SEVEN
publicações acadêmicas

Microbiological evaluation of sunned meat selled at free fairs in the city of Itapetinga-BA

https://doi.org/10.56238/interdiinovationscrese-004

Robson de Queiros Domingues
Master's student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: queirosdomingues@gmail.com

Monique Costa Barbosa
Master's student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: moniquengalimentos@gmail.com

Antônia Cardoso Mendes de Araújo
Master's student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: antoniiaaraujo@gmail.com

Raphael Patury Lins
Doctor student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: raphaelpatury@gmail.com

Carolina da Silva Ponciano
Doctor student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: carol2ponciano@gmail.com

Jaqueline de Jesus Silva
Doctor student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: jaqsali@live.com

Geisa Sales Oliveira
Master's student in Food Science and Engineering, Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: geisasales00@gmail.com

Lígia Miranda Menezes
Doctor Professor, Department of Exact and Natural Sciences, State University of Southwest Bahia, Itapetinga, Bahia.
E-mail: lmenezes@uesb.edu.br

ABSTRACT

Sun-dried meat is a product marketed throughout Brazil, especially in the Northeast region. It does not have its own legislation that supervises its quality standard, which makes it subject to numerous sources of contamination. The objective of this study was to evaluate the hygienic-sanitary conditions of production of sun-dried meat sold in six establishments, open markets, in the municipality of Itapetinga-BA. Microbiological analyses (halophilic mesophiles, Staphylococcus, Salmonella, total and thermotolerant coliforms) were performed at the Food Microbiology Laboratory of UESB. From the results obtained, sample C, in sample 1 considering halophilic mesophilic microorganisms, presented the lowest contamination rate, 7.47 x 102 CFU/g. Sample B presented the highest contamination rate for Staphylococcus aureus, 9.3 x 103 CFU/g. For total coliforms, sample B presented a contamination number of 1.1 x 103 NMP/g. The remaining samples remained within the standard allowed for total coliforms. In the second collection, all samples showed values above those allowed by law for mesophils and Staphylococcus aureus. For coliforms, there was a reduction in the number of contamination to the samples. The presence of Salmonella spp. was found. in all samples on the first day of collection, however, on the second day, only sample C was absent. Therefore, according to the establishments studied, none of them met the criteria established by the current legislation, presenting flaws regarding the safety of the food. It is suggested that establishments adopt the procedures of good manufacturing practices, with the use of preventive measures, in order to ensure the safety of food products.

Keywords: Salted meat, Contamination, Meat products.

1 INTRODUCTION

Meat products are considered one of the main sources of human nutrition, due to their rich nutritional composition, such as high levels of proteins, essential fatty acids, minerals, and vitamins (AMORIM; BANKS; FIUZA, 2019). According to Ishihara *et al* (2017), meat products, especially salty meats, are widespread around the world, presenting variations in flavors, color, and tenderness, the main sensory attributes desired by consumers.

According to the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA), salted meat is defined as an edible product, produced from meat or organs, subjected to the salting process with sodium chloride or addition of mixtures of salts with nitrites, nitrates, condiments and sugars (BRASIL, 2020).

Sun-dried meat, being an artisanal product, does not have its own legislation that characterizes it and identifies the specific quality standard of this product (Brasil, 2017). This is a meat product typical of northeastern Brazil, with wide acceptance throughout the country. The preservation process is carried out in an artisanal way, based on salting and exposing the meat to the open air or ventilated environment, resulting in a slightly salted, semi-dehydrated product with peculiar characteristics. The preparation of this product is done by cutting and salting for its dehydration, which results in a reduction in activity, which consequently limits microbial development (PESSOA *et al.*, 2018).

Salting is one of the main methods of food preservation, conferring peculiar sensory properties, being a simple and inexpensive practice to be carried out, being an effective barrier to the development of microorganisms (NETO *et al*., 2021). However, when the process is not carried out properly, undesirable microorganisms can easily develop, leaving the food with characteristics that are unfit for consumption.

In general, this meat product is not produced on a large scale, and there is no veterinary inspection, which results in inadequate marketing conditions for hygienic-sanitary aspects. Its sale in street markets, municipal markets and butchers takes place inappropriately. Being exposed to the environment without a proper prior protection barrier, being at the mercy of numerous sources of contamination such as handlers, animals, insects, and others (PESSOA *et al.,* 2018).

Therefore, the objective of this study was to evaluate the microbiological quality of sun-dried meat samples from beef coxão, sold in open markets in the municipality of Itapetinga-BA, to identify the hygienic-sanitary standard of this product to make suggestions for improvements in operational procedures.

2 MATERIAL AND METHODS

Six samples of sun-dried meat were collected from three establishments located in the open market of the municipality of Itapetinga-BA, during the month of June. The selection of collection

points was established according to the commercial flow of the region, selecting places that had a greater number of people access.

Data collection was carried out in the morning, occurring on alternate days in order to have a better representation of the microbiological quality of the marketing environment. A total of 200g of sun-dried meat were collected per establishment, and the samples were properly identified, receiving control letters (A, B and C) according to the place of collection. They were packed in isothermal styrofoam boxes containing thermal bags for transport to the food microbiology laboratory of the State University of Southwest Bahia (UESB), preserving their microbiological characteristics found before collection.

In the laboratory, serial dilutions (10-1, 10-2 and $^{10-3}$) of the collected samples (25g) were prepared, following the procedures described by the manual of methods of microbiological analysis of food and water (SILVA et al., 2017). For the analysis of the samples from the second day of collection, serial dilutions were performed up to $^{10-5}$, due to the number of colonies grown in plates in the first collection being countless.

3 MICROBIOLOGICAL ANALYSES

Microbiological analyses were performed to determine the count, expressed in colony-forming unit per gram of sample (CFU), of halophilic mesophilic microorganisms and *staphylococcus aureus*, the determination of the most probable number (MPN/g) of total and thermotolerant coliforms, and the determination of the presence or absence of *salmonella*. All analyses were performed in a unidirectional horizontal flow booth in order to maintain the sterility of the environment, avoiding interference in the analyses due to cross-contamination. All microbiological analyses were performed in triplicate.

To determine the halophilic mesophilic count, 0.1 ml of decimal dilutions were inoculated into sterile glass Petri dishes containing PCA culture medium (Plate Count Agar) plus a concentration of 2% sodium chloride (NaCl). The surface plating method (spread plate) was used, in which the samples were spread over the culture medium using the Drigalski loop. After inoculation, the plates were inverted and incubated in BOD at 35ºC ± 1ºC for a period of 24 to 48 hours.

For *staphylococcus aureus*, the same procedure was adopted, however, the culture medium used was BP agar (Baird-Parker Agar). After inoculation of 0.1 mL of decimal dilutions and scattering of the inoculum on the surface of the culture medium, the plates were inverted and incubated in BOD at 35ºC ± 1ºC for 45 to 48 hours. Subsequently, for the confirmatory test of the presence of *S. aureus*, plaques with 20 to 200 typical colonies were selected for counting, which are smooth, convex circular, black or dark gray colonies with perfect edges, whitish cell mass at the edges, surrounded by an opaque zone and/or a transparent halo extending beyond the opaque zone. according to the manual of methods

of microbiological analysis of food and water (SILVA *et al*., 2017). Five colonies typical of each plate were selected, and gram staining was performed to identify the shape of the colony. Once the presence of globules in arrangements similar to grape clusters was certified, the colony was certified as confirmatory for the presence of *staphylococcus aureus*.

The analysis of total and thermotolerant coliforms was performed according to the multi-tube technique (MPN/g). The determination of coliforms was performed in two stages: presumptive test and confirmatory test. For the presumptive test, 1 ml of decimal dilutions were pipetted into sterile test tubes containing 10 ml of *Lauryl Sulfate Tryptose* (LST) broth with inverted Durhan tubes. The tubes were incubated in BOD at 35ºC ± 1ºC for a period of 24 to 48 hours.

From the LST tubes that showed turbidity of the medium with gas production, a loaded elevation of each culture was transferred to test tubes containing 10 mL of Bright Green Bile Broth 2% (VB) and *E. coli* Broth (EC) with inverted Durhan tubes to perform confirmatory tests for total and thermotolerant coliforms. respectively. For total coliforms, the tubes were incubated in BOD at 35ºC ± 1ºC for a period of 24 to 48 hours. On the other hand, for thermotolerant coliforms, the tubes were incubated at 45ºC ± 1ºC for a period of 24 to 48 hours.

To perform the Salmonella *analyses*, the sun-dried meat samples were pre-enriched, incubating their respective decimal dilutions 10-1 in BOD at a temperature of 35ºC ± 2ºC for a period of 18 to 20 hours, in order to recover injured cells. Subsequently, the pre-enrichment vials were carefully shaken and 1 mL of the dilutions were transferred to test tubes containing 10 ml of Tetrathionate (TT) and Cystine Selenite (CS) Broths for selective enrichment. The tubes were incubated at 35ºC for a period of 24 hours.

For the selective plating of the pre-enriched medium, a striation (depletion striations) of the samples was striated in petri dishes containing the respective culture media Xylose Lysine Deoxycholate Agar (XLD) and Bismuth Sulphite Agar (BS). The plates were inverted and incubated in BOD at 35ºC for a period of 24 hours.

4 RESULTS AND DISCUSSION

The results of the microbiological analyses performed in triplicate in sun-dried meat, referring to the first and second days of collection, according to RIISPOA, are shown in tables 1, 2 and 3. As there is no specific legislation that establishes the standard limit for the presence of microorganisms in sun-dried meat, the microbiological parameters of meat products similar to this product were used, using the NORMATIVE INSTRUCTION - IN Nº 161, of July 1, 2022 (ANVISA, 2022) for beef jerky to compare the results obtained.

According to Table 1, on the first day of collection, sample C showed a lower contamination rate for halophilic mesophilic microorganisms, 7.47 x 102 CFU/g, compared to the other samples.

Sample B was the one with the highest contamination rate (1.15 x 105 CFU/g) and, for this reason, a higher dilution (10-5) was necessary in the second replication in order to enable the count. The legislation does not establish microbiological standards for this microorganism, so the data obtained by Santos and Hentges (2015) were used, who performed a microbiological analysis on beef jerky sold in Medianeira, Paraná, obtained a result of 3.0 x 10^3 CFU/g for halophilic mesophilic microorganisms. Taking this value into account, sample C was the only one that remained within the established standard.

The use of salting in meat products, according to Neto *et al.* (2020), acts as a barrier that limits microbial growth as a result of the reduction in water activity and the increase in the osmotic pressure of the medium. However, the high rates of halophilic mesophilic microorganisms present in the sun-dried meat in samples A and B may be an indication of contamination of the salt used in the salting process, being associated with the reuse of this raw material. In addition, extrinsic factors related to the environment, such as desiccation time and conservation temperature, may have contributed to these results.

Table 1. Results of microbiological analyses on sun-dried meats for the first day of collection in establishments located in Itapetinga-BA, 2023.

Sample	**Halophile Mesophiles (CFU/g)**	***Staphylococcus aureus* (CFU/g)**	**Total coliforms (MPN/g)**	**Thermotolerant coliforms (MPN/g)**
A	3,08 x 104	1,15 x 104	9,3 x 101	4,3 x 101
B	1,15 x 105	9,30 x 104	1,1 x 103	2,1 x 102
C	7,47 x 102	9,0 x 103	2,9 x 102	2,0 x 101

CFU/g: Colony-forming unit per gram of sample; MPN/g: Most likely number per gram of sample.
Source: Authored by the authors, 2023.

Other factors such as: shared utensils, lack of asepsis of the hands frequently, precarious physical structure, use of adornments, buildings in poor conditions, exposure to dust and suspended smoke, improper manipulation of the buyer, and manipulator operating multitasking, were observed during the collection. The performance of multiple tasks by the handlers, such as attending, handling the sun-dried meat and receiving payment, was observed in 2 of the establishments collected.

According to Reolon and Silva (2009), the unpreparedness of handlers performing multiple tasks can point to one of the main causes of food contamination. Establishment B was even under renovation and, as a consequence, the butcher shop environment had abundant dust, which may have directly reflected in this count, since, according to Neves and Santos (2022), *Staphylococcus* exists in the air, in dust, in sewage, water, milk, on surfaces and equipment, humans and animals.

The legislation establishes a standard limit of 10^3 CFU/g for *Staphylococcus aureus,* so none of the samples met the acceptable standards for consumption. These results were similar to those obtained by Assis *et al*. (2019), which when performing a microbiological analysis on sun-dried meat,

obtained 16 (40%) samples with counts above 10^3 CFU/g. *Staphylococcus aureus is* a microorganism that is part of the natural microbial flora of human beings, being found on the skin, nasal mucous membranes, intestine and respiratory tract. Therefore, the high presence of this microorganism may indicate poor hygienic-sanitary conditions of the handler and also of the environment.

In the research of total and thermotolerant coliforms, the legislation establishes a limit of 10^3 NMP/g. The presence of these microorganisms was verified in 100% of the analyzed samples, however, only sample B showed coliform counts at 35ºC (total) with values higher than those established by law, being unfit for consumption. Sample A was the one with the lowest contamination rate for this microorganism, 9.3 x 101 NMP/g, followed by sample C, 2.9 x 102. For thermotolerant coliforms, all samples had counts below the limit established by the legislation.

Coliforms are microorganisms that indicate the hygienic and sanitary quality of processing. The high presence of this microorganism, found in sample B, may be an indication of fecal contamination carried by handlers and equipment.

Contamination may be due to the processing and storage conditions of sun-dried meat, since it is exposed to the environment without proper protection, being subject to contact with contaminants and at the mercy of manipulation by consumers. Hentges (2015) confirms this fact, also emphasizing that contamination may be closely linked to the way in which this product was subjected to processing. Commercialization in open markets, many times, due to the lack of current inspections, ends up being a means of facilitating the occurrence of contamination.

Similar results were obtained by Penha (2017), who found values lower than the standard of 103 MPN/g recommended by current legislation for thermotolerant coliforms (45ºC) and variation in values of <3 NMP/g and 1.1x104 NMP/g for total coliforms (35ºC) in his research. Sousa (2017) also found similar results to the present study, with values lower than 10^3 NMP/g for thermotolerant coliforms in sun-dried meat samples. According to the author, the low count of these microorganisms is due to the practices adopted by food handlers: such as hand asepsis or salting that limits microbiological growth. Despite the desirable result for total coliforms, in some of the samples, the legislation does not assign any microbiological standard to them, therefore, the higher values for thermotolerant coliforms indicate that they are unfit for consumption.

For the sun-dried meat samples on the second day of collection, table 2, considering the halophilic mesophilic microorganisms, all samples were above the established limit of 3.0 x 10^3 CFU/g, being unfit for consumption. There is a reduction in the microbiological quality of halophilic mesophilic microorganisms present in the meat product. According to Abrantes *et al.* (2014), the increase in the rate of halophilic microorganisms in sun-dried meat may be a result of the use of low-quality salt, compromising the characteristics of the final product.

Table 2. Results of microbiological analyses on sun-dried meats for the second day of collection in establishments located in Itapetinga-BA, 2023.

Sample	Halophile Mesophiles (CFU/g)	*Staphylococcus aureus* (CFU/g)	Total coliforms (MPN/g)	Thermotolerant coliforms (MPN/g)
A	2,3 x 105	2,3 x 104	2,4 x 102	2,1 x 101
B	2,1 x105	2,7 x 105	9,3 x 101	9,3 x 101
C	4,3 x 104	1,5 x 104	2,3 x 101	2,9 x 101

CFU/g: Colony-forming unit per gram of sample; MPN/g: Most likely number per gram of sample.
Source: Authored by the authors, 2023.

The same was true for *Staphylococcus Aureus*, there was an increase in the number of contaminating microorganisms for all samples analyzed, from 9.0 x 103 CFU/g and 9.3 x 104 CFU/g for samples C and B, respectively, to 1.5 x 104 CFU/g and 2.7 x 105 CFU/g. 2.3 x 103 CFU/g, remaining in the same range found in the first analysis (Table 1). Thus, all samples are unfit for consumption, according to the standards established by NORMATIVE INSTRUCTION - IN No. 161, of July 1, 2022.

For total and thermotolerant coliforms, there was a reduction in the number of microorganisms for all samples, within the limit allowed by NORMATIVE INSTRUCTION - IN No. 161, of July 1, 2022. The addition of salt in the salting process may have contributed to the reduction of the microbiological load. This data is proven by the studies carried out by Silva (2018) with sun-dried meat subjected to different times of desalting in water, obtaining results like this study, with counts for thermotolerant coliforms lower than 10^3 NMP/g.

According to table 3, for the samples referred to collection 1, the *Salmonella* was detected in all samples. The legislation establishes the absence of these microorganisms in 25 g of sample, the presence of this microorganism makes the samples unfit for consumption. In the second collection, only sample C showed absence of this microorganism. The presence of *Salmonella* in the samples analyzed may be due to inadequate processing practices, related to cross-contamination related to the improper handling of this product, poor storage, and use of inappropriate storage temperature ranges.

Table 3. Presence of *Salmonella spp.*in sun-dried meat sold in establishments located in Itapetinga-BA, 2023.

Sample	***Salmonella spp.***	
	Collect 1	**Gathering 2**
The	Yes	Yes
B	Yes	Yes
C	Yes	No

Source: Authored by the authors, 2023.

These results are like those found by Assis *et al.* (2019), which when carrying out the microbiological, physicochemical characterization and the production and marketing conditions of sun-dried meat from Salinas, Minas Gerais, detected the presence of *Salmonella spp.* in 5% of the samples that were marketed at room temperature. In the research conducted by Menezes *et al.* (2022), the presence of salmonella was also detected in 20% of the analyzed samples of beef jerky sold in public markets in Recife/PE, due to the poor processing and storage conditions of the product.

According to Evangelista (2001), meat products that are exposed to room temperature tend to be more prone to *Salmonella* contamination, a fact observed in the present study. Since the results reflect the inadequate marketing conditions, such as the use of room temperature to expose the products without proper protection. According to Kubota and Alencar (2021), the presence of this microorganism in samples is worrisome, since it is a zoonosis responsible for several diseases that pose risks to the health of humans and animals.

In turn, the absence of *Salmonella spp.*in sample C, obtained in collection 2, it may be due to changes in the handling practices adopted by the establishment. Such as, sanitization and sanitization of the equipment used in the handling of meat products, washing the hands of handlers, increasing the concentration of salt used in salting and reducing excessive handling of the product.

5 CONCLUSION

The establishments in which the samples of sun-dried meat were collected at the open market in the city of Itapetinga-BA showed failures regarding the safety of the food. Among the collection points studied, none of them met the criteria established in the current legislation, about appropriate clothing, handling, handling of utensils and the environment.

Therefore, it can be concluded that the lack of training and instruction of the handlers was the biggest problem observed. The high incidence of microorganisms found reveals the need to adapt the establishments to the regulations established by RDC No. 275/2002, which aims to ensure the procedures of Good Manufacturing Practices. Based on this assumption, it is suggested to adopt preventive measures such as: Constant asepsis of the hands after each operation, sanitization and sanitization of knives, chairs and boards between operations, light-colored uniform, plastic aprons, rubber boots, operator without multiple tasks, weighing of the meat in a plastic bag (without direct contact with the scale), environment for deboning and exposure of the meat without contact with the external environment, non-recycling of salting salt, integrated control of vectors and urban pests, professional training, proper waste management and periodic examinations.

REFERENCES

ABRANTES, M. R.; SOUSA, A. C. P.; ARAÚJO, N. K. S.; SOUSA, Ê. S.; OLIVEIRA, A. R. M.; SILVA, J. B. A. Avaliação microbiológica de carne de charque produzida industrialmente. Revista Arq. Inst. Biol., São Paulo, v. 81, n. 3, p. 282-285, 2014.

AMORIM, J. R. B.; BOTELHO, L. F. R.; FIUZA, A. P. P. Perfil sanitário e microbiológico da carne moída comercializada em hipermercados. Revista COMEIA, v. 1, n. 1, 2019.

ANVISA - Agência Nacional de Vigilância Sanitária. 2022. INSTRUÇÃO NORMATIVA - IN Nº 161, DE 1º DE JULHO DE 2022. Ministério da Saúde - MS, 2022.

ASSIS, D.C.S.; SANTOS, T.M.; ORNELLAS, C.B.D.; DRUMMOND, A.F.; MAGALHÃES, F.C.; SANTOS, W.L.M. Caracterização microbiológica, físico-química e das condições de produção e comercialização da carne de sol de Salinas, Minas Gerais. Revista Arq. Bras. Med. Vet. Zootec. v.71, n.6, p.1985-1992, 2019.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa N° 92 de 18 de setembro de 2020. Brasília, DF, 2020. Evangelista J. Tecnologia de alimentos. 2ªed. São Paulo: Atheneu; 2001.

ISHIHARA, Y. M.; MOREIRA, R. T.; FÉLEX, S. S.; CARVALHO, R. J.; MADRUGA, M. S. Maturação natural de carne-de-sol. Revista B.CEPPA, Curitiba, v. 35, n. 1, 2017.

MENEZES, R. C. C. B.; LIMA, G. E.; SHINOHARA, N. K. S.; VELOSO, R. R. Análise microbiológica e de formaldeído em carnes de charque comercializadas em mercados públicos de Recife/PE. Revista Research, Society and Development, v. 11, n. 4, 2022.

NETO, P. A. S.; LIMA, F. K. S.; MENDES, L. G.; MONTE, A. L. S. Revisão sistemática sobre carnes salgadas e seus processos de qualidade. Revista Research Society and Development, v.10, n.15, 2021.

NEVES, K. L.; SANTOS, E. J. Staphylococcus aureus: informações importantes que você precisa saber. Semear food safety culture, 2022. Disponível em: <https://semearfoodsafetyculture.com.br/staphylococcus-aureus-informacoes-importantes-que-voce-precisa-saber/>. Acesso em: 10 de julho de 2023.

PENHA, J. Q. C. Avaliação da qualidade microbiológica, físico-química e parasitológica da carne bovina salgada comercializada em estabelecimentos e feiras livres na zona norte do município do Rio de Janeiro. 2017. 115 p. Dissertação (mestrado em Vigilância Sanitária) - Instituto Nacional De Controle De Qualidade Em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, 2017.

PESSOA, R. M. S.; GOIS, G. C.; COSTA, D. C. C. C.; SILVA, A. A. F.; SILVA, C. A. P.; SANTOS, R. N. SILVA, A. P. R. Caracterização do perfil dos consumidores de carne de sol comercializada no município de Areia – PB. Revista Semiárido De Visu, Petrolina, v. 7, n. 1, p. 15-23, 2018.

SANTOS, A. M. L.; HENTGES, L. C. Avaliação físico-química e microbiológica de carne seca (charque). 2015. Trabalho de Conclusão de Curso. Universidade Tecnológica Federal do Paraná.

SILVA, L. D. P. Avaliação da qualidade de carne da carne de sol submetida a diversos tempos de dessalga em água. 2018. 44 p. Monografia (Bacharelado em Gastronomia). Universidade Federal Da Paraíba - Centro De Tecnologia E Desenvolvimento Regional, Paraíba, João Pessoa, Paraíba, 2018.

SOUZA, P. V. D. Registro histórico, desenvolvimento e caracterização da carne-de-sol tradicional do município de Picuí - PB. 2017. 56 p. Monografia (Bacharelado em Gastronomia) - Universidade Federal da Paraíba, Centro De Tecnologia e Desenvolvimento Regional, João Pessoa, PB, 2017.