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Physiological quality and antioxidant enzymes activity in eggplant seeds with different ages and resting periods after harvest

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ABSTRACT

Constant changes during the seed maturation process affect its final quality and the post-harvest fruit resting, which can improve seed physiological quality. Therefore, the objective of this study was to evaluate the physiological and enzymatic activities of eggplant seeds extracted from fruits harvested at different ages and resting periods after harvest. Fruits were harvested presenting different colors at 40, 50, 60, and 70 days after anthesis (DAA) and rested for 0 (freshly harvested fruits), 10, and 20 days. The experimental design was set as completely randomized, with four replications and evaluated seeds extracted from six fruits per plot. The resting periods in eggplant fruits harvested at less than 60 DAA improved the mass of 1000 seeds, germination, vigor, and protein content. Seeds with higher germination and vigor presented lower dismutase (SOD), catalase (CAT), and peroxidase (POX) enzymatic activity. The results indicated that the ideal moment of eggplant harvest for seed production is when the fruit reaches the yellowish-brown color corresponding to about 60 DAA; however, if the eggplant fruit is harvested earlier (40 or 50 DAA), it has to rest for about 10 to 20 days to achieve high physiological quality (germination and vigor).

Keywords: *Solanum melongena*, germination, seed vigor, seed maturation.

RESUMO

Qualidade fisiológica e atividade de enzimas antioxidantes em sementes de berinjela com diferentes idades e períodos de repouso após a colheita

Mudanças constantes durante o processo de maturação das sementes afetam sua qualidade final, assim como o repouso pós colheita dos frutos pode melhorar a qualidade fisiológica das sementes. O objetivo deste estudo foi avaliar a qualidade fisiológica e a atividade enzimática de sementes de berinjela provenientes de frutos com diferentes idades e períodos de repouso após a colheita. Os frutos foram colhidos apresentando cores diferentes aos 40, 50, 60 e 70 dias após a antese (DAA) e mantidos em repouso por 0 (frutos recém-colhidos), 10 e 20 dias. Foi utilizado delineamento experimental inteiramente ao acaso, com quatro repetições e foram avaliadas as sementes extraídas de seis frutos por parcela. O repouso dos frutos de berinjela colhidos com menos de 60 DAA possibilitou maiores massas de 1000 sementes, germinação, vigor e teor de proteínas das sementes. As sementes com maior germinação e vigor apresentaram baixa atividade enzimática da superóxido dismutase (SOD), catalase (CAT) e peroxidase (POX). Os resultados indicaram que o momento ideal da colheita é quando o fruto atinge a cor amarelada-marrom correspondente a cerca de 60 DAA; no entanto, se o fruto de berinjela for colhido precocemente (40 a 50 DAA), ele tem que permanecer em repouso por cerca de 10 a 20 dias para as sementes alcançarem alta qualidade fisiológica (germinação e vigor).

Palavras-chave: *Solanum melongena*, germinação, vigor de sementes, maturação das sementes.

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The eggplant (*Solanum melongena*) crop is adapted to hot environments, stopping plant development at low temperatures (10-12°C), and the culture is very sensitive to frost (Gürbüz *et al*., 2018).

The eggplant fruit is usually harvested for consumption before the ideal seed maturation point (Gürbüz *et al*., 2018); however, the fruit must reach maturity for seed production. The appropriate moment of eggplant fruit harvest may result in a high percentage of seed germination and vigor. Due to the indeterminate growth habit of the eggplant plants, the fruits do not ripen simultaneously, resulting in seeds with different maturity stages. This situation can decrease the quality of an eggplant seed lot. However, the post-harvest resting of the fruits can improve seed physiological quality in plants of the Solanaceae family (Nakada-Freitas *et al*., 2018). This period allows the seeds to complete the maturation process and reach high germination and vigor levels. A proper resting period after harvest could allow the early fruit harvest and decrease the time of fruits and seeds exposition to weather and the occurrence of insects and pathogenic microorganisms. However, seed deterioration begins after the fruit physiological maturity, and the fruits should not be harvested too old (Nakada-Freitas *et al*., 2018; Colombari *et al*., 2021).

Seed maturation and deterioration processes are accompanied by physiological, biochemical, physical, and cytological changes (Nakada-Freitas *et al*., 2018; Colombari *et al*., 2021). Biochemical analyses during seed development and maturation can be used as indicators of seed physiological maturity and have been studied in the maturation process of different horticultural species (Colombari *et al*., 2021). During plant development, the occurrence of free radicals in cells can generate lipid hydroperoxides by oxidative reactions; also, excessive levels of reactive oxygen species (ROS) can cause damage to the ribonucleic acid (RNA) and the deoxyribonucleic acid (DNA). Seeds have antioxidant defense systems to reduce the damage caused by ROS. Such a seed defense system is regulated by the enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Saisanthosh *et al*., 2018).

Identifying the best harvest point for fruits and the best resting period after harvest are important for seed producers and researchers to obtain high-quality seeds with high germination and vigor. Thus, the objective of this study was to evaluate the physiological quality and enzymatic activity of eggplant seeds extracted from fruits harvested at different ages and resting periods after harvest.

MATERIAL AND METHODS

The study was conducted in the experimental area of the Faculdade de Ciências Agronômicas (FCA), from Universidade Estadual Paulista (UNESP), located in São Manuel city, São Paulo state, Brazil (22°46'S, 48°34'W, 740 m altitude). Plants were cultivated in an arc-type greenhouse of 7×20 m, 3 m in height, and covered with a low-density polyethylene film (150 µm thickness). The greenhouse sides were kept open to allow the pollinator insects entry.

The Köppen's climate classification of this studied region is Cfa type (temperate, humid mesothermal). The average monthly temperatures (°C) were August = 22.4; September = 24.6; October = 23.3; November = 22.6; December = 24.9; January = 23.8 and February = 23.5.

The soil in the greenhouse is an Oxisol, and the results of soil chemical analysis, presented in the 0-0.2 m soil depth, were (before the planting date): pH $CaCl_2$ = 6.0; organic matter = 10 g/dm^3; P_{resin} = 166 mg/dm^3; H+Al = 19 mmol$_c$/dm^3; K = 4.1 mmol$_c$/dm^3; Ca = 52 mmol$_c$/dm^3; Mg = 9 mmol$_c$/dm^3; sum of bases = 65 mmol$_c$/dm^3; cation exchange capacity = 84 mmol$_c$/dm^3, and V = 80%. The soil was fertilized according to the recommendation of Raij *et al*. (1997), equivalent to 20, 160, and 60 kg/ha of N, P_2O_5, and K_2O, respectively.

Twelve treatments were evaluated in a 4 x 3 factorial scheme: four fruit harvest periods after anthesis [40, 50, 60, and 70 days after anthesis (DAA)] and three post-harvest resting periods (0, 10, and 20 days) before seed extraction. The experimental design was set as completely randomized, with four replications and seeds extracted from six fruits per plot.

Seeds of the F6RC1 line (Napoli group) were acquired from the breeding program of UNESP. Seeds were sown in polypropylene trays (162 cells) containing Carolina Soil® commercial substrate. The seedlings were transplanted to the greenhouse 40 days after sowing date (0.4 m wide and 0.2 m high) spaced 1 m between lines and 0.5 m between plants. All sprouts were removed until the appearance of the first flower. Other crop management activities were carried out in accordance with the recommendations for the eggplant crop (Filgueira, 2013).

Drip irrigation was installed under the black plastic that covered the seedbeds. The side-dress fertilization was divided into six applications every seven days, being included in the liquid solution 1 g of urea (CH_4N_2O) and 1 g of potassium chloride (KCl) per plant, starting 14 days after the seedling's transplant.

Forty-seven days after transplanting (DAT), the flowers were marked on the anthesis day. Plants with diverse fixed fruits were thinned to leave only three fruits per plant. According to the treatment, eggplant fruits were harvested at 40, 50, 60, or 70 DAA. The fruits presented different colors, according to Figure 1. The harvest periods and the corresponding colors were defined from a previous study's knowledge of the line. During the experimental period, the fruits were kept to rest in laboratory conditions (25°C, ventilated area).

The seed extraction followed the same methodology for all treatments. The eggplant fruits were opened manually longitudinally, and the seeds were removed and washed with tap water and distilled water. After washing, the seeds were left to dry in the shade on clay dishes for three days. The seeds were then packed in paper bags and placed in a dry chamber (40% RH, 20°C). After reaching constant water content (8%), the seeds were cleaned removing empty and damaged ones, using equipment that separates seeds by density (model 'De Leo Type 1').

The mass of 1000 seeds, percentage of processed seeds (clean seeds compared to the total seeds before processing), germination (%), and first count of germination (%) were evaluated. The levels of soluble proteins and the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) enzymes were estimated.

The mass of 1000 seeds was determined by weighing 1000 seeds (8% moisture) of each plot, using a scale (0.001 g precision). The germination was tested in the laboratory of vegetable seed production of the Departamento de Produção Vegetal (FCA-UNESP) according to the Seed Analysis Rules (Brasil, 2009), using a plastic box in a seed germinator at 20-30ºC, with the photoperiod adjusted to 8 hours light and 16 hours darkness. Two hundred seeds were evaluated (four repetitions of 50 seeds each). The germination count was based on seedlings considered 'normal' and was made daily until 14 days after sowing. The germination speed index (GSI) was calculated based on the daily germination count until the 14th day after sowing.

The biochemical characteristics were determined in the Laboratory of Ecofisiology Applied to Agriculture (LECA) of FCA-UNESP. The levels of soluble proteins were determined by spectrophotometry (595 nm) and estimated from the standard curve of bovine serum albumin (1 mg/mL) and expressed in mg/g of fresh mass. The superoxide dismutase (SOD) enzyme activity used photoreduced nitrotetrazolium blue chloride (NTB) and was determined by spectrophotometry (560 nm). The results were expressed in units (U) of SOD/mg of protein. The catalase (CAT) and peroxidase (POX) enzyme activities were determined using H_2O_2 and potassium phosphate buffer methodology, using a reading wavelength of 240 and 420 nm, respectively. CAT and POX were expressed as U/mg of protein.

The data were submitted to analysis of variance and Tukey test (5%) to compare fruit post-harvest rest and regression (5%) to verify the effects of maturation periods, using the software Sisvar®. Pearson correlation analysis (5%) among the evaluated characteristics was also performed using the software InfoStat®.

RESULTS AND DISCUSSION

The maximum values for 1000 seeds mass were estimated at 64, 61, and 58 DAA for 0, 10, and 20 rest days after harvest (RDAH), respectively (Figure 2A). During seed maturation, the mother plants transfer many substances to the seeds and consequently accumulate dry mass until the physiological maturity of the seeds (Marcos Filho, 2015). However, the mass of 1000 seeds decreased after those DAA indicated for each RDAH.

Only the mass of 1000 seeds at 40 and 50 DAA of the 10 or 20 RDAH presented higher values than the no-resting period (0 RDAH). After that

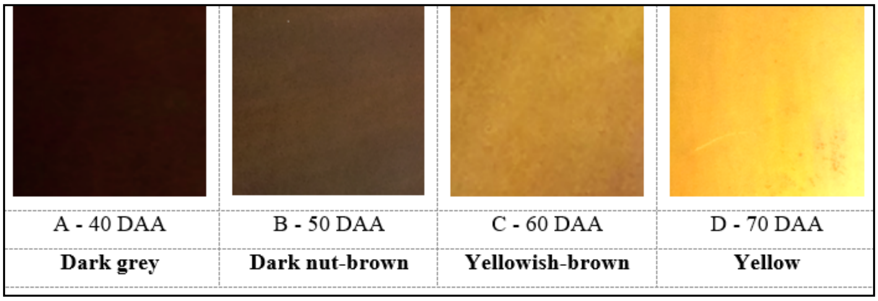

Figure 1. Colors of eggplant fruits harvested at 40 (A), 50 (B), 60 (C), and 70 (D) days after anthesis (DAA) (Photos from Aguilar, 2019). Botucatu, UNESP, 2021.

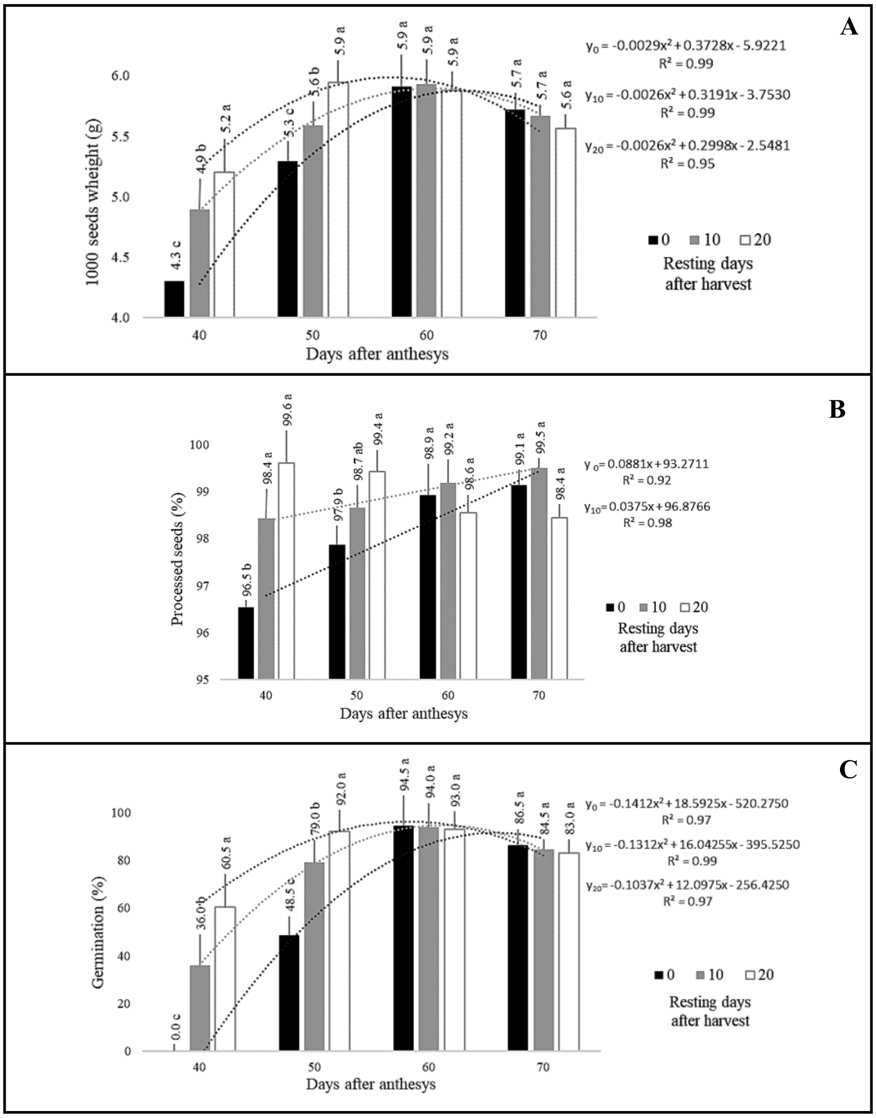

Figure 2. 1000 seed mass (A), percentage of processed seed (B), and seed germination (C) according to eggplant fruit age and times of post-harvest period. Averages followed by distinct letters in the same age (days after anthesis) differ by the Tukey test (5%). Resting period after harvest: 0 days = y0; 10 days = y10; 20 days = y20. Botucatu, UNESP, 2021.

period, at 60 and 70 DAA, the post-harvest did not affect the seed mass for any resting periods (Figure 2A). During the rest after harvest, the not completely mature fruits continued to translocate reserves to the seeds, increasing the 1000 seed mass (Nakada-Freitas *et al*., 2018, 2020; Colombari *et al*., 2022).

Evaluating the mass of eggplant seeds (Ciça hybrid), Nascimento *et al*. (2000) observed the occurrence of lower mass in younger fruits (30 to 40 DAA), results that indicate that seed development is related to fruit maturation. Despite eggplant being a non-climacteric fruit, the seeds might reach maturity within the harvested fruit. Before seed extraction, the fruit resting period allows the seeds to reach physiological maturity because, during this resting period, fruit reserves are still being metabolized and translocated to the seeds, increasing their mass and maturity level (Silva *et al*., 2017).

The older the fruit, the higher the percentage of processed seeds for treatments without a post-harvest period (0 RDAH) and 10 RDAH (Figure 2B). However, at 20 RDAH, there was no difference between the processed seeds in each fruit age, showing that this rest was enough to get dense seeds even in fruits harvested 40 or 50 DAA.

For post-harvest period, the results for processed seeds (Figure 2B) presented similar responses compared to the 1000 seeds mass (Figure 2A), i.e., the percentage of processed seeds increases as the resting period after harvest increases for young fruits (40 and 50 DAA), and no differences for fruits harvested 60 or 70 DAA (Figure 2B).

The processing of seeds was done based on differences in density and, therefore, when the seeds accumulate more dry mass during the fruit maturation

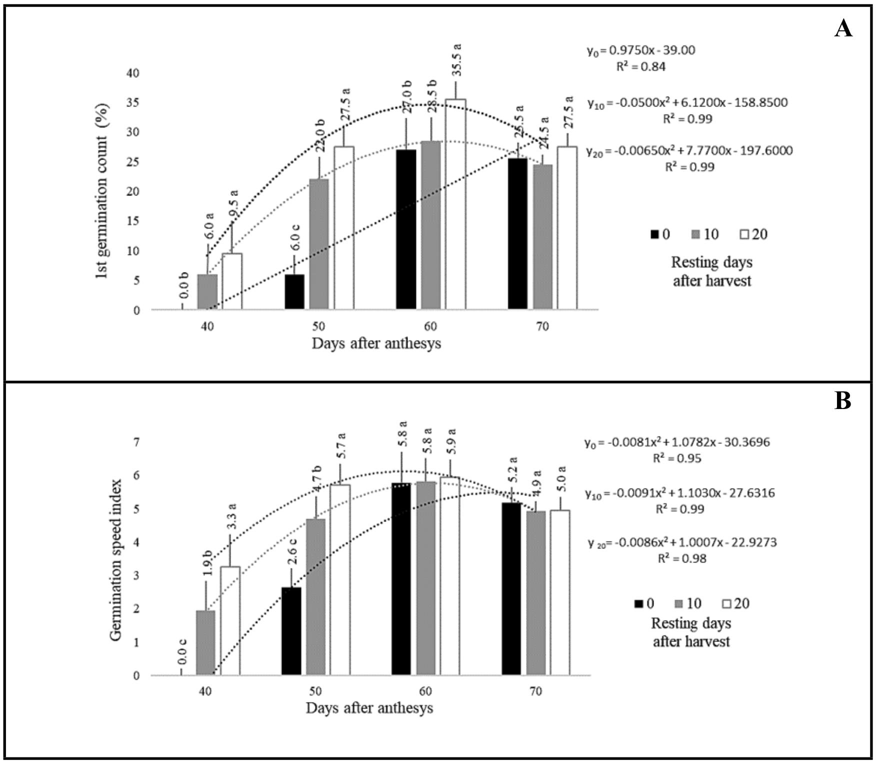

Figure 3. First germination count (A), and germination speed index (B) according to eggplant fruit age and times of post-harvest period. Averages followed by distinct letters in the same age (days after anthesis) differ by the Tukey test (5%). Resting period after harvest: 0 days = y0; 10 days = y10; 20 days = y20. Botucatu, UNESP, 2021.

Table 1. Pearson's correlation coefficient among the physiological and biochemical characteristics of eggplant seeds according to eggplant fruit age and fruit post-harvest period. Botucatu, UNESP, 2021.

Treatments	Proc (%)	MTS	FC	Germ	GSI	Prot	SOD	POX	CAT
Proc (%)	1								
MTS	0.84**	1							
FC	0.72**	0.91**	1						
Germ	0.87**	0.98**	0.93**	1					
GSI	0.84**	0.99**	0.96**	0.99**	1				
Prot	0.80**	0.94**	0.86**	0.92**	0.93**	1			
SOD	-0.78**	-0.82**	-0.57*	-0.77**	-0.75**	-0.80**	1		
POX	-0.70*	-0.84**	-0.80**	-0.81**	-0.84**	-0.89**	0.95**	1	
CAT	-0.90**	-0.94**	-0.81**	-0.95**	-0.93**	-0.86**	0.83**	0.66*	1

**Significant at p<0.01; *Significant at p<0.05; Proc (%) = percentage of processed seeds; MTS = mass of 1000 seeds; FC = first count of germination; Germ = Germination; GSI = germination speed index; Prot = protein content; superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) enzymes activity.

and during the post-harvest period, they become denser and are not discarded during the processing, increasing the rate of vigorous seeds. At 20 RDAH, the seeds from younger fruits continued to gain mass and no longer differed from older fruits for the percentage of processed seeds. At 10 RDAH, the seed processing differences between the 40 and 70 DAA were lower than the differences observed at the no-resting period (0 RDAH), clearly indicating that mass accumulation (translocation) to immature seeds happens in younger fruits during post-harvest.

The maximum seed germination was estimated 91.7, 94.9, and 96.4% at 67, 61, and 58 DAA, for 0, 10, and 20 RDAH, respectively, with a slight decrease after these ages (Figure 2C). The longer the resting period, the greater the estimate of maximum germination and lower the age to achieve this balance.

The tendency for fruit rest after harvest was the same for younger fruits (40 and 50 DAA), as germination increased with the post-harvest resting period. On the other hand, for fruits harvested 60 and 70 DAA, the post-harvest resting period did not affect seed germination (Figure 2C), similar to what was observed for the 1000 seed mass (Figure 2A). The same tendency was observed in other species of the Solanaceae family (Nakada-Freitas *et al*., 2018, 2020; Colombari *et al*., 2021), showing that immature fruits need to rest after harvest, but mature fruits do not.

Seeds extracted in the oldest fruits, 70 DAA, showed a slight germination reduction, which may show the beginning of seed deterioration in the fruit. After reaching a maximum percentage, the reductions in seed germination (Figure 2C) were more pronounced when the fruits rested. After 20 RDAH, the estimate of maximum germination was 96.4% in fruits harvested 58 DAA, reducing to 83.0% for fruits at 70 DAA, a reduction of 13.4%.

Fruits at 40 DAA, with or without a post-harvest resting period, and fruits at 50 DAA with resting periods lower than 20 days, did not reach the minimum germination (80%) determined by the Ministry of Agriculture (Brasil, 2009) for commercialization of eggplant seeds; fruits at 60 and 70 DAA, independently of the post-harvest period, presented germination (%) superior to the standard germination (> 80%) (Figure 2C). The minimum standard germination was achieved when the fruits were harvested at 57, 51, and 46 DAA with 0, 10, and 20 RDAH, respectively.

For fruits with no resting period (0 RDAH), the older the fruit, the higher the value for the first count of germination (Figure 3C). For fruits that rested, the maximum estimated values were 28.4 and 34.6% for fruits harvested at 62 and 60 DAA, for 10 and 20 RDAH, respectively, with reduced values in the oldest fruits with resting. There is no minimum standard for the first germination count; however, the more seeds germinating at seven days, the better the seed lot since the seedlings will be less susceptible to dumping off disease, and the seedlings can be transplanted earlier.

The maximum values estimated for the germination speed index (GSI) were 5.51, 5.79, and 6.18, at 67, 61, and 58 DAA, for 0, 10, and 20 RDAH, respectively (Figure 3B). The longer the fruit's resting period for younger fruits (40 and 50 DAA), the greater the GSI, while for older fruits (60 and 70 DAA), no differences were detected for fruits with or without a resting period (Figure 3B), similar to what was observed for seed germination (Figure 2C) and 1000 seed mass (Figure 2A). Colombari *et al*. (2021) reported similar results in pepper seeds, showing that this is usual in some solanaceous species.

The seed's physical and physiological quality characteristics (1000 seeds mass, percentage of processed seeds, germination, germination first count, GSI) presented a highly significant and positive correlation between each other (Table 1), i.e., the higher the 1000 seeds mass, the higher the percentage of processed seeds, germination, and vigor. These results are consistent since, according to Carvalho & Nakagawa (2012), maximum germination and vigor are usually reached with the maximum dry mass of the seeds.

In the present study, the best seed responses were observed for seeds from fruits between 70 and 80 DAA until the seed extraction (58 and 62 DAA in the plant plus 20 to 10 RDAH). According to Nakada-Freitas *et al*. (2018), the combination of fruit age and post-harvest resting period varies according to the genotype and climatic conditions, mainly temperature. However, the period that seeds remain inside the fruits is the most important factor for harvest determination in plant species with fleshy fruits. The resting periods after fruit harvest positively affected the seed weight and quality up to a certain period from when the seed deterioration started. According to Carvalho & Nakagawa (2012), as the seeds ripen within the fruit (even after harvest), fruit reserves are translocated to seeds, increasing seed mass to a maximum value corresponding to the seed's physiological maturity. After this, seed deterioration starts, that is, a series of physiological, physical, and biochemical changes, starting from physiological maturity in a progressive rhythm, determining the drop in performance potential and culminating in the seed's death. In this study, the beginning of seed deterioration and reduced seed mass, germination, and vigor was observed in seeds from the oldest fruits. It was more pronounced after the post-harvest resting of these oldest fruits.

Eggplant is a plant of indeterminate growth with continuous flowering and fruiting, making it difficult to determine a single harvest of fruits for seed production and the appropriate time for harvest. The color of the fruits can indicate the harvesting moment. Thus, the yellowish-brown color was the most appropriate fruit color to harvest the evaluated cultivar in the present study. This color was reached at about 60 DAA (Figure 1). If the fruit is harvested before that period, it has to rest for about 10 to 20 days to improve the eggplant seed quality. The post-harvest resting in species with indeterminate growth habit and continuous flowering is useful to improve the uniformity of seed quality, reducing the number of harvests and the exposure of fruits to unfavorable environmental conditions.

The maximum total concentration of soluble proteins was estimated at 124.45, 128.54, and 137.97 mg/g of fresh mass at 62, 58, and 55 DAA, for 0, 10, and 20 RDAH, respectively, with reductions in soluble protein concentration after these ages (Figure 4A), showing the probable breakdown of proteins after the maximum seed maturity (highest germination and vigor), that is, the beginning of seed deterioration.

The higher the post-harvest resting period of younger fruits (40 and 50 DAA), the greater the concentration of soluble proteins; no differences were detected on older fruits (60 and 70 DAA), with or without resting period (Figure 4A). These results show a similar tendency for 1000 seed mass (Figure 2A) and for germination (Figure 2C), indicating protein accumulation during the seed maturation, even during the post-harvest rest period.

The lowest SOD concentration was estimated at 0.575 and 1.070 U/mg protein at 60 and 52 DAA, 0 and 20 RDAH, respectively (Figure 4B). At 10 RDAH, the higher the fruit age, the lower the SOD concentration. The comparison among periods of post-harvest resting presented higher SOD enzymatic activity in fruits of 40 DAA at 0 RDAH than for 10 or 20 RDAH. The SOD activity in eggplant fruits at 50 and 60 DAA was not affected by fruit resting. In older fruits (70 DAA) with 20 RDAH, superior SOD activity was observed compared to fruits without post-harvest resting period (0 RDAH).

The superoxide dismutase (SOD) enzyme is related to the plant antioxidant defenses against oxidative reactive species (EROs) (Araújo *et al*., 2018). Greater SOD enzymatic activity was observed in younger fruits (40 DAA) due to the beginning of the seed metabolic activity with a high respiratory rate and increased EROs production (El-Maarouf-Bouteau & Bailly, 2008).

The minimum enzymatic activity of catalase (CAT) was estimated at 0.039 and 0.049 U/mg protein in fruits at 63 and 66 DAA, respectively, at 0 and 10 RDAH (Figure 4C). With 20 RDAH,

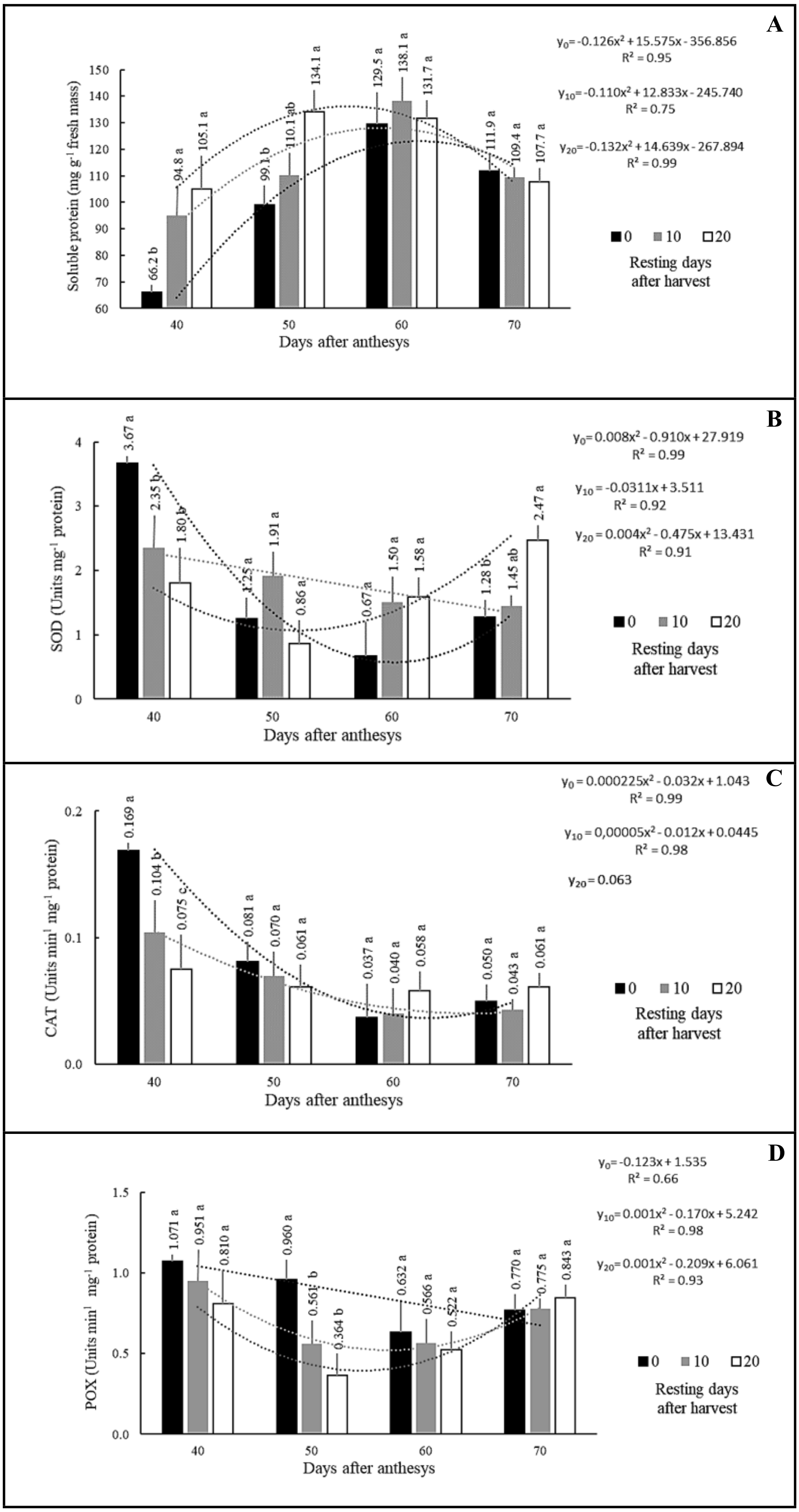

Figure 4. The concentration of soluble protein (A), activity of superoxide dismutase (SOD) enzyme (B), activity of catalase (CAT) enzyme (C), and activity of peroxidase (POX) enzyme (D) according to eggplant fruit age and times of post-harvest period. Averages followed by distinct letters in the same age (days after anthesis) differ by the Tukey test (5%). Resting period after harvest: 0 days = y0; 10 days = y10; 20 days = y20. Botucatu, UNESP, 2021.

the CAT activity was not affected by the fruit age, with an average of 0.063 U/mg protein.

Differences were observed between the post-harvest resting periods for CAT activity in fruits at 40 DAA; the no-resting treatment (0 RDAH) presented superior CAT values compared to 10 and 20 RDAH (Figure 4C). In older fruits (50, 60, and 70 DAA), no CAT activity differences were detected for the fruit post-harvest periods.

The catalase (CAT) enzyme is also involved in the process of cell protection from oxidative damage caused by EROs. Araújo *et al*. (2018) observed pronounced CAT activity in another Solanaceae plant species, peppers seeds from 'Biquinho' and 'Malagueta' cultivars, when harvested at 40 DAA, but this enzymatic activity decreased as the fruit ripens. Colombari *et al*. (2021) also observed a reduction of CAT and SOD activity with the maturation of fruits of sweet pepper until a maturation stage and an increasing activity after this.

As pointed out, both the SOD and CAT enzymes act as a defense mechanism. The SOD primary function is to null the action of superoxide (O_2^-) and to reduce the formation of hydrogen peroxide (H_2O_2), thus avoiding cell oxidation (Saisanthosh *et al*., 2018). After SOD triggers the defense mechanism, the CAT enzyme "breaks" the hydrogen peroxide into water and oxygen. In general, the activity of these enzymes was lower in seeds extracted from mature fruits when compared to seeds from immature fruits, which is possibly related to a reduction in the metabolism, causing less oxidative stress (Araújo *et al*., 2018).

Following the same tendency, the higher the fruit age at harvest, the lower the peroxidase (POX) activity in the no-resting treatment (0 RDAH) (Figure 4D). When fruits rested, the minimum POX activity was estimated at 0.527 and 0.423 U/mg protein for fruits at 57 and 54 DAA, with 10 and 20 RDAH, respectively.

Differences were also observed between the post-harvest fruit resting period for POX activity in fruits at 50 DAA. The no-resting treatment (0 RDAH) presented higher POX activity than at 10 and 20 RDAH (Figure 4D). In matured fruits (60 and 70 DAA), no POX differences were observed between the post-harvest period of fruits.

A significant negative correlation (Table 1) was observed between seed quality variables (processed seeds, 1000 seeds mass, germination, germination first count, GSI) and the SOD, POX, and CAT enzyme activity, i.e., the higher the germination and vigor, the lower the activity of antioxidant enzymes.

Therefore, the activity of these enzymes decreased during the process of seed maturation in the fruits and after resting in immature fruits. The activity of these enzymes is important in seed metabolism to increase the defense mechanisms, avoiding damage to the cell membranes by free radicals (Marcos Filho, 2015).

When the seeds were extracted immediately after harvest (0 RDAH), the antioxidant enzymes (SOD, CAT, and POX) presented superior activity in younger fruits (40 DAA) when seeds were still at the beginning of the maturation process. At this stage, the seeds begin the metabolic activity with high rates of respiration that can increase the production of reactive oxygen species (El-Maarouf-Bouteau & Bailly, 2008). Also, as the seed matures, the activation of the defense mechanisms occurs to prevent the action of superoxide (O_2^-) and the formation of hydrogen peroxide (Saisanthosh *et al*., 2018).

The natural seed maturing process in fruits at 50 and 60 DAA reduced the enzymatic activities until seeds reached physiological maturity. After passing the physiological maturity stage, the seeds from fruits at 70 DAA began the deterioration process and a consequent increase in the activity of these enzymes. In general, the fruit post-harvest period caused the seed enzyme activity; however, in older fruits, the resting period can reduce seed quality and increase the SOD activity. Similar results were related by Colombari *et al*. (2021) in sweet pepper seeds.

The enzymes SOD and CAT expressed a negative correlation with 1000 seed mass, germination, first count in germination test, and GSI (Table 1), indicating that under conditions of high physiological quality of seeds and increase in seed mass, there is less oxidative stress, consequently the low activity of SOD and CAT enzymes and less membrane degradation. The activity of enzymatic antioxidants, such as CAT and SOD, regulates the defense system against ROS that may occur during different stages of seed development. Thus, enzymes act to prevent the progress of seed deterioration (Colombari *et al*., 2021).

The significant correlation between the activity of antioxidant enzymes and the characteristics regarding seed quality demonstrates that the determination of these enzymatic activities can assist in studies of seed maturation, i.e., the increase of enzymatic activity after a period of reduction may indicate early deterioration and losses of seed quality (Saisanthosh *et al*., 2018).

The results indicated that the ideal moment of eggplant harvest for seed production is when the fruit reaches the yellowish-brown color corresponding to about 60 DAA; however, if the eggplant fruit is harvested early (40 or 50 DAA), it has to rest for about 10 to 20 days to achieve high seed physiological quality.

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