Contaminant fungi in processed peanuts

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ABSTRACT

Peanuts (*Arachis hipogea* L) are a plant cultivated in many parts of the planet and are considered a relevant food crop, with many minerals and vitamins in their grains, as well as fats and proteins, which are important for human health. In underdeveloped or developing countries, peanuts represent an important source of income for many families, and can be used by the oil and animal feed industries. Despite being a low-cost food source, peanuts can pose risks to human health, as they are subject to contamination by mycotoxin-producing fungi. Mycotoxins are heat-resistant compounds, which are not completely eliminated after heat treatment, meaning that even when roasted or cooked, peanut kernels can cause inconvenience to those who consume them. Cases involving allergies, necrosis of organs such as kidneys and liver, and even carcinogenesis, caused by mycotoxins, are widely recorded in the medical literature. This research work aimed to evaluate the occurrence and diversity of mycotoxin-producing fungi in peanut kernels, sold in closed packages in the city of Inhumas. BDA medium (potato dextrose agar) was used for the isolation of fungi. The sampling consisted of 200 grains, belonging to five different brands. Half of the samples were disinfected with 1% sodium hypochlorite. Morphological characteristics (macroscopic and microscopic) of the colonies were used to identify the genera to which the isolated fungi belong. The results revealed a high rate of fungal contamination in peanut kernels, with *Fusarium* *being* the predominant fungus. Fungi of the genera *Rhizopus* and *Aspergillus* were also identified in the samples. It is expected that the results obtained will contribute to increase the information on the presence of toxigenic fungi in peanut kernels, helping in the decision-making of regulatory agencies and consumers who use this important food source.

Keywords: *Arachis hypogea*, Mycotoxins, Toxigenic fungi.

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INTRODUCTION

Peanut (*Arachis hypogaea* L) is a species of herbaceous plant, belonging to the Fabaceae family, whose fruits develop in pods (legumes) below the surface of the ground. It was cultivated for the first time, probably in the valleys of Peru (GEETHA *et al*., 2013), thus having South American origin. It belongs to the genus *Arachis*, which contains more than 82 wild species (ROYAL BOTANIC GARDENS, 2022), 66 of which occur in Brazilian territory (FLORA E FUNGA DO BRASIL, 2022).

Approximately two-thirds of the peanuts produced in the world come from China and India, but their production is spread across a hundred countries, especially in Asia and Africa (CHEN *et al*., 2019), with an approximate cultivation area of 30 million hectares (NCUBE; MAPHOSA, 2020), In Brazil, the state of São Paulo concentrates most of the production, having been responsible for more than 90% of the peanuts produced in the 2017/2018 harvest (GRIGOLETO *et al*., 2019).

Peanuts are the fourth main oilseed plant and the 13th most important food crop in the world (ISALAR *et al*., 2021). It has outstanding nutritional characteristics, constituting an important food source for populations in tropical and semi-arid regions of the planet. Its beans contain high percentages of oil, proteins, carbohydrates, phosphorus, calcium, magnesium, amino acids, polyphenols, and vitamins (SINGH *et al*., 2021) and can be consumed, raw, roasted, or cooked, as is the case, for example, in the northeast of Brazil.

Currently, there are several products derived from peanut processing, such as candy, confectionery, paste/cream, butter, oil, flour, as can be easily observed in supermarkets, or even on internet sites. Peanuts can also be used for oil extraction (domestic or industrial use) for soap production, in cooking or even for the manufacture of cosmetics (ABDULLA, 2013). In addition, its grains can also be processed into animal feed, and its waste can be turned into fertilizers (ISALAR *et al*., 2021).

Agricultural products such as peanuts, grThe authors in tropical areas, usually hot and humid, are susceptible to contamination by fungi and bacteria. In addition, the exposure of grains to physical and chemical factors, associated with the absence of good agricultural and manufacturing practices, makes it possible to be attacked by spoilage microorganisms, at any stage of the production chain, (MONYOA *et al*., 2012; EMBRAPA, 201), among them toxigenic fungi, which produce microtoxins.

Mycotoxins are secondary metabolites, produced by numerous species of filamentous fungi and very dangerous to humans and animals, since such microorganisms can infect and develop in food and feed. Such metabolites are generally characterized by being active in extremely low concentrations and being resistant to degradation (MIRABILE *et al*., 2021). Once ingested by the consumer, mycotoxins behave in a toxic way, and can cause acute diseases such as skin rashes, vomiting, diarrhea, headache or chronic diseases such as nephropathies, immunosuppression and carcinogenesis (FREIRE; da ROCHA, 2017).

The mere presence of toxigenic fungi in processed peanut kernels does not necessarily indicate the presence of mycotoxins, but it increases the possibility of their occurrence and points to the low sanitary quality in which the product is found. Thus, the construction of the present work originated from the idea (or intention) of increasing the amount of information on the occurrence of toxigenic fungi in processed peanut samples, aiming to demonstrate to the general public, consumers and regulatory agencies, the state in which the quality of peanuts sold in Inhumas, Goiás, is located. with regard to the presence of these microorganisms.

THEORETICAL FRAMEWORK

PEANUT

Peanuts are a leguminous plant, native to South America, whose cultivation has been occurring in Peru for at least 8,500 years. Portuguese and Spanish explorers, at the time of colonization, would have found Indians growing peanuts in countries such as Mexico, Brazil, Argentina, Paraguay and Bolivia. From these regions, then, peanuts would have been disseminated to Africa, Europe, Asia, the Pacific Islands, and the United States (HAMMONS *et al*., 2016).

Like many other crops, peanuts have acquired greater relevance in regions far from their origin. Notably, its importance is most evident in Asia, where 64% of all world production comes from. In Africa, peanut production represents 26% of global production (FAOSTAT, 2019) and exceeds the combined amount of all other legume grains produced on that continent (BERTIOLI *et al*., 2011).

In Brazil, peanut production is basically concentrated in the state of São Paulo, which totals 92% of national production with 890 tons (FOREIGN AGRICULTURAL SERVICE, 2023). Data from the 2018/2019 agricultural cycle inform that the total of grains of this *commodity* produced in Brazil resulted in a revenue that exceeded the mark of R$ 1 billion, covering all aspects related to the crop, from cultivation to the industrial process (JAMMAL, 2019).

The data mentioned above on production and financial transactions suggest the size of the economic impact of peanut crops, which play an important role in job creation and increased income for families that are directly or indirectly involved in the production of this crop (ALBUQUERQUE; DOS SANTOS; CAVALCANTI, 2010).

It is estimated that approximately 80% of the total population of the African continent depends on agriculture as a means of subsistence (FAO, 2015). As a source of income in periods where drought is predominant, peanuts gain prominence for the generation of jobs in this continent, their manufacture had an estimated growth of 130% between the years 1970 and 2013, with Nigeria being the main producing country with 39% of international production and 7% of global production, producing 4.9 million tons in an area of approximately 5.4 million hectares (LOZANO, 2016).

PEANUTS: GENERAL ASPECTS

Peanuts are of significant importance in several socioeconomic aspects, especially in underdeveloped nations. It is a crop with a low production cost, due to its resistance to pests and diseases, which can be grThe authors in small areas. In addition, the demand for peanuts is high, both for *fresh consumption* and for the production of processed foods, which creates business opportunities and increases the possibilities for job and income generation (MARTINS; VICENTE, 2010).

Chemical and nutritional characteristics of peanuts add value to its production and commercialization (UNICAMP, 2006), since its grains have great gastronomic versatility, and can be consumed raw, roasted, processed or even as part of the elaboration of products such as peanut butter, peanut butter, paçoca, among others (SALVE *et al*., 2020). This is because its grains have significant amounts of various nutrients such as proteins, mineral salts, vitamins, fibers and healthy fats (SOARES; SCALLOP; CARVALHO, 2021).

Peanut kernels are also an excellent source of oil, rich in fatty acids beneficial to human health, having important characteristics for use in the food and cosmetics industries. Unlike other oils, peanut oil has high levels of monounsaturated and polyunsaturated fats (SINGH *et al*., 2021).

The relationship between peanut production and care for the environment is also a relevant topic, as the cultivation of this plant can require a lot of soil care and thus, if there is no proper management, productivity can decrease over time. In addition, the inadequate disposal of production residues can cause land degradation and reduce the potential of local environmental resources (SOUZA *et al*., 2010), thus requiring appropriate strategies to mitigate possible impacts.

FUNGI

The peanut plant is cultivated in tropical and subtropical regions, with low water availability, at latitudes between 10º and 30º south (BELLETTINI; ENDO, 2001; SILVA *et al*., 2018). It can measure between 30 and 50 centimeters in height, have alternate leaves, composed of three oval-shaped leaflets, with yellow flowers, which develop at the base of the stems. Its fruits appear below the surface of the soil, and may have an oval shape, with a protective film of reddish-brThe authors color and between three and four grains inside its pods (KRAPOVICKAS; GREGORY, 1994).

Due to the fact that it is a crop that develops entirely in the soil, peanuts are subject to fungal contamination. Fungi are eukaryotic, heterotrophic, diverse, unicellular or multicellular organisms and can be found in practically all environments on the planet, especially in soil and aquatic environments (TORTORA, 2012). It is rare to find environments free of the presence of these organisms, which use the atmosphere as their main means of survival and can tolerate significant variations in temperature, humidity, pH and oxygen levels. (JUNIOR, 2015).

In general, fungi have spores that appear somewhere in their life cycle. These spores are microscopic reproductive bodies, which commonly enter industrial environments through the contamination of raw materials and can be disseminated through the air, in the form of aerosols or water, and are also carried by animals and insects (BERNARDI; GARCIA; COPETTI, 2019). This is a form adopted by fungi so that they can propagate, colonizing new substrates, thus ensuring their survival (FERRO *et al*., 2019).

Fungi obtain nutrients through other living beings, such as plants and animals, through the decomposition of the organic matter of these organisms through parasitism. They play an important role in the decomposition of organic matter, in the cycling of nutrients, in addition to having great biotechnological potential, being used by the pharmaceutical industry in the production of medicines, personal hygiene products, in the preparation of food and beverages, and in the production of biofertilizers (DE ABREU *et al*., 2015).

Some fungi, such as mushrooms and truffles, are prized for their flavor and nutritional value, while others are used in the production of cheeses, wines, and beers (BAIÃO, 2020). Despite being beneficial in many processes, a significant part of fungi is toxigenic, which indicates that in addition to contaminating food inputs, they are poisonous and can produce dangerous toxins, causing numerous health problems in animals and humans (ROCHA *et al*., 2014).

With regard to peanut fungal contamination, several factors are related, which can occur before, during and after harvest, mainly due to the absence of good practices along the production chain. Thus, inadequate storage conditions, lack of adequate hygiene during harvest, non-conformities during processing and transportation, contaminated soil and irrigation water, and contact with other infected foods represent circumstances that favor the process of contamination of peanut grains by fungi (CAVALCANTE; OLIVEIRA, 2022).

The main genera of fungi frequently found in peanut kernels are *Aspergillus*, *Penicilinium*, *Rhizopus* and *Fusarium* (VISOTTO *et al*., 2008). *Aspergillus* sp. and *Penicilinium* sp. are generally associated with poor physiological quality of the grains, involving problems related to size, color, hull integrity, texture, and flavor (SABBADINI *et al*., 2009).

The genus *Aspergillus* encompasses more than 340 officially accepted species of filamentous fungi (Bennett, 2010). They are organisms recognized for their remarkable reproductive capacity (through the release of conidia) and for their remarkable metabolic versatility. They can thrive easily in environments with low humidity and water activity, spreading across a wide variety of terrestrial habitats (BORGES *et al*., 2012).

Fungi of the genus *Aspergillus* are the main causes of the deterioration of agricultural products. Its species also produce important mycotoxins, posing a risk to human and animal health, especially *A. Flavus*, *A. Parasiticus*, *A. Niger* and *A. Ochraceus* (KLICH, 2002). However, *A. Flavus*, *A. Niger,* and *A. fumigatus* can cause aspergillosis, which is characterized by lung problems, allergies, and systemic impairment (LIRA, 2022).

Penicillum is a genus of fungus comprising approximately 150 described species. Most of them are spoilage, can decompose food and attack plants and animals in low temperature conditions (HECK *et al*., 2021). It has very peculiar characteristics when observed under a microscope, making identification quite complex, mainly due to the number of branches of its hyphae and the size and shape of its conidia (CHALFOUN; BATISTA, 2003).

A particularly interesting characteristic in fungi of the genus *Penicillium* is their ability to produce lipases (SILVA, 2022). Lipases extracted from molds, such as *Penicillium*, have several applications in the medical field, in the production of pharmaceuticals, in the cosmetics and food industries, and also in the production of biodiesel (HECK *et al*., 2021).

The genus *Fusarium* comprises phytopathogenic fungi that attack several species of plants used in human food, causing considerable damage to crops around the world. *Fusarium* sp. are responsible for a disease generically knThe authors as fusariosis, which can affect both the shoots and the roots of plants. Its most common species are *Fusarium oxysporum* and *Fusarium solani*, which affect crops such as tomatoes, potatoes, bananas and corn (PINARIA; LIEW; BURGESS, 2010).

Fusarium *infection* can occur in different ways, including contamination from other infected grains, through the soil, or even from the water used in irrigation (NELSON *et al*., 1992). To control the growth of these fungi, agronomic techniques such as crop rotation, certified grains, and the use of specific fungicides are usually used. However, integrated management is the best strategy for disease prevention and control (LESLIE; SUMMERELL, 2019).

The genus *Fusarium* produces a variety of mycotoxins, the most important being trichothecenes (vomitoxin and T-2 among others), fumonisins, zearalenone, moniliformin and fusaric acid. In general, *Fusarium* species can produce different mycotoxins, depending on the cultivation conditions (SANTIN *et al.*, 2021).

Rhizopus is one of the main groups of fungi that cause black molds in food (fruits, vegetables, and grains). The *fungus Rhizopus* uses conditions such as temperatures between 20º C and 30º C and places with high humidity to develop (SILVA *et al*., 2021). However, they are also easy to find in the environment. These microorganisms, in addition to being food spoilage, can cause diseases in humans, the most important of which is cerebral zygomycosis, which mainly affects the lungs, subcutaneous tissue and gastrointestinal tract, reaching even the blood vessels when it is already very widespread (RODRIGUES, 2009).

MYCOTOXINS

Mycotoxins are secondary metabolites produced by filamentous toxigenic fungi that cause toxic reactions when ingested by humans or animals. *Fusarium*, *Aspergillus* and *Penicillium* are the most abundant fungi that contaminate food and produce mycotoxins before, during and after harvest (BINDER *et al*., 2007).

Mycotoxins can be classified according to the species of fungus that produces, their chemical structure and their mechanism of action. The same species of fungus can produce different mycotoxins, depending on the environmental conditions in which this microorganism develops. On the other hand, a single mycotoxin can be produced by different species of fungi. Although there are numerous fungi capable of producing mycotoxins, the most technically significant genera are *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins can take on various structural conformations (HUSSEIN; BRASEL, 2001). Of the approximately

300 substances identified as mycotoxins, the most studied include aflatoxins, trichothecenes, fumonisins, ochratoxin A, rye spur alkaloids and patulin (BANDO *et al*., 2007).

Mycotoxins can be harmful to human and animal health, and ingestion of contaminated food can cause acute and chronic toxic effects, including liver, kidney and neurological problems. Aflatoxin is considered a human carcinogen and is associated with a higher incidence of liver cancer (COSTA; VERZELETTI; WAGNER, 2014). According to Liu; Wu (2018), aflatoxin may be responsible for inducing hepatocellular carcinoma in a range of 4.6% to 28.2% of cases worldwide.

Numerous studies have shThe authors that the presence of aflatoxin caused by *Aspergillus* sp. in peanuts is a global problem (SANTOS *et al*., 2018; LIEN, 2019; ARISTIL, 2020; QIN *et al*., 2020; MASAKA *et al*., 2022). Alternatively, many studies have demonstrated over the last few years that other mycotoxins can also occur in peanuts, such as ochratoxin A, produced by *Aspergillus niger*, *Aspergillus carbonarius*, (MAGNOLI *et al*., 2006), *Aspergillus Ochraceus* and *Penicillium* (KRSKA *et al*., 2012) and zearalenone by *Fusarium* (SANGARE-TIGORI *et al*., 2006; KRSKA *et al.,* 2012).

In order to minimize mycotoxin contamination in peanuts, it is considerable to implement control measures at all stages of the production chain, from cultivation to processing and storage. Some of these measures include the selection of high-quality grains, the use of appropriate agronomic practices, the control of humidity and temperature during storage and processing, the use of detection and analysis methods to identify the presence of mycotoxins, in addition to good manufacturing practices (SAITA; PANDOLFI, 2019).

PEANUT QUALITY

Ensuring a product with adequate quality for the consumer is a process that involves awareness, commitment and persistence of agents related to the production chain of peanuts and derivatives. The quality of peanuts can be defined by several factors, including the harvesting process, which is an essential step in the quality of the final product (SCALCO; QUEIROZ; MACHADO, 2008).

Costa *et al*. (2019) emphasize that in production systems, harvesting and post-harvest processes are the basis for obtaining high-quality products. The process of handling peanuts, when not carried out properly, can promote the growth of fungi, lead to undesirable fermentations and produce mycotoxins such as ochratoxin (OTA), which can harm the health of humans (ROCHA *et al*., 2014).

The post-harvest stages involve processes that must be carried out properly to prevent peanut grains from being contaminated by fungi, such as *Aspergillus*. Contamination can occur primarily in the soil, which is a great indication of poor quality during processing operations (SUASSUNA *et al*., 2005).

In general, the stages of processing for the quality of the beans involve: harvesting, which must be carried out during the maturation stage of the plant and its pods; followed by drying, which must be carried out as soon as possible. The pods should be placed up, facilitating the absorption of wind and sunlight, minimizing water activity and later humidity. Next, the cleaning stage aims to remove all impurities, which are due to the field (leaves, branches, stones and other materials considered foreign bodies), for this the peanut grains are subjected to processes such as sieving and fans to facilitate the extraction of dirt (ZANUTTO, 2021). After the cleaning process, the peanuts will be submitted to the classification process with different criteria regarding the color, size and quality of the grain, which help to distinguish which are the best lots to sell (SANTOS *et al*., 2009).

The largest crop of peanuts is harvested during the rainy season, which makes it difficult to dry. As the grain must be bagged with a maximum moisture content of 8%, according to IN No. 32, of August 24, 2016 (BRASIL, 2016), after harvesting, it is recommended that it be immediately subjected to drying to avoid moisture problems, which can be carried out naturally or artificially. It is necessary to avoid rewetting the grain and mitigate the development of fungi and other problems related to peanut quality. Once rewetted during the processing stages, the grains are even more susceptible to attack by fungi (AGROBYTE, 2005). In addition, it is important to eliminate broken pods and grains, as they can affect the conservation of peanuts (SILVA, 2005).

The storage of peanuts should be planned in such a way as to avoid deterioration by moisture and to extend the shelf life of the product. According to Santos (2005), it is recommended that the bags be stacked on wooden pallets, facilitating the circulation of air around the grains, in a place with double-layer walls, smooth flooring, efficient protection, dry and with regular evaluation of the product's humidity, in order to ensure that it is within acceptable standards (CASTRO, 2010). In addition, it is necessary to carry out constant monitoring to identify the presence of pests and rodents, taking the necessary measures to control them. If possible, it is recommended to store the peanuts at temperatures between 15 and 25°C (COX, 2012) and during transport to make sure that the conditions of the packaging are adequate to avoid damage that compromises the integrity of the grains and cross-contamination (SILVA, 2005).

Peanut kernels, like several other legumes, even after the storage stage, continue with the metabolism process still active. Even with the decreased respiration rate, there are some conditions that can influence this process, such as temperature and relative humidity conditions that can speed up the deterioration of peanuts (NUNES *et al*., 2021).

SCALCO (2008) emphasizes that quality management in the peanut production chain is of paramount importance, not only to maintain the quality of the product, which is unquestionable, but also to improve the practices and procedures of the activities that make up the entire chain in order to reduce losses, waste and, therefore, cost.

According to SANTOS and FONSECA (2010), the *Codex Alimentarius* highlights the necessary care for food, which in general must have low bacterial contamination, absence of residues of microorganisms and veterinary drugs pathogenic to humans, and minimal contamination of chemical agents or microbial toxins. As peanut kernels develop underground, exposure to attacks by microorganisms becomes greater and, once contaminated, leads to problems related to food safety and the occurrence of aflatoxigenic fungi (ROLLEMBERG *et al*., 2018). Aflatoxin is the name given to a group of very similar substances, toxic to humans and animals, produced mainly by two subspecies of the *fungus Aspergillus*, called *Aspergillus flavus* and *Aspergillus parasiticus*, when the product's moisture, relative humidity and environmental temperature are favorable (BINDER *et al*., 2007).

Consumption of aflatoxin products can lead to problems such as kidney damage, liver cirrhosis, fever, and liver cancer. In African and Asian countries, where foods contaminated with aflatoxins are widely consumed, the incidence of liver cancer is approximately 13 cases per 100,000 inhabitants per year (FONSECA, 2004).

From an economic point of view, the presence of this fungus reduces the composition of the prices paid to producers (LASCA, 2004). In 2001, the Brazilian Association of the Chocolate, Cocoa, Peanut, Confectionery and Derivatives Industry (ABICAB) launched a quality brand that not only has a marketing aspect, but is directly linked to the quality of the raw materials acquired by the industry.

The ABICAB seal emerges as a certification tool, ensuring the excellence of the products of the industries. This seal, an indicator of quality and reliability, rewards products eligible according to strict ABICAB standards, covering ingredient quality, manufacturing processes, food safety, regulatory compliance, in addition to ensuring that products are free of mycotoxins. The certification criteria involve several stages of the production chain, evaluating the origin and quality of ingredients, hygiene, traceability, sanitary standards, socio-environmental responsibility and commitment to excellence, thus bringing benefits to companies and consumers, conferring a reputation for quality, credibility, competitive differential, conquest of new markets and consumer loyalty for companies, while for consumers, it represents the guarantee of safe, authentic, and quality products, promoting the protection of health and satisfaction (ABICAB, 2022).

GENERAL OBJECTIVE

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- To investigate the occurrence of toxigenic fungi in peanut kernels that have undergone the process of drying, roasting, peeling and packed in plastic bags, in commercial establishments in the city of Inhumas.

SPECIFIC OBJECTIVES

- Inoculate peanut seeds of five different brands, marketed raw, with skin, without the pod, with a red film;
- Isolate fungal strains from 200 peanut kernels;
- Purify isolated fungal strains using specific culture media;

Identify the isolated fungi through microculture technique, using morphological, macroscopic and microscopic descriptors.

METHODOLOGY

The experiments were carried out in an aseptic environment, in the laminar flow chamber, of the microbiology laboratory of the Inhumas Campus, of the Federal Institute of Education, Science and Technology of Goiás (Figure 1). 200 grains of processed peanuts, raw, peeled, with only the outer skin, of five different brands, selected according to popularity, sold in plastic packaging, at room temperature and purchased in the city of Inhumas, were used.

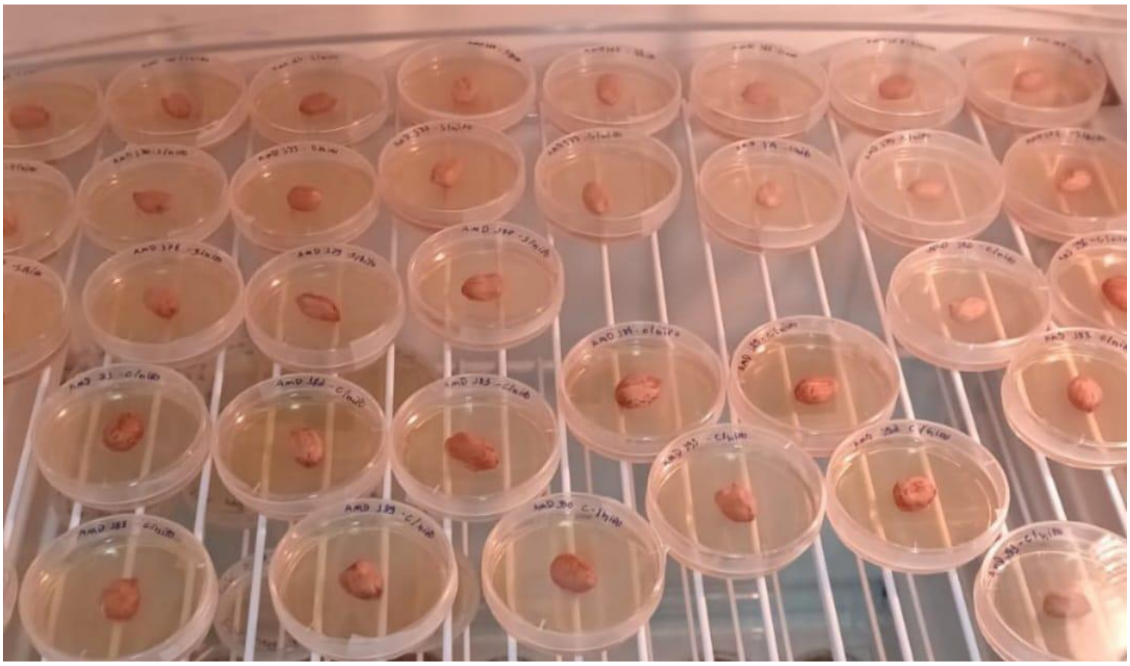
Figure 1 – Aseptic laminar flow chamber, where the inoculation of the sampled peanut grains in culture medium was performed.

Source: The authors (2023)

From each of the selected brands, 40 grains were randomly sampled, which were separated into two lots: in one of the lots (50% of the grains) a superficial disinfestation with 1% sodium hypochlorite was carried out for two minutes. The other batch of grains did not undergo superficial disinfestation with hypochlorite. The differential treatment given to the grains aimed to evaluate the levels of contamination to which the grains were subjected when they went through the processing and storage stages.

The grains treated with sodium hypochlorite were subsequently immersed in 70% ethyl alcohol (1 minute) to remove residual hypochlorite and then immersed in sterile distilled water (1 minute) to remove the residual alcohol. After the disinfestation procedure, all grains were inoculated in polystyrene Petri dishes, containing BDA culture medium. In each plate, only one grain was inoculated. After inoculation, the plates were incubated in a BOD incubator for seven days at a temperature of 28ºC (Figure 2).

Figure 2 - Petri dishes containing BDA medium, inoculated with peanut kernels with and without disinfestation randomly sampled.



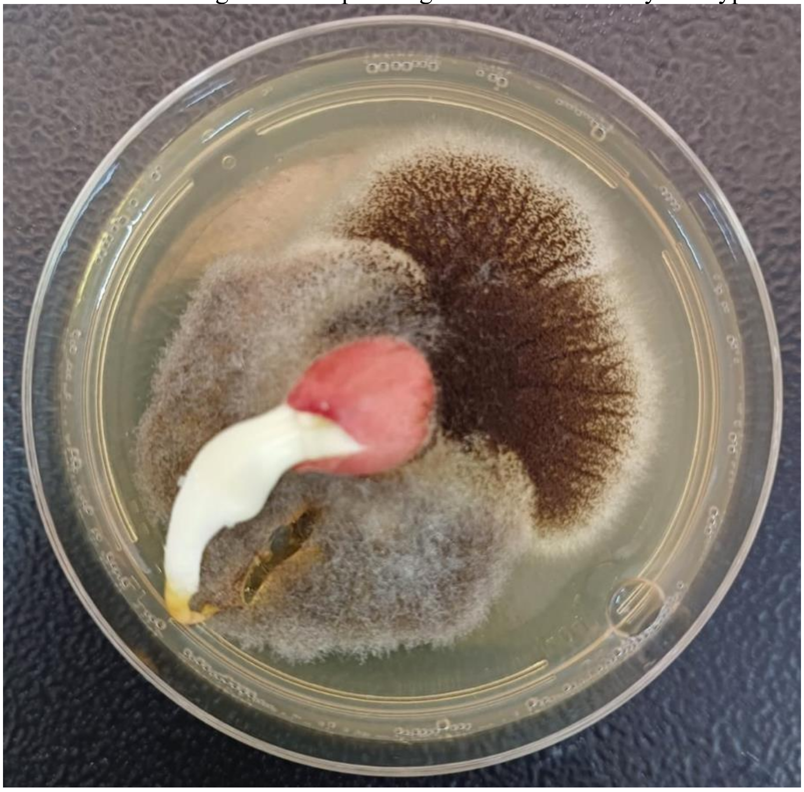
Source: The authors

In some plates, the growth of more than one type of fungus was observed (Figure 3), and it was necessary to perform re-pricking for the purification of the isolates. Peaking was performed with the aid of sterilized tips in an autoclave, transferring them to new Petri dishes containing BDA and incubating again for seven days at 28ºC temperature. The procedure adopted for the isolation of fungi in the present study is described in Rezende *et al*., (2013), and some adaptations were used.

After isolation and obtaining pure cultures, the isolates were identified using the microculture technique (RIDDELL, 1950). The identification of each isolate was carried out through the observation of morphological patterns traditionally used, as described by DE HOOG *et al*., (2000), KERN and BLEWINS (1999) and by PITT and HOCKING (2009).

All objects and utensils used during the experiments were sterilized in an autoclave before being taken to the laminar flow hood. Contamination control to avoid false-positive results was performed using Petri dishes containing BDA. For this, a plate containing the medium was left uncovered in a corner of the laminar flow hood during the inoculums, and was later capped and incubated with the others. This procedure demonstrated that the environment where the experiments were being carried out did not cause external contamination, since all the control plates evaluated did not show fungal growth.

Figure 3 - Plate containing BDA and peanut grain contaminated by two types of fungi

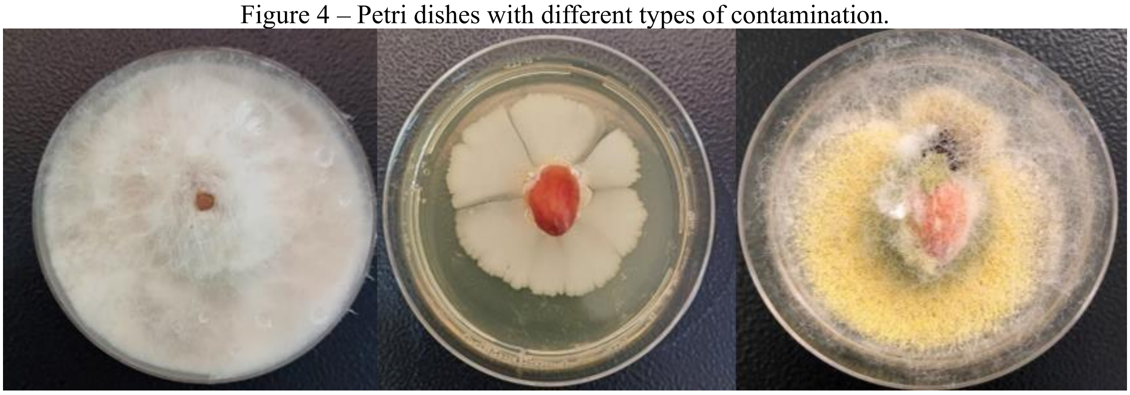


Source: The authors

RESULTS AND DISCUSSION

The samples analyzed in the present study showed high levels of contamination, with the presence of at least one contaminating microorganism in 98% of the grains evaluated. In some plaques, the growth of more than one type of microorganism was observed (Figure 4). The 200 grains evaluated resulted in the obtaining of 102 fungal isolates, of which approximately 23.5% belong to the genus *Fusarium*, 16.5% to the genus *Rhizopus*, 11% to *Aspergillus niger*, the only microorganism identified at the species level, in addition to other findings of unidentified fungi and microorganisms that together add up to 47% (Table 1).

Figure 4 – Petri dishes with different types of contamination.



Source: The authors (2023)

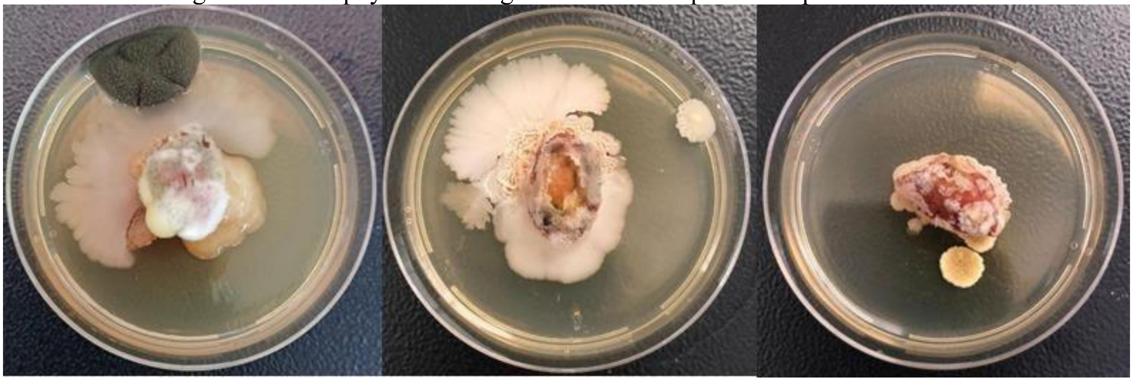
Table 1 – Numbers in % of fungi isolated from the sampled peanut kernels.

Samples	*Aspergillus Niger*		*Rhizopus sp.*		*Fusarium sp.*		Other			
	In the Isolated Sector	%	In the Isolated Sector	%	In the Isolated Sector	%	No. of unidentified fungi	%	No. of Other Microorganisms	%
A1	5	2,5	2	1	5	2,5	3	1,5	25	12,5
A2	10	5	13	6,5	7	3,5	4	2	6	3
A3	3	1,5	10	5	5	2,5	8	4	12	6
A4	3	1,5	7	3,5	15	7,5	3	1,5	12	6
A5	1	0,5	1	0,5	15	7,5	13	6,5	8	4
TOTAL	22	11	33	16,5	47	23,5	31	15,5	63	31,5

Source: The authors (2023).

The treatment adopted for grain disinfestation (described in the methodology) did not result in a difference in contamination levels. However, it was observed that almost all the samples that underwent disinfestation did not show fungal growth, but rather other endophytic microorganisms (Figure 5). These results suggest that most of the fungi identified in the samples were on the surface of the grains and probably infected them during some post-harvest stage.

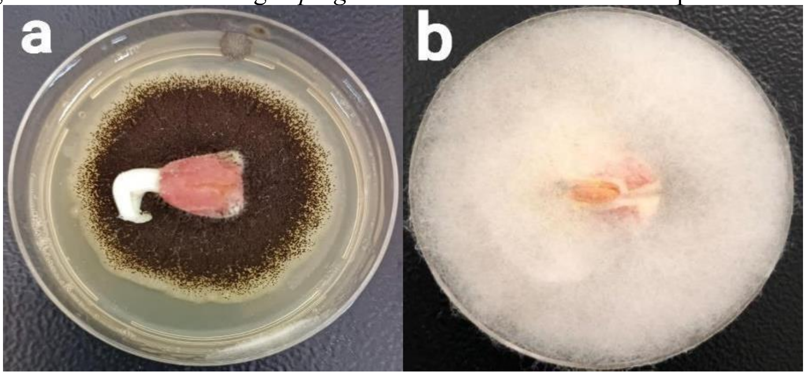
Figure 5 – Endophytic microorganisms found in processed peanut kernels.



Source: The authors (2023)

The analysis of the results also allowed us to observe that, in general, the peanut brands analyzed have a low level of hygienic-sanitary quality, thus being unsuitable for human or animal consumption, since *Aspergillus* and *Fusarium* are toxigenic fungi, producers of mycotoxins and were identified in all the brands analyzed. (Table 1, Figure 6).

Figure 6 – Plates containing *Aspergillus* and *Fusarium* isolates in peanut kernels.



Source: The authors (2023)

In general, fungi of the genus *Aspergillus* are a recurring concern for the food industry, given that their species have the ability to produce a series of mycotoxins, which can cause serious health problems in humans and animals (COSTA, 2014). *Aspergillus niger*, for example, identified in all peanut brands analyzed in the present work, is a species knThe authors to be toxigenic, producing ochratoxins (MIRABILE *et al*., 2021), whose effects on humans and animals include immunosuppression, carcinogenesis, teratogenesis, among others (MAZIEIRO; BERSOT, 2010; HOELTZ *et al*., 2012). Within the group of ochratoxins, ochratoxin A is one of the most studied, given its potential for diffusion and toxicological importance (MIRABILE *et al*., 2021). Ochratoxin A has carcinogenic action and nephrotoxic effects that lead to nephropathy, a condition that affects the functioning of the kidneys (PERAICA; DOMIJAN; SARIĆ, 2008; MANNAA; KIM, 2017).

Other species of the genus *Aspergillus* also produce important mycotoxins, with rich description in the scientific literature. Aflatoxins, produced by *Aspergillus parasiticus* and *Aspergillus* flavus and usually found in stored foods such as the peanut kernels analyzed in this study, can cause liver cancer, chromosomal aberrations, among other serious problems (SMITH *et al.,* 2015; AWUCHI *et al*., 2020; DIOGO *et al*., 2020). Sterigmatocystin, produced by *Aspergillus versicolor, Aspergillus nidulans* and *Aspergillus rugulosus* also causes liver cancer, in addition to being able to chemically transform into eight different compounds capable of inhibiting DNA synthesis (ARRUDA; BERETTA, 2019).

The results obtained in the analyses with peanuts sampled in this work corroborate a series of studies previously carried out in Brazil and described below.

Nóbrega and Suassuna in (2004), carried out a sanitary analysis on 300 peanut grains, from five different cities, in the state of Paraíba. The study revealed the presence of *Aspergillus flavus, Aspergillus niger and Fusarium sp.,* among others, and pointed to contamination in 71.66% of the samples analyzed. The authors emphasized that humidity and temperature control help prevent peanut kernel infection by fungi, both for *fresh* and industrialized products.

Nakai *et al*., (2008), monitored the peanut microflora for 12 months in the region of Tupã, São Paulo. In this work, the authors used both the peanut shell and the peanut kernels as substrate for isolating the fungi. The results of this study demonstrated the prevalence of *Fusarium* sp. in the husk and *Aspergillus* and *Penicillium* in the grains. According to the authors, the results can be explained by the fact that *Fusarium* sp., considered as a field fungus, is directly in contact with the peanut shell in the soil, while *Aspergillus* and *Penicillium*, considered storage fungi, are well adapted to the environmental conditions to which they are subjected during this stage of the production chain. The work of Atayde *et al*., (2012), also pointed out the presence of fungi of the genus *Aspergillus, Fusarium*, *Rhizopus* sp, in addition *to Penicilium*, during the analyses on fungal diversity and the presence of Aflatoxins in different peanut species, from different regions of the state of São Paulo.

Bonifácio *et al*., in (2015), evaluated peanuts sold in bulk in the city of Ji-Paraná, Rondônia. The results revealed high rates of fungal contamination also found in the processed peanut samples analyzed in the present work, such as *Aspergillus* sp. and *Rhizopus* sp. Such levels of contamination, according to the authors, can probably be explained by the adoption of inappropriate practices initiated in the field and also during the post-harvest stages.

In Ijuí, RS, Krahn *et al*., (2020) evaluated different peanut samples, including shelled grains, bulk marketed grains, and shelled industrialized grains, confirming the presence of *A. niger, A. flavus, A. nidulans*. The authors stated that these fungi can be commonly found in almost all stages of the production chain, and pointed out that the presence of these organisms in the product is a consequence of failures in the operational stages of sanitization, facilitating their propagation, which can compromise the health of the consumer through toxic substances, such as Aflatoxins and Ochratoxins.

Like *Aspergillus niger*, isolates of the *genus Fusarium* identified in the present study represent an important finding from the hygienic-sanitary point of view, with regard to the brands of processed peanuts analyzed. Although these organisms are commonly found in peanut samples (GONÇALEZ *et al*., 2008; DE OLIVEIRA; CASTILHO, 2011; SANTOS, 2013), species of *Fusarium* sp. are producers of dangerous mycotoxins and their presence in grains may be due to several factors, such as temperature, humidity, residues of other contaminated plants, as well as inadequate practices along the production chain.

One of the mycotoxins produced by *Fusarium* species is Fumonisin, a secondary metabolite produced by these fungi and associated with health disorders in humans and animals with hepatotoxic, carcinogenic, teratogenic and cytotoxic effects, which can cause leukoencephalomalacia in horses, pulmonary edema in pigs, as well as cancer in humans, the main one being esophageal cancer (ARRUDA; BERETTA, 2019; PRADO, 2017).

Fusarium sp. can also produce other important mycotoxins, listed in the literature, such as Zearalenone, synthesized mainly by *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium graminearum* and *Fusarium roseum* (SMITH and HENDERSON, 1991). Zearalenone is associated with problems such as muscle atrophy, reduced testosterone levels, and infertility (RICHARD, 2007).

The work by Amancio *et al*., (2023), on the growth of mycotoxin-producing fungi in raw peanut kernels in natura, without the pod and roasted peanuts without the pod, carried out in Juiz de Fora, MG, results are similar to those obtained in the present study. *Fusarium* was the most incident group (23.3%), followed by *Aspergillus (*20%), *Penicillium* (13.3%), Alternaria (13.1%) and *Rhizopus* (10%).

The occurrence of mycotoxin-producing spoilage fungi in other agricultural products is relatively common. Sabbadini *et al*., (2009) studied the occurrence of toxigenic fungi in corn and bean grains, commercialized in the municipality of Campo Mourão, finding high rates of infection by storage fungi such as *Aspergillus and Rhizopus*. In coffee, sold in Inhumas, GO and its surroundings, LIMA (2022), observed the presence of fungi of the genus *Aspergillus, Fusarium* and *Rhizopus,* in raw, dried, shelled beans ready for roasting and consumption.

As widely discussed previously, two of the three genera of fungi identified in the present work, *Aspergillus* and *Fusarium* are knThe authors producers of mycotoxins whose harmful effects on humans and animals are widely described in scientific records, in the specialized literature on the subject. *Rhizopus* sp., also identified in the peanut kernels analyzed, produce mycotoxins that have been less studied, without much description in the literature about their effects in humans. These fungi are potential spoilages, which with their saprophytic activity, alter the sensory characteristics of the grains, causing significant economic losses (PINTO, 2000).

CONCLUSION

The five brands of processed peanuts, analyzed in this study, are in hygienic-sanitary conditions that are inadequate for human consumption or even for animal feed composition, since in all of them toxigenic organisms were identified, potentially producing mycotoxins, whose effects on animals and humans can cause serious health problems.

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