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Trolox equivalent antioxidant capacity of *Coffea arabica* L. seeds

Capacidade antioxidante equivalente ao trolox em sementes de *Coffea arabica* L.

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ABSTRACT

The causes of the low desiccation tolerance and low longevity of coffee seeds have not yet been fully elucidated, and a full understanding of their complex physiology is of great interest. Among several alternatives, the loss of antioxidant capacity in seeds may be related to their rapid loss in quality during storage. The objective of this study was to determine the total antioxidant capacity of coffee harvested at different ripeness stages before and after the storage of seeds with different water contents and to relate antioxidant capacity to physiological quality. Seeds in the greenish-yellow or cherry stages, recently harvested or stored for nine months at 10 °C with 40, 30, 20 and 12% water content (wet basis – wb), were submitted to physiological and biochemical quality evaluations, and the Trolox equivalent antioxidant capacity (TEAC) was determined. The germination and root protrusion of coffee seeds from greenish-yellow and cherry fruits were not affected by drying, but seeds harvested at physiological maturity had greater vigor when the moisture content was lower. The quality of coffee seeds decreased during storage, and this decrease was greater in seeds stored with higher water contents. Coffee seeds in the greenish-yellow stage had a higher antioxidant capacity than those in the cherry stage when recently harvested, but there was a substantial reduction in this capacity during storage at both maturation stages. Coffee seed deterioration is related to a reduction in antioxidant capacity, and the isoenzymatic profiles of the antioxidant process are little affected by the seed maturation stage. The deterioration of coffee seeds during storage is related to a reduction in their total antioxidant capacity, regardless of their maturation stage, being more pronounced in the greenish-yellow stage.

Index terms: TEAC; coffee; storage potential; physiological quality.

RESUMO

As causas da baixa tolerância à dessecação e baixa longevidade de sementes de café não foram ainda completamente elucidadas e o entendimento da sua complexa fisiologia é de grande interesse. Dentre várias alternativas, a perda da capacidade antioxidante nas sementes pode estar relacionada à rápida perda de qualidade durante o armazenamento. O objetivo neste estudo foi determinar a capacidade antioxidante total em café colhido em diferentes estádios de maturação, antes e após o armazenamento das sementes com diferentes teores de água, relacionando à qualidade fisiológica. Sementes nos estádios verde-cana ou cereja, recém colhidas ou armazenadas por nove meses em 10 °C com 40, 30, 20 e 12% de teor de água (bu), foram submetidas à avaliação da qualidade fisiológica, bioquímica e à determinação da capacidade antioxidante equivalente ao Trolox (TEAC). Observou-se que a germinação e a protrusão radicular de sementes de café nos estádios verde-cana e cereja não são afetadas pela secagem, mas sementes colhidas na maturidade fisiológica tem maior vigor com a redução da umidade. A qualidade das sementes de café é reduzida durante o armazenamento, sendo esta redução maior em sementes armazenadas com teores de água mais elevados. Sementes de café no estádio verde-cana tem maior capacidade antioxidante do que no estádio cereja quando recém colhidas, porém ocorre redução drástica desta capacidade durante o armazenamento, em ambos estádios de maturação. Em sementes de *Coffea arabica* L., os perfis isoenzimático do processo antioxidativo são pouco influenciados pelos estádios de maturação das sementes. A deterioração das sementes de café durante o armazenamento está relacionada à redução de sua capacidade antioxidante total, independentemente do estágio de maturação, sendo esta mais pronunciada no estágio verde-cana.

Termos para indexação: TEAC; café; potencial de armazenamento; qualidade fisiológica.

INTRODUCTION

The production of arabica coffee seedlings is, in its entirety, obtained by sexual propagation. Thus, the use of seeds with high physiological quality is considered one of the main factors for obtaining vigorous seedlings. Seedlings can be obtained from seeds harvested in the same year or stored from the previous harvest (Carvalho; Almeida; Guimarães, 2014). However, the use of stored seeds is a major challenge, as they may lose viability based on the maturation stage at harvest (Veiga et al., 2007), cherry processing method (Rodriguez; Guzman; Hernandez, 2020), and drying environment and method (Vieira et al., 2007), among other factors.

Coffee fruits mature slowly, requiring six to eight months to complete their cycle and transition to the cherry maturation stage, which is commonly used for seedling production. One way to accelerate the seed and seedling production process is to harvest early, when the fruits are still in the greenish-yellow stage. However, seeds in the greenish-yellow stage usually have lower physiological quality and lower storage potential (Veiga et al., 2007; Vieira et al., 2007; Baliza et al., 2012).

Greenish-yellow coffee fruits have antioxidant activity due to the presence of phenolic compounds, such as chlorogenic acids and related compounds (Farah; Donangelo, 2006). Farah and Donangelo (2006) concluded that genetic factors, maturation stage, environmental conditions and agricultural practices are determinant factors for the composition of chlorogenic acids in greenish-yellow coffee beans and may affect the final composition of the beverage. Montavon et al. (2003) observed that coffee beans in the greenish-yellow stage are more sensitive to oxidation and have significantly higher chlorogenic acid levels than do coffee fruits in the cherry stage and that the lower sensitivity of the beans occurs because the defense mechanisms against oxidative stress become more efficient during maturation.

Coffee seeds are considered intermediate with regard to desiccation tolerance and storage behavior (Ellis; Hong; Roberts, 1990) because, compared to recalcitrant seeds, they withstand drying considerably but not as much as orthodox seeds (Dussert et al., 2006). In addition, several studies have been conducted on the ideal water content for coffee seed storage, but this has not yet been fully elucidated (Hong; Ellis, 1992; Guimarães et al., 2002; Rosa et al., 2005; Veiga et al., 2007; Vieira et al., 2007; Penido et al., 2021).

Coffee seed quality is assessed through official germination and tetrazolium tests (Brasil, 2009) however, such assessments can be complemented by biochemical tests that evaluate enzymatic systems. The evaluation of the activity of enzymes involved in some metabolic pathways can aid in interpreting physiological results and in elucidating which processes are involved in desiccation and storage tolerance (Coelho et al., 2015; Coelho; Rosa; Fernandes, 2017; Abreu et al., 2018; Coelho et al., 2019; Figueiredo et al., 2021).

The activity of free radical scavenging antioxidant system components is usually evaluated in isolation and sometimes may not represent the actual seed quality because these system components have additive effects (Barbosa et al., 2014) and work together to protect the organism during stressful situations caused by the environment (You; Chan, 2015). Thus, a method that allows evaluating the antioxidant pool, such as determining the total antioxidant capacity, may better reflect the physiological potential of seeds. Such methods include the Trolox equivalent antioxidant capacity (TEAC), which employs a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution as a standard and is based on the ability of antioxidants to react with the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$) radical (Gliszczynska-Swiglo, 2006).

Although the quality of coffee seedlings largely depends on the quality of the seeds used, there are few studies on the influence of the seed maturation stage and adequate seed storage methods or on the antioxidant activity of seeds. Given the above, the aim of this study was to determine the total antioxidant capacity before and after the storage of coffee seeds harvested at different maturation stages and dried to different water contents.

MATERIAL AND METHODS

Coffee fruits were harvested at a field belonging to the Federal University of Lavras (Universidade Federal de Lavras - UFLA) and processed in facilities at the Coffee Growing Sector. The physiological evaluation of the seeds was performed at the Central Seed Laboratory of UFLA, and the analysis of the antioxidant capacity was performed at the Department of Horticulture and Crop Science of Ohio State University.

Seeds of *Coffea arabica* L., cultivar Rubi, from fruits harvested at the greenish-yellow or cherry stage, selectively collected from the middle branches of the plants and from the middle part of these branches, were used. After harvest, the cherries were mechanically peeled, and the seeds were demucilaged by fermentation in water

for 24 hours at room temperature and then predried in the shade to remove surface moisture.

The seeds were dried on activated silica gel to water contents of 12, 20, 30 and 40% (wet basis - wb). After reaching the desired moisture levels, the seeds were divided into two portions: one portion was subjected to physiological quality and antioxidant capacity evaluations, and the other portion was packed in hermetic containers and stored in a cold chamber (10 °C and 55% relative humidity) for nine months. After this storage period, the seeds were subjected to the same aforementioned evaluations.

To evaluate physiological quality, the germination test was performed with four replicates of 50 seeds per treatment. The seeds were sown in germination paper rolls moistened with distilled water at an amount of two and a half times the dry paper weight. Acrylic plates were used for sowing, and the paper rolls containing the seeds were kept in a germinator at 30 °C in the presence of light (Brasil, 2009). Fifteen days after beginning the test, the percentage of seeds with primary root protrusion, i.e., those with lengths equal to or greater than 2 mm, was determined. After 30 days, the germination percentage was evaluated, considering only normal seedlings based on the RAS (Brasil, 2009). Forty-five days after beginning the germination test, the percentage of seedlings with expanded cotyledonary leaves was evaluated. Throughout the germination test, daily counts of seeds with primary roots with lengths equal to or greater than 2 mm were performed, and the mean germination time was calculated based on the method described by Labouriau (1983).

The total antioxidant capacity of the seeds was evaluated by determining the TEAC. The seeds were macerated in liquid nitrogen and stored at -80 °C until the evaluation was performed. Three replicates of 200 mg of seeds ground post-lyophilization were used; the replicates showed uniform water contents between treatments after lyophilization. Extractions in methanol were performed in two stages. First, 2 mL of methanol was added to the samples, which were sonicated for 20 minutes and centrifuged for 10 minutes. The supernatants were removed, and the procedure was repeated to improve the extraction efficiency. Then, the collected supernatants were diluted at a 1:10 ratio, and the antioxidant capacity was determined based on the method described by Re et al. (1999) and Cai et al. (2004), with modifications. The $ABTS^{+}$ radical was produced in a buffer solution (pH 7) with 7 mM ABTS and 2.45 mM potassium persulfate and subsequently stored in the dark for 16 hours, generating a free radical solution.

After incubation, the $ABTS^{+}$ radical solution was diluted in buffer until an absorbance of 734 nm was observed. A 75-µL aliquot of the seed extract was added to 2925 µL of diluted $ABTS^{+}$. The solution was carefully homogenized, and after six minutes, the absorbance was evaluated at 734 nm in a spectrophotometer. To generate a standard curve, a solution of Trolox (5 mM) diluted in ethanol was used at concentrations of 1.25 µM, 2.5 µM, 5 µM, 7.5 µM, 10 µM and 15 µM. Seventy-five microliters of each of standard solution was added to 2925 µL of $ABTS^{+}$, in triplicate, and after six minutes, the absorbance was determined at 734 nm, obtaining a standard curve. The absorbances resulting from the extracts were compared with the standard curve, and the results were expressed in terms of TEAC in relation to the dry matter weight initially used (µM Trolox mg^{-1}).

For the biochemical analyses, the method proposed by Alfenas (2006) was used for the extraction, electrophoresis and visualization of the antioxidant enzymes catalase (CAT), esterase (EST) and malate dehydrogenase (MDH). The results of the evaluations before and after nine months of storage were separately subjected to analysis of variance.

The experimental design was completely randomized, in a 2 x 4 factorial arrangement, with two seed maturation stages (greenish-yellow and red) and four water contents (12, 20, 30 and 40% wb), in three replicates for the antioxidant capacity evaluations and four replicates for the physiological quality assessments. The results were subjected to analysis of variance, and the means were compared by the Scott–Knott test at the 5% probability level (Ferreira, 2014). The results obtained for the isoenzymes were interpreted based on the presence/absence and intensity of bands in the gel.

RESULTS AND DISCUSSION

Based on the analysis of variance results, there was a significant interaction between the factors seed maturation stage and water content for all germination test variables, except for the percentage of normal seedlings evaluated before storage. In the evaluation of the physiological quality of the seeds after storage, a significant interaction between factors was observed for all analyzed variables.

Regarding the results for root protrusion evaluated before storage, no significant differences were observed between the seeds at the two maturation stages studied, i.e., greenish-yellow and cherry (Figure 1A). Additionally, the seeds from fruits at the cherry stage showed greater vigor

than did the seeds from greenish-yellow fruits when they were dried to 12 and 20% water content (wb). Based on these results, seeds at the greenish-yellow stage had lower tolerance to desiccation. This behavior was also observed in other studies, such as Veiga et al. (2007), in which the authors observed a higher percentage of root protrusion in seeds from fruits than greenish-yellow fruits. In addition, the same authors found that coffee seeds with higher water content (53%) had lower root protrusion than did seeds subjected to drying up to 12%.

After storage, the seeds from cherry stage, with water contents of 12 and 20% wb, showed higher root protrusion (96 and 94%, respectively). However, in seeds with higher water contents, the percentage of root protrusion was lower than that in the other seeds, reaching 82 and 64% at water contents of 30 and 40% wb, respectively (Figure 1B). For the seeds from greenish-yellow fruits, the lowest root protrusion was observed in the seeds with a water content of 30% wb; the others did not differ significantly from each other. Veiga et al. (2007) did not find significant differences between maturation stages during the storage period; however, in the same study, coffee seeds with lower water content had a higher percentage of root protrusion than those that were not dried.

For the germination percentage of the seeds evaluated before storage, no significant interaction between the factors was observed, with no differences between the maturation stages or among the water contents, with an overall mean of 82% normal seedlings. Similar results were observed by Veiga et al. (2007), who evaluated seeds of *Coffea arabica* cv. Rubi harvested at different maturation stages and subjected to different drying methods.

After storage, the seeds from fruits at the cherry stage had a higher percentage of germination than did seeds from greenish-yellow fruits at all evaluated water contents, except for the seeds stored at 40% water content (wb) (Figure 2). At the greenish-yellow maturation stage, seeds with a water content of 40% (wb) had a higher percentage of normal seedlings, based on the germination test, than did seeds at the other moisture contents studied. Santos et al. (2014) also found similar results for the percentage of normal seedlings at 30 days, which was higher in coffee seeds at the cherry stage than at the greenish-yellow stage. Guimarães et al. (2002) also observed higher germination values for coffee seeds at more advanced maturation stages, close to physiological maturity.

The results for the percentage of seedlings with expanded cotyledonary leaves determined before storage (Figure 3A) indicated that the seeds from greenish-yellow fruits with lower water contents (12 and 20% wb) had lower values than did the wetter seeds (30 and 40% wb), a finding that can be explained by possible damage caused by drying of immature seeds. Coffee seeds harvested at stages more sensitive to desiccation do not tolerate lower moisture levels. Because the seeds from cherry fruits have greater tolerance to desiccation and probably a more developed antioxidant system, they showed higher vigor than those harvested at the greenish-yellow stage, more satisfactorily completing germination and the production of seedlings with expanded cotyledonary leaves.

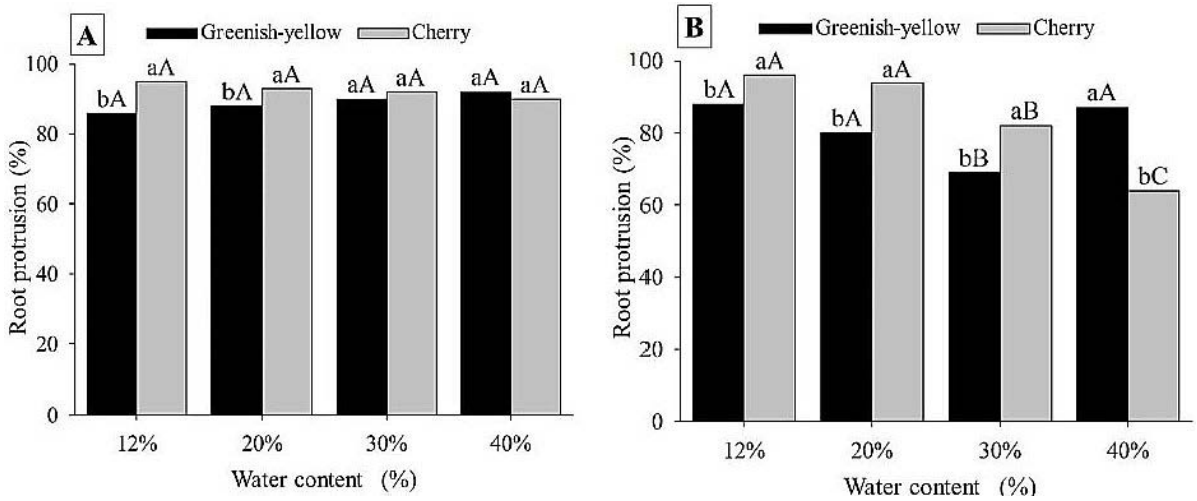

Figure 1: Percentage of root protrusion determined before (A) and after (B) storage, from seeds of *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents. Means followed by the same uppercase letter within each maturation stage and lowercase letter within each water content level do not differ significantly by the Scott–Knott test at the 5% probability level.

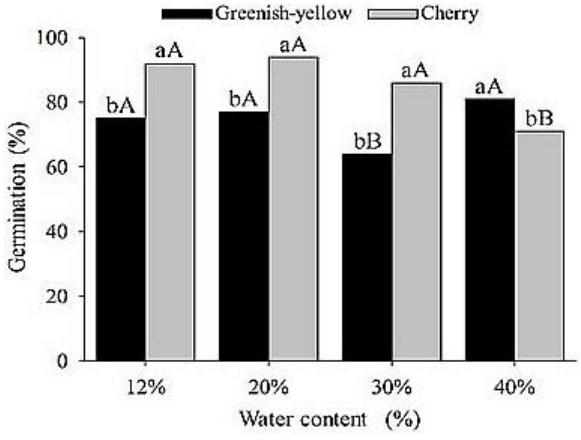

Figure 2: Percentage of germination determined after storage, in seeds of *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents. Means followed by the same uppercase letter within each maturation stage and lowercase letter within each water content level do not differ significantly by the Scott–Knott test at the 5% probability level.

The higher sensitivity to deterioration of seeds with a higher water content during storage at both maturation stages was confirmed by the vigor results. There was a reduction in the percentage of seedlings with expanded cotyledonary leaves as the water content in the stored seeds increased. After storage, the seeds from greenish-yellow fruits had a lower percentage of seedlings with expanded cotyledonary leaves only at a water content of 30% wb. For seeds from cherry fruits, the lowest percentage for the same variable was observed for seeds with higher water contents (30 and 40% wb) (Figure 3B). These data corroborate the results obtained by Coelho et al. (2015), who found a reduction in the number of expanded cotyledonary leaves after storage in a cold chamber, regardless of the seed water content or drying method. These findings can be explained by the high-water content of the stored seeds, which contributes to microorganism proliferation and reduced seed vigor. Clemente et al (2011) emphasized that the germination test in coffee seeds can be affected by the presence of fungi due to the prolonged germination test time of 30 days, which favors conditions for microorganism growth.

The worst physiological performance of the seeds at the two maturation stages occurred after storage of the seeds at a water content of 40% wb (Figure 4B). For the mean germination time, in general, although seeds from cherry fruits had higher means, they required more time to express maximum germination, regardless of whether they were stored or not (Figure 4).

After storage, there was worse performance of seeds with higher water contents, measured by the mean time to maximum germination, and this was even more evident for the seeds from cherry fruits, which showed an increased time to express maximum germination at higher water contents. Water content is the most significant factor in the prevention of deterioration during storage, and in coffee seeds, the optimal water content for storage has not fully defined. Some studies on coffee seeds found that the best moisture level for storing coffee seeds is between 9% and 11% (Hong; Ellis, 1992); however, other studies found acceptable results at moisture levels between 31% and 48% (Guimarães et al., 2002; Rosa et al., 2005). Furthermore, other studies concluded that coffee seeds can be stored both dry and wet (Veiga et al., 2007; Vieira et al., 2007; Penido et al., 2021). Regarding the results of the TEAC evaluation, in general, before (Figure 5-A) and after (Figure 5B) storage, there was an increase in the values as the seed water content was reduced at the two maturation stages. This behavior can be attributed to the fact that while the water content in the seeds is high (before drying), the main source of free radicals is mitochondria, requiring an effective antioxidant defense apparatus to protect this organelle (He et al., 2016). With the reduction in the water content of the seeds, mitochondrial metabolism decreases, initiating the uncontrolled production of free radicals, causing lipid peroxidation (He et al., 2016), which in turns can damage cell membranes, reducing seed quality. Thus, an efficient antioxidant system is essential for the seed to protect itself and maintain its quality, which can be observed in seeds with lower water contents.

According to and Smolikova et al. (2020), lipid peroxidation occurs in all cells, but in fully imbibed cells, water acts as a buffer between free radicals and the macromolecules that are the target of attack, thus reducing the level of damage. Therefore, in seeds with a high water content, it is expected that antioxidant enzymes do not play a protective role, eliminating free radicals, but rather that other mechanisms are involved in this protection.

Seeds from fruits harvested at the greenish-yellow stage showed, before storage, a higher antioxidant capacity than did seeds from fruits harvested at the cherry stage at all water contents (Figure 5A). In general, this lower antioxidant capacity in cherry fruits may be related to the high metabolic rate required to neutralize free radicals during root protrusion, as shown in Figure 1-A. Celli, Pereira-Netto and Beta (2011) commented that as fruits ripen, the content of phenolic compounds, which are considered the main components that determine the antioxidant capacity in plants, is reduced.

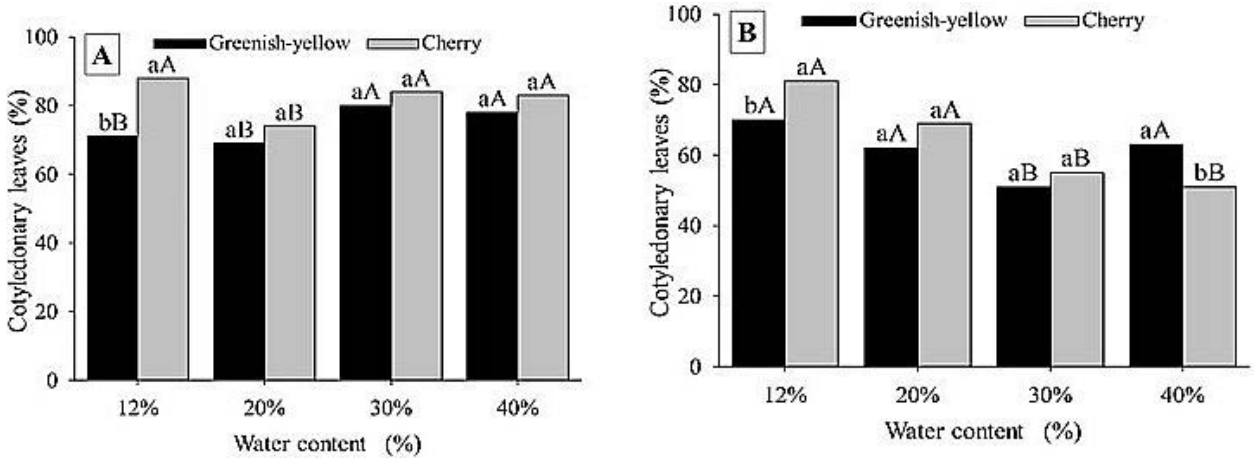

Figure 3: Percentage of seedlings with expanded cotyledonary leaves determined before (A) and after (B) storage in seeds of *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents. Means followed by the same uppercase letter within each maturation stage and lowercase letter within each water content level do not differ significantly by the Scott–Knott test at the 5% probability level.

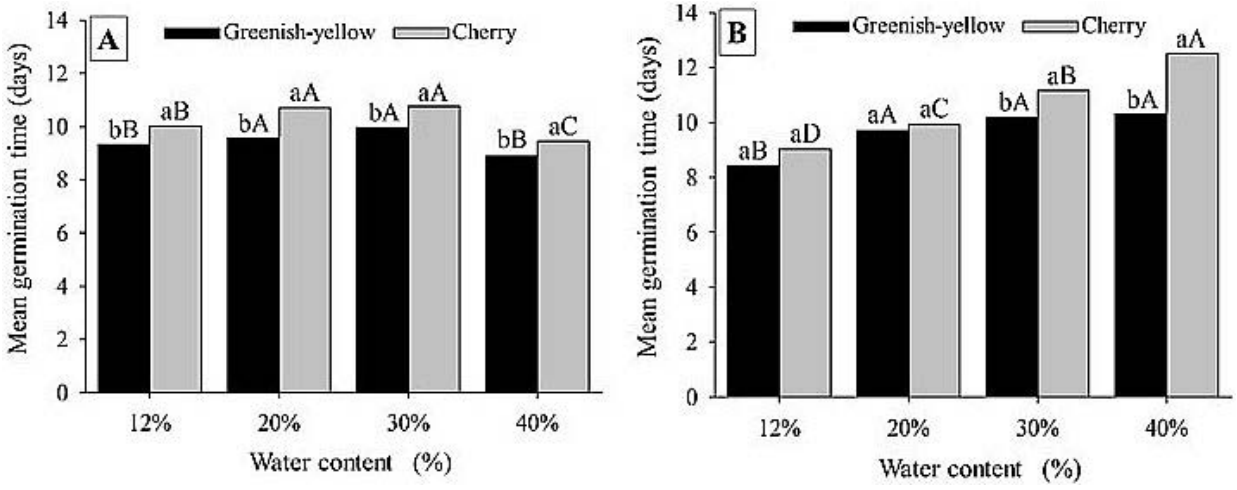

Figure 4: Mean germination time measured before (A) and after (B) storage of *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents. Means followed by the same uppercase letter within each maturation stage and lowercase letter within each water content level do not differ significantly by the Scott–Knott test at the 5% probability level.

However, in stored seeds, there was a marked decrease in the antioxidant capacity at both maturation stages, in addition to an inversion relative to the results before storage, with the seeds from cherry fruits showing higher antioxidant capacity values. The reduction in antioxidant capacity in seeds at both maturation stages and at all water contents studied is most likely due to seed deterioration during storage and a reduction in the antioxidant pool, with this reduction being greater in seeds at the greenish-yellow stage. This can also be attributed to the fact that the seeds from greenish-yellow fruits have not fully developed and established an antioxidant system capable of repairing or minimizing the possible damage caused by deterioration during storage, which may be the determining factor for the lower percentage of root protrusion, germination and seedlings with expanded cotyledonary leaves in stored seeds from greenish-yellow stage (Figures 1B, 2B and 3B).

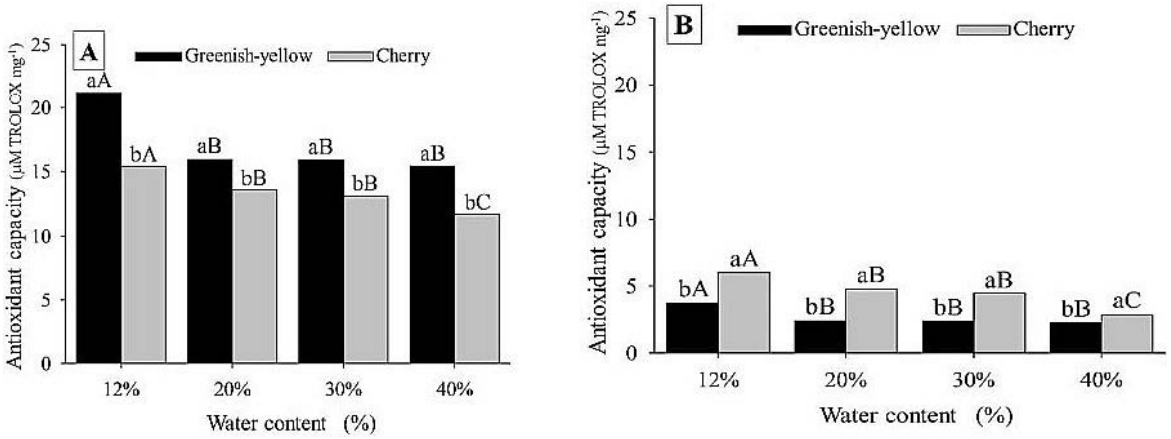

Figure 5: Antioxidant capacity measured before (A) and after (B) storage in *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents. Means followed by the same uppercase letter within each maturation stage and lowercase letter within each water content level do not differ significantly by the Scott–Knott test at the 5% probability level.

Acidri et al. (2020) compared the phytochemical composition of coffee plant organs and their corresponding antioxidant capacity with those of green and roasted coffee beans and found that green coffee beans have higher amounts of phytochemicals, especially chlorogenic acid, sucrose, caffeine and trigonelline. In coffee seeds, the main compound accumulated during fruits maturation is chlorogenic acid, which is involved in the defense against oxidative stress (Acidri et al., 2020; Farah et al., 2008). With the progression of cherry maturation, the chlorogenic acid content decreases by up to 7% or more based on the consolidated development of the antioxidant system and reduction in the activities of polyphenol peroxidase and oxidase, reducing the action of phytochemicals in the defense against reactive oxygen species (Montavon et al., 2003).

The biochemical and physiological basis of the behavior of intermediate seeds during storage was investigated by Dussert et al. (2006), who evaluated the effects of drying and storage on the viability and antioxidant content, represented by lipids and sugars, of coffee seeds. The authors concluded that coffee seed deterioration during storage is also associated with antioxidant loss and oxidation through irreversible structural changes in the cell membrane as well as loss of cell compartmentalization.

Regarding the enzymatic profiles studied before and after storage of the coffee seeds, variations in the intensity of the isoforms were observed as a function of the different water contents and maturation stages (Figure 6). In coffee seeds, enzymatic profiles are commonly used to complement quality evaluations under different stress situations, especially in seeds with different water contents (Brandão Júnior; Vieira; Hilhorst, 2002; Coelho et al., 2015; Coelho; Rosa; Fernandes, 2017; Abreu et al., 2018; Coelho et al., 2019; Figueiredo et al., 2021). It is evident in the isoenzymatic profiles of catalase, esterase and malate dehydrogenase that there was a greater band intensity for seeds with water contents below 30% at both maturation stages.

Catalase expression was affected by the different seed water contents (Figure 6A). This result was also reported by Figueiredo et al. (2021), who found higher catalase activity in coffee seeds dried up to 17%, which maintained their initial high physiological quality after immersion in liquid nitrogen. In the present study, in seeds from fruits harvested at the greenish-yellow and cherry stages, there was an increase in catalase activity as the water content decreased, regardless of storage (Figure 6 A1 and A2). Catalase has been widely investigated in studies on coffee seeds as an indicator of physiological quality, but the results differ in relation to the seed water content studied and their physiological performance (Santos et al., 2014; Coelho et al., 2015; Coelho, Rosa; Fernandes, 2017; Figueiredo et al., 2021).

Catalase is involved in the removal of hydrogen peroxides (H_2O_2) from cells, and its higher activity may be related to the reduction in mechanisms for the prevention of oxidative damage (Nandi et al., 2019). The stress caused to the seeds by drying leads to an increase in the production of reactive oxygen species and stimulates hydrogen peroxide generation. This process most likely stimulated the increased expression of the catalase isoenzyme complex during drying, explaining the higher activity in the driest seeds. Brandão Júnior, Vieira and Hilhorst (2002) observed a decrease in catalase activity in coffee seeds that showed lower physiological performance resulting from desiccation.

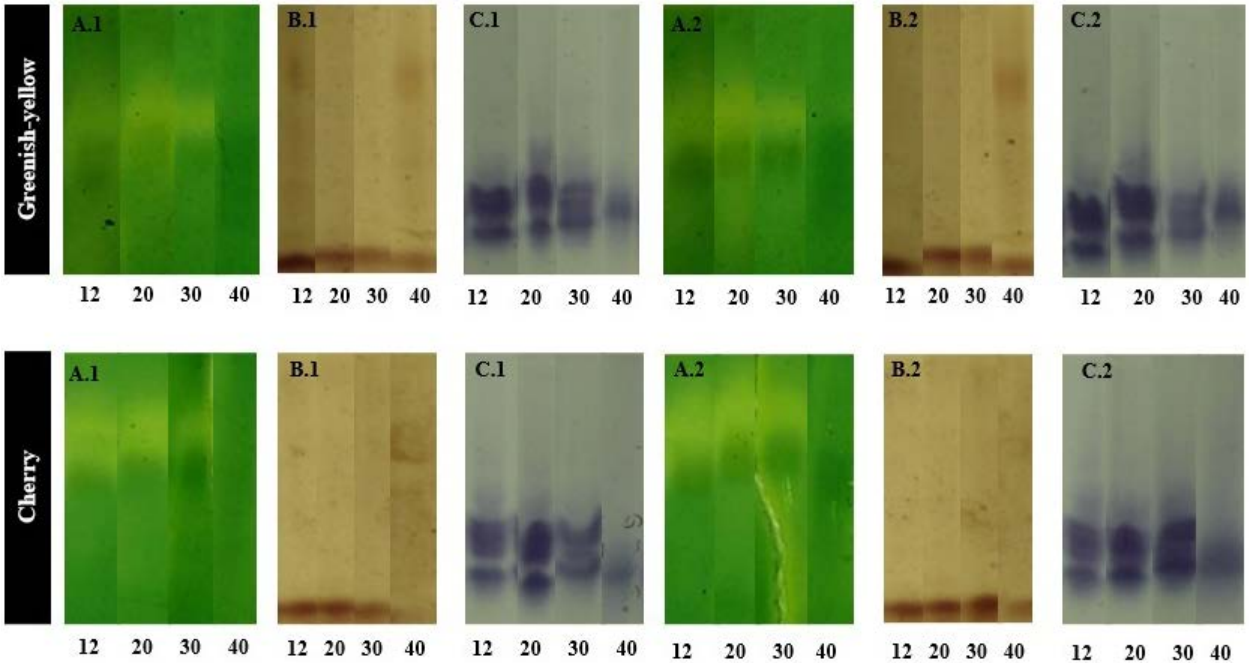

Figure 6: Electrophoretic expression of catalase (A), esterase (B) and malate dehydrogenase (C) before (1) and after (2) storage in seeds of *Coffea arabica* L., cv. Rubi, harvested at two maturation stages and dried to different water contents.

Regarding esterase expression in seeds from fruits harvested at the greenish-yellow and cherry stages, before storage, there was low expression and even an absence of bands at water contents above 30% (Figure 6 B1). However, after storage, as the water content of the seeds decreased, there was an increase in esterase activity, regardless of the maturation stage (Figure 6, B2). Esterase participates in ester hydrolysis reactions and can also act on membrane phospholipids (Taiz; Zeiger, 2013). According to Andrade et al. (2018), esterase is a good indicator of seed deterioration, with an important catalytic function in cell detoxification, and its higher expression may be associated with greater disruption of cell membranes during drying.

Malate dehydrogenase expression was observed at all water contents studied in seeds from fruits harvested at the greenish-yellow stage, regardless of storage. Conversely, in seeds from fruits harvested at the cherry stage, expression was observed at all water contents; however, the highest expression was observed at 30 and 20% wb (Figure 6 C1 and C2). Malate dehydrogenase is part of the respiratory pathway and plays an important role in the catalysis of the conversion of malate to oxalate in the last reaction of the Krebs cycle (Taiz; Zeiger, 2013), and high respiratory activity is related to greater deterioration.

In all enzymatic systems evaluated in the present study, in the treatments characterized by higher seed water contents, enzymatic expression was very low or almost nonexistent because in hydrated cells, water acts as a buffer between free radicals and target macromolecules, thus reducing damage (McDonald, 2004).

The present study involved physiological and biochemical analyses related to the conservation of coffee seeds; these analyses allowed the detection of some transformations that occur during this process. Analyzing all evaluations, better physiological and biochemical results were observed for coffee seeds harvested at the cherry maturation stage, and seed quality was reduced during storage, especially in moister seeds.

CONCLUSIONS

The germination of coffee seeds harvested at the greenish-yellow and cherry stages was not affected by drying, but seeds harvested at physiological maturity had greater vigor after moisture reduction. The quality of coffee seeds decreased during storage, and this reduction was greater in seeds stored at higher water contents. Coffee seeds at the greenish-yellow stage had a higher antioxidant capacity than those at the red stage when

recently harvested, but there was a drastic reduction in this capacity during storage at both maturation stages. The deterioration of coffee seeds during storage is related to a reduction in their total antioxidant capacity, regardless of their maturation stage, being more pronounced in the greenish-yellow stage. In *Coffea arabica* L. seeds, the isoenzymatic profiles of the antioxidant process were little influenced by the seed maturation stage.

AUTHOR CONTRIBUTION

Data Collection, and Data analysis and interpretation, and Writing and editing: Ferreira, I.A. and Fávaris, N.A.B.F.; Conceptual idea, and Methodology design, and Supervision of the experiment, and Co-work of the manuscript: Rosa, S.D.V.F.; Review and approval of the final version of the manuscript: Coelho, S.V.B, Ricaldoni, M.A and Costa, M.C.

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