BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

REVIEW ARTICLE

Fungi and mycotoxins in cassava (*Manihot esculenta* Crantz) and its products

*Fungos e micotoxinas em mandioca (*Manihot esculenta *Crantz) e seus produtos derivados*

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Cite as: Ono, L. T., & Taniwaki, M. H. (2021). Fungi and mycotoxins in cassava (*Manihot esculenta* Crantz) and its products. *Brazilian Journal of Food Technology*, *24*, e2020240. https://doi.org/10.1590/1981-6723.24020

Abstract

Cassava (*Manihot esculenta* Crantz) is a highly consumed food in the world, especially in developing countries. Much of this tuber production comes from small farmers and it can suffer microbial infection during pre-harvest in the field and/or postharvest if stored under inadequate conditions. This review presented cassava production and the processing steps, resulting in products consumed in Brazil and other countries. Studies on fungal occurrence, including toxigenic fungi, presence of aflatoxins and other mycotoxins in cassava and its products carried out in several countries have been revised as well as the used methodologies for mycotoxin detection.

Keywords: Mycobiota; Toxigenic fungi; Aflatoxins; Cassava tubers; Cassava flours; Cassava processing.

Resumo

A mandioca (*Manihot esculenta* Crantz) é um alimento muito consumido no mundo, principalmente nos países em desenvolvimento. Grande parte da produção deste tubérculo vem de pequenos agricultores. A mandioca pode sofrer a infecção microbiana antes da colheita no campo e/ou após a colheita, se estocada sob condições inadequadas. Esta revisão apresenta a produção de mandioca e as etapas de processamento, que resultam nos produtos que são consumidos no Brasil e em outros países. Estudos sobre a ocorrência de fungos, incluindo os fungos toxigênicos, e a presença de aflatoxinas e outras micotoxinas na mandioca e seus produtos, conduzidos em vários países, foram revisados, bem como as metodologias de detecção das micotoxinas.

Palavras-chave: Micobiota; Fungos toxigênicos; Aflatoxinas; Tubérculos de mandioca; Farinhas de mandioca; Processamento de mandioca.

1 Introduction

Cassava (*Manihot esculenta* Crantz) has great socio-economic importance; it is cultivated mainly in developing countries, due to its ability to adapt to adverse conditions and its low cost. The composition of cassava is favorable to the development of fungi and toxins, with a low presence of proteins and a high amount of carbohydrates (Essono et al., 2009). Although few studies have been carried out in Brazil and other developing countries on fungi and toxins in cassava and its products, they are highly consumed in these countries and this issue should be considered for studying.

In 2018, world production of cassava reached more than 277 million tons. In tropical regions, it is considered the third most important crop, after maize and rice. Cassava production in Africa represents more than 60% of world production and among countries, Nigeria is in the first position, followed by Thailand. Brazil is the third-largest cassava producer in the world (Adjovi et al., 2015; Food and Agriculture Organization, 2020).

Generally, cassava production comes mainly from family farming, on a small scale, for local commerce. Thus, it usually does not apply technology, research or studies, which results in lower production and a more heterogeneous product. The highest level of productivity is usually achieved in large-scale production, with the aim to sell to large companies, and which involves investment not only in mechanization, but in the correction of acidity, fertilization, selection of plants, adequate spacing and weed control (Modesto Júnior & Alves, 2014, 2016).

Cassava belongs to the Euphorbiaceae family, is originally from South America, and is believed to have been spread to other continents through Portuguese immigrants. It has approximately 100 species, but only one is commercially cultivated, *Manihot esculenta* Crantz. Mainly small farmers plant cassava, as their cultivation does not require fertile soils or controlled acidity. This crop can withstand great variations in the level of precipitation, temperature and altitude; in addition, its harvest can be carried out from six to twenty-four months (Alves, 2002; Otsubo et al., 2002; Kouakou et al., 2016).

It is estimated that the average consumption of cassava and its products per person, according to data from the Food and Agriculture Organization (FAO) of the United Nations (Food and Agriculture Organization, 2020), in 2017, was 13.81 kg/year. Quantities for the African continent reached 59.45 kg/year and for South America, 27.87 kg/year.

Cassava productivity can be compromised in the event of the development of microorganisms. This contamination occurs mainly under conditions of inadequate practices in the field, transportation and storage. The use of susceptible varieties, contact with poor and contaminated soil, water and air are the main causes of fungal contamination in field. This fact has an economic impact and causes a progressive fall in productivity, since some microorganisms are able to survive in the soil for long periods, which can cause damage in future harvests and produce mycotoxins (Peraica et al., 1999; Gomes & Leal, 2003; Ferreira Neto et al., 2004; Notaro et al., 2013).

This review presented the general aspects of cassava and its main products, mycobiota and occurrence of toxins, aiming to identify possible limitations and indicate where more research could be developed.

2 Production of cassava products

The way cassava can be prepared varies according to the country in which this food is consumed; each region has particularities, resulting in many different products. In addition to being consumed *in natura*, this tuber can be converted into fermented foods such as: *gari*; *fufu*; fermented chips; beers; *beiju;* and *banu*. Also being consumed in non-fermented foods, such as: chips; pellets; and flours such as tapioca (Falade & Akingbala, 2011; Adjovi et al., 2015;).

Below, there is the definition of the cassava products which are found most frequently and a flowchart (Figure 1) showing the processing steps, resulting in products consumed in Brazil and other countries.

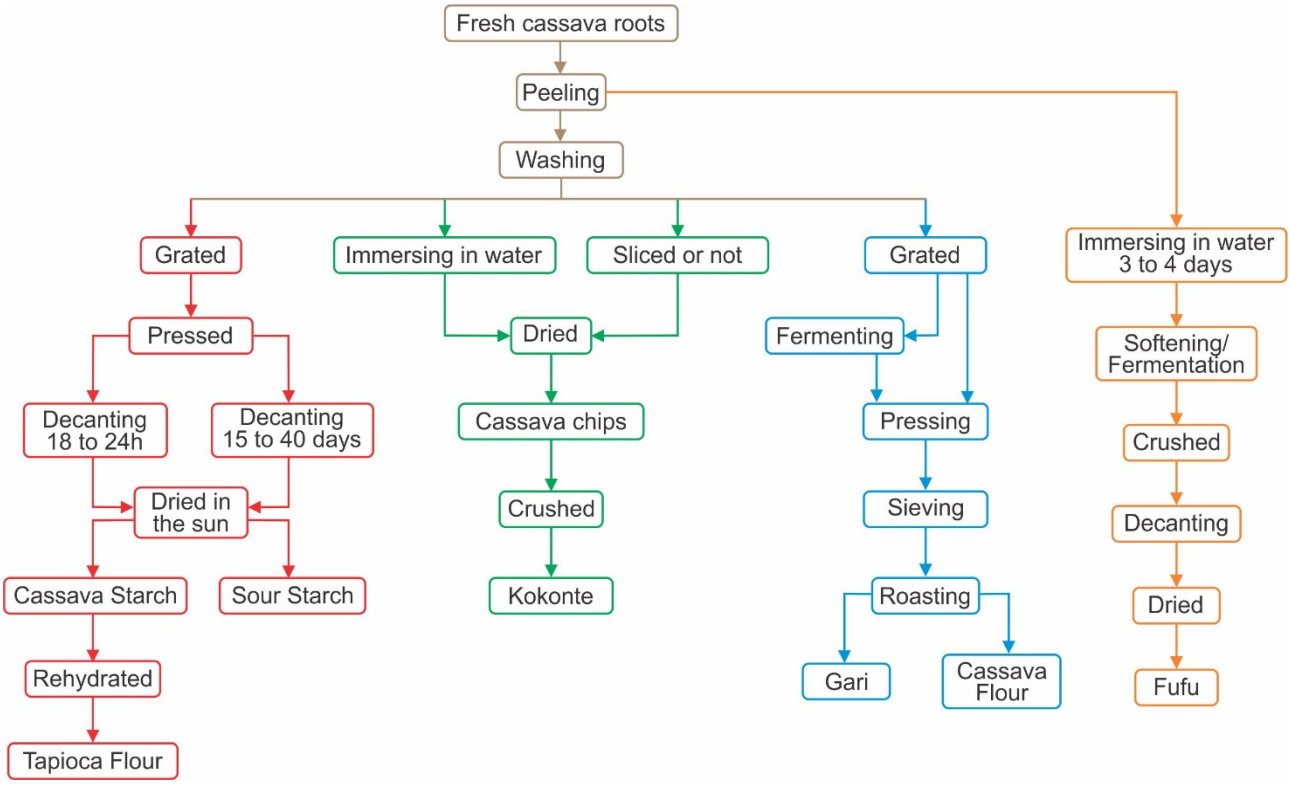

Figure 1. Processing of cassava products.

The most consumed cassava derivative in West Africa is the *gari.* Produced handmade, it is obtained by peeling, scraping, fermenting, pressing, sieving and roasting the cassava. There are several variations for the types of *gari* products; they can be consumed immersed in water, can be drunk or sprayed on other foods, and can also be diluted in water or milk and transformed into a dough (Adjovi et al., 2015; Escobar et al., 2018).

Very popular in Nigeria, Ghana and other parts of Africa, *fufu* is prepared by immersing cassava roots in water for 3 to 4 days. After softening and fermentation, they are crushed and left to stand for decanting and drying. The obtained dough is usually consumed with soups (Ogbuji & David-Chukwu, 2016).

Kokonte is the product obtained through cassava chips, which are traditionally dried in the sun and then crushed, forming flour (Wareing et al., 2001).

The cassava product most used in Africa and most consumed in Brazil, is flour, which can be processed in different ways. In South America, the clean and peeled root is grated and pressed, obtaining a dough, which is sieved, taken to the oven and baked (Cohen et al., 2007; Adjovi et al., 2015).

Other traditional Brazilian products are obtained by natural fermentation of moist starch extracted from cassava tubers that gives rise to cassava starch, sour starch, and tapioca flour. For production, the clean tubers are grated and pressed, and the filtered liquid is used to obtain the starch. In the case of cassava starch, it is deposited at the bottom of the container for 18 to 24 hours, then removed and taken to dry in the sun for about 8 hours. Tapioca flour, in fact, originates from the rehydration of cassava starch. In the manufacture of sour starch, the decanting stage is longer, remaining in the fermentation tanks for 15 to 40 days, which raises the acidity of the final product. Finally, the residue is removed from the tanks and taken to dry in the sun (Cohen et al., 2007; Adjovi et al., 2015).

Cassava products sold in Brazil can have different moisture content and water activity (a_w) ranges. In a study of Ono (2020), fresh cassava tubers presented a_w varied from 0.922 to 0.996, and, because of the high a_w, they can only be stored fresh for a limited time or converted to products which are dried to a lower a_w.

Tapioca flour a_w found in this study (Ono, 2020), 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 ambient temperature. These brands rely on chemical preservatives such as sorbic or propionic acids for their stability.

3 Mycobiota of cassava and its products

Studies carried out in Brazil on cassava mycobiota, analyzed 15 samples of cassava flour in Macapá, noting that 80% of the samples were contaminated by *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium chrysogenum* (Mesquita et al., 2017). Similarly, the genera *Aspergillus* and *Penicillium* were also found in the Amazon by Gomes et al. (2007), when analyzing nine samples of different types of cassava flour. They observed fungal count in all samples and of the total, 40% presented contamination by *Penicillium spp*. and 38% by *Aspergillus spp*. The genera *Rhizopus*, *Cladosporium*, and yeasts, were found in lower proportions.

Cassava is grown in direct contact with the soil, being susceptible to contamination by fungi present in it, which can contaminate the surface and infect its tissues (Adjovi et al., 2015; Paul, 2015). In Nigeria, Sule & Oyeyiola (2012) observed physical-chemical and also mycological characteristics in different types of soils cultivated with cassava. They isolated *Aspergillus*, *Acremonium*, *Brettanomyces*, *Botrytis*, *Byssochamys*, *Cladosporium*, *Doratomyces*, *Geotrichum*, *Gliocladium*, *Humicola*, *Moniliella*, *Mucor*, *Monascus*, *Neuspora*, *Oidiodendron*, *Penicillium*, *Papulospora*, *Piricularia*, *Rhodotorula*, *Rhizopus*, *Saccharomyces*, *Trichoderma* and *Ustilago*.

In Uganda, Kaaya & Eboku (2010) observed fungal counts in all 75 samples of cassava chips, varying between $4.5x10^1$ and $1.0x10^6$ CFU/g with an average of $5.0x10^4$ CFU/g. *Rhizopus spp*. was the most prevalent, and they also identified the genera *Mucor*, *Penicillium, Aspergillus* and *Fusarium*. However, *A. flavus* was the most found toxigenic fungus in 18.5% of the samples. In Nigeria, Jimoh & Kolapo (2008) reported the genera *Rhizopus nigricans, Aspergillus niger* and *Fusarium oxysporum* in cassava chip samples.

Studies in samples of cassava chips in Benin found fungal contamination and the genera found were *Aspergillus, Fusarium, Penicillium, Mucor, Nigrospora* and *Rhizopus* (Gnonlonfin et al., 2008). Later, in the same place, Gnonlonfin et al. (2012) found 14 fungal genera in the samples of cassava chips, with dominance of species *Rhizopus oryzae, Nigrospora oryzae, Chrysonilia sitophila, C. resinae, C. herbarum, A. niger* and *A. flavus*. The mycobiota of cassava products (pellets, flours and gari) analyzed in Nigeria, found *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Neospora* spp., *Choanophora* spp., *Cladosporium* spp., *Rhizopus* spp., *Rhodotorula* spp., *S. cerevisiae*, *F. oxysporium*, *Botrydiplodia theobromae*, *Helminthosporium* spp. and *Trichoderma* spp. Some *Aspergillus* species such as *A.* section *Flavi, A. niger, A. nidulans, A. terreus* and *A. fumigatus* were identified (Aghimien & Ikenebomeh, 2017).

In Ghana, 101 samples of *kokonte* were analyzed and their mycobiota were described by Wareing et al. (2001). It was found that yeasts and *Cladosporium spp.* were the most frequent in the samples, however, other fungi were also isolated, such as *Aspergillus* spp., *Alternaria* spp., *Colletotrichum* spp., *Drechslera* spp., *Fusarium* spp., *Monilia* spp., *N. oryzae, Phoma sorghina, Geotrichum* spp., *Aureobasidium* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Paecilomyces variotii* and *Wallemia sebi*.

In a study on cassava spoilage, from Noon & Booth (1977) in Colombia, 120 cassava samples were analyzed. After 11 days post-harvest, several microorganisms were found as following: *Aspergillus* spp.; *Botryodiplodia* spp.; *Fusarium* spp.; *Mucor* spp.; *Penicillium* spp.; *Rhizopus* spp.; and *Trichoderma* spp..

Table 1 shows a summary of the mycobiota of cassava and its products in different countries.

Table 1. Mycobiota of cassava and its products in different countries.

Samples	Nº samples	Mycobiota	Countries	References
Cassava roots (after 11 days storage)	120	*Aspergillus* spp., *Botryodiplodia* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *Rhizopus* spp. and *Trichoderma* spp.	Colombia	Noon & Booth (1977)
Kokonte	49	*Aspergillus* spp., *Alternaria* spp., *Cladosporium* spp., *Colletotrichum* spp., *Drechslera* spp., *Fusarium* spp., *Monilia* spp., *Nigrospora oryzae, Phoma sorghina, Geotrichum* spp., *Aureobasidium* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Paecilomyces variotii e Wallemia sebi*	Ghana	Wareing et al. (2001)
Cassava flour	9	*Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Rhizopus* spp.	Brazil	Gomes et al. (2007)
Cassava chips	100	*A. flavus, Aspergillus spp., F. verticillioides, P.chrysogenum, P. sorghina, M. piriformis, R. oryzae, N. oryzae*	Benin	Gnonlonfin et al. (2008)
Cassava chips	20	*R. nigricans, A. niger, F. oxysporum*	Nigeria	Jimoh & Kolapo (2008)
Cassava chips	72	*Aspergillus: A. aculeatus, A. candidus, A. clavatus, A. flavipes, A. flavus, A. fumigatus, A. niger, A. nomius, A. ochraceous, A. parasiticus, A. tamarii, A. terreus, A. versicolor*	Cameroon	Essono et al. (2009)
Cassava chips	75	*Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Rhizopus* spp.	Uganda	Kaaya & Eboku (2010)
Cassava chips	60	*Aspergillus* spp., *Cladosporium* spp., *Chrysonilia* spp., *Nigrospora* spp., *Rhizopus* spp.	Benin	Gnonlonfin et al. (2012)
Cassava soil	-	*Aspergillus* spp., *Acremonium* spp., *Brettanomyces* spp., *Botrytis* spp., *Byssochamys* spp., *Cladosporium* spp., *Oratomyces* spp., *Geotrichum* spp., *Gliocladium* spp., *Humicola* spp., *Moniliella* spp., *Mucor* spp., *Monascus* spp., *Neuspora* spp., *Oidiodendron* spp., *Penicillium* spp., *Papulospora* spp., *Piricularia* spp., *Rhodotorula* spp., *Rhizopus* spp., *Saccharomyces* spp., *Trichoderma* spp., *Ustilago* spp.	Nigeria	Sule & Oyeyiola (2012)
Cassava flour	15	*A. niger*, *A. fumigatus* and *P. chrysogenum*	Brazil	Mesquita et al. (2017)
Cassava products (pellets, flour and *gari*)	-	*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Neospora* spp., *Choanophora* spp., *Cladosporium* spp., *Rhizopus* spp., *Rhodotorula* spp., *S. cerevisiae, F. oxysporium, B. theobromae, Helminthosporium* spp. *and Trichoderma* spp.	Nigeria	Aghimien & Ikenebomeh (2017)

4 Occurrence of toxigenic fungi, aflatoxins and other toxins in cassava and its products

Some filamentous fungi are capable of producing secondary metabolites called mycotoxins, and their toxicity can cause diseases and even death in humans and animals. Aflatoxins correspond to the group with the highest occurrence in food and are considered to be grouped into Group 1, i.e., carcinogenic to humans according to the International Agency for Research on Cancer (Peraica et al., 1999; International Agency for Research on Cancer, 2002; Bennett & Klich, 2003).

The most frequently found aflatoxin-producing fungi are *A. flavus, A. nomius* and *A. parasiticus*, although aflatoxins can be produced by other species. Currently, eighteen species in the *A.* section *Flavi* group are recognized as aflatoxin producers: *A. flavus; A. parasiticus; A. nomius; A. pseudonomius; A. novoparasiticus; A. pseudotamarii; A. togoensis; A. pseudocaelatus; A. luteovirescens; A. minisclerotigenes; A. arachidicola; A. sergii; A. transmontanensis; A. mottae; A. aflatoxiformans; A. austwickii; A. pipericola* and *A. cerealis* (Frisvad et al., 2019).

There are more than 20 known aflatoxins, however, the main forms in which they are presented can be associated with AFB_1, AFB_2, AFG_1 and AFG_2, which refer to their fluorescent properties since AFB_1 and AFB_2 show blue fluorescence, whereas AFG_1 and AFG_2 show green fluorescence (Taniwaki & Pitt, 2019).

The occurrence of mycotoxins in cassava and its base products has been reported in several consuming countries, although most reports are on aflatoxins and aflatoxigenic species. In Cameroon, Essono et al. (2009) analyzed 72 samples of cassava chips, evaluating for two months the influence of the product storage time in relation to the quantification of aflatoxins. Initially 13 species of *Aspergillus* were isolated*: A. aculeatus; A. candidus; A. clavatus; A. flavipes; A. flavus; A. fumigatus; A. niger; A. nomius; A. ochraceus; A. parasiticus; A. tamarii; A. terreus* and *A. versicolor*. Of these species, *A. flavus, A. nomius* and *A. parasiticus*, received special attention, for being aflatoxin producers. Of the total, 18 samples showed aflatoxin contamination with a variation between 5.2 and 14.5 µg/kg. The first contaminated sample was detected only after four weeks.

In a study conducted in markets in Tanzania and the Republic of Congo, by Manjula et al. (2009), samples were obtained of 38 different types of cassava root (such as fresh, stored and smoked) and also samples of chips and flour. They quantified aflatoxins AFB_1 in different types of processing and storage time, obtaining results that varied from 0.3 to 4.4 µg/kg for cassava chips and flour. The stored cassava samples showed the highest concentration levels, with a range from 0.1 to 13 µg/kg. The contamination levels between fresh, stored and smoked, presented no difference statistically. In addition, traces of fumonisin were quantified in the samples, with low levels that varied between not detected to 0.07 µg/kg in the chips and flour samples.

In Nigeria, Aghimien & Ikenebomeh (2017) analyzed four types of samples of cassava products: pellets; flours (industrial and local) and *gari*. The products were collected in three main markets in each of the six chosen regions. The highest concentration of AFB_1 was found in the local cassava flours, with levels reaching 83.54 ± 2.95 µg/kg, probably due to the type of processing and lack of good manufacturing practices. However, 75% of the samples obtained results below 20 µg/kg, among them, 6% with values below the detection limit. Investigating the presence of mycotoxins in four samples of cassava flour, in Benin, Ediage et al. (2011) found aflatoxin B_1 (< LOQ - 9 µg/kg) and aflatoxin B_2 (< LOQ - 8 µg/kg). There were fumonisin B_1 (4-21 µg/kg), diacetoxyscirpenol (< LOQ - 6 µg/kg) and zearalenone (< LOQ - 12 µg/kg) in these samples as well. The authors attribute the coexistence of aflatoxins and fumonisin due to possible stress during cassava growth. Although the incidence of aflatoxins has been reported, some authors have not found them in detectable quantities in their work, such as Gnonlonfin et al. (2008). When analyzing the total presence of aflatoxins and fumonisin B_1, they observed the absence of these mycotoxins in samples of cassava chips in Benin.

Muzanila et al. (2000) collected samples of cassava chips, which were later processed into flour, in villages in Tanzania. Analyses were performed to check the production of aflatoxins, but none of the samples showed contamination.

A study of *kokonte*, conducted in Ghana, showed that more than half of 101 samples had at least one toxigenic fungal species with a count greater than 10^4 CFU/g, and these samples were tested for mycotoxins. The samples with toxigenic fungi were tested as follows: *A. flavus* for aflatoxins; *A. versicolor* for sterigmatocystin; *Penicillium* spp. for patulin, penicillic acid and cyclopiazonic acid; *Fusarium* spp. for neosolaniol and T-2 toxin; and *Phoma sorghina* for tenuazonic acid. Neosolaniol, T-2 toxin and aflatoxins were not present in any of the samples analyzed. The most common mycotoxins in *kokonte* samples were sterigmatocystin, patulin and cyclopiazonic acid (Wareing, et al., 2001).

Adjovi et al. (2014) analyzed 36 cassava samples, finding *A. flavus, A. parvisclerotigenus* and *A. novoparasiticus*, with the most toxin-producing strains; however, the presence of aflatoxins was not observed. In this same study, the authors inoculated a highly aflatoxigenic strain of *A. flavus* in cassava, and although the fungus developed, aflatoxins were not produced. The authors reported that possibly cassava may have the capacity to block the production of AFB_1 during pre-harvest; however, in this work it is suggested that after heat treatment this ability may be compromised, since the molecule responsible for inhibition would be thermosensitive. Therefore, it is important to ensure good storage practices for derived products, and in this way, favorable conditions do not arise for the production of toxins.

Table 2 shows data on the occurrence of mycotoxins in cassava and products, mostly from African and Latin American countries. Different methods for mycotoxin analyses have been used such as Thin Layer Chromatography (TLC), Enzyme-Linked Immunosorbent Assay (ELISA), High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Validation of these methods has not always been performed. In addition, the lack of confirmatory tests for mycotoxins put some of these findings in doubt.

Table 2. Occurrence of mycotoxins in cassava and its products from different countries.

Samples	Nº samples	Mycotoxins	Incidence (%)	min-max (µg/kg)	Methods	Countries	References
Cassava chips	54	Total AFs	0	< LOD	TLC	Tanzania	Muzanila, et al. (2000)
Kokonte	2	Total AFs	0	< LOD	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	10	Sterigmatocystin	100	170-1670	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	11	Patulin	36	550-850	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	11	Penicilic acid	45	60-230	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	11	Cyclopiazonic acid	36	80-720	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	18	T-2	0	< LOD	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	18	Neosolaniol	0	< LOD	HPTLC	Ghana	Wareing et al. (2001)
Kokonte	8	Tenuazonic acid	37	20-340	HPTLC	Ghana	Wareing et al. (2001)
Cassava chips	100	Total AFs	0	< LOD	HPLC	Benin	Gnonlonfin et al. (2008)
Cassava chips	20	Total AFs	0	< LOD	TLC	Nigeria	Jimoh & Kolapo (2008)
Cassava chips	6	AFB_1	100	0.4-4.38	ELISA	Congo	Manjula et al. (2009)
Cassava flour	3	AFB_1	100	0.32-1.64	ELISA	Congo	Manjula et al. (2009)
Cassava chips	72	Total AFs	25	< LOD-14.5	ELISA	Cameroon	Essono et al. (2009)
Cassava chips	75	Total AFs	30	0-4.5	HPLC	Uganda	Kaaya & Eboku (2010)
Cassava flour	4	Total AFs	100	< LOQ-9.0	LC-MS/MS	Benin	Ediage et al. (2011)
Cassava flour	4	Diacetoxyscirpenol	75	< LOD-9.0	LC-MS/MS	Benin	Ediage et al. (2011)
Cassava flour	4	Zearalenone	75	< LOD-12.0	LC-MS/MS	Benin	Ediage et al. (2011)
Cassava flour	4	Fumonisin B_1	100	4.0-21.0	LC-MS/MS	Benin	Ediage et al. (2011)
Cassava chips	60	Total AFs	0	< LOD	HPLC	Benin	Gnonlonfin et al. (2012)
Cassava roots	36	AFB_1	0	< LOD	HPLC	Benin	Adjovi et al. (2014)
Pellets, flour and *gari*	72	Total AFs	94	< LOD-83.54	ELISA	Nigeria	Aghimien & Ikenebomeh (2017)

LOD: Limit of Detection; TLC: Thin Layer Chromatography; HPTLC: High Performance Thin-Layer Chromatography; HPLC: High Performance Liquid Chromatography; ELISA: Enzyme-Linked Immunosorbent Assay; LC-MS/MS: Liquid Chromatography-Mass Spectrometry.

5 Conclusion

In fact, significant fungal diversity in cassava tubers and cassava products has been reported and the presence of *Aspergillus* species capable of aflatoxin production is of concern. In this review some studies showed the presence of mycotoxins, especially aflatoxins in cassava and cassava based products. Different methods for mycotoxin analyses have been used, however, validation of these methods has not always been performed and the lack of confirmatory tests for mycotoxins put these findings in doubt. Further studies are needed using more adequate methodologies for fungal identification, in order to determine aflatoxins and mycotoxins with confirmatory steps and identify the compound that can apparently inhibit aflatoxin production in fresh cassava tubers. These findings will contribute to a better understanding in the significance of fungi and toxins in cassava and its products to consumer health.

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Funding: Fundação de Desenvolvimento da Pesquisa do Agronegócio.

Received: Oct. 06, 2020; Accepted: June 22, 2021