



Original Article

## Microbiological characterization of vacuum-packed and conventionally packed sliced turkey hams marketed in Mossoró, Brazil

Caracterização microbiológica de presuntos de peru fatiados embalados a vácuo e em embalagem convencional comercializados em Mossoró, Brasil

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### ARTICLE INFO

#### Article history

Received 19 September 2018

Received in revised form 11 December 2018

Accepted 17 December 2018

#### Keywords:

*Meleagris gallopavo*

Quality

Processed Meat

Public Health

Microorganisms

### ABSTRACT

The consumption of turkey ham has been increasing considerably. This product can be marketed sliced, and thus, it is subjected to intense manipulation and presents a high microbial load, which compromises its quality and safety. The objective of this study was to characterize microbiologically vacuum-packed and conventionally packed sliced turkey hams marketed in Mossoró, state of Rio Grande do Norte, Brazil. The analyses consisted of mold and yeast, viable aerobic mesophilic microorganism, and coagulase positive *Staphylococcus* counts, determination of the most probable number of total coliforms and thermotolerant coliforms, and presence of *Salmonella* spp. Turkey hams present high microbiological counts regardless of their packaging, but conformity for coliforms at 45 °C. Some samples had low quality, denoting that a greater care in the manipulation and conservation of this product is necessary to ensure the safety and absence of risks of this product to public health.

### RESUMO

O consumo de presunto de peru tem aumentado consideravelmente. Esse produto pode ser comercializado fatiado e, portanto, é submetido à intensa manipulação e apresenta alta carga microbiana, o que compromete sua qualidade e segurança. O objetivo desse trabalho foi caracterizar microbiologicamente presuntos de peru embalados a vácuo e em embalagens convencionais comercializados em Mossoró, Rio Grande do Norte, Brasil. As análises consistiram em contagem de bolores e leveduras, microrganismos aeróbios mesófilos viáveis e *Staphylococcus* coagulase positiva, determinação do número mais provável de coliformes totais e coliformes termotolerantes e presença de *Salmonella* spp. Presuntos de peru apresentaram altas contagens microbiológicas, independentemente de suas embalagens, mas conformidade quanto aos coliformes a 45 °C. Algumas amostras apresentaram baixa qualidade, denotando que um maior cuidado na manipulação e conservação deste produto é necessário para garantir a segurança e ausência de riscos deste produto para a saúde pública.

#### Palavras-chave:

*Meleagris gallopavo*

Qualidade

Carne Processada

Saúde Pública

Micro-organismos

### INTRODUCTION

Food safety is an important issue (BASTOS, 2008), since food contamination by pathogens is a public health problem. Several microorganisms are causative agent of toxification in developed countries, such as *Salmonella*

spp.; and result in high economic losses and considerable mortality of contaminated consumers, such as *Escherichia coli*. Thus, identifying these microorganisms is important to evaluate the quality of food and prevent food outbreaks (ZHANG et al., 2017). In this context, meat products require especial care because of their

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potential to carry foodborne pathogens, especially when undergo intense handling (SHAWISH; TARABEES, 2017).

In Brazil, ham is defined as an industrialized meat product obtained from cuts of the hind leg of pig or other animal, which is treated with a thermal process; these products, boned or not, should be called ham followed by the name of the animal used (BRASIL, 2000). Ham can be made from turkey meat (MATHIAS et al., 2010), which has been increasingly found in markets, mainly due to its nutritional characteristics and low fat content, which increases the demand for this product (MATHIAS, 2008).

Ham is a microbiologically stable food, because by the cooking process it is possible to eliminate pathogens like *Salmonella*. However, failures in its production, such as incorrect equipment cleaning, inadequate handling, and inefficient storage, can compromise its quality (FAI et al., 2011; MACEDO et al., 2014).

Sliced ham has become common in markets, mainly because it is convenient to consumers and markets. However, most markets have no rigid inspection control that ensures food safety due to the large quantities of product handled by their slice sectors (WANDERLEY et al., 2016). Thus, some researchers have evaluated the quality of sliced hams packaged in different packages (PEDROSO et al., 2016).

Therefore, subjective and objective methods of control, based on existing health legislations, should be developed to ensure food safety of these products (MARINS et al., 2014); for example, the Resolution RDC No. 12 of January 2, 2001 describes the technical regulation on microbiological standards for food (BRASIL, 2001).

In this context, considering the low number of studies on this subject, the objective of this work was to characterize microbiologically vacuum-packed and conventionally packed sliced turkey hams marketed in Mossoró, state of Rio Grande do Norte, Brazil.

## MATERIAL AND METHODS

The research consisted in qualitative and quantitative evaluations of microorganisms on sliced turkey hams marketed in commercial establishments in Mossoró, State of Rio Grande do Norte, Brazil. Sampling was carried out between August and September 2017, according to the conditions of purchase of the product by the consumer in markets that: market industrialized turkey hams of any brand; market pre-sliced and vacuum-packaged hams (in the commercial establishment) that are taken directly by the consumer from refrigerated displays for purchase; and/or market pre-sliced conventionally packaged hams at the establishment's counter. Ten vacuum-packaged and ten conventionally packaged turkey ham samples were collected for evaluation.

The samples were placed in an ice-cold container and taken to the Laboratory of Food Biotechnology (LABA) of the Federal Rural University of the Semi-Arid Region (UFERSA). Collection and transport procedures followed hygiene practices to minimize external interference (BAPTISTA, 2006).

Microbiological analyses consisted of counting molds and yeasts and viable aerobic mesophilic microorganisms, quantifying coagulase positive *Staphylococcus*, and determining the most probable number (MPN) of total coliforms and thermotolerant coliforms, with observation of growth of typical colonies of *Escherichia coli*, and the presence of *Salmonella* spp. These analyses were performed aseptically, following the methodologies of the American Public Health Association (APHA) (1998), Brasil (2003), and Silva et al. (2007).

Twenty-five grams of the ham samples were diluted ( $10^{-1}$  to  $10^{-7}$ ) in buffered peptone water. Molds and yeasts were counted in 1 mL of each dilution in the surface of Petri dishes containing potato dextrose agar acidified with 10% tartaric acid, in duplicates. The samples were spread in the medium, and the plates were incubated in a biochemical oxygen demand oven at  $25 \pm 1$  °C for five days. This technique was used based on the selective growth of fungi in medium with pH 3.5, using tartaric acid, combined with the incubation temperature.

Standard agar for counting was used as medium for the quantification of viable aerobic mesophilic microorganisms, with spreading performed with a Drigalski loop until complete absorption in the liquid. The plates were incubated inverted in a bacteriological oven at  $35 \pm 1$  °C for  $48 \pm 2$  hours.

Coagulase positive *Staphylococcus* was counted on 1 mL of each dilution in Petri dishes containing Baird-Parker agar and a solution with egg yolk and telluride. This method is based on the microorganism capacity of growing in this medium. The Petri dishes were dried, inverted, and incubated in a bacteriological oven at  $36 \pm 1$  °C for 48 hours. Counting and selection of typical colonies (black colonies) were then performed to identify coagulase positive *Staphylococcus* by the coagulase test. This biochemical test is based on the ability of rabbit plasma to coagulate through the enzyme coagulase produced by the microorganism.

Total coliform growth was evaluated with the multiple tube technique, determining the most probable number. A presumptive test was performed using a 1 mL aliquot of each sample with dilutions of up to  $10^{-3}$  in test tubes with sodium lauryl sulfate broth, followed by culture in a 2% brilliant green bile lactose broth. The total coliforms were then grown in a water bath at  $36 \pm 1$  °C. Positive tubes for total coliforms were subsampled in *Escherichia coli* broth at  $45 \pm 1$  °C in a water bath. Positive results for coliforms were identified by gas formation, confirming the fermentation of the lactose in the medium, and expressed in MPN g<sup>-1</sup>.

Positive samples for coliforms at 45 °C were subsampled in Petri dishes with methylene blue eosin agar. Then, the plates were incubated in a bacteriological oven at 35 °C for 24 hours. Presence of *E. coli* was determined by the growth of colonies of intense metallic green aspect and confirmed by biochemical tests, consisting of citrate, indole, and methyl red tests (SILVA et al., 2007).

*Salmonella* spp. were evaluated in 25 g of each sample diluted ( $10^{-1}$ ) in buffered peptone water. This solution was incubated at 36 °C for 24 hours; then, aliquots of 1 mL were transferred to selective enrichment broths (selenite cystine, tetrathionate, and Rappaport-Vassiliadis) and incubated at 41 °C for 24 hours. Subsequently, they were streaked onto Petri dishes with salmonella-shigella agar and methylene blue eosin agar, and incubated for 24 hours at 36 °C. The typical colonies found on the plates were subjected to biochemical tests

to confirmation according to the methodology described in Brasil (2003).

The data obtained in the counts of mesophilic microorganisms, and molds and yeasts were transformed into  $\log_{10}$ CFU  $g^{-1}$  and subjected to analysis of variance. The means were compared by the Student t test at 5% probability level using the R 3.4.3 program (R DEVELOPMENT CORE TEAM, 2017). Frequency distribution was used for the coliform, coagulase positive *Staphylococcus*, and *Salmonella* spp. data.

## RESULTS AND DISCUSSION

The results of the microbiological analyses of coliforms (total, and thermotolerant) in the turkey hams evaluated are shown in Table 1.

Table 1 – Microbiological characterization of vacuum-packaged and conventionally packaged sliced turkey hams marketed in Mossoró, RN, Brazil.

Packaging	Sample	Total coliforms	Thermotolerant coliforms
		Most probable number (MPN $g^{-1}$ )	
Conventional	1	>1100	<3.0
	2	150	<3.0
	3	3.6	<3.0
	4	<3.0	-
	5	3.6	<3.0
	6	93	<3.0
	7	240	<3.0
	8	>1100	20
	9	1100	3.6
	10	>1100	<3.0
Vacuum	11	>1100	<3.0
	12	>1100	75
	13	210	<3.0
	14	<3.0	-
	15	<3.0	-
	16	<3.0	-
	17	>1100	<3.0
	18	460	<3.0
	19	1100	<3.0
	20	>1100	<3.0

Positive results for total coliform growth were found in 80% of the samples, with counts ranging from <3.0 to >1100 MPN  $g^{-1}$ .

Although the current Brazilian legislation present no specifications for the maximum concentration of microorganism allowed, evaluating total coliforms is important to express the product's post-processing hygienic-sanitary conditions. Microorganisms are easily destroyed by heat treatments, and their counting has been one of the most effective methods to evaluate the safety of food products (SILVA et al., 2007).

Therefore, the total coliforms found in sliced hams indicated contamination of the products after processing, at slicing, packaging, or storing in inadequate temperatures (KAMINSKI; BARRETO, 2013).

The maximum coliform contents at 45 °C in hams should be  $10^3$  MPN  $g^{-1}$  (BRASIL, 2001). The turkey hams evaluated were within the limits allowed by the Brazilian legislation (<3.0 to 75 MPN  $g^{-1}$ ), regardless of the packaging (Table 1). Samples with growth of thermotolerant coliforms (8, 9, and 12) (Table 1) had no development of colonies typical of *Escherichia coli*, indicating the presence of specimens of other genera.

Coliform content at 45 °C determines fecal contamination of the product, mainly represented by *Escherichia coli*, which is a gram-negative bacillus that is part of the normal flora of warm blood animals, associated with feces; some of its lineages can cause foodborne infections (GAVA et al., 2008; ALDSWORTH et al., 2015)

Costa et al. (2017) found similar results for coliforms at 45 °C, with total absence of thermotolerant coliforms in samples of sliced ham marketed in Maceió, AL, Brazil. Fachinello; Casaril (2013) evaluated raw and cooked sliced hams marketed in Francisco Beltrão, PR, Brazil, and found all samples within the recommended standards, as well as Los et al. (2014), who evaluated quality parameters of the cooked ham sold in the Brazilian market.

Mold and yeast counts ranged from 3.58 to 7.65  $\text{Log}_{10}\text{CFU g}^{-1}$ . Although the Brazilian legislation does not establish the maximum fungi content allowed in food products, evaluating such microorganisms is important to ensure the quality of products. These microorganisms can cause unwanted changes of taste and odor and produce mycotoxins or secondary metabolites that accumulate in the body of consumers and can cause health problems due to its toxic and deleterious effects (SOUZA et al., 2017). Bressan et al. (2007) recommend maximum counts of molds and yeasts of  $10^2 \text{CFU g}^{-1}$ , a value that is lower than those found in the present work.

Serio et al. (2009) evaluated the microbiological quality of refrigerated sliced hams marketed in Fortaleza, CE, Brazil, and found similar results for deteriorating molds and yeasts, with counts of  $10^5 \text{CFU g}^{-1}$  for all samples. Macedo et al. (2014) evaluated the microbiological quality of refrigerated sliced hams marketed in Viçosa, MG, Brazil, and found higher values, with samples reaching  $10^6 \text{CFU g}^{-1}$ .

Counts of viable aerobic mesophilic microorganisms ranged from 3.48 to 9.3  $\text{Log}_{10}\text{CFU g}^{-1}$ . Brazilian

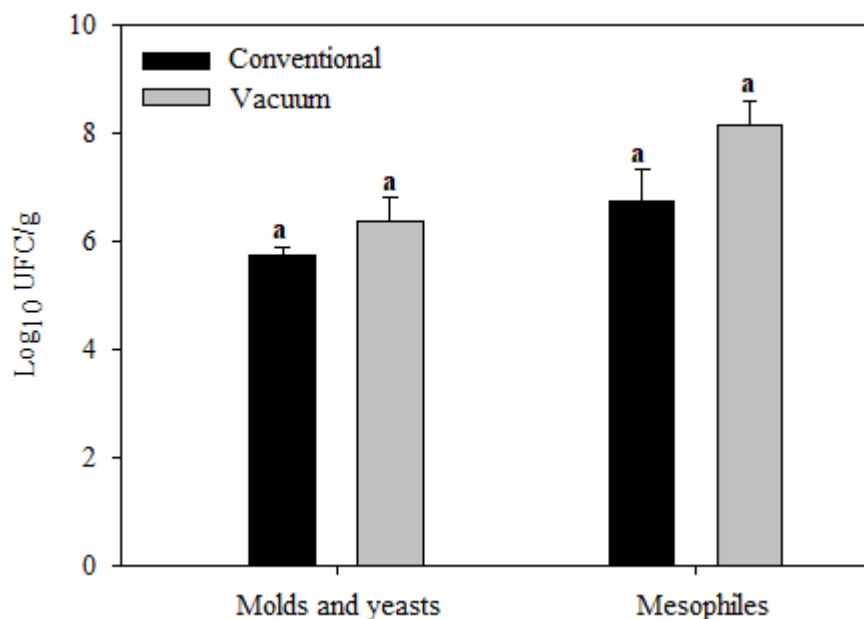
legislation does not establish standards for mesophilic microorganisms in hams (BRASIL, 2001); however, evaluating this microbial group is important because high counts of this microorganisms denote poor hygienic-sanitary quality of food products, from the production to storing stages (PEDROSO et al., 2016). Bressan et al. (2007) recommend maximum counts of mesophilic microorganisms for hams of  $10^3 \text{CFU g}^{-1}$ , a lower limit when compared to the results found in the samples evaluated in the present study.

The high bacterial counts found may be associated to the inadequate storage temperature. Ham is a perishable product due to its processing and composition, requiring adequate refrigeration (BRASIL, 2004), otherwise it allows the development of other microorganisms that may be harmful to humans or to the food characteristics.

Fachinello; Casaril (2013) found counts of aerobic mesophilic microorganisms of  $2.5 \times 10$  to  $2.6 \times 10^4 \text{CFU g}^{-1}$  in sliced hams marketed in Francisco Beltrão, and most of them were inadequate according to the count used by Bressan et al. (2007). Pedroso et al. (2016) found counts of  $10^3$  to  $10^6 \text{CFU g}^{-1}$ , which are similar counts to those found in the present study. Serio et al. (2009) evaluated the microbiological quality of sliced hams marketed in Fortaleza, and also found similar values, with counts of  $10^5$  to  $10^8 \text{CFU g}^{-1}$  for mesophilic microorganisms.

The results of the variance test for counts of molds and yeasts, and mesophilic microorganisms of the vacuum-packaged and conventionally packaged turkey hams is shown in Figure 1.

Figure 1 – Growth of deteriorating microorganisms (molds and yeasts, and viable aerobic mesophilic microorganisms) on samples of vacuum-packaged and conventionally packaged sliced turkey hams marketed in Mossoró, RN, Brazil. Equal letters in the bars indicate absence of significant difference between vacuum-packaged and conventionally packaged turkey ham samples by the Student t test ( $p \leq 0.05$ ).



No significant difference between counts of molds and yeasts, and mesophilic microorganisms and were found in the samples evaluated (Figure 1). However, the use of hygienic-sanitary practices that minimize microbial contamination is required for the handling operations of this product; moreover, the slicer surface can be a source of contamination of this product through deteriorating or pathogenic microorganisms (SERIO et al., 2009).

Coagulase positive *Staphylococcus* growth was observed in 35% of the samples evaluated, with counts ranging from 2.3 to 5.47 log<sub>10</sub>CFU g<sup>-1</sup>. The Brazilian legislation establishes a maximum limit of 3.48 log<sub>10</sub>CFU g<sup>-1</sup> of coagulase positive *Staphylococcus* for turkey hams (BRASIL, 2001). Thus, 25% of the samples were inadequate according to this standard.

*Staphylococcus* in food is associated to processing failures. Some *Staphylococcus*, such as *S. aureus*, can be toxic due to the production of enterotoxins (MARTINS et al., 2009). This pathogen is commonly known as coagulase positive because it coagulates blood plasma (ALDSWORTH et al., 2015).

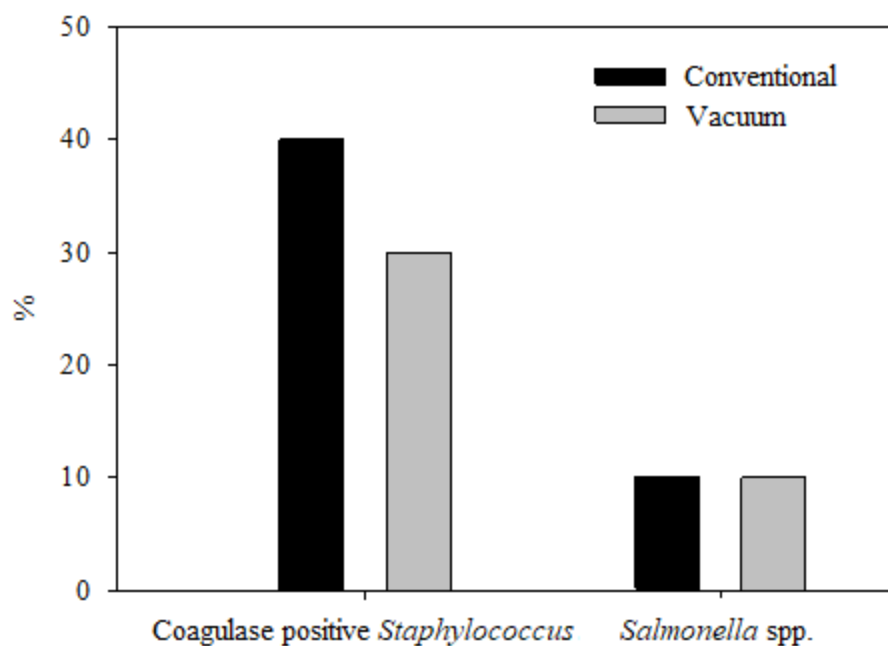
Costa et al. (2017) found coagulase positive *Staphylococcus* in sliced ham samples marketed in Maceió; however, all of them were within the limits established by the Brazilian legislation. Fachinello; Casaril (2013) found no samples with coagulase positive *Staphylococcus*; however, 93% of their sliced ham samples showed growth of *Staphylococcus* spp.

Presence of *Salmonella* spp. was found in 10% of the evaluated hams (2 samples). According to the Brazilian legislation, this pathogen should be absent in 25 g samples (BRASIL, 2001). These results are important because *Salmonella* spp. are microorganisms that concerns public health; its presence indicates post-processing failures, since it can be easily destroyed by applying heat. Excessive manipulation, inadequate thermal control, and poor hygienic conditions favor the growth of this microorganism (FACHINELLO; CASARIL, 2013). Most *Salmonella* spp. are pathogenic to humans; they cause diseases such as typhoid and enteric fevers, and enteric infections, depending on the causative agent (SÁ et al., 2016).

The presence of this pathogen in sliced turkey ham can be attributed to the possible cross-contamination during the inadequate handling, slicing and storage during the marketing process (FAI et al., 2011), which may also be related to the growth of other types of microorganisms found in this food.

Pedroso et al. (2016) evaluated sliced ham marketed in Ribeirão Preto, SP, Brazil, and found different results, with 100% of the samples free from this pathogen. Fai et al. (2011) evaluated the presence of *Salmonella* spp. in refrigerated fat-free pig ham marketed in Fortaleza and found it in 30% of the samples. Contrastingly, Sá et al. (2016) evaluated sliced ham and found absence of *Salmonella* in all samples.

Figure 2 – Percentage of samples of vacuum-packaged and conventionally packaged sliced turkey hams marketed in Mossoró, RN, Brazil, with presence of coagulase positive *Staphylococcus*, and *Salmonella* spp.



Presences of coagulase positive *Staphylococcus*, and *Salmonella* spp. were found in both vacuum-packaged and conventionally packaged ham samples, however, a

higher incidence of *Staphylococcus* was found in the conventionally packaged ham samples (Figure 2).

Pedroso et al. (2016) evaluated vacuum-packaged and conventionally packaged sliced hams and found similar results, with a higher incidence of *S. aureus* in products conventionally packaged by the markets; no *Salmonella* spp. were found, regardless of the packaging; *Escherichia coli* was not found in any sample, which is a similar result to that found in the present study.

Sá et al. (2016) evaluated the presence of *S. aureus*, *E. coli*, and *Salmonella* spp. in sliced hams marketed in Juazeiro do Norte, CE, Brazil, packaged in normal atmosphere packages, and found absence of *Salmonella* spp. and *E. coli*, but significant presence of *S. aureus*.

## CONCLUSION

The microbiological characterization of the vacuum-packed and conventionally packed sliced turkey hams marketed in Mossoró, RN, Brazil, showed the poor quality of some parameters in some samples, with high counts of aerobic mesophilic microorganisms, and presence of coagulase positive *Staphylococcus*, which denotes risks related to its consumption.

The vacuum-packed and conventionally packaged turkey ham samples presented no statistical differences in molds and yeasts, and mesophilic microorganisms. However, a higher percentage of coagulase positive *Staphylococcus* was found in conventionally packaged samples; *Salmonella* was found in one sample of each packaging type (vacuum and conventional).

A careful handling of turkey ham is necessary in all processing and marketing stages, especially regarding the hygiene of manipulators and equipment, and storage conditions.

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