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Effects of processing and factory storage on aflatoxin contamination of *in-shell* brazil nuts

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Abstract

Factors that can improve in-shell Brazil nut quality during processing and storage conditions (related to fungi and aflatoxin - AFL - contamination) were studied in the factory. Samples of *raw* and *dried* in-shell Brazil nuts were collected at different stages (16 samples each stage): at their *arrival* to the factory (Reception); after *heating* treatment (Drying); during *storage* and when they were ready for *shipping* (Expedition). They were analyzed for moisture content - mc, total fungi count and AFLs. Data on the drying process (temperature and time); storage conditions (temperature, relative humidity - RH) and environmental conditions (rainfall index and temperature) were also collected. As expected, the mc and fungi total count of the raw Brazil nuts at Reception were high (x = 22.4 % and 11.5 x 10^2 cfu/g). AFL contamination at that stage was detected only in four of the total (25 %) samples collected and levels varied from 4.8 to 19.2 µg kg^{-1} (x = 11.13 µg kg^{-1}). The quantification limit of the method used was 1.5 µg kg^{-1}. However, after drying in rotary heaters (> 70 °C) the nuts mc reduced to 4.7 % and total fungi count was quite low (0.28 x 10^2 cfu/g). Only two (12.5 %) dried samples were positive for total AFLs (4.8 µg kg^{-1}, each). At the end of storage mc had an increase, reaching 7.9 % and still safe fungi wise. AFL contamination ranged from ND to 8.0 µg kg^{-1} and fungi count was kept low (0.60 x 10^2). At Expedition, where nuts were packed for shipping, AFL levels ranged from ND to 8.2 µg kg^{-1}. There were only two positive samples (3.1 and 8.2 µg kg^{-1}). That difference was probably due to the heterogeneity of AFL contamination per individual nut. Despite of AFLs contamination, high mc and fungi total count in the raw samples, data showed that the temperature of the drying process was efficient to reduce fungi significantly but not for total AFL degradation (resistant to 250 ºC). The factory procedures data showed being effective concerning the Canada, US and Brazil regulations (15, 20 and 30 µg kg^{-1}), but for European Union (4 µg kg^{-1}). The factory recommendations to maintain dried in-shell nuts safety during shipping until getting to destination are to: control temperature and relative humidity (in storage areas); if possible, utilize refrigeration; sort deteriorated/discolorated/florescent/light nuts; and to get mc homogeneous in stored nuts. The crucial point is the quality checking of the raw Brazil nuts accepted for processing. It is necessary to reject batches with high AFL contamination and to fix a maximum level of visible fungi deterioration load on the nuts (indicative of high AFL contamination). Above all, it is necessary to keep quality of the nuts in the forest: from hull collection / primary drying / storage, to river transport (humidity / hygiene / time) to reach factory with low AFL levels.

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Introduction

Brazil nut belongs to the Tree Nut Group of foods, has a high nutritional content and is one of the most important Brazilian commodities. Since 1950 it has been exported to other countries, such as United States, Europe, Australia and China. However, since the contaminations of *Aspergillus flavus* and aflatoxins (AFLs) in Amazon Region were reported, export has been affected, especially to European countries. Brazil nuts come from tropical areas and are collected in the extractivism, with high relative humidity (RH), above 80 % and temperatures (higher than 25 ºC), conditions that unfortunately allowed fungi growth such as *Aspergillus* toxigenic species (Pitt, 1985, Pacheco and Scussel, 2006). Some *Aspergillus* and *Penicillium* species are found being aflatoxigenic especially in nuts (Machida and Saito, 1999, Simsek, et al., 2002). Extractive activities of Brazil nuts involve artisan conditions and indigenous communities from the Amazon forest. They include transportation mostly by boats to the factories were the industrializing process occurs (reception, drying, selection, second drying, classification, polishing and/or cracking (for *shelled* nuts), storage and packaging at expedition). Brazil nuts can be kept *in-shell* or being *shelled* to be exported, or utilized by Brazilian food industries to obtain products, such as snacks and cookies, thus, taking advantage of their high nutritional content, such as protein (sulfurous amino acids), oil (essential fatty acids) and minerals, especially selenium (Pacheco and Scussel, 2006). *In-shell* dried Brazil nut has the highest volume for export, with a short period of storage in the Factories (maximum of 30 days), but a long period of transportation until the final destination to other countries. In fact, the reduction of the Brazilian export to Europe since 1998 shows that the maximum residue level (MRL) of AFL established by European Union (EU, 1998; 2003) became an obstacle for international trade. The AFLs contamination and fungi have been reported in Brazil nuts (Arrus, et al,, 2005a, 2005b; Freire, et al, 2000; Freire and Offord, 2002; Scussel, 2004) however to improve the quality beyond the extractives activities, it is necessary to apply effective control procedures of the Brazil nut processing in the factories. In order to study the factors in the Factory that could prevent/control *in-shell* Brazil nut fungi growth and AFLs formation for export, a work was carried out to evaluate the effects of processing and conditions of storage in the Brazil nuts Factory.

Materials and methods

Material

(a) In-shell Brazil nuts: from the 2006 harvest: (a.1) ***raw*** and (a.2) ***dried*** nuts. Raw nuts are shortly stored (1-2 days) in wooden silos (4 tons each) at Reception and transferred to processing. Dried nut were stored for 30 days (capacity 200 – 300 tons). They were in packs of 40 and 500 kg at the Expedition.

(b) Brazil nut Factory: from the State of Amazonas, Northern Region of Brazil with storage capacity of 200 –300 tons.

(c) Aflatoxin standards: AFB_1, AFB_2, AFG_1 and AFG_2, Sigma. Standard solutions in toluene.

(d) Equipments: chromatovisor UV, 366 nm, Tecnal; spectrophotometer, Femto; moisture oven, Fanem; analytical scale, Gehaka; microbiological oven, Tecnal. For sample collection and preparation: buckets (20 L) and trays (400 x 250 mm) stainless steel; industrial Brazil nut crackers; industrial mill, Caf.

Methods

(a) Drying: nuts were dried in rotary heaters (70 - 102 ºC) until reaching moisture content (mc) of 6 % during 24 to 48 hs (depending on the percentage of mc of nuts at reception.

(b) Storage conditions: nuts were stored in

wooden silos at room temperature. Factory workers registered temperature and RH during the period of storage (30 days -April, 2006).

(c) Environmental conditions: data of (c.1) rain density, (c.2) temperature during the period of storage, and (c.3) RH, were obtained from the Meteorology Brazilian Institute.

(d) Sampling collection: the sampling method (number of sub-sample and size) used was that of the Brazilian Ministry of Agriculture - Mapa (Brazil, 2004).

(a.1) raw nuts: collection was carried out at the factory Reception (when nuts arrived on the ships to the factory harbor or from the factory patio, immediately after disembark). They were collected randomly from each batch arrived. Sub-samples (corresponding to 10 % of total batch - 7 to 10 kg) were collected. They were homogenized manually in clean containers, and a final portion of 1 kg taken with a stainless steel container and packed in 1kg sterilized plastic bags for analysis. Total of 16 samples.

(a.2) dried nuts: were collected after the drying process (when they were taken out of the rotator heater); also at the nuts storage (Day zero and Day 30: after taken them from drying processing area to the storage silos and after completing 30 day); and at packaging at the Factory Expedition (when dried nuts are packed and ready for shipping). Samples were collected as in 2.a.1. Total of 16 samples each stage.

(a.3) Period of collection: *in-shell* Brazil nuts samples were collected from early April (at their arrival to the factory) to late April (at their dispatching).

(e) Sample preparation: 1kg sample were milled in an industrial mill and divided into 300 g portions for fungi total count, mc and AFL analysis.

(f) Laboratorial analysis

(f.1) Fungi total count: methodology by Machida and Saito (1999).

(f.2) Moisture content (m.c): the analysis was carried out using a gravimetric method (AOAC, 2000).

(f.3) Aflatoxin analysis: method by MAPA (Brazil, 2000) utilizing this layer chromatography [detection limit: 1.50 µg kg^{-1} total AFLs].

(g) Statistical analysis: to obtain the single frequency of variable occurrence the descriptive statistics was used and analyzed by Anova method.

Results and discussion

The data, regarding the parameters for in-shell Brazil nuts safety (fungi, AFLs, mc, RH and temperature), obtained from the *raw* nuts at arrival to the factory, up to Expedition of the *dried* ones (when they were ready to be loaded for shipping (after 30 days of monitoring), are shown in Table 1 and Figure 1.

Reception: as expected, the mc and fungi total count of the raw Brazil nuts that were sent to the factory were high. The average was 22.4 % and ranged from 15.4 to 31.6 % and the total fungi count was 11.5 x 10^2 cfu/g. The AFL contamination, at that stage, was detected in four of the total (16) samples collected corresponding to 25 % of samples surveyed. Levels varied from as low as 4.8 to 19.2 µg kg^{-1} (x =11.13 µg kg^{-1}). The high mc of the nuts indicate that the environmental factors such as, high temperature, RH of the extractive (forest) and river transportation time, could have lead to fungi proliferation and AFL formation. Despite of those, the levels were under the Brazilian MRL: 30 µg kg^{-1} (Brazil, 1977). These high mc average and AFLs presence in the four (25 %) samples collected at the factory Reception indicates the need of **good management practices applied previously, in the extractive** and **transportation** activities of Brazil nuts, before their arrival to the factory. That includes improvements on controlling the hygienic conditions during harvesting and transport. Also it is necessary to control the RH and temperature on the boats and ships and a more efficient system of drying in the forest prior to river transportation, even in crops coming from indigenous communities.

Drying Process: that batch of nuts after being

submitted to the process of drying in rotary heaters at temperatures above 70 (70 - 102 ºC) for 48 hs reached an average mc of 4.7 % and a quite low total fungi count of 0.28 x 10^2 cfu/g. As far as AFL are concerned, only two (12.5 %) samples were positive with levels of 4.8 µg kg^{-1}, each. AFLs are resistant to 250ºC, therefore not possible to be destroyed by the heating used; however it was detected reduction (11.13 to 4.8 µg kg^{-1}). Important to emphasize that after this stage, nuts were submitted to manual selection, followed by a short heating treatment, mechanical classification (size), polishing, and then get ready to go storage after cooling (Pacheco and Scussel, 2006).

Table 1 Aflatoxin contamination and total fungi count in *raw* and *dried* in-shell Brazil nuts during factory processing and storage stages.

Brazil nut/ factory stage	Number of contaminated sample[a]	Aflatoxin (mg.kg^{-1}) Total AFLs[b]	AFB_1	AFB_2	AFG_1	AFG_2	Total fungi count CFU/g)[c]
RAW[d]							
Reception	4	19.2	8.2	2.0	7.0	2.0	13.0 x 10^2
		15.2	5.0	3.5	4.2	2.5	2.9 x 10^2
		5.3	2.0	1.3	0.8	1.2	22.0 x 10^2
		4.8	2.4	1.2	1.2	ND[e]	8.2 x 10^2
	Average	11.13	4.4	2.13	3.3	1.43	11.5 x 10^2
DRIED[f]							
After drying	2	4.8	2.4	ND	2.4	ND	0.28 x 10^2
Storage	Day zero	4.8	2.4	ND	2.4	ND	0.28 x 10^2
	Day 30	8.0	4.2	1.1	3.0	ND	0.60 x 10^2
Expedition	2	8.3	4.2	1.1	3.0	ND	0.62 x 10^2
		3.1	1.8	ND	1.3	ND	0.62 x 10^2

[a] total sample collected = 16 samples each stage; [b] aflatoxins: AFB_1+AFB_2+AFG_1+AFG_2; [c] colony formation unity; [d] collected at arrival of nuts to the factory Reception - harbor; [e] not detected [LOQ: 1.5/2 (mg.kg^{-1}) for total AFLs]; [f] dehydrated.

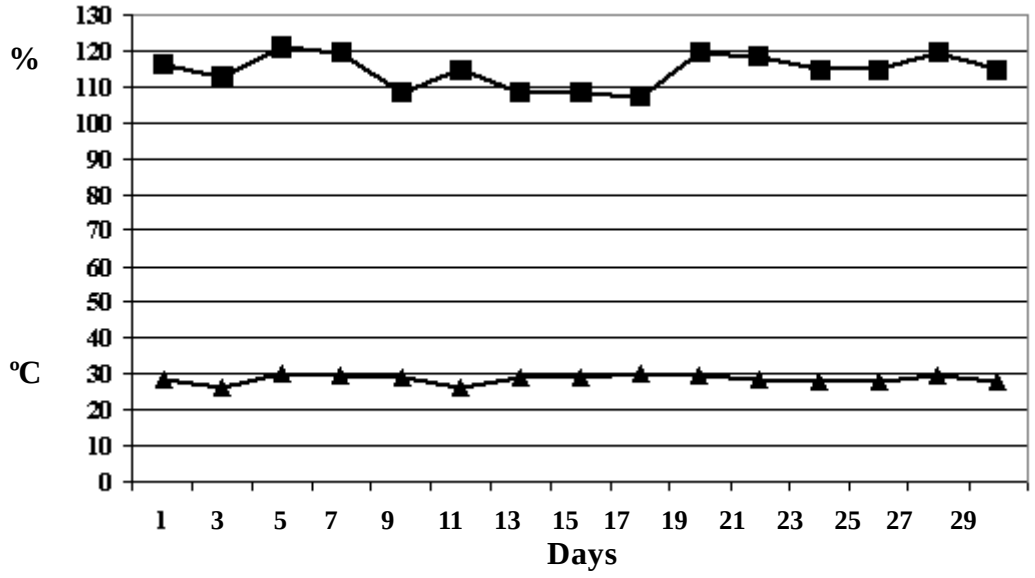

Figure 1. Environmental conditions during the dried in-shell Brazil nuts storage in factory: relative humidity (RH) and temperature. Time: 30 days (in April, 2006).

Storage

During storage an increasing on mc was observed. It reached 7.9 % which is still safe for fungi proliferation. That probably was due to moisture absorption from the environment during the next step (selection/classification/polishing). The RH varied from 77.3 to 95.2 % during that month (x = 87.1 %). See Figure 1. Indeed, that was a month with a rain precipitation index rather high with 496.2 mm. The factory stores nuts at room temperature and its minimum by the time of storage was 24.36 ^{o}C and maximum temperature 29.86 ^{o}C, the average in April was 27.6 ^{o}C. The total fungi count had a slight increase from 0.28 to 0.62 x 10^2. Considering that *in-shell* Brazil nuts is an important Brazilian product for export, mainly for developed countries, the fact that from all samples surveyed, only 18.75 % were contaminated with AFLs, none of them were above the MRL of Canada United States and Brazilian (15, 20 and 30 µg kg^{-1}). On the other hand, the European Union limits to total aflatoxin of 4.0 µg kg^{-1} becomes a limiting factor to export, associated to other factors that reduced the Brazil nut activity such as the decreasing number of Brazil nut trees in the forest, and the increasing international commercial competition with other nuts.

Expedition

At Expedition where nuts are packed getting ready for shipping, AFL levels were from ND to 8.2 µg kg^{-1}. They were detected only in two samples (3.1 and 8.2 µg kg^{-1}). That was probably due to the heterogeneity of AFL contamination per individual nut. The mc average was 7.9 %, attending the factories maximum standards of 8 % maximum.

Despite of the AFLs contamination, high mc and high fungi total count in the raw samples, data shows that at the drying process that utilizes heaters, the temperature was efficient to reduce fungi total count significantly in the Brazil nut samples but not for total AFL degradation. They are resistant up to 250 ºC. As far as the time of storage (30 days) is concerned, it lead to a slightly increase of mc of dried nuts (7.9 % - min 2.6; max 8.8 %), however still safe fungi wise and AFL levels detected were lower (3.1µg kg^{-1}). It is necessary to control Brazil nuts drying process to obtain homogeneous mc throughout the whole drying batch of nuts in the rotary heaters. Also it is recommended to control temperatures of silos and other storage areas as well as aeration with refrigeration (for reducing hot spots thus fungi growth) as well as low RH (environment humidity control). Despite of any improvement that can be carry out on the factory procedures to improve dried nut quality (< mc, fungi and AFLs), if the raw Brazil nuts that get to the factory are already highly contaminated with AFLs or with a high load of fungi deterioration, the probability of getting a final product contaminated is high as drying temperature isn't enough for AFL degradation, even if fungi can be reduced. Unless, the (a) healthy raw nuts are selected prior processing (b) that a good managing practice in the harvesting up to the factory are taken into account. Thus, it is necessary to improve the hulls collection around the trees and the primary storage in the forest, as well as, the conditions of boat transport (time: 50 to 80 days), so that nuts can reach the Factory at AFL low or nil levels.

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