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ORIGINAL ARTICLE

Aspergillus section *Flavi* and aflatoxins in Brazilian cassava (*Manihot esculenta* Crantz) and products

L. T. Ono[1] · J. J. Silva[1] · S. Doná[2] · L. M. Martins[1] · B. T. Iamanaka[1] · M. H. P. Fungaro[3] · J. I. Pitt[4] · M. H. Taniwaki[1]

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Abstract

Aflatoxins are carcinogenic compounds produced by some species of *Aspergillus*, especially those belonging to *Aspergillus* section *Flavi*. Their occurrence in food may start in the field, in the post-harvest, or during storage due to inadequate handling and storage. Because cassava is a staple food for a high percentage of the Brazilian population, we evaluated the presence of aflatoxin-producing species in cassava tubers, cassava products (cassava flour, cassava starch, sour starch, and tapioca flour), and in soil samples collected from cassava fields. In addition, the levels of aflatoxin contamination in cassava products were quantified. A total of 101 samples were analyzed, and 45 strains of *Aspergillus* section *Flavi* were isolated. Among the identified species, *Aspergillus flavus*, *Aspergillus arachidicola*, *Aspergillus novoparasiticus*, and *Aspergillus parasiticus* were found. The majority of strains (73.3%) tested for their aflatoxin-producing ability in synthetic media was positive. Despite that, cassava and cassava products were essentially free of aflatoxins, and only one sample of cassava flour contained traces of AFB_1 (0.35 µg/kg).

Keywords *Aspergillus* · Cassava · Tubers · Cassava products · Aflatoxins · Soil

Introduction

Originally from South America, cassava (*Manihot esculenta* Crantz) has great socio-economic importance, especially in developing countries, where it is mostly cultivated by small farmers (Alves 2002; Otsubo et al. 2002). The average consumption per person of cassava and its products in Brazil in 2017 was 13.8 kg/year, for the African continent 59.5 kg/year, and for South America, 27.9 kg/year (FAO 2020). Brazil is the third largest cassava producer in the world and the biggest in the western hemisphere. Nigeria is the largest producer, followed by Thailand (FAO 2020).

It is estimated that 23% of cassava production can be lost because it is highly perishable and is easily invaded by fungi. Invasion can occur pre-harvest in the field, or post-harvest if stored under inadequate conditions (Wareing et al. 2001; Alves et al. 2005).

Some filamentous fungi are capable of producing mycotoxins, potentially causing diseases or death in humans and animals (Taniwaki and Pitt 2019). Aflatoxins, the most undesirable of mycotoxins, are of great importance in food, as aflatoxin B_1 (AFB_1) has been classified as a Group I liver carcinogen by the International Agency for Research on Cancer (IARC 2002). Indeed AFB_1 is the most potent naturally occurring carcinogen (IARC 2002).

Several species classified in *Aspergillus* section *Flavi* frequently occur in foods, and this section includes most aflatoxigenic species. Currently, eighteen species in *Aspergillus* section *Flavi* are recognized as aflatoxin producers, but the majority is of minor importance. The most commonly occurring species are *Aspergillus flavus* and *Aspergillus parasiticus*, but *Aspergillus nomius*, *Aspergillus novoparasiticus*, and *Aspergillus arachidicola* are of importance in some commodities (Frisvad et al. 2019).

The presence of species from *Aspergillus* section *Flavi* and the formation of aflatoxins in cassava and its products have been investigated mainly in producer countries including Ghana (Wareing et al. 2001), Benin (Gnonlonfin et al. 2008, 2012; Adjovi et al. 2014), Uganda (Kaaya and Eboku

✉ M. H. Taniwaki
marta@ital.sp.gov.br

1 Institute of Food Technology (Ital), Campinas, SP, Brazil

2 Paulista Agribusiness Technology Agency (APTA), Technological Development Center of Agribusinesses in Médio Paranapanema, Assis, SP, Brazil

3 Universidade Estadual de Londrina, Londrina, PR, Brazil

4 Microbial Screening Technologies, Smithfield, NSW, Australia

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2010), and Nigeria (Aghimien and Ikenebomeh 2017). Studies that investigated the occurrence of aflatoxins and toxigenic species in Brazil have been limited and have lacked precision because molecular analyses of species have not been used, limiting the precision of identification, as some are cryptic species (Gomes et al. 2007; Mesquita et al. 2017).

The present study aimed to investigate the diversity of *Aspergillus* section *Flavi* isolated from cassava tubers, products, and soils using molecular markers and to evaluate the frequency of toxigenic strains within the species found. In addition, to assess the occurrence of aflatoxins in samples of cassava and its products produced in Brazil.

Material and methods

Cassava tubers, products, and soil samples

Samples were obtained from producers in the state of São Paulo, in the cities of Assis, Cândido Mota, São Pedro do Turvo, Campos Novos Paulista, Ribeirão do Sul, and Palmital. Samples were also collected from markets in the city of Campinas. A total of 101 samples were collected, 27 of cassava tubers, 22 from soil in which cassava was being cultivated, and 23 from cassava flour, as well as smaller numbers of samples from cassava starch ($n = 11$), sour starch ($n = 9$), and tapioca flour ($n = 9$).

The amount of cassava collected for each sample varied on the number of roots present in each tree and the size of the tubers found. Each tuber from the same tree was collected and considered one sample. For the soil samples, after digging about 10 cm deep, approximately 0.5 kg were collected. For cassava products, a sample of 0.5 kg were purchased from the markets.

Frequency and morphological identification of *Aspergillus* section *Flavi*

Fungi were isolated using the methods described by Pitt and Hocking (Pitt and Hocking 2009). From each sample, approximately 100 g was taken randomly and surface disinfected with sodium hypochlorite solution (0.4%) for 1 min. Fifty pieces of each cassava tuber were then distributed evenly in five Petri dishes containing Dichloran rose Bengal chloramphenicol agar (DRBC) and incubated at 25 °C for 5 to 7 days. Non-particulate samples, i.e., soil, cassava flour, cassava starch, sour starch, and tapioca flour were analyzed by dilution plating. Samples (25 g) were diluted in peptone water (0.1%, 225 mL). Aliquots (0.1 mL) were inoculated onto dichloran glycerol agar (DG18) plates and incubated at 25 °C for 5 to 7 days.

Isolates that had the appearance of belonging to *Aspergillus* section *Flavi* were transferred to Czapek yeast extract agar (CYA) and incubated at 25 °C for 7 days. Morphological identification was carried out using the classification systems of Pitt and Hocking (Pitt and Hocking 2009) and Klich (Klich 2002). The isolated cultures were maintained in silica according to the methodology of Kirsop and Snell (1984).

Molecular analysis of *Aspergillus* section *Flavi*

Initially, forty-two isolates were identified morphologically as belonging to *Aspergillus* section *Flavi*, and later, these isolates were identified to species by molecular methods. For extraction of genomic DNA, the strains were purified by single spore culture and grown in liquid yeast extract sucrose at 25 °C for 3 days in still cultures until the formation of a mycelial film. The mycelia were then removed from the culture medium and macerated using liquid nitrogen. Genomic DNA was purified using a PureLink Plant Kit (Invitrogen, USA) according to the protocol recommended by the manufacturer. DNA was quantified spectrophotometrically using NanoDropTM 2000/2000c (Thermo Fisher Scientific, USA).

The calmodulin gene (*CaM*) was amplified using the CMD5 and CMD6 primer pairs, as previously described (Hong et al. 2006). PCR products were purified using ExoProStar TM 1-Step (GE Healthcare Life Sciences, UK), according to the manufacturer's recommended protocol. Amplicons were sequenced in both directions using the amplification primers and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in a SeqStudio Genetic Analyzer (Applied Biosystems, USA).

CaM gene sequences were aligned with those from type or neotype strains of species in *Aspergillus* section *Flavi* (GenBank) using ClustalW (Thompson et al. 1994). Representatives of all intra and interspecific variability (based on the CaM gene) found in this study were deposited in GenBank and accession numbers can be found in Supplementary Information 1, together with the reference strains of *Aspergillus* section *Flavi* used in the construction of phylogenetic trees. The Tamura-Nei model (Tamura and Nei 1993) with gamma distribution (+G) was selected for maximum likelihood (ML) and Bayesian inference (BI) analysis. MEGA 7 (Kumar et al. 2016) and MrBayes v. 3.2.6 (Ronquist et al. 2012) were used to obtain the phylogenetic inferences based on the respective ML and BI analyses.

To estimate the level of support for the ML tree, bootstrap analysis was performed on 1000 replicates. For BI analysis, the Markov chain Monte Carlo (MCMC) algorithm was run for 5,000,000 generations. Sample frequency was set to 100, with 25% of trees removed as burn-in. Convergence diagnostics were monitored based on an average standard deviation of split frequencies below 0.01, potential scale

reduction factor (PSRF) values close to 1.0, and effective sample size (ESS) values above 200. The trees were visualized using FigTree version 1.4.2, developed by Andrew Rambaut (http://tree.bio.ed.ac.uk/software/figtree).

Water activity

The water activities of cassava and its products were determined using an Aqualab Series 3TE instrument (Decagon, Pullman, WA, USA) at 25 ± 1 °C, in duplicate.

Analysis of aflatoxin production by isolates of *A.* section *Flavi*

Forty-two isolates from *Aspergillus* section *Flavi* were tested for their potential to produce aflatoxins using the methodology described by von Hertwig et al. (2020). The fungi were inoculated into yeast extract sucrose agar. Three small pieces of mycelium were removed (plugs) from the central portion of the colony. The toxin was extracted with 2 mL of methanol and homogenized for 2 min manually. The extract was twice filtered in a 0.22-µm Millex membrane.

The HPLC system used was an Agilent 1260 Infinity model system (Santa Clara, CA, USA) with a fluorescence detector set at 362-nm excitation and 455-nm emission for aflatoxins G_1 (AFG_1) and G_2 (AFG_2) and 425-nm emission for aflatoxins AFB_1 and AFB_2. An ODS (1.8 µm, 40 × 15 mm; Agilent, Santa Clara, CA, USA) guard column and a Zorbax Eclipse Plus C18 column (5 µm, 4.6 × 150 mm; Agilent, Santa Clara, CA, USA) were used. The mobile phase was water:acetonitrile:methanol (6:2:3, v/v/v), containing KBr (119 mg) and nitric acid (4 M, 350 µL/L) at a flow rate of 1 mL/min with injection volume of 20 µL. A post-column derivatization of AFB_1 and AFG_1 was performed with bromine using a KobraCell (R-Biopharm Rhône Ltd., Darmstadt, Germany).

Extraction of aflatoxins from cassava samples

The method used to extract aflatoxins from cassava and products was modified from Stroka et al. (2000). Cassava tuber samples were cut and ground, while cassava flour, starch, sour starch, and tapioca flour were homogenized before weighing. Each sample (25 g) was extracted with sodium chloride (2.5 g) in methanol/water solution (100 mL; 80/20, v/v), then solutions were homogenized in a shaker (New Brunswick Scientific Company, USA) for 30 min. From duplicate filtrations, filtered liquid (10 mL) was diluted with phosphate-buffered saline (PBS; 60 mL) and passed through an Aflatoxin Immunoaffinity column (Easi-Extract Aflatoxin, R-Biopharm, Germany). Finally, the column was washed with distilled water. Aflatoxins were eluted with methanol (1.25 mL) and with deionized water (1.75 mL; Milli-Q system) and then filtered (1 mL) through a 0.22-µm pore syringe filter with 13-mm diameter.

Aflatoxin detection by HPLC

The HPLC system used for aflatoxin detection from cassava samples was an Agilent 1260 Infinity model system (Santa Clara, CA, USA) with a fluorescence detector set at 362-nm excitation and 455-nm emission for AFG_1 and AFG_2 and 425 nm emission for AFB_1 and AFB_2. An ODS (1.8 µm, 40 × 15 mm; Agilent, Santa Clara, CA, USA) guard column and a Zorbax Eclipse Plus C18 column (5 µm, 4.6 × 150 mm; Agilent, Santa Clara, CA, USA) were employed. The mobile phase was water:methanol:acetonitrile (62:22:16, v/v/v), containing KBr (119 mg) and nitric acid (4 M, 350 µL/L) at a flow rate of 1 mL/min with injection volume of 20 µL. Determination of aflatoxins by HLPC-FLD with post-column derivatization of aflatoxins AFB_1 and AFG_1 was performed with bromine using a KobraCell (R-Biopharm Rhône Ltd, Darmstadt, Germany).

Limits of detection and quantification and aflatoxin recovery

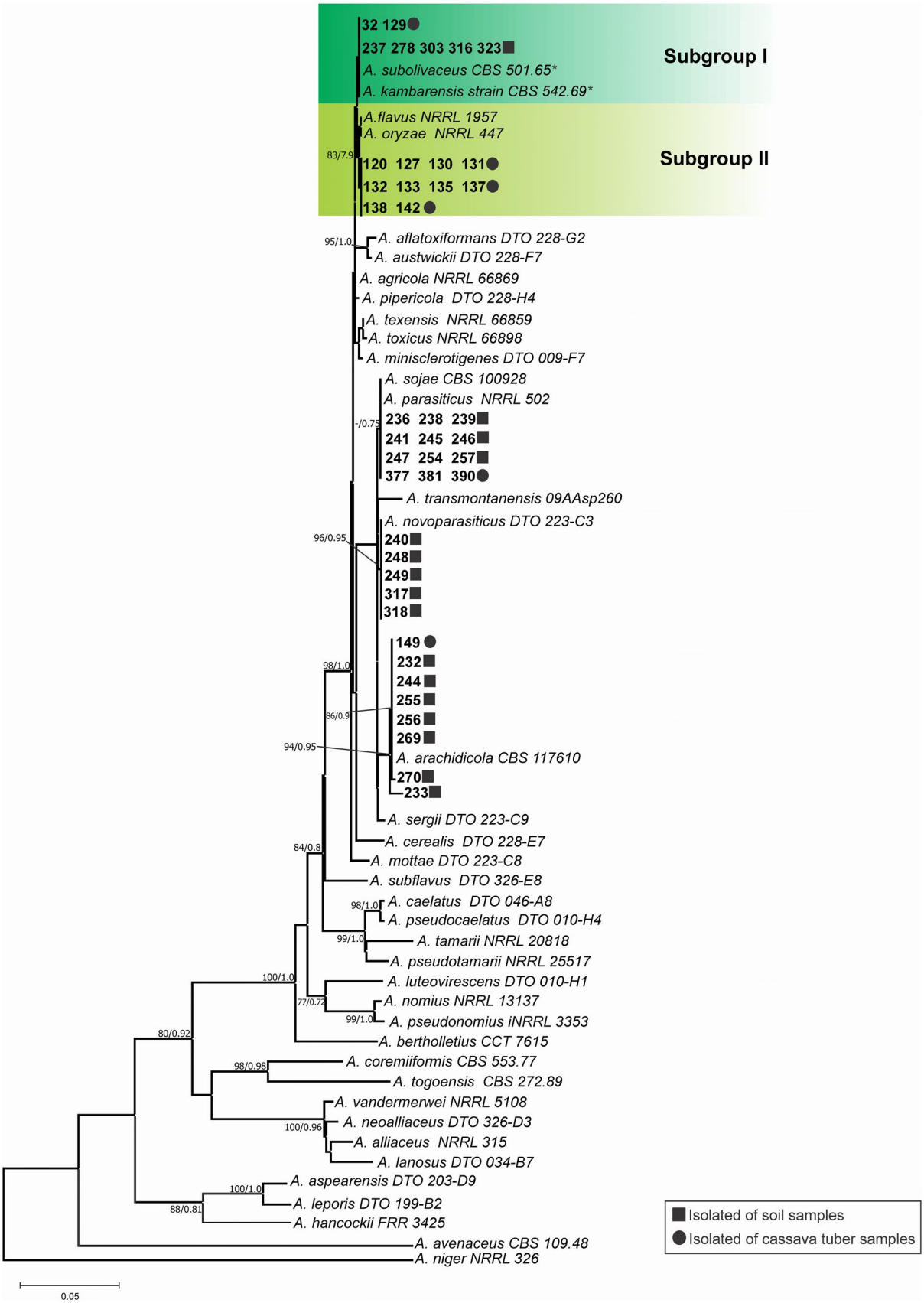
The detection (LOD) and quantification (LOQ) limits were determined according to Magnusson and Örnemark (2014). Eight replicates of cassava tubers artificially contaminated at the levels of 0.20, 0.19, 0.12, and 0.07 µg/kg of AFB_1, AFB_2, AFG_1, and AFG_2, respectively, were analyzed. The standard deviation obtained was used to calculate the LOD and LOQ values. The aflatoxin recovery was determined through a triplicate of low-contaminated samples (0.6 µg/kg of total aflatoxin).

Results

Water activity and diversity of *Aspergillus* section *Flavi*

The water activity of cassava tubers was 0.922 to 0.996, cassava flour 0.082 to 0.677, tapioca flour 0.372 to 0.997, cassava starch 0.317 to 0.598, and sour starch 0.451 to 0.595. Fungi from *Aspergillus* section *Flavi* were present in 13 of the 101 samples examined, only in six samples of cassava tubers and 7 of soil. None was found in samples of cassava flour, cassava starch, sour starch, or tapioca flour.

From the total of 42 isolates of *Aspergillus* section *Flavi* analyzed by molecular technique, four species were identified: *A. flavus* ($n = 17$), *A. parasiticus* ($n = 12$), *A. arachidicola* ($n = 8$), and *A. novoparasiticus* ($n = 5$) (Fig. 1). *Aspergillus flavus*, *A. arachidicola*, and *A. parasiticus* were found in cassava tubers, of which *A. flavus* was the most common.


Subgroup I
Subgroup II
32 129
237 278 303 316 323
A. subolivaceus CBS 501.65*
A. kambarensis strain CBS 542.69*
A.flavus NRRL 1957
A. oryzae NRRL 447
120 127 130 131
132 133 135 137
138 142
83/7.9
95/1.0
A. aflatoxiformans DTO 228-G2
A. austwickii DTO 228-F7
A. agricola NRRL 66869
A. pipericola DTO 228-H4
A. texensis NRRL 66859
A. toxicus NRRL 66898
A. minisclerotigenes DTO 009-F7
A. sojae CBS 100928
A. parasiticus NRRL 502
-/0.75
236 238 239
241 245 246
247 254 257
377 381 390
A. transmontanensis 09AAsp260
A. novoparasiticus DTO 223-C3
96/0.95
240
248
249
317
318
149
98/1.0
232
244
255
86/0.9
256
269
94/0.95
A. arachidicola CBS 117610
270
233
A. sergii DTO 223-C9
A. cerealis DTO 228-E7
84/0.8
A. mottae DTO 223-C8
A. subflavus DTO 326-E8
98/1.0
A. caelatus DTO 046-A8
A. pseudocaelatus DTO 010-H4
99/1.0
A. tamarii NRRL 20818
A. pseudotamarii NRRL 25517
100/1.0
A. luteovirescens DTO 010-H1
77/0.72
A. nomius NRRL 13137
99/1.0
A. pseudonomius iNRRL 3353
80/0.92
A. bertholletius CCT 7615
98/0.98
A. coremiiformis CBS 553.77
A. togoensis CBS 272.89
A. vandermerwei NRRL 5108
100/0.96
A. neoalliaceus DTO 326-D3
A. alliaceus NRRL 315
A. lanosus DTO 034-B7
100/1.0
A. aspearensis DTO 203-D9
88/0.81
A. leporis DTO 199-B2
A. hancockii FRR 3425
A. avenaceus CBS 109.48
A. niger NRRL 326
0.05
Isolated of soil samples
Isolated of cassava tuber samples

◂**Fig. 1** Maximum likelihood tree based on the *CaM* gene showing the relationships between *Aspergillus* section *Flavi* species and isolates of cassava tubers and soil samples. Bootstrap values (BS) and/or posterior probabilities values (pp) higher than 70% and 0.70, respectively, are shown. *Aspergillus niger* is the outgroup. Isolates from this study are in bold. (*) = Synonym of *Aspergillus flavus*

All four species were present in the soil samples, *A. flavus* and *A. arachidicola* being the most frequent (Table 1).

Aflatoxin production in yeast extract sucrose agar

Thirty-one of the 42 strains from *Aspergillus* section *Flavi* here analyzed produced aflatoxins under the conditions used. From the soil samples, 65% of the isolates were able to produce aflatoxins, while for cassava tuber samples, about 94% of the tested isolates were aflatoxigenic (Table 1). All isolates of *A. novoparasiticus* and *A. parasiticus* (Fig. 1) produced aflatoxins of both groups B and G. Out of seven *A. arachidicola* isolates obtained from the soil samples (Fig. 1), only two produced aflatoxins B and G, and the only isolate of this species obtained from cassava tubers was a non-producer (Fig. 1, Table 1). Among the *A. flavus* isolates obtained from cassava tubers (Fig. 1), 100% produced type B aflatoxins, but only one isolate from the soil sample produced aflatoxin group B.

Occurrence of aflatoxins in cassava tubers and products

The averages (8 repetitions) of limit of detection and quantification using the HPLC methodology described for aflatoxins were the following: $AFB_1 = 0.02$ and 0.07, $AFB_2 = 0.02$ and 0.06, $AFG_1 = 0.02$ and 0.06, and $AFG_2 = 0.01$ and 0.02, respectively. The total aflatoxin (AFB_1, AFB_2, AFG_1, and AFG_2) limits of detection for this method were 0.06 µg/kg, and the limit of quantification was 0.21 µg/kg. The average recovery of total aflatoxins was 105%, with an average recovery for AFB_1 of 101%, AFB_2 of 109%, AFG_1 of 101%, and AFG_2 of 110%.

The recovery obtained for both total aflatoxins and individually for AFB_1, AFB_2, AFG_1, and AFG_2 is in accordance with the values recommended by Magnusson and Örnemark (2014), which determines that, for concentrations below 1 µg/kg, the values must remain between 50 and 120%. The occurrence of aflatoxins in cassava tubers and products is shown in Table 2. Only one sample of cassava flour presented a level of AFB_1 of 0.35 µg/kg. AFB_2, AFG_1, and AFG_2 were not detected, and no aflatoxigenic strain was found in this sample.

Discussion

The diversity of mycobiota of cassava and its products has been described by various authors, and isolates from *Aspergillus* section *Flavi* have been reported frequently (Wareing et al. 2001; Gnonlonfin et al. 2008; Adjovi et al. 2014). The occurrence of this fungal group in food is of concern because of aflatoxigenic species. In the present study, isolates from *Aspergillus* section *Flavi* were not found in cassava products examined, cassava flour, cassava starch, sour starch, and tapioca flour, but only in tuber samples and adjacent soil of this food, indicating that soil is the main source of contamination. Heating steps during drying and processing at a temperature of 160 °C for 3 h (SENAR 2018) may destroy most of them and similar asexual fungi in these products. Aflatoxin if present may be reduced but not totally destroyed in this process.

The methods of storage and sale of cassava and its products in Brazil and elsewhere depend on their water activity. Because of their high water activity, cassava tubers can only be stored fresh for a limited time, or converted to products which are dried to a lower water activity. Because of their low water activity, most cassava products are sold at room temperature. Tapioca flour originates from the re-hydration of cassava starch. The a_w found in this study varied from 0.372 to 0.997, among different brands, showing a high vulnerability for fungal growth and mycotoxin production during its shelf life that can last some months in the markets at room temperature. These brands rely on chemical preservatives such as sorbic or propionic acids for their stability.

Aspergillus flavus had the highest frequency of occurrence in all samples (Table 1), but *A. parasiticus* was the species with the highest number of isolates from soil (Fig. 1). *Aspergillus arachidicola* and *A. novoparasiticus* were also isolated from these samples. The regions, where cassava tubers and soil samples were collected, are also used to grow sugarcane, and these two crops can succeed or be intercropped in the same cultivation field. *Aspergillus parasiticus*, *A. novoparasiticus*, and *A. arachidicola* have been reported in sugarcane and in soil cultivated by this crop in Brazil (Iamanaka et al. 2019; Silva et al. 2019), so it is not surprising to find these species in soil planted with cassava.

In this study, *A. arachidicola* was reported for the first time to be associated with cassava culture (soil and tubers). It was unusual to find that only two isolates (of eight) of this species were able to produce aflatoxins, as the majority of *A. arachidicola* isolates have been reported to produce both aflatoxins B and G (Pildain et al. 2008; Calderari et al. 2013; Katsurayama et al. 2018). The identity of the isolates in this group was also confirmed by

Table 1 Presence of *Aspergillus* section *Flavi* species and water activity (a_w) in cassava tubers (27) and soil samples (22) and aflatoxins produced by the strains (number of positive strains/total number of tested strains)

Species	Cassava tubers			Soil		
	No. of occurrence (FO%)	AFB	AFB + AFG	No. of occurrence (FO%)	AFB	AFB + AFG
Aspergillus flavus	3 (11.1)	12/12	-	4 (18.2)	1/5	-
Aspergillus arachidicola	1 (3.7)	-	0/1	4 (18.2)	-	2/7
Aspergillus novoparasiticus	0.00	-	-	2 (9.1)	-	9/9
Aspergillus parasiticus	1 (3.7)	0	3/3	2 (9.1)	-	5/5
Mean a_w	0.987			0.983		

FO frequency of occurrence in % (number of samples that contained a fungal species/total of samples analyzed)

sequencing two other genes *BenA* and *RPB2* (data not shown), that corroborated the previous result. This shows that knowledge about the definition of producing and non-producing species in *A*. section *Flavi* is still in construction. The recent understanding that some strains of *A. flavus* may produce G-type aflatoxins (Frisvad et al. 2019) is another example. Among the isolates from cassava tuber samples, *A. flavus* was the predominant aflatoxigenic species. As can be seen in Fig. 1, there was the formation of two groups within the *A. flavus/oryzae* clade (subgroup I and subgroup II), and subgroup II is composed of the isolates that were grouped together with type strain of *Aspergillus flavus* sensu stricto (NRRL 1957), while the isolates of subgroup I did not group the type strain of *A. flavus*; and formed a new branch together with the type strains of *Aspergillus kambarensis* (CBS 542.69) and *Aspergillus subolivaceus* (CBS 501.65), both considered synonymous with *A. flavus* (see Frisvad et al. 2019). *Aspergillus kambarensis* and *A. subolivaceus* were included in the phylogenetic tree to clarify the taxonomic position of subgroup I isolates and avoid misinterpretations due to the branching within the *A. flavus/oryzae* clade, that occurs naturally due to the great intraspecific variability of *A. flavus*.

Most of the *A. flavus* isolates from subgroup I were obtained from soil samples, but this subgroup was also found in cassava tuber samples and 80% (4/5) of the subgroup I isolates obtained from soil did not produce aflatoxins. As a contrast, all of the isolates obtained from cassava tuber samples were producers of aflatoxins type B. Subgroup II was found only in cassava tuber samples, and all isolates were able to produce aflatoxins type B. Therefore, it is clear that there is no relationship between the aflatoxigenic profile of *A. flavus* and the subgroups found here, but in relation to the origin of the isolates. The community of *A. flavus* from cassava tuber samples showed a higher incidence of aflatoxigenic isolates in relation to those obtained from the soil samples, a fact that weakens the hypothesis of contamination through the soil, at least in the case of *A. flavus*, and raises new questions about the sources of post-harvest contamination.

Despite the well-documented occurrence of *A. flavus* and other potentially aflatoxigenic species in cassava and its products, most reports indicate the absence of aflatoxins in these substrates. We found only one sample of cassava flour with a very low value, close to the detection limit (Table 2). In Benin, Gnonlonfin et al. (Gnonlonfin et al. 2008) studied cassava chips in 20 villages: aflatoxin was not detected in any of the samples, despite the presence of *A. flavus*. Similar results were obtained by Wareing et al. (2001) and Muzanila et al. (2000).

Adjovi et al. (2014) found mostly toxigenic *A. flavus*, *Aspergillus aflatoxiformans* (previously reported as *Aspergillus parvisclerotigenus*), and *A. novoparasiticus* in 36 samples of fresh cassava, but the presence of aflatoxins was not observed. The authors inoculated a highly aflatoxigenic strain of *A. flavus* into fresh cassava, but although the fungus grew, aflatoxins were not produced. The authors suggested that possibly fresh cassava may produce an unidentified compound capable of blocking aflatoxin production in fresh cassava.

Table 2 Occurrence of aflatoxins in cassava tubers and products

Sample	AFB_1	AFB_2	AFG_1	AFG_2	Total
Tuber ($n=27$)	ND	ND	ND	ND	ND
Cassava flour ($n=22$)	ND	ND	ND	ND	ND
Cassava flour ($n=1$)	0.35	ND	ND	ND	0.35
Cassava starch ($n=11$)	ND	ND	ND	ND	ND
Sour starch ($n=9$)	ND	ND	ND	ND	ND
Tapioca flour ($n=9$)	ND	ND	ND	ND	ND
Limit of detection—LOD (µg/kg)	0.02	0.02	0.02	0.01	0.06
Limit of quantification—LOQ (µg/kg)	0.07	0.06	0.06	0.02	0.21
Average recovery (%)*	101	109	101	110	105

ND not detected

*Average recovery at low concentration (0.6 µg/kg)

Several earlier studies reported contamination of cassava and its products by aflatoxins (Bulatao-Jayme et al. 1982; Manjula et al. 2009; Kaaya and Eboku 2010; Aghimien and Ikenebomeh 2017). However, most such reports were in error, relying on thin layer chromatography, where a blue spot running identically with AFB_1 was interpreted as a positive result, or using ELISA tests, both methods without confirmation steps. Cassava extract samples can show more interferences on TLC plates than the fungi extracts in culture media. Therefore, for more reliable results, we recommend using HPLC especially when analyzing food samples.

Isolates from *Aspergillus* section *Flavi* were present in samples of cassava tubers and cultivated soil, but not in its products. Four potentially aflatoxigenic species were found (*A. flavus*, *A. parasiticus*, *A. novoparasiticus*, and *A. arachidicola)*, with *A. flavus* being the dominant species. Despite the presence of these species, only one of the 101 samples analyzed showed low aflatoxin contamination, indicating that aflatoxins are not a problem in Brazilian cassava.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12550-021-00430-2.

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Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

The experiments comply with the current Brazilian laws.

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