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Aflatoxins in Tapioca Gum Marketed in Manaus-AM-Brazil

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Abstract

Introduction: Among the culturally consumed foods in the North and Northeast of Brazil, tapioca gum, derived from cassava, has good popular acceptance, and is used in different culinary preparations. **Objective:** To assess the presence of aflatoxins in samples of tapioca gum from Manaus. **Method:** 24 samples of tapioca gum (n=24) were collected from retailers and fairs in the city of Manaus, for the quantification of AFL by means of high-performance liquid chromatography (HPLC), water activity (Aw) and moisture content (mc%). **Results**: in seven samples, aflatoxin G1 (AFLG1) was detected, representing 29.17% of contamination. The average of Aw was equal to 0.9952 and the mc% was 41.615%. **Conclusion:** this work presents, in an unprecedented way, the presence of AFLG1 in tapioca gum. The mc% correlated with the presence of AFLG1, while Aw had no effect on AFL. It is not possible to determine the risk of the presence of AFLG1 for human consumption due to the lack of legislation for the product, but the results reflect the manufacturing, storage, and transport practices of local businesses, demonstrating the need for intervention.

Keywords: mycotoxins, risk analysis, contamination

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a food with cultural and nutritional relevance in the Brazilian territory, with Brazil being the fourth largest cassava product in the world. It is used for human consumption, development of by-products, flour, starch, or gum and in animal feed. The state of Amazonas stood out in 2022 to produce 890,124 tons of cassava, ranking fifth, preceded by Mato Grosso do Sul-Brazil. Tapioca flour and gum are important products obtained from cassava tuber processing. Obtaining the gum and flour in the family industry results in sale or self-consumption, and the production of gum is considered less complex and involves the processes of peeling, barley, washing, decanting and packaging. Cassava is used as an energy source in developing countries, it has 81.1g of carbohydrates and 17.8% of moisture in each 100g of starch, favorable characteristics for bacterial and fungal growth. The cassava production chain can be contaminated by fungi both pre-harvest and post-harvest if storage and transport conditions are not idea. Certain species of filamentous fungi are capable of producing mycotoxins, and among these, aflatoxins (AFL) can be highlighted, which have a teratogenic, mutagenic, and suppressive effect on the immune system, produced by fungi of the genus *Aspergillus*, with aflatoxin B1 considered carcinogenic to humans. The occurrence of aflatoxins in cassava and cassava products has been investigated in many countries such as the Republic of Congo, Benin, Cameroon, and Nigeria. The climatic conditions of the Amazon Forest of high temperature and relative humidity % reflect contamination by the genus *Aspergillus* and the production of aflatoxins, since, for example, *Aspergillus flavus* is well distributed in hot and humid regions. In this sense, as tapioca gum is a derivative susceptible to fungal contamination and widely accepted and consumed by the inhabitants of the region of the city of Manaus-Amazonas- Brazil, the present study aimed to evaluate samples sold at retail and provide data that support policies preventive measures for health surveillance agencies with retailers and the population.

2. Method

2.1 Sampling

Twenty-four (24) samples of tapioca gum were analyzed, collected in retail stores and fairs in the city of Manaus-Amazonas-Brazil. The samples were kept in original packaging and sent for analysis at the Laboratory

of the Nucleus of Studies in Food Composition and Toxicology (NECTA) at the Federal University of Amazonas.

2.2 Physical-chemical Tests

2.2.1 Moisture Content (mc%)

The mc% was determined using an electronic balance (SHIMADZU, MOC-120H®, Kyoto, Japan) adjusted with an infrared dryer, by drying about 1 g of the sample at a temperature of 105° C. Analyzes were performed in triplicate and results expressed as mean and standard deviation.

2.2.2 Water Activity (Aw)

The Aw was determined using a DECAGON instrument, Aqualab 4TE series, Washington, USA, with internal temperature control at 25°C, by the dew point method. All analyzes were performed in duplicate and results expressed as mean and standard deviation.

2.2.3 Quantification of Aflatoxins

Tapioca gum samples were quantified for AFL B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2) by high performance liquid chromatography (HPLC) using the AOAC method (2016). The method consists of the extraction of Aflatoxins (AFLs) where 50 g of sample were used and extracted with 100 mL of acetonitrile:water (90:10 v/v) and stirred at high speed for 5 minutes in a blender. The extract was filtered through filter paper and 3mL of the filtrate was transferred to a 10 ml culture tube, then the MYCOSEP 226 cleaning column (Romer labs) was applied. 0.5ml of the purified extract was collected and kept in a freezer until analysis by HPLC. For the derivatization of this purified extract, a derivatizing solution composed of Water: glacial acetic acid: trifluoroacetic acid (35:10:5 v/v) was used, where 0.2ml of the purified extract was passed to a derivatization vial with 0.7ml of derivatizing solution with the aid of a 1ml syringe and a filter for a Nylon syringe with a porosity of 0.45µm, this vial was closed and heated at 65°C for 8.5 minutes in a water bath (time required to complete the derivatization of AFLB1 and AFLG1), this procedure was repeated for the 24 samples. The resulting solutions were applied and quantified in the High-Performance Liquid Chromatography (HPLC) system, with: Mobile phase - acetonitrile, methanol, and ultra-pure water (1:1:4), column: X-Terra by Waters, 150x4, 6mm, flow rate of 1.0mL/minute eluting in isocratic mode, with fluorescence detector: λ ex- 360 nm and λ em- 440 nm; injection volume 50µL; 20 minutes running time. Pools of AFL standards B1, B2, G1 and G2 - Sigma Aldrich®, with different concentrations of AFLs B1, B2, G1 and G2, prepared from a pool of stock solution (ng/ml) containing: AFB1 =300; AFB2=50; AFG1=150 and AFG2=50. The pools of standards were subjected to derivatization and analyzed by HPLC, to obtain the chromatogram of the standards at different concentrations. The chromatogram obtained from the tapioca gum samples is then compared each peak with the peak and retention time obtained by each standard (AFB1, AFB2, AFG1 and AFG2). The quantification of the samples was performed from a curve of each Aflatoxin standard obtained from the HPLC reading of different concentrations of the AFL pool.

2.3 Statistical Analysis

The results obtained were analyzed using the R software and SPSS both for correlation analysis. In the statistical analyzes, descriptive statistics tests were used (mean and standard deviation). The Mann-Whitney Test was used for the variables: mc%, Aw and AFL. Spearman's Rho Test was used for mc% and Aw.

3. Results and Discussion

3.1 Physical-chemical Tests

3.1.1 Moisture Content

Table 1 shows the average value of mc% found in tapioca gum, showing variations in its results. The analysis of the moisture content of the tapioca gums showed that the average value of the samples was 41.615%, and they are in disagreement with the limit established in Normative Instruction Nº 23 of 2005 of the Ministry of Agriculture, Livestock and Supply, with maximum allowed value of 14%, as it is a starchy product obtained from the aqueous extraction process of the grated mass of cassava root, with a smaller granulometry, having a greater capacity to adsorb water. This result was also observed by Queiroz and Souza (2020), who carried out moisture analyzes of cassava gum produced and sold in the city of Rio Branco - Acre and obtained an mc% average of 45.51% among five producers.

Table 1. Moisture content (*mc*) in tapioca gum

Number of samples	*mc* % (Mean ± Standard Deviation)	Acceptable limit [a]
24	41.615 ± 1.96	<14

[a] Limit in % established in Brazilian legislation (Brazil, 2005)

The evaluation of the mc% is of great importance, considering that levels greater than 13% can provide microbial growth and deterioration in a short time, the increase in this variable may be related to its manufacturing process. As can be seen in Table 1, tapioca gum has a high mc%, which can favor its faster deterioration and favor alterations of microbial origin. According to Luna et al. (2013) fresh marketed gum has a high mc%, which may favor faster deterioration.

3.1.2 Water Activity (Aw)

Table 2 shows the average value of Aw found in tapioca gum, showing variations in its results. The analysis of the Aw of the tapioca gums showed that the average value of the samples was 0.9952.

Table 2. Water activity level (Aw) in tapioca gum

Number of samples	Aw (Mean ± Standard Deviation)
24	0.9952 ± 0.00275

Pitt and Hocking (2009) describe that Aw levels > 0.70 are necessary for fungal development. In the study by Chisté (2015) with isotherms and mathematical models, two groups of cassava flour, dry and water, presented approximate Aw of 0.53 and 0.45, respectively. In the study carried out by Ono et al. (2021) the water activity obtained from cassava tubers was from 0.922 to 0.996 and from cassava starch from 0.317 to 0.598, values lower than those found in this study. The Aw of 0.60 was cited as the minimum limit capable of allowing the development of microorganism, hence the fact that dehydrated foods, such as cassava flour, are considered microbiologically stable. Thus, the results obtained demonstrate that the samples offer necessary conditions for fungal development.

3.1.3 Quantification of Aflatoxins (AFL)

Figure 1 shows the chromatogram of the standard pool with the highest concentration of AFL (300ng/ml of AFB1; 50ng/ml of AFB2; 150ng/ml of AFG1 and 50ng/ml of AFG2), for visualization of the peak and retention time of each of the AFL, and chromatographic comparison with the analyzed samples. In figure 2 and 3 we can see a chromatogram of a negative sample and another one of a positive sample, respectively. Of the 24 samples analyzed, 07 (29.17%) were contaminated with G1-type AFL, as shown in Table 3.

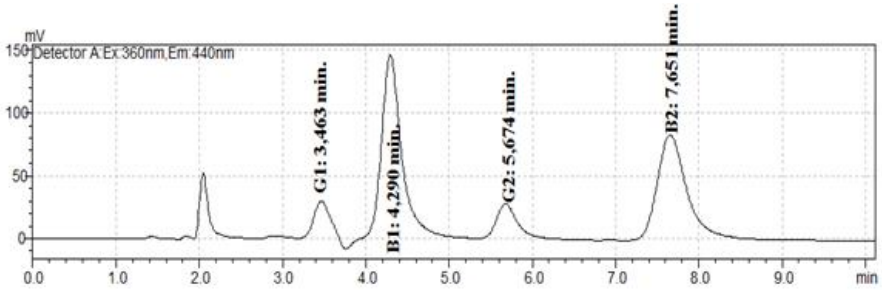

Figure 1. Chromatogram of the standard pool with the highest concentration of each AFL (Retention times: AFG1 = 3.463 min; AFB1 = 4.290 min; AFG2 = 5.674 min; AFB2= 7.651 min)

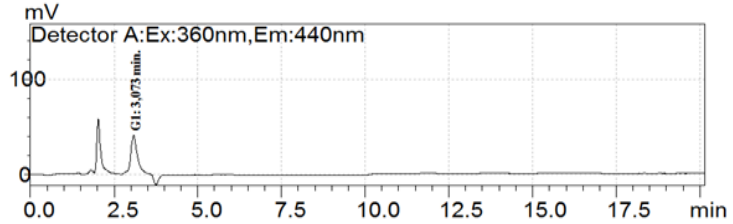

Figure 2. Chromatogram of a negative sample for quantification of aflatoxins.

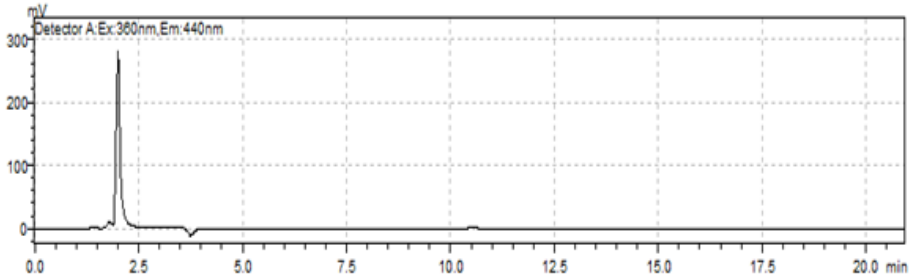

Figure 3. Chromatogram of a sample contaminated with aflatoxin (G1 = 3.073 min)

Table 3. Determination of aflatoxin content in tapioca gum samples

	Number of positive samples	AFLG1 µg/kg (Mean ± Standard Deviation)[a]
24	7	2.161 ± 1.268

[a] The quantification limits of the method were: AFB1= 0.411, AFB2=0.411, AFG1= 0.450, AFG2 = 0.450

About 7 (29.1%) were positive and only AFLG1 was detected. There are few studies related to the occurrence of AFL in tapioca gum, we can mention works focused on other derivatives of cassava. There is the study by Ono et al. (2021) in the state of São Paulo-Brazil, in which no AFL were detected in cassava gum or starch by liquid chromatography. In the study carried out by Morrison et al. (2019) in Guyana using enzyme immunoassay and liquid chromatography, no aflatoxins were detected in the 40 samples of cassava flour and cassava bread; however, after 2 months of storage, AFL were detected at levels below regulatory limits. In contrast, Saleh et al. (2016) performed assays on 36 samples of cassava flour to determine and quantify aflatoxins also using immunoenzymatic assay (ELISA). The results obtained showed that 22 of the 36 were contaminated with total AFL in the range of 2.0 to 7.5 µg/kg. This study indicated that there is a low concentration of aflatoxin in cassava and suggested that cassava flour is safe and good for human consumption. When evaluating the identification of contaminating fungi in products related to cassava, results in which *A. flavus* is present, but no AFL are detected, were also described in the literature which may indicate that, although only seven samples were positive for AFLG1, fungal contamination may still have occurred in the other samples, considering the favorable conditions that the region offers for its growth.

3.2 Statistical Analysis

3.2.1 Mann-Whitney Test

The non-parametric Mann-Whitney test consists of comparing the distribution of two samples. Table 4 presents the descriptive statistics of the results, in Table 5 we can conclude that the non-parametric Mann-Whitney test showed that level (U=23.00; p-value <0.05) influences AFL, while that Aw has no effect on AFL (U=47.00; p-value>0.05).

Table 4. Descriptive statistics

Descriptive statistics							
	Average	Standard deviation	Minimum	Maximum	Percents		
					5th	50th (Mediana)	5th
Mc%	41.61	1.9674	37.79	47.78	0.47	41.81	2.84
Aw	0.99	0.0028	0.99	1.00	0.99	1.00	1.00
AFL	0.29	0.4643	0	1		0	

Table 5. Test statistics

Test statistics		
Test	Mc%	Aw
Mann-Whitney U	23.00	47.00
Asymp. Sig. (2-tailed)	0.020	0.427
Exact Sig. [2*(1-tailed Sig.)]	0.019	0.455

3.2.2 Spearman's Rho Test

Spearman's correlation evaluates the monotonic relationship between two continuous or ordinal variables. The correlation coefficient can vary in terms of value -1 to +1. The higher the absolute value of the coefficient, the stronger the relationship between the variables. Table 6 shows that the correlation between mc% and activity Aw was significant at the 5% level of significance p-value 0.017<0.05. Spearman's Rho Correlation coefficient showed that there is a positive and moderate correlation of 0.481 between mc% and Aw.

Table 6. Spearman correlation

Spearman correlation				
Spearman's rho	Mc%		Mc%	Aw
		Correlation Coefficient	1.000	0.481*
		Sig. (2-tailed)	-	0.017
		N	24	24
	Aw	Correlation Coefficient	0.481*	1.00
		Sig. (2-tailed)	0.017	-
		N	24	24

4. Conclusion

From the results obtained, the occurrence of AFLG1 in the tapioca gum sold in Manaus was verified, but it is not possible to state whether the concentration obtained is safe or not for human consumption, since there is no specific legislation for this basis. However, greater rigor on the part of surveillance is recommended, actions at points of sale that encourage good manufacturing practices, adoption of corrective measures, aimed at improving the production and storage of gum with greater quality and safety with the health of consumers, bearing in mind that the Amazon region has environmental conditions that favor the development of toxigenic fungi and the production of mycotoxins. It is also suggested the analysis and quantification of other mycotoxins that may be present in tapioca gum to support risk management by consumer health protection agencies.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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