

**Research of microorganisms, parasites and dirt in drumsticks sold by street vendors**  
**Pesquisa de micro-organismos, parasitos e sujidades em coxinhas comercializadas por ambulantes**

**Investigación de microorganismos, parasitos y sujetos en baquetas vendidas por vendedores ambulantes**

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### Abstract

It is of great importance to maintain the hygienic-sanitary quality of food and places where it is handled and marketed. This work aimed to evaluate the hygienic-sanitary conditions of Brazilian croquettes with chicken filling sold by street vendors in the city of Mossoró, state of Rio Grande do Norte. Ten samples were subjected to counting aerobic mesophilic bacteria, counting fungi and yeasts, *Salmonella* spp., Coliforms at 35°C and 45°C. The research was into find dirtiness and pathogenic parasites was carried out with the flotation and sedimentation test. Total coliforms were found in 30% (3/10) of the samples evaluated, with values ranging from <3.0 to> 1100 NMP / g and the presence of thermotolerant coliforms in 10% (1/10) with a value greater than > 1100 NMP / g. The count of mesophilic bacteria varied from 3.17 to 6.23 (Log<sub>10</sub> UFC/g). While the mold and yeast count between 3.69 to 5.55 (Log<sub>10</sub> UFC/g). Regarding the analyzes of *Salmonella* ssp., *Escherichia coli* and pathogenic parasites were negative. The results allowed to conclude that, of the total of ten samples analyzed, 10% (1/10) was unfit for human consumption.

**Keywords:** Food quality; Fried food; Contamination.

### Resumo

É de grande importância a manutenção da qualidade higiênico-sanitária dos alimentos e locais onde são manipulados e comercializados. Este trabalho teve como objetivo avaliar as condições higiênico sanitária de coxinhas com recheio de frango comercializado por ambulantes na cidade de Mossoró - RN. Dez amostras foram submetidas à contagem de bactérias aeróbias mesófilas, contagem de fungos e leveduras, *Salmonella* spp., coliformes à 35°C e 45°C. Realizada pesquisa de sujidades, parasitos patogênicos, com a realização do teste de flutuação e sedimentação. Verificou-se presença de coliformes totais em 30% (3/10) das amostras avaliadas, com valores que variam de 1100 NMP/g e presença de coliformes termotolerantes em 10% (1/10) com valor superior a >1100 NMP/g. A contagem de bactérias mesófilas variaram de 3,17 à 6,23 (Log<sub>10</sub> UFC/g). Enquanto a contagem de bolores e leveduras entre 3,69 à 5,55 (Log<sub>10</sub> UFC/g). Quanto as análises de *Salmonella* ssp., *Escherichia coli* e parasitos patogênicos foram negativas. Os resultados permitiram concluir que, do total de dez amostras analisadas 10% (1/10) estava imprópria para o consumo humano.

**Palavras-chave:** Qualidade dos alimentos; Salgados; Contaminação.

## Resumen

Es de gran importancia mantener la calidad higiénico-sanitaria de los alimentos y lugares donde se manipulan y comercializan. Este estudio tuvo como objetivo evaluar las condiciones higiénicas y sanitarias de las baquetas con relleno de pollo comercializadas por los vendedores ambulantes en la ciudad de Mossoró, estado de Rio Grande do Norte. Diez muestras fueron sometidas a conteo de bacterias mesófilas aerobias, contando hongos y levaduras, *Salmonella* spp., Coliformes a 35°C y 45°C. Investigación de suciedad, parásitos patógenos, con prueba de flotación y sedimentación. Se encontraron coliformes totales en 30% (3/10) de las muestras evaluadas, con valores que van desde 1100 NMP / gy presencia de coliformes termotolerantes en 10% (1/10) con un valor mayor a > 1100 NMP / g. El recuento de bacterias mesófilas osciló entre 3,17 y 6,23 (Log10 UFC / g). Mientras que el moho y la levadura cuentan entre 3,69 y 5,55 (Log10 UFC / g). En cuanto a los análisis de *Salmonella* spp., *Escherichia coli* y parásitos patógenos fueron negativos. Los resultados permitieron concluir que, del total de diez muestras analizadas, el 10% (1/10) no era apto para consumo humano.

**Palabras clave:** Calidad alimentaria; Salado; Contaminación.

## 1. Introduction

The brazilian chicken croquettes is a fried food that has a prominent position in the preference of consumption by Brazilians, which represents wide prominence in the food market (Sales, et al., 2015), with increased consumption due to its practicality and affordability financially (Santos, et al., 2018).

The lack of time, ally with the fast pace of life, make people look for practical food, and this ends up resulting in the search for easily accessible and low-priced food (Lima, et al., 2017). However, there is a risk of contamination by microorganisms, dirtiness and parasites, if food is not handled and preserved with good manufacturing practices (GMP), leading to a greater risk of Foodborne Disease (Monteiro, 2015).

The food trade market by street vendors is usually fixed on public roads, with crowds of people (Monteiro, et al., 2017). Not having an adequate infrastructure, the food presents a greater possibility of contamination and spread of pathogenic agents, due to the inadequate conditions of the place and inadequate hygienic handling (Silva, et al., 2017).

During its preparation process, the Brazilian chicken croquette is exposed to different stages of manipulation (Balarak, et al., 2016). Without proper hygienic-sanitary conditions, food can be a vehicle for pathogenic agents (MEDEIROS, et al., 2019) such as parasites and microorganisms.

The evaluation of the microbiological quality of food is a way of verifying hygiene conditions, which can be carried out by analysis of microorganisms that are indicators of health quality that can indicate failures in hygienic-sanitary practices of handling, transport or storage (Silva, et al., 2017).

Another relevant problem is contamination by foreign material which can be macroscopic, or fragments that are difficult to visualize (Aragão, et al., 2018). With division of light dirtiness (like insects and their fragments, mites and animal hair), and heavy dirtiness (wood fragments and animal faeces) (Santos, et al., 2017).

Keeping in mind the importance of maintaining the hygienic-sanitary quality of food, the present study aimed to assess the levels of microbiological contamination, conduct parasitic analysis and research for dirtiness in the street market trading of Brazilian chicken croquettes in the city of Mossoró, state of Rio Grande do Norte.

## **2. Material and Methods**

The food trade market occurs in the street by sellers in the informal labor market in the city of Mossoró, state of Rio Grande do Norte, and over time, it has been establishing itself as a practice of subsistence and disorderly growth, located in specific locations in the city.

The coxinha samples with chicken filling were acquired from ten different vendors selected at random. Right after the acquisition, the samples were placed in an isothermal box, in a plastic bag sterile (individual) from the seller itself. The samples were sent to the Food Microbiology Laboratory, where they were subjected to microbiological analysis, and the parasitic analyzes and dirtiness research were carried out at the Laboratory of Biotechnology Applied to Infectious and Parasitic Diseases at the Federal Rural University of Semi-Arido (UFERSA). All microbiological analyses were performed according to Silva, N. et al (2017), with changes. For microbiological analyzes, dilutions were performed. For the first dilution, 1g of the filling was weighed and added to the 10<sup>-1</sup> dilution, which contained 9 mL of 0.1% peptone saline solution, then homogenized for 10 seconds (sequence followed in all samples), from the central dilution, four serial dilutions of (10<sup>-2</sup> to 10<sup>-5</sup>) were performed, the samples were made in series adding 1 mL of the previous dilution to the next.

The samples were subjected to counting aerobic mesophilic bacteria, counting molds and yeasts, which were performed in duplicate using the surface plating technique, using the medium Plate Count Agar and Potato Dextrous Agar. Right after the plates were incubated in an oven at 37 °C for 48 hours for (mesophilic bacteria) and in a BOD oven with controlled temperature at 25 °C for 7 days (molds and yeasts).

The analyzes of *Salmonella* spp., were performed from a pre-enrichment, with incubation in a bacteriological incubator of the 10-1 dilution, for at least 18 hours at 37 °C. For enrichment, Tetrathionate (TT) and Rappaport (RR) broths were used, incubated together with sample aliquots in tubes at 41 °C in a water bath for 24 hours. From the selective enrichment broths, they were plated on plates of Methylene Blue Eosin Agar (EMB) and incubated in an oven for 24 hours at 36°C. To confirm *Salmonella* ssp., the suspected colonies were subjected to biochemical tests.

For the analysis of total coliforms and thermotolerants, the Resolution of the RDC Resolution N°. 12 of the National Health Surveillance Agency – ANVISA. To detect total coliforms, one mL of each sample was used in tubes containing 2% bright green broth, then they were inoculated in a water bath at 36 ° C for a period of 48 hours, and the presence of coliforms was confirmed by the formation of gas in the Duhan tube. The tubes confirmed by the presence of total coliforms, were transferred to the *Escherichia coli* broth (E.C.) and inoculated at 45°C for 48 hours in a water bath, and confirmed with the presence of gas in the Duhan tube. The results for total coliforms were expressed in NMP/ml and for thermotolerant coliforms by the presence or absence in the sample. To confirm the presence of *E. Coli*, positive samples of thermotolerant coliforms were subjected to Simmons citrate analysis, where they were incubated in an oven at 35 °C for 48 hours.

For parasitological analysis, heavy egg research was carried out using the spontaneous sedimentation technique by the Hoffmann Method (Hoffman, et al., 1934) adapted (Soares et al., 2017). Weighed five grams of the sample with dilution in 30 mL of distilled water and sedimentation process for a time of 30 min. To search for light eggs, the flotation technique was used by the Faust method (Faust, 1938) with the use of hypersaturated saline.

The dirtiness search was carried out by method No. 950.89 b, with description by AOAC - Association of Official Analytical Chemists (2005), adapted (SOARES et al., 2017), where 50g of the sample, 200mL of distilled water and 10mL were added of mineral oil with vigorous stirring for 30 seconds. Subsequently, the content was decanted to form 3 phases (oil, water and dirt). After separation into phases, they were transferred to different containers and the liquid from the dirt container was again placed in the decantation funnel and washed

to achieve translucency of the liquid. Subsequently, the contents were placed on filter paper and, with the aid of a vacuum pump removed the water from the sample. The material contained in the oil container was placed on a filter paper and, with the aid of a vacuum pump, removed all the oil from the sample. Subsequently, they were observed under an optical microscope on 40x objectives.

### 3. Results and Discussion

The results obtained for the microbiological profile of drumsticks sold by street vendors are described in Table 1.

**Table 1.** Microbiological analyses parasites and dirt in brazilian croquettes with chicken sold by street vendors in Mossoró, State of Rio Grande do Norte, Brazil.

Amostra	Coliform 35°C NMP/g	Coliform 45°C NMP/g	Mesophiles (Log10 UFC/g)	Molds (Log10 UFC/g)	<i>Salmonella</i> ssp. e <i>E. coli</i>	<i>Cysts, eggs,</i> <i>larvae and</i> <i>dirt</i>
1	< 3,0	< 3,0	3,17	4,74	Absence	Absence
2	< 3,0	< 3,0	5,32	3,87	Absence	Absence
3	>1100	20	5,81	5,34	Absence	Absence
4	< 3,0	< 3,0	4,81	4,13	Absence	Absence
5	>1100	3,0	6,04	5,53	Absence	Absence
6	< 3,0	< 3,0	4,79	5,19	Absence	Absence
7	< 3,0	< 3,0	4,38	4,48	Absence	Absence
8	< 3,0	< 3,0	4,11	3,69	Absence	Absence
9	< 3,0	< 3,0	5,36	5,46	Absence	Absence
10	>1100	>1100	6,23	5,55	Absence	Absence

Source: Authors (2019).

Of all samples analyzed, was diagnosed the presence of coliforms at 35 °C in 30% (3/10) of the samples, with a range from <3.0 NMP / g to 1100 NMP / g. According to RDC Resolution N°. 12 of the National Health Surveillance Agency - ANVISA, it establishes that

the maximum number of thermotolerant coliforms for bakery and similar products ready for consumption is 102 NMP / g, however the resolution does not establish microbiological values for coliforms totals (Brasil, 2001). Thus, a percentage of 10% (1/10) showed a value higher than this limit for thermotolerant coliforms. The analyzes carried out on the positive samples of thermotolerant coliforms were confirmed by the bacteria *Escherichia coli*, which were negative.

Coliforms are the main indicators of hygienic-sanitary quality of food (Santos, et al., 2015). The presence of total and thermotolerant coliforms in food indicates that the establishment of production and the food handler are not meeting the necessary requirements in complying with Good Handling Practices (Valiatti, et al., 2017). In this sense, the fact that the conditions are unsatisfactory, may bring risks to the population that is exposed to consuming food that is not microbiologically safe, and may cause Foodborne Diseases (Santos, et al., 2014).

The count of the analyzed samples of aerobic mesophilic microorganisms and molds showed contamination in 100% (10/10), with values varying between 3.17 and 6.23 Log<sub>10</sub> UFG / g for mesophilic microorganisms and 3.69 and 5.55 for molds.

Aerobic mesophilic bacteria are indicators of unhealthy environments and contamination at the place of production, which can mean the multiplication of pathogenic bacteria, with the majority of these mesophilic bacteria (Assis, 2014). This is the case with temperature, if the thermal processing is not followed correctly throughout the food process chain, there is a great risk of microbial growth. Bearing in mind that high temperatures are capable of destroying or reducing the growth of microorganisms to an acceptable level (Santos, et al., 2012).

During the collection, it was possible to observe that the stoves were not regulated in such a way that the brazilian chicken croquettes remained at a temperature above 60 ° C, and for the most part, they did not have an adequate infrastructure to commercialize food.

Molds and yeasts are formed by a basic structure called hyphae, which together form a more complex arrangement called mycelium (Costa, et al., 2016). They are considered one of the main food deteriorants, this proliferation happens when the conditions are favorable for their multiplication, as well as they can produce toxins that alter the appearance and the nutritional and organoleptic characteristics of the products in addition to harming human health (Silveste, et al., 2013).

As for the results for parasitic and dirt research, the samples were negative 0% (0/10), for parasites or animal fragments in the 10 samples of chicken drumsticks analyzed.

The absence of parasitic eggs in the Brazilian croquettes with chicken may have occurred due to the type of food being fried. According to Scherr & Ribeiro (2013), foods that go through the cooking process and are subjected to a temperature of 60 °C, for 30 minutes or more, this process has an action to make the larvae unviable and to guarantee the health of the food, that is, to destroy parasite eggs. Parasite eggs may be present in the food due to the direct contact of the handler with the food, which is exposed to different forms of contamination, from its preparation to its commercialization (Rossi, 2014).

In view of the safety and quality of food, the investigation of parasite analysis and the research of dirtiness in fried food is of great importance, the same demonstration on the hygienic conditions of handling, storage, transport and on the risks that the population may be exposed by consuming products with dirt, parasites and larvae (Moura, et al., 2016).

Enteroparasitoses belong to the category of diseases transmitted by food, which represent a serious public health problem, which can cause a series of complications that represent a risk for human consumption, and can bring serious health risks (Ruiz, et al.; 2019).

#### **4. Conclusion**

The results obtained in this study allow us to conclude that, in the total of ten samples analyzed, 10% (1/10) were unfit for human consumption because they presented coliforms at 45°C above the standards established by RDC No. 12 of 2001. Regarding the results of *Salmonella* ssp., as well as the research of cysts, eggs, larvae and dirtiness research, were absent in the analyzed samples.

From the data analysis, growth of aerobic mesophilic bacteria, molds and yeasts was observed in 100% of the samples. This indicates that temperature control, cleaning and thermal.

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