


Microbiological Quality of Food

 <https://doi.org/10.56238/colleinternhealthscienv1-001>

Igor Franklin Silva

Moura Lacerda University Center, São Paulo, Brazil
E-mail: igorfrankribs@gmail.com
ORCID: <https://orcid.org/0000-0002-5745-8632>

Naiá Carla Marchi de Rezende-Lago

Moura Lacerda University Center, São Paulo, Brazil
E-mail: naiarezendelago@gmail.com
ORCID: <https://orcid.org/0000-0001-8178-7527>

Patricia Gelli Feres de Marchi

Federal University of Acre, Brazil
E-mail: patriciamarchi.ufac@gmail.com
ORCID: <https://orcid.org/0000-0001-7940-4478>

Cassio Toledo Messias

Federal University of Acre, Brazil
E-mail: ctoledeomessias@gmail.com
ORCID: <https://orcid.org/0000-0003-1423-1267>

Lidianne Assis Silva

Federal University of Acre, Brazil
E-mail: lidys.assis@gmail.com
ORCID: <https://orcid.org/0000-0003-1976-0141>

ABSTRACT

Currently, the microbiological analysis is of extreme relevance in the food industry, because with it, it is possible to know the conditions under which the food was processed, the risks that it can offer to the final

consumer, if the product has the desired useful life, besides to verify that microbiological standards for food standards are being followed properly. The present work aims to verify the sanitary quality of various foods from the presence of indicator microorganisms, such as mesophiles, total coliforms and thermotolerant coliforms, which indicate the possibility of fecal contamination. Among the foods analyzed, many were highly contaminated, pointing to poor sanitary conditions, making them unfit for consumption. Samples of sausage indicated an acceptable microbiological standard, not reaching high populations of mesophiles and coliforms that could disadvantage the product. This is no longer the case with the cheeses analyzed, since they were the most contaminated samples of the present project, reaching numbers greater than 107 NMP/g. With the work, it was possible to observe that costumers are subject to purchase a products considered to be unapproved for consumption and, unfortunately, in Brazil, there is not always a regulation that limits a maximum quantity of microorganism indicators, thus not preventing the commercialization of contaminated foods, and can cause harm to the health of those who consume them. In order for such an act to be avoided at least, health authorities should act by better monitoring of products and guiding handlers on good sanitary practices.

Keywords: Diseases, Fecal coliforms, Indicator microorganisms, Mesophiles, Public health.

1 INTRODUCTION

The presence of pathogenic microorganisms or their toxins in food represents a risk to public health because they can cause serious damage to the health of those who consume them, besides generating economic losses and also decreasing the shelf life of these products. Microbial contamination, then, is directly associated with the world's public and economic health, and should be avoided through good handling practices (Souza et al., 2003). For Franco and Almeida (1992), many microbial contaminations are undesirable and harmful. To know the existence of possible hygienic deficiencies,

groups of microorganisms called indicators are researched, as well as the pathogens that can develop in food.

Besides the fact that there are several vehicles for food contamination, there are several factors that contribute to increase the probability of food contamination. The hygiene of the handler and everything that comes in contact with the food must be adequate and is of utmost importance for the safe and innocuous production of the final product. Deficiency in hygiene poses a health risk to consumers (Hattori & Klaus, 2013).

According to Hayes (1995), the practice of exams to detect pathogenic microorganisms in food is unusual due to the lack of adequate equipment. To reverse this obstacle, foodstuffs are searched for the presence of microorganisms capable of causing infections. The presence of these microorganisms can be detected by the search for indicators, which indicate the potential contamination present in the food, and are of great significance when evaluating the microbiological safety and quality of food.

For Ribeiro (2008), indicator microorganisms reveal a lot about the microbiological quality of food, because they can indicate the presence of fecal contamination, the degree of product deterioration, how the food was processed and stored, indicate errors in sanitary conditions, reveal the shelf life, and indicate the presence of potentially pathogenic bacteria. In general, indicator microorganisms are used to evaluate aspects of food quality and sanity.

According to Doyle, Beuchat, and Montville (1997), an indicator microorganism should be easy to detect, should be distinguishable from other microorganisms of the food microbiota, should not be present in the natural contamination of the food, should have as its habitat only the mammalian intestinal tract, and should have high resistance to the extra-intestinal environment.

Indicator microorganisms can be classified into two groups, according to the International Commission on Microbiological Specifications for Foods (ICMSF, 1994): I) microorganisms that do not pose a health risk such as mesophiles, psychrotrophs, thermophiles, molds and yeasts. II) microorganisms that cause low or indirect health risk: total and thermotolerant coliforms.

Several factors contribute to the contamination of food, but the main ones are: poor hygiene conditions of the place where it is produced, lack of adequate equipment and utensils, and lack of personal hygiene, especially among the handlers. The conservation and hygiene of the facilities and equipment is extremely important, as well as a high level of knowledge and preparation of the handlers to avoid contamination of the food. Handling errors and non-compliance with hygienic norms favor contamination by pathogenic microorganisms, which, in turn, can multiply in sufficient numbers to cause illnesses to the consumer (Ferreira, 2006).

Pinheiro and Wada (2010) say that equipment and utensils with poor sanitation have been responsible, alone or associated with other factors, for outbreaks of foodborne diseases or alterations in processed foods. For Freitas (1995), cold cuts, vegetable slicers, trays, plates, cutlery, trays, handling

plates, meat tenderizers, among others, must constantly undergo microbiological evaluation to control the efficiency of the sanitization procedure, avoiding contamination of the food produced.

In food production, the quality of the raw material and its proper processing are indispensable factors to guarantee a safe final product that does not present risks to the consumer's health (Pinheiro and Wada, 2010).

Microbiological analysis in food can then be performed in order to investigate the presence or absence of microorganisms in the analyzed product, to quantify the microorganisms present, and to identify and characterize different microbial species (Ribeiro, 2008).

According to their behavior in relation to the optimum temperature for multiplication, bacteria are subdivided into groups. Among these groups are the mesophilic bacteria, which are able to multiply between 10 °C and 45 °C, 30 °C being the optimum temperature. They include most contaminants of food of animal origin, and can reach high counts when the food is kept at room temperature. This is the group of microorganisms most often used to determine food contamination, because they are the most frequent and the largest in number. They are able to indicate whether cleaning, disinfection, and temperature control during the industrial processing, transport, and storage processes were done properly. This determination also allows information to be obtained about incipient alteration of the food, its probable shelf life, lack of control in the thawing of food or deviations in the established refrigeration temperature (Silva, 2002).

Thermophilic microorganisms are those that have the capacity to resist extremely high temperatures, reaching up to 90 °C, although their ideal multiplication temperature is around 55 °C. Although they are not frequent in milk, their presence can cause serious damage when kept at high temperature (ICMSF, 1994). They assume greater importance in foods that have been thermally processed, since they can withstand the processing temperature.

The psychrotrophs present an optimum temperature for multiplication between 20 and 30 °C, although some are capable of reproduce at temperatures below 10 °C. Pasteurization is sufficient to eliminate them, but products from their metabolism, such as spores and enzymes, can resist and cause changes in the abnormal characteristics of the food. The most important enzymes produced by these bacteria are protease and lipase. These bacteria are usually found in water and in containers that have not been properly washed (Oliveira et al., 2012).

According to Franco (2003), total coliforms are a group capable of fermenting lactose and producing gas when incubated at 36 °C for 48 hours. This group includes predominantly bacteria belonging to the genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella*. Only *Escherichia coli* has the intestinal tract as its primary habitat, since the others are also found in other environments such as vegetation and soil. Consequently, the presence of total coliforms in food does not necessarily indicate recent fecal contamination.

Thermotolerant coliforms correspond to total coliforms that have the ability to continue fermenting lactose with gas production when incubated at temperatures of 44-45 °C. They are the closest to a fecal indication, however, the search for the presence of *Escherichia coli* is useful when it is desirable to determine whether there was fecal contamination (Franco, 2003).

Enterococci are very heat resistant and can survive pasteurization or heat treatment of milk. Enterococci are not usual indicators of fecal contamination, because they are found in other environments besides the intestinal tract, such as soil, vegetables and food, and also because they are more resistant, surviving unfavorable temperatures and the action of bactericides. Their presence in high numbers in foods indicates inadequate sanitary practices or exposure of food to conditions that allow their development (Franco, 2003).

The objective of this work was to evaluate the microbiological quality of various foods of animal origin by researching the most common indicator microorganisms, which are: mesophilic microorganisms, total coliforms, and thermotolerant coliforms.

2 METHODOLOGY

The samples consisted of five types of foods of animal origin, of different brands and found in retail supermarkets, as they were exposed for marketing, totaling 30 samples. The foods analyzed were: ground beef; chicken meat; fresh sausage; fresh Minas cheese (without inspection service identification); and bulk sausage.

Immediately after acquisition, the samples were transported in their commercial packages in isothermal boxes with ice to the Microbiology Laboratory of the Moura Lacerda University Center and immediately analyzed. The researches performed were the mesophilic bacteria count (except for minas frescal cheese) and determination of the Most Probable Number (MPN) of total and thermotolerant coliforms.

For the quantification of mesophilic bacteria, decimal dilutions of the samples were made and 1 ml of each dilution was added to sterile Petri dishes in duplicates. Next, 15 to 20 ml of standard counting agar (PCA) was poured at a temperature near 42 °C and then homogenized in a figure of eight ("8"). After solidification of the agar, the plates were inverted and incubated in a bacteriological oven at 35 °C for 48 hours. After the period, colonies were counted from the plates that presented between 25 and 250 colony forming units (CFU), whose result was multiplied by the dilution value to obtain the count per gram (g) of sample (Brazil, 2003).

For the determination of total coliforms, the multiple tube technique was used. Thus, 1 ml of each dilution was transferred to test tubes containing sodium lauryl sulfate broth with an inverted Durham tube in triplicates. After inoculation, the material was incubated in a bacteriological oven at 35 °C for 24 to 48 hours. In the presence of suggestive results (turbidity of the medium with gas bubbles inside the Durham tube), an aliquot was taken with the help of a seeding loop and transferred to bright green lactosate broth

with an inverted Durham tube. It was then incubated at 35 °C for 24 to 48 hours, and positive results were considered total coliforms. To determine the most probable number, a table was used for this purpose (Brazil, 2003).

For the determination of thermotolerant coliforms, the same procedure as for total coliforms was repeated, except that the medium used was EC broth (instead of lactated bile green brilliant broth), which was incubated in a water bath at 45 °C for 24 to 48 hours (Brazil, 2003).

3 RESULTS AND DISCUSSION

Thirty samples of food of animal origin were analyzed. The results obtained are shown in Table 1.

Table 1 - Results of samples taken from supermarkets in 2017.

SAMPLES	Mesophiles (CFU/g)	Total Coliforms (NMP/g)	Thermotolerant Coliforms (NMP/g)
Sausage 1	9,8x10 ²	<3,0	<3,0
Sausage 2	1,2x10 ³	<3,0	<3,0
Sausage 3	2,6x10 ³	<3,0	<3,0
Sausage 4	3,8x10 ³	<3,0	<3,0
Sausage 5	5,5x10 ³	<3,0	<3,0
Sausage 6	6,2x10 ³	3,6	3,6
Sausage 7	9,3x10 ³	3,6	<3,0
Sausage 8	1,0x10 ⁴	21	<3,0
Sausage 1	1,0x10 ³	<3,0	<3,0
Sausage 2	1,81x10 ³	3,6	<3,0
Sausage 3	1,1x10 ⁴	7,4	<3,0
Sausage 4	2,2x10 ⁴	<3,0	<3,0
Sausage 5	3,2x10 ⁴	3,6	<3,0
Sausage 6	4,2x10 ⁴	<3,0	<3,0
Sausage 7	2,9x10 ⁵	110	<3,0
Sausage 8	3,0x10 ⁵	46	<3,0
Chicken 1	5,8x10 ⁴	43	<3,0
Chicken 2	3,5x10 ⁵	4.300	<3,0
Chicken 3	3,2x10 ⁵	9,2	<3,0
Chicken 4	3,0x10 ⁶	2.300	<3,0
Chicken 5	3,4x10 ⁶	4.300	<3,0
Ground beef 1	1,2x10 ⁵	9,2	3,6
Ground beef 2	3,8x10 ⁵	150	<3,0
Ground beef 3	8,5x10 ⁶	>1.100	<3,0
Ground beef 4	4,4x10 ⁶	1400	<3,0
Ground beef 5	4,5x10 ⁷	15.000	<3,0
Cheese 1	-	>11.000.000	<3,0
Cheese 2	-	>11.000.000	<3,0
Cheese 3	-	>11.000.000	<3,0
Cheese 4	-	21.000	<3,0

Source: Authors.

The plate count method is a general method capable of identifying mesophilic aerobes when the temperature and incubation time are correct (Brazil, 2003). Jay (1998) explains that the procedure is based on the premise that each microbial cell present in a sample will form a separate, visible colony when fixed with medium that allows it to grow. Franco and Landgraf (1996) show that the plate count technique is the most widely used in food analysis laboratories. However, mesophilic microorganism counts are not

performed in cheeses, because lactic cultures are used in their manufacture, and therefore cheeses are naturally contaminated foods.

The criteria for the establishment of microbiological standards can be considered by the characterization of microorganisms and/or their toxins, by the classification of foods according to epidemiological risk, by the methods of analysis that allow the determination of microorganisms and other criteria, when scientific evidence justifies it. These microbiological standards show us the tolerable limits of microorganisms for a given food, condemning products that exceed this limit, ensuring the health of the community (Brazil, 2011).

Regarding the thermotolerant coliforms (which indicate a high chance of contamination by feces), it is observed that no sample showed a high population that disfavors the product.

The Sanitary Code of the State of São Paulo (São Paulo, 1992) determines, for some foods analyzed in the present work, that the microbiological characteristics are as follows:

Raw meat (beef, pork, chicken), prepared or not, offal:

- Standard plate count: $\leq 3.0 \times 10^6$ CFU/g
- Coliform bacteria of fecal origin (MPN): $\leq 3.0 \times 10^2$ /g²

Fresh prepared sausage meats:

- Standard plate count: $\leq 10^6$ CFU/g

For total and thermotolerant coliforms, ANVISA, by Resolution RDC No. 12, of January 2, 2001, determines that the maximum number should be 10^3 for all products in this work.

Table 1 shows the populations of mesophiles, total and thermotolerant coliforms found in the samples of food. It is found that:

- Mesophilic population:
 - Sausage: ranged from 10^2 to 10^4 CFU/g
 - Sausage: ranged from 10^3 to 10^5 CFU/g
 - Ground beef: ranged from 10^5 to 10^7 CFU/g
 - Chicken: ranged from 10^5 to 10^6 CFU/g.

We noticed that three samples of ground beef (60%) and two of chicken (40%) presented mesophilic population higher than 10^6 CFU/g, showing very poor hygienic-sanitary conditions of meat food, bringing risk to the consumer's health. In the sausage and frankfurter samples, the population can be considered low, mainly because they are cooked products (sausage), which eliminates many microorganisms that are more sensitive to heat, and because of the presence of nitrite and preservatives (frankfurter), which reduces the microbial multiplication rate.

- Total coliforms:
 - Sausage: ranged from <3.0 to 21 MPN/g

- Sausage: ranged from <math><3.0</math> to 110 MPN/g
- Ground beef ranged from 9.2 to 15,000 MPN/g
- Chicken: ranged from 9.2 to 4,300 MPN/g
- Cheese: ranged from 21000 to >11,000,000 MPN/g

It can be seen that in the sausage samples no large populations of total coliforms were found. Of the 30 samples analyzed, eight (27%) did not meet the legislation (Anvisa, 2001). In the sausage samples, 7 and 8 indicated a higher population when compared to the sausage samples. It can be observed that in three samples of chicken breast (60%), three of ground beef (60%) and all of cheese, the total coliform population was higher than 1000 NMP/g, which is considered very high, potentially dangerous and unfeasible for consumption.

The following figures indicate the frequency of foods considered contaminated, classified as "disapproved or "approved", using the microbiological limit of 10^6 CFU/g for mesophiles and 1000 MPN/g for total and thermotolerant coliforms, based on the Resolution RDC No. 12 of January 2, 2001 and the sanitary code of the State of São Paulo of 1992.

Figure 1 - Ratio between approved and failed sausage samples for mesophiles.



Source: Authors.

Carvalho et al. (2005) found results for mesophiles in sausage samples ranging from $<1.0 \times 10^1$ to 2.0×10^2 CFU/g. In this study, the results were higher, ranging from 9.8×10^2 to 1.0×10^4 (Figure 1). Cardoso et al. (2000) did not detect the presence of mesophiles in sausages produced in slaughterhouses in the city of Descalvado/SP.

Figure 2 - Ratio between approved and failed sausage samples for total and thermotolerant coliforms.



Source: Authors.

Similar to this research (Figure 2), Carvalho et al. (2005) also did not detect large populations of total and thermotolerant coliforms. Cardoso et al. (2000) also did not detect the presence of thermotolerant coliforms, but 25% of their samples were positive for total coliforms.

Figure 3 - Ratio between approved and disapproved sausage samples for mesophiles.



Source: Authors.

In Figure 3, the values obtained are lower than those found in the analyses performed by Sabioni et al. (1999), in that most of the samples exceeded 10^6 CFU/g, and were also lower than those obtained by Falcão et al., cited by Sabioni et al. (1999), in which 90% of the samples exceeded 10^6 CFU/g. Silva (2002) found in sausage a minimum of 2.9×10^5 , the maximum number found in the present study. Carvalho et al. (2005) detected equivalent values to this work, with results between $8,0 \times 10^2$ to $6,5 \times 10^4$. Cardoso et al. (2000) did not detect mesophiles in their study.

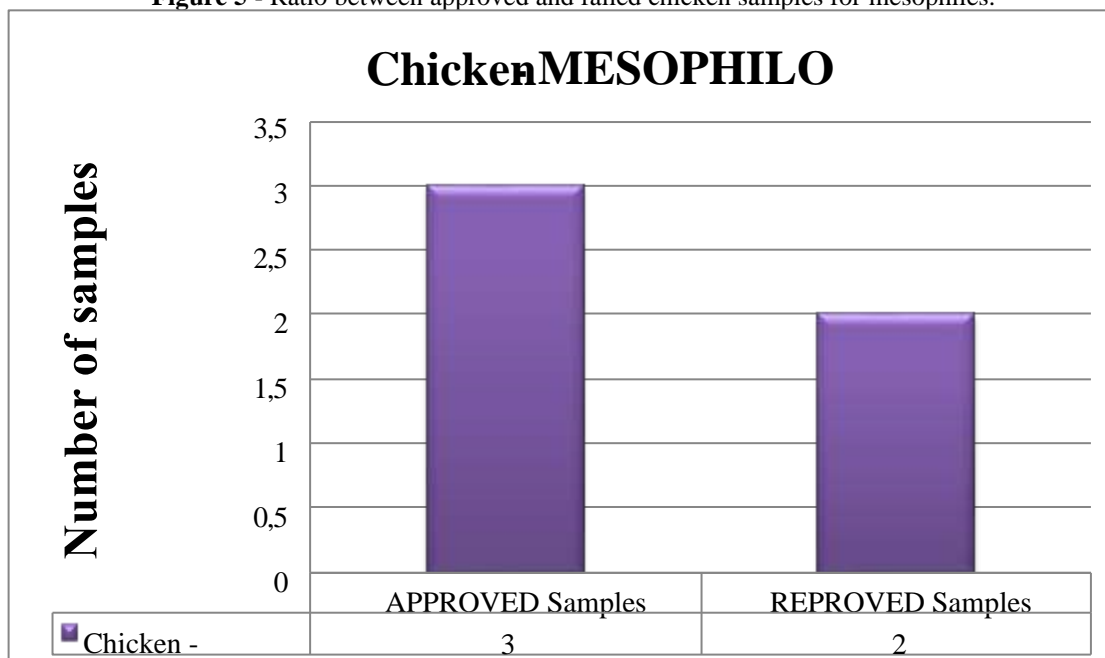
Figure 4 - Ratio between approved and disapproved sausage samples for total and thermotolerant coliforms.



Source: Authors.

Sabioni et al. (1999) found more than 80% of the fresh sausage samples had counts of total coliforms lower than 10^5 NMP/g, in the present study, all samples presented values below 10^5 NMP/g (Figure 4). These values are within the range found by Vasconcelos and Iaria (1991), 2.3×10^1 to 9.3×10^4 NMP/g. Silva (2002) found values below 10^2 NMP/g, values equal to those found in the present research. Carvalho et al. (2005) detected higher values for total coliforms (TC) and thermotolerant coliforms (TTC) when compared to this study, reaching 1.1×10^5 for TC and 4.6×10^4 for TTC. Cardoso et al. (2000) did not detect thermotolerant coliforms in sausages, but 12.5% of the samples were positive for CT.

Figure 5 - Ratio between approved and failed chicken samples for mesophiles.

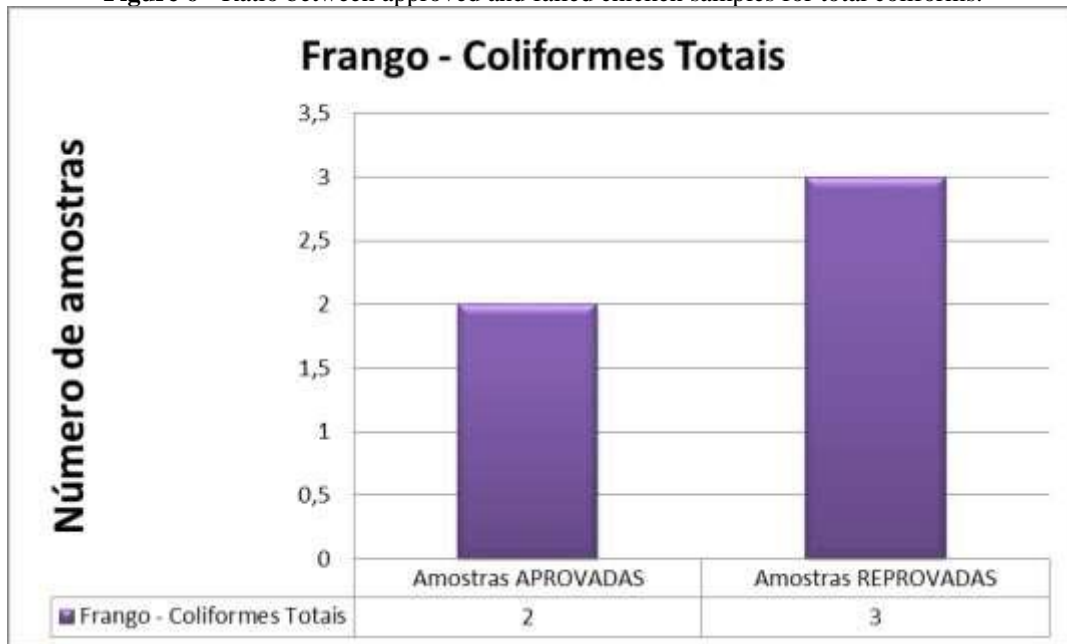


Source: Authors.

The mesophil results obtained in chickens by Hoffmann et al. (1995) ranged from 5.8×10^3 to 2.3×10^4 CFU/g. Silva (2002) found results between 2.0×10^6 and 3.0×10^6 . Therefore, it can be concluded that in the present study, the results found were higher than those of the first author and three samples were lower than

those of the second author cited (Figure 5). The results obtained by Hoffmann et al. (1995), are within the acceptable limit of 10^6 , which is not the case with those of Silva (2002). Cardoso et al. (2000) did not detect mesophiles in broiler chickens.

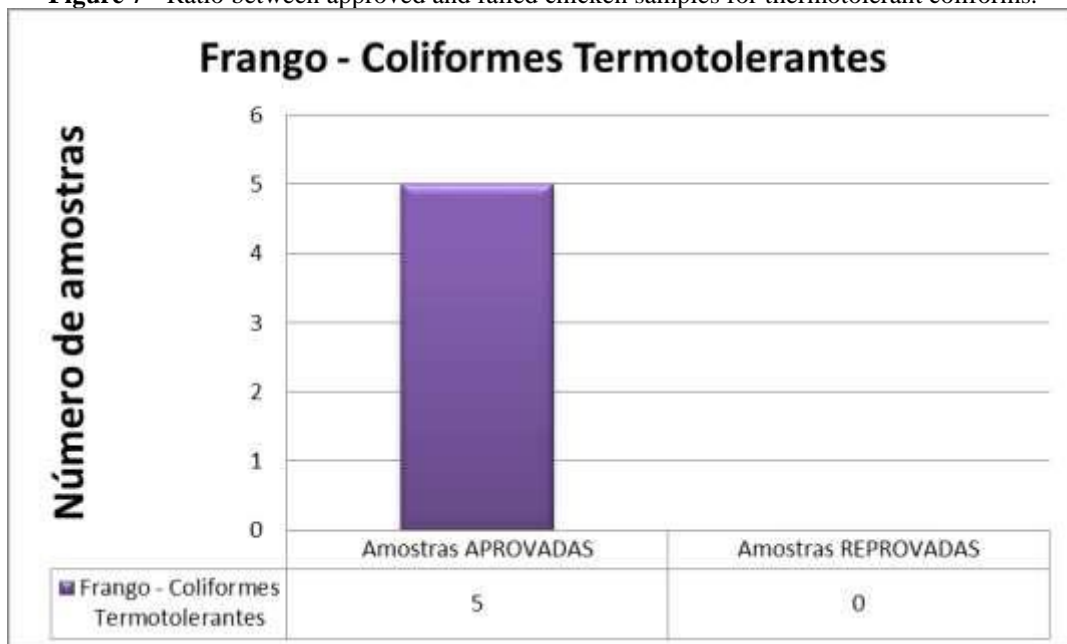
Figure 6 - Ratio between approved and failed chicken samples for total coliforms.



Source: Authors.

The values obtained in two samples in the present work were close to those obtained by Hoffmann et al. (1995) which were within the range of 9.0 MPN to 9.3×10^1 MPN/g, the values obtained by Vieira and Teixeira (1997) in chicken were 2.3×10^1 to 2.4×10^3 MPN/g, close to the sample of this work, two samples were higher than 2.4×10^3 MPN/g (Figure 6).

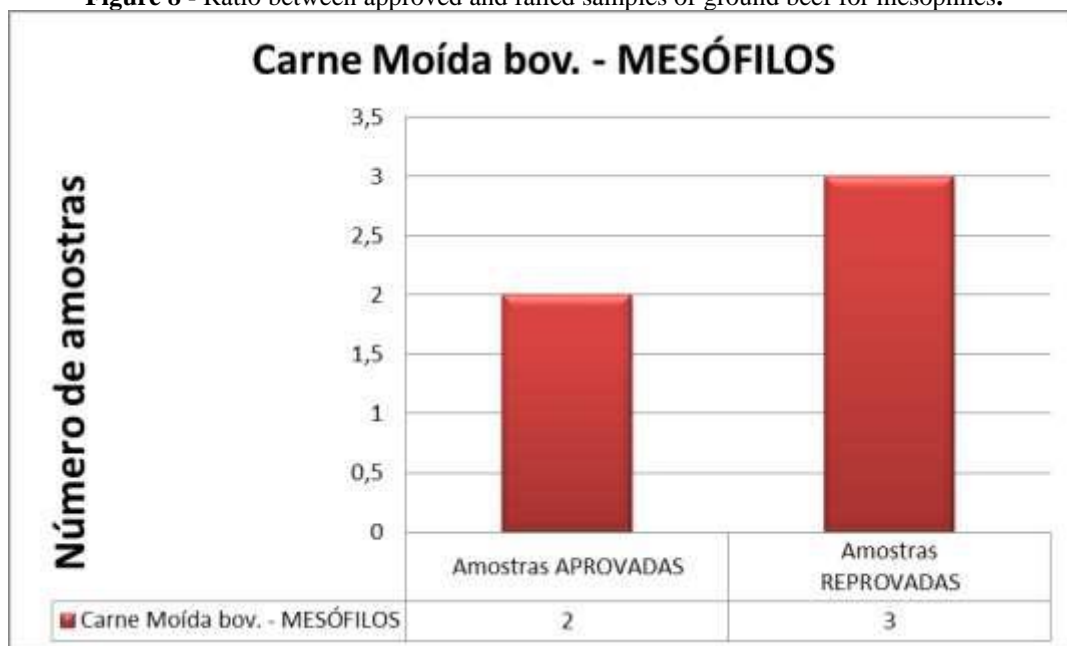
Figure 7 - Ratio between approved and failed chicken samples for thermotolerant coliforms.



Source: Authors.

Cardoso et al. (2000), researching in slaughterhouses, did not observe the presence of thermotolerant coliforms in chicken meat, as in this research, indicating good handling practices and extinguishing any chance of contamination by feces (Figure 7)

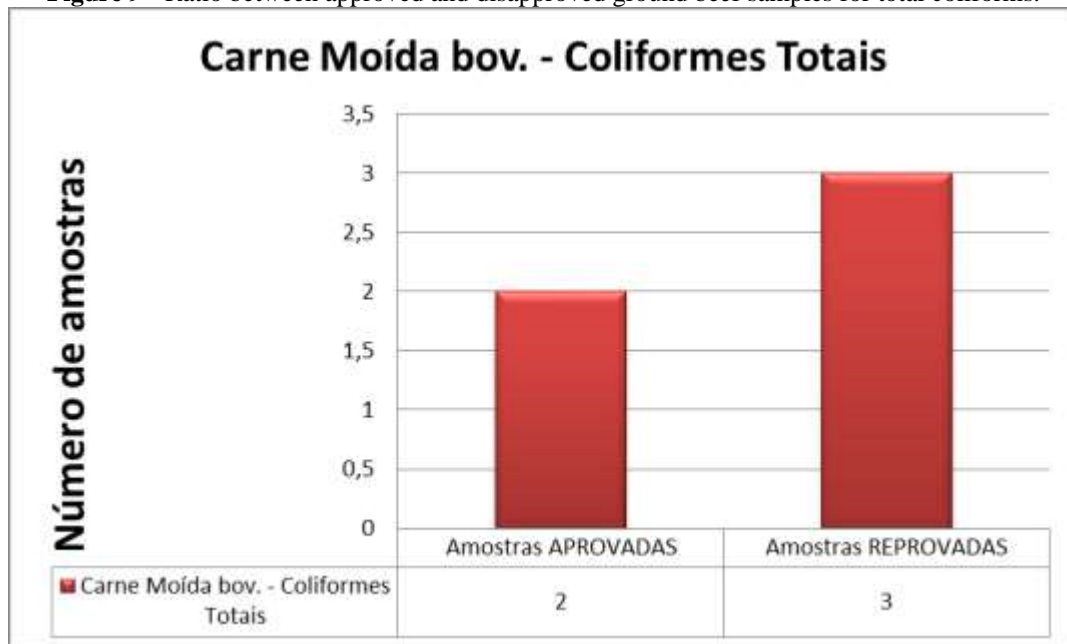
Figure 8 - Ratio between approved and failed samples of ground beef for mesophiles.



Source: Authors.

Rossi Jr. et al. (1990) found in beef, counts of 8.1×10^3 CFU/g and 6.9×10^3 CFU/g, while Hoffmann et al. (1998) found counts of 1.0×10^3 to 4.6×10^3 CFU/g in ground beef. These values are lower than those of the present work (Figure 8). Silva (2002) found results close to those of the present study, using the same methodology. Cipriano (2021) compared aerobic mesophilic microorganism counts between hypermarkets and neighborhood markets, for hypermarkets the minimum value was 2.9×10^5 CFU/25g and the maximum 1.3×10^7 CFU/25g. While in neighborhood markets the minimum value was 2.8×10^5 CFU/25g and the maximum was 4.4×10^7 CFU/25g.

Figure 9 - Ratio between approved and disapproved ground beef samples for total coliforms.



Source: Authors.

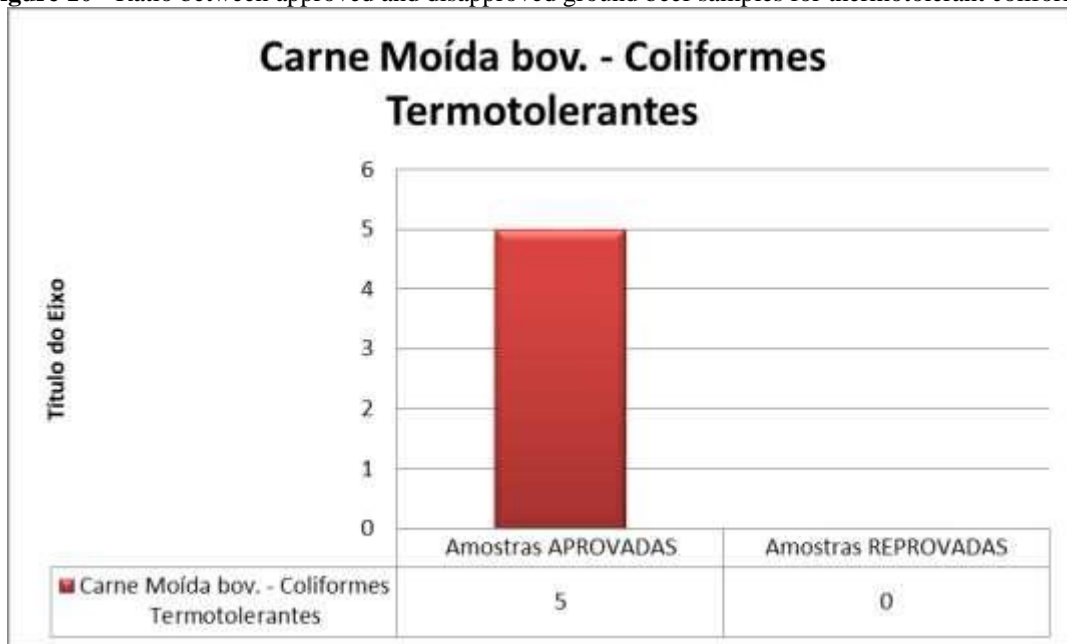
Hoffmann et al. (1998), for ground beef, found from 4.0 MPN to 75 MPN/g in the samples analyzed, values are close to two of the samples in this study. According to Silva (2002), meat has its microbial load increased as it goes through new cuts or mincing and, therefore, the values found in the present study for samples of beef in a single cut are considered high when compared to the values found by the cited researchers (Figure 9).

Rossi et al. (1990) found lower counts for total coliforms, 59 NMP/g (table deboning) and 35 NMP/g (aerial deboning). Almost half of the beef samples analyzed by Tanaka et al. (1997) had values between 10 and 1000 MPN/g, while approximately 40% were above 1000 MPN/g, similar to the present study.

Hangui et al. (2015) observed that for thermotolerant coliforms, 100% of the samples presented values below the limit established by law, which is 10^3 NMP/g. The highest value found was 2.6×10^2 NMP/g. This does not happen in the present study, because all samples presented values lower than 3.6 MPN/g (Figure 10).

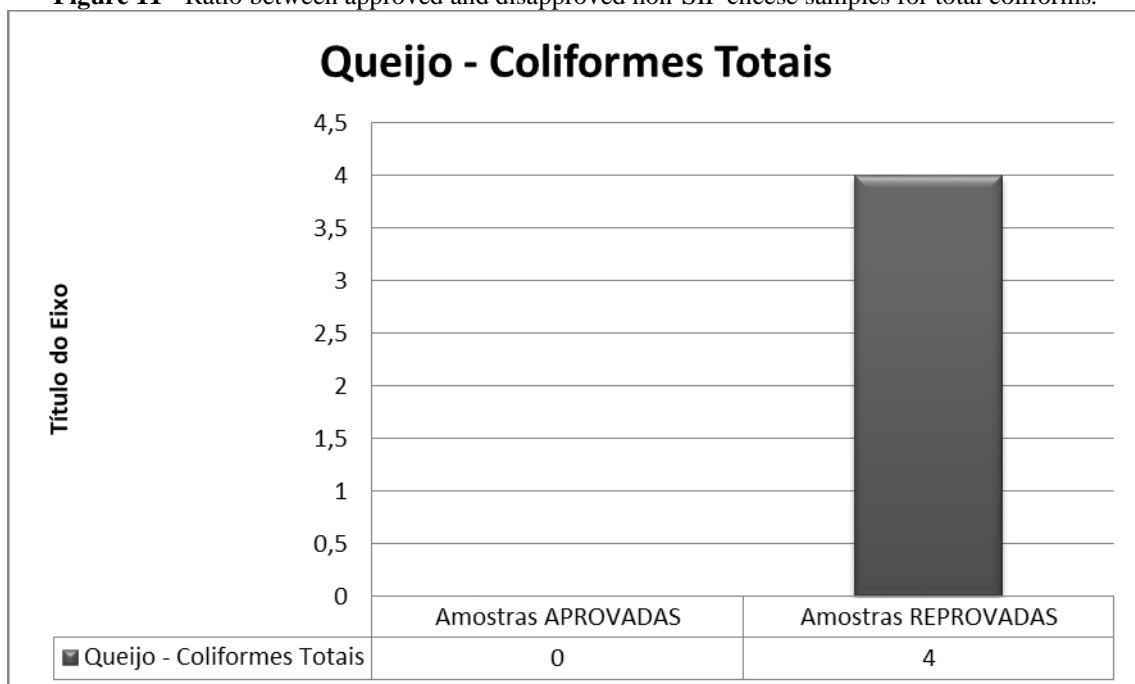
Nascimento et al. (1985) also found from absence, as Silva (2002) also found, to the value maximum of 1.1×10^8 NMP/g. Therefore, the samples of the present study are in agreement with the author mentioned above (Figure 11). Santana et al. (2008) found an average value of 1.07×10^3 , which is much lower than the value found in the present study. The hygienic-sanitary quality of fresh Minas cheese, produced by hand and sold in street markets, is very precarious, constituting risks to consumer health due to inadequate quality of raw materials and/or improper conditions of processing, storage and marketing (Ferreira et al., 2011).

Figure 10 - Ratio between approved and disapproved ground beef samples for thermotolerant coliforms.



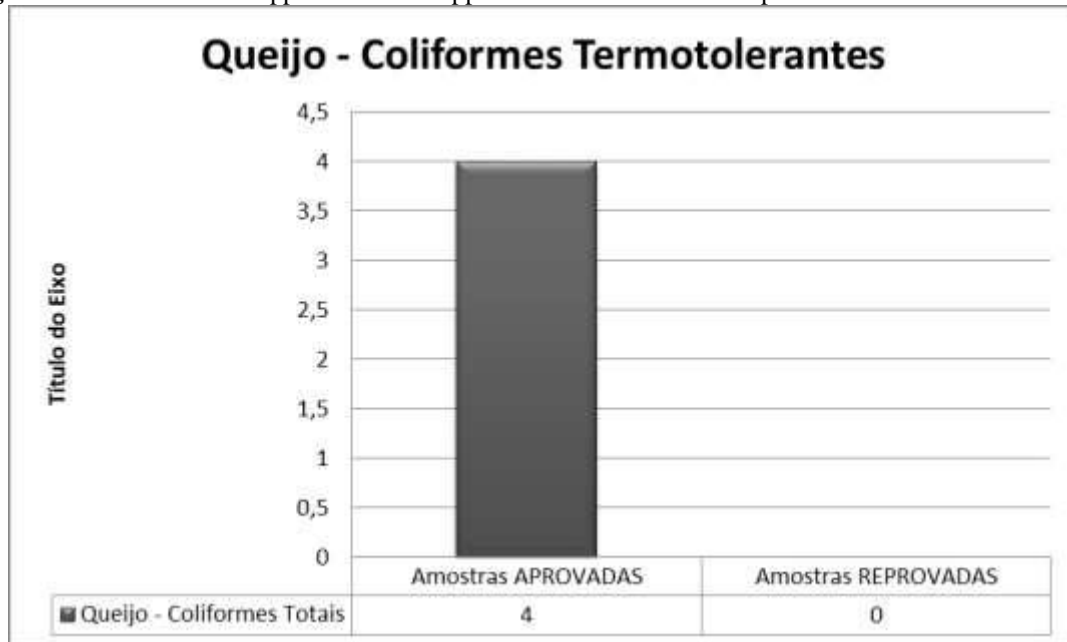
Source: Authors.

Figure 11 - Ratio between approved and disapproved non-SIF cheese samples for total coliforms.



Source: Authors.

Figure 12 - Ratio between approved and disapproved non-SIF cheese samples for thermotolerant coliforms.



Source: Authors.

Ferreira et al. (2011) found in their research values of $< 2.0 \times 10^2$ to $\geq 1.1 \times 10^4$ NMP/g for coliforms, Santana et al. (2008) found a mean value of 8.58×10^2 , values different from the present study, where no sample was detected positive for this microorganism (Figure 12).

Based on all the exposed results, it is suggested that training on contamination and hygiene should be done periodically to learn the manipulators who will work in the food sector, as well as the rules of this training should be adopted by the collaborators and carried out rigorously. The commitment of the team in the food sector is essential for the goal to be achieved and for the good handling practices to be successful. Through these educational ways, the food obtained by consumers in supermarkets will have its microbiological load reduced, making quality and healthy food, free of pathogens.

Unfortunately, the legislation that limits the amount of mesophiles in food is state legislation, leaving it up to each state to define the tolerable microbial load, which can create doubts regarding the limit to be used by each company and can also serve as a mechanism to circumvent the law, taking advantage of this variation of microbial load that each state defines. The National Health Surveillance Agency, which is a federal agency, by Resolution RDC No 12, January 2, 2001, says that meat foods should only be free of *Salmonella* and should have a limit of 10^3 of total and thermotolerant coliforms. It is then up to the health authorities a better supervision and orientation of food handlers to ensure public health.

3 CONCLUSION

Based on the results obtained and the conditions in which the present work was carried out, it can be concluded that not all foods offered to consumers present adequate hygienic-sanitary quality. It is up to the authorities to develop efficient laws and constant inspection of the food exposed for sale to ensure

food safety. Furthermore, it is concluded that more processed foods present less microbial contamination than unprocessed ones.

It is necessary to have more research lines in the area of food inspection, which assesses the entire production process, from obtaining the raw material to the arrival of the final consumer, both products and by-products of animal origin that are marketed, to evaluate the procedure performed and the hygienic-sanitary quality of the final product, in order to ensure food safety to those who will consume it.

REFERENCES

- Brasil (2003). Instrução Normativa nº 62 de 26 de agosto de 2003, Métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água. Diário Oficial [da União], Brasília, seção1, p.14. <http://extranet.agricultura.gov.br/sislegis-consulta/servlet/VisualizarAnexo?id=6078>
- Brasil (2001). Resolução RDC nº 12, de 2 de janeiro de 2001. Aprova o “Regulamento técnico sobre padrões microbiológicos para alimentos”. Órgão emissor: ANVISA-Agência Nacional de Vigilância Sanitária. www.anvisa.gov.br =>.
- Cardoso, A. L. S. P., Tessari, E. N. C., Castro A. G. M. & Kanashiro, A. M. I. (2000). Pesquisa de Salmonella SP., coliformes totais, coliformes fecais e mesófilos em carcaças e produtos derivados de frango. *Arquivo do Instituto de Biologia*, 67(1), 25-30.
- Carvalho, A. C. F. B., Cortez, A. L. L., Salotti, B. M., Burger, K. P. & Vidal-Martins, A. M. C. (2005). Presença de microrganismos mesófilos, psicrotróficos e coliformes em diferentes amostras de produtos avícolas. *Arquivo do Instituto de Biologia*, 72(3), 303-307.
- Cipriano, L. C., de Sousa, L. B., de Godoy Siqueira, H. P., Lima, E. F., Messias, C. T., de Marchi, P. G. F., de Medeiros, E. S., Hoppe, I. B. A. L. & de Siqueira, AB (2021). Vida útil de carne bovina moída comercializada no Município de Boa Vista – Roraima. *Research, Society and Development*, 10(2), e19010212282-e19010212282.
- Doyle, M. P., Beuchat, L. R. & Montville, T. J. (1997). *Food Microbiology: Fundamentals and Frontiers*, Editora.
- Ferreira, H., Lima, H & Coelho, T. (2014). Microrganismos indicadores em alimentos de origem animal. Programa de Pós-graduação em Ciência Animal – PPGCA da Universidade Federal Rural do Semiárido – UFERSA. <http://www2.ufersa.edu.br/portal/view/uploads/setores/126/Resumo%20MO%20indicadores.%20Heider,%20Hiagos,%20Thiago.pdf>
- Ferreira, M. S. F. (2006). Contaminação de alimentos ocasionada por manipuladores. Programa de Pós-graduação Lato Sensu, Curso de Especialização em Qualidade em Alimento, Universidade de Brasília.
- Ferreira, R. M., Spini, J. D. C. M., Carrazza, L. G., Sant’ana, D. S., de Oliveira, M. T., Alves, L. R. & Carrazza, T. G. (2011). Quantificação de coliformes totais e termotolerantes em queijo Minas Frescal artesanal. *PUBVET*, 5 (5), Ed. 152, Art. 1022.
- Franco, B. D. G. M & Landgraf, M. (1996). *Microbiologia de alimentos*. Atheneu.
- Franco, B. D. G. M. (2003). *Microbiologia dos Alimentos*. Atheneu.
- Franco, R. M. & Almeida, L. E. F. (1992). Avaliação microbiológica de queijo ralado, tipo parmesão, comercializado em Niterói, RJ. *Revista Higiene Alimentar*, 6(21), 33-36.
- Freitas, L. H. (1995). Sistema especialista para diagnóstico de toxinfecções alimentares de origem bacteriana. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) - Universidade Federal de Viçosa, Viçosa.
- Hangui, S. A. R., Pereira, A. F., Dourado, A. T. D. S., Martins, J. D., Vargem, D. D. S. & da Silva, J. R. (2015). Análise microbiológica da carne moída comercializada na cidade de Anápolis, Goiás, Brasil. *Revista Eletrônica de Farmácia, Anápolis* REF-ISSN1808-0804, 12(2), 30–38.
- Hattori, N. A & Klaus, I. C. (2013). Avaliação microbiológica e higiênico-sanitária em uma panificadora no município de Missal-PR. Paraná. Trabalho de conclusão de curso da Universidade Tecnológica Federal do Paraná, curso de Tecnologia em Alimentos, campus Medianeira.
- Hayes, P. R. (1995). *Food microbiology and hygiene*. (2a ed.), Chapman and Hall.
- Hoffmann, F. L., Garcia-Cruz, C. H. & Vinturim, T. M. (1995). Estudo higiênico sanitário de frangos comercializados na cidade de São José do Rio Preto-SP. *Higiene Alimentar*, 9(35), 31-33.
- Hoffmann, F. L., Garcia-Cruz, C. H & Vinturim, T. M. (1998). Qualidade microbiológica de amostras de carnes e de presunto. *Higiene Alimentar*, 12(58), 52-57.
- ICMSF (International Commission On Microbiological Specifications For Foods) (1994). *Microrganismos de los alimentos*. 1. Técnicas de análisis microbiológico. Acribia.
- Jay, J. M. (1998). *Modern food microbiology*. (5a ed.), Aspen Publishers.

Nascimento, D., Sabioni, J. G., Pimenta, N. & Xandó, S. R. (1985). Avaliação microbiológica de queijos tipo Minas–frescal da cidade de Ouro Preto-MG. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, 19(2), 120-129.

Oliveira, D. T., Moreira, A., Urnau, L., Noskoski, L. & Cereser, N. D. (2012). Psicotróficos na indústria de laticínios. XV Amostra de Iniciação Científica. UNICRUZ, RS.

Pinheiro, M. B., Wada, T. C. & Pereira, C. A. M. (2010). Análise microbiológica de tábuas de manipulação de alimentos de uma instituição de ensino superior em São Carlos-SP. São Carlos, SP. Centro Universitário Central Paulista (UNICEP).

Ribeiro, F. B. (2008). Avaliação da contribuição para a área de Vigilância Sanitária de Alimentos de pesquisas realizadas em Programas de Pós-Graduação Strictu Sensu da Universidade de São Paulo. São Paulo. Dissertação (Mestrado) - Faculdade de Saúde Pública da Universidade de São Paulo.

Rossi, J. R. O. D., Iaria, S. T., Santos, I. F. & Berchieri, J. R. A. (1990). Carne mecanicamente separada de origem bovina: I – Influência de dois sistemas de desossa manual sobre as características microbiológicas do produto recém obtido. *Revista de Microbiologia*, 21(4), 324-330.

Sabioni, J. G., Maia, A. R. P. & Leal, J. A. (1999). Avaliação microbiológica de linguiça frescal comercializada na cidade de Ouro Preto – MG. *Higiene Alimentar*, 13(61), 110-113.

Santana, R. F., Santos, D. M., Martinez, A. C. C. & Lima Á. S (2008). Qualidade microbiológica de queijo-coalho comercializado em Aracaju, SE. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, 60(6), 1517-1522.

São Paulo (Estado) (1992). Secretaria da Saúde. Código sanitário: Decreto no 12.342 de 27 de setembro de 1978: regulamento da promoção e recuperação da saúde no campo da competência da Secretaria de Estado da Saúde (revisto e atualizado até dezembro de 1990). (5a ed.), IMESP.

Silva, M. C. (2002). Avaliação da qualidade microbiológica de alimentos com a utilização de metodologias convencionais e do sistema SimPlate. Dissertação (Mestrado) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo.

Souza, R. R., Germano, P. M. L. & Germano, M. I. S. (2003). A manipulação inadequada dos alimentos: fator de contaminação. *Revista Higiene Alimentar*.

Vasconcelos, J. C. & Iaria, S. T. (1991). Condições microbiológicas (higiênico-sanitárias) das linguiças frescas comercializadas em feiras livres no município de São Paulo - SP. Indicações de contaminação. *Boletim do Centro de Pesquisa e Processamento de Alimentos*, 9(1), 64-75.

Vieira, C. R. N & Teixeira, C. G. (1997). Condições higiênico-sanitárias de carcaças resfriadas de frango comercializadas em Poços de Caldas – MG. *Higiene Alimentar*, 11(48), 36-40.