



## Bacteriological Characteristics of Fresh Ostrich Sausage (*Linguíça*)

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### ■ Keywords

Ostrich meat, bacteriological analysis, vacuum-packing.

### 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  $5 \pm 2^\circ\text{C}$ . 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  $\omega$ -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*



*al.*, 2000; Hautrive *et al.*, 2008; Hoffman & Mellett, 2003; Lee & Kang, 2003; Mastromatteo *et al.*, 2009; Mckenna *et al.*, 2003).

*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 46°C,  $3 \times 10^3$  ( $3.48 \log_{10}$ ) CFU/g; and *Salmonella* spp., undetectable/25 g sample.

Modern meat-packing techniques aim at maintaining the microbial and sensory quality of the products. Product shelf life may be extended by inhibiting or delaying the growth of undesirable microorganisms, which is achieved by manipulating the meat microenvironment. Vacuum and modified-atmosphere packaging (MAP) techniques are used by the food industry to increase the shelf life of products (Seydim *et al.*, 2006).

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  $5 \pm 2^\circ\text{C}$ .

## **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  $2^\circ\text{C}$  for 24 hours (h), the meat was removed from the carcasses

of the birds, packed, boxed, and frozen at  $-35^\circ\text{C}$ , after which, it was stored at  $-18^\circ\text{C}$  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  $-18^\circ\text{C}$ . At the time of sausage preparation, meats and lard were thawed in a refrigerator (Brastemp® 360) at  $7^\circ\text{C}$  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, 0.1% sucrose, 0.2% garlic, 0.05% black pepper, 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  $5 \pm 2^\circ\text{C}$  (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  $36 \pm 1^\circ\text{C}$  for 48 h for mesophilic bacteria and at  $7^\circ\text{C}$  for 10 days for psychrotrophic bacteria. Sulfite-reducing Clostridia were cultured on Sulfite-Polymyxin-Sulphadiazine (SPS) Agar (Himedia®). The plates were anaerobically incubated at  $46^\circ\text{C}$  for 24 h. Coagulase-positive Staphylococci were cultured on Baird Parker Agar (Himedia®) with egg-yolk tellurite emulsion, and the plates were incubated at  $36 \pm 1^\circ\text{C}$  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  $36 \pm 1^\circ\text{C}$  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  $\mu\text{L}$  of the BHI cultures were incubated in test tubes containing the same volume of reconstituted plasma fibrinogen (Larboclin® Coaguplasma) at  $36 \pm 1^\circ\text{C}$  for 18 to 24 h.

*E. coli* was enumerated using a miniaturization method adapted from Merck (2005). From the sample dilutions, a volume of 100  $\mu\text{L}$  was incubated in three series of three Eppendorf microtubes containing 1000  $\mu\text{L}$  of Fluorocult LMX Broth Modified (Merck®) at  $36 \pm 1^\circ\text{C}$  for 48 h. The cultures whose broth showed a colour change from yellow to blue-green were considered positive for coliforms and exposed to long-wave UV light (4W/366 nm) (Merck®). One drop of Kovacs' reagent was added to cultures presenting blue fluorescence. The cultures that formed a red ring on their surface were considered positive for *E. coli*.

The detection and isolation of *Salmonella* was performed using the rapid method proposed by Pignato *et al.* (1995). Samples (25 g) were homogenized for 60 seconds with 225 mL of Salmosyst Broth Base in a stomacher and then pre-enriched by incubation at  $36 \pm 1^\circ\text{C}$  for 6 hours. After that period, 10 mL of the pre-enrichment broth was transferred to a test tube containing 1 mL of Salmosyst Selective Supplement and incubated at  $36 \pm 1^\circ\text{C}$  for 18 h. Following incubation, a loopful of selective broth culture was streaked for isolation on Rambach Agar (Himedia®) and on Hektoen Enteric Agar (Himedia®) plates and incubated at  $36 \pm 1^\circ\text{C}$  for 24 h. A typical colony from each plate was picked and inoculated on Triple Sugar Iron (TSI) Agar (Himedia®) slant by streaking the slant

and stabbing the butt. The tubes were incubated at  $36 \pm 1^\circ\text{C}$  for 24 h. Presumptive isolates of *Salmonella* spp. were subjected to the serological polyvalent flagellar (H) test.

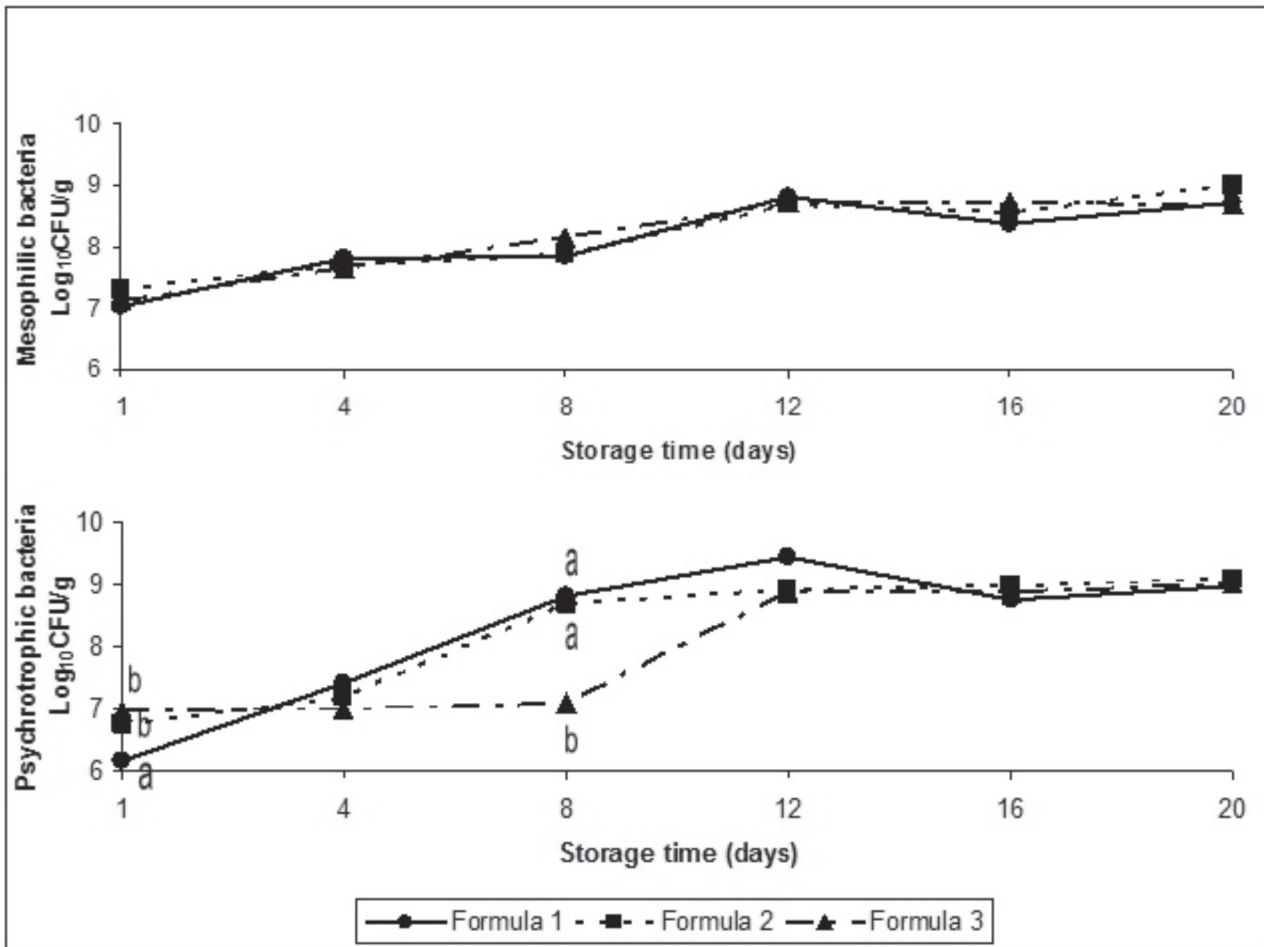
### Statistical analysis

Each parameter was tested in quintuplicate, on days 1, 4, 8, 12, 16 and 20 of storage time. Statistical analyses were performed using SPSS for Windows 14 (SPSS, Sao Paulo, Brazil). Differences ( $p < 0.05$ ) among the formulations were evaluated by Kruskal-Wallis test. The Mann-Whitney test was performed to show which formulations were different from each other. The Friedman test was used to determine differences during the storage time, and the Wilcoxon test was used to show which days were different from each other.

## RESULTS AND DISCUSSION

High initial mesophilic bacteria counts (Fig. 1) suggest the exposure of food to excessive temperature during processing, storage, transportation, distribution, or retail display of the raw materials (Alonso-Calleja *et al.*, 2004; Mastromatteo *et al.*, 2009). Furthermore, grinding the meat through comminution increases its surface area, which favors microbial growth (Milani *et al.*, 2003). This high initial count was similar to those reported by Alonso-Calleja *et al.* (2004) in vacuum-packed retail ostrich meat purchased within 3 to 7 days after packing and Capita *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 Seydim *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



**Figure 1** – Mesophilic and psychrotrophic bacteria counts in different types of ostrich linguíça (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 ( $p < 0.05$ ).

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 than 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.

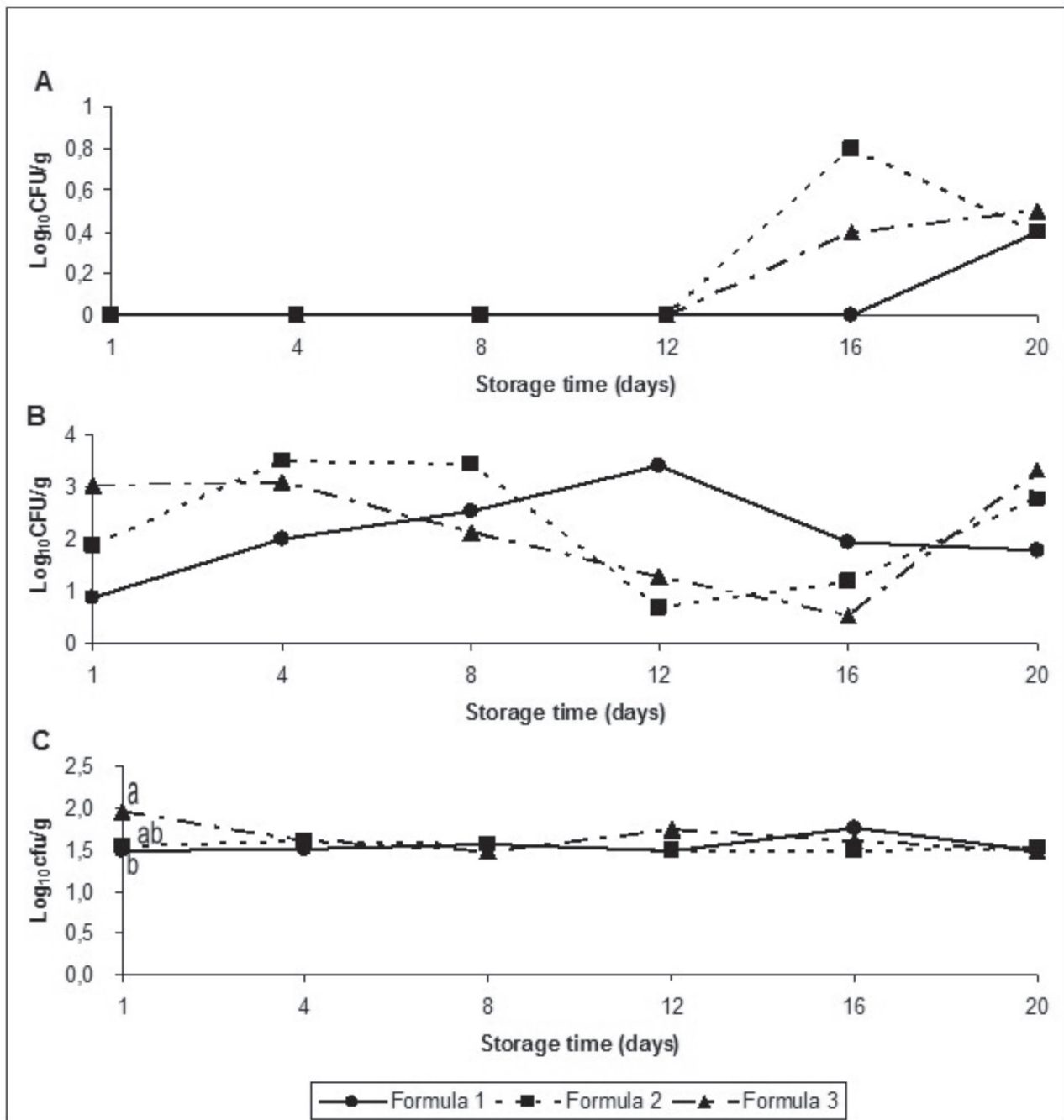
Sulfite-reducing Clostridia growth was only observed after day 16 in Formulas 2 and 3, and day 20 in Formula 1. All counts (average of 3.48 log<sub>10</sub> CFU/g) remained below the limit established by legislation (Brazil, 2001) during the evaluated storage time. There were no differences ( $p > 0.05$ ) 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,  $3.7 \log_{10}$  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  $2.38 \log_{10}$  CFU of *Staphylococcus aureus*/cm<sup>2</sup> on ostrich carcasses post-chilling processed in an exports-approved South African plant.



**Figure 2** – Sulfite-reducing Clostridia (A), and coagulase-positive Staphylococci (B), and *E. coli* (C) counts in different types of ostrich linguíça (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  $3.7 \log_{10}$  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  $2.15 \log_{10}$  CFU/g. Gill *et al.* (2000) found an average *E. coli* count lower than  $0.02 \log_{10}$  CFU/g in 25 dressed ostrich carcasses at a small processing plant. Gill (2007) obtained average *E. coli* counts of approximately 0 and  $2 \log_{10}$  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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