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Nutritional quality of Brazil nuts from different trees and under different storage conditions

Qualidade nutricional da castanha-da-Amazônia em diferentes castanheiras e condições de armazenamento

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ABSTRACT

The Amazon nut is a naturally organic food, considered a functional food for promoting good nutrition and health of the body. Its regular consumption has already been associated with reducing several diseases due to the high antioxidant and anti-inflammatory activities of some phytochemicals, mainly Selenium (Se). The objectives of this study were: 1) to evaluate whether Brazil nut trees that present higher fruit production also have higher Se content, 2) to check for alterations in Brazil nut (2021 harvest) seeds after one year of storage under different controlled conditions; 3) to check for alterations in the proximate composition of seeds (2022 harvest) after pre-drying and storage in the field. The average Se content was 143 $mg\ kg^{-1}$, varying from 33 to 544 $mg\ kg^{-1}$, with higher values in Brazil nuts collected from trees with lower fruit production. Seeds stored in their fruit for one year had higher moisture content (21%) and water activity (0.91) than those in the climate-controlled cold room and laboratory room, in addition to more carbohydrates and less lipids. This proved the efficiency of seed storage in fruit to maintain germination viability. In general, no significant changes were found in the proximate and nutritional composition of Brazil nuts taken to storehouse of pre-drying and the local buyer's warehouse, proving that these conditions enable the quality maintenance of fresh Brazil nuts. These results can support commercialization, improvement, and valorization actions in the Brazil nut production chain that favor its quality as a functional and nutraceutical food.

Keywords: *Bertholletia excelsa*; Brazil nut; selenium; proximate composition; "plant-based meat".

RESUMO

A castanha-da-amazônia é um alimento naturalmente orgânico, considerado funcional por promover boa nutrição e saúde do organismo. Seu consumo regular já foi associado com a redução de várias doenças, graças à elevada atividade antioxidante e anti-inflamatória de alguns fitoquímicos, principalmente do Selênio (Se). Os objetivos deste estudo foram: 1) avaliar se matrizes superiores de castanheiras, que apresentam maior produção de frutos, também possuem maior teor de Se; 2) analisar se castanhas (safra 2021) sementes são alteradas após um ano armazenamento em diferentes condições controladas; 3) verificar se a composição centesimal das castanhas (safra 2022) é alterada após pré-secagem e armazenamento no campo. O teor médio de Se foi 143 $mg\ kg^{-1}$, variando de 33 a 544 $mg\ kg^{-1}$, com maiores valores nas amêndoas de castanhas coletadas em matrizes com menor produção de frutos. As amêndoas armazenadas nos próprios frutos durante um ano apresentaram teor de umidade (21%) e atividade de água (0,91) superiores aos daquelas armazenadas na câmara fria e na sala do laboratório, além de mais carboidratos e menos lipídeos. Isso comprovou a eficiência do armazenamento das sementes nos ouriços para manter sua viabilidade germinativa. De modo geral, não houve alterações significativas na composição centesimal e nutricional de castanhas que passaram pelos paióis de pré-secagem e pelo armazém do comprador local, comprovando que nessas condições é possível manter a qualidade da castanha fresca. Esses resultados podem subsidiar ações de comercialização, melhoramento e valorização da cadeia produtiva da castanha que favoreçam sua qualidade enquanto alimento funcional e nutracêutico.

Palavras-chave: *Bertholletia excelsa*; castanha-do-brasil; selênio; composição centesimal; "carne vegetal".

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Introduction

The Brazil nut tree (*Bertholletia excelsa* Bonpl. 1808, Lecythidaceae) is native to the Amazon, which originates multiple products and has high economic importance (de Souza et al., 2023). In areas related to Environmental Sciences in the Amazon, the Brazil nut tree is considered the symbol species of the region and an example that the best strategy for protecting the biome and keeping the forest standing is conservation through use (Guedes et al., 2014; Fonseca et al., 2021). The formation of Brazil nut tree stands in the aggregate can be related to the actions of original populations and agroextractivists aimed at using the ecosystem.

In the eastern Amazon, Brazil nut trees occur in clusters, in true Brazil nut tree groves, probably originating from human action (Levis et al., 2018). Brazil nut tree groves under more intense collection (Scoles and Gribel, 2012) and areas of shifting cultivation (Cotta et al., 2008; Guedes et al., 2014) present a higher density of seedlings and saplings Brazil nut trees, confirming that the agroextractivist collection and use contribute to maintaining the viability of populations and conserving the species.

The species also has an important social role as it is a source of subsistence for thousands of families in the region. In the south of the State of Amapá, extractivists mainly consume fresh nut "milk," especially during the harvest period (January to April). In addition to Amapá and the Brazilian Amazon, the Brazil nut tree occurs in Venezuela, Colombia, Peru, Bolivia, Suriname, Guyana, and French Guiana, with higher numbers in the Brazilian Amazon (Tourne et al., 2019). The Brazil nut tree can reach over 60 m in height and diameter at breast height (DBH) above 3 m, taken 1.30 m above the ground (Peres et al., 2003). It is a century-old species (Caetano Andrade et al., 2019) that can live for more than 1,000 years (Vieira et al., 2005), maintaining its fruit production capacity.

The fruit, popularly known as *ouriço*, is very lignified and can contain 10 to 25 seeds (nuts), known worldwide as "Brazil nut." There is high variation in the number of fruits produced between years, depending on the region where the population occurs (Kainer et al., 2007; Neves et al., 2016). In addition to the quantitative aspect, it is also important to understand the factors associated with nut quality, as the market for functional foods like Brazil nuts is highly demanding (Safraid et al., 2022). According to ANVISA (Brasil, 2003), functional foods are composed of fatty acids, omega-3, carotenoids, dietary fiber, probiotics, and phytosterols, playing a key role in quality of life and long-term benefits (Safraid et al., 2022).

As a functional food, due to the high antioxidant and anti-inflammatory activity of phytochemicals, mainly selenium (Se), the regular consumption of Brazil nuts is associated with the reduction of cardiovascular diseases and cancer (Danielski et al., 2020). Brazil nuts are also important for the immune system, reducing viral infections and possible oxidative stress or inflammatory conditions, also acting on thyroid hormone metabolism (Rayman, 2012).

The Brazil nut is considered one of the plant foods with the highest content of Se (Silva et al., 2013; Silva Junior et al., 2017). The Se content in its kernel can vary from 8 to 252.3 mg kg^{-1}, depending on the concentration in the soil (Duarte et al., 2019) and genetic variability. Brazil nuts from the States of Amazonas and Amapá have higher Se contents than those from other Amazon states (Silva Junior et al., 2017). In addition to Se, the Brazil nut has elevated content of proteins with high biological value, fiber, and essential lipids (omega 3 and 6) (Pacheco and Scussel, 2006). Brazil nut lipids improve HDL cholesterol, as they are also rich in fatty acids, such as oleic acid, and contain phytosterols that inhibit cholesterol synthesis in the liver (Costa et al., 2020). Brazil nuts are also a source of vitamin E, phosphorus, potassium, magnesium, and calcium (Chunhieng et al., 2004).

The Brazil nut is present in one of the most important projects in terms of human nutrition in Brazil —Brazilian Food Composition Table (NEPA, 2011). NEPA (2011) presents the composition of the main foods consumed in Brazil, including the Brazil nut, based on representative sampling and analysis in laboratories with proven analytical capacity. The presence of Brazil nuts in this table demonstrates the importance of this food for Brazilians.

Although Brazil nuts are one of the non-timber forest products (NTFPs) in the Amazon with potential for commercialization and consumption, variations in their production chain are not well understood yet. Some important steps for maintaining product quality and valuation, such as storage and drying, vary greatly throughout the Amazon.

Drying is one of the main stages of this chain (Barroncas, 2020), as it provides food with characteristics preserved for longer periods (Alves and Nicoleti, 2016), in addition to facilitating transport and reducing the proliferation of fungi. Drying and storage conditions can also alter the quality of seeds for seedling production and the quantification of their constituent elements during laboratory analysis.

Studies on factors influencing the Se content, composition, and nutritional quality of the Brazil nut are necessary to support research on tree selection and marketing strategies, considering the Brazil nut as a functional food. Determining the Se content per nut tree is an important attribute to be used in the production of seedlings selected for genetic improvement, as well as understanding the variations in its composition is essential to guide consumers on the ideal amount of Brazil nut intake.

In this context, the objectives of this study were: 1. to evaluate whether Brazil nut trees that present higher fruit production also have higher Se content, 2. to analyze whether the properties of nut seeds are changed after storage under different controlled conditions, and 3. to check for alterations in the proximate composition after drying in the field in storehouse of pre-drying rooms and the local buyer's warehouses.

Material and Methods

Characterization of the study area

The study region is located at the Cajari River Extractive Reserve (*Resex Cajari*). The Resex has an area of 501,771 ha in the extreme south of the State of Amapá. The region is divided into Upper, Middle, and Lower Cajari, with Brazil nut trees occurring in the Upper Cajari, which is crossed by BR 156 (Brasil, 1990) (Figure 1).

A detailed description of the vegetation, soils, and climate of the study region can be found in Guedes et al. (2014) and Neves et al. (2016).

Collection and analysis of nut samples

To test the hypothesis related to **objective 1**, in March 2021, fruit were collected from 55 Brazil nut trees with different production capacities distributed across three permanent plots in the Upper Cajari, according to Guedes et al. (2017) (Figure 1). These nut trees have had their fruit/seed production monitored annually since 2010. The survey and measurement of tree diameters are usually carried out every 2 or 3 years. The diameter of the trees was measured in 2020, at 1.30 above the ground (DBH), with a tape measure accurate to 1 mm.

From each of the 55 trees (19 in the Cláudio plot, 18 in each of the other two plots), ten fruits were collected, of which five were opened shortly after arrival at the Embrapa Amapá laboratory, and samples of, approximately, 1 L were taken for Se analysis and proximate composition of fresh Brazil nuts. These samples were sent to the UFPA Analytical Chemistry laboratory, but only 19 were analyzed due to the pandemic and lack of resources and personnel.

The rest of the seeds from the opened fruits and the five fruits taken to the laboratory and not opened were used in **objective 2**. The five fruits kept closed were stored in plastic bags at room temperature. A portion of the remaining samples from the opened fruits were placed in plastic trays and taken to the cold room (15°C) of the Seed Laboratory, and the other part was left in trays on the bench at room temperature, aiming to evaluate the proximate composition and quality of seeds after storage in the fruits or under controlled conditions for 12 months.

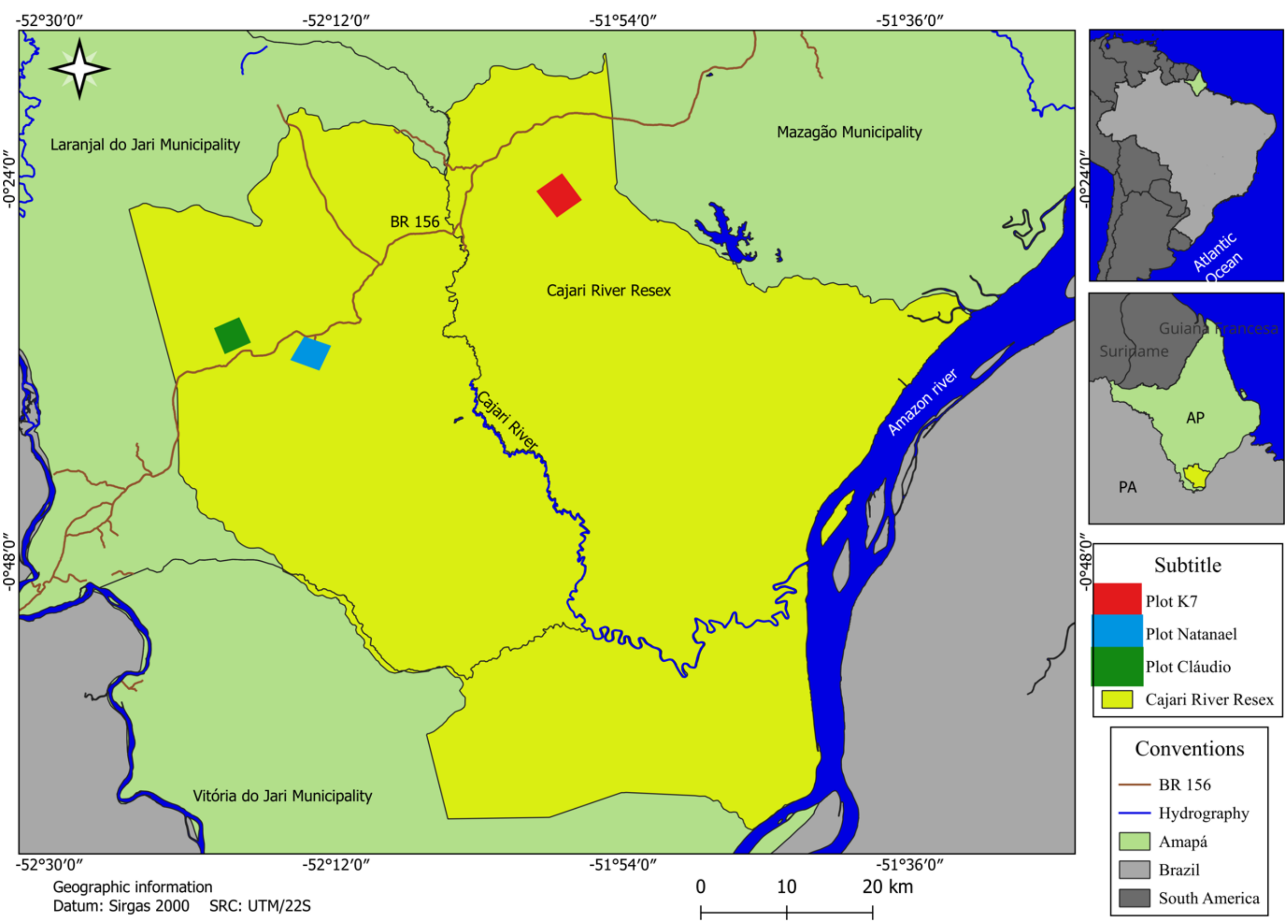

Figure 1 – Location map of the Cajari Resex, southern State of Amapá, eastern Amazon, and the permanent plots where fruits and nuts were collected in 2021.
Source: prepared by the authors based on IBGE (2010), ICMBIO (2023).

The second sampling was carried out in March 2022, when samples of 1 L of Brazil nuts in the shuck were collected directly from the extractivists' production field to meet objective 3. Six samples of fresh seeds were taken before being sent to storehouses of pre-drying, plus six samples after three days in each warehouse.After Brazil nuts are washed, drying is carried above an area with a suspended screen (*girau*) covered with transparent tiles. Three additional samples of seeds stored in Brazil nut buyers' storage warehouses were also collected after being stored for approximately one month. In the warehouse, the seeds are stored in bulk in large piles that fill the structure until they are close to the roof, which has an opening for air to escape.

Determination of Se content in Brazil nuts

Se content was measured using Atomic Absorption Spectrometry with Zeeman background correction and an EDL lamp for Se; AAnalyst™ 800 AAS, Perkin Elmer. A standard solution containing 1 g Se kg^{-1} (98% purity, Fluka, Buchs, Switzerland) was used to prepare the calibration curve for the determination of Se. Data were described based on dry weight (DW), expressed in mg kg^{-1}. Three seeds were shelled and weighed individually, on the ground, and 0.5 g was taken from each sample for digestion. The analyses were carried out following an adaptation of the methodologies of Moraes de Brito et al. (2019) and Silva Junior et al. (2017).

Proximate composition and minerals present in Brazil nuts

Proximate and nutritional composition analyses were carried out in triplicate, according to the methodologies of Anderson et al. (1988), Silva and Queiroz (2002), Brasil (2003), Nogueira and Souza (2005), and IAL (2008). Water activity was determined through a digital device (NOVASINA, Lab Touch – aw), and pH was measured using a potentiometer (Bel Engineering, W3b).

Statistical analysis

Descriptive statistics were calculated for the diameter measured in 2020 and fruit production of Brazil nut trees (n=19) in the 2021 harvest, and for the seven proximate composition variables analyzed in the same samples. Non-parametric statistics were calculated to check the significance of the effect of the predictor variables (nut trees, storage, and pre-drying conditions) on the response variables (Se content, proximate composition attributes, and nutrients), as they did not present homogeneity of variance and/or normality, or independence of residuals.

The relationship between Se content, fruit production, and the seven proximate composition variables was evaluated using Spearman's correlation. Kruskal-Wallis analyses (H test) were carried out to comparisons of different pre-drying and storage conditions in the field at Resex (fresh seeds, after pre-drying in a storehouse and storage in a warehouse) and under experimental conditions at Embrapa Amapá (in a controlled cold room, in a room at natural temperature and in the fruit itself).

Results and discussion

Se content and proximate composition of fruit from different Brazil nut trees

The diameter of the 55 Brazil nut trees varied from 74.5 cm to 286.5 cm, indicating adult trees with productive potential (Table 1). The average fruit production per tree in the 2021 harvest was 208 fruits, with the most productive tree producing 519 fruits (Table 1). There was high variation in fruit production and Se content. The Coefficient of Variation (CV) for tree diameter was 35%, and for fruit production, 76%. In general, according to the triplicate analyses to evaluate the proximate composition of these fresh Brazil nuts, there were no significant variations in seeds between the different trees, which can be proven by the low deviation values. The component that presented the highest CV was carbohydrate content, with 16%.

The average protein content was 13%, ranging from 11 to 15% (Table 1). The average lipid content was 72%, confirming the oleaginous characteristic of Brazil nuts (Stachiw et al., 2016). The CV for Se content was 85%, with an average content of 143 mg kg^{-1} and a range from 33 to 544 mg kg^{-1}. This variability in the average selenium content found is higher than that reported by Silva Junior et al. (2017), who observed an average Se concentration in Brazil nuts from the State of Amapá of 50.93 mg kg^{-1}. This value differs from that verified by Silva Junior et al. (2022), who found lower values, 48.6 µg Se kg^{-1}, in a study on the accumulation and localization of Se using different spectroanalytical techniques.

Table 1 – Descriptive statistics (mean, standard deviation, minimum, and maximum) of the diameter measured in 2020 and fruit production of Brazil nut trees (n=19), selenium content - Se (dry matter basis) and proximate composition of Brazil nuts, collected in March 2021, in the native Brazil nut forest of Resex Cajari, southern Amapá, eastern Amazon. Analyses carried out in the UFPA analytical chemistry laboratory.

Am.	DBH	FP	[Se]	M	Ash	Lipids	Prot.	Fiber	C
	cm	n	(mg kg^{-1})	(%)	(%)	(%)	(%)	(%)	(%)
Mean	149.0	208	142.8	5.1	3.2	72.2	12.8	3.5	6.8
SD	52.7	158	120.9	0.3	0.3	1.8	1.2	0.5	1.1
Min.	74.5	30	33.26	4.55	2.86	69.24	11.00	2.65	5.44
Max.	286.5	519	544.41	5.92	3.96	74.57	15.21	4.52	9.20

DBH: diameter measured in 2020; FP: fruit production in 2021; M: moisture, C: carbohydrates.

The variation in the Se content in the seeds of Brazil nut trees is probably related to differences in the concentration of Se in the soil, which varies according to the region, i.e., in a given location, selenium is abundant in the soil, which is not the case at other sites (Pieczyńska and Grajeta, 2015). Brazil nuts from Amapá (50.93 mg kg^{-1}) and Amazonas (68.15 mg kg^{-1}) presented an average concentration 20 times higher than Brazil nuts from Acre and Mato Grosso, and five times higher than those from Roraima (Silva Junior et al., 2017). The high variability in Se content among nuts collected at the same location was also reported by other authors (Silva Junior et al., 2017). Selenium distribution within the plant occurs in different ways and depends on plant species, development stages, concentration in the soil, and other environmental conditions (Li et al., 2008; Renkema et al., 2012).

The correlation analysis showed that the Brazil nut trees that produce the largest number of fruit are not those whose fruit have the highest Se content (Figure 2), and there is a significant inverse relationship between these variables (r=- 0.49; =0.064).

The highest Se values were observed in seeds from trees with lower fruit production. This may be an indication that increased production can lead to a drain of nutrients and Se, reducing their concentration due to the dilution effect. Even though it is not an essential element for plants, Se can be absorbed and accumulated in plant tissues, which makes it an important source of nutrients in animal diets (Dumont et al., 2006).

Although there is this inverse relationship between Se content and productive trees, which may seem incompatible with the objective of selecting superior trees, both in quantitative and qualitative terms, this association becomes interesting when considering that Se can cause toxicity called selenosis (Nóbrega, 2015), when ingested in high quantities.

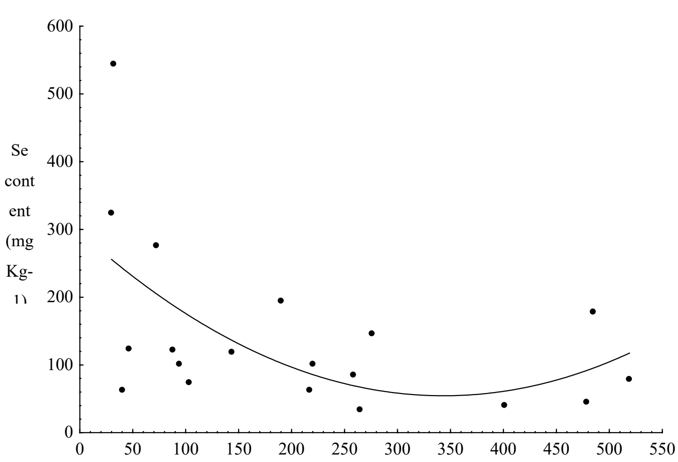

Figure 2 – Relationships between the Se content (dry matter basis) in Brazil nut seeds and the fruit production of each tree (n=19; r=- 0.49; p=0.064) in 2021, in a Brazil nut tree grove located in the Cajari River Extractive Reserve, southern State of Amapá, eastern Amazon.

Thus, when it is verified that the most productive trees have lower contents of Se, the consumption of more nuts per capita can be recommended, based on the recommended daily intake, which, for adults of both sexes, is 55 µg Se (IOM, 2000), or 34 µg in Brazil (Brasil, 2005). The maximum tolerable daily intake of Se is up to 400 µg, with the limit for toxicity being 850 to 900 µg (Maeda et al., 2014).

Some recent studies have shown that excess Se in the soil may be correlated with plant species that accumulate high content of Se (El Mehdawi et al., 2011; Reynolds et al., 2020), referred to as Se hyper-accumulators. Plants propagate this nutrient in the soil through leaf litter and roots (Zhao et al., 2010), thus increasing the available Se content. In this way, hyper-accumulating species can change the Se content of the local soil and obtain a higher amount of resources for reproductive survival, in addition to negatively affecting non-Se-accumulating species around them (Chang et al., 2022).

The highest content of Se is generally found in reproductive organs, such as flowers, fruits, and seeds (Harvey et al., 2020), which indicates that this element has an important role in the plant reproductive biology. Thus, the predominance of higher Se content in seeds of Brazil nut trees that produce fewer fruits may be a strategy of the species to increase the probability of reproductive success of trees that produce fewer propagules. Se can protect the seed against biotic and abiotic stresses or even play important roles during germination (Silva Junior et al., 2022).

The results are also important when there is interest in the production of seedlings for plantations that generate Brazil nuts with high Se content to supply the functional food market. In this sense, Brazil nut trees with lower fruit production and higher Se content can also become interesting as matrices. Forestry improvement is increasingly concerned with selecting superior matrices, not only in quantitative terms but also based on the characterization of the quality of the products.

Even with a sample survey in the same region and nearby nut trees, this variability in the selenium content indicates a sufficient variation for the selection of superior tree matrices, both in quantitative terms related to production and in qualitative terms associated with the nutritional contents in the seed. Both Se content and fruit production varied in nearby trees, which may indicate that the variation may also depend on attributes of the trees themselves, and not only the soil.

Proximate composition and nutritional value in Brazil nuts stored under controlled conditions

In general, changes in the homogeneous groups of the proximate composition were greater than in the nutrients found in seeds, when considering the variations in storage in the fruit, cold room, or room, for 1 year. The highest and most significant values of moisture (Kruskal-Wallis test: H (2, n=26)=13.85897; p=0.001), water activity (H (2, n=26)=8.09142; p=0.0175) and carbohydrate (H (2, n=26)=11.8981; p=0.0026) in seeds kept in the fruit are important results regarding the use of nuts as seeds for seedling production. On the other hand, the content of lipids (H (2, n=26)=15.09463; p<0.001) and proteins (H (2, n=26)=7.12765; p=0.028) were significantly reduced in seeds stored in the fruit (Table 2).

In recent years, a low-cost method for producing Brazil nut tree seedlings by storing the seeds in the fruit themselves has been developed at Embrapa Amapá (Guedes et al., 2023). These seeds start germination in the fruit, making it easier to break the nut to remove the seed without damaging it, which is essential to accelerate and increase the germination percentage. The efficiency in seedling production from storing seeds in the fruit can be explained by the results listed in Table 2.

The increase in less recalcitrant compounds, such as carbohydrates, associated with the reduction in compounds more difficult to break down, such as fats, can facilitate germination. The higher carbohydrate content in seeds stored in the fruit for one year may be due to the physiological transformations that occurred at the beginning of the germination process. In some fruits, seeds were observed with their radicles already emerging when they were opened. Toniol et al. (2009) reported that *Qualea grandiflora* seeds accumulate a type of reserve carbohydrate that can be immediately mobilized by the seed during the resumption of metabolism for germination and initial seedling growth.

Therefore, maintaining higher carbohydrate content in seeds stored in the fruit, associated with greater water availability, are the most interesting properties to maintain seed viability.

Table 2 – Average results of nutritional and proximate composition analysis of Brazil nuts stored in the fruit (*ouriço*), in the cold room, and in the Embrapa Amapá laboratory room for 1 year.

	Storage		
	Fruit *(Ouriço)*	Room	Cold room
**Moisture (%)	20.78	2.91	3.50
*Total crude protein (%)	12.2	15.1	15.4
**Lipids (%)	32.7	54.8	61.1
n.s. Ash content (%)	2.5	3.1	3.1
**Total carbohydrates (%)	31.9	24.1	16.9
*Enevery value (kcal 100 g^{-1})	470	650	679
*Water activity (Aa)	0.91	0.43	0.42
n.s. pH	6.6	6.7	6.5
*ATT acidity (%)	3.70	2.56	3.54
*Ca (g kg^{-1})	1.91	2.58	2.35
*P (g kg^{-1})	0.40	0.47	0.49
*Fe (mg kg^{-1})	35.3	41.8	43.4
*Cu (mg kg^{-1})	22.1	30.1	63.7
*Zn (mg kg^{-1})	44.7	50.5	74.7
*Mn (mg kg^{-1})	5.3	18.5	9.9

**Significant at 1% probability; *significant at 5% probability; n.s.: not significant.

Figure 3 shows the mean values and variations around the means of the main changes occurring in seeds due to storage under controlled conditions.

A study that evaluated the effect of storage in moist sand on the germination of Brazil nut seeds reported that storage, in addition to conserving the seeds and their moisture, allows the continuation of the embryo formation process and the onset of germination inside the seed with shell (Silva et al., 2009). With regard to the germination of seeds of *Guizotia abyssinica*, an oilseed species, the water content of the seeds interfered with the germination and growth of seedlings (Gordin et al., 2014). It is essential that the seeds, in addition to moisture, can also maintain water activity, which explains the use of Brazil nuts that were stored for 12 months in black plastic bags inside the fruit. This activity is very important, as it represents free or available water for chemical reactions to take place. There is no point in the seed having high total moisture with low water activity.

Proximate composition and nutritional value of Brazil nut seeds before and after pre-drying in the field and storage in a warehouse

One of the most important projects in terms of human nutrition in Brazil is the TACO project (NEPA, 2011), whose objective is to generate data on the composition of the main foods consumed in Brazil, including Brazil nuts, based on a sampling design that guarantees representative values, analyzed by laboratories with proven analytical capacity. The presence of the Brazil nut seed in this table demonstrates the importance of this food for Brazilians.

The Brazil nut is recognized worldwide as an important functional food rich in nutrients and vitamins, with high energy value. The energy from Brazil nut seeds is associated with the quantities of homogeneous groups of lipids and carbohydrates, which are part of the proximate composition of the food. In general, the composition and nutritional capacity of food depends on the time it is stored and the temperature to which it is subjected (Celestino, 2010).

During the collection and processing of seeds along the production chain, the Brazil nut is subjected to different storage and drying conditions, which can alter its value as a functional food. Table 3 presents the analysis of Brazil nuts stored in a buyer's warehouse at the Cajari Resex, and before and after pre-drying in the storehouse close to the Brazil nut trees.

The majority of responses evaluated showed no significant differences between pre-drying and storage in the field. Below are the statistics for the variables related to the proximate composition: **proteins** H (2, n=15)=0.52500 - p=0.769; **lipids** H (2, n=15)=0.29167 - p=0.864; **carbohydrates** H (2, n=15)=0.01818 - p=0.991; total energy value H (2, n=15)=1.2250 - p=0.542. The content of macro- and micronutrients present in the Brazil nut seed also showed no significant differences: **Ca** H (2, n=15)=0.29167 - p=0.864; **P** H (2, n=15)=1.7250 - p=0.422; **Fe** H (2, n=15)=0.1500 - p=0.928; **Cu** H (2, n=15)=0.81667 - p=0.665; **Zn** H (2, n=15)=0.3500 - p=0.839; **Mn** H (2, n=15)=1.0667 - p=0.587.

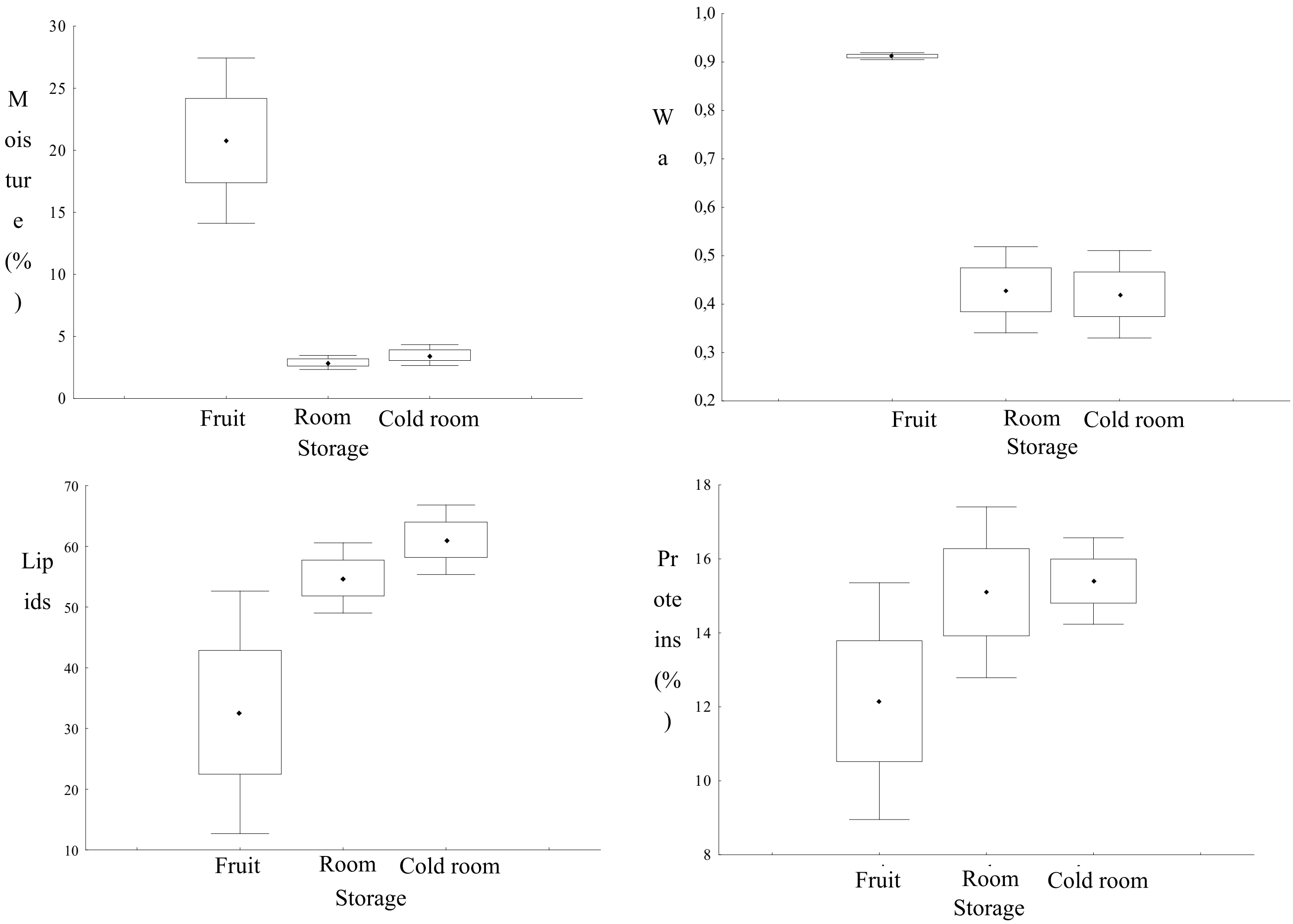

Figure 3 – Means, deviations, and confidence interval of moisture, water activity, and concentrations of lipids and proteins in Brazil nut seeds stored in the fruit, in the cold room and in the room at the Embrapa Amapá laboratory for one year.

In general, there were no significant changes in variables representing the proximate and nutritional composition. This is important to prove that under the evaluated pre-drying and storage conditions, it is possible to maintain the quality of the fresh Brazil nut seed. In the local market, fresh nuts are sold more often because they are tastier. Fresh Brazil nut seeds are also widely used by families to extract fresh nut "milk" used, mainly, to cook game meat and fish and to prepare "*chimbereba*". This drink is a mixture prepared with fresh nut "milk" and the juice of some acidic fruit, usually cupuaçu or taperebá, which extractivists take to the forest to consume while collecting Brazil nut fruits.

Another study that evaluated the proximate composition of Brazil nut seeds before and after drying, at a temperature that reached 45°C in the State of Acre also found no effect of drying on total crude protein, lipid, total crude fiber, and total carbohydrates, indicating that this drying does not interfere with the physical-chemical characteristics of the nuts (Costa, 2012). The only variable that showed a significant difference was ash content. The author points out that the increase in ash content may have occurred from the reduction in moisture content, favoring a greater concentration of minerals in the nuts.

The Brazil nut seed samples showed no changes in their mineral content (Ca, P, Fe, Cu, Zn, and Mn) due to pre-drying (Table 3). In the present study, among the macrominerals, the one with the highest value was Ca, and for the microminerals, the highlight was Zn, followed by Fe, Cu, and Mn (Table 3). Lopes (2021) verified a higher value for P [7,082.73 (mg/100 g)] compared to Ca [2,260.47 (mg/100 g)], and for micronutrients, the author found the following order Fe [69.22 (mg/100g)]>Zn [57.73 (mg/100 g)]>Mn [22.81 (mg/100g)]>Cu [22.22 (mg/100 g)]. Another study that evaluated water-soluble extracts of Brazil nut seeds observed that phosphorus (1,053.25 mg/100 g) was the most abundant mineral, followed by potassium (965.00 mg/100 g), sodium (320.00 mg/100 g), selenium (274.96 mg/100 g), magnesium (265 mg/100 g), and calcium (110.00 mg/100 g) (NEPA, 2006).

The differences in water activity were also not significant H (2, n=15)=2.1500 - p=0.341, despite the significant difference in moisture content H (2, n=15)=10.1667 - p=0.007. Another significant difference detected was related to TTA (total titratable acidity): H (2, n=15)=6.4833 - p=0.039. Figure 4 illustrates that these two variables with significant differences are related to each other and present the same pattern of variation.

Table 3 – Mean values of nutritional and proximate composition analysis of Brazil nuts, under different storage and drying conditions, in March 2022, at the Cajari Resex, southern State of Amapá.

Drying	Before storehouse	After storehouse	Warehouse
**Moisture (%)	26.21	22.10	14.67
n.s.Total crude protein (%)	12.4	12.8	13.5
n.s.Lipids (%)	45.1	42.9	40.6
n.s.Ash content (%)	2.3	2.3	2.6
n.s. Total carbohydrate (%)	14.0	19.8	28.5
n.s. Energy value (kcal 100 g^{-1})	511	517	534
n.s.Water activity	0.91	0.91	0.89
n.s. pH	6.5	6.6	6.8
*ATT (%)	6.38	4.65	3.15
n.s Ca (g kg^{-1})	2.29	2.89	2.26
n.s. P (g kg^{-1})	0.33	0.38	0.32
n.s Fe (mg kg^{-1})	22.4	21.4	22.1
n.s. Cu (mg kg^{-1})	16.8	17.1	16.4
n.s. Zn (mg kg^{-1})	38.3	41.2	40.4
n.s. Mn (mg kg^{-1})	8.7	8.8	9.4

**Significant at 1% probability; *significant at 5% probability; n.s.: not significant.

Fresh Brazil nut seeds, before being taken to the storehouse, had higher moisture content and total titratable acidity, while nuts stored for approximately one month in the buyer's warehouse had lower moisture and acidity. The acidity in fresh nut seeds may be related to the dark liquid normally present inside the fruits (*ouriços*), which may have medicinal functions and protective properties of the seeds.

Pre-drying in the room for three days at a temperature of around 32°C resulted in a decrease in moisture content by 15.7%, which is a lower value when compared to Costa (2012), who obtained an average reduction of 39.6% in a 6-hour drying process at 45°C using a prototype natural convection air dryer. The loss of moisture and the protection of the seeds from rain in the room allow for weight reduction, making work easier and reducing transportation costs.

The maximum temperature in the room during pre-drying was 32°C, lower than higher temperatures, around 65°C when the protein denaturation begins. During the sampling of this study, the days were cloudy and rainy, as normally occurs during the Brazil nut collection period. On less rainy days, the temperature in the room can reach 39°C (Pimenta et al., 2015), but it remains within the range for maintaining the quality and flavor of fresh Brazil nut seeds. The authors also report that Brazil nut seeds dried in the room are considered suitable for processing and use for the production of cookies (Pimenta et al., 2015).

Values of ash, proteins, and lipids in the present study are lower when compared to other studies with Brazil nuts (Table 4). This may be related to the moisture content of the seeds evaluated, as it appears that the fresh seeds in the present study had high moisture content. The lower moisture values in other studies show that the seeds evaluated were subjected to some drying or storage process that did not maintain moisture, which can cause the concentration of lipids and proteins when the water leaves them.

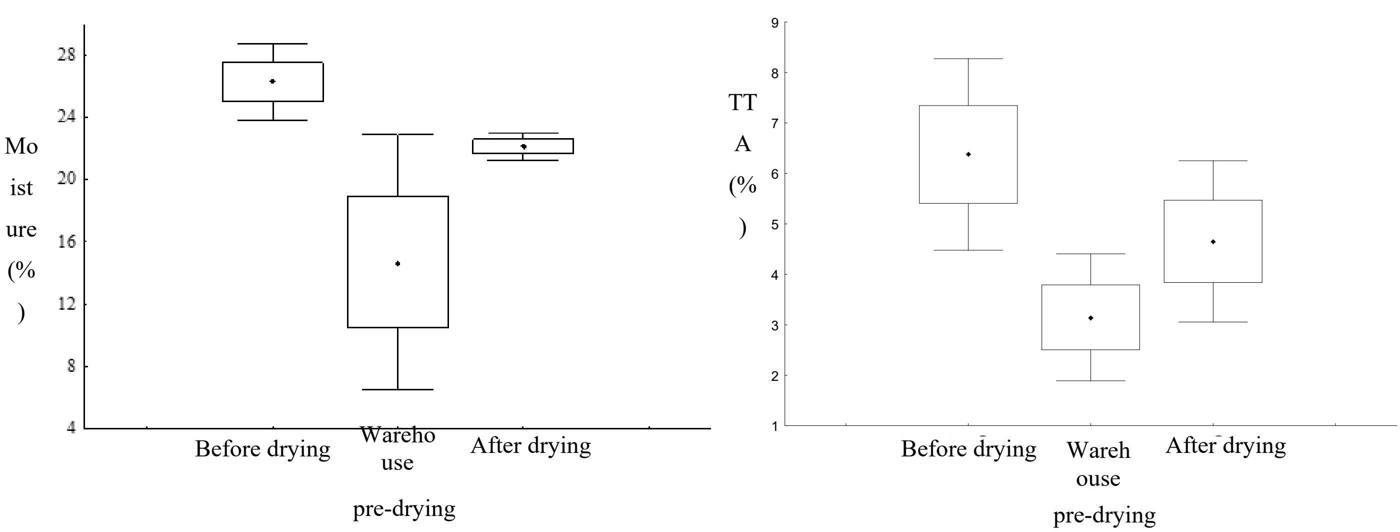

Figure 4 – Means, deviations, and confidence interval of moisture content and total titratable acidity in Brazil nut seeds (2022 harvest) from nut trees at the Cajari Resex, southern State of Amapá, eastern Amazon, before and after pre-drying in a storehouse close to the forest and a warehouse for Brazil nut's buyers.

Table 4 – Mean values of proximate composition, in percentage, of Brazil nut seeds analyzed by several Brazilian authors.

	Ash	Moisture	Protein	Lipids	Carbohydrate
Present study	2.3	26.21	12.4	45.1	14.0
Lopes (2021)	3.46	1.56	15.53	56.05	13.43
Silva et al. (2021)	3.57	11.54	15.48	65.32	–
Santos (2015)	3.29	–	13.75	66.19	16.74
Freitas and Naves (2010)	3.56	3.10	14.11	64.94	06.27
NEPA (2006)	3.4	3.4	14.5	63.5	15.1

The carbohydrate content in the present study was higher than those given by Lopes (2021) and Freitas and Naves (2010), and lower than those registered by NEPA (2006). This leads us to believe that, in addition to differences in the drying and storage conditions of the seeds, the soil and climate conditions of the Brazil nut tree groves and/or their genetic variability may favor higher or lower values for the proximate composition variables. Freitas and Naves (2010) point out significant variations in the proximate composition of different cultivars of true nuts, as in the case of Brazil nuts, hazelnuts, and pistachios. For the authors, this variation can be explained by differences in climate, soil, agricultural practices, and genetic characteristics of the seeds analyzed. Therefore, data on the nutrient content of these foods must be obtained taking into account variables such as geographic origin, environmental conditions, and varietal characterization of edible nuts and seeds.

Conclusions

- The highest content of Se is found in nuts from less productive Brazil nut trees, which can also be selected as matrices when considering the qualitative aspect of the nut as a functional food.
- Brazil nut seeds stored in the fruit (*ouriço*) retain properties of fresh seeds, such as higher moisture content and water activity, favoring their use as seeds for seedling production.
- The proximate composition and quality of the nuts are not altered after pre-drying in storehouses near the groves or in warehouses used by local buyers.

The present study brings new information, such as the possibility of Se content well above the highest values reported in the literature. New sampling and analyses are underway to confirm this result. Further studies are suggested to test the hypothesis that the Brazil nut tree is a hyper-accumulating species of Se and investigate the mechanisms involved in the element's uptake and concentration in Brazil nut seeds.

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Authors' contributions

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