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Evaluation of the microbiological quality of *Manihot esculenta* Crantz gum commercialized in the city of Manaus, Amazonas state, Brazil

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Abstract

Among the most frequently consumed regional foods in the world is cassava (*Manihot esculenta* Crantz). It is consumed in the forms of flour and gum (cassava starch), both of which are rich in energy and calories. Considering the popularity of this food in the northern region of Brazil, and that it is commonly marketed in fairs, shops, bakeries and supermarkets, there is the possibility of contamination in the different stages of the harvesting of the raw material, in the elaboration, and in the packaging of the product. Therefore, microbiological testing of 12 duplicate samples of tapioca gum corresponding to 12 of the 18 sectors of the city of Manaus was performed. The analyses were performed at the Food Microbiology Laboratory, at the Faculty of Agricultural Sciences of the Federal University of Amazonas, and took into account the Brazilian legislation for this product: standard plate count (PCA) for aerophilic mesophilic bacteria (such as *Bacillus cereus*), coliforms, *Salmonella* spp., *Staphylococcus aureus*, molds and yeasts. The results showed the absence of *B. cereus* and *Salmonella* in all samples, 14 of the 24 samples were within the limits established for thermotolerant coliforms and diverged from the legislation for PCA, while 12 of the 24 samples did not present *S. aureus* and 19 samples were above the limits for molds and yeasts. These results support the importance of microbiological analysis for tapioca gum, and good production practices to ensure quality in the finished product, in addition to avoiding health problems for consumers.

Keywords: microbiological control, cassava gum, tapioca.

Avaliação da qualidade microbiológica da goma de *Manihot esculenta* Crantz comercializada na cidade de Manaus, Amazonas, Brazil. Entre os alimentos mais consumidos no mundo está a mandioca (*Manihot esculenta* Crantz), nas formas de farinha e goma (amido) que são ricos em energia e calorias. Levando em consideração a popularidade desse alimento na região norte do Brasil, e que ele é comumente comercializado em feiras, comércios, padarias e supermercados, existe a possiblidade de ocorrer contaminação nos diferentes estágios da colheita da matéria prima, na elaboração e no empacotamento do produto. Realizou-se, portanto, a análise

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microbiológica de 12 amostras em duplicata, da goma de tapioca correspondente à 12 dos 18 setores da cidade de Manaus, AM. As análises foram realizadas no Laboratório de Microbiologia de Alimentos, na Faculdade de Ciências Agrárias da Universidade Federal do Amazonas, levando em consideração as legislações brasileiras para este produto: contagem padrão em placas (PCA) para bactérias mesófilas aerófilas (tais como *Bacillus cereus*), coliformes, *Salmonelas* spp., *Staphylococcus aureus*, bolores e leveduras. Os resultados mostraram a ausência de *B. cereus* e *Salmonella* em todas as amostras, 14 das 24 amostras estavam dentro dos limites estabelecidos para coliformes termotolerantes e em desacordo com a legislação para PCA, 12 das 24 apresentaram ausência de *S. aureus* e 19 amostras estavam acima do limite para a presença de bolores e leveduras. Esses resultados suportam a importância das análises microbiológicas para produtos minimamente industrializados, das boas práticas de produção para garantir uma melhor qualidade no produto final, além de evitar problemas de saúde aos consumidores.

Palavras-chaves: controle microbiológico, goma de mandioca, tapioca.

1. Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the *Euphorbiaceae* family and is cultivated by farmers in more than 100 countries, both tropical and subtropical. It is classified as sweet or bitter, depending on the amount of cyanogenic glycosides found in it (FAO, 2013; DÓSEA et al., 2010). Cassava is a very important source of carbohydrates and is present in the diet of about 800 million people worldwide. Although the African continent is the largest producer of this tuber, followed by the Asian continent and Latin America, with a total production of approximately 200 million tons, its origin is in the Brazilian Amazon, (KHUMAIDA et al., 2015).

According to the Brazilian Institute of Geography and Statistics (IBGE), in May 2021, the northern region of Brazil possessed 442,751 hectares of harvested area. The state of Amazonas alone has a total of 87,250 hectares of harvested area (IBGE, 2021). Cassava is considered the fourth most important food crop in the world and the main crop in tropical regions. Studies point to cassava as being the crop with the highest calorie productivity, the crop with the highest biological efficiency as an energy producer and, also, one that has the best adaptation to nutrient-deficient soils (NASSAR, 2006).

The roots of this tuber are characteristically rich in carbohydrates; therefore, they are an important source of energy. Starch obtained from cassava can be used in different applications in the food and pharmaceutical industries (FAO, 2013). Among the foods that can be produced, there is cassava gum, which is usually manufactured in an artisanal manner and produced from starch through the decanting process of the liquid part (SHINOHARA et al., 2018). In the artisanal form of production, the riverine populations and the Amazonian indians leave the roots to soak for about 3 to 5 days until they absorb enough water and become soft enough to be peeled and grated. The mass obtained is compacted to form a semi-dry biomass that will be roasted to become the flour, and the liquid part is used to obtain two by-products: the starch (gum) and the liquid (called "tucupi" by the local population).

Cassava gum, also known as cassava starch, tapioca gum, starch, or sweet starch, is a white, fine, and odorless powder. The lighter the color of the starch, the better the quality, since the color is responsible for indicating whether the cassava used is old or not. It also indicates

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the level of cleanliness under which the gum was processed. In addition, it is worth noting that hygiene is another parameter that must be considered when considering the quality of the product. These parameters make it possible to determine the microbiological quality of the product (SUFRAMA, 2003; LIMA et al., 2007).

The centesimal composition of the gum reveals, moisture content and ash, lipids, proteins and carbohydrates, 54.7%, 0.15%, 0.66%, 0.67% and 43.81%, respectively. (DE LUNA et al., 2013). In the dry matter, it contains 1.06% of digestible starch and 21.34% of resistant starch (HOLLAND and OLIVEIRA, 2015).

Taking into account that much of this starch is produced by hand and then commercialized, and that not all producers observe adequate hygiene standards, there is a need to verify the quality of the product (QUEIROZ and SOUZA, 2020). The commercialization of tapioca gum is common in the northern region and, in the city of Manaus, it is possible to find it in markets, shops, bakeries and supermarkets. Production is manual, most of the times, but there are industrialized tapioca gums. It is worth noting that in both cases contamination can arise in various forms, such as during the harvesting of the raw material or in the transport and storage of the finished product. Therefore, the present study sought to analyze samples of tapioca gum marketed in different sectors of the city of Manaus to determine the microbiological quality of this regional food.

2. Materials and Methods

2.1 Location and collection of samples

This study was conducted at the Food Microbiology Laboratory (FML) of the Faculty of Agricultural Sciences – UFAM. A total of 12 samples of cassava gum from 12 of the 18 sectors of the city of Manaus with weights ranging between 500 g and 1 kg were analyzed in duplicate. Samples were obtained from markets to supermarkets, as shown in Table 1. The samples were acquired the day before each analysis and were packed in a thermal bag for transport to the laboratory. Microbiological control was performed in a Class II, Type A2 biological security cabin (PA420, Pachane, Brazil). The Software QGIS 2.18.5, with the Sirgas 2000 coordinate reference system, Brazil Mercator EPSG: 5641, was used for the preparation of the map showing the collection areas (Figure 1).

2.2. Presumptive and confirmatory analyses

According to national and international standards for microbiological control of food, all the samples were of at least 200 g and, from these, 25 g was separated and added to 225 mL of 0.1% peptone water. This was stirred for 5 min in a vortex mixer (Velp Scientifica) and two more serial dilutions were made. For the presumptive test, 1 mL of the solution was transferred to screw-capped test tubes containing an inverted Durham tube and 9 mL of lauryl sulfate tryptose (LST) broth. This was performed in triplicate for the three dilutions (CODEX ALIMENTARIUS COMMISSION, 2003). According to the method of Brasil (2003), 50 µL of the third dilution was sown in triplicate in specific media (Plate Count agar – PCA, Baird Parker – BP, Sabouraud agar – SAB, *Salmonella* and *Shiguella* agar - SS) for the quality indicator microorganisms (Figure 2).

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Table 1 – Samples of tapioca gum with their respective sectors, neighborhoods, locations of the collections and origin.

SAMPLE	SECTOR	NEIGHBORHOOD	COLLECTION LOCATION	ORIGIN
S1 S2	5	Coroado	Agroufam Market	Careiro da Várzea
S3 S4	1	Centro	Adolpho Lisboa Market	Janauacá
S5 S6	2	Compensa	Modelo Market	Janauacá
S7 S8	3	Cachoeirinha	DB Supermarket	Vacuum-packed/NI
S9 S10	4	Educandos	Panair Market	Janauacá
S11 S12	12	Nossa Senhora das Graças	Pátio Gourmet	Vacuum-packed/NI
S13 S14	13	São Geraldo	Dorval Porto Market	Careiro da Várzea
S15 S16	11	Flores	Carrefour Supermarket	Vacuum-packed/NI
S17 S18	9	São José Operário	São José 2 Market	NI
S19 S20	14	Alvorada	João Sena Municipal Market	Janauacá
S21 S22	17	Novo Israel	Sepror Market	Rio Preto da Eva
S23 S24	10	Nova Cidade	Nova Cidade Market	NI

Key: Sx = Sample x, NI – no information.

2.2.1. Test for total coliforms and thermotolerant coliforms

After 24 to 48 hours, for the positive tubes in the presumptive test (that were clouded by microbial growth), a sample was transferred with the aid of a platinum loop to test tubes with a screw cap containing an inverted Durham tube and 9.9 mL of brilliant green broth (BG) for the confirmatory test of total coliforms and then incubated in a BOD chamber at 36 ± 1 °C. A similar process was performed with test tubes with a screw cap that contained *E. coli* (EC) culture medium. These were placed in a water bath for 24 hours with a temperature of 45 ± 1 °C for the confirmatory test for thermotolerant coliforms (BRASIL, 2003). For the tubes that tested positive for thermotolerant coliforms, the procedure of cross-striation depletion was performed using eosine methylene blue (EMB) agar medium, and the plates were incubated in a BOD chamber at a temperature of 36 ± 1° C. The appearance of colonies of a metallic green color confirmed the presence of *E. coli*. Figure 2 shows the microbiological control scheme for each sample and analysis.

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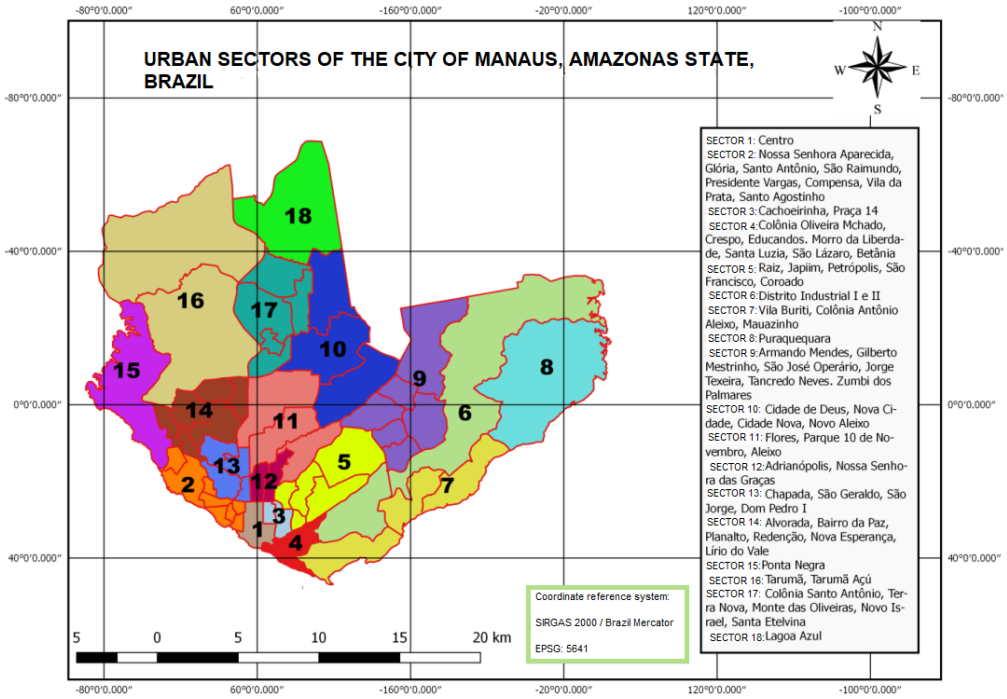

Figure 1-- Location of the study area.

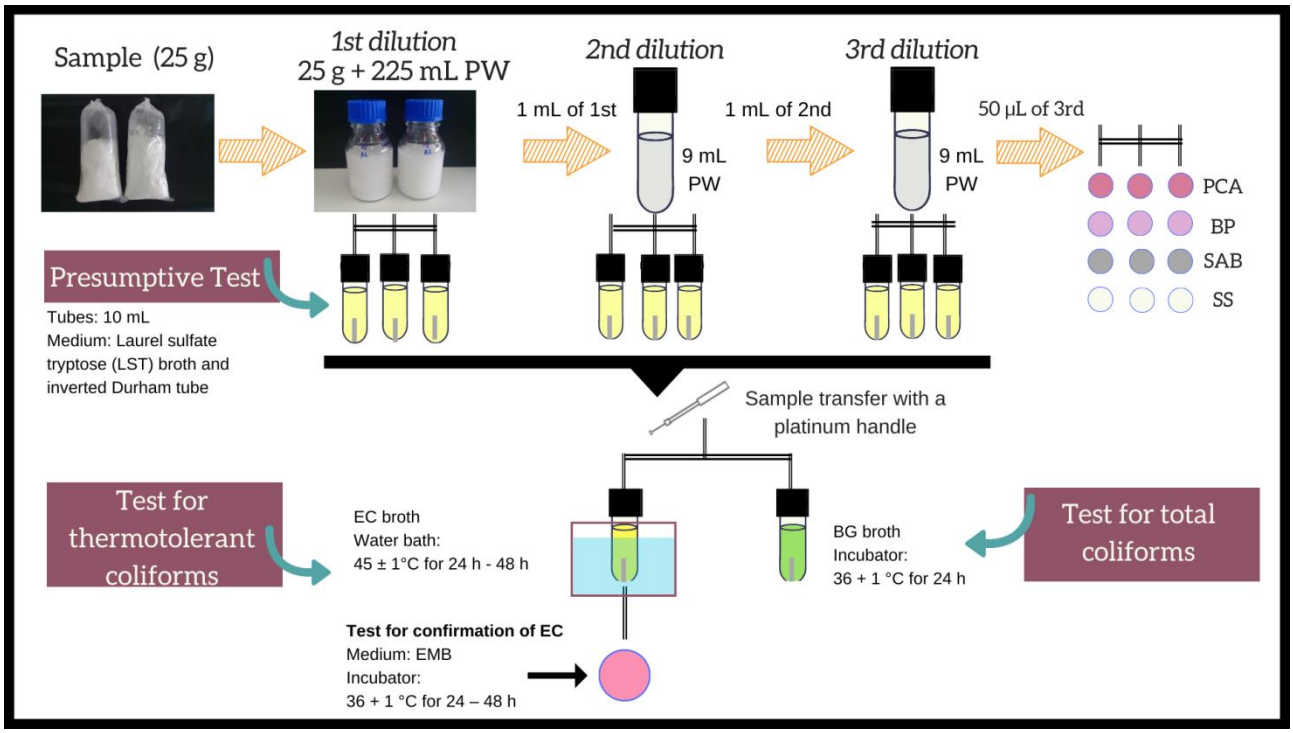

Figure 2 - Microbiological control scheme for each sample. (Source: adapted from Silva et al., 2007).
Key: PW: peptone water; PCA: Plate Count agar culture medium; BP: Baird Parker culture medium; *SS*: *Salmonella,* SAB: Sabouraud culture medium; EC: *E. coli* culture medium; BG: brilliant green culture medium and EMB: eosin methylene blue agar culture medium.

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2.2.2. Test for aerophilic mesophilic bacteria

The standard Plate Count agar (PCA) medium was used to identify aerophilic mesophilic bacteria and indicate colony-forming units (CFU/g) and compare their levels with current legislation (BRASIL, 2003).

2.2.3. Test for coagulase-positive *Staphylococcus* sp. and *Bacillus cereus*

An aliquot of 50 µL was inoculated in triplicate, which corresponded to the third dilution sample with peptone water in the specific Baird-Parker Agar medium. Then, the plates were inverted and incubated at a temperature of 36 ± 1 °C for 30 to 48 hours in a BOD chamber for the subsequent colony count and identification. The colonies of *S. aureus* presented characteristic colonies of a black color (BRASIL, 2003). For *B. cereus*, 100 µL of the third dilution sample with peptone water was inoculated in triplicate in the plates with Mannitol egg yolk polymyxin (MYP) medium. Afterwards, the plates were inverted and incubated at 30 ± 1 °C for 30 to 48 hours. The reading was performed by counting and checking to see whether the colonies were surrounded by an opaque halo of precipitation on a pink background (BRASIL, 2003).

2.2.4 Confirmation of coagulase-positive *Staphylococcus*

We used the slide catalase test for the colonies in the Baird-Parker medium to verify the confirmation. A portion of the culture was placed on top of the slide and afterwards a drop of 3% hydrogen peroxide was added. The presence of air bubbles indicated a positive result for catalase, i.e., the presence of *Staphylococcus* (ANVISA, 2004).

2.2.5. Test for *Salmonella* spp.

For the study of this microorganism, pre-enrichment was performed following the ISO 6579 (2007) method, in which the first dilution, 25 g of the sample plus 225 mL of peptone water (PW) was homogenized. After incubation, selective enrichment was performed, in which 1 mL was transferred to Muller Kauffmann tetrathionate broth and 0.1 mL to Rappaport-Vassilidis broth, both were placed in a BOD chamber at the temperature of 36 ± 1◦C for 24 hours. After the incubation period of the two broths, the drainage procedure was performed by making cross-striations on the plates with xylose lysine deoxycholate (XLD) agar medium and, shortly after the minimum incubation period, the presence of *Salmonella* colonies was verified.

2.3 Data analysis

The results of the analysis were evaluated according to the microbiological criteria provided in Normative Instruction No. 60 of December 23rd, 2019, from the Brazilian National Health Surveillance Agency – ANVISA/MS (BRASIL, 2019), and the Normative Instruction of the National Committee on Norms and Standards for Food (CNNPA) No. 12 of 1978 (BRASIL, 1978), since in the ANVISA resolution there is no limit for the presence of aerophilic mesophilic bacteria or yeast and molds.

3. Results and Discussion

It can be observed in Table 1 that a significant part of the tapioca gum sold in markets in Manaus originate in Janauacá. The municipalities that supply the city are the nearby municipalities and most of the gum produced is sold either wholesale, retail by local commerce or by street vendors (SILVA, 2014). Janauacá, is a municipality located close to Manaquiri and Careiro, and is recognized for its

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cassava production (ERAZO, SILVA and PEREIRA, 2018).

In the presumptive phase, the presence of *B. cereus* and *Salmonella* ssp. were not detected, as shown in Table 2 below. Depending on the conditions where the genus *B. cereus* are found, they can germinate and multiply, and thus contribute to food spoilage. Contamination by *B. cereus* occurs mainly in cereals and their derivatives, dairy products, and meats (MARTINS et al., 2014). The production of amylase by this genus makes them a degrading source for rice and other starch-based products, and for this reason we focused on detecting them.

Table 2 – Values found in the analysis compared with the limits of NI N° 60/2019, ANVISA/MS

Sector	Sample	Standard: NI 60/2019	
		Total coliforms NMP/g	Thermotolerant coliforms NMP/g
		No reference in the standard	Limit: 10
5	S1	>1100	>1100
5	S2	120	>1100
1	S3	<3.0	<3.0
1	S4	<3.0	<3.0
2	S5	3.6	75
2	S6	<3.0	20
3	S7	3.6	75
3	S8	15	20
4	S9	<3.0	<3.0
4	S10	<3.0	<3.0
12	S11	<3.0	<3.0
12	S12	<3.0	<3.0
13	S13	7.4	15
13	S14	23	3.6
11	S15	<3.0	<3.0
11	S16	<3.0	<3.0
9	S17	>1100	1100
9	S18	>1100	>1100
14	S19	240	<3.0
14	S20	36	<3.0
17	S21	<3.0	<3.0
17	S22	<3.0	<3.0
10	S23	>1100	93
10	S24	35	9.4

Key: Sx = sample x. NI = normative instruction

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According to Rubio (2015), like cassava starch, raw rice has a low water content in its composition, i.e., ranging from 12 to 14%. This amount does not allow a proliferation of *B. cereus*; however, vegetative cells of this microorganism can survive, and the presence of spores of these can be observed for long periods of time. Nevertheless, our tests did not identify *B. cereus* cells in the analyzed samples.

The study by Lima et al. (2007), which evaluated the hygiene standards of 12 samples of tapioca flour and 15 samples of gum produced and marketed in the state of Paraíba also did not detect the presence of *Salmonella* in their analyses. The same occurred with Dósea et al. (2010), who conducted research into the microbiological quality of cassava flour and starch in traditional production units known as "casas-de-farinha" and model units that are built in technical and research institutes in the municipality of Lagarto, Sergipe state, via a total of 81 samples, of which 36 samples were of starch. This microbiological analysis also did not identify the presence of *B. cereus* and *Salmonella*. Therefore, they were all found to be within the standard established by current Brazilian legislation, as were the samples analyzed in this project. The authors Shinohara et al. (2018) performed a microbiological analysis of 16 samples of industrialized tapioca starch commercialized in the metropolitan region of Recife, Pernambuco state, and did not detect the presence of colony forming units (CFUs) for *B. cereus* or *Salmonella* in their samples.

With regard to thermotolerant coliforms, in our study, it was observed that ten samples had a most likely number value (MLN) greater than 10 MLN/g (BRASIL, 2019); samples S1 and S2, S5 to S8, S13, S17, S18 and S23 were above what is allowed by the NI No. 60/2019, ANVISA/MS. Among them, samples S7 and S8 were industrialized gums (Figure 3) from Sector 3, which presented confirmation of *E. coli*. Therefore, it can be observed that even industrialized products may be contaminated. For total coliforms, the values obtained ranged from >1100 to <3.0, the maximum and minimum, respectively; however, there is no limit and/or reference value established by Brazilian legislation.

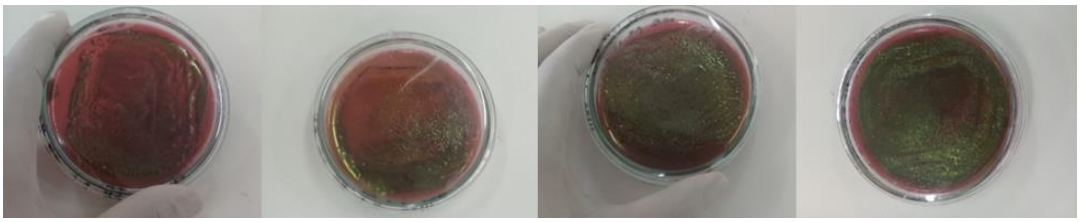

Figure 3 - Bright green colonies in EMB culture medium confirm *E. coli* in samples S7 and S8 from Sector 3.

When there are thermotolerant coliforms present, i.e., fecal coliforms, it is a sign that there is a lack of hygiene, especially during the process of manipulation (QUEIROZ and SOUZA, 2020). In addition, a high number of coliforms does not always indicate direct contamination with fecal material, since it may also be related to improper handling procedures, which may involve poor hygiene of the handler, contamination during transportation, as well as inadequate packaging of the product.

Foodborne diseases (FBDs) can be caused by biological and chemical agents, such as bacteria, viruses, parasites (in these cases infections), toxins and pesticides (in these cases intoxications).

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These problems occur when there is an intake of contaminated food or water, and the symptoms consist of nausea, vomiting, and diarrhea, which may or may not be accompanied by fever (BRASIL, 2018) in the case of infections, and may reach the central nervous system and can cause muscle paralysis, among other symptoms, in the case of poisonings. In both cases, FBDs can be life-threatening, especially to children. When the preparation of food occurs in large quantities or in advance, in conjunction with prolonged exposure to room temperature, it often contributes to the proliferation of contaminating microorganisms and, when food is subjected to insufficient heating, cooking or reheating occurs, this can also contribute to the survival of pathogens (MELO et al., 2018).

According to Erazo, Silva and Pereira (2018), who researched the work processes in the artisanal family production of cassava flour and gum in the region of Janauacá, after the cassava is removed from the ground, it is taken via canoes to the flour-making houses. These houses either float on the river or are close to the river and, according to the authors, this form of production allows accessibility to water for washing the product. When the cassava arrives at these houses, it is trampled and wetted constantly until the peel is removed so that the gum can be manufactured. Water is big part of the production of cassava gum, thus it is a major factor in contamination of the product.

In addition, most of the time, the cassava gum that is sold in the city markets is stored in large plastic or aluminum basins and the handlers do not use uniforms, caps, gloves or masks to avoid contamination of the product. While purchasing our samples, it was often observed that the hand used to put the gum in the plastic bags is the same one that gives the change to the consumer, thus being another point of contamination.

People working with food handling have great responsibility in preventing the occurrence of food poisoning during food production and distribution, since handlers can contaminate both the raw ingredients and finished products (WALKER, PRITCHARD and FORSYTHE, 2003). Outbreaks of foodborne diseases are commonly found. An outbreak occurs when two or more people have the same symptoms after eating food form the same source (NUNES et al., 2017). Outbreaks, such as that of *Shigella sonnei* in 2016 in Northern Ireland (O'BRIEN et al., 2020) and the outbreaks caused by *B. cereus* in the European Union between 2000 and 2013 (OSIMANI, AQUILANTI and CLEMENTI, 2018), can occur all over the world, though mainly with greater frequency and severity in less developed regions, as observed in Latin America, Africa and China.

The authors Abreu et al. (2011) conducted a microbiological analysis of the hands of food handlers in the municipality of Santo André, São Paulo and found the presence of coliforms in most of the samples that were analyzed. Therefore, the conditions in which the food was prepared were considered unsatisfactory in this case. In addition, the presence of 62.5% of thermotolerant coliforms was also found in the samples.

For the standard plate count, i.e., the aerophilic mesophilic bacteria *S. aureus* for the molds and yeasts, the limits established by the National Commission of Norms and Standards for Food (CNNPA) No. 12 of 1978 were used as a basis. The results obtained are shown in Table 3.

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Table 3 – Values for the samples compared with the values for the quality standard according to the National Commission of Norms and Standards for Food (CNNPA) Standard No. 12 of 1978.

Sector	Sample	CNNPA Standard 12/1978		
		Standard plate count	***Staphylococcus aureus***	**Molds and yeasts**
		Limit: $5x10^5$ UC/g	**Limit: Absent 0.1g**	**Limit: 10^3 UFC/g**
5	S1	Un	$58x10^3$	$3.33x10^2$
5	S2	$53x10^3$	$35.33x10^3$	0
1	S3	$27x10^4$	0	$6.67x10^2$
1	S4	$19x10^4$	0	$3.33x10^2$
2	S5	$22x10^4$	$4.33x10^3$	$43x10^3$
2	S6	$17x10^4$	$4.67x10^3$	$39.33x10^3$
3	S7	Un	$32x10^3$	$23.23x10^3$
3	S8	Un	$78x10^3$	$19.53x10^4$
4	S9	$94.33x10^3$	$8.67x10^3$	$23.67x10^3$
4	S10	$95x10^3$	$8.33x10^3$	$41.33x10^3$
12	S11	$18x10^4$	0	$2.33x10^3$
12	S12	$24x10^4$	0	$6x10^3$
13	S13	Un	<10	$99x10^3$
13	S14	Un	<10	$14.33x10^3$
11	S15	Un	0	$7x10^3$
11	S16	0	0	0
9	S17	Un	0	$9.33x10^3$
9	S18	Un	0	$7x10^3$
14	S19	Un	<10	$23x10^4$
14	S20	Un	$2.33x10^3$	$21x10^4$
17	S21	Un	0	$23.33x10^3$
17	S22	Un	0	$30.66x10^3$
10	S23	Un	0	$4.67x10^3$
10	S24	Un	0	$1.67x10^3$

Key: Sx = sample x, Un = uncountable

In the standard plate count, the majority of the samples (S1, S7, S8, S13 to S15 and S17 to S24) presented uncountable colonies that were higher than the established limit, this being $5x10^5$ CFU/g, for bacterial counts. For industrialized gum, the study of Shinohara et al. (2018) found a result of $4.5x10^5$ CFU/g, which is close to the established limit. Of the three (in duplicate) minimally industrialized tapioca gums that were analyzed in this work, only the samples S11, S12 and S16 were in accordance with the law, while the samples S7, S8 and S15 were

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over the established limit. It is worth noting that what Shinohara et al. (2018) classifies as industrialized, may be what we classify as minimally industrialized, since the only difference between this and the raw product that is sold in the markets is that they are weighed in front of the consumer, while those sold in grocery stores and supermarkets are vacuum packed elsewhere.

However, it can be seen that half of the samples were within the standard values of colony forming units, ranging from $53x10^3$ CFU/g to $27x10^4$ CFU/g, maximum and minimum, respectively. In the study of Oliveira, Sampaio and Biscosin (2017), who carried out a microbiological evaluation of the cassava starch produced in the state of Rondônia, Brazil, values ranging from $1.55x10^3$ CFU/g to $5.17x10^4$ CFU/g were found, which were acceptable and within the limits, as were those obtained in the present study.

For molds and yeasts, 79.17% of the analyzed samples, from S5 to S24 (with the exception of sample S16), presented values above the limit established by CNNPA NI No. 12 of 1978, with extremes of $1.67x10^3$ CFU/g and $23x10^4$ CFU/g. In Figure 5, it is possible to visualize the molds and yeasts found in samples S6, S7, S11 and S12. The work of Oliveira, Sampaio and Biscosin (2017) also found higher results than the established limits, with values ranging from $1.55x10^3$ CFU/g to $5.17x10^4$ CFU/g. The same occurred in the study by Shinohara et al. (2018), in which the authors found values that were above the permissible limits, since they ranged from $2.5x10^3$ CFU/g to $4.7x10^4$ CFU/g, maximum and minimum, respectively. Lima et al. (2007) obtained values from 10^3 to $9.3x10^4$ CFU/g. In other words, all the studies show results above the established limit.

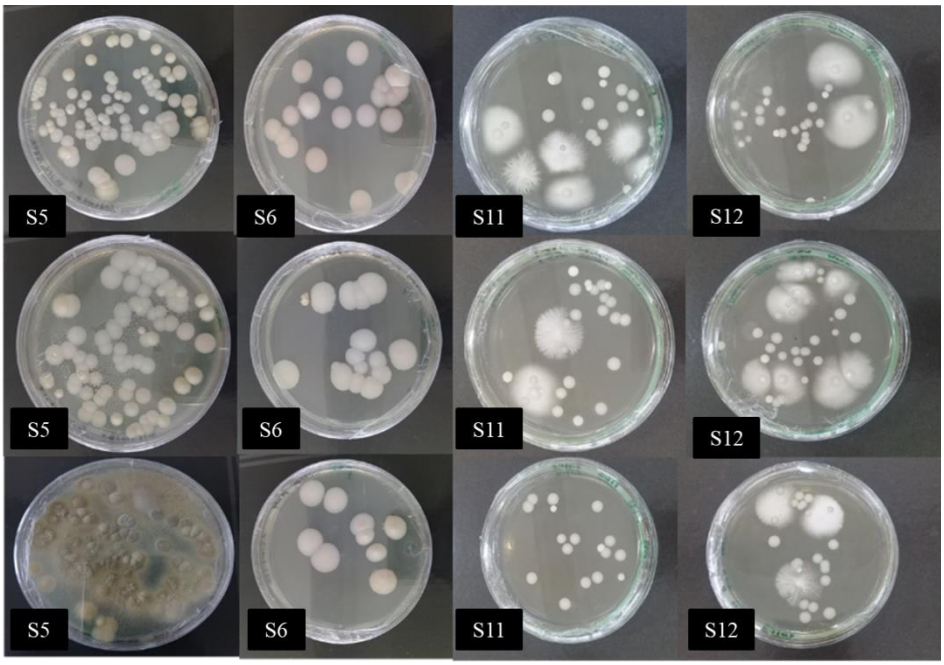

Figure 5 – Molds and yeasts found in samples S5 and S6, S11 and S12.

Most of the samples were in accordance with the established standards regarding the presence of the microorganism *S. aureus*, since the CNPPA standard No. 12 of 1978 establishes absence as being less than 0.1 g, and the samples S3, S4, S11, S12, S15 to S18, S21 to S24 did not show any colony growth. Table

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3 shows that 50% of the samples analyzed presented the presence of this microorganism, with colony-forming units presenting extremes ranging from 4.33x10^3 CFU/g to 78x10^3 CFU/g. Lima et al. (2007) also found variations from 0 to 6.3x10^3 CFU/g, which is above what is allowed by the current legislation (CNNPA NI No. 12 of 1978). Species of this genus of bacteria, *Staphylococcus*, are part of the human microbiota since they colonize mainly the skin, which facilitates contamination during manipulation of food and can act as opportunistic pathogens, thus causing infections (ANDRADE JÚNIOR et al., 2017).

It is important to highlight that contamination by microorganisms can arise at different stages of production, from harvesting to final processing. It was observed during this research that in several markets in the city cassava gum was often exposed for hours or even days to the environment and there are cases where the gum is sold in large containers and packaged on site by merchants without use of suitable clean utensils and/or personal protective equipment required for working with food, such as a mask, hat and gloves. The water used in the manufacture of cassava gum can be another problem if it is not properly treated for human consumption. In the traditional method of production, the roots are submerged in water from rivers or water tanks, so there are intrinsic and extrinsic factors that also contribute to the presence of these contaminating microorganisms.

4. Conclusion

The present study showed an abundant and diverse microbiota in the gum samples obtained in the city of Manaus. According to the parameters of ANVISA (normative instruction No. 60/2019) for the presence of *Salmonella* spp. and *B. cereus*, all samples were within the tolerances of the standard, and 3 of the 12 samples did not present values higher than the limits for thermotolerant coliforms. For 8 of the 12 samples, the count of aerophilic mesophilic bacteria in Petri dishes containing the PCA culture medium did not comply with CNNPA legislation No. 12 of 1978, and 10 of the 12 showed no presence of *S. aureus*; however, 9 (75%) samples were above the limit for the presence of molds and yeasts. Therefore, in order to avoid possible health problems for consumers, it is necessary to adopt measures, such as the use of good manufacturing practices, to prevent contamination and maintain a quality finished product.

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References

ABREU, E. S.; MEDEIROS, F. S.; SANTOS, D. A. 2011. "Análise Microbiológica De Mãos De Manipuladores De Alimentos Do Município De Santo André." *Revista Univap* 17 (30): 39. Accessed on: <http://revista.univap.br/index.php/revistaunivap/article/view/24> https://doi.org/10.18066/revunivap.v17i30.24.

ANDRADE JÚNIOR, F. P. et al. 2017. "*Staphylococcus aureus* como agente etiológico de doenças transmitidas por alimentos: dados epidemiológicos, principais alimentos, fatores causais, toxinas, sintomatologia e medidas profiláticas". In: II Congresso Brasileiro de Ciências da Saúde, Campo Grande. Accessed on: <https://editorarealize.com.br/revistas/conbracis/trabalhos/TRABALHO_EV071_MD1_SA3_ID439_01052017134401.pdf>.

Scientia Amazonia, v. 10, n. 4, CA10-CA23, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

ANVISA. Agência Nacional de Vigilância Sanitária. 2004. "Descrição dos meios de cultura empregados nos exames microbiológicos." Accessed on: <http://www.anvisa.gov.br/servicosaude/microbiologia/mod_4_2004.pdf> Acesso em: 29 abr 2019.

BRASIL. Ministério da Saúde (MS). 1978. "Comissão Nacional de Normas e Padrões para Alimentos." Resolução - CNNPA nº 12, de 1978.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). 2003. "Instrução Normativa nº 62, de 26 de agosto de 2003, que oficializa os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água." Accessed on: <https://www.defesa.agricultura.sp.gov.br/legislacoes/instrucao-normativa-sda-62-de-26-08-2003,665.html>.

BRASIL. Ministério da Saúde. "Informações Técnicas." Brasília, 2018. Accessed on: <http://www.saude.gov.br/saude-de-a-z/doencas-transmitidas-por-alimentos/informacoes-tecnicas>.

BRASIL. Ministério da Saúde. 2019. "Instrução Normativa nº 60, de 23/12/2019, da Agência Nacional de Vigilância Sanitária. Listas de padrões microbiológicos para alimentos." Diário Oficial da União, Brasília, 2019.

CODEX ALIMENTARIUS COMMISSION. 2003. "Recommended international code of practice. General principles of food hygiene." CAC/RCP 1-1969, Rev. 4-2003. *Codex Alimentarius Commission*, Washington, DC.

DE LUNA, A. T., et al. 2013. "Estudo físico-químico, bromatológico e microbiológico de Manihot esculenta Crantz (mandioca)." *Revista Interfaces: Saúde, Humanas e Tecnologia* 1 (088).

DÓSEA, R. R. et al. 2010. "Qualidade Microbiológica Na Obtenção de Farinha e Fécula de Mandioca Em Unidades Tradicionais e Modelo." *Ciencia Rural* 40 (2): 441–46. https://doi.org/10.1590/s0103-84782009005000241.

ERAZO; R. L.; SILVA, L. J. S.; PEREIRA, H. S. 2018. "Processos de Trabalho Na Indústria Familiar Artesanal de Farinha e Goma de Mandioca Da Região de Janauacá, Amazonas." *Revista Terceira Margem Amazônia* 3: 214–29. Accessed on: <http://www.revistaterceiramargem.com/index.php/terceiramargem/article/view/221> https://doi.org/10.36882/2525-4812.2018v3i10p%25p

FAO. "Produzir mais com menos: Mandioca." 2013. Accessed on: <http://www.fao.org/3/a-i2929o.pdf>.

HOLLAND, N.; OLIVEIRA, J. J. O. "Composição centesimal e amido resistente de goma de mandioca e tapioca" 2015. In: ANAIS DO SIMPÓSIO LATINO AMERICANO DE CIÊNCIAS DE ALIMENTOS. *Anais eletrônicos...* Campinas, Galoá, 2015. Accessed on: <https://proceedings.science/slaca/slaca-2015/papers/composicao-centesimal-e-amido-resistente-de-goma-de-mandioca-e-tapioca->.

IBGE. "Levantamento Sistemático da Produção Agrícola" – SIDRA. 2021. Accessed on: <https://sidra.ibge.gov.br/tabela/1618>.

ISO. International Organization for Standardization. 2007. "Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp."

KHUMAIDA, N. et al. 2015. "Cassava (Manihot Esculenta Crantz.) Improvement through Gamma Irradiation." *Procedia Food Science* 3: 27–34. https://doi.org/10.1016/j.profoo.2015.01.003.

LIMA, C. P. et al. 2007. "Presença de Microrganismos Indicadores de Qualidade Em Farinha e Goma de Mandioca (Manihot Esculenta, Crantz)." *Revista APS* 10 (1): 14–19. Accessed on: <https://www.ufjf.br/nates/files/2009/12/Qmandioca.pdf>

MARTINS, W. F. et al. "Pesquisa de *Bacillus cereus* em produtos farináceos e a base de soja." In: Anais do XX Congresso Brasileiro de Engenharia Química – COBEQ 2014. São Paulo. *Anais eletrônicos...* São Paulo, 2014. Accessed on: <http://pdf.blucher.com.br.s3.amazonaws.com/chemicalengineeringproceedings/cobeq2014/0053-27308-172388.pdf>.

MELO, E. S. et al. 2018. "Doenças Transmitidas Por Alimentos e Principais Agentes Bacterianos Envolvidos Em Surtos No Brasil." *Pubvet* 12 (10): 1–9. https://doi.org/10.31533/pubvet.v12n10a191.1-9.

NASSAR, N. M. A. 2006. "Mandioca: Opção Contra a Fome." *Ciência Hoje* 39 (231): 30–39. Accessed on: <http://www.geneconserve.pro.br/mandioca_ciencia hj.pdf>

NUNES, S. M. et al. 2017. "Surto de Doença

Scientia Amazonia, v. 10, n. 4, CA10-CA23, 2021
Revista on-line http://www.scientia-amazonia.org
ISSN:2238.1910

Trasmitida Por Alimentos Nos Municipios de Mauá e Ribeirão Pires - SP." *Hig. Aliment* 31: 97–102.

O'BRIEN, S. et al. 2020. "Investigation of a Foodborne Outbreak of Shigella Sonnei in Ireland and Northern Ireland, December 2016: The Benefits of Cross-Border Collaboration and Commercial Sales Data." *Public Health* 182: 19–25. https://doi.org/10.1016/j.puhe.2020.01.008.

OLIVEIRA, B. N. K.; SAMPAIO, F. P.; BISCOSIN, A. 2017. "Avaliação Microbiológica Da Goma de Mandioca Produzida No Estado de Rondônia." *Higiene Alimentar* 31 (266/267): 3224–27.

OSIMANI, A.; AQUILANTI, L.; CLEMENTI, F. 2018. "Bacillus Cereus Foodborne Outbreaks in Mass Catering." *International Journal of Hospitality Management* 72 (January): 145–53. https://doi.org/10.1016/j.ijhm.2018.01.013.

SILVA, D. M. 2014. "Da goma de mandioca a fécula industrializada no município de Tefé – AM". In: VII CONGRESSO BRASILEIRO DE GEÓGRAFOS – CBG, 2014, Vitória. *Anais eletrônicos...* Vitória. Accessed on: <http://www.cbg2014.agb.org.br/resources/anais/1/1404408847_ARQUIVO_Dagomaafeculaindustrializadanomunicipiodetefeam.pdf>.

SILVA, N.; JUNQUEIRA, V. C. A.; SILVEIRA, N. F. A.; TANIWAKI, M. H.; SANTOS, R. F. S. dos; GOMES, R. A. R. Manual de métodos de análise microbiológica de alimentos. 3. ed. São Paulo: Varela, 552 p., 2007.

SUFRAMA. "Amido de mandioca." 2003. Accessed on: <http://www.suframa.gov.br/publicacoes/proj_pot_regionais/amido.pdf>.

QUEIROZ, A. M. de; SOUZA, L. G. S. 2020. "Resumo Diagnóstico de Qualidade Da Fécula de Mandioca Comercializada Em Rio Branco - AC Quality Diagnosis of Cassava Starch Sold in Rio Branco - AC Abstract Diagnóstico de Calidad Del Almidón de Yuca Vendido En Rio Branco - AC Resumen Introdução." *Applied Research & Agrotechnology* 6305: 1–10. Accessed on: <https://revistas.unicentro.br/index.php/repaa/article/view/6305> https://doi.org/10.5935/PAeT.V13.e6305.

RUBIO, G. A. M. "Ocorrência de Bacillus cereus em arroz cru vitaminado e cinética de multiplicação do patógeno no arroz cozido." (Dissertação) Mestrado. Universidade Federal de Viçosa, Viçosa, 2015. Accessed on: <https://www.locus.ufv.br/handle/123456789/6835#:~:text=Locus%20Reposit%C3%B3rio%20Institucional%20da%20UFV,do%20pat%C3%B3geno%20no%20arroz%20cozido&text=Abstract%3A,meio%20de%20cultura%20para%20bact%C3%A9rias>.

SHINOHARA, N. K. S. et al. 2018. "Microbiological Analysis in Industrialized Cassava Gum." *Journal of Environmental Analysis and Progress* 3 (2): 226–31. Accessed on: <http://www.journals.ufrpe.br/index.php/JEAP/article/view/1854> https://doi.org/10.24221/jeap.3.2.2018.1854.226-231

WALKER, E.; PRITCHARD, C.; FORSYTHE, S. 2003. "Food Handlers' Hygiene Knowledge in Small Food Businesses." *Food Control* 14 (5): 339–43. Accessed on: <https://www.sciencedirect.com/science/article/abs/pii/S0956713502001019?via%3Dihub>https://doi.org/10.1016/S0956-7135(02)00101-9.