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How to store araticum seeds and maintain their physiological quality

Edvan Costa da Silva[1], Fabíola Villa[1]*, Daniel Fernandes da Silva[2], Jean Carlo Possenti[3], Michel Anderson Maseiro[4], Maria Soraia Furtado Vera Cruz[1]

[1]Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, PR, Brasil
[2]Universidade Federal do Paraná, Curitiba-PR, Brasil
[3]Universidade Tecnológica Federal do Paraná, Dois Vizinhos-PR, Brasil.
[4]Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brasil
*Corresponding author, e-mail: fvilla2003@hotmail.com

Abstract

The purpose of seed storage is its preservation; in this sense, the storage period and types of packaging materials utilized in this process interfere with the conservation of seed vigor. The objective here was to evaluate the physiological potential of araticum seeds in different types of packaging materials and storage periods. The work was developed at the State University of Western Paraná Seed Technology Laboratory (Unioeste) - Campus Marechal C. Rondon - from February to August 2020. The seeds were packed in different packaging materials for three months (February, March, and April / 2020), kept in a cold chamber (16° C and 40% RH). The experimental design used was completely randomized, in a 5 x 7 factorial scheme [five packages (glass, plastic bag, burlap bag, kraft paper, and pet bottle) x seven storage periods (0, 15, 30, 45, 60, 75 and 90 days)]. It is important to note that araticum seeds have dormancy, so they were submerged in 500 mg L^{-1} of gibberellic acid (GA_3) for 24 hours before the setting up of the germination test. Evaluating the physiological aspect of the germination and vigor. The kraft paper packaging is the most suitable for storing araticum seeds. The physiological potential of seeds of this species decreases when its storage period is increased.

Keywords: *Annona sylvatica (A. St.-Hil) Mart.*, conservation, germination, seed vigor

Introduction

Araticum (*Annona sylvatica* (A. St.-Hill.) Mart) is a native species belonging to the family Annonaceae, with geographic distribution from Pernambuco to Rio Grande do Sul. Seeds of this species have a recalcitrant behavior, showing a short period of viability under uncontrolled environmental conditions, not exceeding 90 days (Carvalho, 2008; Lorenzi, 2016).

Knowledge about the behavior of plant species seeds under different storage conditions is essential for the rational management of species (Guedes et al., 2012a). The germplasm of endangered plant species can be conserved from storage (Léon-Lobos & Ellis, 2018). However, the seeds must be stored under appropriate conditions to obtain positive results (Becerra-Vázquez et al., 2020).

Seed quality cannot be improved by storage mode. However, it can be preserved with as little deterioration as possible, through appropriate methods to maintain vigor and germination power over a longer period (Goldfarb & Queiroga, 2013). Seeds start the irreversible process of deterioration after physiological maturity, which results in a decrease in vigor and culminates in their death. The speed with which this process will occur is directly related to storage conditions (Marcos Filho, 2015).

The association of packaging with storage conditions and water content directly influences seed longevity of several species, such as fruit, forestry, horticultural, and floricultural species (Menegaes et al., 2020). The type of packaging used during storage interferes with seed vigor conservation (Smaniotto et al., 2020). The period of storage of seeds of native and forest species, associated with different types of packaging, are important points for the maintenance of viability. These species can present completely different behaviors

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under the same storage conditions (Souza et al., 2011).

The physiological potential of forest seeds for the production of quality seedlings supports work with the use of different packaging and storage. Some works with seeds of fruit and forest species under Brazilian conditions were carried out, using packaging and storage, such as: *Annona squamosa* L. (Morais et al., 2014), *Plinia cauliflora* (Mart.) Kausel (Hossel et al., 2019), *Jatropha curcas* L. (Pinto Junior et al., 2012), *Tabebuia caraiba* Mart. (Guedes et al., 2012b), *Myracrodruon urundeuva* Allemão (Guedes et al., 2012a; Inô et al., 2019), *Amburana cearensis* (Allemão) A. C. Smith (Araújo et al., 2017) e *Caesalpinia ferrea* (Silva et al., 2019).

Information regarding the types of packaging and storage is still scarce considering the relevance of the quality of Annonaceae seeds. Thus, this study aimed to evaluate the physiological potential of araticum seeds with types of packaging and storage periods.

Material and Metods

The study was carried out from February to August 2020 in the Laboratory of Seed Technology at the Western Paraná State University (Unioeste), Marechal Cândido Rondon *Campus*, Paraná State, Brazil. Araticum (*Annona sylvatica* (A. St.-Hill.) Mart) seeds from ripe fruits collected in February 2020 at the Experimental Farm belonging to the Unioeste Experimental Stations Nucleus from four native plants, named accessions A1, A2, A3, and A4, were used.

The local climate is classified, according to Köppen, as *Cfa*, subtropical, with an average temperature of the coldest month below 18 °C (mesothermal) and an average temperature of the hottest month above 22 °C (Alvares et al., 2013). The summers are hot, frosts are infrequent in the winters, with a tendency to concentrate the rains in the summer months, but without a defined dry season. The average annual rainfall varies from 1600 to 1800 mm (Caviglione et al., 2000).

The fruits were taken to the Laboratory of Seed Technology immediately after collection, the epicarp was removed, placed in 5-L plastic buckets, and left to rest for 48 hours. Subsequently, the seeds were washed in running water over a sieve (06 mesh, 23 BWG thread, and 65 cm rim) until the mucilage was completely removed. Then, the seeds were placed to dry in a dry, shaded, ventilated place at a room temperature of 25 ±2 °C.

All seeds were disinfected after homogenization, according to the methodology adapted from Silva et al. (2007). Thus, they were immersed for 10 minutes in a sodium hypochlorite solution (2.5% active chlorine) at a proportion of 10 mL for 1000 mL of water. Then, they were rinsed in running water for the total washing of the integument and dried on paper towels again at a room temperature of 25±2 °C.

After this step, the seeds were packed in different packages for three months (February, March, and April 2020) and maintained in a cold chamber (16 °C and 40% RH). **Figure 1** shows the temperature and relative humidity data during seed storage.

The experimental design was completely randomized in a 5×7 factorial scheme (five packages: glass, plastic bag, cotton waste bag, kraft paper, and PET bottle) × seven storage periods: 0, 15, 30, 45, 60, 75, and 90 days), with four replicates and 25 seeds per replicate. The araticum seeds were submerged in 500 mg L^{-1} of gibberellic acid (GA_3) for 24 hours before performing the germination test due to their dormancy.

Seed viability was also verified before storage (considering this as a zero-storage period), through a germination test, which evaluated: first germination count

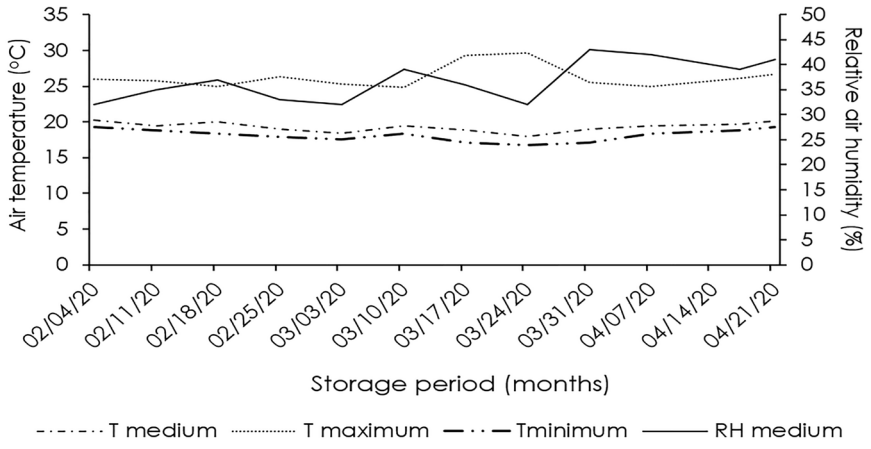

Figure 1. Air temperature and relative humidity observed during the storage period of *Annona sylvatica* seeds.

(PC), germination percentage (G), germination speed index (GSI), mean germination time (MGT) and electrical conductivity (EC). Before storage, the water content of the seeds was also determined.

The water content of the seeds was determined by the oven method at 105 ± 3°C, for 24 hours (Brasil, 2009), in two replications of 50 seeds in each test. After this time, the samples were removed from the oven, cooled in a desiccator containing silica gel and weighed again on a precision analytical balance with four decimal places, model Bel M254-A.

The first germination count test (PG) was performed together with the germination test, with the number of seeds with primary root protrusion being recorded on the 15th day after the experiment was set up, and the results were expressed in percentage (%) of germinated seeds. The germination test (G) was carried out with four replications of 25 seeds per treatment, distributed on a germitest paper moistened with distilled water 2.5 times its weight and placed in gerbox boxes. Subsequently, they were arranged in a BOD with a constant temperature of 25±2 °C, relative humidity of 80–85%, and photoperiod of 12 h. The evaluations were carried out from the 15th day of the experiment to the 105th day. The botanical criterion was adopted in the germination analysis, that is, the seed was considered germinated when it presented protrusion of the primary root, with results expressed in percentage (%).

The germination speed index (GSI) was performed concomitantly with the germination test. The evaluations were carried out weekly from the first day after sowing until the 105th day. The germination speed index was calculated from the daily values of germinated seeds, according to Maguire (1962). The average germination time was used to stipulate the value in days to reach the maximum germination, using the formula of Edmond and Drapala (1958).

The electrical conductivity (EC) test was performed according to the methodology described by Vieira and Krzyzanowski (1999). Fifty seeds were used for four subsamples of each treatment, being weighed accurately to three decimal places (0.001 g). The samples were placed to soak in plastic cups with 75 mL of deionized water and kept in a BOD with a controlled temperature of 25 °C for 24 h. The solutions with the products were lightly stirred to standardize the leachates and immediately read in a portable digital conductivity meter (Microprocessed Bel W12D). The results were divided by the mass and expressed in μS cm^{-1} g^{-1} seeds.

The data obtained were submitted to Shapiro-Wilk normality analysis (p>0.05), verifying the need for arcsene transformation √x/100 for the first germination count. Once the model's assumptions were met, ANAVA tested the effects of isolated factors and their interaction. The comparison of the means of the packaging factor variables were analyzed by the Tukey test, at 5% error probability. For the storage period factor, polynomial regression curves were fitted. The statistical program used was SISVAR (Ferreira, 2011), to perform the statistical analyses.

Results and Discussion

Table 1 shows that the physiological seed quality was not affected by the interaction between packaging and storage periods. However, a significant result was observed when analyzing only the individual effect of each factor.

Figure 2 shows that the water content of stored seeds decreased with the storage period, with a higher variation in the water content of seeds packed in kraft paper and cotton waste bags (14.7 to 8.0% and 8.1%, respectively). **Table 2** shows a significant difference between the packages for first germination count (PC) and germination (G), with kraft paper statistically differentiating as the best (3.38 and 6.32%, respectively), followed by cotton waste bag (2.64 and 5.41%, respectively) and PET bottle (1.86 and 4.44%, respectively).

The germination speed index (GSI) (Table 2) showed that kraft paper (1.18) stood out as the best packaging, followed by cotton waste bag (1.12), plastic bag (1.08), PET bottle (1.07), and glass (1.07).

A significant difference was found for the

Table 1: Summary of analysis of variance containing the mean square values for the variables: first germination count (PC), germination percentage (G), germination speed index (GSI), mean germination time (MGT) and electrical conductivity (EC) as a function of storage periods (P) and packaging (E).

F.V	G.L.	PC [(a)]	G	GSI	MGT	EC
P	5	120.164*	658.56*	0.06*	109.53*	18.29*
E	4	481.93*	1698.66*	1.16*	747.32*	3.44*
E x C	20	34.64[ns]	30.02[ns]	0.14[ns]	47.80[ns]	0.45[ns]
Erro	90	33.40	59.73	0.63	29.83	0.74
Total	119					
CV(%)	30.80	29.73	36.41	14.03	5.14	8.14

*Significant at 5% probability of error, ns = not significant, FV = variation factor, GL = degrees of freedom. (a) Data transformed to arc sine (√x/100). CV = coefficient of variation.

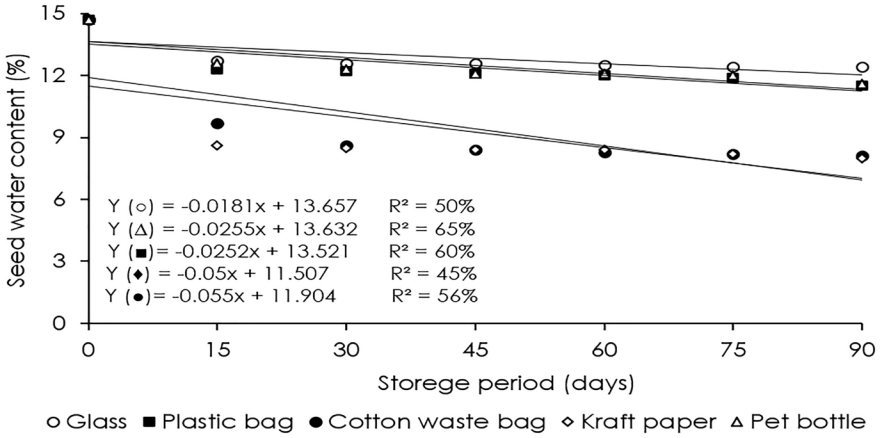

Figure 2: Water content of *Annona sylvatica* seeds during the storage period.

Table 2: Mean data of first germination count (PC), percentage of germination (G), germination speed index (GSI), mean germination time (MGT) and electrical conductivity (EC) of seeds of *Anonna sylvatica* (A. St.-Hil) Mart. In different packaging.

Packaging	PC (%)	G (%)	IVG	TMG (days)	CE (μS cm^{-1} g^{-1})
Glass	2.04 bc*	4.46 c	1.07 c	44.14 c	16.68 ab
Plastic bag	2.01 bc	4.69 bc	1.08 c	41.45 c	16.81 ab
Cotton waste bag	2.64 b	5.41 b	1.12 b	36.58 b	17.44 b
Kraft paper	3.38 a	6.32 a	1.18 a	30.29 a	16.41 a
PET bottle	1.86 c	4.44 c	1.07 c	42.41 c	16.94 ab
CV(%)	30.80[(a)]	29.73	36.41	14.03	5.14

**Means in the column followed by the same letters do not differ statistically by Tukey's Test, at 5% probability of error. CV = coefficient of variation. (a) Data transformed by arc sine (√x/100).

average germination time (MGT) (Table 2), in which kraft paper was the best packaging (30.29 days), followed by cotton waste bag (36.58 days), plastic bag (41.45 days), PET bottle (42.41 days), and glass (44.14 days), with no statistical difference compared to each other.

For electrical conductivity (EC) (Table II) it is observed that between the packages studied there was a significant difference only when comparing the kraft paper (16.41μS cm^{-1} g^{-1}) and the cotton waste bag (17.44 μS cm^{-1} g^{-1}), with kraft paper considered the best statistically.

Figure 3A shows PC of araticum seeds as a function of storage periods. A quadratic behavior is observed, reaching a maximum point of 4.98% at the beginning of storage. The data fit the decreasing linear regression model for G and GSI of araticum seeds (Figures 3B and 3C), reaching the lowest value of 4.11 % in the period of 90 days for G. There was a reduction in germination potential of the order of 61.31% compared to that recorded at the beginning of storage (6.63%). GSI reached the lowest value (1.05) in the period of 90 days, also being 15.23% lower than the index recorded for the beginning of storage.

Figure 3D shows that the data for MGT fit the quadratic regression model, with an increase in results up to 41.10 days, with a maximum point of 63.16 days.

A linear increase was observed in EC (**Figure 4**), reaching the highest value for the period of 90 days (18.09 μS cm^{-1} g^{-1}), being 19.56% higher than the electrical conductivity recorded for the beginning of storage (15.13 μS cm^{-1} g^{-1}).

Seeds are composed of organic substances as a reserve and structural macromolecules that give them the characteristic of hygroscopicity in relation to water. Thus, seeds are permanently exchanging water vapor with the ambient air to a greater or lesser degree, gaining moisture or undergoing desorption as a function of variations in relative humidity, always tending to reach hygroscopic equilibrium. The results of the present study are in line with what was stated by the authors, as the place of storage consisted of a cold chamber, which allowed the maintenance of relative humidity of around 35% (Figure 1). Figure 2 shows that the seeds packed in packages that allowed gas exchange had a reduction in their water content, tending to reach hygroscopic equilibrium with the low relative humidity of the place.

Thus, an important factor in storage is the packaging (Hossel et al., 2016). According to Smaniotto et al. (2014), a reduction in seed water content is related to the permeability of the packaging in which they were packed. They also demonstrated that water vapor exchanges with the environment are allowed depending on the type of packaging due to the hygroscopic properties of seeds, which culminate in the maintenance

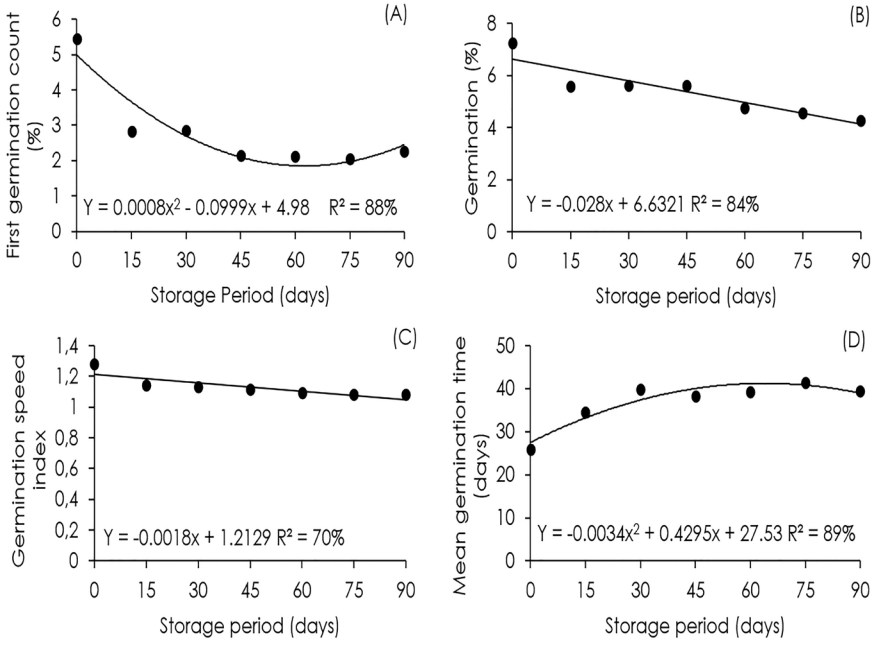

Figure 3. First germination count (A), germination (B), germination speed index (C) and average germination time (D) of *Anonna sylvatica* seeds stored for different periods.

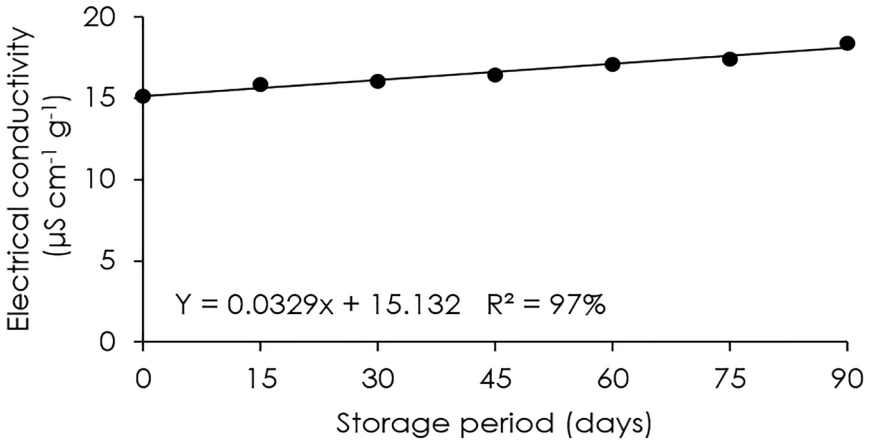

Figure 4. Electrical conductivity of *Anonna sylvatica* seeds stored for different periods.

of water content in equilibrium with the relative humidity of the storage environment and the air temperature. Morais et al. (2014) also found that the water content of sugar apple (*Annona squamosa* L.) seeds is reduced when stored in paper bags for three months and stored in a refrigerator (6–8 °C).

Paper packaging is permeable and allows the exchange of water vapor with the environment (Marcos Filho, 2015; Amaral et al., 2019). Possibly, the seeds of the studied species do not have a totally recalcitrant behavior, showing that they are more orthodox or intermediate.

Reducing the water content of seeds decreases leads to a reduction in their metabolism. Some species tolerate this desiccation more and can maintain their viability for a longer period. The same authors classified orthodox seeds as those that withstand desiccation at water contents lower than 10% (Guollo et al., 2018).

Morais et al. (2009) studied the influence of different types of packaging and environments on the germination of sugar apple (*Annona squamosa*) seeds and found that paper packaging was considered the most suitable regardless of the environment. The results in the literature vary according to the species. Oliveira et al. (2012) tested the storage of red beadtree (*Adenanthera pavonina*) seeds using two types of packaging (plastic bag and paper bag) and found no differences in the percentage of germination. Morais et al. (2014) reported

that plastic packaging was unsuitable for storage under refrigeration conditions, as it reduced the germination capacity of sugar apple seeds.

Silva et al. (2019) studied the storage and conservation of Brazilian ironwood (*Caesalpinia ferrea*) seeds under different storage and packaging environments and concluded that thick plastic was a more viable alternative for seed packaging and storage than kraft paper.

Similarly, Morais et al. (2014) evaluated the storage conditions of sugar apple (*Annona squamosa*) seeds to maintain their vigor. The authors concluded that the highest vigor is obtained with seed storage using paper packaging under natural conditions, while plastic packaging is inadequate to store sugar apple seeds because they have high water contents. In this case, the plastic packaging did not allow the exchange of water vapor between the seeds and the environment, favoring the respiratory process and their degradation.

MGT represents the growth and progress in the following stages of the development of the species (Queiroz et al., 2016), reflecting the average time required for germination to occur, that is, the shorter this time, the faster the speed of the seed germination process.

According to Marcos Filho (2015), the electrical conductivity test is related to the ability of membranes to reorganize during soaking and the greater the number of exuded ions in this test, the higher the seed deterioration. Morais et al. (2009) observed that the vigor measured through the electrical conductivity test of sugar apple seeds was affected by the time and type of storage packaging, with the paper packaging maintaining seed vigor because the electrical conductivity value was reduced during storage.

These results show a decrease in the vigor of araticum seeds with increasing storage time, corroborating with information found in the literature, which also reported a decrease in vigor in Paraná pine (*Araucaria angustifolia*) and cambará (*Vochysia divergens*) seeds as the storage period increased (Garcia et al., 2014; Oliveira et al., 2018).

Strenske et al. (2015) reported that the shorter the storage period, the faster the germination speed of quinoa (*Chenopodium quinoa*) seeds, with a decrease in their germination potential when it is increased. Knowing the physiological seed quality during the storage period is essential due to the particularities of each species regarding the conditions for their conservation (Masetto et al., 2013).

According to Silva et al. (2014), the deterioration process of stored seeds is inevitable and, when this occurs, the seeds lose vigor and are more susceptible to stress during germination, losing the potential to originate normal seedlings. Sabry et al. (2012) observed that an increase in the average germination time of a lot of seeds led to a reduction in its vigor, with an increase in deterioration processes.

The electrical conductivity data demonstrate the deleterious effect of storage time on the cell membrane system of seed embryos, allowing the release of solutes (Taiz et al., 2017), negatively affecting their vigor (Marcos Filho, 2015).

The increase in electrical conductivity means a decrease in seed vigor, indicating that the seeds are regressing, as observed with the increased electrolyte leakage (Akter et al., 2014). The evaluation of the physiological potential of forest seeds showed a positive correlation between the germination test and electrical conductivity, which is appropriate as an alternative for a quick evaluation of seed vigor (Guollo et al., 2017).

The present study showed that the physiological potential of araticum seeds decreases with an increase in the storage period and kraft paper stood out among the studied packages as the most appropriate for seed storage. The effect of this type of packaging for araticum seeds is interesting. In addition, this material made it easier for the seeds to reduce their water content at low temperatures, as in the case of the cold chamber, with low values of relative humidity, reducing their deterioration.

Further studies are required due to the lack of research focusing on the storage of seeds of this species and others of the family Annonaceae. Moreover, new investigations to test other water content ranges in seeds, packaging, environments, and storage periods are needed aiming to prolong their longevity and subsidize the planning for the commercial seedling production.

Conclusions

Kraft paper packaging is the most suitable for storing araticum seeds.

The physiological potential of seeds of the species decreases with increasing storage period.

Low water contents in araticum seeds during cold storage were better for reducing their deterioration.

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