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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·cm^{-2} or log CFU·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·cm^{-2} or log MPN·g^{-1} (FDA, 2002).