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Amazonian peach palm seeds methods comparation of water content determination by quadratic plateau regression[1]

Comparação de métodos de determinação do teor de água de sementes de pupunha por meio de modelo de regressão quadrática-platô

Bruna Ariane da Silva[2], José Luiz Nogueira[2], Thomas Bruno Michelon[2]*, Elisa Serra Negra Vieira[3], Maristela Panobianco[2]

ABSTRACT - Given the recalcitrant nature of peach palm seeds, which results in high sensitivity to desiccation, more effective approaches to seed quality monitoring are extremely important and justify the need to provide an efficient method to determine their moisture content. For seeds which are considered to be larger (thousand seed weight > 200 g), seed preparation is required, but it is impractical owing to endocarp hardness. The objective of this work was to compare methods of evaluating the moisture content of peach palm seeds, aiming to identify an straightforward method for the species. Different seed preparation and drying methods (oven method at 105 ± 3 °C, low temperature at 103 ± 2 °C, and high temperature at 130 ± 2 °C) were tested. For all methods tested, the beginning of weighing took place after the first hour, and continued until the mass of each repetition became constant for two consecutive measurements. The data were analyzed by nonlinear quadratic plateau regression to evaluate the stabilization period of water content. Thus, the oven method at 130 °C for four hours (with whole seeds) is recommended.

Key words: *Bactris gasipaes*. Oven method. Recalcitrance. Water content. Drying period.

RESUMO - O comportamento altamente recalcitrante das sementes de pupunheira justifica a adequação de um método eficiente para determinação do seu teor de água, uma vez que estas são sensíveis à dessecação. Visto que as sementes de pupunha são consideradas grandes (peso de mil sementes > 200 g) os métodos atuais de determinação do teor de água exigem preparo das sementes, o que é inviabilizado devido a resistência do endocarpo. O objetivo deste trabalho foi comparar diferentes métodos de determinação do teor de água de sementes de pupunheira, visando identificar o mais eficiente para a espécie. Foram testadas diferentes formas de preparo da semente e métodos de secagem. Para todos os tratamentos testados, o início da pesagem ocorreu após a primeira hora, continuando até que a massa de cada repetição se tornasse constante por duas medidas consecutivas. Os dados foram analisados por uma regressão não linear quadrática em platô para avaliar o período de estabilização do teor de água. Assim, o método de estufa a 130 ºC durante quatro horas (com sementes inteiras) é recomendado.

Palavras-chave: *Bactris gasipaes*. Método estufa. Recalcitrante. Teor de água. Dessecação.

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*Author for correspondence
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[1]Part of the third author’s doctoral thesis presented at the Federal University of Paraná (UFPR)
[2]Department of Plant Science, Federal University of Paraná (UFPR), Paraná-PR, Brasil, bruna_ariane2@hotmail.com (ORCID ID 0000-0002-6127-3868), nogueira.ufpr@gmail.com (ORCID ID 0000-0001-7089-073X), thomasbrunomichelon@gmail.com (ORCID ID 0000-0002-7437-5062), maristela@ufpr.br (ORCID ID 0000-0002-9990-2172)
[3]Embrapa Florestas, Paraná-PR, Brasil, elisa.vieira@embrapa.br (ORCID ID 0000-0002-0799-7654)

INTRODUCTION

Peach palm (*Bactris gasipaes* Kunth), belonging to the Arecaceae family, is a palm tree native to the Amazon region. Its seeds are increasingly used in commercial planting, encouraged by a high demand for its products, especially fruits (peach palm) and palm heart in both domestic and export markets (SILVA *et al.*, 2013; VIEIRA *et al.*, 2021). Moreover, there is increased development of other by-products (BOLANHO; DANESI; BELÉIA, 2015; MARTÍNEZ-GIRÓN; FIGUEROA-MOLANO; ORDONEZ-SANTOS, 2017; MELO-NETO *et al.*, 2018; MUJICA *et al.*, 2017).

Commercially, the species is planted by means of seminal seedlings, and those are considered to be recalcitrant. Recalcitrant seeds have relatively high lethal limits to desiccation (BARBEDO; CENTENO; RIBEIRO, 2013). Peach palm seeds are considered as having a recalcitrant behavior and critical moisture content of 38.0% (FERREIRA; SANTOS 1992); below such percentage rate, there is a reduction in viability, which may be lethal (BOVI; MARTINS; SPIERING, 2004). For forests species, as well as recalcitrant seeds, studies have proposed mathematical models to predict seed desiccation tolerance and methodologies to determine moisture content, which is particularly important during the storage period to monitor the loss of germination (PELISSARI *et al*., 2018; SILVA *et al.*, 2020a, b).

International Rules for Seed Testing (INTERNATIONAL SEED TESTING ASSOCIATION, 2020) establish the procedures for moisture content assessment, and recommend two procedures: oven method at low temperatures at 101-105 °C (17 h) for the introduction of new species; and oven method at high temperatures at 130-133 °C (1 h ± 3 minutes, 2 h ± 6 minutes, or 4 h ± 12 minutes). In turn, the Rules for Seed Analysis (BRASIL, 2009) also consider the oven method at 105 °C (24 h) for all species and with whole seeds. For large seeds of forest species (thousand seed weight > 200 g), they both recommend cutting the seeds into parts smaller than 7.0 mm, and exposure of the sample to the environment must not exceed four minutes, but this period may be considered to be short to perform the cuts depending on the hardness of the endocarp.

Those methods may be excessive for stabilization and non-practicable as well when applied to peach palm seeds, given the difficulty in pre-processing them as recommended by the rules. Therefore, the objective of this work was to compare methods of assessing the moisture content of peach palm seeds by quadratic plateau regression, aiming to identify a straightforward method for the species.

MATERIAL AND METHODS

Peach palm seeds used in this study were collected in the Brazilian municipality of Ouro Preto do Oeste, RO, latitude 10°44’53” S and longitude 62°12’57” W, altitude 280 m, and Aw climate according to the Koppen classification (Figure 1).

Thousand seed weight was measured using eight sub-samples of 100 pure seeds that were weighed on a precision balance, and calculated by multiplying the average mass obtained in subsamples of 100 seeds per 10, adopting a coefficient of variation ≤ 4 (INTERNATIONAL SEED TESTING ASSOCIATION, 2020).

Moisture content measurement: four replicates of five seeds each were used, testing: ways of preparing the material – whole seeds, seeds cut into four parts, seeds cut into two equidistant parts and seeds cut into fragments smaller than 7.0 mm (Figure 2). For the processed seeds (cut into two and four parts and the seeds cut into fragments), the time limit of four minutes was used, as suggested by International Seed Testing Association (2020).

Procedures for extracting water from the material (INTERNATIONAL SEED TESTING ASSOCIATION, 2020): oven method at 105 °C; oven method at low temperatures at 101-105 °C and oven method at high temperatures at 130-133 ºC.

For all methods tested, the beginning of weighing took place after the first hour, continuing until the mass of each repetition became constant for two consecutive measurements. The percentage of water was calculated on the wet basis. The data collected to determine moisture content were analyzed according to a completely randomized design 3 x 4 (three drying temperatures and four ways of preparation) with four replications. For drying time, a nonlinear quadratic plateau regression for water content against drying time in hours was adjusted to evaluate time for water content stabilization. The regression can be described as:

$$f(x) = \left\{ \begin{array}{l} \theta_0 + \theta_1 x_i - \dfrac{\theta_1 x_i^2}{2\theta_b} if, x_i \leq \theta_b \\ \theta_0 + \dfrac{\theta_1 \theta_b}{2} \ldots \cdots if, x_i > \theta_b \end{array} \right\} \quad (1)$$

Where $f(x)$ represents the water content, x the time in hours, θ_0 the y-intercept, θ_1 the angular coefficient and θ_b the x value of the break point (the beginning of plateau), which could be interpreted as the value in hours for water content stabilization.

The temperature and treatment were evaluated by testing reductions on the saturate model (model with both effects on all parameters) using the likelihood ratio test (LEWIS; BUTLER; GILBERT, 2011) and the final model adjustment was evaluated by R^2.

All the analyses were performed in the R software, version 3.5.2, using the nls() and anova() functions from stats package, version 4.2.0.

Figure 1 - Location of the municipality where peach palm seeds were produced (*Bactris gasipaes* Kunth) - Ouro Preto do Oeste, Roraima, Brazil

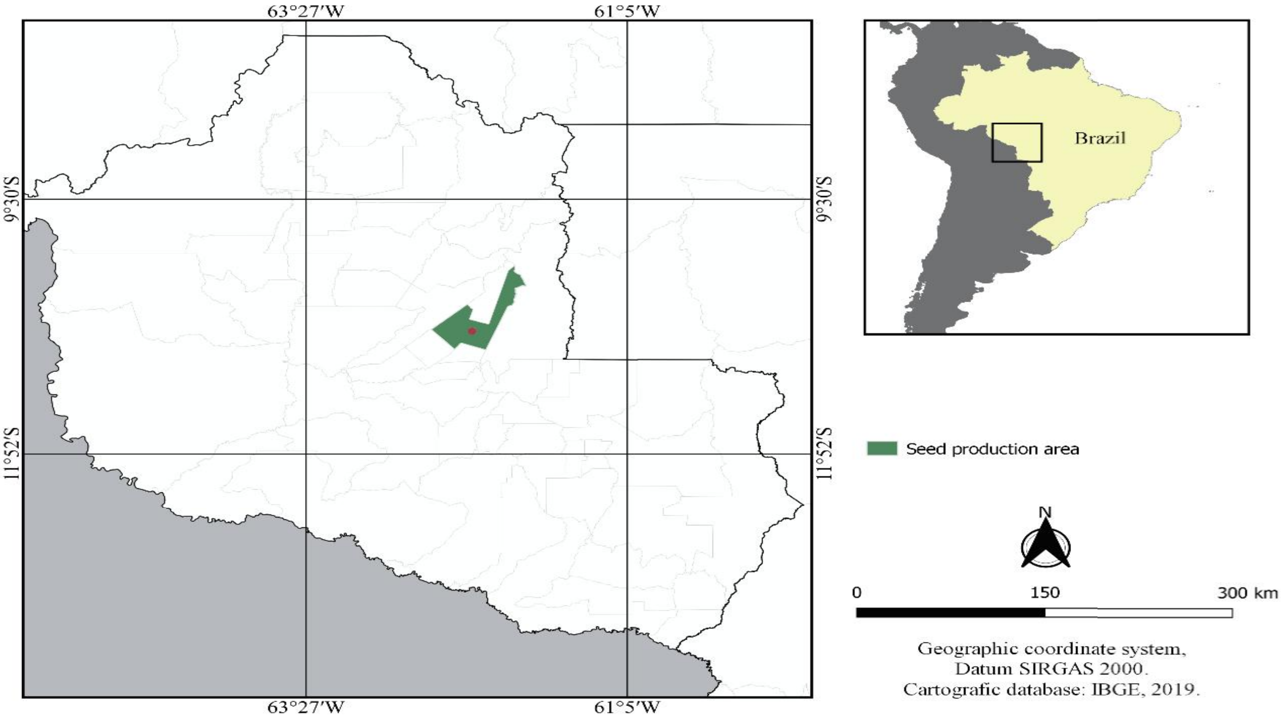
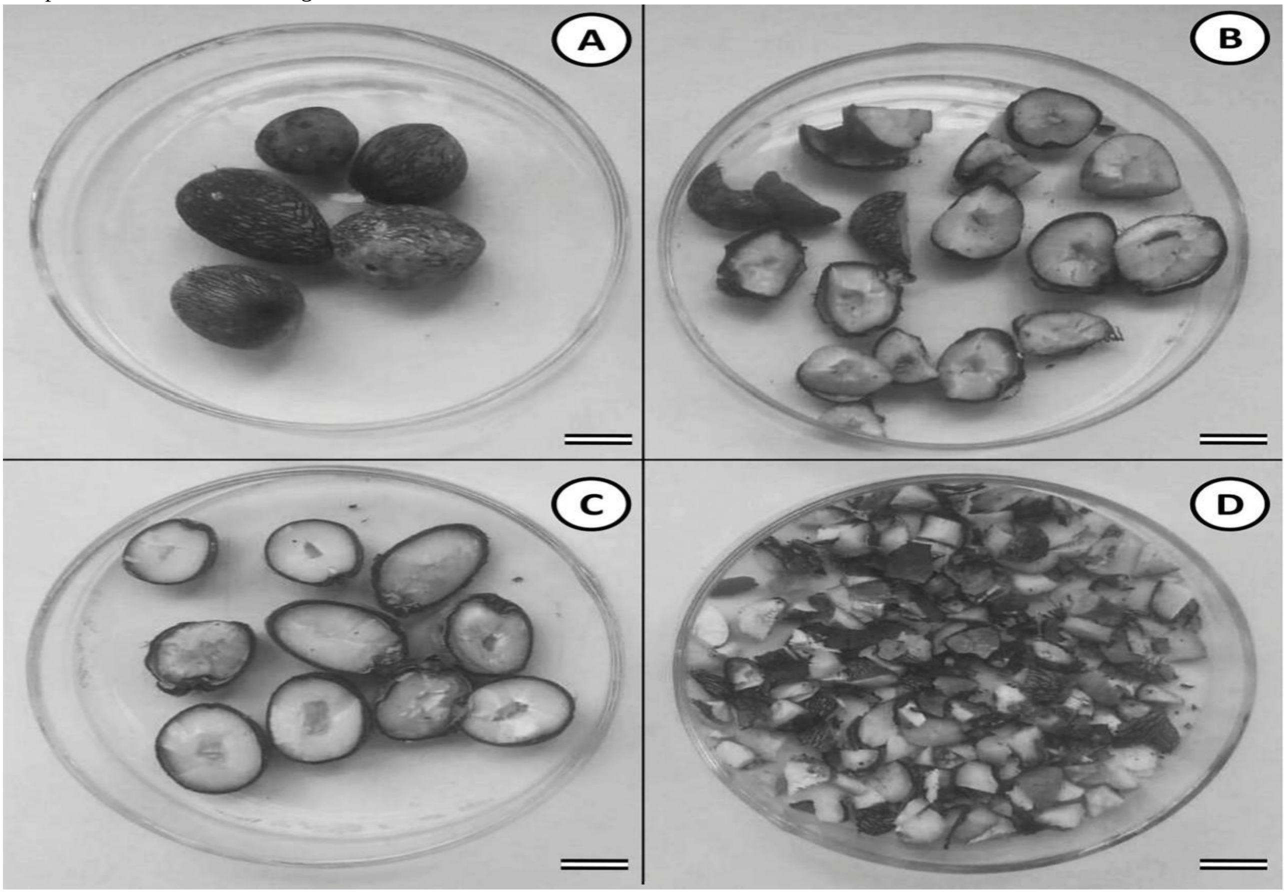


Figure 2 - Ways of preparing peach palm seeds (*Bactris gasipaes* Kunth): A. Whole seed; B. Seeds cut into four parts; C. Seeds cut into two parts; D. Seeds cut into fragments smaller than 7.0 mm. 1 cm scale bar

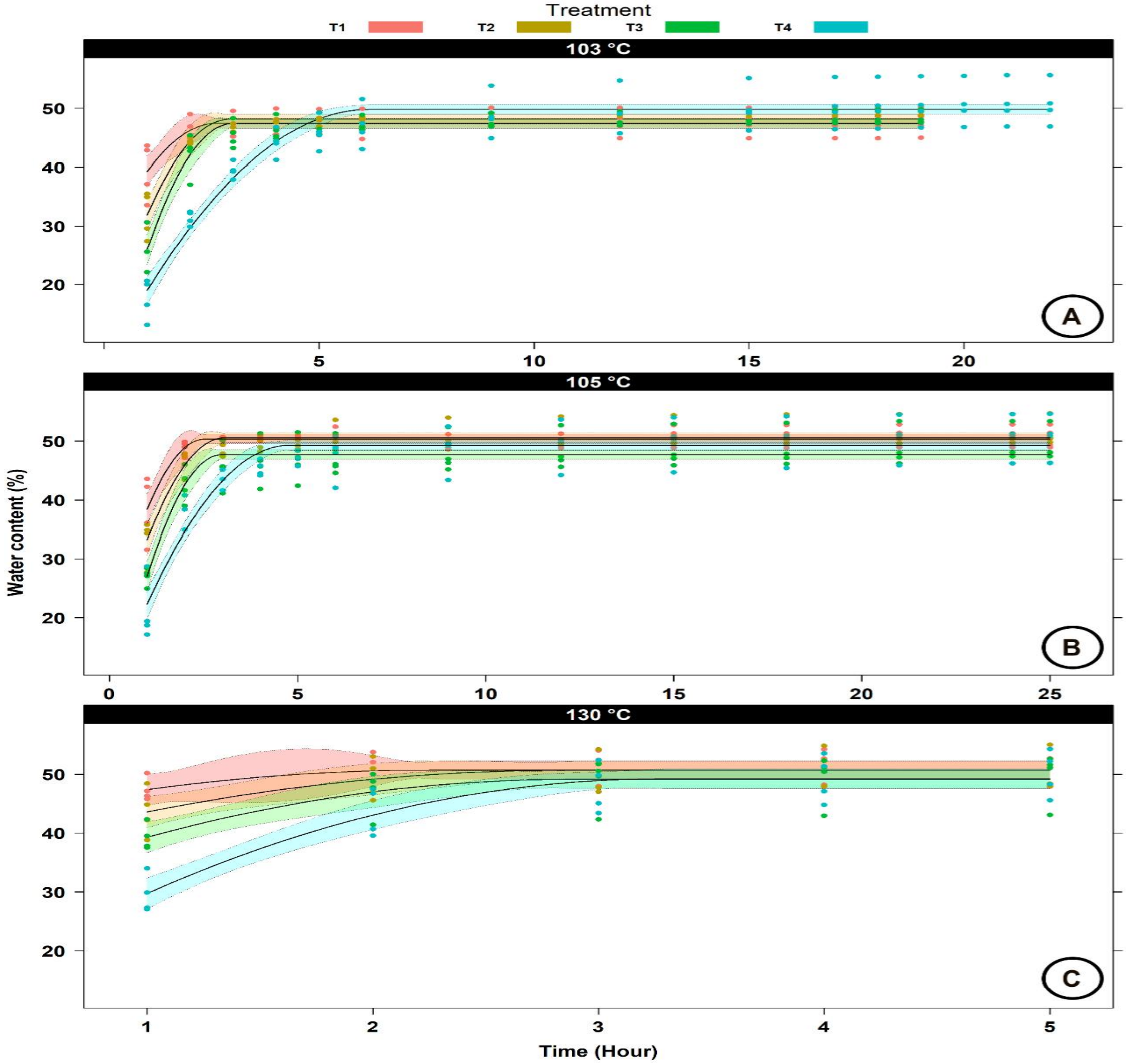


RESULTS AND DISCUSSION

Thousand seed weight was 2199.16 g, indicating the presence of 455 seeds per kg. According to the instructions for analysis of forest seeds (BRASIL, 2013), the seeds of this species have thousand seed weight values ranging between 2,000 g and 4,000 g. Among the species that represent the Arecaceae family, there is great variation in thousand seed weight (BRASIL, 2013; LOPES *et al*., 2011; SILVA *et al*., 2009).

For all parameters, there was significance both in the effect of temperature and preparation ways because the complete model could not be reduced by the likelihood ratio test. The complete model shows a satisfactory adjustment with R^2 = 0.84 (based on averages) and a residual standard deviation of 2.692. Thus, depending on the temperature and treatment, the seeds present different levels of initial water content (parameter θ_0), probably owing to the loss of water to the air during the time it takes to cut the seeds, variation in humidity rates (parameter θ_1) and stabilization periods (parameter θ_b). Therefore, as the effect of temperature and processing was effective, regression analysis for was performed each treatment (Figure 3).

Figure 3 - Values found and adjusted to the quadratic plateau regression for the water content of peach palm seeds (*Bactris gasipaes* Kunth) in relation to the drying period in hours under three different temperatures: A – 103 °C (101-105 °C), B – 105 °C, C – 130 °C (130-133 °C); and four types of cutting: T1 - seeds cut into fragments smaller than 7.0 mm, T2 - cut into four parts, T3 - cut into two parts, T4 - whole seed. The strips represent the confidence bands (95%)



In the drying method at a low temperature of 103 °C (Figure 3A), it was found that all types of seed preparation that were used were effective to determine moisture content, and water loss stabilized within 17 hours (INTERNATIONAL SEED TESTING ASSOCIATION, 2020). Thus, according to the parameter θ_b (Table 1), in cutting into fragments smaller than 7.0 mm (T1), into four parts (T2) and into two parts (T3), stabilization occurred in about three hours after the beginning of drying. The treatment which required a somewhat longer period for stabilization (6.19 hours) was the one with whole seeds (T4).

Based on the results when using the oven method at 105 °C (Figure 3B), it was found that in all ways of seed preparation, water loss within the maximum time recommended by the literature for completion of the analysis, i.e., after 24 h, had already taken place (INTERNATIONAL SEED TESTING ASSOCIATION, 2020). Treatments with fragments smaller than 7.0 mm stabilized after the measurement of 2.54 hours, whereas seeds cut into two and four parts took 3.07 and 2.99 hours, respectively, showing no difference between the same treatments at 103 °C. By comparison, whole seeds took almost 5 hours to stabilize; however, the fact that seed preparation (cutting) was unnecessary makes it worth using whole seeds because they are easier to use in routine analyses.

Figure 2C shows the seed water extraction curves for the oven method at a high temperature at 130-133 °C over five hours of drying. The period recommended for this method in the International Seed Testing Association (2020) may range from 1 h ± 3 minutes to 4 h ± 12 minutes, depending on the species. Treatments with fragments smaller than 7.0 mm and cut seeds were the ones which more quickly lost water, stabilizing at 2.24 hours after measurement. However, it should be noted that the bands of this parameter suggest that they does not differ from the seeds cut into two parts (2.87 hours) and four parts (2.85 hours), as well from the whole seed (3.29 hours). This temperature is very promising because it offers faster results and less work to prepare the material.

Seed drying involves two processes: the first one is the removal of water lying on the seed surface (water output is faster), and the second one consists of the movement of the water from the inside to the surface. In the second phase, there is a reduction of motion to the environment, since water is inside the seed and moves slowly to the surface by different mechanisms, such as by capillary action, water diffusion, vapor pressure gradients, and gravity and vaporization of water (GARCIA *et al*., 2004). It is worth noting that although the Rules for Seed Analysis (BRASIL, 2009) recommend the 24-hour period at 105 °C recommended for all species and 17 hours at 103 °C for new ones, by such periods are excessive for peach palm, since even the whole seeds present moisture stabilization after five and six and half hours, respectively. Therefore, for peach palm seeds, one does not need to wait the recommended period and the test can be completed earlier.

Table 1 - Quadratic plateau regression and estimated parameters for the water content of peach palm seeds in relation to the drying period in hours under three different temperatures: A – 103 °C (101-105 °C), B – 105 °C, C – 130 °C (130-133 °C)); and four types of cutting: T1 - seeds cut into fragments smaller than 7.0 mm, T2 - cut into four parts, T3 - cut into two parts, T4 - whole seed. θ_0 represents the intercept, θ_1 the angular coefficient and θ_b the value in hours for which the water content stabilizes

Treatments	Coefficients		
Temperature 103	θ_0	θ_1	θ_b
T1	26.32 (-11.19; 63.83)	16.02 (-29.74; 61.77)	2.64 (-0.51; 5.78)
T2	10.57 (-20.78; 41.92)	25.75 (-9.84; 61.33)	2.92 (1.14; 4.69)
T3	-0.93 (-31.27; 29.42)	32.34 (-1.56; 66.25)	2.99 (1.59; 4.38)
T4	6.01 (-5.81; 17.83)	14.14 (7.03; 21.26)	6.19 (4.42; 7.97)
Temperatura 105			
T1	17.76 (-23.52; 59.04)	25.71 (-26.18; 77.61)	2.54 (0.45; 4.62)
T2	12.52 (-16.67; 41.7)	24.78 (-7.24; 56.81)	3.07 (1.27; 4.87)
T3	0.9 (-29.44; 31.24)	31.34 (-2.55; 65.24)	2.99 (1.55; 4.42)
T4	6.07 (-9.23; 21.38)	18.07 (6.5; 29.63)	4.78 (3.15; 6.42)
Temperatura 130			
T1	39.9 (-35.23; 115.02)	9.62 (-95.87; 115.12)	2.24 (-7.41; 11.9)
T2	33.88 (1.23; 66.54)	11.76 (-26.07; 49.59)	2.85 (-1.33; 7.04)
T3	25.97 (-6.34; 58.28)	16.13 (-21.13; 53.39)	2.87 (-0.16; 5.91)
T4	8.97 (-17.5; 35.44)	24.5 (-3.49; 52.5)	3.29 (1.37; 5.21)

Cutting into fragments smaller than 7.0 mm required a 10-minute preparation time for peach palm, which has a hard endocarp, thus not meeting the requirements prescribed in the International Seed Testing Association (2020) for large seeds of forest species (i.e., thousand seed weight > 200 g). In the latter case, a maximum of four minutes is recommended to cut the seeds of each repetition, as there may be an environmental water loss, underestimating the actual amount of moisture. Thus, this treatment is not suitable for determining seed water content for this species, and such finding is corroborated by other authors that worked with forest seeds with hard endocarps (SILVA *et al*., 2020a, b).

For routine analysis, it is essential to use methodologies that are practical and compatible with the laboratory's hours of operation (RÍOS; ZOUGAGH; AVILA, 2012). Thus, by evaluating all treatments tested, it appears that the use of whole (intact) seeds in the oven method at a high temperature at 130-133 °C perfectly meets these requirements because it does not require material preparation (cutting); moreover, it has simple implementation, with stabilization of seed water loss in about 3 hours of drying.

Through this study, we evidenced that extracting water from peach palm seeds is a very rapid process, with mass stabilization occurring in a few hours, while drying times of 24 hours or 17 hours are unnecessary, although recommended in the Rules for Seed Analysis and International Seed Testing Association (2020), respectively, for most species. We hope that our study may aid in greater equipment optimization in the laboratory to enable faster results. The correct determination of seed moisture content is important to evaluate the physical and physiological quality of seeds, and it is even more relevant for species with recalcitrant behavior (BARBEDO, 2018; PELISSARI *et al*., 2018), such as peach palm.

CONCLUSION

The oven method at 130 °C by the period of four hours (with whole seeds) is recommended.

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