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Influence of cutting and deboning operations on the microbiological quality and shelf life of buffalo meat

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ABSTRACT

Considering the specific biochemical composition of buffalo (*Bubalus bubalis*) meat (high iron content, high biological value proteins and essential fatty acids, low amounts of fat and cholesterol), we evaluated the influence of cutting and deboning operations on the microbiological quality and shelf-life of vacuum-packed buffalo meat stored under refrigeration. On the processing day, samples were collected from carcass, deboning room surfaces and meat cuts. Samples from meat cuts were evaluated weekly for two months. On the processing day, higher counts of *Pseudomonas* spp. were observed in samples from meat cuts compared with the hindquarters and the processing surfaces. For thermotolerant coliform scores, the averages were −0.5 log MPN·cm^{-2}, −0.4 log MPN·cm^{-2} and 0.9 log MPN·g^{-1}, respectively. Higher counts of *Pseudomonas* spp. and LAB in meat cuts were observed on the processing day and after the first week of storage, respectively, remaining constant during shelf life. *Listeria grayi* was identified in two samples of hindquarters and meat cuts during storage. *Listeria innocua* was identified in one meat cut. In conclusion, cutting and deboning operations influence the microbiological quality and shelf life of vacuum-packed buffalo meat stored under refrigeration.

1. Introduction

Buffalo (*Bubalus bubalis*) herds have grown progressively and significantly, to meet an increase in the demand for their meat (ABCB, 2001; Brasil, 2014). Expansion in buffalo meat consumption is related to its nutritional advantages compared to beef. It is richer in iron, contains a higher amount of high biological value protein and essential fatty acids, along with lower quantities of fat and cholesterol (Cannarsi et al., 2008; Giuffrida-Mendoza et al., 2015; Lira et al., 2005; Ziauddin, Mahendrakar, Rao, Ramesh, & Amla, 1994). Although these features contribute to the appreciation of this food as a healthy product, buffalo meat presents a slow decline in post-mortem pH (Neath et al., 2007) and its high moisture content and water holding capacity (Lira et al., 2005; Tateo, De Palo, Quaglia, & Centoducati, 2007) can provide ideal conditions for microbial growth, different from that observed in beef.

In Brazil, buffalo meat is mainly sold in vacuum packs. Deboning, i.e., meat removal from bones and preparation of meat cuts, is an extremely important step regarding the microbiological quality of the final product. During deboning, meat is exposed to processing plant environment, equipment, utensils and manipulation, facilitating cross-contamination with spoilage and pathogenic microorganisms; thus, meat cuts are potential vehicles of foodborne diseases (Nel, Lues, Buys, & Venter, 2004). Considering the potential risks during deboning, key factors for sanitary control in the food industry should be noted. The main key factors are: (1) equipment cleaning; (2) handlers' personal hygiene and; (3) general cleaning conditions of work environment (Jullien et al., 2008).

When packed in the presence of oxygen, meat cuts have limited shelf life, due to the growth and biochemical activity of aerobic microorganisms such as bacteria from the *Pseudomonas* genus, which are considered the main ones responsible for deterioration processes in refrigerated beef (Lambert, Smith, & Doods, 1991; Oussalah, Caillet, Saucier, & Lacroix, 2006). These bacteria are responsible for reactions that culminate in meat discoloration and slime, as well as gas and enzyme (lipases and proteases) production. Enzymatic reactions provoke rancidity and bitter flavor (Oussalah et al., 2006) in beef.

Vacuum packaging is efficient in prolonging the shelf life of meat cuts, maintaining their desirable characteristics (Brightwell, Clemens, Adam, Urlich, & Boerema, 2009; Singh & Singh, 2005). Besides, Borch, Kantmuermans, and Blixt (1996) and Brightwell et al. (2009) stated that the storage conditions in this type of packaging favor the growth of lactic acid bacteria (LAB), whose metabolites are already recognized for their antimicrobial activity against spoilage and pathogenic

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microorganisms (Djenane et al., 2005; Jones, Hussein, Zagorec, Brightwell, & Tagg, 2008; Matamoros, Pilet, Gigout, Privost, & Leroi, 2009). However, this group of bacteria can also cause undesirable changes in the meat cuts, such as premature degradation and, consequently, reduced shelf life (Borch et al., 1996).

In addition, some authors pointed out that the use of vacuum packing at refrigerated temperature could promote the growth of different *Listeria* spp. during shelf life. These microorganisms are able to persist in food industry facilities due to their ability to produce biofilms on the processing surfaces, ensuring their survival, with high potential to contaminate food (Chae, Schraft, Hansen, & Mackereth, 2006; Farber, Warburton, Gour, & Milling, 1990).

Buffalo meat has a different composition from beef and is commonly processed in the same facilities. Considering this and the lack of studies with this product, the present study was designed to evaluate, throughout shelf life, the impact of microbiological contamination during the cutting of vacuum-packed buffalo meat cuts stored under refrigeration (5 °C).

2. Materials and methods

2.1. Sample collection

Samples were collected in a cattle and buffalo slaughterhouse. Aiming to evaluate the buffalo carcass contamination before entering in the deboning line, samples were obtained from hindquarters after the elaboration of the primary cuts. This step occurred after the chilling period (24 h), when the internal temperature reached 4 °C. These samples were collected in January (n = 6), March (n = 10), June (n = 10) and September (n = 9), totaling 35 carcasses sampled. The number of carcass sampled was equivalent at 10% of the number of slaughtered animals in the day. Forequarters were not sampled because they were not processed in the facility, and sent directly to expedition. Slaughtered buffalos ages ranged from 36 to 48 months old and the average carcass weight was 222.6 kg.

To evaluate the hygienic-sanitary conditions at deboning room, seven different processing surfaces, which had direct contact with the meat, were sampled in each collection period. Samples were collected from two plastic monoblocks, a waiting table for cuts and cleaning, two processing tables and two transport carts, totaling 28 samples. To evaluate microbiological conditions of the product during shelf life, after deboning, in each collection (n = 4), two meat cuts were randomly selected, divided in nine equal pieces, and vacuum-packed. Samples were isothermally transported to Laboratório de Inspeção de Produtos de Origem Animal da Universidade Federal de Pelotas for the subsequent microbiological evaluation.

2.1.1. Samples from hindquarters

The surface of the hindquarters (n = 35) was sampled in five (lower sirloin, rump cover, tenderloin, upper/lower sirloin and flank sirloin) 25 cm^2 points (125 cm^2/carcass), using previously sterilized swabs. After collection swabs were kept in 25 mL of 0.85% saline until analysis (Silva, Junqueira, & Silveira, 2010). To ensure collection from both sides of the carcass, each point was alternated between right and left sides, i.e., when the first sample was collected from the right lower sirloin, the subsequent samples were from left rump cover, right tenderloin, left upper/lower sirloin and right flank sirloin.

2.1.2. Samples from processing surfaces

Sample collections were performed before the beginning of daily activities in the deboning room. In each collection, samples were collected from the surface of seven pieces of equipments that were to be in contact with the meat cuts, i.e., two plastic monoblocks, a waiting table for cuts and cleaning, two processing tables and two transport carts. Five random 25 cm^2 points were sampled (125 cm^2/equipment) using previously sterilized swabs. After collection swabs were kept in 25 mL of 0.85% saline until analysis (Silva et al., 2010).

2.1.3. Samples from meat cuts

In each collection, two buffalo meat cuts, randomized from each side of the carcass, were collected at the end of the deboning line and divided into nine pieces of around 200 g each (Brasil, 2001). Meat cuts were packed in 50 μm thermo-shrinkable polyethylene (Ecofriendly®, Deltaplam, Brasil) with oxygen permeability rate lower than 10 cm^3/m^2/day at 23 °C/1 atm/65% RH. Vacuum packaging were performed in Duplavac® Inox 2-62 (Selovac, Brasil), with 99.8% of vacuum reached, and storage under refrigeration (5 °C) for two months. Before microbiological analyses, each sample was evaluated for 'blown pack' spoilage characteristics (Rossi Júnior, Felipe, Martineli, & Mesquita, 2011). Analyses were performed every week in duplicate.

2.2. Microbiological analysis

2.2.1. Listeria spp.

Samples were first subjected to the pre-enrichment phase in *Listeria* enrichment broth (UVM, Acumedia®) at 30 °C for 24 h, followed by selective enrichment in Fraser broth (Acumedia®) at 30 °C for 48 h, and finally to selective-differential isolation in Oxford agar (Acumedia®) and Palcam (Acumedia®) at 30 °C for 48 h. Typical colonies were transferred to trypticase soy agar supplemented with yeast extract (YE-TSA, Acumedia®) and incubated at 30 °C for 24 h (Farber & Daley, 1995). For *Listeria* genus confirmation, bacterial DNA was extracted using a methodology adapted from Sambrook and Russel (2001), followed by Polymerase Chain Reaction (PCR) using a pair of primers (For: GCTGAAGAGATT GCGAAAGAAG, Rev.: CAAAGAAACCTTGGATTTGCGG) for the identification of the *prs* gene, specific for *Listeria* strains.

PCR amplification was performed in a 25 μL volume, using 12.5 μL of Gotaq® Green Master Mix (Promega®), 25 pmol *prs* primer set (Eurofins®) and 10 ng (2 μL) of DNA template. A reaction mixture without DNA template was included as negative control. The PCR program was performed in a thermocycler (Bioer®) and consisted of 94 °C for 3 min, 35 cycles of 94 °C for 40 s, 53 °C for 1 min and 15 s, 72 °C for 1 min and 15 s, and a final cycle of 72 °C for 7 min (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004). After the end of all cycles, the products generated by PCR were subjected to electrophoresis at 80 V for 1 h in 1.5% agarose gel. The amplified product was stained with GelRed™ and visualized in a transilluminator (Loccus®). For biochemical differentiation of the species, the following tests were performed: motility test in sulfite indol motility agar (SIM, Merck®), hemolysis verification on blood agar plates (Columbia, Micro Med®) and fermentation of rhamnose (Vetec®), xylose (Vetec®) and mannitol (Vetec®).

2.2.2. Pseudomonas spp. quantification

Serial decimal dilutions of the samples were performed in 0.85% saline, from which aliquots of 0.1 mL were seeded on the surface of plates containing cetrimide base agar (Acumedia®) and incubated at 25 °C for 48 h (Nel et al., 2004). The results were expressed in log $CFU \cdot cm^{-2}$ or log $CFU \cdot g^{-1}$.

2.2.3. Thermotolerant coliform quantification

From the sample dilutions, 1 mL aliquots were transferred to a series of three test-tubes with inverted Durham's tube and Sodium Lauryl Sulfate broth (SLS, Micro®), and incubated at 37 °C for 48 h. From each SLS positive reacted tube, a sample was transferred to another test-tube containing inverted Durham's tube and *Escherichia coli* broth (EC, Micromed®), followed by incubation in a water bath at 45 °C for 48 h. The results were expressed in log $MPN \cdot cm^{-2}$ or log $MPN \cdot g^{-1}$ (FDA, 2002).

2.2.4. Lactic acid bacteria (LAB) quantification

From the sample dilutions, 0.1 mL aliquots were transferred to plates containing De Man, Rogosa and Sharpe agar (MRS, Acumedia®) and incubated anaerobically at 35 ± 2 °C for 96 h (Jones et al., 2008). The results were expressed in log CFU·g^{-1}.

2.3. Statistical analysis

Microorganism counts were statistically evaluated with Statistica 7.0 (Statsoft, 2004). Experiment was conducted in a completely randomized design, using analysis of variance (ANOVA) with significance level of 5% ($p < 0.05$). Significant differences were verified by Fisher's Least Significant Difference — LSD test ($p < 0.05$).

3. Results and discussion

The *Pseudomonas* spp. count on hindquarter surfaces prior to deboning was higher at first collection when compared to second and fourth collections (Table 1). These results, which are similar to those described in other papers, reflect a lack of standardization in operational hygiene procedures performed during the primary cuts of carcasses, and this can favor the spread and multiplication of pathogenic and spoilage bacteria related to the reduction in product shelf life (Gill & Jones, 1999; Yashoda, Sachindra, & Rao, 2000).

Pseudomonas spp. counting was similar ($p > 0.05$) between the deboning room processing surfaces: 2.8 log CFU·cm^{-2} in the processing tables; 3.2 log CFU·cm^{-2} in the waiting table; 3.0 log CFU·cm^{-2} in the transport carts; and 2.4 log CFU·cm^{-2} in the plastic monoblocks. However, evaluating the different collection days, as observed on the surface of hindquarters, the average *Pseudomonas* spp. count for processing surfaces was higher ($p < 0.05$) for collections 1 and 3 (Table 1). These findings demonstrated that the hygienic quality during meat manipulation was inferior in these days. The presence of viable microorganisms on the food processing surface has a potential hazard of cross-contamination, because they could multiply and colonize the surface, or just remain viable, unable to multiply, due to adverse environmental conditions (Veran, 2002). *Pseudomonas* genus bacteria are able to fixate on food processing surfaces and form biofilms, creating a persistent source of contamination for the final product (Orgaz, Lobete, Puga, & Jose, 2011; Sofos & Geornaras, 2010). According to Wirtanen, Husmark, and Mattila-Shandholm (1996), 5 log CFU·cm^{-2} adherent cells are sufficient for biofilm formation, as can be observed in our results (collection 3), and this value can be encountered in deboning rooms. These differences observed in the bacterial count before the beginning of daily activities in the deboning room were attributed to a lack of standardization in the facilities' cleaning and disinfection procedures.

Regarding thermotolerant coliform counts (Table 2), there was no difference between collections neither in the hindquarter surfaces nor in the processing surfaces. The average MPN found on hindquarter surface was −0.4 log MPN·cm^{-2}. According to Silva Júnior (1995), the microbiological standard for food production utensils indicates that they should be free of thermotolerant coliforms. In this study, the average thermotolerant coliforms found in processing surface samples was −0.4 log MPN·cm^{-2}. Although in low numbers, this result indicates contamination of fecal origin, and raises concern due to the participation of *E. coli*, an important cause of foodborne disease (Newell et al., 2010), in this group of microorganisms.

Vacuum packs are widely used to increase products' shelf life by reducing the oxygen concentration inside the pack (Brightwell et al., 2009; Singh & Singh, 2005). As described in Table 1, different *Pseudomonas* spp. counts on hindquarters and processing surfaces did not alter this parameter in vacuum-packed buffalo meat cuts. During the shelf life, the count was significantly lower on the processing day (average of 3 log CFU·g^{-1}), when compared with subsequent weeks. After the first week, counts remained constant (Fig. 1). Nel et al. (2004) stated that the packing of beef cuts in anaerobic packing partially inhibits the growth of *Pseudomonas* spp., reporting that microorganism counts ranged from 4 log CFU·g^{-1} to 5.9 log CFU·g^{-1} for two months of refrigerated storage, with no significant difference between weeks of storage.

According to Ordóñez et al. (2005), vacuum-packed meats maintain a certain rate of residual oxygen within the pack during the first days of refrigerated storage, but this gas is rapidly depleted by the metabolic activity of muscle and microorganisms. Thus, although these packs will help to reduce the growth of aerobic microorganisms, the residual oxygen inside keeps them still viable, which may explain why the growth of *Pseudomonas* spp. is significantly higher on the processing day and during the first week of storage, as well as why count values are maintained during the shelf life. Another possible explanation for this finding is that, because *Pseudomonas* spp. possess strictly aerobic metabolism, some species are able to grow under anaerobic conditions (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Certain characteristics of *Pseudomonas* spp. isolated from meat, which are not easily observed in vitro, can be correlated with its rapid growth on this substrate (Labadie, 1999). As these bacteria need a certain amount of iron for their metabolism, and the buffalo meat is rich in this nutrient, *Pseudomonas* spp. growth occurs rapidly as it saves energy at low temperatures to meet its requirement of iron from different sources available in the product. Furthermore, many materials from which vacuum packs are manufactured are sufficiently permeable to oxygen (Marsh & Bugusu, 2007) and thus the respiration and growth of aerobic microorganisms could not be inhibited. Vacuum packs can be made out of a large variety of materials, since they provide a humidity barrier and the correct permeability to gases, to maintain a constant environment inside the pack during the storage period (McMillin, 2008).

LAB counts were similar to those for *Pseudomonas* spp. from the second week of storage. In other words, the scores recorded on the processing day were significantly lower than scores after the first week of storage, and both were significantly lower than every other week of shelf life (Fig. 1). Jones (2004) also noted the increase in LAB counts

Table 1
Average *Pseudomonas* sp. counts in samples of hindquarters, deboning room processing surfaces and vacuum-packed meat cuts.

Collection	*Pseudomonas* sp.		
	Hindquarters surface (log CFU·cm^{-2})	Processing surfaces (log CFU·cm^{-2})	Meat cuts* (log CFU·g^{-1})
1	3.4^{a} ± 2.9 (n = 6)	3.4^{a} ± 1.7 (n = 7)	3.7 ± 0.6 (n = 2)
2	1.2^{b} ± 0.7 (n = 10)	1.2^{b} ± 0.5 (n = 7)	3.9 ± 2.2 (n = 2)
3	2.5$^{a, b}$ ± 2.0 (n = 10)	5.3^{a} ± 0.6 (n = 7)	1.0 ± 0.0 (n = 2)
4	1.2^{b} ± 0.5 (n = 9)	1.8^{b} ± 0.9 (n = 7)	3.4 ± 1.9 (n = 2)
Average	2.1 ± 1.8	2.9 ± 1.9	3.0 ± 1.5

Data are presented as mean ± standard deviation (SD).
Different letters in columns represent values that significantly differ (Fisher's Least Significant Difference — LSD test, $p < 0.05$).
* At processing day.

Table 2
Average thermotolerant coliforms counts in samples of hindquarters, deboning room processing surfaces and vacuum-packed meat cuts.

Collection	Thermotolerant coliforms		
	Hinquarters surfaces (log PMN·cm^{-2})	Processing surfaces (log PMN·cm^{-2})	Meat cuts* (log PMN·g^{-1})
1	−0.2 ± 0.7 (n = 6)	0.5 ± 0.4 (n = 7)	2.2 ± 0.7 (n = 2)
2	−0.5 ± 0.4 (n = 10)	0.9 ± 0.5 (n = 7)	0.5 ± 0.0 (n = 2)
3	−0.7 ± 0.2n (n = 10)	0.3 ± 0.0 (n = 7)	0.5 ± 0.0 (n = 2)
4	−0.5 ± 0.4 (n = 9)	0.5 ± 0.2 (n = 7)	0.5 ± 0.0 (n = 2)
Average	−0.5 ± 0.4	0.5 ± 0.3	0.9 ± 0.7

Data are presented as mean ± standard deviation (SD).
Different letters in columns represent values that significantly differ (Fisher's Least Significant Difference — LSD test, $p < 0.05$).
* At processing day.

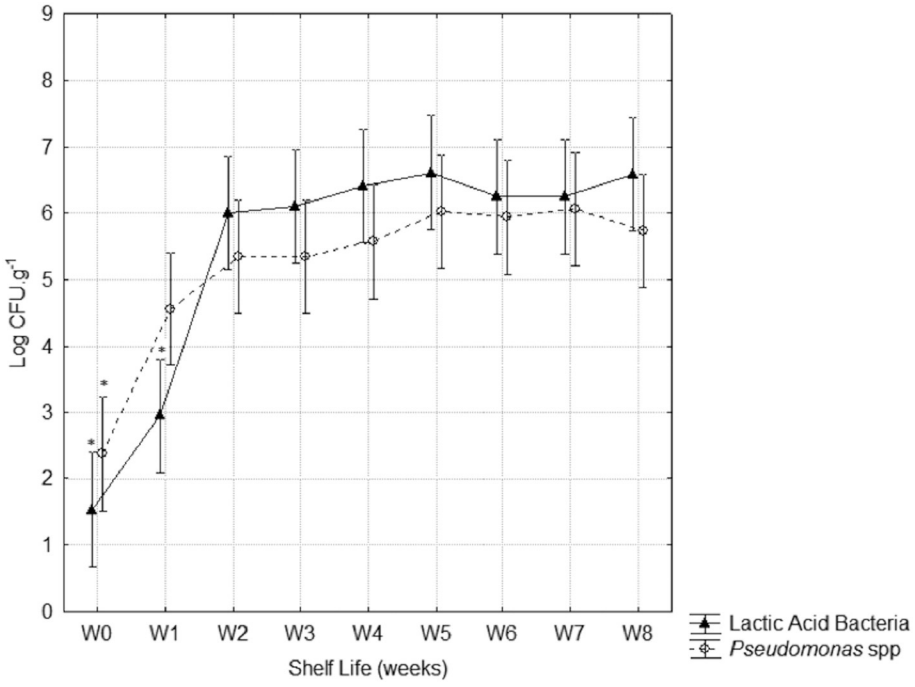

Fig. 1. Average counts (n = 4) of *Pseudomonas* spp. and lactic acid bacteria on vacuum-packed buffalo meat cuts during shelf life, stored at 5 °C. *Significant difference compared with other points in graph (Fisher's Least Significant Difference — LSD test, $p < 0.05$).

during the storage of vacuum-packed beef cuts for 16 weeks at −1.5 °C, where the average score on the day of processing and at the end of the storage period were, respectively, 3.3 log CFU·g^{-1} and 8.4 log CFU·g^{-1}. This tendency for LAB counts to increase during the storage of vacuum packed meat was also observed by Lavieri and Williams (2014), where the mean count in BAL samples of ground beef increased from 2 log CFU·g^{-1} on the processing day to 5.6 log CFU·g^{-1} after 25 days of refrigerated storage. The results obtained in the present study are also similar to those described by Lorenzo and Gómez (2012), who found differences in *Pseudomonas* spp. and LAB counts in vacuum-packed fresh foal meat during 2 weeks' storage at 4 °C, where the average counts of these microorganisms on day zero was 4.2 log CFU·g^{-1} and 3.3 log CFU·g^{-1}, while at the end of storage they were 4.4 log CFU·g^{-1} and 6.9 log CFU·g^{-1}, respectively.

Van Impe, Poschet, Geeraerd, and Vereecken (2005) suggested a process of self-limiting multiplication of microbial species interactions, assuming that deceleration in simultaneous populations occurs due to the exhaustion of essential nutrients, the accumulation of growth-detrimental metabolic products and/or decrease in pH due to acid production. This model could explain the maintenance of *Pseudomonas* spp. and LAB counts in meat cuts from the first and second week of refrigerated storage, respectively, until the end of shelf life.

Thermotolerant coliform counts were similar between collections, as well as during shelf life, i.e., eight weeks. Average scores were 0.9 log MPN·g^{-1} at the time of processing and 1 log MPN·g^{-1} at the end of shelf life. According to Resolution No. 12 of the Brazilian National Health Surveillance Agency (ANVISA) (Brasil, 2001), thermotolerant coliform scores in unmatured vacuum-packed meat are acceptable up to 4 log MPN·g^{-1}. In this paper, all samples showed levels below those allowed by Brazilian law. Counts at processing day reflect the sanitary conditions during product development. The pre-operating and the operating procedures, if improperly conducted, even associated with the ambient temperature required by Brazilian law (up to 16 °C) (Brasil, 2000), provide the maintenance and multiplication of pathogenic and spoilage microorganisms in the processing environment by exerting direct influence on the microbiological quality of the final product.

In our experiment, during the refrigerated storage, a loss of vacuum in the packs was also noted, with subsequent gas accumulation. In addition, the meat cuts presented sensory changes, such as greenish color, fetid odor, exudates and loose consistency, probably due to proteolytic activity (Rossi Júnior et al., 2011). As buffalo meat presents a pH increase during the storage period (Vishnuraj, Kandeepan, & Shukla, 2014) and the microorganisms responsible for fetid odors develop better in meats with pH above 6 (Neath et al., 2007; Vishnuraj et al., 2014), this specific characteristic of buffalo meat could be the factor responsible for the changes.

In most foods, the deterioration process begins when the counts of microorganisms reach levels of 6 log CFU·cm^{-2} (Dainty & Mackey, 1992), and these changes can be attributed to *Pseudomonas* spp. and LAB metabolism. Deterioration occurred as microorganism counts remained constant, with an average 5.9 log CFU·g^{-1} and 7.0 log CFU·g^{-1}, respectively, and these bacteria are responsible for the production of extraneous flavors and odors, exudative meat with greenish tinge, and slime in the pack (Cayré, Vignolo, & Garro, 2003; Nychas, Dillon, & Board, 1988; Zhang, Kong, Xiong, & Sun, 2009). In addition, some hetero-fermentative LAB are capable of producing gas inside a vacuum pack when stored at refrigerated temperatures (Hanna, Smith, Hall, & Vanderzant, 1979). Therefore, as observed in other meats, the bacterial populations found in the buffalo meat cuts may have contributed to the changes seen in the pack.

Other authors mention that the interaction between LAB and enterobacteria enhances the degree of product deterioration (Borch et al., 1996). Indeed, this may also be associated with the changes observed in the present study because thermotolerant coliform counts, even in low amounts, were present in buffalo meat cuts during the entire period of refrigerated storage. Another point to be considered is that *Listeria* spp. and LAB are among the best adapted microorganisms to multiply at low temperatures (Cornu, Billoir, Bergis, Beaufort, & Zuliani, 2011), and both populations may be in competition in refrigerated food with long shelf life, such as vacuum-packed meat cuts.

Among the 35 hindquarter samples evaluated previous to deboning, *Listeria grayi* was isolated in only two of them (5.7%). Dimic et al. (2010) analyzed *Listeria* spp. in 29 samples of refrigerated meat (chicken, pork and beef) and verified the presence of the bacterial genus in 82.7% of samples. It was confirmed that *Listeria welshimeri* was the species with the highest incidence (31%), followed by *Listeria monocytogenes*

(27.6%) and *Listeria innocua* (24.1%). In the samples from meat processing surfaces, *Listeria* spp. were not found, differing from the study presented by Barros et al. (2007), which found the presence of this bacterial genus in samples of equipment (76/148; 51.4%) and facilities (23/65; 35.4%) of meat processing plants and slaughterhouses. Renier, Hébraud, and Desvaux (2011) stated that it is common to find *Listeria* spp. on food processing surfaces, as well as *Pseudomonas* spp., because these bacteria produce biofilms, which allow for their persistence within industries and subsequent cross-contamination of the final products.

L. monocytogenes was also not found in meat cut samples, but *L. innocua* and *L. grayi* were present in different samples during the storage period, not being isolated on the processing day. Specifically, during the shelf life, *L. grayi* was detected after four weeks in the first collection, four and five weeks in the second collection, and after five and eight weeks in the third collection. *L. innocua* was only detected after seven weeks in the third collection. Our results suggest that in the processing day, as the number of this microorganism was too low, it was undetected by the phenotypic method used. The storage conditions allowed bacterial proliferation, permitting then the detection. Working with vacuum-packed beef cuts under refrigeration, Nel et al. (2004) identified the presence of *L. monocytogenes* in 52% (26/50). In a study that examined *L. monocytogenes* in cold smoked salmon, the growth of a minority population (*Listeria* spp.) was partially inhibited after the LAB population reached the stationary phase, suggesting that only the pathogen metabolism was affected by the limiting factor and/or inhibited by the residual factor product that led the LAB to stop growing (FAO-WHO, 2004). This behavior may explain the absence of *L. monocytogenes* in buffalo meat cuts during refrigerated storage and the presence of other bacterial genera, while the counts of LAB remained constant. Devlieghere et al. (2001) also affirmed that the multiplication of the minority population tends to slow when the growth of the main population reaches its maximum.

McLauchlin (1997) stated that the presence of any kind of *Listeria* in food can be an indicator of poor hygiene and can be interpreted as indicating suitable conditions for the presence of the major pathogen of the genus *L. monocytogenes* (Vitas, Aguado, & García-Jalón, 2004). Therefore, a vital goal within industries is to inhibit this microorganism genus, maintaining controlled conditions that prevent its multiplication in food. As observed, this statement should also be true for buffalo meat processing.

Some authors support the claim that the *Pseudomonas* spp. metabolism, through its ability to hydrolyze proteins, can release stimulating factors for the growth of *Listeria* spp. (Borezee, Pellegrini, & Berche, 2000; Marshall, Andrews, Well, & Farr, 1992; Verheul, Rombouts, & Abee, 1998). Such a proposition justifies the isolation of this bacterial genus throughout the storage period of buffalo meat when the counts of *Pseudomonas* spp. increase and then stabilize. Lebert, Robles-Olvera, and Lebert (2000), evaluating the *Pseudomonas* spp. and *Listeria* spp. interactions in meat products, noted that no growth of *Listeria* was observed until *Pseudomonas* reached the stationary phase of growth, and this characteristic was also observed in buffalo meat.

4. Conclusion

Cutting and deboning operations performed during buffalo meat production influence the microbiological quality and shelf life of the vacuum-packed and refrigerated product. This finding highlights the importance of a rigid and systematic hygienic-sanitary control during these operations.

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