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Maturation and Resting of Sweet Pepper Fruits on Physiological Quality and Biochemical Response of Seeds

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HIGHLIGHTS

- Completely yellow sweet pepper fruits have higher physiological seed quality.
- Enzymatic activity is an indicator of physiological quality of sweet pepper seed.
- Post-harvest resting affects the quality of sweet pepper seeds.

Abstract: The post-harvest resting of the fruits can improve seed physiological quality ,once it allows the seed to complete the maturation process, so it has been a common practice in vegetable seed companies, however, there are a few studies of this technique in sweet pepper. The objective of this research was to evaluate physiological quality, and biochemical response of sweet's peppers in regarding on the stage of maturation and the post-harvest rest of the fruits. The experimental was conducted in a 4x2 factorial, being the first factor comprised four maturation stages (35, 50, 65 and 80 days after anthesis) and, the second the post-harvest management of fruits, with and without a temporary storage of seven days. Seeds were evaluated for water content, weight of thousand seeds, germination, vigor, superoxide dismutase, catalase and peroxidase activities, lipid peroxidation and hydrogen peroxide content. Fruit harvest time indicated is 80 days after anthesis (fruits 100% yellow) when seeds showed maximum germination and vigor. The post-harvest resting of the fruits was beneficial to seed physiological quality, weight of one thousand seeds and

to reduce hydrogen peroxide content. Seeds of higher physiological quality showed lower superoxide dismutase and catalase enzyme activity, so they can be used as a marker of physiological quality in sweet pepper seeds.

Keywords: *Capsicum annuum* L.; germination; lipid peroxidation; reactive oxygen species; seed vigor.

INTRODUCTION

Pepper (*Capsicum annuum* L.) is the third most important cash crop whitin to the family of solanaceae, it is a perennial crop with an undetermined habit [1,2]. This vegetable crop is one of the most consumed fruit vegetables in the country, due to its nutritional value and high profits in production [3]. Among the several factors that interfere in its production is the use of quality seeds.

The properties that make up attribute physiological seed quality such as germination and vigor are acquired during the seed maturation process [4]. The harvest of sweet pepper seeds is performed in the final process of fruit maturation, when the fruits are ripe. However, the delay harvest of the seeds can lead to seed germination within the fruit or start deterioration and the early harvest can be responsible for seeds with low physiological potential [5].

In sweet pepper, the process of flowering and fertilization are continuous, because it is an indeterminate habit crop, enabling the species to produce fruits and seeds at different stages of maturation. Thus, it is almost impossible that all fruits reach maturity at the same time [5].

In fleshy fruit species, such as sweet pepper, even after harvest, the seeds can reach physiological maturity when they remain inside the fruits for a few days [6], as the high initial water content in the seed allows the synthesis of reserve to continue, enabling maturity uniformity [7,8]. Thus, keeping the fruits in rest for a period of seven to ten days before seed extraction can avoid this problem of irregular maturity [9]. That procedure allows early harvesting, reducing the time that the fruits are exposed to unfavorable climatic conditions, such as the attack of pests and diseases. Moreover, the resting allows enables the simultaneous harvesting of fruits at various stages of ripening [10].

The deterioration of seed process begins when the seed reach physiological maturity, which in sweet peppers coincides with the beginning of the fruit color change, usually from green to yellowish spots, in addition to the stabilization of water content by the amount of dry matter [9,11]. This process is accompanied by a series of physiological, biochemical, physical and cytological changes, resulting in reduced quality. Thus, the changes that occur in the seeds that are related to the deterioration process are the reduction of the respiratory activity and the synthesis of proteins, nucleic acids degradation and inactivation of enzymes, and the loss of integrity of the cell membranes due to lipid peroxidation [6]. Therefore, variations in enzyme activity, lipid peroxidation and free radical removal can also be an efficient marker to monitor biochemical changes resulting from seed deterioration [4].

Enzymes are part of the antioxidant system and are activated by seeds to protect against reactive oxygen species (ROS) generated when exposed to various stresses during maturation, germination, storage and aging. Enzymatic antioxidant system is represented by several enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) [7].

Seed physiology quality is important for producer and the seed companies, so identify the harvest ideal time when seeds have completed their development and physiological maturity is important [8]. The objective of this research was to evaluate physiological quality, and biochemical response of sweet's peppers (*Capsicum annuum* L.) in regarding on the stage of maturation and the post-harvest rest of the fruits.

MATERIAL AND METHODS

Research was conducted in a protected environment in São Manuel - SP (22° 46 'S, 48° 34 'W and altitude of 740 m). The soil used was fertilized and corrected with limestone as it is recommended by Bulletin 100 [12]. Moreover, the soil was fertilized by fertigation according to Trani and coauthors [13], using venturi system weekly. Irrigation was carried out by dripping with flexible tape and flow of 1.6 L ha^{-1}.

Chemical characteristics of the soil used were: pH($CaCl_2$): 4,4; organic matter.: 5 g dm^{-3}; P (resin): 2 mg dm^{-3}; H+Al: 26 $mmol_c$ dm^{-3}; K: 1,1 $mmol_c$ dm^{-3}; Ca: 33 $mmol_c$ dm^{-3}; Mg: 4 $mmol_c$ dm^{-3}; sum of bases: 39 $mmol_c$ dm^{-3}; capacity of exchange cation: 64 $mmol_c$ dm^{-3} and base saturation: 60%.

Sowing was carried out in polypropylene trays of 162 cells, on July 24, 2017, using substrate Carolina Soil, placing one seed per cell. Seedling transplants at 47 days after sowing (DAS), when the crops had four true leaves stages. It was used the cultivar 1730, an inbreeding line from Sakata Seeds.

The managements were the withdrawal of sprouts until the appearance of the first flower (147 DAS), pest and disease control when needed a plant tutoring.

Eight treatments, in a 4x2 factorial scheme, were evaluated in randomized complete block design with four replications. The first factor comprised four maturation periods 35, 50, 65 and 80 days after anthesis (DAA) and, the second the post-harvest management (with and without a temporary fruits storage for seven days after harvesting). Ten plants were evaluated per plot and all fruits fixed on the plants were harvested, without thinning. Harvest was carried out manually, using scissors to separate the fruit from the mother plant.

To determine the maturation period, all flowers of all plants were marked on the day of their anthesis. The harvests were performed when the fruits had the maturity stage corresponding to 35, 50, 65 and 80 days after anthesis, based on fruit coloring and crop cycle, (Figure 1) and half of the fruits had their seeds extracted on the day of harvesting (without rest) and the other half remained at post-harvest resting on laboratory bench (40% RH and 20°C). At harvest time the visual appearance of the fruits was: fully green fruits at 35 DAA; fruits with transient coloration from green to yellow at 50 DAA; fruits with 75% bright yellow color at 65 DAA and at 80 DAA the fruits were 100% yellow, but opaque and with less pulp firmness.

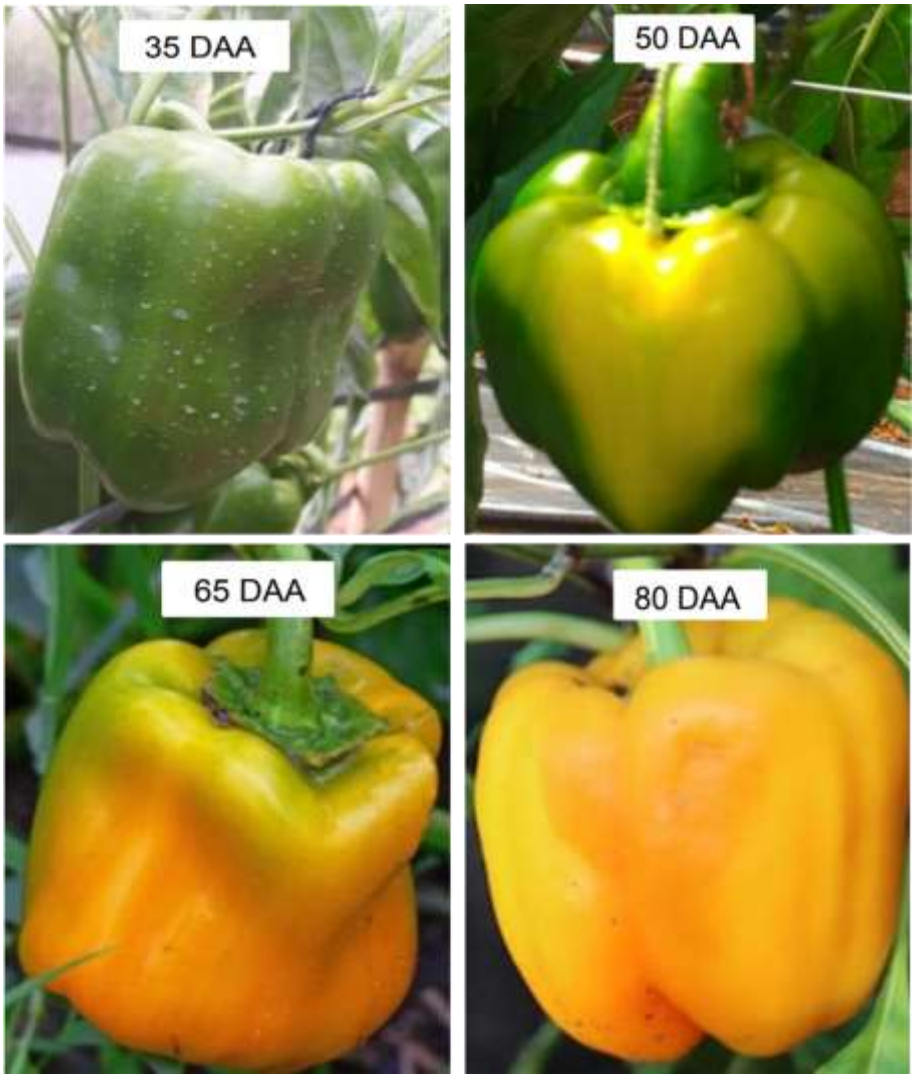

Figure 1. Visual aspects of sweet pepper fruit at maturation stage (35, 50, 65 and 80 days after anthesis).

The characteristics evaluated in the seeds were water content, weight of thousand seeds (WTS), germination, vigor (first germination count and speed of germination), the activity of the enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), lipid peroxidation (LIP) and hydrogen peroxide content (H_2O_2). All evaluations were performed without seed processing.

Seed water content was determined immediately after extraction from fruits by the oven method at 105 ± 3 ºC 24 hours, using 10g of seeds [14]. After this process, the seeds were put in a dry chamber (40% relative humidity and 20 ºC), to reduce seed water content to approximately 8% for storage.

For the weight of a thousand seeds, eight repetitions of 100 seeds per plot were weighing through a precision scale of 0.0001, expressed in grams [14].

Germination test was evaluated with four replicates of 50 seeds. The seed were placed in gerbox with two filter paper moistened using destiled water (two times paper weight). The gerbox were storage at 20-30 ºC alternating temperatures. The germination percentage was scored after 14 days by counting normally seedlings [14].

First germination count was evaluated concomitantly to germination test, counting normally seedlings after seven days of sowing [14]. Speed of germination was conducted concomitantly with the germination test, with daily calculation of the normally seedling. The germination speed index was calculated by the sum of the number of seeds germinated each day, divided by the number of days elapsed between the seeding and germination, according to the Maguire formula [15]:

GSI= G1n1 + G2n2 + … + Gini

Where GSI is seedlings' germination speed index, G is number of seeds germinated each day and N is number of days elapsed from the seeding until the last count.

For biochemical analysis, seeds were frozen in liquid nitrogen, in order to paralyze all reactions, and then kept in an ultra-freezer at a temperature of - 80ºC. Enzymatic extraction was carried out according to the methodology proposed by Kar and Mishra [16]. To calculate the activity of the SOD, CAT and POD enzymes it was necessary to determine the content of soluble proteins totals that was done through the method proposed by Bradford [17].

SOD was determined according to the methodology proposed by Beauchamp and Fridovich (1971), adapted by Bor and coauthors [18]. The reading was performed on a spectrophotometer at 560 nm. A unit of SOD is defined as the activity of the enzyme necessary for the inhibition of 50% of the photo-reduction of nitroblue tetrazolium (NBT). To calculation the specific activity of the enzyme, the percentage of inhibition obtained, the volume of the sample and the concentration of protein in the sample, the activity were expressed in U mg^{-1} of protein.

CAT was determined according to the methodology proposed by Peixoto and coauthors [19]. The readings were performed on a spectrophotometer at 240 nm from 0 to 80 seconds, in order to verify how much there decrease in absorbance. To calculate the specific activity of the enzyme, the molar extinction coefficient of H_2O_2 (39.4 mmol L^{-1} cm^{-1}) was used and the activity expressed in nmol of H_2O_2 consumed min^{-1} mg^{-1} protein.

POD was determined according to the methodology proposed by Teisseire and Guy [20]. The reading was performed on a spectrophotometer at 430 nm. To calculate the specific activity of the enzyme, its molar extinction coefficient (2.5 mmol L^{-1} cm^{-1}) and the activity expressed in µmol of purpurogalin min^{-1} mg^{-1} of protein.

The LIP was determined according to the methodology of Heath and Packer (1968), cited by Rama Devi and Prasad [21]. For the calculations, the molar extinction coefficient of malondialdehyde (MDA) (155 mmol L^{-1} cm^{-1}) was used and the results were expressed in MDA nmol g fresh $mass^{-1}$.

The H_2O_2 content was determined through the reaction of potassium iodide (KI) according to the methodology of Alexieva [22]. The determination of the H_2O_2 content was performed in a spectrophotometer at 390 nm and the elaboration of a standard calibration curve constructed from the H_2O_2 solution and the results expressed in µmol g fresh $mass^{-1}$.

The data were submitted to Lilliefors tests, for normality, and Cochran and Bartlett tests, for homogeneity of variances and, when significant, analysis of variance was performed to compare post-harvest fruit rest, and regression ($p>0.005$) to verify the effects of maturation periods. Pearson correlation analysis ($p<0.01$ and $p>0.05$) among the evaluated characteristics was also performed.

RESULTS AND DISCUSSION

According to results obtained in the analysis of variance, there was no interaction between the sources of variation studied for the seed quality tests, which they were analyzed separately.

Seed water content were adjusted to the decreasing linear model as a function of maturation stage, the high values in fruits were obtained at 35 DAA and it reducing over the time to a minimum of 56 and 53% at 80 DAA, without and with fruit rest, respectively (Figure 2). Inside of the fruit, seeds at physiological maturity keep high water content (35 to 40%) [4], however, the values found in this research were higher.

Nogueira and coauthors [23], evaluating the water content in 'Magda' sweet pepper seeds, found minimum water content of 54% at 70 DAA. Besides that, Vidigal and coauthors [24] also achieved a reduction in water content in yellow sweet pepper, with a minimum of 47% at 75 DAA, without fruit rest. The reduction in seed water content as a function of fruit maturation stage was also reported in other species of Capsicum genus [6,25].

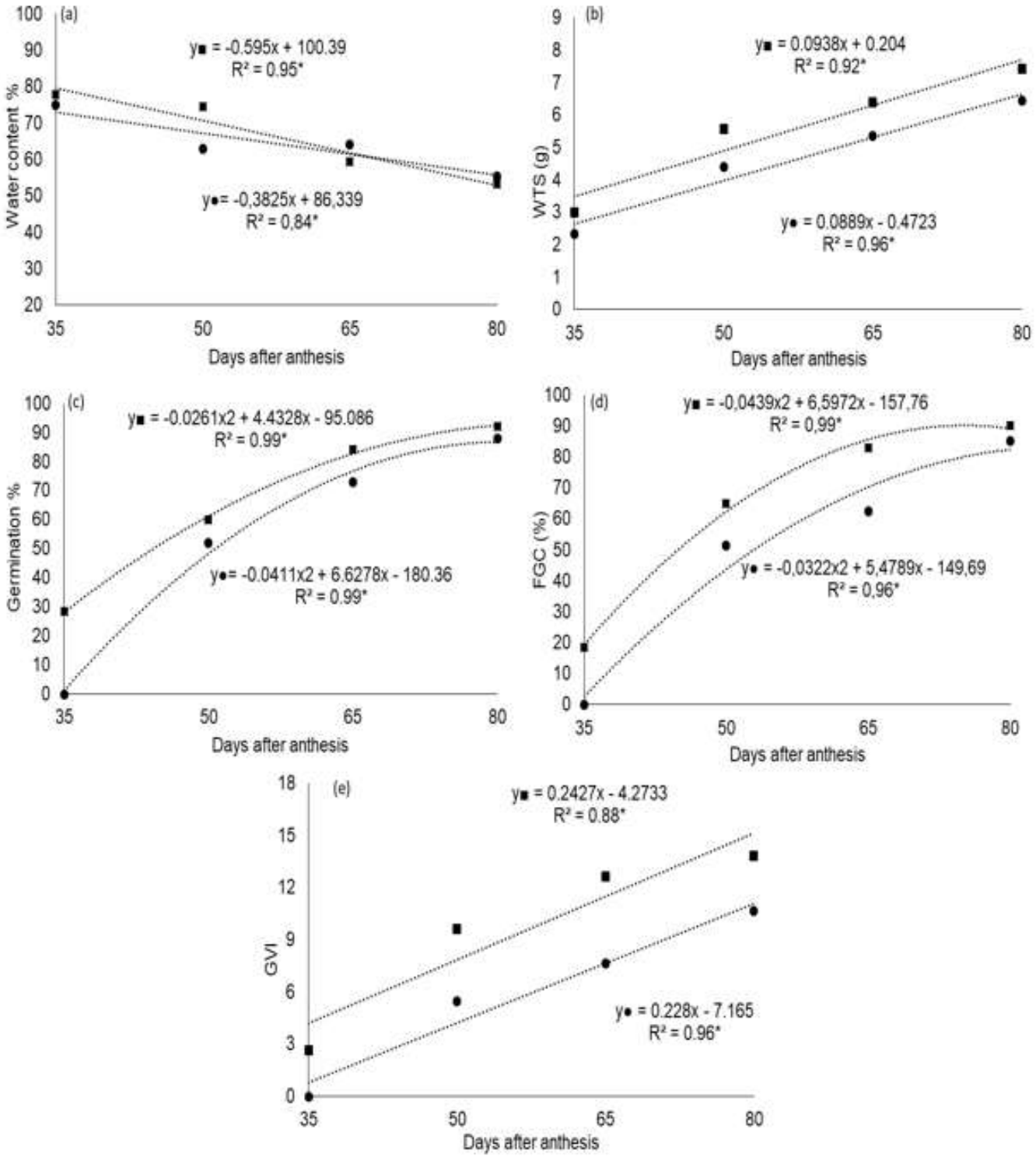

Figure 2. Water content (a), weight of a thousand seeds (b, WTS), germination (c), first germination count (d, FGC) and speed of germination (e, SG) in sweet pepper seeds for maturation stage (35, 50, 65 and 80 days after anthesis), without (●) and with (■) seven days of fruits post-harvest rest.

It was observed linear increases in the weight of one thousand seeds (WTS) with fruit maturation, reaching maximum values of 6.6 and 7.7 g at 80 DAA, in seeds without and with post-harvest rest, respectively (Figure 2). In yellow sweet pepper also there was increases in WTS up to 75 DAA [24]. This is

because seeds tend to increase the dry weight until physiological maturity during the maturation process [4].

The beginning of seed development was characterized by the relatively slow accumulation of dry mass, since in this phase predominate the cell division and expansion, which are responsible for the constitution of the adequate structure to receive the substances transferred from the mother plant. Soon after, the replacement of water content by dry matter begins after the initial seed growth [8].

For germination and FGC, the data were adjusted to the quadratic model as a function of the maturation stage (Figure 2). At 35 DAA, in fruit without post-harvest rest, germination was zero and even in fruit with rest, the germination did not reach 30% at this age. Maximum values were estimated at 87 and 83% at 80 DAA for germination and FGC, respectively, without fruit rest. However, the maximum value for the fruit with rest were estimated at 93 and 90% at 80 and 75 DAA. The SG, linear increases were obtained with maximum values estimated at 80 DAA of 11.1 and 15.1, without and with fruit rest, respectively (Figure 2).

In yellow sweet pepper seeds the maximum germination (88%) and FGC (56%) occurred at 75 DAA [24], and in 'Malagueta' pepper seeds the maximum germination percentage (93%) and SG (2.1) happened between 60 and 62 DAA, with fruit rest for 10 days [26]. Habanero pepper seeds showed maximum germination (72%) when were obtained from fruits harvested at 67 DAA. However, when the seeds were extracted from fruits in early developmental periods (50 and 60 DAA), they practically don't germinate, even after 7 days of post-harvest rest [25]. Therefore, literatures show that the age of harvest can change according to species, cultivar and, cultivation and environmental conditions.

Seed water content was not affected by post-harvest rest (Table 1). Although water content of the seeds decreases during maturation, the reduction in freshy fruit seeds is less pronounced than in dried fruits, and the water content in freshly fruit are usually between 30 and 50% [4].

Table 1. Average values of water content (WC), weight of a thousand seeds (WTS), germination, first germination count (FGC) and speed of germination (SG) in sweet pepper seeds without and with seven days of fruits post-harvest rest.

Post-harvest rest	WC	WTS	Germination	FGC	SG
	--%--	--g--	----------%--------		
Without	64.4a	4.6b	54b	50b	5.9b
With	66.2a	5.6a	66a	64a	9.7a
CV (%)	8.1	4.4	8.2	12.7	22.8

*Averages followed by the same letter in the columns do not differ from each other by the Tukey test at 5% probability.

For the WTS, germination, FGC and SG, higher values were obtained after post-harvest rest (Table 1). These results emphasize the importance of the post-harvest rest for the production of sweet pepper seeds. Pereira and coauthors [6] studying the effects of postharvest rest in *Capsicum baccatum* L. also obtained improvement in seed physiological quality. Similar results were reported by Vidigal and coauthors [24] researching the effect of post-harvest rest on *Capsicum annuum* L. fruits, in which they found a higher percentage of germination, first germination count, controlled deterioration and electrical conductivity when the fruits were stored.

Post-harvest temporary storage of fruits before extraction allows the seeds to complete their physiological maturation [23]. Thus, the reserves continue to be metabolized and translocated to the seeds, allowing increases in weight and improvement of seed physiological quality. Post-harvest resting of fruits is especially important in species with indeterminate growth habit that produce fleshy fruits, such as pepper, cucumber, tomato, and others species. The post-harvest resting species with indeterminate growth habit is useful to improve the uniformity generated by continuous flowering, reducing the number of harvests and the exposure of seeds to unfavorable field conditions [9].

The activity of the enzymes SOD, CAT and, LIP adjusted to the quadratic model as a function of fruit age (Figure 3), showing high enzymatic activities, as well as high membrane degradation, at 35 DAA, which decreased to a certain stage of fruit maturation. The minimum estimated averages were 5.2 mg^{-1} protein, 0.06 µmol H_2O_2 consumed $minute^{-1}$ g^{-1} protein and 6.9 MDA nmol g fresh $mass^{-1}$ at 66, 78 and 73 DAA for

SOD, CAT and LIP, respectively, in the treatment without fruit rest. With post-harvest fruit rest the lowest averages were 4.4 mg^{-1} protein, 0.08 µmol H_2O_2 consumed $minute^{-1}$ g^{-1} protein and 8.1 MDA nmol g fresh $mass^{-1}$ at 66, 73 and 67 DAA, respectively for SOD, CAT and LIP. Activity of the POD enzyme increased with fruit age, with maximum values at 80 DAA estimated at 0.32 and 0.22 µmol purpurogaline min^{-1} mg^{-1} protein in treatments with and without fruit rest, respectively (Figure 3).

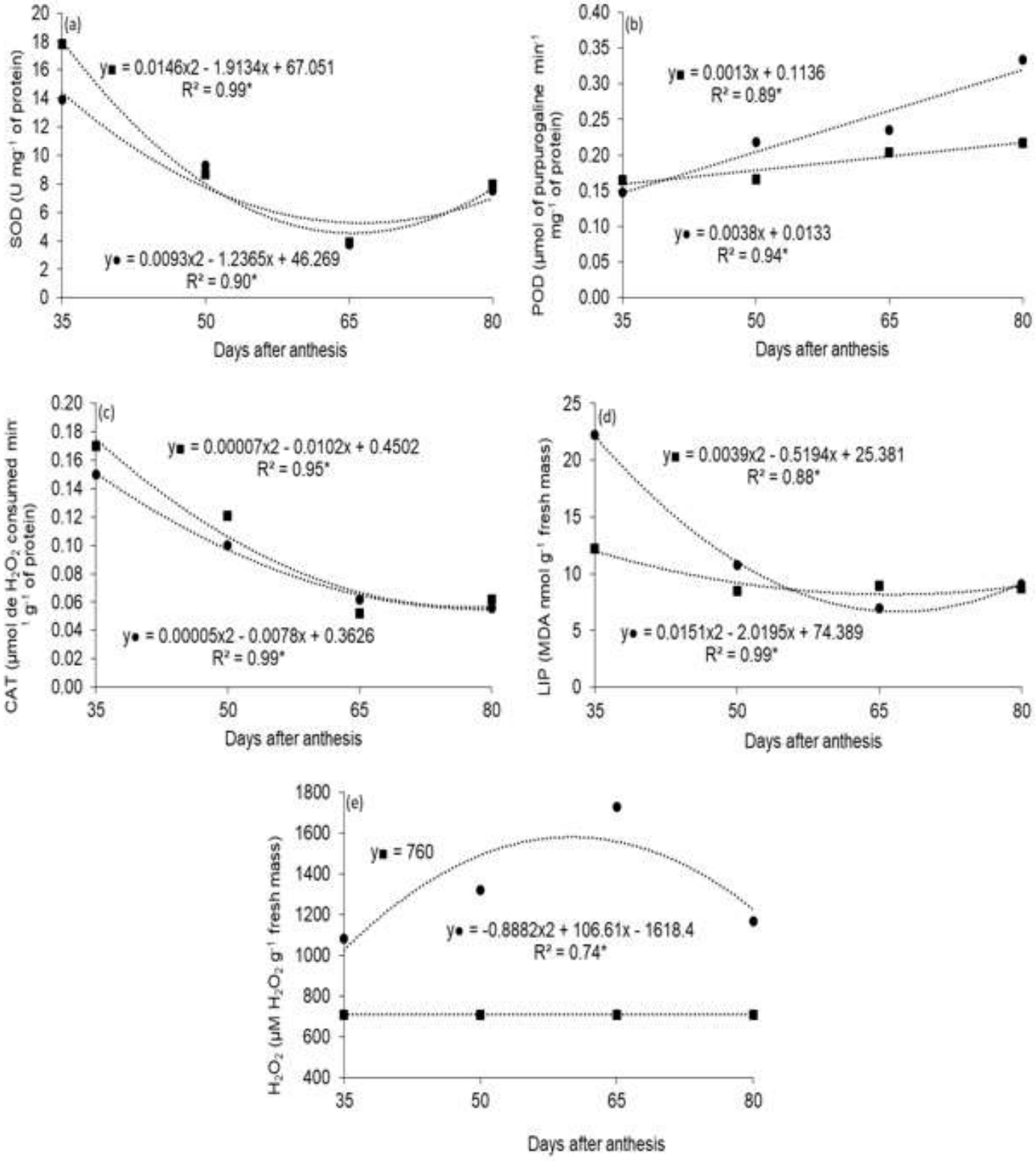

Figure 3. Activity of superoxide dismutase (a, SOD), peroxidase (b, POD) and catalase (c, CAT), lipid peroxidation (d, LIP) and hydrogen peroxide (e, H_2O_2) enzymes in sweet pepper seeds for maturation stage (35, 50, 65 and 80 days after anthesis), without (●) and with (■) seven days of fruits post-harvest rest.

Increases in SOD and POD activities, especially in the early stage of seed maturation, indicate increased oxidative stress to regulate ROS level [27], as well as increases in CAT activity represents a greater ability to eliminate H_2O_2 [1], because it is the main enzyme involved in hydrogen peroxide removal [28]. However, this enzyme is only actived at high concentrations of H_2O_2 [27], thus, it can be supposed that the enzyme that acted during the seed maturation process from 50 to 80 DAA was possibly POD.

H_2O_2 content adjusted to the quadratic model without fruit rest, with a maximum estimated at 1580.7 µM H_2O_2 g fresh mass^{-1} at 60 DAA (Figure 3). There was no difference in maturation stage considering the post-harvest rest (760 µM H_2O_2 g fresh mass^{-1}). Thereby, for the cultivar of sweet pepper studied, the H_2O_2 content of the seed with fruit rest is stable among the maturation stages.

Similar results of the enzymes were obtained in immature pumpkin seeds 'Jabras' (15 DAA), in which they showed high activity of the enzymes SOD, CAT and POD. 'Omega' cucumber seeds also showed increased CAT and SOD activity from 30 to 45 DAA [29], probably because the seeds still were in process of formation and, according to the authors, by the fact the dehydration process is considered a stress factor and provides free radical formation.

In 'Amarela Comprida' pepper seeds, at 40 and 50 DAA, there was no difference in the enzymatic response, which could be associated with the maturation stage, however, there was a slight increase in SOD activity in seeds obtained from fruits harvested from 50 DAA and stored for 6 days [9]. This enzyme, in 'Habanero' pepper seeds, showed higher and lower enzyme activity when fruits that were harvested with the first signs of yellowing and completely ripe, respectively [30].

Analyzing the post-harvest rest of the fruits, it was showed less activity of the enzymes SOD and CAT without the rest, in contrast, there was less membrane degradation and lower H_2O_2 content with the fruit rest (Table 2). Post-harvest resting enabled less oxidative stress, probably due to the low H_2O_2 content and less membrane damage compared to the treatment without rest. Post-harvest resting allows the seeds to have more time to complete their maturation, improving the physiological quality and preserving the quality of mature seeds [31].

Table 2. Activity of the enzymes superoxide dismutase (SOD) (U mg^{-1} de protein), peroxidase (POD) (µmol de purpurogaline min^{-1} mg^{-1} de protein) and catalase (CAT) (µmol de H_2O_2 consumed minute^{-1} g^{-1} protein), lipid peroxidation (LIP) (MDA, nmol g fresh mass^{-1}) and hydrogen peroxide content (H_2O_2) (µM H_2O_2 g fresh mass^{-1}) in sweet pepper seeds without and with seven days of fruits post-harvest rest.

Post-harvest rest	SOD	POD	CAT	LIP	H_2O_2
Without	8.3b	0.24a	0.08b	12.7a	1325.5a
With	9.4a	0.18b	0.10a	9.3b	760.5b
CV (%)	6.7	3.9	8.7	6.4	4.4

*Averages followed by the same letter in the columns do not differ from each another by the Tukey test at 5% probability.

There was negative correlations between seed water content and germination percentage, FGC, SG and WTS (Table 3), it happens because when the water content decreases, the physiological quality of the seeds and the weight of one thousand seeds, increases. This fact is already known in seed physiology, where germination, vigor and dry matter content practically reach maximum at the same point where seed water content decreases rapidly [4].

SOD, CAT and LIP enzymes showed a positive correlation with seed water content (Table 3), just because during the seed maturation period, the dehydration process is considered a stress factor that causes the formation of free radicals [29], and increased antioxidant system enzyme activity capable of eliminate ROS. The enzymes SOD and CAT and LIP expressed negative correlation with germination, FGC, GVI and WTS (Table 3), indicating that under conditions of high physiological quality of seeds and increase in WTS there is less oxidative stress, consequently low activity of SOD and CAT enzymes and less membrane degradation.

Table 3. Correlation between water content (WC), germination (G), first germination count (FGC), speed of germination (SG), weight of a thousand seeds (WTS), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzyme activities, lipid peroxidation (LIP) and hydrogen peroxide (H_2O_2) content in sweet pepper seeds.

	WC	G	FGC	SG	WTS	SOD	POD	CAT	LIP
G	-0.71**								
FGC	-0.68**	0.97**							
SG	-0.62**	0.91**	0.94**						
WTS	-0.71**	0.95**	0.96**	0.93**					
SOD	0.58**	-0.77**	-0.78**	-0.69**	-0.75**				
POD	-0.67**	0.67**	0.63**	0.48**	0.58**	-0.50**			
CAT	0.78**	-0.88**	-0.86**	-0.76**	-0.86**	0.90**	-0.70**		
LIP	0.48**	-0.85**	-0.81**	-0.72**	-0.77**	0.64**	-0.49**	0.65**	
H_2O_2	-0.21	0.05	-0.03	-0.18	-0.09	-0.37*	0.42*	-0.3	-0.08

** and * = significant at 1% and 5%, respectively, by Pearson correlation analysis.

The activity of enzymatic antioxidants, such as CAT and SOD, regulate the defense system against ROS that may occur during different stages of seed development. Thus, enzymes act to prevent the progress of seed deterioration and changes in their activity may indicate loss of quality [(32].

LIP also showed a positive correlation with SOD and CAT enzymes (Table 3), so, whenever membrane damage is occurring, enzyme activity will be high to try to combat degradation and prevent seed from deteriorating. The enzyme POD was positively correlated with germination, FGC, SG and WTS, indicating that this enzyme was the one that acted to keep the ROS levels low during the seed maturation process, because CAT is only active at relatively high concentrations of H_2O_2 [33].

H_2O_2 content was positively correlated to the enzymes SOD and negatively correlated to POD (Table 3). Although a positive correlation has been obtained with SOD, this is not the main enzyme involved in H_2O_2 removal. The results that enzyme activities changes during fruit maturation and post-harvest fruit rest are closely related to seed maturation process and can be used as indicators of seed maturity.

CONCLUSION

The best age for harvesting fruits is at the stage when the fruits were 100% yellow, however, opaque and with less pulp firmness, stage that has higher seed quality of sweet pepper.
The post-harvest rest of the fruits increase seed quality.
The enzymatic activities can be used as marker of physiological quality in sweet pepper seeds.

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