INTRODUCTION

Although chicken feet are considered to be byproducts in Brazil, they are exported as cuts to other countries, with the same commercial value as other edible parts. This product has been exported to Eastern countries for several years; however, with the opening of the Chinese market in recent years, this process has been intensified. The export of chicken feet from Brazil to China amounted to 66.91% of the total chicken exports in 2010 (USDA/FAS, 2010).

As there is no specific legislation for chicken feet, the microbiological quality of chicken feet was evaluated as a product for human consumption at different steps of the technological processes. Eighty broiler feet from 20 lots at 4 steps of processing were collected for quantitative analysis, total count of aerobic mesophilic bacteria, and determining the most probable number of coliforms and fecal coliforms. Thirty-eight pools of 15 broiler feet each from 19 lots were used for qualitative analysis and the isolation of Salmonella enterica spp. and Escherichia coli O157:H7. Escherichia coli O157:H7 was not found in any of the samples. Salmonella spp. were isolated in 68% (13/19) of the lots. The Salmonella Schwarzengrund serotype was found in 12 of the 13 lots of positive samples and the Salmonella Anatum and Salmonella Corvallis serotypes were identified in the remaining lot. Processing is effective in reducing contamination by mesophilic bacteria, coliforms, and Salmonella spp. in these products. This work constitutes the first study in Brazil on microbiological quality of chicken feet.

Key words: chicken feet, Salmonella spp., Escherichia coli, coliform, mesophilic

MATERIALS AND METHODS

Chicken feet were collected in a poultry processing plant under Federal Inspection Service, at Minas Gerais State (Brazil) during 5 visits from May to July 2009. Eighty broiler feet from 20 separate lots were collected individually from each processing step to determine the total count of aerobic mesophilic bacteria (TA), the most probable number of coliforms (TC), and the most probable number of fecal coliforms (FC). The sampling was performed at 4 steps: after cutting at the tibia-metatarsus joint (P1), after scalding between 65 and
70°C for 28 s and withdrawing of the cuticle (P2), after chilling (P3), and after freezing at a temperature of approximately −30°C (P4). All samples were packed in plastic bags and isothermal boxes and transported to the laboratory.

A portion of 25 g was taken from each foot, rinsed in 250 mL of 0.1% buffered peptone water and manually shaken for 1 min. The pour plate technique was used for TA and the most probable number (MPN) technique was used for TC and FC (USFDA, 1998).

For *Salmonella* spp. and *E. coli O157:H7*, isolation was conducted as described by USFDA (1998). From each of the 19 lots studied (1 lot was missed), 15 feet were collected and tested as a single composite unit. For each step, at P1 and at P4, a total of 570 feet was rinsed in 400 mL of buffered peptone water in a plastic bag and manually shaken for 1 min. After isolation procedures, the antigenic characteristics of *Salmonella* spp. were analyzed with somatic and flagellar antisera (Oswaldo Cruz Institute, Rio de Janeiro, Brazil).

*Escherichia coli* strains were tested serologically for the O157 somatic and H7 flagellar antisera (Denka Seiken Co., Tokyo, Japan). The isolates that were H7-negative were examined for motility by agar stab methods to confirm nonmotile variants (Fields et al., 1997). *Escherichia coli* O157 culture supernatant toxicities for Vero cell were evaluated for the specific cytopathic effects. Ten, 100, and 1,000 50% cytotoxic doses of control Stx2 per mL were added to the filtrates to confirm that no inhibitors of cytotoxicity were present (Gentry and Dalrymple, 1980). To detect the O157 *stx1*, *stx2*, *eae*, *ehxA*, and *saa* genes, a multiplex PCR was performed according to the procedure described previously (Paton and Paton, 2002), and amplified DNA fragments were resolved by gel electrophoresis using 2% agarose, and then stained with ethidium bromide.

The results of TA, TC, and FC were expressed in logarithms to base 10 (log 10) and subjected to Bartlett’s test for variance similarities as prerequisites of further use of parametric or nonparametric tests. The values of TA were subjected to ANOVA, followed by Tukey’s test to differentiate among steps, whereas for TC and FC we used Kruskal-Wallis nonparametric analysis followed by Dunn’s test for differentiation among steps, both at the significance level of 5%. A Student’s *t*-test at the 5% level was also used to confirm step similarities in the case of the nonparametric test. The chances of contamination by *Salmonella* spp. at the P1 and P4 steps of processing were quantified by calculating the odds ratio and its CI at 5% significance. All statistical analyses followed procedures described previously (Thrusfield, 1995). The program InStat, version 3.1 (GraphPad, 2009) was used for the calculations.

**RESULTS AND DISCUSSION**

The results of Bartlett’s test revealed that the variances of the TA averages at steps P1 to P4 were similar (*P* > 0.05), meaning that the data followed a normal distribution (Thrusfield, 1995), whereas for TC and FC, variances at the same steps were different (*P* < 0.05).

Regarding TA, the contamination level decreased after processing (*P* < 0.05) from P1 to P4 (Figure 1), but...
there was only a statistical difference between P1 and the other steps (Table 1).

Before scalding, Kotula and Pandya (1995) found a TA average for chicken feet of 6.2 ± 0.1 cfu/g, which is lower than the value we found in our present study. Higher values were obtained by Lopes et al. (2007) with chicken carcasses when compared with the steps after scalding and after chilling. Göksoy et al. (2004), working with chicken carcasses, found a higher value after scalding, but a similar result after chilling. These differences between results obtained in the analysis of feet and carcasses might be credited to different technological processing of these products, such as the higher temperatures used for feet scalding and the larger predisposition to contamination of carcasses because of evisceration.

For the first time in Brazil, chicken feet for human consumption were examined microbiologically. The numbers for Salmonella spp., TA, and coliforms were low and similar to those obtained by Bilgili et al. (2003). There is no legislation in Brazil to regulate the maximum limit for the TA in poultry meat, but the legislation of China set a maximum limit of 1.0 × 10⁶ cfu/g for fresh poultry and 5.0 × 10⁵ cfu/g for frozen poultry (USDA/FAS, 2006). Therefore, both fresh and frozen feet analyzed in this study showed values in accordance with Chinese regulations (Figure 1).

In the Kruskal-Wallis test, the TC averages were higher at the beginning of the feet processing, before scalding (P1), but only with statistical differences (P < 0.05) between this step and the others. After scalding (P2) and after chilling (P3), the averages decreased but did not differ significantly (P > 0.05) among themselves (Table 1). The Kruskal-Wallis test was also significant (P < 0.05) for differences among FC samples. By the Dunn test, samples collected at P1 were different from those at the other steps. The P2 and P3 steps were equal (P > 0.05), and P3 differed from P4 (P < 0.05), but P4, judged the same as P2, was found different by the Student’s t-test (P < 0.05), used as confirmatory (Table 1).

Lopes et al. (2007) and our group found similar values for TC and FC at step P2 by analyzing chicken carcasses after scalding. All samples studied in this work were in accordance with the national standard for fecal coliforms in fresh and frozen chicken meat (Figure 1; Ministério da Saúde, 2001), which is the only existing microbiological standard in Brazil for chicken meat.

When compared with Chinese standards, presented by USDA/FAS (2006), the average of FC in fresh feet samples were higher than the allowed value. However, all samples were in accordance with the Chinese standards for FC analysis on frozen poultry, even being 100 times lower than the Brazilian standard (Figure 1).

The values found for TC and FC in the feet studied showed a more expressive reduction than the TA values during technological processing. Between P1 and P4, a reduction of 5.6 log₁₀ MPN/g was observed for TC and 5.4 log₁₀ MPN/g for FC, but only 3.4 log₁₀ cfu/g was found for TA. One possible explanation is the lower resistance of these bacterial groups to sudden temperature changes during processing at the steps of scalding (P2), chilling (P3), and freezing (P4).

The results for E. coli O157:H7 yielded 2 strains that were positive against the O157 antiserum, among the samples collected in P1. None was confirmed as O157:H7 strains. Similarly, Silveira et al. (1999) did not find this serotype among 17 strains of E. coli O157 isolated in hamburgers produced in Brazil. Doyle and Schoeni (1987) reported the isolation of E. coli O157:H7 from chicken meat in the United States. However, in research conducted in Brazil, positive results for this bacterium have not been found (Silva et al., 2001; Jakabi et al., 2004; Silveira, 2010).

Of the 19 lots of feet investigated for Salmonella spp., 68% (13/19) were positive before scalding. This result is similar to that found by Kotula and Pandya (1995), who reported that 55% of feet were contaminated by this organism.

After processing, Salmonella spp. was isolated from only 1 lot (5.26%), which was also positive before processing. The value of the odds ratio was 39.00 (P < 0.05), which means that the chance of the bacteria being present before processing was 39 times greater than that after processing (CI = 4.17–363.64). Bilgili et al. (2003) analyzed processed chicken feet and found 2.78% positivity for Salmonella spp. Data from the Brazilian National Program for Monitoring the Prevalence and Resistance of Bacteria in Chicken indicate a Salmonella spp. prevalence of 4% in frozen chicken carcasses marketed at retail (Ministério da Saúde, 2008), whereas a prevalence of 2.3% was found in several products of poultry originated from Europe (EFSA, 2010).

Other research has demonstrated a higher prevalence of Salmonella spp. in chicken carcasses, such as 19% (17/90) reported by Cason et al. (1997), 39.3% (24/61) by Ribeiro et al. (2007), and approximately 59% by Chen et al. (2010). Consequently, the contamination

**Table 1.** Mean total count of aerobic mesophilic bacteria (TA) and most probable number (MPN) of coliforms (TC) and fecal coliforms (FC) of feet samples collected at 4 steps of processing

<table>
<thead>
<tr>
<th>Step</th>
<th>TA²</th>
<th>TC³</th>
<th>FC³</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>7.1 ± 0.2ᵃ</td>
<td>6.2 ± 0.2ᵃ</td>
<td>5.8 ± 0.2ᵃ</td>
</tr>
<tr>
<td>P2</td>
<td>5.6 ± 0.2ᵇ</td>
<td>3.7 ± 0.2ᵇ</td>
<td>3.7 ± 0.3ᵇ</td>
</tr>
<tr>
<td>P3</td>
<td>5.0 ± 0.2ᵇ</td>
<td>2.5 ± 0.2ᵇ</td>
<td>2.1 ± 0.2ᵇ</td>
</tr>
<tr>
<td>P4</td>
<td>3.7 ± 0.1ᵇ</td>
<td>0.6 ± 0.1ᶜ</td>
<td>0.4 ± 0.1ᶜ</td>
</tr>
</tbody>
</table>

ᵃ Means within a column lacking a common superscript differ (P < 0.05).
ᵇP1 = after cutting at the tibia-metatarsus joint; P2 = after scalding and withdrawal of cuticle; P3 = after chilling; and P4 = after freezing.
ᶜn = 20; values are means ± SE (log₁₀ MPN/g).

³n = 20; values are means ± SE (log₁₀ cfu/g).
rates for chicken feet are smaller than those for chicken carcasses. *Salmonella* Schwarzengrund was found in 12 of the 13 positive lots investigated. This serotype was the second most prevalent in research conducted by Chen et al. (2010). *Salmonella* Anatum and *Salmonella* Corvallis were both isolated from the remaining positive lot. This demonstrates the possibility of contamination by different serotypes in the same sample, and the importance of serotyping all suspicious colonies.

Juneja et al. (2003) studied the thermal resistance of *Salmonella* spp. and showed that a temperature of approximately 65°C is not enough to eliminate these bacteria in 28 s, which is the time used to scald the feet. According to the authors, 71°C would be a sufficient temperature for a 7.0 log10 reduction of *Salmonella* spp., considering the processing time used. In Brazil, there are no legal requirements for time and temperature for scalding chicken feet, but legislation (Ministério da Agricultura, Pecuária e Abastecimento, 1952) suggests a temperature range of between 80 and 90°C; however, this range is not used by the industry, in order to save on steam. After processing, there was a 92.31% reduction in *Salmonella* spp. occurrence in the lots studied. Considering the indicator microorganisms, a 99.98% reduction (3.4 log10) occurred with TA, and a 99.99% reduction occurred (5.6 log10 and 5.4 log10) with TC and FC, respectively. From these results, it appears that, despite the different quantities, there was a trend of reducing contamination.

**Conclusions**

Technological processing of chicken feet used by the poultry industry was effective for the reduction of TA, TC, FC, and *Salmonella* spp. As there is no standardization for the technological processing of chicken feet, other studies that determine the appropriate processing techniques and define the limits to be followed are demanded. Thus, a raw product with a high hygienic-sanitary standard can be obtained, even with low-cost processing, as was used in this work.

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**REFERENCES**


