Bacteriological Characteristics of Fresh Ostrich Sausage (Linguiça)

ABSTRACT

The aim of this study was to evaluate bacteriological characteristics and shelf life of three formulations of ostrich sausages (linguiças), only differing in lean meat percentage: Formula 1, 100% ostrich meat; Formula 2, 75% ostrich meat + 25% pork; and Formula 3, 50% ostrich meat + 25% pork + 25% chicken. All linguiças were vacuum-packed and stored at 5 ± 2°C. Mesophilic and psychrotrophic bacteria, sulfite-reducing Clostridia, coagulase-positive Staphylococci, and Escherichia coli were enumerated and Salmonella spp were isolated and identified. Initial mesophilic and psychrotrophic bacteria counts were high. During storage time, sulfite-reducing Clostridia, coagulase-positive Staphylococci, and Escherichia coli counts never reached the tolerance limit established by the Brazilian legislation. However, Salmonella was isolated from a Formula 2 sample on day 1, therefore, it was considered inappropriate for consumption. The shelf lives of Formulas 1 and 3 were below 12 and 8 days, respectively. If initial bacterial counts had been lower, the shelf life of the evaluated formulas would probably be longer. This study showed that ostrich meat trimmings can be successfully used in the production of ostrich linguiças, and that the formula containing ostrich meat as the only source of lean meat presented the longest shelf life.

INTRODUCTION

Ostrich (Struthio camelus) meat is considered a delicacy and it is usually served cooked, grilled, or dried (biltong is the South African version of dried and cured ostrich meat, which is similar to beef jerky) (Böhme et al., 1996). It is marketed in Western societies as a healthy alternative to other red meats due to its nutritional properties, such as low cholesterol and intramuscular fat content and a high percentage of ω-3 polyunsaturated fatty acids (Fisher et al., 2000; González-Montalvo et al., 2007). Ostrich meat’s relatively high pH makes it highly perishable and further research on the use of ostrich meat in value-added products is clearly needed, especially on fresh meat products (Fernández-López et al., 2006).

Approximately one-third of the lean meat separated from the ostrich carcass consists of lean trimmings (Harris et al., 1993), which are a challenge for processors to market as it is less noble and valuable than the ten major muscles. In order to increase the profitability of the ostrich industry these lean trimmings should be used as a raw material for ostrich meat products (value-added products) (Mckenna et al., 2003). Several authors have demonstrated the successful use of ostrich meat in several meat products (Böhme et al., 1996; Capita et al., 2006b; Cavalheiro et al., 2010; Souza et al., 2012; Dicks et al., 2004; Fernández-López et al., 2003; Fernández-López et al., 2006; Fisher et
Linguiça is one of the most produced and marketed meat products in Brazil, possibly because sophisticated technology is not required in its production (Milani et al., 2003). It is a meat product that has recognizable texture, color, flavor, and aroma. Linguiça is a type of cured sausage, fresh or smoked, made of meat, fat (optional) and other ingredients, which are stuffed into natural or artificial casings and submitted for processing. The ingredients required include livestock meat and salt, and optional ingredients include fat, water, vegetable or animal protein, sugar, plasma, intentional additives, flavors, spices, and condiments (Brazil, 2000).

The microbiological standards for linguiça established by Resolution of the Collegiate Directorate (RDC) No. 12 of January 2, 2001, of the National Agency of Health Surveillance (ANVISA) (Brazil, 2001) establishes the analyses of the following pathogens and acceptable limits per sample as follows: Escherichia coli, $5 \times 10^3$ (3.7 log$_{10}$) Colony Forming Units (CFU)/gram (g); coagulase-positive Staphylococci, $5 \times 10^3$ (3.7 log$_{10}$) CFU/g; sulfite-reducing Clostridia at 46ºC, $3 \times 10^3$ (3.48 log$_{10}$) CFU/g; and Salmonella spp., undetectable/25 g sample.

The aim of this study was to evaluate the bacteriological characteristics and the shelf life of three formulations of fresh ostrich linguiças, vacuum-packed and stored at 5 ± 2ºC.

**MATERIALS AND METHODS**

**Raw material**

Ostrich meat trimmings were obtained from deboning the carcasses of 12- to 14-month-old ostriches slaughtered in a processing plant under the Federal Inspection Service (SIF) of the Brazilian Ministry of Agriculture (MAPA), Brazil. After chilling at 2ºC for 24 hours (h), the meat was removed from the carcasses of the birds, packed, boxed, and frozen at -35ºC, after which, it was stored at -18ºC until use.

The pork used in the sausages derived from the loin, and the chicken meat from the leg. Lard was also used. These raw materials were purchased in a retail store (supermarket) and derived from processing plants with Official Inspection Service, and were stored in a freezer at -18ºC. At the time of sausage preparation, meats and lard were thawed in a refrigerator (Brastemp® 360) at 7ºC for 18 h.

**Linguiça formulation, vacuum packing and storage**

Three formulations were elaborated with three different lean meat percentages. Formula 1 contained 100% ostrich meat; Formula 2, 75% ostrich meat + 25% pork; and Formula 3, 50% ostrich meat + 25% pork + 25% chicken. The sausages were prepared according to a traditional formula, with 78.95% lean meat, 15.68% pork fat, 2.15% sodium chloride, 0.03% chili powder, 6 mL white wine/kg, 0.05% nutmeg, 2% iced water, 1.25 g/kg Prague® powder, and 1.25 g/kg sodium erythorbate.

Meat and lard were separately ground using a 106-mm meat grinder (Ibrasmack® MC 106); with a 10-mm grinding plate, and mixed with the other ingredients in a mixer (Incomaf® MT 200) for 15 minutes. The mixed product was vacuum-stuffed (Incomaf® RS 1040) into natural sheep casings. The sausages were vacuum-packed (Selovac® 300 B) and stored at 5 ± 2ºC (Brastemp® 360) until the analyses, performed on days 1, 4, 8, 12, 16, and 20.

**Bacteriological analyses**

In compliance with RDC No. 12 (Brazil, 2001), the following bacteriological analyses were performed: sulfite-reducing Clostridia, coagulase-positive Staphylococci and $E.\ coli$ enumeration, and $Salmonella$ spp. isolation and identification.

In order to monitor product spoilage status, mesophilic and psychrotrophic bacteria were also counted.

The methodology recommended by Normative Instruction No. 62 of 26 of August of 2003 (Brazil, 2003) was applied to enumerate mesophilic and psychrotrophic bacteria, sulfite-reducing Clostridia, and coagulase-positive Staphylococci. Each sample (25 g) was aseptically removed from its package, placed in a stomacher bag, diluted with 225 mL sterile 0.1% buffered peptone water, and homogenized in a
Seward stomacher 80 (Seward®) at normal speed for 1 min. Samples were then serially diluted using the same diluents. Mesophilic and psychrotrophic bacteria were cultured on Plate Count Agar (Himedia®). Plates were incubated at 36 ± 1°C for 48 h for mesophilic bacteria and at 7°C for 10 days for psychrotrophic bacteria. Sulfite-reducing Clostridia were cultured on Sulfite-Polymyxin-Sulphadiazine (SPS) Agar (Himedia®). The plates were anaerobically incubated at 46°C for 24 h. Coagulase-positive Staphylococci were cultured on Baird Parker Agar (Himedia®) with egg-yolk tellurite emulsion, and the tubes were incubated at 36 ± 1°C for 48 h. Three typical and three atypical colonies were selected, transferred to test tubes containing “Brain Heart Infusion” (BHI) broth (Himedia®), and incubated at 36 ± 1°C for 24 h. Gram stains were prepared from the cultures and observed microscopically (Olympus® BX41). Gram-positive cocci cultures were selected for the catalase test, using 3% hydrogen peroxide. The catalase-positive cultures were subjected to the coagulase test: 200 µL of the BHI cultures were incubated in test tubes containing a mixture of chicken, turkey, and ostrich meat. On the other hand, the initial count was higher than those reported by Alonso-Calleja et al. (2006b) in ostrich chorizo. However, it was lower than that reported by Mastromatteo et al. (2009) in poultry patties containing a mixture of chicken, turkey, and ostrich meat. On the other hand, the initial count was higher than those reported in ostrich meat and its products by other authors (Capita et al., 2006a; Capita et al., 2006b; Fernández-López et al., 2006; Fernández-López et al., 2008; Otremba et al., 1999). Although Seydím et al. (2006) stated that mesophilic counts between 6 and 8 log_{10} CFU/g are associated with meat spoilage, which is characterized by off-odors and possible slime development, none of the samples showed any signs of spoilage until day 8.

There were no mesophilic count differences (p > 0.05) among the evaluated formulas, which is consistent with the results reported by Fernández-López et al. (2006) in ostrich burgers. Storage time did not significantly affect mesophilic counts, despite they tended to increase with storage time until day 12. This was probably due to the high initial counts, because in this case, microbial growth lag and most of the log
phases of microbial growth were most likely missed, as microbial growth was only observed at the end of the log growth period and in the stationary phases. In contrast, other authors (Capita et al., 2006a; Fernández-López et al., 2006; Fernández-López et al., 2008; Mastromatteo et al., 2009; Otremba et al., 1999) found increasing counts during storage time in vacuum-packed ostrich meat and its products.

The high initial psychrotrophic bacteria counts observed in the present study were different (p < 0.05), with Formula 1 presenting lower initial counts that the other formulas. On the other hand, Fernández-López et al. (2006) did not find any differences (p > 0.05) among ostrich burger formulas. The initial counts were similar (Formulas 2 and 3) and lower (Formula 1) to those found by Alonso-Calleja et al. (2004) in vacuum-packed retail ostrich meat. The initial count found in Formula 1 linguiça sausages was similar to those found in ostrich chorizos by Capita et al. (2006b). However, other authors (Capita et al., 2006a; Capita et al., 2006b; Fernández-López et al., 2006; Fernández-López et al., 2008; Otremba et al., 1999) found lower bacteria counts, while Mastromatteo et al. (2009) reported higher counts than those observed in the present study. There were no differences (p > 0.05) in psychrotrophic counts due to storage time, despite the trend of increase until day 12, whereas other authors (Capita et al., 2006a; Fernández-López et al., 2006; Fernández-López et al., 2008; Mastromatteo et al., 2009; Otremba et al., 1999) observed increasing counts during storage in vacuum-packed ostrich meat and its products.

Sulfite-reducing Clostridia growth was only observed after day 16 in Formulas 2 and 3, and day 20 in Formula 1. All counts (average of 3.48 log$_{10}$ CFU/g) remained below the limit established by legislation (Brazil, 2001) during the evaluated storage time. There were no differences (p > 0.05) due to formulation or storage time. These results may be explained by the inclusion of nitrite in the formulas, which inhibits the growth of Clostridium spp., as stated by Jay (2000). According to that author, Clostridium botulinum
cannot grow or produce toxin when competing with a large number of other microorganisms.

*Linguiça* formulation and storage time did not influence \((p > 0.05)\) coagulase-positive Staphylococci counts. The tolerance limit established by legislation (Brazil, 2001) for this microorganism, \(3.7 \log_{10} \text{CFU/g}\), was never reached during storage time. Therefore, the shelf life of the evaluated fresh *linguiça* formulas expired when the counts began to decrease, as this indicates the decline of bacterial growth, which occurred on day 8 in Formulas 2 and 3 and day 16 in Formula 1. According to Valero et al. (2009), *Staphylococcus aureus* is an indicator of poor hygiene in food processing, and therefore, the presence of *Staphylococcus aureus* in the samples indicates that there may have been failures in maintaining proper hygiene and/or storage temperature of the raw materials. Karama (2001) found an average of \(2.38 \log_{10} \text{CFU/cm}^2\) of *Staphylococcus aureus* on ostrich carcasses post-chilling processed in an exports-approved South African plant.

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**Figure 2** – Sulfite-reducing Clostridia (A), and coagulase-positive Staphylococci (B), and *E. coli* (C) counts in different types of ostrich linguiça (Formula 1, 100% ostrich meat; Formula 2, 75% ostrich meat and 25% pork; and Formula 3, 50% ostrich meat, 25% pork, and 25% chicken). No differences \((p > 0.05)\) due to storage time were detected. a-c Values bearing different letters are significantly different \((p < 0.05)\).
E. coli counts were different among the evaluated formulas only on day 1, with higher counts in Formula 3 compared with Formulas 1 and 2, which were similar to each other. During storage time, the counts remained below the limit established by legislation (Brazil, 2001), which is 3.7 log10 CFU/g. Therefore, the formulas shelf life ended when the counts began to decrease, which occurred on day 8, in Formulas 2 and 3, and day 12, in Formula 1. There were no differences (p > 0.05) due to storage time. Karama (2001) found E. coli in 43% of 30 ostrich carcasses slaughtered in an export-approved South African abattoir, in these E. coli positive samples the mean count was 2.15 log10 CFU/g. Gill et al. (2000) found an average E. coli count lower than 0.02 log10 CFU/g in 25 dressed ostrich carcasses at a small processing plant. Gill (2007) obtained average E. coli counts of approximately 0 and 2 log10 CFU/g in carcasses of ostriches slaughtered in two different plants.

Salmonella spp. was detected only in a sample of Formula 2 ostrich linguiças. The standard established by Brazilian legislation (Brazil, 2001) requires the absence of Salmonella spp. in 25 g of sample, and therefore, this standard was not complied by Formula 2 fresh linguiças. Salmonella spp. was not detected on any other day in Formula 2 linguiças or the other formulas. Considering the 90 samples analyzed in the present study, this amounts to 1.11% of the samples in which Salmonella was isolated. The presence of Salmonella was most likely due to the contamination of raw materials. Karama (2001) found Salmonella in 23.3% of 30 ostrich carcasses slaughtered in an exports-approved South African processing plant. Gopo & Banda (1997) did not detect Salmonella in any of 120 samples of fillet and meat-and-bone meal from ostriches slaughtered in an ostrich processing plant. According to Gill (2007), while Salmonella was recovered from more than 20% of ostrich carcasses in one processing plant, samples from more than 100 carcasses from eight US plants only yielded one case of Salmonella contamination. Freitas Neto et al. (2009) did not find Salmonella spp. in any of 90 ostrich carcasses derived from a single processing plant.

CONCLUSION

The fresh ostrich linguiça formulas evaluated in the present study showed high initial counts of mesophilic and psychrotrophic bacteria. During storage time, the tolerance limits established by legislation for sulfite-reducing Clostridia, coagulase-positive Staphylococci, and Escherichia coli was never exceeded. However, Salmonella was isolated from a Formula 2 sample on day 1. Formula 1 presented the best shelf life (less than 12 days), followed by Formula 3 (less than 8 days), while Formula 2 was considered inappropriate for consumption. Further research on raw materials, ingredients, additives, and technological process steps may reveal possible sources of contamination. This study showed that ostrich meat trimmings can be used for the production of ostrich linguiças, and that the formula elaborated with ostrich meat trimmings as the only source of lean meat presented the longest shelf life.

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