



Brazil nuts: Benefits and risks associated with contamination by fungi and mycotoxins

Otniel Freitas-Silva^{a,b,*}, Armando Venâncio^a

^a Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal

^b Embrapa Food Technology, Av das Américas, 29501, 23.020-470, Rio de Janeiro, Brazil

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ABSTRACT

The monotypic type genus *Bertholletia* produces commercially nutritionally harvested edible seeds, Brazil nuts. It is an important product from the Amazon forest in the food production chain, with a 2008 annual world production of 78,000 tonnes, being Brazil responsible for approximately 40% of it. Although there are beneficial nutritional properties, the prevailing mycobiota of Brazil nuts include fungi that are producers of aflatoxins, such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. Aflatoxins have deleterious effects in consumption considering the global distribution chain, affecting major exporting countries. The present review is focused on the importance of Brazil nuts for the Amazon rainforest, emphasizing on the social and environmental impact of its production, on the mycobiota contamination of seeds, and on the presence of mycotoxins and related food safety aspects.

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1. Introduction

The Amazon rainforest consists of multiple ecosystems containing a huge biodiversity. The forest has an important role in the global weather balance and it provides shelter and livelihood for many local communities (Vieira, Toledo, Silva, & Higuchi, 2008). The production and extractivism of the Brazil nut (*Bertholletia excelsa* Humb. & Bompl.) is observed only in the Amazonian biome. This is an important activity for forest people in countries where the trees grow, stimulating the sustainable use of renewable natural resources while combining social development and conservation (Silvertown, 2004). The gathering and commercialization of Brazil nuts by traditional population is an activity that neither destroys the forest nor threatens the ecological balance and the environment (Wadt, Kainer, Staudhammer, & Serrano, 2008). With effect, *B. excelsa* trees are an essential part of the rainforest, as they help maintain the balance in the relationship between flora and fauna (Wadt, Kainer, & Gomes-Silva, 2005).

Brazil nuts are the main commodities from the Amazon rainforest extractivism. The nuts are destined to national or international trade. Gatherers pick and store the fruits; they are responsible for the initial handling and processing, which is still done in the forest (Codex Alimentarius Commission, 2010). The main stages in Brazil nut production are: production and collection in the forest (cleaning paths between trees, gathering the fruit, opening the fruit and transporting

them to the camp), and processing (cleaning, drying and soaking, peeling the nuts, drying the peeled nuts) and commercialization in the packing house (FAO, 2006).

Contamination by aflatoxins (AF) is a major problem for tree nuts, as well as for other stored grains, milk and dry fruits, especially because the causal fungi, *Aspergillus flavus* group, occurs as a natural contamination (Molyneux, Mahoney, Kim, & Campbell, 2007). Industries and producers have endeavored considerable efforts over the last 15 years to minimize fungal growth and AF production in tree nuts, particularly in the case of Brazil nuts, due to the hot and humid climatic conditions found in the Amazon environment, with an average temperature of 26 °C and relative humidity of 80–95%, which favors the production of these toxins. In addition, the extractivism characteristics (temperature and relative humidity during gathering and handling) are hard to be controlled, having a direct or indirect effect on toxigenic fungi and on the production of AF. Since contamination is usually associated with shelled nuts, proven processing/treatments that reduce AF levels in Brazil nuts include shelling or sorting by size, specific gravity, color or damage (De Mello & Scussel, 2009; Pacheco, Lucas, Parente, & Pacheco, 2010).

This review considers the benefits of Brazil nuts and many aspects concerning AF in Brazil nuts, including the occurrence of the fungi, the mycotoxins production and its control.

2. Economic, social and ecological aspects of Brazil nuts

Despite the importance of the Brazil nut tree, the same is still currently classified as vulnerable to extinction (IUCN, 2010). Native from Amazonian region, the Brazil nut tree (*Bertholletia excelsa* HBK)

* Corresponding author at: Permanent address: Embrapa Food Technology, Av das Américas, 29501, 23.020-470, Rio de Janeiro, Brazil. Tel.: +351 253604400, +55 21 3622 9600; fax: +351 253678986, +55 21 3622 9713.

E-mail addresses: ofreitas@deb.uminho.pt, ofreitas@ctaa.embrapa.br (O. Freitas-Silva).

is among the highest specimens in the tropical forest, easily reaching 50 m and living up to 1200 years old. The pod, which weighs about a pound and may contain 15 to 24 seeds, is known as the fruit of the Brazil nut (Mori & Prance, 1990). Brazil nuts are a typical non-timber forest product (NTFPs) and, for that reason, have been increasingly valued in the market, also for its characteristic flavor, high nutritional value and their association with environmental conservation (Yang, 2009). It is the only seed internationally traded that is gathered in the forest (Peres et al., 2003). Its origin comes from the Amazon region, mostly in the north of Brazil and neighbour countries (Peru, Guyana, Venezuela, Suriname and Bolivia), but only Bolivia, Brazil and Peru export this nut. In 2008, Bolivia was responsible for 53% of in-shell Brazil nut world production, compared with 39.35% and 0.37% from Brazil and Peru, respectively (FAOSTAT, 2011).

The majority of attempts to cultivate Brazil nuts for commercial purposes inside and outside the Amazon region failed because no attention was given to pollinator agents, since the Brazil nut tree is a predominantly pollinating species, with a very low autogamy level. The tree produces fruits only with the presence of its natural pollinators, the bees. According to Motta-Maués (2002), the main pollinators of Brazil nut trees are carpenter bees and bumble bees, from the Apidae and Anthophoridae families, e.g. *Xylocopa frontalis*, *Epicharis rustica*, *E. affinis*, *Epicharis* sp., *Eulaema nigrata*, *Bombus transversalis* and *B. brevivilus*. This author reinforces the importance of the development of management programs for main pollinators so as to allow the cultivation of Brazil nuts in wide scale. This is to avoid the decrease of the natural population of pollinators in commercial plantations and, consequently, a low fruit production, even in an agroforestry system or managed forestry.

Brazil nuts, although exported since 1800, only had a place in the exports agenda of NTFPs after the beginning of the twentieth century (Emmi, 2003). After the decline of rubber production (*Hevea brasiliensis*), Brazil nuts became the primary extraction product for export purposes in the Northern Region of Brazil, and its exploitation has a key role in the socio-economic organization of large areas of natural forest.

Sustainable exploitation is widely advocated as a strategy for reconciling economic pressures on natural habitats by means of nature conservation (Silvertown, 2004). The extraction of Brazil nuts has always been seen as an example of sustainable exploitation of the Amazon forest. But this view was shattered by the publication of results from studies made by Peres et al. (2003) showing that there is no regeneration of Brazil nut trees, i.e. they undergo an ageing process and the ageing rate of the population does not follow the occurrence of new plants, which might mean, in the long term, a collapse caused by over gathering. The concern raised by Peres et al. (2003) has been challenged by some studies such as one conducted in Bolivia, assessing the population structure of Brazil nut trees in a forest with and without gathering activities, which was also compared to a secondary (15 years old) forest. This research showed that gathering did not affect regeneration rate, and that the differences observed favoring the secondary forest are due to the positive influence of light on the recruitment of seedlings (Zuidema & Boot, 2002). Such ecological characteristics of Brazil nuts are corroborated also with arguments of Cotta, Kainer, Wadt, and Staudhammer, (2008). These authors reinforce the theory of Balée (1989) in which the occurrence and distribution of Brazil nut trees in the forestry is not only the result of natural actions, as it has been thought so far, but rather the result of anthropogenic activities of native Amazon populations in the forest environment. In other words, “castanhais” are a sort of cultural forest, and their long-term sustainability will depend on its proper management by humans and appropriate environmental interactions.

Over the last 15 years in Brazil, Brazil nuts have experienced a significant decrease in exports. According to a comparative assessment, one of the explanations for the crisis caused by such decrease in the exports in the Brazilian Amazon was the entry of Bolivia into the

international market in 1996 (Coslovsky, 2005), as well as a high incidence of AF in the nut recorded in Brazil. Until 1990, most of Bolivia's production was sent to Brazil, where the nuts were processed, industrialized and exported. From 1990, the exportation of Brazil nuts by Bolivia was fostered mostly by grants from the World Bank and by a low taxation system (Coslovsky, 2005). This caused the product supply to reach prices below those charged by the market, inhibiting the Brazilian exporter, who preferred to wait for the Bolivian stock to end in order to sell their products.

The crash and crisis can still be justified by non-tariff barriers imposed by the European Community since 1998, EC regulation 1525/98 and EC decision 493/2003 (European Communities, 1998; European Union Commission, 2003). Because of those decisions, Brazilian exports of Brazil nuts in shell to Europe fell by almost 90% between 2000 and 2004. The first regulation reduced the acceptable limit of total aflatoxins (AFT) in Brazil nuts to 4 µg/kg and 2 µg/kg for AFB₁ and rejected contaminated consignments from Brazil (Silva, 2010). While the second one imposed special conditions on the import of Brazil nuts in shell originating in or consigned from Brazil. Moreover, the domestic market absorbs only 10% of the Brazil nut production, because what is left in Brazil is the lower quality product and no marketing strategy for Brazilians consumers was established.

The Brazil nut production is currently recovering in Brazil; as of 2009 (Fig. 1) the national production of it was of 37,467 tonnes, representing an increase of 21.6% compared to the production of 30,815 tonnes achieved in 2008. The main producer is the State of Amazonas, concentrating 42.7% of the total gathered, following by the States of Acre (27.5%), Pará (18.7%) and Rondônia (6.9%) (IBGE, 2009).

The existence of a market for rainforest products, therefore, is not enough on its own to prevent the destruction of habitats or overexploitation of the resource. Moreover, a more sophisticated approach to sustainability is required, translated as the development of an ethically-traded Brazil nut market (Silvertown, 2004).

Brazil nut can be processed or unprocessed. The nuts can be sold in their shell, semi dried or dehydrated in bulk (without treatment). Shelled nuts are obtained manually by cracking them to open, and they can be sold with or without their pellicle. The storage and conservation of Brazil nuts are one of the most important issues in their commercialization (Arrus, Blank, Clear, Holley, & Abramson, 2005; Pacheco & Scussel, 2009).

In addition to being a foodstuff, the residue of Brazil nuts has several applications. Their pods and shell can be used as fuel and to produce recycled wood and handicraft, and the oil can be used for culinary purposes or in the cosmetic industry (Pacheco & Scussel, 2006). From processed Brazil nut it can be obtained the Brazil nut milk, processed kernels also can be used in variety of food industry products (mix with others nuts, cereals bars, chocolate and the meal to flour preparations), (Souza & Menezes, 2004).

In trade, Brazil nut can be classified in ready-to-eat (RTE) or those destined for further processing (DFP) (Codex Alimentarius Commission, 2010). RTE Brazil nuts are those which are not supposed to undergo any additional processing/treatment before reaching the final consumer. Both,

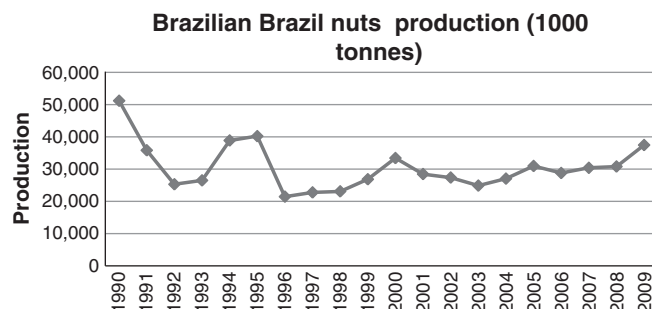


Fig. 1. Annual fluctuation in the production of Brazilian Brazil nuts (IBGE, 2009).

RTE and DFP Brazil nuts can be traded shelled or unshelled and both are susceptible to mycotoxins.

3. Health benefits

Among all nuts, Brazil nuts are regarded as one of the most nutritious human foods, due to their high contents of proteins, carbohydrates, unsaturated lipids, vitamins and essential minerals. Brazil nuts have been of interest due to their exceptionally high, by a substantial factor, level of Selenium (Se) (Chunhieng, Petritis, Elfakir, Brochier, Goli, & Montet, 2004; Chunhieng, Hafidi, Pioch, Brochier, & Montet, 2008; Welna, Klimpel, & Zyrmicki, 2008) and other important minerals as calcium, magnesium, phosphorus and potassium (USDA, 2008; Yang, 2009; INC, 2010).

Concerning Se, an important antioxidant, it is an essential trace element consumed in sub-milligram amounts, primarily in organically bound forms, in the diet. Gross deficiency leads to a range of diseases or disorders, the most well-known being the cardiomyopathy, Keshan disease (Verkerk, 2010). This antioxidant is required for the function of a number of key selenium-dependent enzymes (selenoproteins) necessary for a wide range of metabolic processes, including thyroid hormone regulation, immune function and reproduction (Pacheco & Scussel, 2007). Studies suggest that proper selenium intake is correlated with a reduced risk of breast, lung and prostate cancer (Reeves & Hoffmann, 2009). These findings need more investigation to correlating selenium with reduced risk of various cancers (Verkerk, 2010). As a result, Brazil nuts are sometimes recommended as a protective measure.

According to Rodrigues, Araújo, Azevedo, and Machado (2005), broken nuts lose 40% of their commercial value, being used to produce mechanically pressed oil. The byproduct of Brazil nut oil (cake), due to its nutritional characteristics, can be used in the food industry: an extract called “milk” and a protein concentrate and isolate are obtained from the defatted cake, after extraction using hexane or ethanol (Freitas, Freitas-Silva, Miranda, & Coelho, 2007). The vegetable oil is composed of a complex mixture of triglycerides, and the remaining constituents are usually composed of free fatty acids, monoglycerides, diglycerides and other fats at small concentrations: tocopherols, carotenoids and sterols (Rodrigues et al., 2005).

Dry Brazil nuts are about 18% protein, 13% carbohydrates, and 69% fat. The relation among saturated, monounsaturated and polyunsaturated oil in percentile are:25:41:34 (USDA, 2008), this represents that content of saturated fat in Brazil nuts is higher than in any other nut. Brazil nuts also are a rich source of vitamin B1 and vitamin E, a good source of niacin and calcium, and a source of iron (USDA, 2008).

4. Contamination routes of aflatoxins in Brazil nut chain

4.1. Toxicological aspects of aflatoxins

Mycotoxins are natural contaminants produced by fungi and are commonly found in a wide variety of food products including cereals, legumes, nuts and their products (Paterson & Lima, 2010). Whenever the consumption of these products, including Brazil nuts, is significant in the diet of populations, such infection poses a high risk of chronic exposure (Jolly, Bayard, & Vodouhe, 2009). Mycotoxins can cause adverse effects to health. Among more than 300 types of mycotoxins, the AF are the most studied ones due to their toxic potential (Klich, 2009). The ingestion of AF may increase the risk of lung and liver cancer and it can also suppress the immune system, increasing the risk of infections. Additionally, it may cross the placental barrier (Jolly, Bayard, & Vodouhe, 2009; Meggs, 2009; Partanen, El-Nezami, Leppänen, Myllynen, Woodhouse, & Vähäkangas, 2010). There are no reports on the existence of susceptibility to AF, although there are differences in susceptibility from one species to another.

AFB₁ is responsible for carcinomas in animals, showing a strong relationship with the incidence of cancer in humans (Jolly, Bayard, & Vodouhe, 2009; Meggs, 2009). AF are a group of related structurally

oxygenated heterocyclic compounds produced under favorable conditions by several species of *Aspergillus*. The most known naturally occurring AF are named B₁, B₂, G₁, and G₂ (Fig. 2). AFB₁ occurs in the highest amounts in contaminated commodities; AFB₂, AFG₁, and AFG₂ (Soares, Rodrigues, Freitas-Silva, Abrunhosa, & Venâncio, 2010) are generally not reported in the absence of AFB₁. Total aflatoxins (AFT) refer to the sum of the four related compounds.

In 1998, JECFA considered the carcinogenic potential of AF and the potential risks associated with their intake. No tolerable daily intake was proposed, since these compounds are genotoxic carcinogens. The risk estimative for human liver cancer resulting from exposure to AFB₁ was derived from epidemiological and toxicological studies (FAO/WHO, 2008).

The JECFA reviewed a wide range of studies conducted with both animals and humans that provided qualitative and quantitative information on the hepatocarcinogenicity of AF. The carcinogenic potency of AFB₁ is substantially higher in carriers of hepatitis B virus (about 0.3 cancers/year/100,000 persons/ng of AFB₁/kg bw/day), as determined by the presence in serum of the hepatitis B virus surface antigen (HBsAg⁺ individuals), than in HBsAg[−] individuals (about 0.01 cancers/year/100,000 persons/ng of AFB₁/kg bw/day). The JECFA also observed that vaccination against hepatitis B virus would reduce the number of carriers of the virus, and thus reduce the potency of the AF in vaccinated populations, leading to a reduction in the risk for liver cancer (FAO/WHO, 2008).

4.2. Fungi contamination in Brazil nuts

Fungal infection in Brazil nut kernels has been known since the beginning of last century. One of the first reports described in literature about the loss of quality of Brazil nuts was made by Spencer (1921). This author described a percentage of losses caused by microbiological contamination between 10 and 25% in Brazil nuts exported from Amazon to the U.S.A. At that time, he found that post-harvest losses of Brazil nuts were predominantly due to fungal contamination. The author, who has described the presence of *Aspergillus flavus* in Brazil nut kernels, also described other fungi infecting the kernels. Among them, *Penicillium* spp., *Pellionella macrospora*, *Cephalosporium bertholletianum*, *Fusarium* spp. and *Phomopsis bertholletianum* were detected. Other authors, who later studied the fungal mycobiota in Brazil nuts, also highlighted the diversity of species, with the predominance of mitosporic fungi. Since *Aspergillus* is notably the predominant fungus,

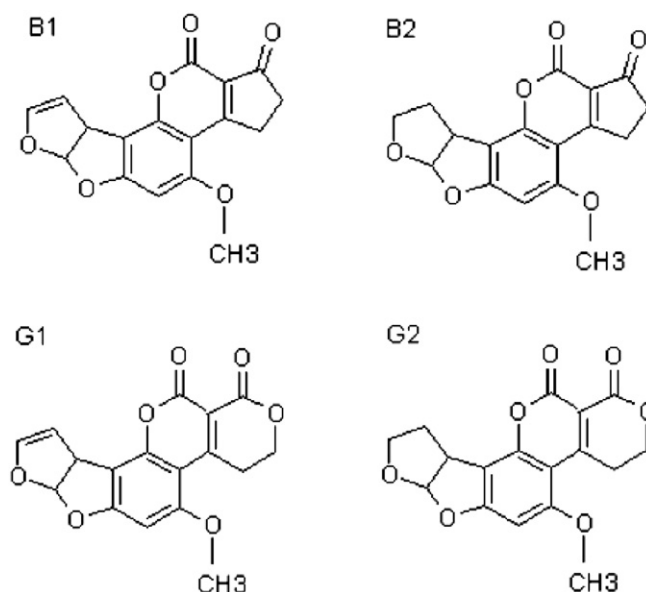


Fig. 2. Chemical structure of aflatoxins AFB₁, AFB₂, AFG₁ and AFG₂.

especially species in section *Flavi*, there is great concern with the production of AF in this product. Subsequent studies also demonstrated the predominance of *A. flavus*. It was reported that among the seventeen species found in Brazil nuts, *A. flavus* was the dominant species, followed by *A. niger*, *Penicillium citrinum* and *Penicillium glabrum* (Freire, Kozakiewicz, & Paterson, 2000). According to this study, some species were primarily isolated from Brazil nuts, including *Acremonium curvulum*, *Cunninghamella elegans*, *Exophiala* sp., *Fusarium oxysporum*, *Pseudoallescheria boydii*, *Rhizopus oryzae*, *Scopulariopsis* sp., *Thielavia terrestris* and *Trichoderma citrinoviride*. The species found by Freire, Kozakiewicz, and Paterson (2000) included fungi associated with deterioration and potentially toxigenic species, including *A. flavus* and *P. citrinum*, producers of AF and citrinin, respectively. The presence of these fungi may be a sign of inadequate drying, processing and storage.

To answer some questions concerning fungal distribution, abundance, specificity and implications of these distribution patterns and concerning the control of AF contamination in Brazil nuts, Bayman, Baker, and Mahoney (2002) studied different tree nuts groups. These authors noted that the fungal incidence in Brazil nuts occurred in the following descending order: *A. flavus*, *Penicillium* spp., *A. tamarii*, *A. niger*, *A. nidulans*, *A. fumigatus*, and *Rhizopus* spp. These authors stated that the frequencies of *A. flavus*, *A. tamarii*, *A. niger*, *Penicillium* and *Rhizopus* in Brazil nuts were the same ones observed in sterilized and unsterilized samples. In this way, they concluded that the internal colonization rate in Brazil nuts was much higher than in other tree nuts they had studied. However, *A. niger* was less common in Brazil nuts than in other nuts. Since Brazil nuts come from wild collection, being stored and transported under conditions that favor fungal growth, those fungi may have more opportunities to colonize the inner tissues of Brazil nuts than of other nuts. This point of view is contrary to the one proposed by Freire et al. (2000), considering that penetration, spore germination and infection takes place during inflorescence or during formation of the seeds, which suggests an invasion and colonization of premature young tissues.

Fungal infections can both occur in harvest and post-harvest stages (Fig. 3). Fungi may develop during storage and transportation of the product and subsequent kernel contamination is frequent in all stages of a process flow diagram. However, there are bands of moisture and water activity (a_w) which are ideal for the production of AF. The moisture content recommended for in-shell and shelled Brazil nuts to prevent the growth of toxigenic fungi are 5 and 4.5%, corresponding to an a_w of 0.75 and 0.68, respectively (Arrus, Blank, Clear, Holley, & Abramson, 2005).

In fact, the most influent parameters in toxin production are temperature and humidity. Storage temperatures between 25 °C and 30 °C and a relative humidity of 97%, as occur in Amazon environment, favor the production of toxins during storage. Decreasing temperature or relative humidity is not feasible in the Amazon context; so, drying of nuts prior to storage is one possible action (Arrus, Blank, Clear,

Holley, & Abramson, 2005). Additionally, these authors recommended in-shell Brazil nuts storage, since the intact shell acts as a barrier to fungal growth on its surface. This should be considered at appropriate storage conditions, when there is the possibility of cross-contamination, with spores from other lots or environmental contamination. Therefore, if the period during which the product (shelled and in-shell Brazil nuts) is under critical conditions is minimized, the probability of contamination is also reduced (Johnsson, Lindblad, Thim et al., 2008). In practice, this usually means reducing the moisture content of Brazil nuts as soon as possible to levels below that required for the activity of fungi.

4.3. Aflatoxins in Brazil nuts

Qualitative and quantitative analyses are conducted by a number of available analytical methods to determine AF in nuts. Although most of them have not been specifically developed for the Brazil nut, they are used in-house after validation. In general, the methods include solvent extraction followed by a cleanup step using liquid-liquid partition and a solid phase extraction, using silica, florisil, C18, aluminum oxide and immunosorbents. Thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) with UV detector or fluorescence is commonly used for the detection and quantification of AF. More recently, HPLC with mass spectrometry detection (Pacheco & Scussel, 2009) has been used. Its advantage is that there is no need for clean-up and additional confirmation stages. The limit of quantification (LOQ) for each AF depends on the detection method, and usually ranges from 0.1 to 1 µg/kg (Gilbert & Vargas, 2003; Marklinder, Lindblad, Gidlund, & Olsen, 2005; Sobolev, 2007). AF ELISA kits are mainly used for screening purposes.

Among mycotoxins, AF are the most commonly observed in Brazil nuts and, additionally, there are reports of other mycotoxins such as chaetoglobosin C and spinolosin found in Brazil nuts with low sanitary quality (Freire et al., 2000).

The production of AF is enhanced by environmental conditions (temperature and relative humidity) and storage conditions. Other factors include agricultural product characteristics, such as water activity, moisture content in food and substrate, in addition to the damage caused by insects (Arrus, Blank, Abramson, Clear, & Holley, 2005).

Although *A. flavus* and *A. parasiticus* are undoubtedly the most common species and the most important producers of AF in foodstuffs (Rodrigues, Venâncio, Kozakiewicz, & Lima, 2009), there are other species in the same section (*A. arachidicola*, *A. avenaceus*, *A. bombycis*, *A. caelatus*, *A. lepore*, *A. minisclerotigenes*, *A. nomius*, *A. oryzae*, *A. parvisclerotigenus*, *A. pseudotamarii*, *A. sojae*, *A. tamarii* and *A. toxicarius* (Ito, Peterson, Wicklow, & Goto, 2001; Samson, Hong, & Frisvad, 2006; Pildain, Frisvad, Vaamonde, Cabral, Varga, & Samson, 2008). It should be pointed out that the identification of new species and restriction of the taxon in new groups are the result of a polyphasic taxonomic approach (Samson, Hong, & Frisvad, 2006; Klich, 2007; Godet & Munaut, 2010).

Currently, there is a controversy over which species contribute most to AF production in Brazil nuts. It is well known that mycotoxins can exist in products without the fungi associated with the mycotoxin being detected, and vice versa. Therefore, the detection of AFG₁ and AFG₂ in Brazil nuts is often surprising, where only strains of *A. flavus* had been previously detected. One hypothesis to support this controversy is the erroneous attribution to the taxon, especially after the recent taxonomic guidelines in Section *Flavi* (Samson, Hong, & Frisvad, 2006). In this case, it is assumed that there is a greater contribution by *A. nomius* (Olsen, Johnsson, Möller, Paladino, & Lindblad, 2008) in the AF contamination of Brazil nuts, since *A. flavus* produces only B₁ and B₂, while *A. parasiticus*, *A. nomius*, *A. arachidicola*, *A. bombycis*, *A. minisclerotigenes*, *A. oryzae*, *A. parvisclerotigenus* and *A. toxicarius* additionally produce AFG₁ and AFG₂ (Samson et al., 2006; Rodrigues, Venâncio, Kozakiewicz, & Lima, 2009).



Fig. 3. Brazil nuts in bad stored conditions infected by *Aspergillus flavus*.

The incidence and frequency of Brazil nut contamination by AF had been monitored by the Ministry of Agriculture of Brazil since 1998. The data concerning the occurrence of AF in Brazil nut samples obtained from export batches and batches rejected by importing countries between 2005 and 2006, analysing only the edible portion (kernels), demonstrated that about 85% of 294 samples showed no detectable levels of AFB₁. In AF-positive samples, the lower AFT limit ranged from 0.4 to 2.42 µg/kg and only 13 samples (4.4%) showed levels above 20 µg/kg (Codex Alimentarius Commission, 2010). These results are favorable, in comparison with previous years. In 2003, Brazil was audited by the European Union with regard to the production and processing of Brazil nuts. Exports of the product were totally restricted since the publication of Decision 2003/493/EC (European Union Commission, 2003). This publication established criteria and conditions for Brazil in order to export in-shell Brazil nuts to the EU. This included the implementation of good manufacturing practices, appropriate sampling plans, favorable analysis conditions and certification of the final product (European Union Commission, 2003). This measure represented the suspension of Brazilian exports, which caused an enormous socioeconomic impact on the entire production chain and led to a stoppage of Brazil nut exports to the EU.

According to the European Commission Directorate General for Health and Consumer Affairs, through the Rapid Alert System for Food and Feed (RASFF), the number of notifications on AF in 2009 (638 notifications) has significantly decreased compared to 2008 (902). AF findings in nuts, nut products and seeds generated 518 notifications. Brazil received 16 notifications, 7 of which concerned Brazil nuts (4 notifications on in-shell nuts and 3 notifications on Brazil nut kernels from Bolivia) (RASFF, 2010).

These findings have resulted in changes in EU legislation. With the adoption of Commission Regulation (EC) No. 1152/2009 of 27 November 2009 imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by AF and repealing Decision 2006/504/EC, the control frequencies at import were increased, kept or decreased mainly based on the findings reported through the RASFF. The control frequency of imports remained unchanged for in-shell Brazil nuts from Brazil (100%) (RASFF, 2010).

These numbers reflected a little progress on the production chain of Brazil nuts in Brazil, on the steps represented by an implementation of good manufacturing practices, appropriate sampling plans, favorable analysis conditions and certification of the final product.

5. Aflatoxins regulation

AF are the most regulated mycotoxins and many countries have specific legislation for AF (van Egmond, Schothorst, & Jonker, 2007). The AF limit for Brazil nuts in Brazil is 30 µg/kg of AFB₁ + AFG₁ (BRASIL, 1977). This limit is currently under review in order to meet food safety standards, since the consumption of contaminated Brazil nuts may represent a risk to the health of consumers both internally and externally, in addition to causing serious losses to the economy and agribusinesses due to a rejection of the contaminated lots by the market.

Although the limits imposed by the European Union are too restrictive, there was an attempt in recent regulations (European Union Commission, 2010) to abide by the maximum permitted limits of the Codex Alimentarius concerning tree nuts. An AFT limit of 10 and 15 µg/kg, for RTE and DFP, respectively, was established for Brazil nuts (Table 1). Under these regulations, this relaxation in the legislation does not result in increased consumer exposure to AF (EFSA, 2009).

6. Possibilities of controlling the occurrence of aflatoxins in foods

Currently there is no available technology to eliminate contamination of food by mycotoxins (Halász, Lásztity, Abonyi, & Bata, 2009;

Shapira & Paster, 2004). Most current strategies for reducing mycotoxins are based on prevention, either at the pre or post-harvest stage, e.g. on the segregation of contaminated grains after harvest (Venâncio & Paterson, 2007). Strategies for the removal or inactivation of mycotoxins are applied on a case by case approach (Sant'Ana, Rosenthal, & Massaguer, 2008). Biological, physical, or chemical methods, for instance, are examples of control methods that do not have a broad application yet (Freitas-Silva & Venâncio, 2010). The lack of practical solutions for controlling mycotoxin contamination in field, at harvest and in processed products leads to the search for alternatives to their partial or total elimination. At the moment, the application of ozone is one of the most promising tools available to ensure food safety (Freitas-Silva & Venâncio, 2010). The application of ozone in low doses can directly protect Brazil nuts from contamination, by reducing the growth of pathogenic microorganisms and decay and, consequently, ensuring product quality.

Compliance with legal limits can also be achieved by investing in inherent natural resistance factors of nuts. This observation was recently evidenced by the addition of natural antioxidants found in tree nuts, such as hydrolysable tannins, flavonoids and phenolic acids (Mahoney et al., 2010). Tannic, caffeic and gallic acids found in almond peel were added to culture media containing *Aspergillus flavus* and the production of AFT was reduced by up to 99.8%. There is evidence that the oxidative stresses of AF are exacerbated by the fungus and hence compounds that alleviate oxidative stress can limit the accumulation of AF. Therefore, increasing the antioxidant activity of compounds in the almond itself may restrict the ability of AF biosynthesis by *A. flavus* and, consequently, achieve compliance with regulatory limits (Mahoney et al., 2010). In the same line of research, the use of biological agents to control mycotoxins is possible; it is notorious the action that many lactic acid bacteria have, inhibiting mould growth, mycotoxin synthesis and/or mycotoxin degradation (Haskard, El-Nezami, Kankaanpää, Salminen, & Ahokas, 2001; Mokoena, Cheule & Gqualeni, 2006; Gratz et al., 2006).

Brazil nut, being a tropical crop, is so easily subjected to contamination by AF, due to the natural susceptibility of the host. Additionally, Amazon has the ideal climatic conditions for mold growth during flowering and pod collection. Due to these conditions, the control of the harvest time is an impractical measure. However, it is possible to reduce the time between collection and processing of Brazil nuts, by an adaptation to drying condition standards in order to reduce moisture and water activity of the kernels, thus decreasing the critical cause of contamination. The effects of relative humidity (RH) and temperature on the production of AF have been evaluated after inoculation of Brazil nuts with an aflatoxigenic *Aspergillus* strain (Arrus, Blank, Clear, Holley, & Abramson, 2005). The maximum level of AF occurred in shelled Brazil nuts which were stored at 25–30 °C and at a relative humidity of 97%, while in-shell Brazil nuts presented lower levels of AF. There was no production of AF in batches stored at 10 °C (97% RH) or 30 °C (RH 75%). According to Arrus et al. (2005), the

Table 1
Current EU regulations on aflatoxins in nuts.

Current maximum EU aflatoxin levels ^a		Aflatoxin B ₁ (µg/kg)	Total aflatoxin (µg/kg)
Almonds, apricot kernels and pistachios	For further processing ^b	12	15
	Ready-to-eat ^c	8	10
Hazelnuts and Brazil nuts	For further processing	8	15
	Ready-to-eat	5	10
Other nuts	For further processing	5	10
	Ready-to-eat	2	4

Data adapted from European Union Commission (2010).

^a Applied as of March 9, 2010.

^b For further processing: to be subjected to sorting or other physical treatment, before human consumption or use as an ingredient in foodstuffs.

^c Ready-to-eat: intended for direct human consumption or use as an ingredient in foodstuffs.

constraining percentage of the moisture content and a_w that is necessary to control the production of AF (less than 4 µg/kg) at 30 °C for up to 60 days of storage is of 4.57% (0.70 a_w) for in-shell Brazil nuts and 4.50% (0.68 a_w) and 5.05% (0.75 a_w) for shelled and sliced Brazil nuts, respectively. These authors state that above these values, the production of AF may increase significantly. These results suggest that the growth of fungi after harvest can be minimized by an appropriate control of temperature and water activity, which are important strategies to be adopted to prevent the production of AF in the Brazil nut chain.

7. Final considerations

The commercial extraction of NTFPs can be an important component of rainforest conservation strategies. According to this, if the forest becomes economically productive, deforestation will be discouraged. For Amazon areas where *Bertholletia excelsa* occurs, the Brazil nut trade will be particularly positive for the preservation of the forest. Given the profitability of the product and the symbiotic relation of Brazil nut trees with their forest environment, it is argued that this environment will be preserved in order to guarantee a continuous supply of Brazil nuts in Amazon.

A proper management of biodiversity in forestry and agroforestry systems will not allow pests and diseases to reach damage levels characteristically observed in monocultures, because of its equilibrium.

The experience of Mycotoxicology in the last five decades indicates that the current levels of food consumption can be maintained by quantitatively increasing food supply in general. However, in terms of food provision, the task of producing mycotoxin-free products with nutritional quality becomes a challenge, especially in complex production chains of developing countries, such as it is observed in the Brazil nut, where this is more difficult to be achieved.

With regard to AF, the management of the entire Brazil nut chain is still a challenge. This wild commodity needs to be safely offered to consumers, especially for the ones who expect to eat not only a nut but also a functional food, because of its health-related appeal, and mostly because of its oil characteristics and high selenium content. Prevention of contamination by *Aspergillus* spp. through good handling practices is still the best measure to avoid AF in Brazil nuts and to ensure the quality and safety of this product.

Conflict of interest statement

The authors have declared no conflict of interest.

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