MICROBIOLOGICAL ASSESSMENT AND PH MEASUREMENT IN GROUND BEEF AVAILABLE IN SUPERMARKETS IN SOUTHERN BAHIA

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ABSTRACT - Assessment of the microbiological quality and pH of foods available in supermarkets is vital to guarantee food safety, offering consumers essential information about the integrity and freshness of products. Therefore, the objective of this study was to evaluate the microbiological quality and pH of the beef most sold in supermarkets in southern Bahia. Forty supermarkets that sell fresh ground beef were randomly selected in four counties in the south of the state of Bahia, Brazil, to collect and prepare "ground beef" samples. 20 simple samples of 100 g of ground meat from non-specific cuts were collected (different cuts in the ground mixture, e.g.: duckling, shoulder, tough leg, chuck, breast or flank steak), as is common in regional trade, collected in each of the establishments. Researched, totaling 800 simple samples. The simple samples were homogenized, constituting a composite sample per establishment, totaling 40 samples, which were then identified and transported to the Laboratory in isothermal boxes, properly refrigerated. Once the samples were in possession, the pH was measured and microbiological analyzes were carried out, using techniques to identify mesophilic aerobic bacteria, molds and yeasts. The most likely number of total coliforms ranges from 21 to 2.7×10^4 MLN g^{-1} (average 3.5×10^3 MLN g^{-1}), and coliforms at 45ºC or thermotolerant <3 to 5×10^3 MLN g^{-1} (average 1.3×10^3 MLN g^{-1}). The presence of *Escherichia coli* was confirmed in 70% of the samples. No *Salmonella* was detected in any of the samples. Most samples were within the standards determined by current legislation, with the absence of *Salmonella* in 25g of sample. The average PAN of coliforms at 35°C and 45°C complied with national and international legislation, however, the verification of the presence of *E. coli* in 16 samples (40% occurrence) shows a medium risk of colibacillosis occurrence transmitted by meat. Even so, the risk of transmission of these diseases cannot be rejected, since the presence of *E. coli* is independent of the number of coliforms and the national legal standards established for the group of thermotolerant coliforms. Regarding the pH, one sample (2.5%) showed characteristics of beginning of decomposition, as it had a pH above 6.4.
Keywords: microbiological quality, pH value, beef *in natura* ground, hygienic-sanitary conditions.

EVALUACIÓN MICROBIOLÓGICA Y MEDICIÓN DEL PH EN CARNE MOLIDA DISPONIBLE EN SUPERMERCADOS DEL SUR DE BAHÍA.

RESUMEN - La evaluación de la calidad microbiológica y el pH de los alimentos disponibles en los supermercados es vital para garantizar la seguridad alimentaria, ofreciendo a los consumidores información esencial sobre la integridad y frescura de los productos. Por tanto, el objetivo de este estudio fue evaluar la calidad microbiológica y el pH de la carne molida de res vendida en los supermercados del sur de Bahía. Se seleccionaron aleatoriamente 40 supermercados que venden carne molida fresca en 4 municipios del sur del estado de Bahía, Brasil, para recolectar y preparar muestras de "carne molida". Se recolectaron 20 muestras simples de 100 g de "carne molida" de cortes no específicos (diferentes cortes en un mismo molido, por ejemplo: patito, paleta, pierna dura, espaldilla, pechuga o arrachera), como es común en el comercio regional. , recolectadas en cada uno de los establecimientos encuestados, totalizando 800 muestras simples. Las muestras simples fueron homogeneizadas, constituyendo una muestra compuesta por establecimiento, totalizando 40 muestras, las cuales luego fueron identificadas y transportadas al Laboratorio en cajas isotérmicas, debidamente refrigeradas. Una vez en posesión de las muestras, se midió el pH y se realizaron análisis microbiológicos, utilizando técnicas de identificación de bacterias aerobias mesófilas, mohos y levaduras. El número más probable de coliformes totales osciló entre 21 y $2,7 \times 10^4$ NMP g^{-1} (promedio $3,5 \times 10^3$ NMP g^{-1}), y coliformes a 45ºC o termotolerantes de <3 a 5×10^3 NMP g^{-1} (promedio $1,3 \times 10^3$ NMP g^{-1}). g-1). Se confirmó la presencia de *Escherichia coli* en el 70% de las muestras. No se detectó la presencia de Salmonella en ninguna de las muestras. La mayoría de las muestras estuvieron dentro de los estándares determinados por la legislación vigente, observándose ausencia de Salmonella en 25g de muestra. El NMP promedio de coliformes a 35°C y 45°C cumplió con la legislación nacional e internacional, sin embargo, la verificación de la presencia de E. coli en 16 muestras (40% de ocurrencia) muestra un riesgo medio de ocurrencia de colibacilosis transmitida por la carne. Aun así, no se puede descartar el riesgo de transmisión de estas enfermedades, ya que la presencia de E. coli es independiente del número de coliformes y de los estándares legales nacionales establecidos para el

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grupo de coliformes termotolerantes. En cuanto al pH, una muestra (2,5%) presentó características de inicio de descomposición, al tener un pH superior a 6,4.
Palabras-clave: calidad microbiológica, valor de pH, carne molida fresca, condiciones higiénico-sanitarias.

INTRODUCTION

Beef is one of the most consumed foods of animal origin by the Brazilian population, due to its flavor and nutritional potential (ALVES et al., 2011). Among the meat products, ground beef is a food that stands out among the others, since it is well accepted by the consumer and is one of the most consumed, due to its practicality, affordable price and can be used in different ways in cooking (DEMINICIS et al., 2023; MENDONÇA and SILVA, 2012).

However, beef can be easily contaminated by microorganisms and its handling during processing can represent an increase in spoilage counts, in addition to having a high potential for pathogen proliferation, as it is obtained from other pieces of meat already handled and sometimes due to exposure to inadequate temperature, high amount of water and favorable pH, making it easy to be contaminated by microorganisms and pathogens such as *Salmonella*, *Stapylococcus aureus* and *Escherichia coli* (SOARES et al, 2015; GOMES et al., 2017). These pathogens are the main responsible for public health problems derived from food contamination, which is a constant concern for health agencies (WHO, 2018).

In this way, the importance of analyzing the microbiological quality of this type of meat is emphasized, as it is a product widely consumed by the population, and because it poses a risk to consumers, given that it is a food possibly, but not necessarily, of greater contact surface and coming from the grinding of more than one type of meat cut.

Meat quality is a broad and complex term that can be defined as a combination of characteristics that account for the product as a whole (RAMOS and GOMIDE, 2012). ANVISA Resolution RCD n° 12/2001 (BRASIL, 2001) recommends the absence of *Salmonella* sp. in 25 g. However, Brazilian legislation does not have standards for total coliforms and Escherichia coli, nor does ANVISA Resolution - Resolution of the Collegiate Board of Directors - RDC n°126 establish indicators for these microorganisms, but the Sanitary Code of the State of São Paulo regulates the standards for coliforms at 45°C or maximum thermotolerants of 3.0×10^2 g^{-1}. In this work, the microbiological quality and pH of fresh ground beef sold in supermarkets in the extreme south of the state of Bahia were evaluated.

MATERIAL AND METHODS

We randomly selected 40 supermarkets that sell ground beef in natura, which are registered with Visa in the respective municipalities of Teixeira de Freitas, Eunápolis and Itabuna and Ilhéus, between May 2016 and April 2018. checklist (check list) based on ANVISA Resolutions n° 275/2002 and n° 216/2004. The data obtained in the checklist were tabulated in the SPSS version 17 statistical program, and a descriptive analysis of the data was performed.

Based on the legal basis of Resolution ANVISA RCD No. 216/2004 (BRASIL, 2004), corresponding to the Good Practices for Food Services and SVS/Ministry of Health Ordinance No. 326/1997 (BRASIL, 1997a) - Corresponding to the General Principles of Hygiene for food, the practical application of the inspection form for butchers and fishmongers was carried out. An "Indispensable" item is considered to be one that meets good manufacturing and control practices, which can have a critical influence on the quality or safety of products and processes. "Necessary" is the one that meets and that can influence in a less critical degree the quality or safety of products and processes. "Recommended" is the one that meets and that may reflect in a non-critical degree on the quality or safety of products and processes.

In the period from April 2018 to May 2019, 20 simple samples of 100 g of "ground beef" were collected from non-specific cuts (different cuts in the same grind, example: duckling, shoulder, hard thigh, chuck, breast or diaper), as is common in regional commerce, collected in each of the surveyed establishments, totaling 800 simple samples. The simple samples were homogenized, constituting a sample composed by establishment, totaling 40 samples, which were then identified and transported to the Laboratory in isothermal boxes, properly refrigerated.

In the laboratory, they were homogenized and diluted using 0.1% peptone saline solution. The analyzes were initiated soon after the reception of the samples, using the techniques recommended by Vanderzan and Splittstoesser (1992). Mesophilic aerobic bacteria, molds and yeasts, determination of the Most Probable Number (MPN) of total coliforms and Coliforms at 45°C or thermotolerant, *Escherichia coli*, *Salmonella* and coagulase-positive *Staphylococcus* count were performed. To determine the pH, 10 g of each sample were used, and the sample was diluted in a beaker with the aid of 100 mL of water, the contents being stirred until the particles were uniformly suspended. Then the pH was measured using a digital potentiometer (Digimed DM-22), previously calibrated with pH 4 and 7 buffer solutions (IAL, 2005).

RESULTS AND DISCUSSION

The results obtained in the survey (Table 1) reveal that in 100% of the samples the presence of total coliforms was verified, with 6 samples (60%) presenting counts above 1.1×10^3 MPN g^{-1}. The presence of mesophilic bacteria was observed in 100% of the samples, with levels that did not exceed 104 CFU g^{-1}.

As for the levels of molds and yeasts found in the samples, 90% had a level equal to 10^3 CFU g^{-1} and only samples 5, 23 and 29 had a level of 6.4×10^4 CFU g^{-1}. The Sanitary Code of the state of São Paulo (SÃO PAULO, 1998), establishes as a standard for molds and yeasts in fresh meat, the acceptable maximum of 10^3 CFU g^{-1}. According to this legislation, 90% of the samples surveyed in this work

are in accordance with the Special Technical Norms Relative to Food and Beverages for the State of São Paulo (SÃO PAULO, 1978).

The results for positive *Staphylococcus coagulase* were <100 CFU g^{-1} in all samples analyzed, however the presence of *Salmonella* was not detected. As for *Escherichia coli*, its presence was confirmed in 76% of the samples, and as for Coliforms at 45ºC or thermotolerant, all samples (100%) contained the microorganism. As for pH values, 90% of the samples complied with Brazilian legislation for this parameter (BRASIL, 2017), considering that a pH of 6.4 is the critical limit for meat consumption (BRASIL, 1997b).

In 100% of the samples, the presence of total coliforms was verified and 60% had a count above 1.1 x 10^3

MPN g^{-1}, a result also found by Arçari et al. (2011), in which analyzing 25 samples of ground beef from five supermarkets in the city of Vitória (Espírito Santo State, Brazil), they found positive results in 100% of the samples. However, Brazilian legislation does not establish acceptable limits for the group of total coliforms in ground beef, but the presence of this microorganism shows that the hygienic-sanitary conditions are deficient. Which puts the health of consumers of these products at risk. This indicator of coliforms in ground meat is probably linked to the factor of inadequate refrigeration and also the exposure of contaminated meat at room temperature (FERREIRA and SIMM, 2012).

TABLE 1 - Microbiological analyzes and pH of ground beef sold in supermarkets in Teixeira de Freitas, Eunápolis, Itabuna and Ilhéus (BA), between 2018-2019.

Sample	*Staphylococcus* coagulase	*Escherichia coli*	*Salmonella*	Total coliforms PAN	Fecal coliforms PAN	Mesophilic bacteria UFC g^{-1}	Molds and yeasts UFC g^{-1}	pH
1	<100	A	A	3.5 x 10	5 x 10^3	9.0 x 10^4	2.7 x 10^3	6.1
2	<100	2 x 10	A	3.1 x 10^3	4.6 x 10^2	6.7 x 10^4	<10	5.8
3	<100	A	A	2.7 x 10^4	2.8 x 10^3	7.4 x 10^4	<10	5.9
4	<100	7 x 10	A	4.6 x 10^2	4.6 x 10^2	2.4 x 10^4	1.7 x 10^3	6.4
5	<100	4.6 x 10	A	2.4 x 10^3	2.4 x 10^3	2.7 x 10^4	6.4 x 10^4	6.4
6	<100	2 x 10	A	93	<3	8.7 x 10^4	8.7 x 10^2	6.2
7	<100	A	A	2.4 x 10^3	2.4 x 10^3	1.0 x 10^4	<10	6.4
8	<100	7 x 10	A	35	38	4.8 x 10^4	3.8 x 10^3	6.3
9	<100	< 10	A	36	21	4.3 x 10^4	<10	5.9
10	<100	<10	A	21	6.1	6.7 x 10^4	6.5 x 10^2	6.1
11	<100	A	A	3.0	9.2	3.0 x 10^4	2.7 x 10^3	6.1
12	<100	A	A	3.6	<3.0	4.7 x 10^4	1.8 x 10^3	6.0
13	<100	A	A	3.6	<3.0	7.3 x 10^4	3.7 x 10^3	6.0
14	<100	A	A	3.6	3.6	4.3 x 10^4	6.4 x 10^4	5.9
15	<100	7 x 10	A	3.6	3.6	6.3 x 10^4	8.9 x 10^2	5.8
16	<100	7 x 10	A	3.6	3.6	2.7 x 10^4	6.3 x 10^2	5.9
17	<100	2 x 10	A	7.4	3.6	1.3 x 10^4	<10	6.3
18	<100	2 x 10	A	15	3.6	4.1 x 10^4	<10	6.3
19	<100	5.6 x 10	A	23	<3.0	4.8 x 10^4	<10	6.4
20	<100	A	A	23	23	7.7 x 10^4	<10	6.2
21	<100	A	A	43	33	9.0 x 10^4	<10	5.8
22	<100	A	A	43	<3.0	6.7 x 10^4	1.7 x 10^3	5.9
23	<100	A	A	15	5 x 10^3	7.4 x 10^4	6.4 x 10^4	6.1
24	<100	A	A	9.2	4.6 x 10^2	2.4 x 10^4	8.7 x 10^2	6.2
25	<100	A	A	>1.1x10³	2.8 x 10^3	2.7 x 10^4	<10	5.8
26	<100	2 x 10	A	4.6x10²	4.6 x 10^2	8.7 x 10^4	2.7 x 10^3	7.0
27	<100	A	A	15	2.4x10^3	1.0 x 10^4	1.8 x 10^3	6.1
28	<100	A	A	93	<3	4.8 x 10^4	3.7 x 10^3	6.2
29	<100	A	A	3.6	2.4 x 10^3	4.3 x 10^4	6.4 x 10^4	6.4
30	<100	A	A	>1.1 x 10³	38	6.7 x 10^4	8.9 x 10^2	6.3
31	<100	A	A	3.6	21	9.0 x 10^4	6.3 x 10^2	5.8
32	<100	A	A	3.6	6	6.7 x 10^4	<10	5.8
33	<100	A	A	9.2	9.3	7.4 x 10^4	<10	5.8
34	<100	7 x 10	A	9.2	<3.0	2.4 x 10^4	<10	5.9
35	<100	2 x 10	A	23	<3.0	2.7 x 10^4	<10	5.9
36	<100	5,6 x 10	A	1,1x10³	<3,0	8,7 x 10^4	8,7 x 10^2	5,9
37	<100	A	A	1,1x10³	<3,0	1,0 x 10^4	<10	6,1
38	<100	A	A	1,1x10³	<3,0	4,8 x 10^4	3,8 x 10^3	6,2

Continuation of table 1 - Microbiological analyzes…

| 39 | <100 | A | A | $1,1x10^3$ | <3,0 | $4,3 \times 10^4$ | <10 | 6,2 |
| 40 | <100 | A | A | $1,1x10^3$ | <3,0 | $6,7 \times 10^4$ | $6,5 \times 10^2$ | 6,4 |

A = absent.

Even if Brazilian legislation does not establish a standard for the total count of mesophilic bacteria in raw meat, according to Delazari (1979) meats containing bacterial concentrations around 10^7 CFU g^{-1} already have their quality compromised in relation to aroma and flavor. According to Silva (1997), this process occurs because of this concentration, the supply of glucose is exhausted and the bacteria begin to use amino acids as a substrate for their growth. The degradation of these compounds causes the appearance of hydrogen sulfide odors and acid esters. Results for coagulase positive *Staphylococcus* were <100 CFU g^{-1} in all samples collected. Soares et al. (2015) found high counts of *Staphylococcus* sp. in the analyzed steak samples, with values in CFU g^{-1} ranging from <3 to >7.30 $\log_{10}$ CFU g^{-1}.

Silvestre et al. (2014) detected *Staphylococcus* in all samples of fresh beef evaluated in Alexandria-RN. Almeida et al. (2010) found that 60% and 40% of samples of ground chuck steak and tenderloin steaks, respectively, contained high levels of *S. aureus*. Sousa et al. (2000) detected the presence of this pathogen in 26.6% of ground beef collected in butcher shops in Macapá-AP. Silva Júnior et al. (2018) analyzed the microbiological characteristics of ground beef sold in supermarkets, also in Macapá, and found the absence of *Salmonella* spp. was $2.3x10^3$ to $2x10^5$ CFU g^{-1}.

According to Germano and Germano (2008), bacteria of the genus *Staphylococcus* are common inhabitants of the skin and mucous membranes of food handlers, and therefore, their high levels in food implies contamination during processing, due to lack of hygiene on the part of handlers. And although Brazilian legislation does not impose limits on the presence of this pathogen in beef, it is worth noting that staphylococcal toxin is capable of generating clinical manifestations at doses below 1 mg, which is a toxin level reached.

The results for *Staphylococcus coagulase* and *Salmonella* corroborate the results found by Abreu et al. (2011) in which they found that all meat samples evaluated for the presence of *Salmonella* spp. in Umuarama-PR were in accordance with the standard required by Anvisa Resolution - Resolution of the Collegiate Board of Directors - RDC nº 12/2001, which was also reported by Sousa et al. (2000), in ground beef from butchers. Soares et al. (2015) detected *Salmonella* spp. in 8.3% meat samples. The presence of *Salmonella* spp. in some of the meats evaluated in eight different cities in the state of Rio Grande do Norte, possibly associated with contamination during slaughtering, processing and packaging operations.

Silvestre et al. (2014) analyzed the presence of *Salmonella* spp. in fresh beef sold in Alexandria-RN and verified the presence of this pathogen in some samples. These authors verified that in 11.4% of the samples the presence of this microorganism was detected. Alcântara et

al. (2012) detected the presence of this pathogen in 27.77% of the beef evaluated in their study, with counts ranging from 2.08 to 4.40 $\log_{10}$CFU g^{-1}. Almeida et al (2010) in Diamantina-MG, did not detect *Salmonella* spp. in soft top steaks, however, they found the presence of this pathogen in 20% of the ground chuck steak samples. Becker and Kiel (2011) found *Salmonella* spp. in beef from one of the four evaluated supermarkets in Cascavel-PR.

The confirmation of the presence of *Escherichia coli* in most samples allows us to infer that there is high contamination, mainly because *E. coli* is the most prevalent enterobacteria in meat (Hernandéz-Macedo et al., 2011; Hernandéz-Macedo et al., 2011; Rossi Júnior et al., 2011) and its contamination usually starts during slaughter, by contact of the animal's skin impregnated with feces residues. Rosina and Monego (2013), evaluating the hygienic-sanitary conditions of ground beef from refrigerated benches sold in Canoinhas-SC, found that 90% presented a presumptive result for *E. coli* (values greater than 3.0 PAN g^{-1}) and 15% presented values greater than 1.1 x 10^3. Similar results were obtained by Souza et al. (2020) and Oliveira et al. (2017), who found that most samples (90.0%) presented values of up to 10^2 MPN g^{-1} and 5.0% presented *E. coli* values greater than 10^3 MPN g^{-1}.

Brito et al. (2011) analyzed 30 samples of pre-ground meat from five butcher shops and five supermarkets in different districts of Ilha do Governador, Rio de Janeiro, highlighting the presence of thermotolerant coliforms in 100% of the samples. Silva et al. (2004), evaluating the sanitary quality of ground beef sold in João Pessoa-PB, verified the contamination by thermotolerant coliforms, with an index of 10^3 NMP g^{-1}, in 100% of the samples collected. Mcnabb et al. (2009), conducting a study in Dracena-SP, detected *E. coli* contamination in 53% of ground beef samples. According to the Sanitary Code of the State of São Paulo, which regulates the standards for coliforms at 45°C or maximum thermotolerants of $3.0x10^2 g^{-1}$, 77.5% of the analyzed samples were found to be out of standard, since they presented results above $2.4x10^3$ PAN g^{-1}.

As for the pH values, the majority (97.5%) of the samples were in compliance with the Brazilian legislation for this parameter (pH between 5.8 and 6.4). Thus, from the pH data found in the present study, it appears that this analysis performed individually is not reliable to indicate the level of deterioration of the meats, because although most samples showed a high level of contamination by microorganisms' mesophiles and psychrotrophs, only one sample had a pH above 6.4, corroborating the results measured by Soares et al. (2015).

Assis et al. (2019), studying the hygienic-sanitary and technological conditions (HST) of production and commercialization of dried meat in the municipality of Salinas, in the northern region of the state of Minas Gerais,

using 40 samples in the local market, found two samples (5%) positive for *Salmonella* spp. and 16 (40%) had *S. aureus* counts above 10^3CFU g^{-1}. The values found in the analysis of aerobic mesophiles, molds and yeasts and total and thermotolerant coliforms were 2.50×10^7 CFU g^{-1}, 1.73×10^4 CFU g^{-1}, 9.1 PAN g^{-1} and 4.3 PAN g^{-1}, respectively.

CONCLUSIONS

Most samples were within the standards determined by current legislation, with the absence of *Salmonella* in 25g of sample. The average PAN of coliforms at 35°C and 45°C complied with national and international legislation, however, the verification of the presence of *E. coli* in 16 samples (40% occurrence) shows a medium risk of colibacillosis occurrence transmitted by meat. Even so, the risk of transmission of these diseases cannot be rejected, since the presence of *E. coli* is independent of the number of coliforms and the national legal standards established for the group of thermotolerant coliforms. Regarding the pH, one sample (2.5%) showed characteristics of beginning of decomposition, as it had a pH above 6.4.

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