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Physiologically meaningful moisture content determination for Brazil nut (*Bertholletia excelsa* Bonpl.) seeds

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Brazil nut (*Bertholletia excelsa* Bonpl.; Lecythidaceae) seeds are large, and the oily embryo (edible part) is covered by a thick, woody seed coat. Difficulties in seed storage may be due to desiccation sensitivity of the seeds, and prior variable results may be related to procedures for seed moisture content (MC) determination. This study first compared 3 methods of preparation of whole seeds, prior to MC determination: (i) intact seeds; (ii) chopped (<7 mm) and (iii) cut lengthwise. Drying at 105°C for 24 h was sufficient to remove free water in all three procedures, with results within tolerance limits. Secondly, seeds in equilibrium with relative humidity had a high variation in MC. This could be explained to some extent by the increased proportion of seed coat in smaller seeds, with 48% of seed weight average total. Seed coat MC was always higher than embryo MC. Linear regression revealed an increasing overestimation of embryo MC with desiccation. Considering 10% MC of the whole seed, embryo MC was 5.3% (a 1.9-fold overestimation). In this respect, it is physiologically meaningful to determine embryo MC and not whole seed MC. The approach may be applied to other species with similar seed morphology.

Key words: *Bertholletia excelsa*, drying, Lecythidaceae, recalcitrant seeds, seed moisture content, seed testing.

INTRODUCTION

Bertholletia excelsa Bonpl. (Lecythidaceae), popularly known as Brazil nut, is native to the Amazon region (Flora do Brasil, 2018). Seeds are the main product of this multiple-use tree of great socioeconomic importance for the Amazon region (Calvi and Ferraz, 2014). In 2014, about 37,500 tons of seeds were produced in Brazil, generating approximately USD 33.8 million on the national market or for export (IBGE, 2014). Seed

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collections are done primarily in native populations (IBGE, 2014) which reduce the demand for plantations. However, some studies indicate that this species is suitable for plantations (Ferreira and Tonini, 2009), or as restoration of degraded areas (Costa et al., 2009), and presents higher production in stands with clayey to very clayey soil texture (Guerreiro et al., 2017).

Research on seed technology of Brazil nut is scarce, which can be observed based on the lack of publications in recent years. The main studies were published in books and technical reports during the 1980s and 1990s. Brazil nut seeds measure 4 to 7 cm, are triangular in cross section and weigh 6.7 to 16.7 g (Müller et al., 1995; Ferreira et al., 2006). The seed coat is lignified, hard, rough, permeable to water (Müller et al., 1995), and offers mechanical restriction to germination (Müller and Freire, 1979). The embryo is mainly a large, reserve-storing hypocotyl, no distinction of cotyledons is visible with an ellipsoid shape being the shoot pole is sharper than the root pole on the opposite end (Prance and Mori, 1978).

Brazil nut seeds are not viable when stored for a long time (Figueirêdo et al., 1990a, b; Figueiredo and Carvalho, 1994; Camargo et al., 1997). This may be due to desiccation sensitivity of the seeds. However, the relation between seed moisture content (MC) and germination gives no conclusive results so far. Seeds had been classified as desiccation sensitive (recalcitrant), because germinability was reduced when dried to values lower than 14% seed MC (Figueirêdo et al., 1990c). However, seeds were also considered as intermediate, based on 42% germination with 5% MC (Figueirêdo and Carvalho, 1994) or 16% viability (tetrazolium test) with 4.5% MC (Camargo et al., 1997). As seed storage behaviour is based on seed tolerance to desiccation, the method used to determine seed MC is extremely important and can be the reason for these differences.

According to seed testing prescription of that time (Brasil, 1976, 1992), seed MC was determined with whole seeds, dried under 3 possible conditions (105°C for 24 h, 103°C for 17 h or 130°C for 1 h). Comparing these conditions, no difference was found with whole Brazil nut seeds (Camargo, 1997). However, due to the large seed size, the prescribed time might not have been enough for complete loss of free water. In the current edition for seed testing, the 3 temperature conditions continue to be valid and, in addition, large tree seeds (weight of 1,000 seeds > 200 g), with very hard integument and/or high oil content, should be cut in pieces smaller than 7 mm (Brasil, 2009; ISTA, 2015).

In the special case of Brazil nut, the woody seed coat may contribute around 50% of the whole seed weight (Müller et al., 1995; Ferreira et al., 2006). Seed MC determination is necessary for deciding adequate procedures to maintain seed quality during seed handling and commercialization. If the objective of seed MC determination is related to viability, as during seed development, maturation and storage, the analysis should determine MC of the physiologically active part (embryo and its seed reserves), and exclude all other seed components, such as dispersal aids, protective coats, etc.; in this way, the aim of this study was to evaluate the contribution of seed coat to seed weight and its effect on MC values and to propose a new protocol for seed MC determination of Brazil nut, in comparison with seed testing procedures of the last decades.

MATERIALS AND METHODS

Fruits of *B. excelsa* were collected in 2 municipalities of Amazonas State (Brazil), in Itacoatiara and Autazes, about 176 and 108 km from Manaus, respectively. Climate is tropical wet, type Am Köppen classification, with an annual average of 28.2°C; 82.7% relative humidity (RH) and 2,240 mm precipitation (INMET, 2016). Fruits were collected during natural dispersal between January and February. In the Seed Laboratory in Manaus, the indehiscent woody fruits were opened with a machete. Seeds were rinsed in running water to remove impurities and empty seeds eliminated after floating. Subsequently, seeds were superficially dried for 40 min under ventilation at room temperature (27 ± 3°C and 70 ± 3% RH). Until the beginning of the experiments, whole seeds were kept in double polyethylene bags (130 µm thick) in a cold chamber (15 ± 1°C, 96 ± 3.5% RH) to equilibrate moisture.

The Itacoatiara collection was used to compare 3 preparation methods of the seeds prior to moisture determination: (i) cutting into pieces smaller than 7 mm, following the most recent recommendations for seed testing (Brasil, 2009; ISTA 2015), (ii) using whole seeds according to Brazilian Seed Testing Rules of 1992 (Brasil, 1992), and (iii) cutting lengthwise with a knife and the help of a hammer. For each preparation, 60 seeds were used, considering each seed as a repetition. Following the Seed Testing Rules (Brasil, 1992; 2009), the samples were weighed using a balance with 0.001 g precision scale and dried in an oven at 105 ± 3ºC for 24 h. To verify if all free water was evaporated, the samples returned to the oven and were weighed every 24 h for another 6 days. Seed MC was calculated as percent of fresh seed weight (Brasil, 1992, 2009; ISTA, 2015).

The Autazes collection was used (i) to describe variation in seed size and the contribution of seed coat and embryo to the whole seed weight based on 1,100 seeds; (ii) to compare whole seed MC with the MC of the seed parts, that is, the embryo and the seed coat; thereafter, 1,700 seeds were tested through a desiccation gradient. Desiccation was done by spreading out the seeds in one layer in an air-conditioned room at 25 ± 3 °C and 68 ± 3% RH. After different periods of drying, seeds were packed in polyethylene bags (130 µm thick) and maintained at 15 ± 1 °C for at least a month in order to equilibrate moisture between seed coat and embryo and throughout each desiccation level. Seed MC was determined for each individual seed, separating the seed coat from the embryo with a longitudinal cut. Samples were dried at 105 ± 3°C for 24 h and seed MC was expressed as a percentage of fresh weight. Whole seed MC was obtained by summing the weight of the seed parts.

Differences in MC between preparation methods and between values during the 7 days of drying were evaluated according to Seed Testing Rules, based on the allowed levels of tolerance for seed moisture (Bonner, 1984; Brasil, 2009) and analysed by one-way ANOVA (Sisvar® version 5.3). The comparison between whole seed mass with embryo and seed coat mass was shown with linear regressions (SigmaPlot® version 11.0). Relation between MC of whole seeds and embryo and seed coat MC through a desiccation gradient was also determined with linear regressions.

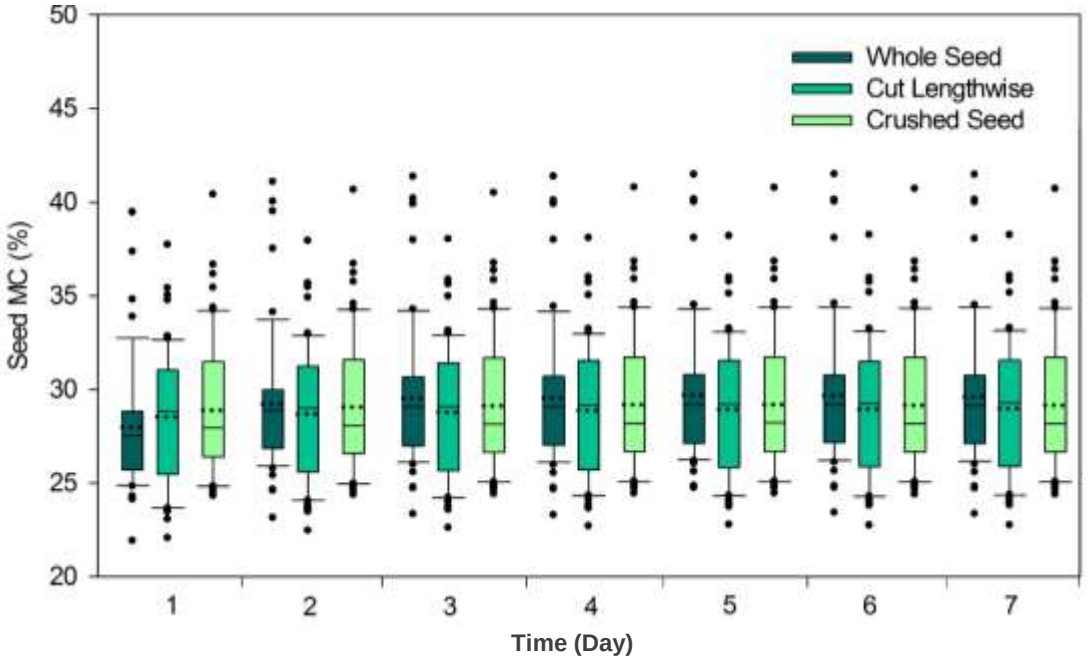

Figure 1. Boxplot of Seed Moisture Content (MC) of *Bertholletia excelsa* Bonpl. comparing three preparation methods: whole seeds, seeds cut lengthwise and crushed seeds (small pieces of ≤ 7 mm). Median (solid line) and mean value (dotted line) of 60 seeds for each treatment. Samples were dried at 105 ± 3°C and reweighed every 24 h, during seven days.

RESULTS

Average seed MC determined by the 3 methods was 28.0% (whole seed), 28.5% (cut lengthwise) and 28.9% (crushed) all with the same standard deviation (σ = 3.5). Seven days of drying increased these values slightly to 29.6, 29.0 and 29.2%, respectively (Figure 1). However, no statistical difference was detected between the methods and the length of drying time (P = 0.724). The findings are also consistent with the standards for seed quality analysis, as up to 2.5% difference between the results of 2 replicates are tolerated for large tree seeds with a high MC (> 25%) (Brasil, 2009). In this respect, all 3 methods can be used for MC determination of *B. excelsa* seeds, and 24 h at 105°C gave satisfactory results for the evaporation of free water. However, in all 3 seed preparations, MC between the individual seeds showed a high variation (Figure 1). The range between the extremes was 25 to 35% (excluding outliers). Considering that all seeds had been maintained under the same environmental humidity for at least 30 days before the MC determination, factors other than equilibrium moisture content may have affected these results; therefore, seed size and the percentage seed coat *vs.* embryo were measured.

Individual seed weight ranged from 3.76 to 20.35 g, an average of 8.80 g. The majority (66.6%) had between 6.55 and 11.05 g (Figure 2). The relation between seed coat and embryo mass in relation to whole seed mass is shown in Figure 3. It is possible to determine that, in smaller seeds, the percentage of seed coat and embryo is quite similar and, with an increase in size, seed coat proportion is reduced (Figure 3). On average, the woody seed coat in fresh seeds of *B. excelsa* accounts for 42.3% of the seed weight, complemented by 57.7% for the embryo. This relation changes to 40.4% for the seed coat and 59.6% for the embryo, by considering only dry matter.

Brazil nut embryos are primarily oily and the seed coat is woody and fibrous. The correlation between whole seed MC with embryo MC and seed coat MC was linear as attested by R^2 of 0.97 for the embryo and R^2 = 0.91 for the seed coat (Figure 4). Embryo MC was always lower and seed coat MC higher than MC determined with the whole seed. Consequently, if MC is determined using whole seeds, the MC of the living part is overestimated (Figure 4). Linear regression reveals that overestimation of embryo MC is augmented in drier seeds. Considering 10% MC of whole seeds, embryo MC will be 5.3% (a 1.9-fold overestimation), while seed coat MC will be 16.5% (a 0.6-fold underestimation). Values for embryo and seed coat MC will be similar only when whole seed MC reaches 43% (Figure 4).

DISCUSSION

Determination of seed MC aims to evaluate free water in

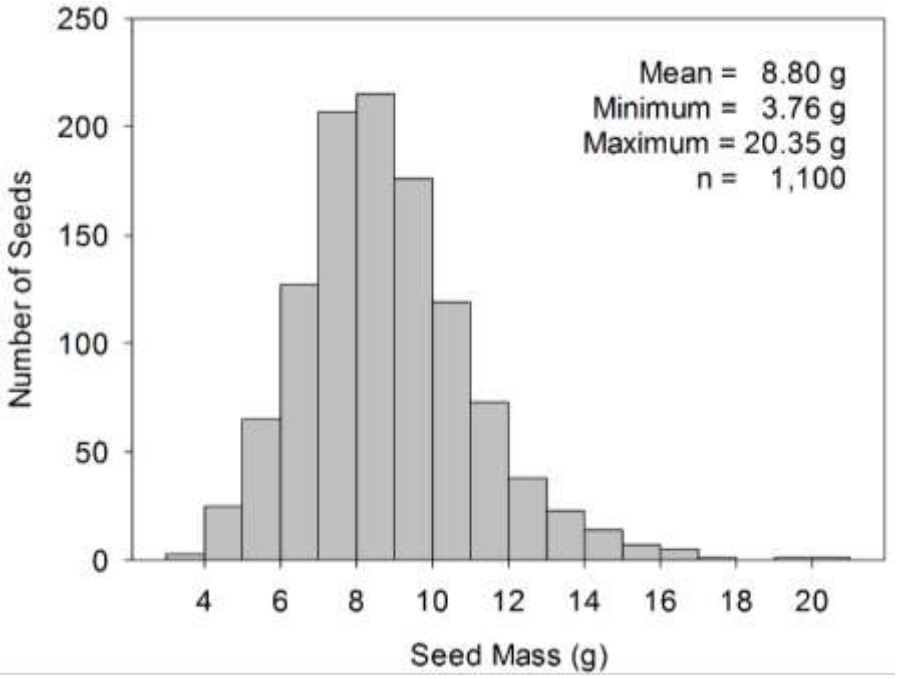

Figure 2. Variation in fresh seed weight of *Bertholletia excelsa* Bonpl.

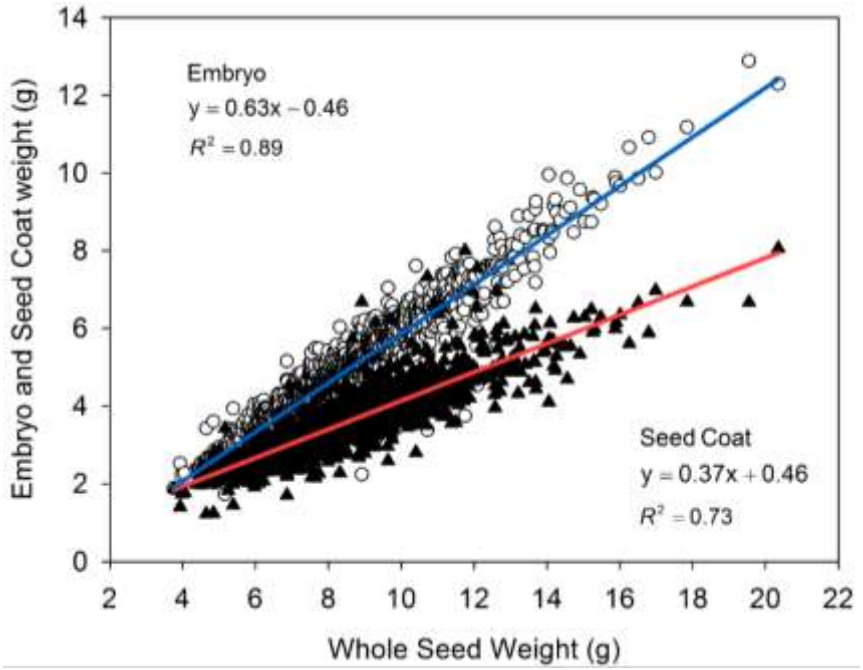

Figure 3. Relation between weight of whole seeds and the weight of seed components: embryo (open circles; blue line) and seed coat (closed triangles; red line) of *Bertholletia excelsa* Bonpl. (n = 1,100).

the seeds. According to Seed Testing Rules (Brasil, 2009), the drying period at 105°C should be completed in 24 h. In the special case of Brazil nut, the seeds are very large and contain about 67% lipids (Ferreira et al., 2006; Balbi et al., 2014); thus, the drying period should permit evaporation of free water and avoid or minimize

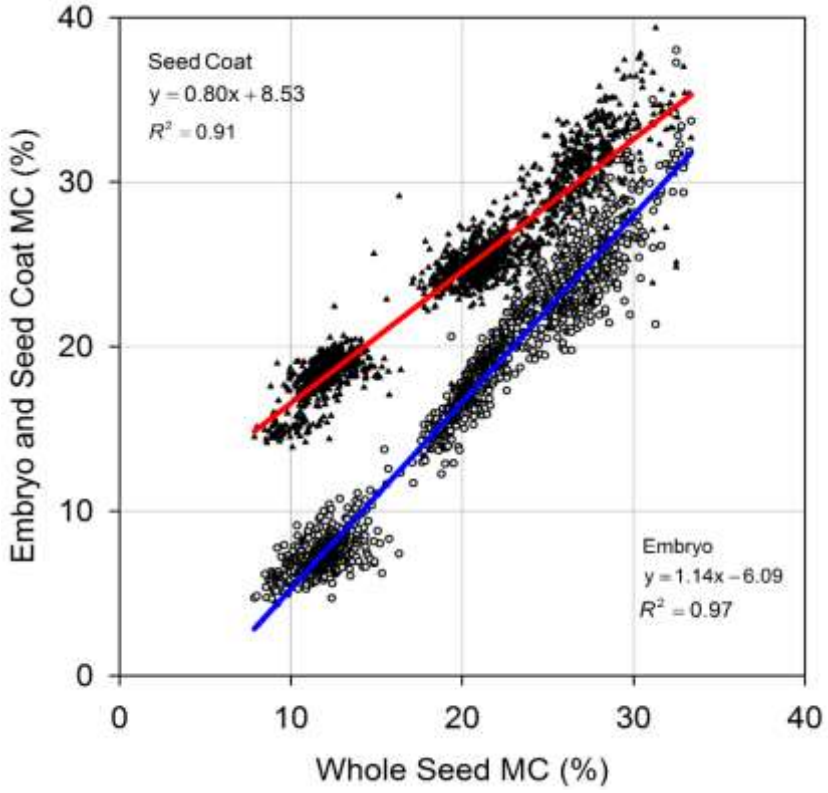

Figure 4. Relation between whole seed Moisture Content (MC) and the MC of seed components: embryo (open circles; blue line) and seed coat (closed triangles; red line) through a desiccation study of *B. excelsa* seeds. Data represent individual seed measurements (n = 1,700).

evaporation of volatile compounds and oils. This study showed that 24 h of drying provide results within the tolerance levels given for seed testing, which recommended whole seeds (Brasil, 1992), recently crushed seeds (Brasil, 2009; ISTA, 2015), and the lengthwise cut tested here. Grinding seeds cannot be recommended as the procedure resulted in significantly reduced values in comparison to whole seeds (Camargo, 1997). The extended drying period of up to seven days kept MC constant in all three preparation methods. In this way, 24 h of drying is an adequate desiccation time at 105°C for Brazil nut seeds and it can be assumed that the loss of volatile substances or oils under this condition is not significant for seed quality testing.

On the other hand, a large variation in MC between seeds in equilibrium with RH was detected. Variation could be explained to some extent by increasing percentage of the seed coat in smaller seeds, and by variation in seed coat weight of seeds with the same embryo size and shape. As Brazil nuts are collected after natural fruit release, further studies are suggested to evaluate embryo MC of the same fruit and between fruits, and if seed moisture is further affected during the transport of the indehiscent woody fruits.

Divergent results of Brazil nut seeds considering desiccation tolerance were probably based on the procedures for MC determination. As the protective seed coat holds a considerable percentage of seed weight, MC determination with the whole seed overestimates embryo MC. Differences of MC between the seed coat and the embryo was observed earlier in a seed storage study of Brazil nut by Kainer et al. (1999). These authors treated the seeds as desiccation-sensitive and stored them in moist sand. After six months, seed coat MC had increased from 29 to 40%, while embryo MC increased only from 22 to 28%. This result is contrary to what usually happens in most species, where the seed coat contains less moisture than in the reserves and/or embryo (Schmidt, 2000).

A linear regression to estimate embryo MC with MC of whole seeds is presented in this study, using seeds from Autazes. Average seed size (8.8 g) was slightly smaller and had a greater range (3.8 to 20.4 g) than seeds from other regions. Brazil nut seeds from Amapá State (Brazil) had an average weight of 10.5 g (ranging from 6.7 to 16.7 g) (Ferreira et al., 2006) and from Pará State (Brazil), had a weight of 11.9 g (Müller et al., 1995). These authors reported an even higher percentage of the seed coat in total seed weight, with 52% (Ferreira et al., 2006), and 51% of total (Müller et al., 1995), compared to 42.3% in

this study. Differences in the percentage of woody seed coat to whole seed mass, due to provenance, will hinder the direct application of the regressions presented here.

Conclusions

Seed viability of Brazil nut can only be guaranteed if embryo MC is maintained above a tolerable level. Considering this aspect, it is physiologically meaningful to determine embryo MC and not whole seed MC. Seed coat removal is easier using a longitudinal seed cut and, if desired, both parts can be evaluated. Although this is a case study of one species, the approach may be applied to other species with similar seed morphology.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

ACKNOWLEDGEMENTS

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