# Filamentous fungi, bacteria and yeasts associated with cashew kernels in Brazil[1]

## Fungos, bactérias e leveduras associados a amêndoas de castanhas de cajueiro no Brasil

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**ABSTRACT**

During the past ten years a study has been conducted on the occurrence of fungi, bacteria and yeasts associated with cashew kernels in Brazil. Over a 1,000 samples from farmers and retailers have been examined. This paper aims to list the microorganisms so far isolated. A total of 79 fungal, 18 bacterial and 1 yeast species have been confirmed. Members of *Aspergillus* and *Penicillium* are dominant. Species potentially toxigenic such as *Alternaria alternata, Aspergillus clavatus, A. flavus, A. parasiticus, A. ochraceus, A. ustus, Penicillium citrinum* and *P. oxalicum* were frequently isolated. Among the bacteria those of the genus *Bacillus* were dominant. *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. were identified in some small processing plants. *Pichia guillermondii* was the only yeast confirmed. Measures aiming to reduce microbial contamination of kernels during processing and to improve hygienic conditions in big plant as well as in small processing units are discussed.

***Index terms:*** cashew kernels, microorganisms, spoilage, postharvest losses.

**RESUMO**

No presente trabalho são relacionados os organismos associados a amêndoas de cajueiro no Brasil. Um total de 79 fungos, 18 bactérias e 1 levedura já foi confirmado. Dentre os fungos filamentosos, as espécies de *Aspergillus* e *Penicillium* são as mais freqüentes. Espécies potencialmente toxigênicas tais como *Alternaria alternata*, *Aspergillus clavatus*, *A. flavus*, *Aspergillus parasiticus*, *A. ochraceus*, *A. ustus*, *Penicillium citrinum* e *P. oxalicum* foram comumente isoladas. Com relação às bactérias, aquelas do gênero *Bacillus* foram as mais freqüentes. *Escherichia coli*, *Staphylococcus aureus* e *Salmonella* sp. foram detectadas em algumas amostras coletadas em minifábricas no Estado do Ceará. Apenas a levedura *Pichia guillermondii* foi encontrada em associação com as amêndoas de cajueiro. Práticas para reduzir a deterioração fúngica das amêndoas, bem como para melhorar as condições higiênicas durante o processamento das amêndoas de cajueiro no Brasil são discutidas.

***Termos para indexação:*** amêndoas de cajueiro, microrganismos, deterioração, perdas pós-colheita.

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## Introduction

Cashew nut (*Anacardium occidentale* L.) is an extremely importante source of income for thousands of people in the Brazilian Northeastern region. Commercially, it accounts for an annual turnover of 170 million dollars, besides providing employment for more than 100,000 people. Approximatelly 700,000 hectares are planted with this crop in Brazil. The processed kernels are the principal commodity exported to USA, European countries and Japan (Paula Pessoa et al., 1995). Due to its high nutritional value cashew kernels are subjected to microbial contamination pre-, postharvest, and during precessing. Plant pathological surveys conducted in the last seven years have shown that over 10% of the annual crop is unfit for human consumption as a result of microbial biodeterioration and therefore cannot be exported, with an accompanying loss of revenue. Fungi, in a greater extend, are the responsible for these losses. Over 60 species have been identified. Many others are now included (Freire et al., 1999; Freire and Barguil, 2001; Freire and Cardoso, 2003). Occurrence of bacteria and yeasts on cashew kernels have also been recorded by several Brazilian authors (Freire and Offord, 2002; Lemos, 2003; Muniz, 2004).

This paper aims to present an updated survey of all filamentous fungi, bacteria and yeasts so far recorded on cashew kernels in Brazil.

## Fungi

Filamentous fungi are an ubiquitous group of organisms which explore almost all ecological niches on earth. They are estimated to be responsible for the spoilage of up to 25% of all plant-derived foods produced annually (Geisen, 1998). Many of these food-borne-fungi are also responsible for a high global incidence of mycotoxins, which are secondary metabolites produced by fillamentous fungi and involved in a toxic response called mycotoxicosis in human and higher animals when contaminated foodstuff is ingested (Bhat and Miller, 1991). Mycotoxins are considered to be among the most carcinogenic natural substances known. Over 300 such compounds have been isolated, with approximately 20 posing risk to public health (Geisen, 1998; Smith et al., 1995). Mycotoxin contamination of foods and feeds, however, is not a particular problem to the developed world, although heavy economic costs are incurred in ensuring low concentrations of mycotoxins (Mannon and Johnson, 1985). Data indicate that between 25% and 50% of world crops, in particular staple crops, are contaminated with mycotoxins. In developing countries such contaminations have more serious consequences, affecting agricultural economies, reducing annual production and good quality exports and seriously affecting the health of the population (Bhat and Miller, 1991). In these countries usually the good quality products are exported while the substandard produce unacceptable to foreign buyers (because they exceed regulatory limits for mycotoxin content) is sold to the domestic market (Dawson, 1991).

Apparently, the first report on fungal infection of cashew kernel in Brazil was provided by Ponte et al. (1975) who isolated *Penicillium digitatum* ( now confirmed as *P. citrinum*). During a survey in several states of the Brazilian Northeastern region Freire et al. (1996) found 25 fungal species associated with cashew kernel deterioration. The number of species has increased as the survey continued in the last 5 years (Freire and Barguil, 2001). Nowadays, a total of 79 fungal species have been isolated from cashew kernels in Brazil (Table 1).

A number of environmental factors has been linked to the infection of developing cashew kernels in Brazil. Laboratory and field studies on the mode of entry of fungi into cashew kernels have confirmed three different routes of infection. Indeed, many fungi have been isolated from stigma and anthers of cashew flowers, apparently growing down the style and infecting ovaries, remaining associated with these tissues until harvest. On the other hand, most the fungi isolated from kernels are endophytic in cashew tissues, so that their ingress into ovary tissues during seed maturation does not seem improbable (Freire et al., 1996). Finally, wounding by insects may provide infection courts and also allow the invasion to developing kernels. Indeed, several fungi have been isolated from external parts of bugs (Hemiptera: Crinoceridae) when feeding upon young nuts in field (unpublished results). Fungal invasion may take place immediately, if the insects carry fungal innocula or if conidia and mycelium are present on the nut surfaces.

There are other compreensive studies on the occurrence of filamentous fungi in cashews traded in diffrent countries. In Thailand, major fungi isolated comprise *A. flavus* (60%), *N. oryzae* (58%), *A. niger* (53%), *C. globosum* (47%) and *Eurotium chevalieri* (40%) (Pitt et al., 1993). Similar findings

**Table 1 -** Incidence of filamentous fungi isolated from Brazilian cashew kernels*.

| | | | |
|---|---|---|---|
| *Acremonium roseolum* (G.Sm.) W. Gams | < 1% | *Fusarium pallidoroseum* (Cooke) Sacc. | 1% |
| *Acremonium* sp. | < 1% | *F. solani* (Mart.) Sacc. | 2% |
| *Absidia corymbifera* (Cohn) Sacc. & Trott. | 6% | *Geotrichum candidum* Link. | < 1% |
| *Alternaria alternata* (Fr.) Keissl. | 2% | *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. | 1% |
| *Ashbya gossypii* (S. Ashby & W. Nowell) Guilliermond | < 1% | *Macrophomina* sp. | < 1% |
| *Aspergillus candidus* Link | 1% | *Microascus cinereus* (Emile-Weil & L. Gaudin) Curzi | 1% |
| *A. clavatus* Desm. | 1% | *Mucor racemosus* Fresen. | 2% |
| *A. flavipes* (Bain. & Sart.) Thom & Church | < 1% | *Nematospora coryli* Peglion | < 1% |
| *A. flavus* Link | 21% | *Nigrospora oryzae* (Berk. & Br) Petch | 5% |
| *A. fumigatus* Fresen. | 2% | *Ophiostoma* sp. | < 1% |
| *A. japonicus* Saito | < 1% | *Paecilomyces variotii* Bainier | 2% |
| *A. niger* Tiegh. | 56% | *Penicillium brevicompactum* Dierckx | 13% |
| *A. ochraceus* Wilhelm | 10% | *P. citrinum* Thom | 8% |
| *A. parasiticus* Speare | 2% | *P. glabrum* (Wehmer) Westling | 10% |
| *A. sydowii* (Bainier & Sartory) Thom & Church | 1% | *P. implicatum* Biourge | 1% |
| *A. tamarii* Kita | 8% | *P. minioluteum* Dierckx | 1% |
| *A. terreus* Thom & Church | 3% | *P. oxalicum* Currie & Thom. | 1% |
| *A. ustus* (Bainier) Thom & Church | 2% | *P. thomii* Maire | < 1% |
| *A. versicolor* (Vuil.) Tirab. | 3% | *P. purpurogenum* Stoll | 2% |
| *Aureobasidium pullulans* (de Bary) G. Arnaud | 1% | *Pestalotiopsis guepini* (Desmaz.) Steyaert | 4% |
| *Beltrania rhombica* Penz. | < 1% | *Phaeotrichoconis crotalariae* (M.A. Salam P.N. Rao) Subram. | < 1% |
| *Chaetomium funicola* Cooke | 1% | *Phellinus* sp. | < 1% |
| *C. globosum* Kunze:Fr. | 2% | *Phoma* sp. | 1% |
| *Choanephora* sp. | 5% | *Pithomyces* sp. | < 1% |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 13% | *Poitrasia circinans* | < 1% |
| *C. herbarum* (Pers.) Link. | 4% | *Rhizopus oryzae* Went & Prinsen Geerligs | 5% |
| *C. sphaerospermum* Penz. | 1% | *R. stolonifer* (Ehrenb.:Fr.) Vuill. | 16% |
| *Cylindrocladium parvum* P.J. Anderson | 1% | *Sarcopodium* sp. | 1% |
| *Cladobotryum* sp. | < 1% | *Scopulariopsis gracilis* | 1% |
| *Colletotrichum gloeosporioides* (Penz.) Sacc. & Penz. | < 2% | *Scytalidium* sp. | < 1% |
| *Cunninghamella elegans* Lendn. | 3% | *Spegazzinia tessarthra* (Berk. & Curt.) Sacc. | 1% |
| *Curvularia lunata* (Wakker) Boedijn | 6% | *Spiniger* sp. | < 1% |
| *C. senegalensis* (Speg.) Subram. | 2% | *Syncephalastrum racemosum* Cohn ex J. Schröt. | 3% |
| *C. tuberculata* Jain | 1% | *Talaromyces trachyspermum* | 1% |
| *Dactylaria* sp. | < 1% | *Talaromyces* sp. | < 1% |
| *Drechslera* sp. | < 1% | *Torula herbarum* f. *quartenella* Sacc. | 1% |
| *Drechslera* cf. *papendorfii* (Aa) M.B. Ellis | < 1% | *Thielavia terricola* | < 1% |
| *Emericella nidulans* (Eidam) Vuill. | 11% | *Trichoderma atroviridae* | 1% |
| *E. rugulosa* (Thom & Raper) C.R. Benjamin | 7% | *Tritirachium* sp. | < 1% |
| *Eurotium amstelodami* Mangin | 12% | *Xylaria* sp. | < 1% |
| *E. chevalieri* Mangin | 8% | | |

* Incidence expressed as a percentage of 1,000 samples analysed.

have been made with Brazilian cashews, although *A. niger* had been the dominant species (60%). As for *A. flavus*, the second most frequently isolated species (21%), all isolates were non-sclerotial forms. Dominant penicillia were *P. brevicompactum* and *P. glabrum* (13% and 8%,respectively). Potentially micotoxigenic species were frequently isolated from cashews in Brazil although only small amounts of mycotoxins, specially $G_2$ aflatoxin had been detected in substandard samples (Freire et al., 1999). Fungi have also been reported in different cashew processing units of India (Krishnaswamy et al., 1973). A wide range of fungi, representing several genera and species, has been reported associated with deterioration of cashew kernels in Saudi Arabia and Nigeria (Abdel-Gawad and Zohri, 1993; Adebajo and Diyaolu, 2003).

## Bacteria

Although not directly involved in cashew kernel deterioration, growth of bacteria on kernels may take place during processing. Environmental damp conditions, problems with equipments and workers may also increase the levels of contamination. Despite being an excellent substrate for microorganisms growth, occurrence of serious bacterial contamination has rarely been reported in Brazilian cashews. Counts of aerobic mesophile bacteria on raw kernels from main exporters of Ceará State ranged from <10 CFU/g to 8.0 x $10^3$ CFU/g. As for oil-roasted and salted kernels the counts ranged from <10 CFU/g to 9,0 x $10^2$ CFU/g. Neither *Salmonella* nor *Staphylococcus aureus* was detected. Population of *Escherichia coli* in one sample of raw kernel and in two samples of oil-roasted and salted kernels exceeded the limit permited by the international food legislation (Lemos, 2003). Total population of aerobic mesophile bacteria of raw cashew kernels from small processing modules in the same state ranged from 4.0 to 7.0 log CFU/g. Coagulase-positive staphylococci were found in about 27% of samples from one of the six modules evaluated, ranging from 2.0 to 2.78 log CFU/g. Contamination by *Enterobacteriaceae* occurred in samples of three modules. Coliforms at 35$^o$C was detected in 13% to 40% of samples from three modules. Coliforms at 45$^o$C were only confirmed in samples from one module. *Escherichia coli* was not detected in any of the samples tested, although presence of *Salmonella* sp. had been confirmed in 18 samples (Muniz, 2004). A total of nine genera of bacteria was detected in surface sterilized and unsterilized raw Brazilian cashew kernels obtained from farmers of Ceará State. The genus *Bacillus* with six species (*B. cereus*, *B. macerans*, *B. megaterium*, *B. mycoides*, *B. pumilis* and *B. subtilis*) was the most common. Counts for this genus ranged from 0.08 x $10^2$ to 1.7 x $10^2$ CFU/sample. Other bacterial species included *Acinetobacter baumannii* (0.10 x $10^2$ cfu/sample), *Enterobacter cloacae* (0.08 x $10^2$ CFU/sample), *E. sakazakii* (0.20 x $10^2$ to 0.38 x $10^2$ CFU/sample), *Gordona bronchialis* (1.2 x $10^2$ CFU/sample), *Klebsiella pneumoniae* (0.60 x $10^2$ CFU/sample), *Micrococcus luteus* (0.10 x $10^2$ to 0.15 x $10^2$ CFU/sample), *Rhodobacter capsulatus* (0.10 x $10^2$ CFU/sample), *Staphylococcus aureus* (1.7 x $10^2$ CFU/sample), *S. hominis* (0.10 x $10^2$ CFU/sample) and *Xanthomonas maltophilia* (0.10 x $10^2$ to 0.14 x $10^2$ CFU/sample) (Freire and Offord, 2002).

From cashews purchased in South Africa Wehner and Rabie (1970) found *Bacillus* spp. as the most frequently isolated bacteria. Aerobic mesophile bacteria have also been reported from cashews elsewhere (Candlish et al., 2003).

## Yeasts

Information on the identity of yeasts from cashew kernels in Brazil is meagre. These organisms are usually evaluated together with the filamentous fungi (molds) without preocupation of identifying the isolated species. Counts of yeasts are in most the cases below the limits of the Brazilian and the international food legislation. They have been isolated from roasted and salted as well as from raw kernels processed either by big exporters or by small producers (Lemos, 2003; Muniz, 2004). Analyses of yeasts in raw Brazilian cashew kernel revealed de presence of *Pichia guillermondii* (3.2 x $10^2$ cfu/sample), the only species identified so far on this commodity (Freire and Offord, 2002). As yeasts are neither common agents of deterioration of cashews nor toxins producers their actual importance on this commodity has not been thoroughly studied. However, certain foodborne yeasts may be hazardous because of their ability to elicit allergic reactions (Beuchat and Cousin, 2001). Undentified yeasts were also isolated from cashews at different stages of processing in small processing plants of India (Krishnaswamy et al., 1973).

## Concluding remarks

Fungi enumeration of nuts have frequently shown the presence of field and storage molds, especially *Aspergillus* and *Penicillium*. The mold composition changes from field fungi to storage fungi through processing to storage, as nuts are inadequatelly stored. The high humidity and storage temperatures may contribute to continued growth of the field fungi and possibly micotoxin production. Despite cashew nuts often are subject to microbial contamination, they are seldom vehicles in food poisoning. Although filamentous fungi may grow under damp conditions, cashew processing is normally a dry process so bacterial and yeasts growth is not important (King and Jones, 2001). Exhaustive attempts to identify micotoxins from substandard Brazilian cashews revealed the presence of peaks which corresponded to aflatoxins. Only trace levels of aflatoxin $G_2$ could be confirmed. However, the samples analysed were not fit for human consumption. Presence of aflatoxin $B_1$ (0.35 ug/kg) has recently been reported in cashews from retail outlets in Lodz, Poland (Leszczynska et al., 2000).

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