Effect of chitosan coating on contamination of fresh bovine meat sold in the open market

Efeito de revestimento com quitosana na contaminação de carnes bovina fresca comercializadas em feira livre

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ABSTRACT - This study aimed to evaluate the sanitary status of fresh bovine meat sold at the open market in Jiquiriça municipality, Brazil and validate the use of chitosan as an antimicrobial coating to control the bioburden. In total, we evaluated 30 samples of fresh beef for the presence of coagulase-positive staphylococci and Salmonella spp. Furthermore, stores selling fresh meat products were evaluated for compliance with sanitary regulations set by the Ministry of Health in Brazil. To evaluate the antimicrobial activity of chitosan two sets of meat samples were tested, the test group consisted of contaminated meat coated with 2% chitosan solution whereas the control group consisted of untreated contaminated meat. It was found that fresh bovine meat at market stores is exposed to a number of contaminants, including several pathogens. All of the tested samples contained coagulase-positive staphylococci (3.8-6.1 log CFU g⁻¹) and Salmonella spp. were detected in 30% of all samples. When assessing the sanitary conditions of the meat stores, non-compliance was highest in the marketing and display category (79%), followed by the hygiene of manipulators (73%) and the facilities, equipment and utensils categories (54%). Meat treated with 2% chitosan solution showed significant lower (p<0.01) microbial load of Salmonella enterica and Staphylococcus aureus than the control samples. These findings suggest that applying a 2% chitosan coating on fresh meat is a feasible method for controlling pathogenic microbial load.

Key words: Salmonella. Staphylococcus. Internal meat temperature. Antimicrobial activity.

RESUMO - Este estudo teve como objetivo avaliar as condições higiênicas sanitárias da carne bovina in natura comercializada na feira livre do município de Jiquiriça, Brasil e validar o uso de quitosana como revestimento no controle da carga microbiana. No total, foram avaliados 30 amostras de carne in natura quanto à presença de estafilococos coagulase positiva e Salmonella spp. Além disso, os boxes que comercializavam carne fresca foram avaliados quanto ao cumprimento dos regulamentos sanitários estabelecidos pelo Ministério da Saúde. A atividade antimicrobiana da quitosana foi testada usando dois conjuntos de amostras de carnes, o grupo teste que consistiu da carne contaminada intencionalmente e revestida com solução de quitosana a 2% e o grupo controle que consistiu da carne contaminada não tratada. Verificou-se que a carne in natura comercializada no mercado está exposta a vários contaminantes, incluindo vários patógenos. Todas as amostras testadas continham estafilococos coagulase positivo (3,8-6,1 log UFC g⁻¹) e Salmonella spp. em 30% das amostras. Na avaliação das condições sanitárias das carnes, o não conformidade foi maior na categoria comercialização e exposição (79%), seguida da higiene dos manipuladores (73%) e das categorias instalações, equipamentos e utensílios (54%). A carne tratada com solução de quitosana a 2% apresentou carga microbiana significativamente menor (p<0,01) de Salmonella enterica e Staphylococcus aureus do que as amostras controle. Esses achados sugerem que a aplicação de um revestimento de quitosana a 2% na carne in natura é um método viável para controlar a carga microbiana patogênica.

INTRODUCTION

Beef contains proteins of high nutritional value, fats, fat soluble vitamins A, D, E, and K as well vitamins of the B complex, water and minerals (mainly iron, potassium, sodium, magnesium and zinc) (OLIVEIRA; SILVA; CORREIA, 2013). Although the levels these nutrients may vary according to breed age, sex, feeding, and meat cutting (PARDI; LEWIS; WHITTLAKER, 2005). Nutrients found in beef have important roles in human health, including the formation of new organic tissues, energy production and regulation of physiological processes, amongst others (OLIVEIRA; SILVA; CORREIA, 2013).

The abundance of nutrients in beef favors the growth of bacteria (WELKER et al., 2010). The microorganisms found in the meat are present on the animal itself or may contaminate it during the slaughter and marketing process (LUNDGREN et al., 2009). Meat products are among the foods most commonly involved in food outbreaks (SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO, 2014).

Food-borne illnesses are caused by biological or chemical agents, which enter the human body through ingestion of contaminated food or water. Biological contamination is considered the main danger to public health, with bacterial infection being the main cause of food poisoning (GERMANO; GERMANO, 2011).

The Salmonella genus represents the primary cause of food poisoning in several countries during the last 100 years (LEE et al., 2015). In Brazil, in the last decade this bacterium was responsible for 38% of reported food-borne illnesses (SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO, 2014). S. aureus has been the second most common etiological agent in bacterial food poisoning largely due to its ability to produce thermostable enterotoxins (SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO, 2014). During slaughter and handling bacteria present in the hands, nose, and mouth of the manipulators as well as on the skin of the animals can contaminate the meat products.

The sanitary quality in the handling of meat is cause for concern in all stages of production, especially in commercial establishments, due to structural faults which is common in fairgrounds. Fresh beef marketed at fairs presents a high risk of microbial contamination (LUNDGREN et al., 2009), leading to a decrease in the shelf life of the product and increasing the risk of foodborne diseases (BARROS; VIOLANTE, 2014).

Chitosan is a polysaccharide obtained from the partial deacetylation of chitin. Chitin is a non-toxic and biodegradable biopolymer, widely distributed in nature, found mainly in exoskeletons of crustaceans and in the cell walls of some fungi (KUNIYOSHI, 2012).

In recent years, the use of chitosan as a biocointainer in foods has received increased attention due to reports of its antimicrobial activity in vitro (RAMÍREZ et al., 2011). Chitosan has been used as an edible coating of minimally processed products (SOARES et al., 2011), and fish fillets (FERNÁNDEZ-SAIZ et al., 2013), as well as in the post-harvest treatment of fruits (CHONG; LAI; YANG, 2014).

Bearing in mind that beef is the fifth most common food type involved in food poisoning in Brazil, being responsible for 365 cases of 9,942 outbreaks reported in the period 2000-2014 with leading pathogens being Salmonella spp. and S. aureus (SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO, 2014), the present study aimed to evaluate sanitary conditions of meat sold at a free market and verify the antimicrobial activity of the chitosan coating on meat.

MATERIAL AND METHODS

Samples of fresh beef were obtained between April to December 2014 from 53% of the stores (n = 19) that sold fresh meat at the free market in Jiquiriçá, Brazil. The selection of stores was systematic and completely randomized in order to construct the real focus of microbial contamination. This study is of the cross-sectional type of quantitative and observational character.

The analysis of the sanitary aspects of the meat trading (facilities, equipment and utensils, hygiene of the manipulators, commercialization and exhibition of the product for sale) was carried out by means of visual observation and application of checklist adapted from the Resolução da Diretoria Colegiada, RDC/275 (BRASIL, 2002) e RDC/216 (BRASIL, 2004).

Three meat samples were collected on Saturdays during the morning shift, and 10 samples of 300 g were collected in each collection, constituting a total of 30 meat samples from different cuts for sale. After checking the temperature, the samples were placed in plastic bags of first use, labelled and transported in an isothermal box to the Laboratório de Microbiologia de Alimentos e Ambiental - LABMAA, da Universidade Federal do Recôncavo da Bahia - UFRB.

Coagulase-positive staphylococci were counted by surface inoculation on Baird-Park agar and the typical colonies submitted to catalase and coagulase tests (SILVA et al., 2010). For the Salmonella tests, we used the methodology proposed in Silva et al. (2010). The samples went through the pre-enrichment stage in lactose
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broth, selective enrichment (tetrathionate and rappaport broths), and streaked onto selective plates (xylose-lysine deoxycholate agar and Salmonella Shigella agar). Strains with presumptive identification of Salmonella were submitted to biochemical tests (TSI, LIA, urease, indole, citrate and malonate) and serological testing using polyvalent somatic serum (antigens O e Vi) of Probac®.

To test for the antimicrobial effect of chitosan, beef cuts were subdivided into 10 parts to test each bacterium (S. enterica and S. aureus), weighing on average 100 g. High molecular weight chitosan and 90% deacetylation grade was obtained commercially from Polymar Indústria e Comércio LTDA. Meat cuts were intentionally contaminated with bacterial suspension of 6 log CFU mL$^{-1}$ of each bacterium to be tested. After 30 minutes, 5 units were submitted to treatment with chitosan coating solution which consisted of immersing the cuts of meat in 500 mL of 2% chitosan solution for 10 seconds. The other units, that formed part of the control group were immersed in sterile distilled water also for 10 seconds. All samples were kept refrigerated at 4 °C for 72 hours. After this period, microbiological analysis of the test group and control group were performed to establish the microbial load of the pathogens (SILVA ET AL., 2010).

The results were analyzed using Analysis of Variance (ANOVA) according to the completely randomized design, in duplicate and three replicates. The mean of the microorganism counts and internal temperature of the meat in natura were analyzed using Tukey’s test. SISVAR version 4.0 was the statistical software used to compare mean values and the minimum level of significance considered was 5%.

RESULTS AND DISCUSSION

All of the evaluated stores showed a high percentage of non-compliance (54-79%), with no statistically significant difference between the stores ($p>0.05$). The marketing and display category presented the highest percentage of non-compliance, followed by the hygiene of the manipulators category and equipment and utensils (Figure 1). The high proportion of stores complying with the regulations in the facilities equipment and utensils category was due to the presence of sinks for hand washing, refrigerated counters and worktops with smooth surfaces that were waterproof and easy to sanitize (BRASIL, 2002).

The precarious conditions in which the meat is marketed at the Jiquiriçá market exposes the food to pathogenic microorganisms as well as environmental contamination and pollution (LUNDGREN ET AL., 2009). Even though stores were equipped with refrigerated counters marketing and display of the meat was carried out on the counters at room temperature, in order to facilitate the direct contact of the customer with their cut of choice. Another source of contamination was the simultaneous handling of cash and meat cuts by the traders without hand washing between operations. When hands were washed it was with a cotton cloth that was also used for cleaning surfaces and equipment, resulting in cross-contamination.

With regards to the hygiene of the manipulators, none of them used light-colored uniforms suited for the activity. They did not appear to have clean hands and clean and short nails with 60% wearing personal ornaments (i.e. rings, watches, bracelets etc.) and 20%

Figure 1 - Compliance with hygienic-sanitary regulations of fresh meat stores at the market in Jiquiriçá, Brazil, during April to December, 2014

<table>
<thead>
<tr>
<th>Category</th>
<th>Adequacy</th>
<th>Inadequacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marketing and display of meat</td>
<td>78.9%</td>
<td>21.1%</td>
</tr>
<tr>
<td>Hygiene of the manipulators</td>
<td>73.0%</td>
<td>27.0%</td>
</tr>
<tr>
<td>Facilities, equipment and utensils</td>
<td>54.0%</td>
<td>46.0%</td>
</tr>
</tbody>
</table>
had hand injuries. In 90% of the stores, there were long conversations between the traders and customers. According to the RDC N° 216 (BRASIL, 2004), such transgressions should be observed by the consumer at places of business where products of animal origin are marketed, especially since meat handlers have been shown to be major contributors to food contamination. Miranda e Evangelista-Barreto (2012) reported a similar situation in different establishments (supermarkets, markets, butchers and minimarkets) of Cruz das Almas, Bahia, reporting that 91.6% of the handlers had hand adornments and only 41.6% had their hands sanitized. Moreover, Lundgren et al. (2009) observed non-compliance with the legislation when studying 67 points of sale of beef in open markets and public markets in the municipality of Joao Pessoa, Brazil.

The Decree-Law N° 207 (BRASIL, 2008) states that meat and meat derived products must be protected from direct exposure to sunlight, dust or any other external contaminant, and contact with the public. In addition, meat and associated products need to be refrigerated at temperatures between -2 and 7 °C or frozen at below -12 °C, with a maximum tolerance of 3 °C.

The internal temperature of the samples tested (Table 1) was suitable for proliferation, of most microbes, ranging from 5° - 60 °C (BRASIL, 2004). Report of abuse of the time/temperature binomial by Barros and Violante (2014) at free trade fairs in the Bahia Recôncavo, demonstrates the lack of intervention of by inspection agencies to allow the marketing of perishable products at room temperature.

The microbial analyses reflected unhygienic conditions at the meat market. Elevated coagulase-positive *Staphylococcus* counts (>3.8 log CFU g⁻¹) were observed in all stores, with no statistically significant difference by ANOVA F test (p>0.05) (Table 2). Although Brazilian legislation does not establish limits for the counts of coagulase-positive staphylococci in fresh meat, the determination of maximum limits for the counting of this bacterium is necessary, since the bovine meat is often consumed without undergoing a heat treatment capable of inactivating the thermostable toxins produced by some strains of this group.

Staphylococcal toxins promote increased intestinal peristalsis, causing inflammation throughout the gastrointestinal tract, with the most severe lesions in humans forming in the stomach and upper part of the small intestine of humans (GERMANO; GERMANO, 2011). Considering that these toxins are produced between 10° and 45 °C (BRASIL, 2004), the temperatures at which the meat is marketed at the Jiquiriçá market favors the proliferation of the bacteria. In Brazil, 20% of the total food outbreaks reported from 2000 to 2014 were caused by *S. aureus* (SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO, 2014).

In meat products, a microbial load of 10⁷ CFU cm⁻² is sufficient for the smell of putrefaction to be detected, whereas counts of 10⁸ CFU cm⁻² are sufficient for the formation of mucus, mainly caused by colonies of *Pseudomonas*, *Bacillus* and *Streptococcus*. Meat changes due to oxidation of oxymyoglobin (red) to methamoglobin (brown) (VASILE et al., 2014).

*Salmonella* spp., which makes the meat unfit for commercial use (BRASIL, 2001), were detected in 50% of the stores (Table 2). One of the problems preventing the improvement of hygienic-sanitary control of products of animal origin marketed in open marks in Brazil is the resistance on the market traders in adjusting their hygienic practices. These hygienic habits and practices are passed down and transmitted by generations of traders. It would be difficult to change these practices by the determination of normatives, the provision of occasional courses or the carrying out of coercive actions of supervision and control. These actions would have little influence in changing the hygiene habits of the traders because their perception of contamination is associated with the change in the visible characteristics of the product and not the presence of a biological or chemical contaminant. In this sense, the market is a space of beliefs that needs

<table>
<thead>
<tr>
<th>Collections</th>
<th>Internal meat temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
</tr>
<tr>
<td>1 collection</td>
<td>25</td>
</tr>
<tr>
<td>2 collection</td>
<td>26</td>
</tr>
<tr>
<td>3 collection</td>
<td>27</td>
</tr>
</tbody>
</table>

S = stores tested in this study.
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Table 2 - Mean count of coagulase-positive staphylococci and presence of Salmonella spp. in fresh beef traded at the free market in Jiquiriçá, Brazil, during the period from April to December 2014

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS (Log CFU g⁻¹)</td>
<td>5.8±0.07</td>
<td>5.6±0.12</td>
<td>5.6±0.06</td>
<td>5.6±0.04</td>
<td>4.7±0.03</td>
<td>6.1±0.02</td>
<td>5.8±0.07</td>
<td>4.9±0.03</td>
<td>5.3±0.07</td>
<td>5.4±0.03</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- = Absence of Salmonella spp. + = Presence of Salmonella spp. CPS = coagulase-positive staphylococci S = stores tested

Conclusions

We found that fresh beef marketed at the Jiquiriçá free market is exposed to several sources of contamination, being handled and marketed under unhealthy conditions with the presence of pathogens such as Salmonella spp. The standardization of acceptable limits for the counts of coagulase-positive staphylococci in meat bovine in natura is necessary in order to improve the safety of this food. The 2% chitosan coating was effective in reducing microbial counts of pathogens such as S. enterica and S. aureus, improving food safety.

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REFERENCES
