ABSTRACT The objective of this study was to evaluate the correlation between the levels of bioactive amines and the microbiological quality of liquid pasteurized egg stored under refrigeration. Pasteurized whole egg liquid was obtained from 2 types of different raw materials, fresh eggs, and commercial fertile eggs. They were stored under refrigeration over a period of 21 d. The treatments were arranged in a completely randomized split plot, with the plots being the 2 types of liquid pasteurized egg, and the subplots being the 4 storage periods (1, 7, 14, and 21 d). The storage period did not contribute to the increase (P > 0.05) of contamination by mesophilic aerobic microorganisms and total coliforms in commercial liquid pasteurized egg. However, for fertile eggs, the storage period led to an increase (P < 0.05) in the numbers of microorganisms. Levels of the amines putrescine, cadaverine, and tyramine were detected only in fertile liquid pasteurized egg, and the storage period contributed to the increase (P < 0.05) in the levels of these amines. There was a high correlation between total coliform most probable number and cadaverine levels, and a moderate correlation between the numbers of aerobic mesophilic microorganisms and tyramine levels. It was concluded that the most contaminated liquid pasteurized eggs were the fertile liquid pasteurized eggs and this caused the highest levels of bioactive amines in them compared with all the eggs that had been subjected to pasteurization and refrigerated storage.

Key words: liquid pasteurized egg, quality, storage, bioactive amine, microbiology
Enterobacteriaceae family, especially the genera *Escherichia*, *Enterobacter*, *Salmonella*, *Shigella*, and *Proteus* alongside species of the genera *Achromobacter*, *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Pedicioccus*, *Streptococcus*, *Propionibacterium*, and *Clostridium* (Russell and Snyder, 1968; Halász et al., 1994; Lima and Glória, 1999).

Because the deterioration of food is associated with increased levels of some of these amines, their use as an index of chemical quality has been proposed for some foods such as fish (Veciana-Noguès et al., 1997) and eggs (Saito et al., 1992; Bardóczi et al., 1995; Okamoto et al., 1997; Oliveira et al., 2009). Knowledge of the levels of bioactive amines in liquid pasteurized egg is important because amines are thermally stable, making their quantification an indication of the quality of the final product.

The objective of this study was to evaluate the correlation between the levels of bioactive amines and the microbiological quality of liquid pasteurized whole egg obtained from 2 different types of raw materials, commercial eggs and fertile eggs, which were subjected to pasteurization and refrigerated storage.

### MATERIALS AND METHODS

#### Samples and Experimental Conditions

Twelve batches of liquid whole egg subjected to pasteurization and refrigerated storage were analyzed. Commercial eggs (fresh eggs disqualified due to weight or being cracked or broken) and fertile eggs (disqualified for hatching due to low weight or surplus production) were used as raw materials for pasteurization. After the pasteurization of 6 batches of commercial eggs, a 1-L sample of each batch was collected. Six 1-L samples were also collected from the 6 batches of fertile liquid pasteurized egg. Hence, each sample of liquid pasteurized egg represented one batch or one repetition and each batch of both eggs had 54,546 units. All eggs were broken in an automatic machine Diamond 120s model (Moba, Barneveld, the Netherlands) and soon after were subjected to high-temperature, short-time pasteurization following the binomial time-temperature 60°C for 3.5 min (USDA, 2008) using an on-plate heat exchanger (Inoxil, Guarulhos, Brazil). Hydrogen peroxide was not added to the eggs.

All samples of liquid pasteurized egg were divided into 4 equal portions and placed into 4 sterile containers of 250 mL each. The 4 containers of each batch of samples were dated and stored under refrigeration at 3.5°C for up to 21 d. Refrigerated liquid pasteurized egg was analyzed at 1, 7, 14, and 21 d.

#### Methods of Analysis

**Microbiological Analyses.** The most probable numbers (MPN/mL) of total and thermotolerant coliforms were determined in accordance with Hitchins et al. (2001). For the presumptive test, 1 mL of liquid pasteurized egg (dilutions of 10⁰, 10⁻¹, and 10⁻²) was incubated (48 ± 2 h at 35°C) in lauryl tryptose broth (Difco, Sparks, MD). For the confirmatory test for coliforms and thermotolerant coliforms, a loop of suspension from each lauryl tryptose test tube producing gas was transferred to test tubes containing brilliant green, lactose bile broth 2%, and EC broth (Difco), which were then incubated at 48 ± 2 h at 35°C and 48 ± 2 h at 45.5°C, respectively. For the total aerobic mesophilic count (cfu/mL), 1 mL of liquid pasteurized egg (dilutions of 10⁻², 10⁻³, and 10⁻⁴) was placed in a sterile dish of Petrifilm Aerobic Count Plate (3M, St. Paul, MN) and incubated 48 ± 2 h at 35°C (AOAC, 1990).

**Determination of Bioactive Amines.** Amines were extracted from 3-g samples with 20 mL of 50 g/L trichloroacetic acid (TCA) in 3 successive extractions (7, 7, and 6 mL). After agitation for 10 min in a vortex mixer, the slurries were centrifuged at 10,000 × g at 4°C for 21 min and the supernatants were collected, combined, and then filtered through 13-mm-diameter and 45 m.M HAWP pore membranes (Millipore, Bedford, MA). The amines were separated by ion-pair HPLC using a μBondapak C18 column, 300 × 3.9 mm, 10 mm (Waters, Milford, MA). They were quantified by fluorescence at 340 and 445 nm of excitation and emission, respectively, after postcolumn derivation with o-phthalaldehyde (Moreira et al., 2008). The quantification of amines was achieved by interpolation in an external standard curve.

The amine standards (sperrmine 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 of chromatographic grade.

#### Statistical Analyses

The treatments were arranged in a completely randomized split plot, with the plots being the 2 types of liquid pasteurized egg, and subplots being the 4 storage periods (1, 7, 14, and 21 d), making a total of 8 treatments with 6 replicates (n = 48). The microbiological variables and levels of bioactive amines were subjected to nonparametric statistical analysis. The Kruskal-Wallis test was applied to the plot, and the Friedman test was applied to the subplot. The Pearson correlation at 5% probability was also performed to correlate the microbiological results at the level of bioactive amines.

### RESULTS AND DISCUSSION

**Microbiological Analyses**

Commercial liquid pasteurized egg showed no significant differences (P < 0.05) for counts of mesophilic aer-
obic microorganisms (Table 1) with increasing storage period. However, for samples of fertile liquid pasteurized egg, numbers of mesophilic aerobic microorganisms were higher with increasing storage period. Their counts reached above 6.40 log cfu/mL on d 21.

Because the fertile egg remains in contact with the nest and the birds longer, it is therefore exposed to more contamination and can carry a larger number of microorganisms on the inside. However, the pasteurization temperature should be sufficient to inactivate these microorganisms. Therefore, the primary hypothesis regarding fertile liquid pasteurized egg samples suggested for the present study is that raw material before pasteurization was excessively contaminated, considering that the other factors such as control of the binomial time-temperature, packaging, and transport were controlled.

Table 2 shows the microbiological results for total coliforms. During the entire commercial liquid pasteurized egg evaluation period, only one sample (4.1%) on d 7 was contaminated by total coliforms. However, all fertile liquid pasteurized egg samples were positive for total coliforms on d 21. In this experiment, MPN of total coliforms ranged from <0.3 to 110.

All commercial liquid pasteurized egg samples were negative for thermotolerant coliforms. However, on the first day of storage, thermotolerant coliforms were detected in 1 (4.1%) fertile liquid pasteurized egg sample.

### Bioactive Amines

Of the 10 amines evaluated, none was detected in commercial liquid pasteurized egg. However, in fertile liquid pasteurized egg, low levels of the amines putrescine, cadaverine, and tyramine (Table 3) were detected on d 14 and 21 of storage. The 3 amines found in fertile liquid pasteurized egg are biogenic amines whose formation is due to the decarboxylation of amino acids by microbial enzymes (Gloria, 2005).

With the lengthening of the storage period, there was an increase ($P < 0.05$) in the levels of putrescine. At 21 d, this value reached 15.3 mg/kg of sample. Saito et al. (1992) observed putrescine level equal to 15.2 mg/kg in deteriorated eggs. However, Bardócz et al. (1995) found 0.35 mg/kg of putrescine in boiled eggs, and Oliveira et al. (2009) observed only 0.17 mg/kg of putrescine in eggs after 40 d of refrigerated storage. Figueiredo et al. (2013) did not observe the presence of this amine in eggs stored for up to 28 d either at room temperature or refrigerated.

Cadaverine (2.9 mg/kg) and tyramine (9.1 mg/kg) were detected in samples of fertile liquid pasteurized egg at 21 d of storage. Cadaverine was found in all 6 samples of fertile eggs and tyramine in 4 samples. Saito et al. (1992) observed higher levels of cadaverine (55.2 mg/kg) in deteriorated eggs. Nishimura et al. (2006) found 26.6 mg/kg of cadaverine in egg yolk. In contrast, Ramos et al. (2009) observed decreased levels of cadaverine in egg yolk (10.80 mg/kg to 7.39 mg/kg) after 26 d of refrigerated storage.

The literature contains no reports on amine research in liquid pasteurized egg or on the detection of tyramine either in shell eggs or in boiled eggs. According to Halász et al. (1994), tyramine is responsible for food poisoning, and its detection is therefore important in food safety programs. In the present study, low tyramine content was detected (9.1 mg/kg) in liquid pasteurized egg.

Although the levels of toxic bioactive amines have not been clearly established yet, ten Brink et al. (1990) consider levels from 100 to 800 mg/kg of tyramine in foods to be potentially hazardous to human health.

### Table 1. Mean counts of mesophilic aerobic microorganisms (log cfu/mL) in fertile and commercial liquid pasteurized egg stored under refrigeration for up to 21 d

<table>
<thead>
<tr>
<th>Type of egg</th>
<th>Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Commercial</td>
<td>3.4 ± 0.28a,x</td>
</tr>
<tr>
<td>Fertile</td>
<td>2.52 ± 0.42b,x</td>
</tr>
</tbody>
</table>

*a,b*Means followed by different letters in the same row are different ($P < 0.05$; Kruskal-Wallis test).

*x,y*Means followed by different letters in the same column are different ($P < 0.05$; Friedman test).

### Table 2. Means of total coliforms (most probable number/mL) in fertile and commercial liquid pasteurized egg stored under refrigeration for up to 21 d

<table>
<thead>
<tr>
<th>Type of egg</th>
<th>Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Commercial</td>
<td>0a,x</td>
</tr>
<tr>
<td>Fertile</td>
<td>0.12b,x</td>
</tr>
</tbody>
</table>

*a,b*Means followed by different letters in the same row are different ($P < 0.05$; Kruskal-Wallis test).

*x,y*Means followed by different letters in the same column are different ($P < 0.05$; Friedman test).
pharmacological effects of tyramine in the human body are peripheral vasoconstriction, increased cardiac output, salivation and lacrimation, increased respiratory rate, increased blood sugar levels, release of noradrenaline by the sympathetic nervous system, and migraine. Cadaverine poisoning causes hypotension, bradycardia, exaggerated contraction of the jaw muscles, paralysis of the extremities, and potentiation of the toxicity of other amines (Shalaby, 1996).

The increased levels of the aforementioned 3 amines with increasing storage period (P < 0.05), particularly in fertile eggs, confirm that bioactive amines are related to egg deterioration, which was bacterially mediated. Emphasis should be placed on the possibility of using the analysis of amine levels as a food quality index.

Using the Pearson correlation to 5% probability, a high positive correlation was found (0.84) between the MPN of total coliforms in fertile liquid pasteurized egg and cadaverine levels, and a moderate positive correlation (0.72) was found between the counts of mesophilic aerobic microorganisms and putrescine levels. These results are similar to those of Lima and Glória (1999) and Jay et al. (2005) who reported that cadaverine is produced mainly by members of the Enterobacteriaceae family.

The quality of refrigerated liquid pasteurized egg is influenced by the type of raw material (commercial or fertile eggs) used to make it. Fertile eggs produce a lower quality end product than commercial eggs. The fertile eggs used to make it. Fertile eggs produce a lower quality end product than commercial eggs. Using the analysis of amine levels as a food quality index.

### ACKNOWLEDGMENTS

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### REFERENCES


References to other amines (Shalaby, 1996).


### Table 3. Means of amine levels (mg/100 g) in whole fertile liquid pasteurized egg stored under refrigeration for 21 d

<table>
<thead>
<tr>
<th>Amine content (mg/kg)</th>
<th>Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Putrescine</td>
<td>NDc</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>NDb</td>
</tr>
<tr>
<td>Tyramine</td>
<td>NDb</td>
</tr>
<tr>
<td>Total</td>
<td>ND</td>
</tr>
</tbody>
</table>

Means followed by distinct letters differ by the Friedman test (P < 0.05). ND = not detected (amine content <0.05 mg/kg).
ity of egg from Dekalb hens under different storage conditions. Poult. Sci. 88:2428–2434.