 0.77	1.84 ± 1.20	1.47 ± 0.24	0.89 ± 0.34	8.08 ± 2.00	4.84 ± 1.22	2.23 ± 2.00	0.74 ± 1.22	7.63 ± 0.26	7.39 ± 0.30

[a] Mean and standard deviation of the results from five different brands analysed in duplicate ($n = 10$).

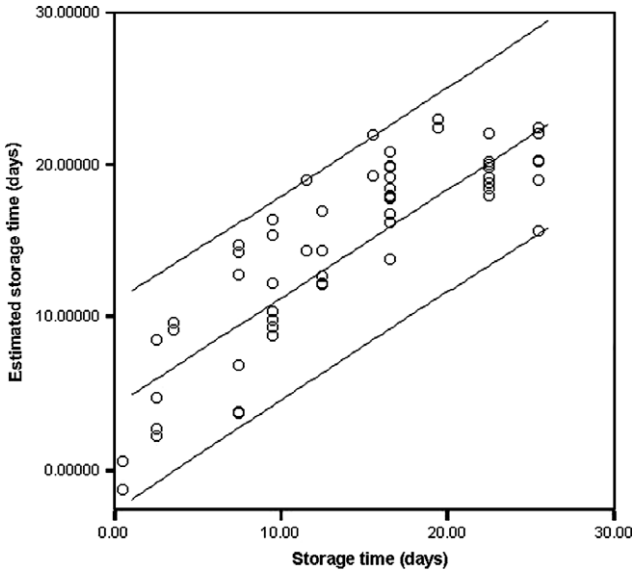

Fig. 3. Estimation of shell egg storage days. y = shell egg storage (days), x = biogenic amine concentrations, SE = standard error. Linear Regression with 95.00% Individual Prediction Interval; Estimated storage days = 4.20 + 0.71 * storage days; R-square = 0.71.

ethylamine, propylamine, putrescine, cadaverine) could be considered different or not. Two-way ANOVA not only assessed time and temperature effect, but also whether there is an interaction between the two parameters. The balanced design of experiment 2, involving five different brands of avian eggs was chosen for that purpose. The data obtained as values, for each group, presented normal distribution (Shapiro–Wilk Test) and homocedasticity of variances (Levene Test) was observed. It was concluded, with 95% confidence, that storage time during shelf-life presented significant differences for ethanolamine, ethylamine, propylamine, putrescine, cadaverine contents ($p < 0.01$). No significant differences were observed for the same amines at two different temperatures for $p > 0.01$. However, differences were significant for ethanolamine and propylamine for $p > 0.05$. No interactive effect was observed between storage time and temperature ($p > 0.01$). Thus, a significant decrease of biogenic amine contents during shelf-life was observed.

Silversides and Budgell (2004) evaluated a total of 2123 hen eggs and verified that with storage, egg and albumen weights decreased, whereas yolk weight increased. The increase of egg yolk may justify the decrease of biogenic amines during shelf-life. However, migration of biogenic amines to egg albumen and chemical modifications can also occur, because ethanolamine, ethylamine and putrescine falling faster then propylamine and cadaverine.

3.3. Estimation of storage time during shelf-life

Shell egg storage time during its shelf-life was estimated with stepwise variable selection involving the 5 biogenic amine concentrations as variables by multiple linear regression analysis. All data obtained from experiments 1 and 2 for different storage days and two temperatures (25 and 5 °C) were used. The general formula of the estimation equation is as follows:

$$y = a_1x_1 + a_2x_2 + \cdots + a_nx_n \quad (1)$$

where y is shell egg storage days and $x_1, x_2, \ldots, x_n$ are biogenic amine concentrations. The equation was:

$$y = 25.083 - 0.711 \times \text{putrescine} - 0.892 \times \text{ethylamine} - 0.508 \times \text{propylamine} \quad (2)$$

$$R = 0.842; \quad p < 0.01. \quad (3)$$

The correlation between the measured and estimated values is shown in Fig. 3. The product storage time can be estimated with 3 variables: putrescine, ethylamine and propylamine, as well as a constant. The estimation error is 3.9 d. The estimation of shell egg age on the basis of biogenic amine parameters has not been described previously.

4. Conclusions

The evolution of yolk biogenic amine contents during shelf-life of hens shelled eggs was assayed by RP-HPLC/UV using dabsyl derivatization. Five biogenic amines, ethanolamine, ethylamine, propylamine, putrescine, cadaverine were quantifies in egg yolk samples. Storage time during shelf-life presented significant differences for its contents, it decreased, specially putrescine, ethanolamine and ethylamine. On the contrary, storage temperature did not presented a significant effect on the levels of the mentioned amines. Shell egg storage time during its shelf-life was estimated with stepwise variable selection involving the five biogenic amine concentrations as variables by multiple linear regression analysis.

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