

Antioxidant enzyme activity and physiological potential of *Capsicum baccatum* var. *baccatum* seeds as a function of post-harvest storage of fruit

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ABSTRACT: Post-harvest storage of fleshy fruit is a strategy used for improving seed quality. The aim of this study was to evaluate post-harvest storage of the fruit of *C. baccatum* var. *baccatum* (*cumari* pepper) and relate it to antioxidant enzyme activity and seed physiological potential. Red-colored fruit (65 days after anthesis) was gathered and stored at 20 °C for 0, 5, 10, 15, and 20 days. The seeds were removed and evaluated for moisture content, physiological quality, antioxidant enzyme activity, and protein content. The means were fitted to regression equations and then passed through multivariate analysis (PCA). Seed moisture and 100-seed weight decreased through post-harvest storage. The 5- and 10-days storage periods led to an increase in the germination. Electrical conductivity was greater for the 0- and 20-day storage periods. Dry matter and protein increased in the 5- and 10-days. Enzymes exhibited reductions of activity comparing 0 and 20 days. The post-harvest storage of *C. baccatum* fruit for 5 and 10 days improves seed physiological potential and is related to physiological maturity. The incomplete maturation of *cumari* seeds obtained from unstored fruit (0 days) is related to greater enzyme activity, greater oxidative stress, and low germination and vigor. The lack of post-harvest storage of fruit and storage of fruit for longer than 10 days contributes to a lower physiological potential of *cumari* pepper seeds.

Index terms: germination, oxygen reactive species, *cumari* pepper, physiological quality, vigor.

Ação enzimática antioxidativa e potencial fisiológico de sementes de *Capsicum baccatum* var. *baccatum* em função do armazenamento pós-colheita dos frutos

RESUMO: O armazenamento pós-colheita de frutos carnosos é uma estratégia usada para melhorar a qualidade das sementes. O objetivo deste estudo foi avaliar o armazenamento pós-colheita de frutos de *C. baccatum* var. *baccatum* (pimenta cumari) e relacioná-lo à atividade de enzimas antioxidantes e ao potencial fisiológico das sementes. Frutos de cor vermelha (65 dias após a antese) foram colhidos e armazenados a 20 °C por 0, 5, 10, 15 e 20 dias. As sementes foram removidas e avaliadas quanto ao grau de umidade, qualidade fisiológica, atividade de enzimas antioxidantes e conteúdo de proteínas. As médias foram ajustadas às equações de regressão e depois avaliadas por análise multivariada (PCA). A umidade e o peso de 100 sementes diminuíram com o armazenamento pós-colheita. Os períodos de armazenamento de 5 e 10 dias levaram a um aumento na germinação. A condutividade elétrica foi maior nos períodos de armazenamento de 0 e 20 dias. A matéria seca e o teor de proteína aumentaram aos 5 e 10 dias. As enzimas exibiram reduções de atividade comparando 0 e 20 dias. O armazenamento pós-colheita de frutos de *C. baccatum* por 5 e 10 dias melhora o potencial fisiológico das sementes e está relacionado à maturidade fisiológica. A maturação incompleta de sementes de cumari obtidas a partir de frutos não armazenados (0 dias), está relacionada a maior atividade enzimática, maior estresse oxidativo e baixa germinação e vigor. O não armazenamento pós-colheita dos frutos e o armazenamento por mais de 10 dias contribui para o menor potencial fisiológico de sementes de pimenta cumari.

Termos para indexação: germinação, espécies reativas de oxigênio, pimenta cumari, qualidade fisiológica, vigor.

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INTRODUCTION

Worldwide production of peppers has continued to grow, reaching approximately 40 million tons (FAOSTAT, 2019). Among the pepper genera, *Capsicum* has around 35 species with color, pungency, and aroma traits that promote human consumption (Carrizo-García et al., 2016; Aguiar et al., 2018). In addition, the species of this genus have notable levels of capsaicinoids, which are secondary metabolites that give rise to the pungency of these species, offer diverse benefits for health, and have industrial applications (Naves et al., 2019). Among the species of the *Capsicum* genus, *C. baccatum* var. *baccatum*, known in Brazil as “cumari”, has small, upright, rounded fruit with a mild aroma and high pungency. These traits make it a good option for producing preserves (EPAMIG, 2006).

Germination and vigor are important characteristics for seed-propagated species, for which uniform stand is indispensable for obtaining satisfactory production (Rajjou et al., 2012). In general, peppers are propagated by seeds that have irregular germination, mainly due to dormancy (Quintero et al., 2018), which is a characteristic from its evolutionary development and intrinsic to the genotype. In that regard, post-harvest storage of fleshy fruit is a strategy adopted by producers for the purpose of making the maturity stage more uniform and, in many cases, it can assist in improving the quality and/or breaking the dormancy of seeds in many crops. Recently, many studies report the beneficial effect of post-harvest storage of fruit on the quality of different pepper species (Araújo et al., 2018; Gonçalves et al., 2018; Martínez-Muñoz et al., 2019; Medeiros et al., 2020).

When observed, the deleterious effects of fruit storage are mainly related to seed deterioration, which intensifies after seed physiological maturity (Shaban, 2013). The deterioration process of seeds under different conditions is directly related to incomplete or partial reduction in oxygen, leading to the formation of reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^{\cdot}) (Gupta et al., 2015). Although ROS perform important cell functions, when they are above basal levels, they cause damage that can lead to cell death through oxidative stress (Choudhury et al., 2017). With the aim of neutralizing the deleterious effects of excess ROS on cells, antioxidant mechanisms should be regulated, above all in the seed embryo (Oracz and Karpinski, 2016). In this respect, enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) are part of the enzymatic antioxidant system and play a crucial role in neutralization of these ROS (Kapoor et al., 2019).

Evaluation of antioxidant enzyme activity can provide important responses regarding seed physiological potential under different conditions. Araújo et al. (2018) evaluated antioxidant enzyme activity in *C. chinense* and *C. frutescens* pepper seeds in different maturity groups and observed that CAT showed considerable potential for monitoring seed physiological quality. In *C. annum* seedlings, high correlation was observed between SOD activity and tolerance to water deficit (Sahitya et al., 2018). Vidigal et al. (2009) observed that SOD enzyme had greater activity in seeds obtained from fruit in more advanced maturity stages of *C. annum*.

Information regarding the effect of post-harvest storage of fruit on antioxidant enzyme activity and its relation to physiological potential in *C. baccatum* var. *baccatum* seeds is very limited. Such information may assist in defining strategies regarding adequate management of fruit after harvest for the purpose of obtaining better quality seeds. In light of the above, the aim of this study was to evaluate the effect of post-harvest storage of fruit on the physiological potential and antioxidant activity of *C. baccatum* var. *baccatum* seeds.

MATERIAL AND METHODS

The experiment was conducted in the Department of Plant Science of the *Universidade Federal de Viçosa*, Minas Gerais, Brazil. *C. baccatum* var. *baccatum* (“cumari verdadeira”) plants were produced in a greenhouse and grown according to recommendations for production of pepper seeds.

Gather and storage of fruits: Ripe fruit was gathered from 20 different plants when it reached intense red coloring, corresponding to approximately 65 days after anthesis. The fruit was stored in *kraft* paper bags and kept in a laboratory

environment (20 ± 2 °C; 60% relative humidity) for 5, 10, 15, and 20 days, which constituted the post-harvest storage treatments. For the control treatment (without storage; 0 days), the seeds were immediately removed from the fruit after it was gathered (Figure 1).

After each storage period, the fruit was cut with a utility knife; the seeds were removed, washed in running water for five minutes, and placed to dry at ambient temperature until reaching approximately 15% moisture. The following analyses were made:

Moisture content: This was determined immediately after removal of the seeds from the fruit (without washing in running water). The seeds were dried by the laboratory oven method at 105 ± 3 °C for 24 hours. Four replications of 50 seeds were used, and the results were expressed in % (Brasil, 2009).

100-seed weight: Eight replications of 100 seeds were weighed in a precision balance and the results were expressed in grams (g) (Brasil, 2009).

Electrical conductivity: Seeds were dried in the shade until reaching constant weight. After this period, four replications of 50 seeds previously weighed and immersed in 50 mL of distilled water for 24 hours at 25 °C (Brasil, 2009). Posteriorly, electrical conductivity was read on a digital conductivity meter, and the results were expressed in $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$.

Germination: Four replications were made of 50 seeds distributed on two sheets of paper towel, moistened with water in the amount of 2.5 times the weight of the dry paper, in “gerbox” boxes. The boxes were kept in BOD with temperatures alternating between 20 °C and 30 °C and 8 h photoperiod; the lighted period corresponded to the higher temperature (Brasil, 2009). After preliminary tests were performed, the time of 30 days was defined for evaluation of germination, and the results were expressed in percentage of normal seedlings.

First germination count: This was performed together with the germination test, evaluating the percentage of normal seedlings obtained 14 days after sowing.

Seed dry matter: Four replications of 50 seeds were weighed and then placed to dry at 105 °C for 24 h and weighed once more. The difference between wet weight and dry weight was defined as the seed dry matter content. The results were expressed in $\text{g} \cdot \text{seeds}^{-1}$.

Antioxidant enzyme activity: The seeds were kept in moistened paper for 48 h at 20-30 °C. After that, they were frozen in liquid nitrogen and stored at -20 °C until the time of evaluation. The activity of the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) were determined through crude enzyme extracts, which were obtained by maceration of 0.2 g of seeds in liquid nitrogen, followed by addition of 2 mL of extraction medium, potassium phosphate buffer (0.1 M, pH 6.8), containing ethylenediamine tetraacetic acid (EDTA) (0.1 mM), phenylmethylsulfonyl fluoride (PMSF) (1.0 mM), and 1% polyvinylpolypyrrolidone (PVPP) (w/v) (Peixoto et al., 1999). The homogenate was centrifuged at 14.000 rpm for 15 min at 4 °C.

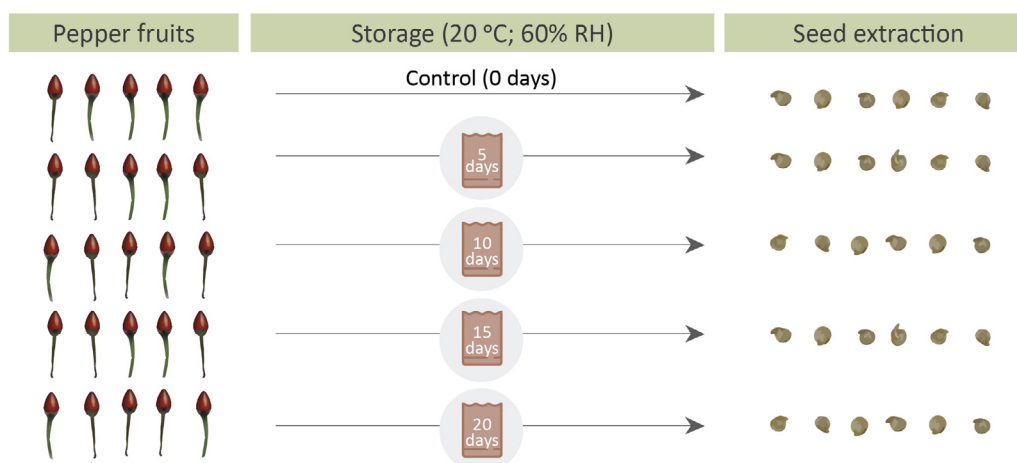


Figure 1. Fruit storage and seed extraction of *C. baccatum* var. *baccatum*.

The SOD activity was evaluated by the addition of the crude enzyme extract to 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μ M nitro blue tetrazolium (NBT), 0.1 mM EDTA, and 2 μ M riboflavin (Del Longo et al., 1993). The nitroblue tetrazolium (NBT) photoreduction, according to Giannopolitis and Ries (1977). One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50% (Beauchamp and Fridovich, 1971). The CAT activity was determined by incubating 50 mM potassium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2 (Havir and McHale, 1987). The enzyme activity was calculated using the molar extinction coefficient of $36 M^{-1}.cm^{-1}$ degradation in 240 nm (Anderson et al., 1995). The results were expressed in μ mol $min^{-1} mg^{-1}$ protein. The APX activity was determined by incubating 50 mM potassium phosphate buffer (pH 7.8), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.2 mM H_2O_2 . The enzyme activity was calculated using the molar extinction coefficient of $2.8 mM^{-1}.cm^{-1}$ at 290 nm (Nakano and Asada, 1981). The results were expressed in $nmol.min^{-1}.mg^{-1}$ protein. The POX activity was assessed by the addition by incubating 25 mM potassium phosphate buffer (pH 6.8), 20 mM pyrogallol, and 20 mM H_2O_2 (adapted from Kar and Mishra, 1976). Purpurogallin production was measured using the molar extinction coefficient of $2.47 M^{-1}.cm^{-1}$ at 420 nm (Chance and Maehly, 1955).

Protein content: This was determined using bovine serum albumin (BSA) as a standard (Bradford, 1976). Enzyme extract in the amount of 50 μ L was added to 1 mL of Bradford reagent, followed by shaking. After 20 minutes, absorbance of the sample was read in a spectrophotometer at 595 nm. The results were expressed in $mg.g^{-1}$ of fresh matter.

Experimental design and statistical analysis: The experiment was conducted in a completely randomized experimental design, with four replications. The data passed through analysis of variance. The mean values obtained for each treatment were fitted to regression equations, and the regression coefficients were evaluated by the *t*-test at 5% probability. Multivariate principal component analysis (PCA) was performed for all the characteristics evaluated. A “*n x p*” matrix was obtained, where “*n*” corresponds to the number of treatments (*n* = 5) and “*p*” is the number of variables analyzed (*p* = 11). The eigenvalues and eigenvectors were calculated from the covariance matrices and displayed on a biplot generated from the Factoextra package (Kassambara and Mundt, 2016). The R statistical software was used in all the analyses (R Core Team, 2019).

RESULTS AND DISCUSSION

Post-harvest storage of the fruit caused gradual reduction in the moisture content of *C. baccatum* var. *baccatum* seeds. Comparing the control treatment (0 days of storage) with the longest period of storage (20 days), this reduction was approximately 20 percentage points (p.p.) (Figure 2A).

During the seed maturation process, the moisture content is high and it decreases as seeds complete their development (Marcos-Filho, 2016). The reduction in moisture content leads to multiple changes in the seeds, including transcriptional, post-transcriptional, and metabolic processes (Angelovici et al., 2010). As seeds mature (and dries) their content of ABA often declines, especially in non-dormant seeds, as does the sensitivity of the seed to the hormone, thus permitting germination upon imbibition of the mature seed (Bewley and Nonogaki, 2017). In this context, abscisic acid (ABA) has been proved to play a leading role in the regulation of seed maturation, being the balance of this hormone with gibberellic acid (GA) considered the key for seed germination through the dormancy breaking, enzymatic activation, mobilization and transport of reserves, and others (Yan and Chen, 2016; Bewley and Nonogaki, 2017).

Just as for moisture content, there was a reduction in 100-seed weight over the fruit post-harvest storage time. The difference in weight between the seeds obtained from the unstored fruit (0-days) and those stored for 20 days was approximately 1 g. The seeds obtained from fruit stored for 5 days had the maximum 100-seed weight among all the treatments, at approximately 2.4 g (Figure 2B). Nevertheless, it is important to highlight that both for moisture content (Figure 2A) and for 100-seed weight (Figure 2B), this reduction was especially more accentuated beginning at 10 days of fruit storage. Therefore, the reduction in the seed weight is clearly related to the loss of water from the storage seeds inside the fruits, which is commonly observed in different pepper species (Vidigal et al., 2009; Ricci et al., 2013; Pereira et al., 2014; Araújo et al., 2018).

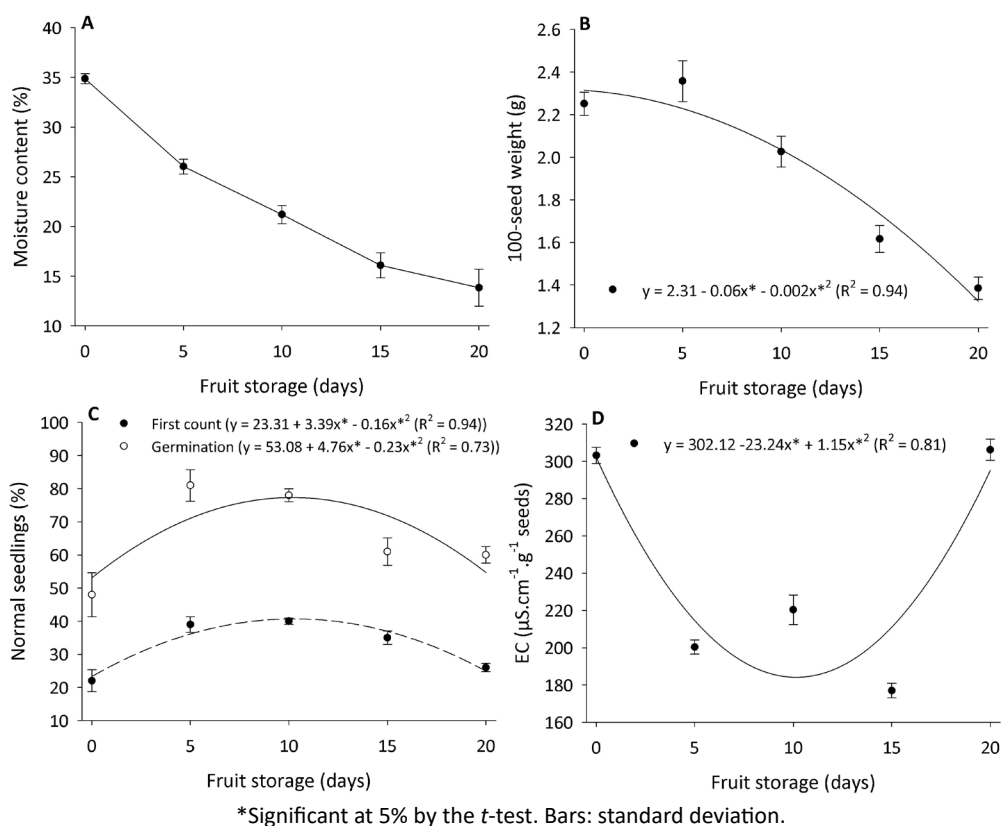


Figure 2. Moisture content (A), 100-seed weight (B), germination and first germination count (C), and electrical conductivity (D) of *C. baccatum* var. *baccatum* seeds under different fruit post-harvest storage times.

The first germination count and germination percentage showed a similar quadratic tendency. In general, there was a notable increase in germination up to 10 days of storage and a reduction at 15 and 20 days. The highest percentages of first germination count and germination were observed in the seeds obtained from the fruit stored for 5 days, approximately 39% and 81%, respectively. In contrast, the lowest percentages of first germination count and germination were obtained in the seeds from the unstored fruit (0 days), with values of 22% and 48%, respectively (Figure 2C). Based on the results of the germination test, it can be said that the seeds of the fruit stored for 5 and 10 days had similar physiological quality. Therefore, the low germination in non-stored fruits (0 days) is probably due to dormancy, a common process in pepper species when harvested before the physiological maturity (Queiroz et al., 2011; Ricci et al., 2013; Medeiros et al., 2020). Considering the fruit stored for 20 days, it was observed a similar germination percentage to the seeds of the unstored fruit (0 days) (Figure 2C). In this context, these results can be associated with the seed deterioration process, which involves many cellular events such as lipid peroxidation, oxidative stress, and mitochondria changes, inducing the reduction of germination and vigor (Ebene et al., 2019).

The seeds obtained from the fruit stored for 5, 10, and 15 days had lower values of electrical conductivity, of approximately 200, 220, and 180 $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$, respectively. In contrast, the seeds from fruit stored for 0 and 20 days had higher electrical conductivity, with values of approximately 300 $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ (Figure 2D). The electrical conductivity test is based on organization of cell membranes, and it is directly related to seed physiological quality since higher values indicate lower integrity of cell membranes (Prado et al., 2019). Thus, the electrical conductivity values reinforce the results observed in germination and evidence the lower membrane integrity of the seeds obtained from the unstored fruits (0 days) (probably due to the incomplete maturation) and stored for 20 days (due to the seed deterioration). It is important to emphasize that although the 15 day storage time led to a lower conductivity value (Figure 2D), this result

was not accompanied by high germination rates (Figure 2C).

The seed dry matter showed an increase in the treatments of 5 and 10 days in relation to the control (0 days), followed by reduction in the treatments of 15 and 20 days. Storage of the fruit for 20 days resulted in the lowest value of seed dry matter (Figure 3A). Just as observed for dry matter, the results observed for protein content help reinforce that physiological maturity was reached between 5 and 10 days of fruit storage, with degradation of these proteins occurring from 15 to 20 days of fruit storage. Comparing the treatments of 10 and 20 days, the difference in protein content was approximately 1 mg.g⁻¹ of fresh matter (Figure 3B).

According to Kijak and Ratajczak (2020), the desiccation tolerance (DT) acquisition in orthodox seeds (such as peppers) is very complex and involves numerous genes encoding proteins involved in gene expression, metabolic shutdown, and storage material accumulation. Among the cellular mechanisms and alterations involved in DT, these authors cite the accumulation of protective late embryogenesis abundant (LEA) and heat shock (HSP) proteins, activation of the antioxidative system, accumulation of sugars, hormonal balance (mainly the degradation of abscisic acid (ABA) and accumulation of gibberellin (GA)), and others. Thus, the sum of these many factors will allow the desiccation, breaking dormancy, storage, and quality maintenance of the seeds (Dekkers and Bentsink, 2015; Kijak and Ratajczak, 2020).

Considering the seed weight, it is a characteristic that can be related to physiological quality, since the stored fruit tends to continue to deposit reserves in the seeds, leading to improvement in physiological quality through the balance between gibberellin (GA) and abscisic acid (ABA). Therefore, based on seed dry matter results, it is possible to confirm the physiological maturity of *cumari* seeds between 5 and 10 days of fruit storage, evidenced by the maximum dry matter accumulation (Marcos-Filho, 2016). In addition, the deterioration of the seeds stored for 20 days is also reinforced by the lower protein content (Figure 3B), indicating greater consumption of the reserves, affecting germination and vigor under these conditions (Figure 2).

In a similar way, Socolowski et al. (2011) observed that *Xylopia aromatica* seeds with greater concentrations of proteins manifested a greater speed of germination and formation of normal seedlings. In contrast, the seeds obtained from unstored fruit (0 days) also had high levels of protein, but with a low germination percentage, which could be associated with dormancy and greater concentration of ABA (Bewley and Nonogaki, 2017). The ABA is a hormone that directly controls the induction and maintenance of primary seed dormancy through complex crosstalk with other plant hormones, such as GA (Matilla et al., 2015). In this sense, as has been shown in many studies, pepper seeds obtained from recently gathered fruit have lower germination rates compared to seeds from fruit stored after harvest. As already mentioned, this fact is mainly associated with achieving the point of physiological maturity and breaking dormancy as

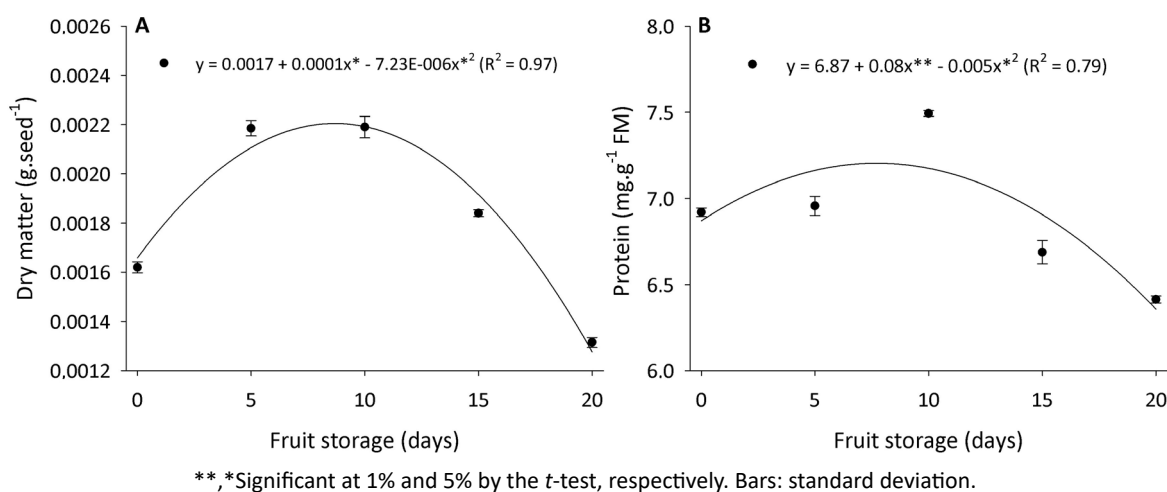


Figure 3. Dry matter (A) and protein content (B) of *C. baccatum* var. *baccatum* seeds from fruit under different post-harvest storage times.

well as with uniformity of the seed lots (Pereira et al., 2014; Gonçalves et al., 2018; Medeiros et al., 2020).

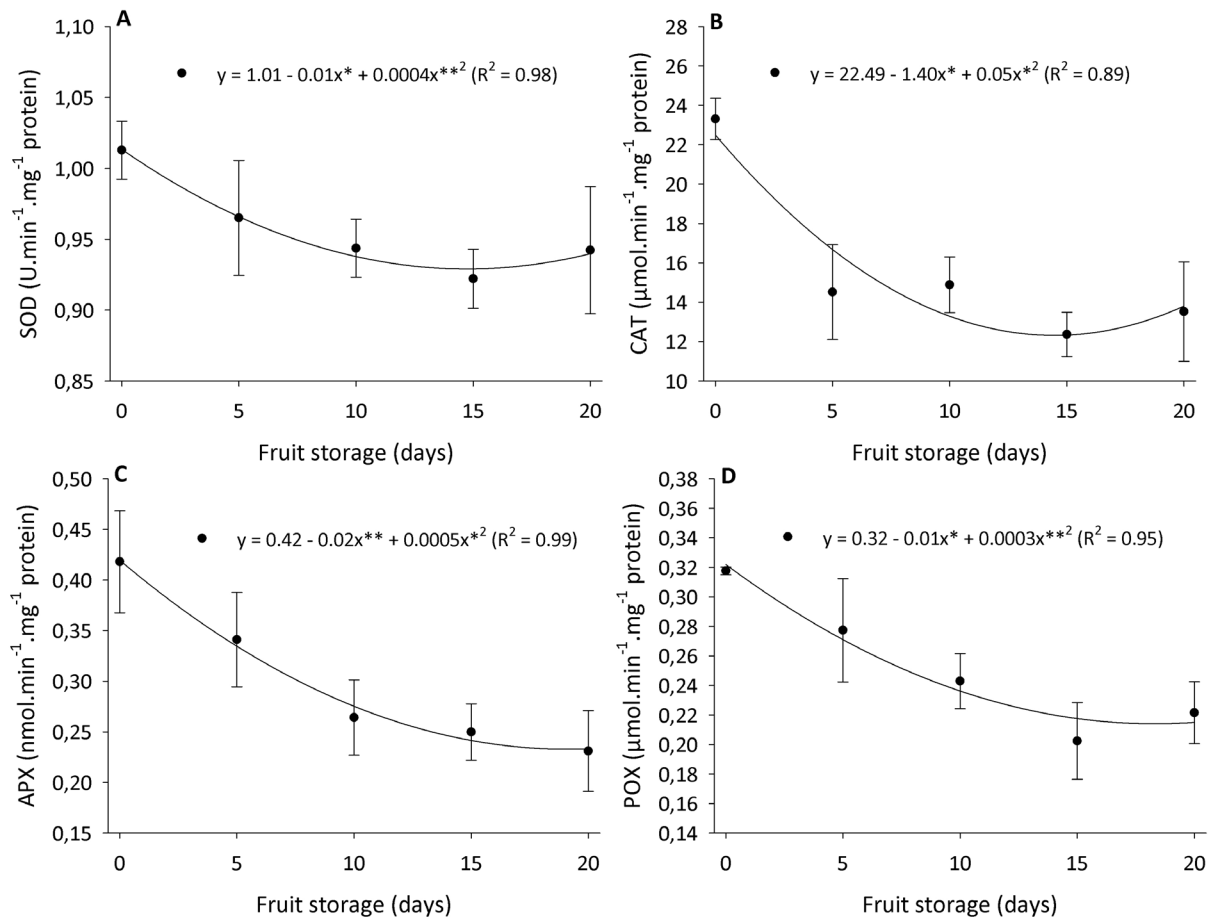
In summary, the continuous loss of water (Figure 2A) was directly reflected in the reduction in the weight of seeds (Figure 2B) obtained from stored fruit, above all in the seeds obtained from the fruit stored for 20 days. As already mentioned, considering the seeds obtained from the fruit stored for 5 and 10 days, the similarity among these treatments in regard to reduction in moisture content (Figure 2A), 100-seed weight (Figure 2B), germination and first germination count (Figure 2C), and electrical conductivity (Figure 2D) may be related to the seed physiological maturity observed in these treatments (Figure 3A).

The deterioration process is directly affected by factors such as genotype and environment (Ellis, 2019), and generally intensifies after the point of seed physiological maturity (Shaban, 2013). Thus, at the time of 20 days of post-harvest fruit storage, there was a reduction in 100-seed weight (Figure 2B) along with a reduction in germination (Figure 2C), an increase in electrical conductivity (Figure 2D), and reduction in dry matter and protein content (Figure 3), confirming an intensification in the deterioration process of the seeds obtained mainly after 10 days of fruit storage.

The post-harvest storage of fleshy fruit is a strategy adopted by producers for the purpose of making seeds more uniform and improving the physiological quality of the seeds after harvest. As also found in this study, *C. baccatum* seeds obtained from fruit with intense red coloring and kept at rest for 10 days achieved their maximum physiological potential (Pereira et al., 2014; Gonçalves et al., 2018). It is related that *C. chinenses* seeds obtained from yellow and orange fruits exhibit higher physiological quality, as well as low seed dormancy. Furthermore, the storage of yellow and orange fruits after harvested per 7 or 14 days before seed extraction is a good alternative to improve the seed physiological quality of this specie (Medeiros et al., 2020). For *C. annum*, depending on the level of fruit maturity, storage for 10, 14, 15, and up to 20 days contributed to better expression of seed germination and vigor (Alan and Eser, 2008; Vidigal et al., 2009; Martínez-Muñoz et al., 2019). In contrast, *C. pubescens* seeds removed soon after fruit harvest had better germination and vigor compared to seeds kept in the fruit for up to 17 days. This variation in results shows the wide genetic variability among different species of the *Capsicum* genus (Keyhaninejad et al., 2014).

Regardless of the antioxidant enzyme evaluated, the greatest activities were observed in the seeds obtained from the unstored fruit (0 days), followed by reduction in activity up to 20 days of post-harvest storage of the fruit. These reductions in enzyme activity were generally less accentuated in SOD (Figure 4A) and more accentuated in the enzymes CAT (Figure 4B), APX (Figure 4C), and POX (Figure 4D).

According to Kranner et al. (2010), the response to stresses in seeds can basically be defined as post-translational modifications and signaling of stress through cross-talk between reactive oxygen and nitrogen species and seed hormones, which result in changes in the transcriptome. These authors also mention that when the protection and repair mechanisms fail, depending on the dose and the time of exposure to stress, the result is the death of the seeds. Another important concept to be considered is the “oxidative window for germination”, that basically defines the critical levels of ROS threshold. In this sense, when above or below the “oxidative window for germination” (weak or high amounts of ROS), germination cannot occur (Bailly et al., 2008). Therefore, the ROS are related to secondary metabolism and, at high concentrations (upper limit of the oxidative window), they affect cell homeostasis and cause serious damage, especially in lipids, proteins, and nucleic acids (Li et al., 2017). However, at basal concentrations (within the oxidative window), the ROS are considered to be important molecules that act in processes such as cell signaling, gene expression, control of redox status, and, that way, the imbalance between the production of ROS and the antioxidant mechanisms is what in fact characterizes oxidative stress (Bailly et al., 2008; Mittler, 2017; Kapoor et al., 2019). In this context, although the high activity of antioxidative enzymes (indicating the metabolic activation), the unstored seeds (0 days) presented low germination and vigor (Figure 2). In addition to the probable dormancy of these seeds (as already discussed), these results indicate a level of reactive oxygen species (ROS) in which the enzymes were not able to neutralize. Therefore, greater antioxidant activity, together with lower germination and vigor, could be related to greater oxidative stress in the seeds obtained from the unstored fruit (0 days) (Figure 4).



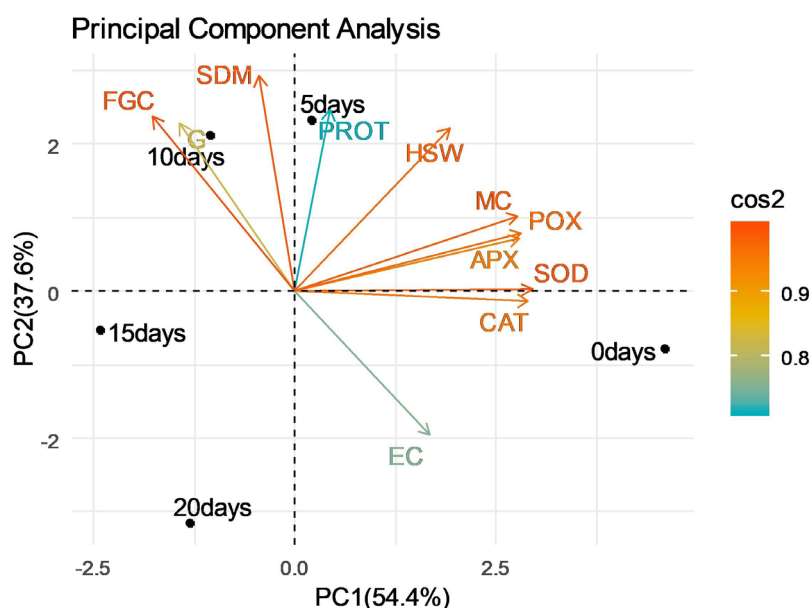
** , *Significant at 1% and 5% by the t-test, respectively. Bars: standard deviation.

Figure 4. Activity of the enzymes superoxide dismutase (SOD) (A), catalase (CAT) (B), ascorbate peroxidase (APX) (C), and peroxidase (POX) (D) in *C. baccatum var. baccatum* seeds from fruit under different post-harvest storage times.

Moreover, the intermediate antioxidant activity in the seeds obtained from the fruit stored for 5 and 10 days, together with greater germination and vigor, indicate the balance between the ROS and these mechanisms, where the seeds are able to express their maximum physiological potential. In contrast, the low antioxidant activity in the 15- and 20-day treatments indicates inefficiency of the antioxidant mechanisms in neutralizing the excess of ROS, with a direct reflection on reduction in germination (Figure 2C), in electrical conductivity (Figure 2D), in dry matter (Figure 3A), and in protein content (Figure 3B) in these treatments. These results are similar to those observed by Araújo et al. (2018) in *C. frutesces* and *C. chinense* seeds, where post-harvest storage of the fruit resulted in reduction in CAT activity, which, for its part, was highly related to the physiological quality of the seeds. Another fact that may be related is the significant reduction in moisture content during fruit storage (Figure 2A), especially at 15 and 20 days, which is generally associated with greater ROS production, causing cell damage in seeds during the seed drying process (Bewley and Nonogaki, 2017).

Principal component analysis (PCA) shows that component 1 (PC1) and 2 (PC2) explained 54.4% and 37.6%, for a total of 92% of the total variability of the data (Figure 5). It can be considered efficient for explaining the total variability of the data observed, since the sum of the PC1 and PC2 components was greater than 80% (Jolliffe and Cadima, 2016).

The treatments corresponding to the seeds obtained from the fruit stored for 5 and 10 days were in the region of the positive scores of PC2, near the corresponding vectors of first germination count, germination, seed dry matter, and protein content. The treatments corresponding to the seeds from the fruit stored for 15 and 20 days were in the



PC1 – Principal component 1; PC2 – Principal component 2; MC – moisture content; HSW – 100-seed weight; EC – electrical conductivity; G - germination; FGC – first germination count; SDM – seed dry matter; PROT – protein content; SOD – superoxide dismutase; CAT – catalase; APX – ascorbate peroxidase; and POX – peroxidase.

Figure 5. Biplot obtained from the linear combination of the physiological and biochemical variables in *C. baccatum* var. *baccatum* seeds from fruit under different post-harvest storage times.

region of the negative scores of PC2, near the corresponding region of the electrical conductivity vector, indicating lower seed vigor under these conditions. In a similar manner, seeds from the unstored fruit (0 days) were also in the region of the negative scores of PC2; however, they were nearer to the vectors corresponding to the antioxidant enzymes. In addition, there is a greater distance of the 20 days treatment from the vectors corresponding to physiological quality and the vectors corresponding to the antioxidant enzymes compared to the other treatments (0, 5, 10, and 15 days) (Figure 5).

In general, the proximity of the vectors of germination, first germination count, dry matter, and protein content of the 5 and 10 days treatments confirm the greater physiological quality of the seeds obtained from these treatments. In contrast, the presence of the 0, 15, and 20 days treatments in the negative scores of the PC2, together with the electrical conductivity vector, show that these conditions (both the lack of storage and storage for more than 10 days) negatively affect the physiological quality of the seeds (Figure 5).

Thus, PCA clearly illustrate the results observed, reinforcing not only the beneficial effects of post-harvest storage of the fruit on the quality of *C. baccatum* var. *baccatum* seeds, but also the 5 and 10 days times as ideal for this storage. These results can serve as a basis for future studies on *C. baccatum* var. *baccatum*, such as studies on seed maturation and dormancy, as well as on different conditions of post-harvest storage (such as temperature and relative humidity) of fruit and evaluation of non-enzymatic antioxidant mechanisms.

CONCLUSIONS

Post-harvest storage of ripe fruit of *C. baccatum* var. *baccatum* for 5 and 10 days at 20 °C improves the physiological potential of its seeds and it is also related to their physiological maturity.

The incomplete maturation of *cumari* pepper seeds obtained from unstored fruits (0 days) is related to greater antioxidative enzyme activity, greater oxidative stress, and low germination and vigor.

The lack of post-harvest storage of fruit and storage of fruit for longer than 10 days contribute to reduction in the physiological potential of *cumari* pepper seeds.

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