INTRODUCTION

The need for production of high-quality, fresh, ready-to-eat foods containing only natural ingredients has steadily grown due to the new consumer lifestyles (Katz, 1999; Conte Junior et al., 2010). Shredded cooked chicken breast fillet is a very practical product because it can be consumed directly or used to make other products. However, there is a risk of contamination during the meat processing of this product because comminution increases the exposed surface area and facilitates contamination and consequent deterioration (Cortez-Vega et al., 2012; Huda et al., 2012). It is important to note that chicken is highly perishable and susceptible to changes due to physical and chemical alterations and bacterial growth (Tsola et al., 2008). The storage temperature and type of packing, as well as species and number of psychotropic bacteria, are the primary factors affecting poultry deterioration (Tuncer and Sireli, 2008).

New preservation methods are being studied to reduce the growth of microorganisms that appear before or during chicken processing (Conte Junior et al., 2010; Fraqueza and Barreto, 2011; Novaes et al., 2012; Ahn et al., 2013). The effect of atmospheric oxygen and the growth of aerobic microorganisms are important factors that influence the shelf-life of perishable foods kept in aerobiosis (Parry, 1993; Sarantópoulos et al., 1998; Mano et al., 2002). Thus, fresh food preservation, particularly meat in modified atmosphere packaging (MAP), has greatly improved during the past 20 yr (Mano et al., 2000; Lopes et al., 2004; Mantilla et al., 2009). Modified atmosphere packaging consists of sub-
stituting the atmosphere surrounding the product by an alternative atmosphere (e.g., a gas or a mixture of gases) that is especially prepared for each type of food, allowing for improved control of chemical, physical, and microbiological reactions and avoiding or minimizing the primary types of deterioration that can occur during storage (Parry, 1993; Ordoñez, 1996; Monteiro et al., 2012).

Modified atmosphere packaging is very important for food given that it may extend product shelf life; particularly, the use of carbon dioxide should be enhanced because it has a degree of bacteriostatic or bactericidal effects on certain microorganisms (Mano et al., 2000; Jeremiah and Gibson, 2001; Malavota et al., 2006). Although the use of MAP has increased during the last 10 yr, optimizing the gas mixture, composition, and concentration to ensure food quality and safety for each product remains a challenge (Narasimha and Sachindra, 2002; Novaes et al., 2012). Thus, the purpose of the present study was to determine the shelf life of shredded cooked chicken breast fillet stored under refrigeration and packed in modified atmospheres with different CO₂ concentrations and to evaluate the influence of the concentration of this gas on the behavior of mesophylic and psychrotrophic bacteria, enterobacteria, and lactic acid bacteria (LAB).

MATERIALS AND METHODS

Samples

Approximately 6.3 kg of chicken breast fillet (m. pectoralis major) were cooked at 100°C and 2 kgf/cm² pressure for 20 min. The fillets were immediately shredded using a stainless-steel crusher machine (Cozix, Equipamentos e Serviços Industriais Ltda., Minas Gerais, Brazil) and cooled at 4 ± 2°C. This stage was performed in a slaughterhouse located in the state of Rio de Janeiro. The shredded cooked fillets were then packed with ice (1 ± 1°C) and sent to the laboratory to be packed in the modified atmosphere (treatments) and aerobiosis (control) conditions. In addition, the bacteriological tests, pH measurements, and gas composition determination within the packages was conducted.

Sample Treatment

One hundred five samples of approximately 60 g of shredded cooked chicken fillet were packed into multilayer high barrier plastic bags (Cryovac BB4L) with diffusion coefficients, according to the supplier, of 150 cm³/24 h·m²·bar of CO₂, 35 cm³/24 h·m²·bar of O₂, and 1.4 cm³/24 h·m²·bar of N₂ at 22°C. The bags were filled with approximately 1 L of the following atmospheres: T1 (aerobiosis packaging, control), T2 (vacuum packaging), T3, T4, T5, T6, and T7 (packaging in a modified atmosphere with 10, 30, 50, 70, and 90% CO₂, with the remaining volume filled with N₂). The samples packed in aerobiosis conditions were placed in expanded polystyrene trays wrapped with polyvinyl chloride film. All of the samples were stored at 4 ± 2°C for 28 d. The intervals for pH and bacteriological tests and evaluation of the gas composition inside of the packages were established based on the evolution of the results that were observed for each parameter (Monteiro et al., 2012).

Bacteriological Analysis

The method established by the American Public Health Association (APHA, 2001) was followed for aerobic heterotrophic mesophyll bacteria (AHMB) and aerobic heterotrophic psychrotrophic bacteria (AHPB); the samples were cultured on plate count agar, incubated inverted at 35 ± 1°C and read after 48 ± 2 h, or incubated at 7 ± 1°C and read after 10 d, respectively. A LAB count was performed using the pour plate method using De Man-Rogosa-Sharpe agar and incubated at 30 ± 1°C for 120 h (APHA, 2001). For the Enterobacteriaceae count, the double-layer pour plate method was used with crystal violet neutral red bile glucose agar. The incubation was performed at 35 ± 1°C for 18/24 h (APHA, 2001).

pH Measurement

After the microbiological tests, the pH was measured in triplicate using the potentiometric method with a digital pH meter (Digimed, DM-32 model, São Paulo, Brazil) according to technique described by the Association of Analytical Chemists (AOAC, 2005).

Determination of the Interior Package Gas Concentration

The gas concentration inside of the packages with modified atmosphere was determined using gas analysis equipment (PBI-Dansensor, Check Pointer O₂/CO₂, Ringsted, Denmark) and expressed as O₂% and CO₂%. The remaining gas inside of the packages was N₂ (Esmer et al., 2011).

Statistical Analysis

The bacterial growth curves were adjusted using the DMFit 2.0 (IFR, Norwich, UK) statistical program based on predictive microbiology and idealized by Baranyi and Roberts (1994). The pH results were statistically modeled with a second-order polynomial linear regression. Pearson correlations were used to examine the relationship between the CO₂ concentration and AHMB doubling time in the T3, T4, T5, T6, and T7 groups. When a significant F was found, additional post-hoc tests with the Bonferroni adjustment were performed. Statistical significance was set at a 0.001 level of confidence. All of the analyses were performed using a commercially available statistical package (GraphPad
RESULTS AND DISCUSSION

Variations in the gas composition during the 28-d storage are shown in Figure 1. Carbon dioxide reduction during the first 24-h storage was observed in all of the treatments. This behavior can be explained because CO₂ dissolves in the aqueous and fatty phases of the product, resulting in package volume contraction (Jakobsen and Bertelsen, 2002; Rotabakk et al., 2008). However, no further reduction in volume was observed after the initial hours in storage. The CO₂ concentration remained stable after the first storage day, and a significant variation of atmosphere composition was not observed in any of the treatments during the remaining storage period. This result can be explained by the decrease in CO₂ dissolution in the food matrix and the increase in bacterial growth, with resultant CO₂ production (Esmer et al., 2011). Similar results were described by Friedrich et al. (2008) and Degirmencioğlu et al. (2012). The residual oxygen percent available inside of the packages submitted to a modified atmosphere decreased during storage and reached values below 1% at the end of the storage period for all of the treatments with CO₂. This result is related to the fact that most microorganisms present in meat, such as Brochothrix thermosphacta and LAB, which produce carbon dioxide as a metabolite (Nychas, 1994; Patsias et al., 2006; Gallas et al., 2010), use the available residual oxygen. In addition, meat biochemical activity and plastic impermeability help to maintain the reduced O₂ concentration inside the packages (Mano et al., 2002).

The pH initial value of the shredded cooked chicken breast fillets was 6.3 (Figure 2). This pH is in accordance with values reported by Balamatsia et al. (2007) in fresh chicken muscle. The average pH value of the samples in aerobicosis conditions presented an increasing trend during the storage period, indicating a decrease in sample quality. According to bacterial count increase, intense metabolic activity occurs in the food, which led to a production of alkaline products that increase the pH of the food matrix (Fang and Lin, 1994). In the present study, an increased pH accompanied the growth of AHMB (T1).

The average pH values that were obtained for the other groups (T2–T7) decreased during storage; this decrease was more evident between d 12 and 16. Several authors (McMullen and Stiles, 1993; Gill, 1996; Leygonie et al., 2011; Gómez and Lorenzo, 2012) observed a decrease in pH values as a consequence of CO₂ solubility in the food matrix and an increase in the number of LAB. The T2–T7 groups exhibited a significant increase in LAB (the dominant AHMB population) and a decreased pH, with values between 5.8 and 6.0.

The average values of the AHMB, AHPB, Enterobacteriaceae, and LAB counts are presented in Figure 3. For all of the treatments, we found coefficients of deter-
mination and respective ranges of 0.95 to 0.99, 0.96 to 1.00, 0.46 to 1.0, and 0.93 to 1.00, respectively.

The AHMB count for the shredded cooked chicken breast fillet was 5.1 log cfu∙g−1 (d 0; Figure 1A). The high initial count is related to the processing stages, especially during the comminution and manual packaging, during which there is increased risk of contamination. The AHMB count reached 7 log cfu∙g−1, which is considered to be the upper acceptable limit for fresh poultry as defined by the Commission on Microbiological Specifications for Foods (ICMSF, 1988).

The AHMB were present at 7.0 log cfu∙g−1 in T1 on the 9th storage day. The bacterial growth curve in T3 was similar, achieving the same value at the 10th storage day. Similar values were determined for the vacuum packaging (T2) and the 30/70 Co2/N2 packaging (T4), which had a shelf life of 13 or 14 d.

The effect of carbon dioxide in reducing bacterial growth rate was evident in the T5, T6, and T7 samples, which presented shelf lives of 21, 23, and 28 d, respectively. Table 1 shows that the duplication time (h) was inversely proportional to the Co2 concentration. For instance, T7 presented a log phase of 3.6 h, whereas the bacterial population of samples with 10% Co2 (T3) doubled in 1.2 h. This appears to be the growing stage where carbon dioxide has a bacteriostatic function. At the completion of the experiment, the AHMB count in the T7 treatment (90% CO2) was 107 log cfu∙g−1, whereas the T3 treatment (10% CO2) achieved values of 109 log cfu∙g−1. These results reveal the effect of CO2 on AHMB growth. This effect is due to (i) oxygen restriction inside of the package and (ii) Co2 bacteriostatic and bactericide activity due to its alteration of the cell membrane functions, directing enzyme inhibition, and reducing the speed of enzymatic reactions (Daniels et al., 1985; Church, 1993; Sarantópoulos et al., 1998).

Figure 3B shows that the initial count of AHPM was 5.0 log cfu∙g−1. The bacterial growth curves were similar for all of the treatments examined. However, growth was slower in treatments with Co2-enriched atmospheres than for the control group. It should be noted that the existence of strict aerobic psychrotrophic genera resulted in better development in an O2-rich atmosphere (Gram and Huss, 1996). The comparison of treatments T1 and T7 shows that it was more difficult for this bacterial group to grow in products that were packaged in CO2-enriched atmospheres.
The initial *Enterobacteriaceae* count was approximately 10^5 log cfu·g⁻¹ (Figure 3C). The presence of this bacterial group is traditionally associated with the hygienic and sanitary quality of foods (del Río et al., 2007). *Enterobacteriaceae* growth presented variable behavior depending on the composition of the packaging atmosphere; the T1, T2, and T3 groups presented a significant increase in growth, treatments T4 and T5 did not exhibit an increase, and the T6 and T7 treatments exhibited decreased growth during storage.

The LAB increased in number during storage (Figure 3D); however, LAB were not the dominant AHMB group in treatments with low CO₂ concentration. The LAB performance in treatment T7 was similar to that for AHMB (Figures 3A), indicating that LAB prevailed in this treatment. In an anaerobic environment, lactic acid-producing bacteria thrive given that they are more tolerant to CO₂ than are pseudomonads or *Enterobacteriaceae* (Stiles, 1991; Dainty and Mackey, 1992). According to Vermeiren et al. (2004), LAB thrive in the deterioration process of meat products stored under refrigeration and anaerobic conditions, such as MAP.

A shelf life increase at high CO₂ concentrations occurs because the deterioration that is caused by LAB occurs later than for aerobic bacteria, such as *Pseudomonas* spp. Generally, one of the alterations caused by LAB is described as acidification of the product, which differs from the putrefaction that is caused by AHMB (Stiles, 1991; Smolander et al., 2004). In the present study, a pH decrease was also observed in the vacuum treatment and all of the treatments with CO₂.

These bacteriological tests and pH results demonstrate that the use of MAP extended the shelf life of the product. The bacterial groups examined presented a lower growth rate in the treatment conditions that were enriched with CO₂ compared with the remaining treatments. However, specific pathogens should also be assayed. The best preservation, with respect to microbiological spoilage, was obtained in the packages with high CO₂ concentrations. Although other quality parameters, such as sensory attributes, should be considered when evaluating the commercial life of the product, we suggest the use of a 90% CO₂ atmosphere for the preservation of ready-to-eat shredded cooked chicken breast fillets given that this condition resulted in the greatest shelf life compared with the other examined treatments.

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**Table 1.** The growth parameters of aerobic heterotrophic mesophyll bacteria (lag phase, doubling time, count in the stationary phase, and shelf life) in shredded cooked chicken breast fillet packed under different treatments at 4 ± 2°C for 28 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lag phase (d)*</th>
<th>Doubling time (h)*</th>
<th>Stationary phase (log cfu·g⁻¹)</th>
<th>Shelf life2 (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (aerobiosis)</td>
<td>1.8</td>
<td>1.1</td>
<td>9.1</td>
<td>9</td>
</tr>
<tr>
<td>T2 (vacuum packaging)</td>
<td>1.9</td>
<td>1.8</td>
<td>8.8</td>
<td>13</td>
</tr>
<tr>
<td>T3 (10% CO₂/90% N₂)</td>
<td>1.8</td>
<td>1.2</td>
<td>7.4</td>
<td>10</td>
</tr>
<tr>
<td>T4 (30% CO₂/70% N₂)</td>
<td>2.5</td>
<td>1.8</td>
<td>7.7</td>
<td>14</td>
</tr>
<tr>
<td>T5 (50% CO₂/50% N₂)</td>
<td>0.5</td>
<td>2.5</td>
<td>7.6</td>
<td>21</td>
</tr>
<tr>
<td>T6 (70% CO₂/30% N₂)</td>
<td>2.1</td>
<td>2.9</td>
<td>7.2</td>
<td>23</td>
</tr>
<tr>
<td>T7 (90% CO₂/10% N₂)</td>
<td>2.6</td>
<td>3.6</td>
<td>7.0</td>
<td>28</td>
</tr>
</tbody>
</table>

*The lag phase, doubling time, and stationary phase were assessed using the DFMFit 2.0 (IFR, Norwich, United Kingdom) statistical program.

2The shelf life of meat was defined as the number of days needed to reach 10^7 cfu·g⁻¹.


