

## Effect of chitosan coating on contamination of fresh bovine meat sold in the open market<sup>1</sup>

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<sup>-1</sup>) 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<sup>-1</sup>) e *Salmonella* spp. em 30% das amostras. Na avaliação das condições sanitárias das carnes, a 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.

**Palavras-chave:** *Salmonella*. *Staphylococcus*. Temperatura interna da carne. Atividade antimicrobiana.

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## 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; WHITTAKER, 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 biocontainer 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

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<sup>-1</sup> 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%.

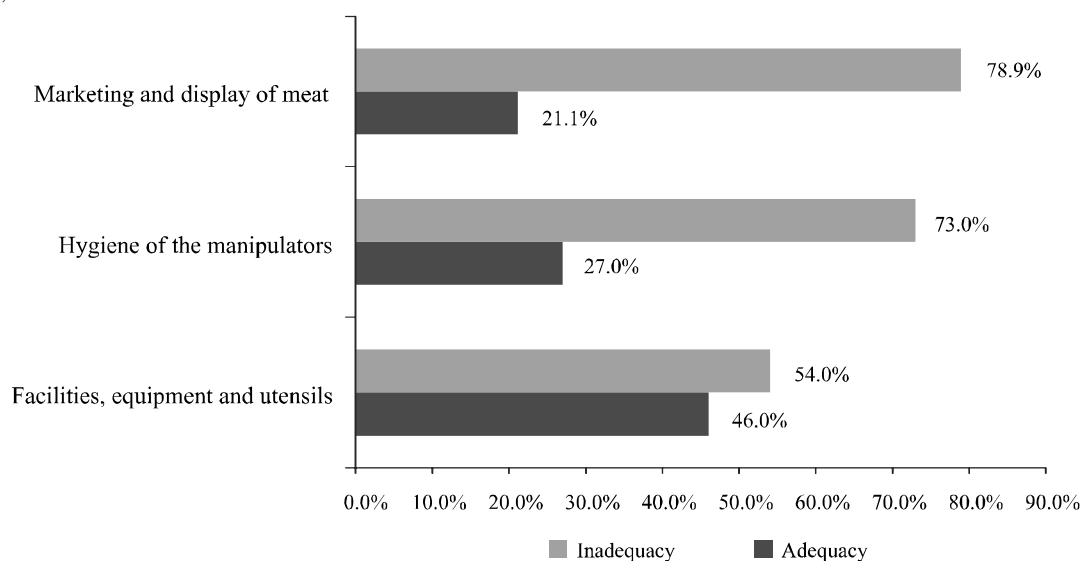
## RESULTS AND DISCUSSION

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



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 ( $>3.8 \log \text{CFU g}^{-1}$ ) 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  $10^7 \text{CFU cm}^{-2}$  is sufficient for the smell of putrefaction to be detected, whereas counts of  $10^8 \text{CFU cm}^{-2}$  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 1** - Temperature of fresh beef sold at Jiquiriçá, Brazil, during the period from April to December 2014

Collections	Internal meat temperature (°C)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
1 collection	25	27	24	25	26	25	25	27	28	26
2 collection	26	25	28	27	26	27	28	24	25	27
3 collection	27	24	25	28	27	24	26	25	27	28

S = stores tested in this study

**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

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

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

to be understood so that viable health interventions can be implemented.

For many sellers, the cleanliness of their place is used as a marketing strategy and not as a health aspect. They believe that cleanliness must be maintained only to attract customers, disregarding the fact that lack of hygiene contaminates food and causes disease (MINNAERT; FREITAS, 2010). These beliefs and practices contribute to spread of contamination through meat, thereby placing a burden on public health instead of bringing health benefits to the consumer (OLIVEIRA; SILVA; CORREIA, 2013).

In this sense, the performance of health services and health surveillance is extremely important. It is essential to include educational projects in the food sector, since most outbreaks occur due to the lack or little knowledge of the manipulators regarding the consequences of improper handling (OLIVEIRA *et al.*, 2008). Current sanitary laws are not effective as most of the time, the municipal fiscals adopt only coercive and punitive measures, to the detriment of a dialogue with the traders (ALMEIDA; PENA, 2011).

The meat treated with 2% chitosan presented reduction of 6 log cycles of the microbial load of *S. aureus* and *Salmonella* spp. when compared to the control (Table 3). The use of the chitosan coating in the animal food industry is promising as it significantly reduces ( $p < 0.01$ ) pathogenic bacteria, such as *S. enterica* and *S. aureus*, involved in food poison outbreaks.

Chitosan has been used in the control of dyslipidemia (BESSA-JUNIOR; GONÇALVES, 2013)

**Table 3** - Mean reduction of microbial load of beef coated with 2% chitosan solution for 72 hours at 4 °C

Treatments	Microorganisms (Log CFU g <sup>-1</sup> )	
	<i>S. enterica</i>	<i>S. aureus</i>
With chitosan	6.39±0.01 b	6.69±0.02 b
Without chitosan	0.10±0.00 a	0.06±0.00 a

\* Averages followed by the same letter do not differ by the Tukey test at 5% probability; \* Means followed by different letters present statistical difference by the F test at 1% probability

and may have additional health benefits considering that beef is a source of cholesterol (SANTOS *et al.*, 2013). Besides controlling microbial growth in fresh meat products coating meats with this polysaccharide could inhibit the absorption of cholesterol by the human organism.

Another benefit of using chitosan as a coating, besides not altering the organoleptic characteristics of the product, is that it maintains the bright red natural coloration of the meat (OLIVEIRA; SILVA; CORREIA, 2013) as a result of the chelating action of chitosan (PARK *et al.*, 2010). Through the use of chitosan, chemical preservatives such as nitrite, a red color enhancer in the meat, can be minimized.

## CONCLUSION

We found that beef fresh meat 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 reduction microbial counts of pathogens such as, *S. enterica* and *S. aureus*, improving food safety.

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