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Characteristics of In-Shell Brazil Nuts and Their Relationship to Aflatoxin Contamination: Criteria for Sorting

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External characteristics of in-shell Brazil nuts were evaluated for dimensions (length and face width), weight, chromaticity, and shell thickness. The internal characteristics evaluated were moisture content (mc), aflatoxin contamination (analyzed by LC-MS/MS), and shell/nut ratio. According to their length, Brazil nuts were classified in three groups: I, II, and III, corresponding to large, medium, and small sizes, respectively. It was possible to establish the following parameters as standards for normal/healthy nuts: length (53.2, 43.9, and 36.6 mm), weight (12.9, 8.8, and 6.3 g), and shell chromaticity components (L*, 38.3, 39.5, and 41.6; a*, 8.0, 7.9, and 7.8; and b*, 17.6, 18.0, and 18.7), for the three groups, respectively. The mean of shell thicknesses were 1.92 and 2.68 mm taken from each face and nut top. The nuts, classified as small (Group III), presented aflatoxin B_1 contamination at a level of 5.62 μg/kg. The Groups shell/nut ratios were 1.2, 1.2, and 1.3 for normal whole and healthy nuts. No aflatoxin was detected in Groups I and II. The data obtained from the Brazil nut measured characteristics can help to distinguish healthy/safe and deteriorated nuts and will be useful for Brazil nut sorting and machine development.

KEYWORDS: Brazil nuts; shell; aflatoxins; dimensions; weight; color; deterioration

INTRODUCTION

Brazil nut (*Bertholletia excelsa* H. B. K.) belongs to the Lecythidaceae family and is naturally grown in the Amazon Region of Brazil, Bolivia, and Peru (*1*). Its fruit is a wooden sphere (pod) that holds ca. 12–25 seeds (nuts) inside. The Brazilian production and number of trees per area represent 80–90% of the whole South American Amazon basin production (*2, 3*).

In-shell nuts for export are currently classified as large, medium, and small by the number of nuts in a fixed weight (453 g) (*4*). However, that classification does not give information on the individual nut characteristics. Standards of Brazil nut parameters such as length, width, weight, shell thickness, and shell/nut ratio are important tools that can be used for further development of methodology for sorting quality. Despite nut classification, aflatoxin contamination has been of concern for Brazil nut exporters, especially since 1998, when the European Union (EU) reduced the maximum residue level of total aflatoxins to 4 μg/kg (*5*) and rejected contaminated batches. Aflatoxin contamination may occur either in the forest or during nut storage prior to processing (*6*). Fungal contamination can lead to nut deterioration and toxin formation. The aflatoxigenic strains of *Aspergillus flavus* can grow in nuts (*7*) and are directly related to the Amazon forest climatic conditions of high humidity and heat during harvesting in the wet season from December to May. Thus, it is necessary to control fungal proliferation and reduce toxin contamination (*1, 8*).

Some deterioration characteristics of Brazil nuts, which can be detected visually or physically by consumers (light weight, discoloration, irregular forms, rattled nut, visible mould, slimy, and soft consistency) may be used for manual nut sorting (*9*). However, for commercial sorting of large quantities of nuts, this method is not feasible, and it is necessary to develop more effective technologies. Studies have reported deterioration characteristics of contaminated nuts and grains to develop methods for sorting. Pearson et al. (*10*) evaluated the physical properties of pistachio nuts to determine distinguishable characteristics (weight, length, width, and thickness) from early split to normal pistachio nuts. The early split pistachio nuts were more susceptible to fungal contamination, and they were significantly different from the normal healthy ones. Consequently, they could develop a system for sorting the normal from the early split pistachio nuts by computer image detection (*11*).

Fungal contamination and subsequent mycotoxin production in corn can affect some characteristics such as color, weight, protein structure, and oil content. In a work carried out by Dowell et al. (*12*), the authors found that grains that presented discoloration had lower weight (0.13 g) and higher fumonisin concentration (476.7 mg/kg). Pearson and Wicklow (*13*) reported in 2006 that corn contaminated by fungi had lower mass

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than the noncontaminated corn, while thickness and length were not significantly different.

Other characteristics that may be selected visually by consumers are their shell color. Some studies have identified defects in nuts and grains by measuring their color alteration, some caused by fungi (*14–17*). Apart from color, another way of sorting deterioration in nuts (pistachio, peanuts, cashew nuts, almonds, and macadamia) and grains (corn, soybean, and wheat) is by detecting toxin fluorescence (*18–20*) and also by detecting the bright greenish yellow fluorescence, a fluorescence produced by kojic acid from fungi in the kernel (*18, 19, 21–23*). However, the latest and very promising method for inner nut and grain deterioration detection is by near infrared (NIR) spectrophotometry (*12, 13, 24, 25*).

Sorting machines for nuts and grains have been developed, reported, and widely used in factories and industries for improving the quality of final products (*12, 26, 27*). The use of sorting machines could also be an indirect way of sorting Brazil nuts with possible aflatoxin contamination. Thus, Brazil nut characterization is essential to establish parameters for its development. To date, there are no sorting machines for Brazil nuts.

Although some studies have been carried out on aflatoxin contamination in Brazil nuts (*7, 8, 28, 29*) and on visual, external characteristics (*9*), none have actually measured their external characteristics and related them directly to classification and safety. Therefore, this study was carried out to establish the external parameters of in-shell Brazil nuts and their relationship to fungal deterioration and aflatoxin contamination and also to compare the TLC and LC- MS/MS method performance for the Brazil nut matrix. This is preliminary work for the development of a Brazil nut sorting machine.

MATERIALS AND METHODS

Samples. In-shell, processed (dry) Brazil nuts (65 kg = ca. 7137 nuts), 15 kg from the 2005 harvest and 50 kg from the 2006 harvest, were collected in a Brazil nut factory in the state of Amazonas, Brazil.

Solvents, Reagents and Other Materials. Acetonitrile (HPLC grade), MilliQ water, chloroform, acetone, and sulfuric acid (analytical grade) were obtained. Chromatoplates, Si-G60, 20 × 20, were obtained from Merck.

Aflatoxin Standards. AFB_1, AFB_2, AFG_1, and AFG_2 were obtained from Sigma.

Equipment and Apparatus. Digital caliper (6 inches) was from Lee; semi-analytical model 440-53 was from Kern; analytical scale model AB204-S was from Mettler; and amplifying lenses were from Olympus. Ultraviolet cabinet, λ 257 and 356 nm. LC-MS/MS system: liquid chromatograph, model 1100, was from Agillent and mass/mass detector API 4000 with ESI and APCI from Applied Biosystems MDS SCIEX. Column: C_8, particle size 5 μm, 4.6 mm, 150 mm, Zorbax. Sphere spectrophotometer, with dynamic rotational sampling, model SP60, was from X-RITE. Brazil nut cracker was provided by the CIEX factory/Manaus, Brazil.

External Characteristics. The in-shell nuts were measured for length, width (three faces), weight, shell thickness, and shell chromaticity. For that purpose, the length was designated as A and the three faces as B, C, and D (**Figure 1A**).

Length. Nuts were separated into three groups (Groups I, II, and III for long, medium, and short sizes, respectively) by measuring their length as follows: the length of each nut was measured with a caliper from top (A1) to bottom (A2) extremities and reported in millimeters (mm). (A1 = the nut top that was attached to the pod center and presents a small hole, and A2 = the opposite top.)

Face Width. The width of each of the three faces (B, C, and D) of each nut was measured at half of each face length and reported in mm (B = the curved face that touches the pod inner surface; C and D = the right and left faces from B, respectively).

Weight. The nuts, previously divided into three groups, were weighed individually and the data registered in grams (g).

Shell Thickness. The measurements were taken from each face (B, C, and D), top (A1) and bottom (A2) of each shell. The nuts of each group were carefully opened, the nut was taken out, and the shell thickness was measured on the three points of each face (A1, A2, and center of nut face) with a caliper.

Shell Chromaticity Analysis. The chromaticity values (achromatic component L*, relative darkness or lightness; chromatic component a*, green to red; chromatic component b*, blue to yellow) were obtained by photo-colorimetric readings (opening diameter 8 mm) on the three faces of each nut: B, C, and D. This was carried out by measuring two points of each face (40 nuts per Group). Faces were divided in two halves, the color measured in the center of each half (point one and point two), and the mean calculated (**Figure 1B**). Prior to the chromaticity colorimeter analysis, each Brazil nut used in this study was visually classified according to its shell color aspects, by a group of consumers, as edible/safe and not edible/unsafe. After this, these two portions were submitted to chromaticity analysis as described above.

Internal Characteristics. Apart from external measurements, nuts were analyzed for moisture content, aflatoxins, and shell/nut ratio.

Moisture Content. Moisture content was measured by gravimetric analysis (*30*).

Aflatoxins. Pools of in-shell nuts of each group were submitted to aflatoxin analysis in triplicate. The methodology used was TLC with UV detection at 365 nm (*31*) and LC-MS/MS with atmospheric pressure chemical ionization–APCI in the positive mode (*31*). LC conditions: column (C_8), flow rate (1 mL/min), mobile phase of methanol/water (55:45, v/v) held for 3 min then changed to methanol/water (70:30, v/v) from 3 to 5 min. MS/MS: the APCI source operated at a dissolvation temperature of 700 °C. Curtain gas was 15.0 psi, nebulizer gas at 55.0 psi, corona discharge needle current of 5 μA, and collision-activated dissociation (CAD) 10. The parent (and the two daughters) ions (*m/z*) were selected for each toxin. For aflatoxin B_1, 313.1 *m/z* (241.10; 285.10); aflatoxin B_2, 315 *m/z* (259.09; 287.20); aflatoxin G_1, 329.1 *m/z* (200.05; 243.05); and aflatoxin G_2, 331.2 *m/z* (245.07; 231.20). TLC LOD and LOQ obtained were 0.21, 0.26, 0.23, 0.25, and 0.42, 0.52, 0.46, and 0.50 for aflatoxin B_1, B_2, G_1, and G_2, respectively. For the LC-MS/MS, the LOD and LOQ were obtained as follows: Brazil nuts were finely ground, homogenized, and spiked prior extraction with aflatoxins at five concentrations ranging from 1 to 10 μg/kg. Portions of 25 g were taken for extraction by adding 100 mL acetonitrile/water (80:20 v/v) to the sample, mixed for 2 h, and filtered (*33*). The LOD method was defined by 3 times the signal/noise ratio and LOQ by 6 times the signal/noise. Five points were used to build an analytical curve, in order to obtain the *R* values for LOD and LOQ. Each point corresponded to a mean of five injections of each extract.

Shell and Nut Ratio. This was carried out by weighing normal (healthy) nuts in-shell and shelled Brazil nuts (n = 200) and calculating the shell/nut ratio in order to help predicting deterioration when detecting different ratios. Depending on the extent of fungal deterioration, it can lead to nut mass reduction (lower weight of the edible part).

RESULTS AND DISCUSSION

From the data obtained with the external and internal characteristics of Brazil nuts, it was possible to set some parameters for normal, healthy (standard), and defective (out of standard) nuts. **Table 1** summarizes the characteristics of in-shell Brazil nuts.

External Characteristics of Nuts. The determination of nut length, face width, weight, shell thickness, and chromaticity allowed them to be classified into three groups of normal and healthy in-shell nuts and establish standard characteristics that can help nut sorting.

Length. Brazil nuts were classified by size into Groups I, II, and III for large, medium, and small nuts, respectively, according to their length. Group I: nuts with lengths greater that 50 mm; Group II: from 40 to 50 mm; Group III: lower than 40 mm. The longest nut was from Group I with 74.1 mm and the shortest

Table 1. External Characteristics of In-Shell Brazil Nuts

	dimensions (mm)					shell thickness (mm)					
	length	face width				top	bottom	faces[a]	chromaticity values		
Brazil nut group	A	B	C	D	weight (g)	A1	A2	B/C/D	L*[b]	a*[c]	b*[d]
					I						
mean	53.2	21.7	29.4	29.7	12.9	2.8	2.8	2.0	38.3	8.0	17.6
max	74.1	28.9	37.3	36.3	18.0	4.4	3.7	3.1	50.7	20.8	24.9
min	50.0	14.4	23.2	24.4	6.0	1.7	2.0	1.2	4.4	3.3	7.5
SD[e]	2.9	2.5	2.3	2.2	1.9	0.6	0.4	0.4	4.6	2.9	3.2
RSD[f]	5.4	11.6	7.9	7.6	14.8	23.1	14.00	18.6	12.1	36.3	18.2
					II						
mean	43.9	19.9	26.5	26.3	8.8	2.9	2.9	2.0	39.5	7.9	18.0
max	50.0	33.8	39.9	37.6	17.0	4.0	4.1	3.0	50.6	13.3	24.8
min	38.6	12.1	16.0	13.9	2.0	1.7	1.7	1.2	29.4	3.7	11.1
SD[a]	2.7	2.6	2.9	2.8	2.1	0.6	0.6	0.4	4.0	2.5	2.6
RSD	6.1	13.00	10.8	10.7	23.6	19.7	21.2	19.8	10.1	31.9	14.4
					III						
mean	36.6	18.8	23.5	23.5	6.3	2.4	2.4	1.8	41.6	7.8	18.7
max	40.0	31.7	31.2	31.3	11.3	3.7	3.9	2.7	54.1	12.3	24.3
min	14.0	11.7	15.0	13.2	1.0	1.4	1.3	0.9	25.5	3.1	7.2
SD	2.8	2.5	2.8	2.6	1.7	0.5	0.5	0.4	3.9	2.5	2.5
RSD	7.7	13.3	11.6	11.1	27.2	21.6	22.6	21.3	9.4	32.7	13.5

[a] $n = 200$ (per group). Mean of the measurements taken from the center of the faces B, C, and D. [b] Achromatic component (relative darkness or lightness). [c] Chromatic component (green to red). [d] Chromatic component (blue to yellow). [e] Standard deviation. [f] Relative standard deviation (%).

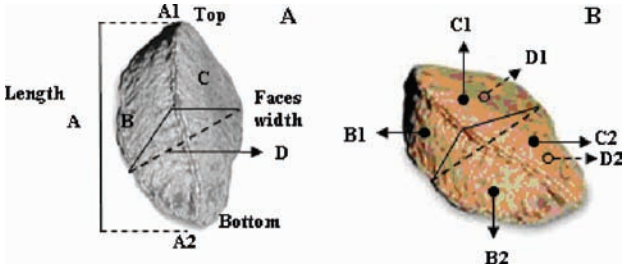

Figure 1. In-shell Brazil nut points taken for the [**A**] nut dimensions measurements, (A) length and (B, C and D) face width, and [**B**] shell chromaticity analysis, face B (point B1 and B2); C (C1 and C2); and D (D1 and D2).

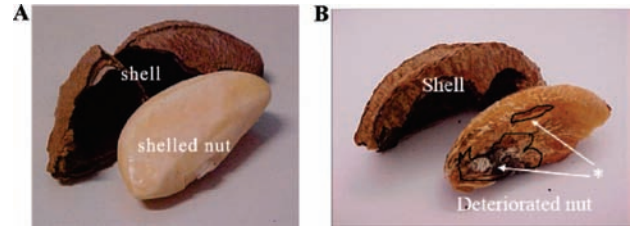

Figure 2. Shelled Brazil nuts. [**A**] normal and healthy nut ($F < 1.5$); [**B**] nut deteriorated by fungi with mass reduction (*) ($F > 1.5$).

(Group III) with 14.0 mm. As expected, the variation of nut length was high among all nuts surveyed. However, the RSD for Groups I, II, and III were 5.4, 6.1, and 7.7%, respectively. After measuring the nut lengths and faces widths, weights were then evaluated for each Group.

Face Widths. The three nut faces (B, C, and D) presented a measured width pattern for Groups I, II, and III (**Table 1**). The nut width range for face B was 11.7 to 33.8 mm, for C from 15.0 to 39.9 mm, and for D from 13.2 to 37.6 mm. The width of face B had consistently lower values when compared to those of C and D, for all groups. It was observed that the face B of Brazil nut shows a more curved shape. In fact, it is the face that touches the pod inner surface. Neither face C or D has such a curved shape as face B. The nut faces C and D each touch other nuts in the pod. The edge between both faces is the nut part that links it to the pod center. Both faces had their width decreased according to the Groups I, II and III i.e., ca. 29.3 and 23 mm.

Weight. the weight range obtained for the Groups I, II and III were 6 to 18 g; 2 to 17 g and 1 to 11.3 g, respectively. Some important weight data was gathered for the respective Groups that could be related to fungi and aflatoxins. Although most of the largest nuts (Group I) had also the heaviest weight (max. Eighteen g), some of them presented lighter weight (min 6 g) in the same Group. It was observed that a high percentage of light nuts were in Group III, probably due to the fungal deterioration inside (**Figure 2**). Indeed, these were the nut batches that presented aflatoxin contamination.

Shell Thickness. As far as shell thicknesses are concerned, no significant differences among the nut groups were observed, either for the nut top, bottom, or half-face measurements. The thickness means for Groups I, II, and III were 2.8/2.8/1.9, 2.9/2.9/2.0, and 2.4/2.4/1.8 mm for the shell points A1/A2/faces, respectively. As expected, the shell top and bottom were thicker than the faces. Face B (the face that touches the pod and has a curved shape) was consistently thinner than faces C and D. The thinner the face, the higher the possibility of cracking when the nuts fall from trees to the soil even if they are still inside the pods as the trees can reach 50 m height. In addition, insects and rodents can more easily enter the pods and attack cracked in-shell nuts allowing fungal spore proliferation. The RSD% of the weight and thickness measurements of the size II and III groups presented the highest variation (**Table 1**).

Chromaticity. Quite a wide variation was detected among the components responsible for the nut color intensity (L, a*, and b*).

*Component L**. The shell chromaticity mean values obtained in this study for the lightest (maximum) and darkest (minimum) achromatic component L* were 54.1 and 4.4, respectively (**Table 1**). The nuts of Group III were the ones that showed higher L* values corresponding to lighter shell chromaticity. In fact, those nuts were contaminated by aflatoxin B_1. This data corroborates the work reported by Marklinder et al. (*9*), where the

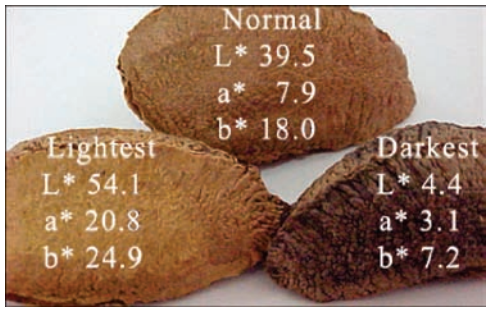

Figure 3. In-shell Brazil nut color variation.

consumers visually selected, among different nut external characteristics, the light color as not edible or not safe. However, nuts classified in Group I had the lowest L* values and no aflatoxin detected in this study.

Although the aflatoxin contaminated nuts show the lightest color, it was observed that deteriorated nuts can show color alteration either darker or lighter than normal ones. Therefore, it was assumed for this work that the darkest and lightest values of the Brazil nuts should be discarded (considered out of standard) in order to reduce the probability of contaminated ones (**Figure 3**). Despite this, some shell color alteration can be a result of other factors such as the conditions of harvest, storage, or by contact with oil during transportation by boat. Thus, it is recommended that these standard L* value nuts to be used *shelled* after quality checking.

Components a and b**. The chromatic components: *a** (green to red) and *b** (blue to yellow) of the Brazil nut shell minimum and maximum were 3.1 and 20.8, respectively, for *a** and 7.2 and 24.9, respectively, for b*. Considering the nut brown color and the chromacity values a* and b*, the data more often showed mean chromaticity mix-values on the color system chart (*33*) of yellow + red = brown. **Figure 3** shows the chromaticity variation of the Brazil nut shells evaluated in this study. The shell chromaticity components (L*, a*, and b*) that were considered as acceptable color were in the range 31.5–48.6; 4.1–10.1 and 11.9–23.0, respectively.

Internal Nut Characteristics. The evaluation of the internal characteristics of the Brazil nut comprised analyzing parameters that are directly related to fungi deterioration, i.e., moisture content, aflatoxin contamination, and shell/nut ratio. Despite this, the nut can also deteriorate becaus of tissue reactions such as hydrolysis and oxidation, and they are the second cause of low quality nuts. These reactions can lead to sensory deterioration (rancid flavor by lipid auto-oxidation; texture alteration by protein hydrolysis). However, the main deterioration in Brazil nuts occurs because of fungal proliferation leading to stained shell and/or rotten nuts, and also because of weight reduction of nuts (nut rattling inside).

Moisture Content. The obtained moisture content of nuts was low, ranging from 3.6 to 8.3%. The mean was ca. 6% (5.9, 6.0, and 5.9%) for Groups I, II, and III, respectively. These values are considered safe as far as fungal growths are concerned. Arrus et al. (*29*) proposed that processed in-shell Brazil nuts (dry nut) should be dried to a moisture content of approximately 5.0%, when stored for 30 days at 95% and 30 °C. However, according to A. Pacheco (personal communication), they can still be considered safe up to 8%.

Aflatoxin Contamination. Aflatoxin B_1 contamination was detected only in nuts from Group III when using the LC-MS/MS methodology. These nuts presented the highest variation (RSD%) and the lowest weight (mass reduction) from the standard ones, set for the same group. Besides, these nuts

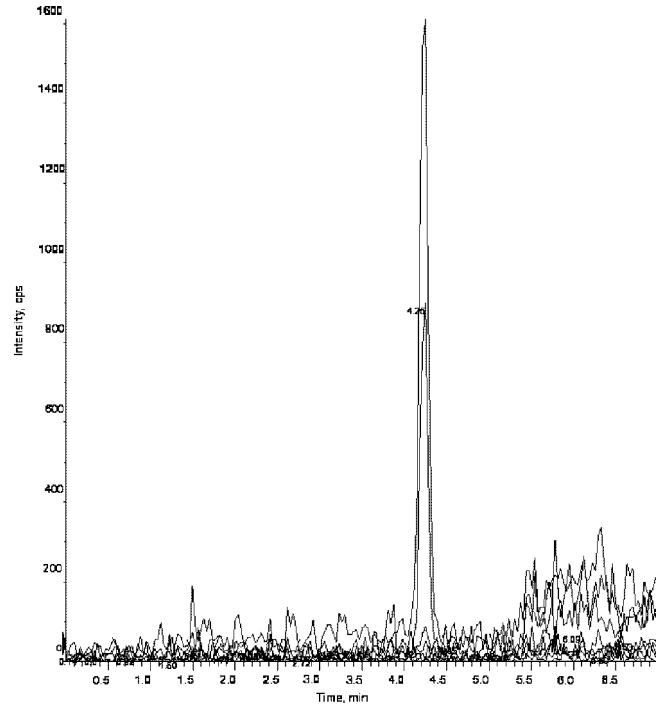

Figure 4. LC-MS/MS-APCI chromatogram of an in-shell Brazil nut naturally contaminated with aflatoxin FB_1.

showed more number of shells with lighter color, observed visually and corroborated by the high L*, a*(red), and b* (yellow) chromaticity measurements. Therefore, nut weight and color, apart from nut external quality, may be an indirect way of identifying fungal and mycotoxin contamination and a tool to be used for sorting machine development. As far as the results obtained from the two different analytical methods are concerned, they showed differences in the levels of aflatoxin detected. When nuts were analyzed by TLC, the aflatoxins B_1, B_2, G_1, and G_2 were not detected in any of the samples up to the LOQ method: 0.42 μg/kg. However, when the nuts were analyzed by LC-MS/MS, aflatoxin B_1 was detected at a level of 5.62 μg/kg only in the nuts from Group III (**Figure 4**). The LOQ of the LC-MS/MS method utilized for the aflatoxin analysis was quite low at levels of 0.08, 0.09, 0.1, and 0.12 μg/kg for AFB_1, AFB_2, AFG_1, and AFG_2, respectively, and their recoveries from Brazil nuts reached 92.4, 72.5, 99.8, and 97.1% of the four toxins, respectively. In fact, the LOQ of LC-MS/MS for aflatoxin B_1 is much lower than the TLC value (0.08 μg/kg). No matrix effect was observed. The advantage of this method is the sensitivity at ppt levels, the reduced time of extraction, and the lack of the need for clean up for Brazil nuts (*32*). The fact that aflatoxin B_1 was not detected using the TLC method even when its LOQ is 0.42 μg/kg is probably due to the contamination heterogeneity of nuts.

It is important to emphasize that the moisture content detected may not be the cause of aflatoxin contamination during the drying process. The detected aflatoxin contamination in the nuts may have come from the raw nuts prior to processing and may have occurred either in the forest after falling into wet ground or during the primary nut storage, prior to their transfer by boat to the factory. These are factors that contribute to fungal proliferation (*6, 1, 8*).

Shell and Nut Ratio. The measurements of shell and shelled nut weights can help to set standards of shell/nut ratio for predicting whole in-shell Brazil nut variation. The literature mentions that the percentage of shell to edible nut of an individual Brazil nut is 50%. However, the exact measurements

Table 2. Brazil Nut Ratio Shell/Nut Weight

Brazil nut groups	weight			
	total	shell	nut	shell/nut ratio
		I		
mean	15.0	7.8	6.6	1.2
max	16.7	9.0	9.0	1.8
min	11.5	6.6	3.8	0.7
SD	1.6	0.9	1.3	0.3
RSD	10.5	11.4	20.5	0.2
CI	14.0–16.0	7.2–8.4	5.8–7.2	1.0–1.4
		II		
mean	10.5	5.4	4.6	1.2
max	13.2	7.6	6.5	1.8
min	5.7	3.1	2.4	0.8
SD[a]	2.7	1.5	1.4	0.3
RSD[b]	25.7	28.2	30.6	0.2
CI[c]	8.8–12.2	4.5–6.4	3.8–5.5	1.0–1.4
		III		
mean	6.9	3.5	2.9	1.2
max	8.5	4.4	3.7	1.9
min	5.8	3.2	2.0	0.9
SD	0.8	0.4	0.5	0.3
RSD	12.2	11.2	19.5	0.2
CI	6.4–7.4	3.3–3.8	2.5–3.2	1.1–1.4

[a] Standard deviation. [b] Relative standard deviation (%). [c] Confidence interval.

obtained in this study found that they were 53 to 47%, corresponding to 4.2 to 3.8 g for an 8 g nut, for shell to nut, respectively. The shell/nut ratio means were 1.2, 1.2, and 1.3 for the three nut groups, respectively (**Table 2**). From the confidence interval, obtained from the mean, it was possible to establish a shell/nut factor (F) for healthy and deteriorated nuts ($F = 1.5$) corresponding to a ratio of 60% shell to 40% nut (equation 1). According to this proportion, it was observed that nuts that have $F < 1.5$ were considered healthy and nuts with $F > 1.5$ were considered deteriorated. In conclusion, nuts that have less than 40% of their total weight, corresponding to the edible part, were considered contaminated and were discarded.

$$F = \frac{\text{Shellweight}}{\text{Totalweight-Shellweight}} \quad \begin{matrix} F < 1.5 & \text{healthly nuts} \\ F < 1.5 & \text{deteriorated nuts} \end{matrix} \tag{1}$$

Therefore, 1.5 was considered the standard ratio for normal healthy whole Brazil nuts, despite overall sizes and values < 1.5. Reduction of the mass of the edible nut by fungi or dehydration indicates deterioration. In addition, a higher ratio compared to standard may indicate deteriorated inner nuts, which could help to reject the lightest ones, especially when NIR spectrophotometry methods are used for confirmation (*25*, *26*). Fungi contamination may provoke some alteration in nut structure related to weight, color, protein structure, and oil content. Protein structure and oil content can be detected by an NIR sensor, which can pass through the shell material reaching the inner (edible part of) nut and is able to detect chemical component alteration. The use of this methodology will be the next step carried out by our group.

Characteristics of in-Shell Brazil Nuts and Their Relation To Aflatoxin Contamination: Criteria for Sorting. It was possible to establish size and weight parameters that would differentiate normal and healthy in-shell Brazil nuts (**Figure 5**) from nuts that do not meet the standard . Also, setting the shell/nut ratio can be very helpful as far as the detection of nut deterioration with mass reduction due to fungi are concerned. Face thickness differences were observed, which can help to understand which nut face is more susceptible and vulnerable for cracking and fungal spore invasion when the nut falls to the ground.

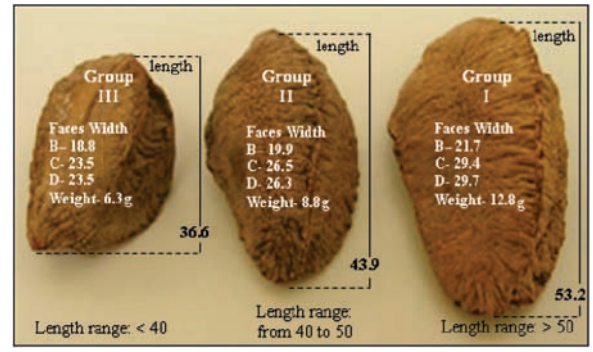

Figure 5. In-shell Brazil nut Groups I, II, and III and mean of standard dimensions of length (mm), width (mm), and weight (g).

The shell chromaticity components (L*, a*, and b*) in the range 31.5–48.6, 4.1–10.1, and 11.9–23.0 can help to discard the nuts that do not meet the standard and establish an acceptable color range.

In addition, in this study, the nuts classified as small (Group III) were the only ones that showed aflatoxin contamination (5.62 μg/kg aflatoxin B_1). The level was slightly higher than the maximum residue limit (MRL) allowed for aflatoxin B_1 (5 μg/kg) by the USA (*34*) and southern South America-Mercosur (*35*). However, it was two times higher than the EU MRL of 2 μg/kg for aflatoxin B_1, a more restrictive regulation. It is important to emphasize the difference of detection between the two methodologies used. While no aflatoxin was detected by TLC, the LC-MS/MS was able to detect contamination. This shows the feasibility of the semiquantitative method currently used in Latin American laboratories, when high sensitivity is necessary for export. Considering the low MRL, either for total aflatoxins or aflatoxin B_1, established by importing countries that have more sensitive methodology, exporting countries should be more careful as to the methodology used.

These data will be extrapolated for building vibrating trays with specific sized and shaped sections for large/medium/small nut classification; setting compressed air devices with adjusted flow for blowing off deteriorated, low weight nuts; and photoelectric cells for color chromaticity detection of stained nuts. Nuts that do not meet the standard can be sent to the shelling section of the factory, after having their nut quality (deterioration and aflatoxin contamination) inspected by the quality control laboratory.

The visual characteristics of Brazil nuts (size, weight, and color) observed by consumers may indirectly indicate nut deterioration. However, when using exact measurements of these characteristics, one can establish standards to facilitate the selection of healthy nuts and the rejection of deteriorated and/or contaminated ones. The data obtained in this study, either of external or internal characteristics of Brazil nuts, will be used for mechanically setting nut size classification and developing a specific sorting machine. This is the first work carried out on setting physical method parameters for Brazil nuts. More data are being gathered on nut varieties from different Amazon regions. Further studies using NIR will be advisable for the detection of chemical compounds responsible for deterioration.

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