Bioactive amines and quality of egg from Dekalb hens under different storage conditions

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ABSTRACT The objective of this study was to investigate the possibility of using bioactive amines as an index of quality of fresh and stored eggs. Large white eggs from 24-wk-old Dekalb layers were randomly distributed in 3 groups: (i) 10 freshly laid eggs, (ii) 60 eggs stored at 25 ± 1°C and 60% RH for 30 d, and (iii) 50 eggs stored at 6 ± 1°C and 60% RH for 50 d. The eggs were weighed and the internal quality was evaluated by Haugh units (HU), pH of albumen, total solids, total volatile bases, and bioactive amines in the albumen and yolk. The fresh eggs had, on average, 56.85 g, 98.55 HU, albumen pH of 8.02, total solids of 12.17 g/100 g in the albumen and 52.43 g/100 g in the yolk, and absence of volatile bases. None of the 10 amines investigated were detected in the albumen; however, the yolk contained 0.37 mg/kg of spermidine. Throughout storage, there was a significant decrease in the weight of the egg and HU and a significant increase in the pH and in the total solids of the albumen. The decrease in HU and the increase in the total solids of the albumen were faster at 25 ± 1°C compared with 6 ± 1°C. At 50 and 30 d of storage at 6 ± 1 and 25 ± 1°C, respectively, significant levels of total volatile bases were detected. The levels of spermidine in the yolk increased significantly at the 40th and 15th days of storage at 6 ± 1 and 25 ± 1°C, respectively. At these storage times, the presence of putrescine and agmatine was also detected. Therefore, the presence of other amines besides spermidine or spermine levels higher than 1.0 mg/kg in the yolk could be used as an index of quality of fresh eggs and throughout storage.

Key words: egg, quality, storage, bioactive amine, Haugh unit

INTRODUCTION

Eggs are a good source of high-quality protein. They provide important sources of iron, vitamins, and phosphorus. Eggs provide a unique and well-balanced source of nutrients for persons of all ages. Low caloric value, ease of digestibility, and high nutrient content make eggs valuable in regular and many therapeutic diets. Availability, modest cost, ease of preparation, popular taste appeal, and low caloric value give eggs a primary advantage for human nutritional needs (Theron et al., 2003; Jones and Musgrove, 2005).

It is well known that the quality of eggs is affected throughout storage. Furthermore, it is known that the lower the storage temperature, the longer the shelf life of eggs. However, in several countries, refrigeration of eggs is not required during distribution and commercialization (Jones and Musgrove, 2005; Xavier et al., 2008). It is important, therefore, that those concerned with the handling of eggs are knowledgeable about egg quality to have a rational basis for day-to-day marketing decisions. Furthermore, they must be able to use an objective means to evaluate egg quality.

The internal quality of eggs can be evaluated by means of physical, chemical, biological, and functional characteristics. Factors like lineage, age, and feeding of the layers associated with temperature, air RH, and storage conditions and time influence the quality of eggs. Some alterations of the albumen and the yolk are verified along storage and the speed of the changes is affected by the storage temperature. Among the chemical reactions that take place inside the eggs during storage, there is the transformation of dense into liquid albumen. This change possibly involves H2CO3, one of the components of the buffering system of the albumen, which is dissociated into water and CO2 gas. There is a decrease in the total solids content of the albumen.
There is also an increase in the pH and a decrease in the weight of the egg (Brake et al., 1997; Alleoni and Antunes, 2001; Xavier et al., 2008).

Therefore, during storage, the internal quality of eggs may be determined by the increase in pH of the albumen, the decrease in the weight of the eggs, and by the decrease in the total solids content of the albumen. Based on some of these phenomena, a quality index was developed—Haugh units (HU). It is calculated using the height of the dense albumen and the weight of the egg (Scott and Silversides, 2000; Silversides and Budgell, 2004). According to the USDA (2000), HU values higher than 72 represent excellent egg quality (AA), values from 60 to 72 represent high quality (A), and values lower than 60 represent inferior quality (B). However, the evaluation of eggs should encompass other indices or characteristics.

Several indices of chemical quality based on the contents of bioactive amines have been proposed for foods, such as tuna fish (Veciana-Nogués et al., 1997), chicken (Silva and Glória, 2002; Moreira et al., 2008), and beer (Loret et al., 2005). Bioactive amines are organic bases of low molecular weight. They may be classified as polyamines and biogenic amines. The first are growth factors, whereas the latter are formed by the decarboxylation of amino acids by microbial enzymes (Glória, 2005). The quantity and the type of amines in a food depend on the nature of the food and its microbiota. Some microorganisms, namely enterobacteria, lactobacilli, pediococci, and enterococci are particularly active in the production of biogenic amines. The increase in these microorganisms and in the contents of biogenic amines during the storage of foods may represent loss of quality, mainly of products with a high content of protein (Coutts et al., 1986; Silva and Glória, 2002; Theron et al., 2003; Jones et al., 2004).

Very few studies have been undertaken on bioactive amines in eggs. Bardócz et al. (1995) found 0.26 to 0.35, 0 to 0.14, and 0.20 to 0.60 mg/kg of putrescine, spermidine, and spermine, respectively, in boiled eggs (n = 3). Okamoto et al. (1997) reported in boiled eggs (n = 2) 0.4 mg/kg of putrescine, 1.0 mg/kg of spermine, 0.5 mg/kg of cadaverine, 0.5 mg/kg of histamine, and 1.4 mg/kg of spermidine. Saito et al. (1992) determined the levels of bioactive amines in spoiled eggs and found 15.2 mg/kg of putrescine and 55.2 mg/kg of cadaverine. Therefore, information on the types and levels of amines in eggs when just laid is needed. Furthermore, information on the changes and the factors that may affect amine profile and levels in eggs is also important. These changes may be used as an objective and reliable index of quality for eggs, which could be used to better access egg quality.

The objective of this study was to investigate the possibility of using amines as a quality control index for the quality of eggs. Fresh eggs from 24-wk-old Dekalb hens were characterized with regard to bioactive amines and other physicochemical attributes. The influence of storage time and temperature on the levels of bioactive amines and on the physicochemical characteristics of Dekalb eggs was also investigated.

**MATERIALS AND METHODS**

**Materials**

One hundred twenty large (from 55 to 60 g) white eggs from 24-wk-old Dekalb laying hens were used. The hens were housed in the same laying facility (Fazenda Experimental Professor Hélio Barbosa, Escola de Veterinária, Universidade Federal de Minas Gerais) and were fed a commercial laying hen diet and water ad libitum.

The amine standards—spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, serotonin hydrochloride, β-phenylethylamine hydrochloride, agmatine sulfate, tyramine, and tryptamine—were purchased from Sigma Chemical Co. (St. Louis, MO). The reagents were of analytical grade, and HPLC solvents were chromatographic grade.

**Methods**

After classification, the eggs were placed in paper cartons and randomly distributed into 3 groups: (i) 10 eggs were analyzed immediately to determine the quality of the eggs immediately after being laid, (ii) 50 eggs were stored under refrigeration in a BOD incubator (Tecnal, Piracicaba, São Paulo, Brazil) set at 6 ± 1°C and 60% RH for 50 d, and (iii) 60 eggs were stored at room temperature (25 ± 1°C) and 66% RH for 30 d. Every 10- and 5-d intervals, 10 eggs stored at 6 ± 1 and 25 ± 1°C, respectively, were also analyzed. The eggs were weighed before and after storage. The internal quality of the eggs was analyzed by HU, pH, and total solids content of albumen, total solids of the yolk, total volatile bases, as well as bioactive amines present in both albumen and yolk.

**Methods of Analysis**

**Determination of the Weight Loss, HU, pH, Total Solids Content, and Total Volatile Bases.** The weight loss of the eggs during storage was calculated by weighing the samples before and after storage.

Haugh units were determined by using SAO equipment (Sistema de Análise de Ovos, Gil Fabricação e Projetos Especiais, Ribeirão Preto, São Paulo, Brazil), which determined the height of the albumen. The HU values were calculated from egg weight (W) and albumen height (H), considering the following formula: $HU = 100 \log (H + 7.57 - 1.7W^{0.5})$ as described by Silversides and Budgell (2004).

The pH of the albumen was determined using a pH meter (DM 20, Digimed, São Paulo, Brazil) previously calibrated with buffer solutions pH 7.0 and pH 10.0 (Brasil, 1999).
Total solids contents were determined in both albumen and yolk. Five grams of albumen and yolk was weighed separately in previously dried porcelain weighing dishes. Approximately 4 g of calcinated, washed, and dried sand was added to the capsule for the analysis of the albumen. Samples of albumen and yolk were kept in the oven at 105 ± 2°C (Quimis Q-317N22, Diadem, São Paulo, Brazil) for 5 and 12 h, respectively (MAPA, 1991).

Total volatile bases of the albumen and the yolk were determined separately. One gram of sample was placed in a Kjeldahl distillation unit (Tecnal TE-036/1) along with 20 mL of distilled water and 2 g of MgO. The distillate was collected in a flask containing 20 mL of 0.1 N H₂SO₄ and 5 drops of 0.1% methyl red. The final solution was titrated with 0.1 N NaOH (Brasil, 1999).

### Determination of Bioactive Amines

Amines were extracted from 3-g samples with 20 mL of 50 g/L of trichloroacetic acid in 3 successive extractions. After agitation for 10 min in a vortex mixer, the slurries were centrifuged at 10,000 × g at 4°C, and the supernatants were collected, combined, and then filtered through 13-mm-diameter and 45-μm pore HAWP membranes (Millipore, Bedford, MA). The amines were separated by ion-pair HPLC using a μBondapak C18 column, 300 × 3.9 mm i.d., 10 μm (Waters, Milford, MA). They were quantified by fluorescence at 340 and 445 nm of excitation and emission, respectively, after postcolumn derivatization with o-phthalaldehyde (Moreira et al., 2008). The quantification of amines was achieved by interpolation in an external standard curve.

### Statistical Analysis

The experiment was designed as a totally randomized sampling. The data were submitted to ANOVA and the means were compared by the Tukey test at 5% probability. The Kruskal-Wallis test was used to compare means of the contents of amines at 5% probability. Linear regression models were determined during storage, and the results were expressed as equations, slopes, and correlation coefficients (Sampaio, 2002).

### RESULTS AND DISCUSSION

#### Physicochemical Characteristics and Levels of Bioactive Amines in Fresh Eggs

The results obtained (Table 1) represent the quality of the eggs immediately after they were laid. The eggs weighed on average 56.85 g (54.60 to 60.56 g). Even though the weight of eggs can vary widely depending on many factors, such as the environmental temperature, the breed, and the age of the layer, the values found were typical of fresh eggs of Dekalb laying hens of similar age (Wu et al., 2005, 2008) and also of fresh eggs of Lohmann hens (Narváez-Solarte et al., 2005).

With regard to the internal quality of the fresh eggs, the HU values were, on average, 98.55. These values are much higher than 72, which is considered by the USDA (2000) characteristic of good quality eggs. The values were higher than those reported by Tharrington et al. (1999) and Silversides and Budgell (2004) for eggs of laying hens with similar ages but from different lineages. They were also higher than HU values (71.37) described by Wu et al. (2008) for hens of the same lineage. However, Narváez-Solarte et al. (2005) and Xavier et al. (2008) found similar HU values (94.57 to 103.13) for fresh eggs of Lohmann and Hy-Line hens, respectively.

The pH values of the albumen varied from 7.85 to 8.52, with an average of 8.02. According to the literature (Alleoni and Antunes, 2001), the pH of fresh eggs is approximately 7.8. However, lower pH values were reported by Silversides and Budgell (2004)—7.31 to 7.78 for 2 different lineages of laying hens but from different lineages. They were also higher than HU values (71.37) described by Wu et al. (2008) for hens of the same lineage. However, Narváez-Solarte et al. (2005) and Xavier et al. (2008) found similar HU values (94.57 to 103.13) for fresh eggs of Lohmann and Hy-Line hens, respectively.

#### Physicochemical characteristics and bioactive amine contents of fresh eggs of 24-wk-old Dekalb laying hens

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weight</td>
<td>54.60</td>
<td>60.56</td>
<td>56.85</td>
<td>3.5</td>
</tr>
<tr>
<td>Haugh units</td>
<td>95.22</td>
<td>105.59</td>
<td>98.55</td>
<td>3.0</td>
</tr>
<tr>
<td>Albumen pH</td>
<td>7.85</td>
<td>8.52</td>
<td>8.02</td>
<td>2.5</td>
</tr>
<tr>
<td>Total solids (g/100 g)</td>
<td>11.36</td>
<td>12.82</td>
<td>12.17</td>
<td>3.5</td>
</tr>
<tr>
<td>Total volatile bases (mg/100 g)</td>
<td>ND²</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Bioactive amines (mg/kg)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Yolk Total solids (g/100 g)</td>
<td>51.63</td>
<td>52.95</td>
<td>52.43</td>
<td>0.9</td>
</tr>
<tr>
<td>Total volatile bases (mg/100 g)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Spermidine (mg/kg)</td>
<td>0.22</td>
<td>0.84</td>
<td>0.49</td>
<td>6.1</td>
</tr>
</tbody>
</table>

1\(n = 10\).

2ND = not detected (<0.05 mg/kg for each bioactive amine).
literature. Wu et al. (2008) found 11.89% of total solids for the albumen and 50.70% for the yolk of Dekalb hens. Ahn et al. (1997) found 12.72 and 50.69% of total solids contents in the albumen and yolk, respectively, for 28-wk-old laying hens. Tharrington et al. (1999) described a variation of total solids content from 11.60 to 11.90% in albumens and from 50.73 to 51.53% in yolks of 28-wk-old layers of different lineages.

The total volatile bases were not detected in either albumen or yolk, indicating that the fresh egg does not contain ammonia and other volatile bases (Yao et al., 1998).

The contents of total bioactive amines detected in the eggs are also indicated in Table 1. Only spermidine was detected in the yolk, at levels ranging from 0.22 to 0.84 mg/kg. None of the 10 amines investigated were found in the albumen. However, a compound showing a high peak and time of retention of approximately 31 min was detected in the albumen, though it could not be identified. This compound certainly contains 1 primary amine group in its chemical moiety because the derivatization reagent used for the detection of the amines—o-phthalaldehyde—is specific for that type of substance (Salazar et al., 2000). The compound could be either a primary amine or an amino acid; however, the elution of amino acids during the chromatographic run generally occurs before the amines, during the first 5 min of the run. No information regarding the profile of bioactive amines in eggs, when just laid, was available in the literature. Therefore, it has been reported for the first time.

### Influence of Storage Temperature and Time on the Quality of the Eggs

The changes in the quality of the eggs during storage at 6 ± 1°C (refrigeration temperature) for 50 d and at 25 ± 1°C (room temperature) for 30 d are indicated on Tables 2 and 3, respectively. There was a significant decrease in the weight of the egg and in HU, the pH and the total solids of the albumen increased, whereas the total solids content of the yolk decreased and then increased. However, the rates of the changes were faster, the higher the storage temperature.

The weight loss was affected by the storage temperature; it was higher at 25 ± 1°C compared with 6 ± 1°C. Five percent weight loss was observed at 40 d of storage at 6 ± 1°C and at 15 d of storage at 25 ± 1°C. The influence of the temperature on the weight of the egg can be confirmed by comparing the slopes of the linear regression $y = −0.044x + 56.21$ ($R^2 = 0.673$) for 6 ± 1°C and $y = −0.127x + 56.26$ ($R^2 = 0.907$) for 25 ± 1°C. At the higher temperature, the rate of weight loss was 2.7 times faster than at the lower temperature. Similar results were observed by Cepero et al. (1995). They observed weight loss of 1.5, 3.5, and 8.5 g in eggs after storage at 4, 18, and 32°C, respectively. Therefore, the higher the storage temperature, the higher the weight loss of the eggs.

The values of HU significantly decreased with storage time, irrespectively of the temperature. However, the decrease was greater at 25 ± 1 than 6 ± 1°C. This result can be confirmed by the slopes of the regression

### Table 2. Changes on the physicochemical characteristics of eggs from 24-wk-old Dekalb laying hens during storage at 6 ± 1°C for 50 d

<table>
<thead>
<tr>
<th>Storage (d)</th>
<th>Egg wt (g)</th>
<th>Haugh unit</th>
<th>pH Albumen</th>
<th>Total solids (g/100 g) Albumen</th>
<th>Yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>56.85 ± 1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.55 ± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.02 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.17 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.43 ± 0.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>55.25 ± 1.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.78 ± 5.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.89 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.36 ± 0.45&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>51.99 ± 1.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>55.21 ± 2.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.04 ± 4.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.23 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.02 ± 0.90&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>56.07 ± 0.85&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>54.88 ± 2.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.71 ± 5.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.23 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.36 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.71 ± 1.61&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>53.78 ± 1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.43 ± 2.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.16 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.08 ± 0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.75 ± 4.13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>54.72 ± 2.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.74 ± 4.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.13 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.47 ± 0.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.29 ± 0.74&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a–d</sup>Mean values ± SD with different letters in the same column are significantly different ($P \leq 0.05$, Tukey test).

<sup>1</sup>n = 10.

### Table 3. Changes on the physicochemical characteristics of eggs from 24-wk-old Dekalb laying hens during storage at 25 ± 1°C for 30 d

<table>
<thead>
<tr>
<th>Storage (d)</th>
<th>Egg wt (g)</th>
<th>Haugh unit</th>
<th>pH Albumen</th>
<th>Total solids (g/100 g) Albumen</th>
<th>Yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>56.85 ± 1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.55 ± 2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.02 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.17 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.43 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>54.93 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.11 ± 8.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.03 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.58 ± 0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.81 ± 0.47&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>55.15 ± 2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.13 ± 11.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.39 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.92 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.44 ± 1.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>53.90 ± 1.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.18 ± 2.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.30 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.15 ± 0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.40 ± 0.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>54.03 ± 1.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.26 ± 4.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.40 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.35 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.90 ± 1.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>53.12 ± 1.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.53 ± 3.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.48 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.01 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49.81 ± 0.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>52.49 ± 2.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.39 ± 5.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.41 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.83 ± 1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.73 ± 2.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>**</sup>Mean values ± SD with different letters in the same column are significantly different ($P \leq 0.05$, Tukey test).

<sup>1</sup>n = 10.
lines \[ y = -0.493x + 93.025 \] \( (R^2 = 0.904) \) at 6 ± 1°C and \[ y = -2.675x + 91.86 \] \( (R^2 = 0.980) \) at 25 ± 1°C, which means that the decrease on the HU was 5.4 times faster at 25 ± 1°C compared with 6 ± 1°C. Alleoni and Antunes (2001) found HU values of 60.63 in eggs stored for 21 d at 8°C. Jones and Musgrove (2005) analyzed eggs stored at 4°C for 10 wk and the HU mean value was 67.43 at the end of the storage. According to these results, the storage temperature significantly affects HU values. Based on the criteria established by the USDA (2000), the eggs of 24-wk-old Delkab layers could be stored for up to 50 d at 6 ± 1°C or up to 10 d at 25 ± 1°C before the eggs reached HU values that represent low-quality eggs (HU = 60).

The pH of the albumen increased significantly (P ≤ 0.05) at the beginning of storage, reaching a value that did not change further with time. At 6 ± 1°C, the pH changed significantly up to 20 d of storage, reaching a value of 9.20, which remained constant until the end of the experiment (50 d). During storage at 25 ± 1°C, the pH changed significantly up to 10 d of storage, reaching a value of 9.40, which also remained constant until the end of the experiment (30 d). Based on these results, the pH increased up to a value that was higher, the higher the storage temperature. An increase in albumen pH along storage time has been reported by Scott and Silversides (2000) and Silversides and Budgell (2004). However, according to Xavier et al. (2008), the most significant increase occurs in the beginning of the storage period.

Total solids in the albumen increased during storage at both temperatures up to a point, which remained constant until the end of the experiment. The increase in total solids content of the albumen is expected because a loss of moisture of the albumen through the eggshell and to the egg yolk happens during storage (Ahn et al., 1997). The change at the higher temperature was faster when compared with the lower temperature. This result may be confirmed by the slopes of the equations of linear regressions [\( y = 0.05258x + 12.265 \) \( (R^2 = 0.906) \) for 6 ± 1°C and \( y = 0.059x + 12.259 \) \( (R^2 = 0.973) \) for 25 ± 1°C], which was 2 times faster at 25 ± 1°C, compared with 6 ± 1°C.

For the total solids of the yolk, a decrease was observed at 6 ± 1°C throughout storage time, whereas a decrease followed by an increase was recorded at 25 ± 1°C.

During storage at 6 ± 1°C, the total volatile bases were present at significantly higher levels at 50 d of storage (54.9 ± 2.7 and 73.2 ± 32.9 mg/100 g in the yolk and albumen, respectively). During storage at 25 ± 1°C, the total volatile bases were detected at 20 and 30 d with values, respectively, of 20.1 ± 16.7 and 50.5 ± 34.8 mg/100 g of the yolk and 33.1 ± 38.8 and 44.0 ± 20.7 mg/100 g for the albumen. These results indicate that significant changes in the volatile bases were observed late throughout the storage period—30 d at 25 ± 1°C and 50 d at 6 ± 1°C.

Among characteristics evaluated, most of them showed significant changes at the beginning of the storage period, remaining constant afterwards (pH, total solids, and egg weight). On the contrary, total volatile bases and HU changed significantly at a later stage of storage. Therefore, the attributes evaluated did not provide a reliable method to gradually evaluate loss of quality in eggs throughout storage.

### Influence of Storage Temperature and Time on the Profile and Levels of Bioactive Amines of the Eggs

In the albumen, the unknown peak was also detected throughout storage at both temperatures. No other amine was detected in the albumen throughout storage. In the yolk, however, among the 10 amines investigated, only spermidine, spermine, agmatine, and putrescine were detected (Table 4). The changes in amines during storage did not follow normal distribution; therefore, the data were submitted to nonparametric statistics and the means were compared by the Kruskal-Wallis test. Spermidine levels increased during storage at both temperatures, reaching a significantly higher concentration at 15 and 40 d, when the eggs were stored at 25 ± 1 and 6 ± 1°C, respectively. When the maximum concentration of spermidine was observed, agmatine and putrescine were also detected. Spermine was only detected in yolks on the 30th day of storage at 25 ± 1°C. The presence of putrescine and agmatine along storage may represent the onset of spoilage because they are biogenic amines, which are produced by the decarboxylation of ornithine and arginine, respectively (Glória, 2005).

The profile of amines throughout storage of eggs was reported for the first time. The low levels of amines found in the yolks at both temperatures may be explained by the presence of its shell, which works as a physical barrier and protects against the invasion of microorganisms. Furthermore, the shell membranes and the albumen also prevent contamination of the eggs (Theron et al., 2003; Jones et al., 2004). The presence of enzymes in the albumen possessing antimicrobial activity may have impaired the development of microorganisms and formation of amines (Brake et al., 1997). Moreover, the optimal pH for decarboxylase activity is lower than the typical pH of the albumen (8.02 to 9.41) throughout storage (Coustt et al., 1986).

Among studies on bioactive amines in eggs, Bardóczi et al. (1995) and Okamoto et al. (1997) worked with a small number of samples (n ≤ 3) of boiled eggs, whereas Saito et al. (1992) evaluated spoiled eggs. Several amines were detected, suggesting poor egg quality. The presence of cadaverine in the spoiled samples could indicate contamination with enterobacteria, which are able to decarboxylate lysine (Glória, 2005). The presence of histamine may also indicate contamination of...
the egg with a microorganism capable of decarboxylating histidine.

During storage under refrigeration (6 ± 1°C), the profile of amines in the yolk changed significantly only on the 40th day. This result indicates that when the levels of spermidine changed significantly, the values obtained for HU (75.43) were still representative of egg of excellent quality (USDA, 2000), the pH (9.16) had already increased significantly and was stable since the 20th storage day, and the total solids of the albumen and of the yolk had already changed significantly.

During storage at room temperature (25 ± 1°C), the profile of amines in the yolk changed significantly on the 15th day. This result indicates that when the levels of spermidine changed significantly, the values obtained for HU (46.18) were representative of eggs of inferior quality (USDA, 2000), the pH (9.30) had already increased significantly and was stable since the 10th storage day, and the total solids of the albumen and of the yolk had just changed significantly.

These results suggest that temperature affects significantly the physicochemical changes that occur during storage of eggs. Based on these results, there was no consensus on the changes observed for the amines with changes observed on the other characteristics, which are representative of egg quality.

### Amines as a Quality Index of Eggs

Fresh eggs (immediately after they were laid) were observed to contain only spermidine in the yolk at low levels, which were lower than 1.0 mg/kg. Therefore, the presence of other amines in fresh eggs, or spermidine levels higher than 1.0 mg/kg in the yolk, may indicate that the eggs are not fresh or that they have undergone microbial contamination.

The increase in the levels of spermidine could be used to follow changes of eggs during storage; however, the changes on spermidine levels did not coincide with changes in other indices considered representative of egg quality. Compared with pH, amines would be a better index because the pH increases significantly in the beginning of storage (Xavier et al., 2008), remaining unchanged afterwards.

During storage at room temperature, the significant change in spermidine was concomitant to the significant changes in the total solids of the albumen and of the yolk. However, the values obtained for HU were representative of eggs of inferior quality. On the other hand, during storage at refrigerated temperatures, the significant changes in spermidine levels preceded significant changes in HU to inferior quality eggs and were late compared with the significant changes in the total solids of the albumen and of the yolk. Therefore, further studies are needed to investigate which characteristic can better represent the changes in the quality of eggs, which would assure consumer acceptability with regards to sensory evaluation, functional properties, and safety to consumers’ health.

Overall, fresh eggs from 24-wk-old Dekalb hens had, on average, weight of 56.85 g and a HU value of 98.55. The albumen had a pH of 8.02 and total solids content of 12.17 g/100 g, whereas the yolk had total solids content of 52.43 g/100 g. No amine was detected in the albumen, whereas spermidine was the only amine detected in the yolk at low levels (≤1.0 mg/kg).

During storage of the eggs, independent of the storage temperature, there was a significant decrease in egg weight and in HU and a significant increase in the pH and in the total solids content of the albumen. The total solids content of the yolk decreased with storage at 6 ± 1°C, whereas it decreased followed by an increase during storage at 25 ± 1°C. The decrease in pH and the increase in the total solids content of the albumen were faster at 25 ± 1°C compared with 6 ± 1°C. During storage, the levels of spermidine in the yolk increased

### Table 4. Influence of storage temperature and time on the levels of bioactive amines in yolks of eggs of 24-wk-old Dekalb laying hens

<table>
<thead>
<tr>
<th>Storage time (d)</th>
<th>Spermidine (mg/kg)</th>
<th>Spermine (mg/kg)</th>
<th>Agmatine (mg/kg)</th>
<th>Putrescine (mg/kg)</th>
<th>Total (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 ± 1°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.49 ± 0.03bc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.49 ± 0.03c</td>
</tr>
<tr>
<td>10</td>
<td>0.34 ± 0.04bc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.34 ± 0.04d</td>
</tr>
<tr>
<td>20</td>
<td>0.58 ± 0.04b</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.58 ± 0.04e</td>
</tr>
<tr>
<td>30</td>
<td>0.49 ± 0.04bc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.49 ± 0.04f</td>
</tr>
<tr>
<td>40</td>
<td>1.77 ± 0.09b</td>
<td>ND</td>
<td>0.46 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td>2.21 ± 0.11a</td>
</tr>
<tr>
<td>50</td>
<td>0.27 ± 0.02</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>25 ± 1°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.49 ± 0.03bc</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.49 ± 0.03c</td>
</tr>
<tr>
<td>5</td>
<td>0.26 ± 0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.26 ± 0.015</td>
</tr>
<tr>
<td>10</td>
<td>0.82 ± 0.06</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>1.35 ± 0.07</td>
<td>ND</td>
<td>0.16 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>2.88 ± 0.05c</td>
</tr>
<tr>
<td>20</td>
<td>0.71 ± 0.05</td>
<td>ND</td>
<td>0.38 ± 0.05</td>
<td>1.60 ± 0.05c</td>
<td>2.74 ± 0.05c</td>
</tr>
<tr>
<td>25</td>
<td>0.34 ± 0.02</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>0.62 ± 0.05bc</td>
<td>1.99 ± 0.29</td>
<td>ND</td>
<td>ND</td>
<td>2.40 ± 0.01c</td>
</tr>
</tbody>
</table>

**Note:** Mean values ± SD with different letters in the same column and storage temperature are significantly different ($P \leq 0.05$; Kruskal-Wallis test).

**Footnotes:**

1. Mean values ± SD with different letters in the same column and storage temperature are significantly different ($P \leq 0.05$; Kruskal-Wallis test).
2. $n = 10$.
3. ND = not detected (<0.05 mg/kg for each bioactive amine).
significantly at the 40th and 15th days of storage at 6 ± 1 and 25 ± 1°C, respectively, decreasing afterwards. At these storage times, other amines were also detected in the yolks—putrescine and agmatine. Therefore, the presence of other amines besides spermidine or spermine levels higher than 1.0 mg/kg in the yolk could be used as an index of quality of eggs.

The time at which the changes in the levels and profile of amines occurred preceded changes in HU and was posterior to changes in pH and total solids content of the albumen during storage at refrigerated temperatures. However, during storage at room temperature, the changes in spermidine levels occurred after changes in HU, preceded changes in the pH of the albumen, and coincided with changes in the total solids contents of the albumen and yolk. Further studies are needed to ascertain which quality index would be more representative of the quality and safety of eggs for consumption, which could be applied to a wider range of storage temperatures.

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