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Physiological quality of habanero pepper (*Capisicum chinense*) seeds based on development and drying process

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The objective in this research was to study the physiological and biochemical alterations in habanero pepper during the development and after the natural and controlled drying. The experiments were conducted at Seeds Laboratory and in the experimental area of Agriculture Department at Universidade Federal de Lavras (UFLA). The fruits were harvested in four stages of development, being these E1-49, E2- 56, E3- 63 and E4- 70 days after anthesis. After harvest, parts of fruits were kept at rest for seven days. The seeds extracted from the fruits in different periods of harvest were submitted to two methods of drying: artificial drying at 35°C, and natural drying on shadow until 9% of water content. The performance of seeds under different treatments was evaluated through germination, emergence, electrical conductivity and water content tests and through the activity of the isoenzyme systems; esterase (EST), catalase (CAT), superoxide dismutase (SOD) and heat-resistant proteins. With the results, it was concluded that habanero pepper seeds from fruits harvested and submitted to post-harvest have higher physiological quality and less dormancy. Habanero pepper seeds, when harvested next to maturity points (E3 and E4) and dried in controlled conditions (35ºC) in constant air flux, induces the synthesis of heat-resistant proteins.

Key words: *Capsicum chinense* Jacquin, seeds formation, vigor of seeds.

INTRODUCTION

Habanero pepper is the most Brazilian between all the species being sourced in the amazon region and is extremely appreciated by the unmistakable flavor and spiciness. Like the other peppers species, the offer of quality seeds of habanero pepper is limited, mainly by lack of knowledge about the better seeds harvest stage and the utilization of adequate dry methods, which aim to increase the potential of storage and the establishment of plants in field (Queiroz, 2014) In the production process, the harvest and drying influences significantly on seeds quality should being performed in the adequate moment and following the technique recommendations to reduce

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at maximum the possible qualitative and quantitative losses (Faria et al., 2003). For the most part of species, the most appropriate time for seeds harvest is closest to the maturity physiological point, which corresponds to the time of greater weight, germination and vigor. When the harvest is realized in the optimum point, the preservation of physiological potential of seeds is favored, because when seeds are kept in field after the physiological maturity, occurs the process of deterioration (Abud et al., 2013).

In the case of fleshy fruits like habanero pepper, the maturity of seeds generally coincides with the beginning of coloration change, when the fruits present red color. Beyond the fruits coloration, other indicators of physiological maturity are the vigor, moisture content, mass and the size (Dias et al., 2006; Vidigal et al., 2011). Caixeta et al. (2014) reported that the better harvest time of habanero pepper for seeds production, varies between 60 to 67 days after anthesis, phase characterized by the changes of fruits coloration. However, researches have shown that even before the complete maturation of fruits, seeds already achieve the physiological maturity (Zanin, 1990).

It is known that the understanding about the changes that occurs in seeds during the different development stages when occurs loss of water, is important for the choose of the methodology which must be used in the drying process of seeds harvested with high water contents, once, with progressive water loss, seeds become tolerant to high temperatures of indicating that the events occurs together with the reduction of water content (Rosa et al., 2000).

Therefore, knowing the ideal moment of harvest and the adequate drying method for seeds is very important to guarantee the maximum quality and vigor in the field. With this, the objective of this work was to evaluate the effect of drying and harvest time of fruits on the quality of habanero peppers seeds, aimed at the maximum quality of these seeds.

MATERIALS AND METHODS

This research was conducted in the Central Laboratory of Seeds and in the experimental area from the Agricultural Department of Universidade Federal de Lavras (UFLA), in Lavras, MG. The city is located at southern region of Minas Gerais, coordinates 21°14'S of latitude and 40°17'W of longitude, and at 918.8 m of altitude. Also, varieties of yellow habanero pepper seeds (*Capsicum chinense* Jacquin) were used in this research.

Seeds were soaked in plastic trays with 72 cells with commercial substrate Plantmax, used for seedlings formation. These cells were transplanted to the experimental area after 45 days after soaking.

The tests for the seeds production were conducted in the experimental area of the Agricultural Department at UFLA, in dark red latosol (LE), clayey texture and conventionally prepared. The transplanting of seedlings was realized in the second week of December. Each splot was composed by 2 lines of 10 m of length, with 12 plants. The fertilizations, as well as the others cultural practices, were realized according recommendations for this culture (Filgueira, 2003; Pinto et al., 2006). Was used the randomized block design (DBC) with four replications, being the fruits harvested in four different period of harvest in each block: E1 (49 days after anthesis, fruits completely green), E2 (56 days after anthesis, fruits with first signals of yellowing), E3 (63 days after anthesis, yellow fruits with green signals) and E4 (70 days after anthesis, mature fruits characterized by the orange color). Part of the fruits was maintained in resting for 7days after the harvest.

Seeds were manually extracted with the aim of stylus. After the extraction, seeds were disinfected with solution of 1% of sodium hypochlorite for one minute. Following, were done tests to evaluate the seeds quality.

Seeds extracted from fruits in different periods of harvest with and without resting for seven days, were submitted to two methods of drying: M1 (artificial drying at 35°C, until 9% of water content) and M2 (natural drying at shadow, until 9% of water content).

After drying, the quality of seeds was evaluated by the germination, emergence and electric conductivity tests.

The water content of seeds was evaluated in greenhouse, at 105 ± 3ºC during 24 h, using two subsamples for each treatment, according the Regras para Análise de Sementes – RAS (Brasil, 2009). The results were expressed in medium percentage by treatment.

In the germination test, the sowing was realized in gerboxes with two papers moistened with water, in the proportion of three times the weight of the dry substrate. The boxes were maintained in chamber germination under alternate temperature and light (20°C/16 h in dark and 30°C/8 h in light presence). At 7 and 14 days, was realized the evaluation according to Brasil (2009). Each treatment was composed of four subsamples of 50 seeds. The results were expressed in percentage of normal seedlings.

Seeds which not germinated were submitted to tetrazolium test. These seeds were immersed for coloration in solution of 2,3,5 triphenyl tetrazolium chloride at 0.075%, during 3 h in dark at 35ºC. After this period, seeds were washed in current water and submersed in cold water until the evaluation. In the second phase, the embryos were individually analyzed, after opening lengthwise with stylus, verifying their external and internal parts. The interpretation was made with aim of magnifying glass with fluorescent lighting, to verify if the seeds were dead.

In emergence test, the sowing was realized in multicellular trays, containing commercial substrate (Plantmax®). The trays were kept in greenhouse with intermittent nebulization system, at temperature of 28°C. For each treatment were used four subsamples of 50 seeds. Were realized daily evaluations from the beginning of emergence, computing the number of emerged plants until the stabilization on the stand. Was computed the percentage of normal seedlings at 21 days.

For the electrical conductivity were used four subsamples of 50 seeds with known masses, immersed in 25 mL of distillated water and kept in BOD chamber, at 25ºC for 24 h (Vidigal et al., 2008). After this period, the electric conductivity of each solution was determined in conductivimeter, and the results were expressed in $\mu S.cm^{-1}.g^{-1}$ of seeds.

For the enzymes analyses, two samples of 100 seeds of each treatment were macerated in recipient with liquid nitrogen. Were removed subsamples of 100 mg, which were added extraction buffer (Tris HCl 0.2 M, pH 8) in the quantity of 2.5 times the weight of each sample and 0.1% of β-mercaptoethanol. The material was homogenized in vortex and kept in refrigerator during 12 h followed by the centrifugation at 14000 rpm for 30 min at 4ºC and them, applied in polyacrilamide gel. The electrophoretic run was realized in a discontinuous polyacrylamide gel system at 7.5% (separating gel) and 4.5% (concentrating gel) using Tris-glycine pH 8.9 as standard buffer in the gel electrode system. In each gel channel, was applied 50 µL of the sample supernatant and the running was performed at 150 V for 5 h. At the end of running, the gels were revealed for the enzymes superoxide dismutase (SOD-EC.1.15.1.1.), catalase (CAT- EC.1.11.1.6.) and esterase (EST- EC

Table 1. Germination percentage in function of development stages (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

Development stages	Drying			
	Without resting		With resting	
	Natural	Controlled	Natural	Controlled
49	23^{dA}	3^{bB}	43^{cA}	30^{bA}
56	48^{cB}	73^{aA}	80^{aA}	78^{aB}
63	64^{bA}	60^{aA}	69^{bA}	72^{aA}
70	72^{aA}	67^{aA}	80^{aB}	85^{aA}
CV(%)		19.39		

Means followed by the same lower.

3.1.1.1.) according the protocols established by Alfenas (2006).

For extraction of heat-resistant proteins, 100 seeds of each treatment were macerated in appropriate recipient with liquid nitrogen. Were separated 100 mg in microtubes for the application of 1000 µL of buffer solution (Tris-HCl (pH 7.5); 500 mM NaCl; 5 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride (PMSF)). The homogenate was centrifuged at 14,000 rpm, at 4°C, for 30 min. and supernatant was incubated in water bath at 85°C, for 15 min and again centrifuged by 30 min like above related. The supernatant was poured into microtubes and pellet was discarded. Before the application to gel, the tubes with samples containing 70 µl of protein seed extract + 40 µl of sample buffer solution, composed by 5 ml of glycerol; 2.5 mL of buffer solution of concentrating gel, 2.5 mg of Bromophenol Blue (BPB) completing the volume to 25 mL of distillated water, were placed into boiling water bath for 5 min. Subsequently, on each groove of polyacrylamide SDS-PAGE gel at 12.5% (separating gel) and 6% (stacking gel) were applied 50 µl of the extract containing the heat resistant proteins + the sample buffer solution.

Following the methodology described by Alfenas (2006), electrophoretic run was performed at 150 V, for 12 h and subsequently gels were stained with solution of coomassie brilliant blue at 0.05% and discolored with 10% acetic acid solution.

Completely randomized experimental design was used in a factorial scheme of (4x2x2), being the factors; periods of harvest (E1, E2, E3 and E4), conditions of the fruits (with and without resting for seven days) and dry methods (M1 and M2).

Analyses of variances for all the tests were realized with the aid of SISVAR® statistical program (Ferreira, 2011). For the comparison between the averages, was used the Scott-Knott test at 5% of probability. The evaluation of the enzymatic patterns was made according to the intensity of the bands, using a surface of a transilluminator.

RESULTS AND DISCUSSION

The effect of fruits harvest time and the dry conditions was significant ($p<0.05$), by the F test, in relation to the germination and vigor of habanero pepper seeds.

The values of water content in seeds harvested in different times after drying varied from 8.8 to 9.1%. The percentage of seeds germination harvested after 70 days after anthesis (DAA) and which were kept inside the fruits by seven days were higher than those harvested at 70 DAA, but that were not kept in resting inside the fruits. Seeds harvested in initial time of development, at 49, 56 and 63 DAA, presented low germination when compared to the time of 70 DAA (Table 1). In different stages were possible to verify variations in germination taxes before and after drying, showing that the pepper seeds can be included in a group described by Kermode and Bewley (1989), for which the desiccation represents a signal which diverts the development program for a germination program, since the drying was not harmful to the germination even in the stage of 56 DAA, where the factors like high content of ABA and resistance of tissues, which surround the embryo, could affect the germination and cause that, can confuse the degenerative effects of drying (Marcos, 2005).

The resting after harvest caused an increase of seeds germination, being the maximum value (85%) found in the treatment where seeds were dried with temperature control and at 70 DAA.

These results are in agreement with the results found by Sanchez et al. (1993) which, studying the resting after harvest of bell pepper, concluded that the resting showed to be beneficial to seeds harvested in the stage 1 (green) that initially presented germination of 60% and, after nine days of resting, achieve around 90% of germination.

According with Santos et al. (2015), also working with habanero pepper seeds, the period of fruits resting of seven days promotes the germination, with higher potential germinative was obtained when the period of fruits resting was superior to nine days.

This result comes to reinforce the necessity of post-harvest resting of pepper fruits above 56 DAA, when the germination achieves 60%, once that during the post-harvest resting period, seeds not yet fully mature would complete their maturation stage, while those already mature have their quality preserved for keep in osmotical equilibrium inside the fruit.

In Table 2, the percentage of viable seeds evaluated by the tetrazolium test is shown. Accordingly, the results revealed that the drying temperature of 35°C promoted lower values of dormant seeds at 70 DAA, with resting of fruits. The vigor evaluated by the first count of germination increased during the post-harvest resting in treatments where the fruits were harvested at 56, 63 and 70 DAA

Table 2. Average results (%) of viable seeds evaluated by the tetrazolium test in habanero pepper seeds in function of development stages (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

Development stages	Drying			
	Without resting		With resting	
	Natural	Controlled	Natural	Controlled
49	18^{aA}	23^{aA}	16^{aA}	15^{aA}
56	12^{bA}	08^{cB}	05^{bB}	07^{bA}
63	18^{aA}	20^{aA}	14^{aB}	15^{aB}
70	10^{bB}	12^{bA}	05^{bA}	02^{cB}
CV(%)	4.13			

Means followed by the same lower case letter in the column and capital letter in line do not statistically differ by the Scott-Knott test at 5% significance.

Table 3. Percentage of normal seedlings in the first count of germination in function of development stages (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

Development stages	Drying			
	Without resting		With resting	
	Natural	Controlled	Natural	Controlled
49	9^{bA}	3^{aA}	6^{cA}	5^{cA}
56	2^{bA}	4^{aA}	32^{aA}	22^{bB}
63	6^{bA}	4^{aA}	23^{bA}	6^{cB}
70	21^{aA}	9^{aB}	25^{bB}	53^{aA}
CV(%)	29. 54			

Means followed by the same lower case letter in the column and capital letter in line do not statistically differ by the Scott-Knott test at 5% significance.

(Table 3).

The treatment of 70 DAA differs of the others with germination values superior in the first count, what can infer that for be the treatment harvested in the stage where the fruit is completely yellow, implies that the seeds were with complete formation when compared to the fruits harvested in other stages.

These results are in agreement with studies realized by Teixeira et al. (2005) with bell pepper cv. 'Tico' indicating that the fruits harvest of bell pepper for extraction of seeds can be realized when these fruits achieve the orange coloration, being recommended the post-harvest resting of seven days.

The percentage of seedlings emergence in trays (Table 4) presented, in general, lower values than the percentage of germination. This difference could be cause in BOD chamber, the temperatures is alternate of 20 to 30ºC and the trays are kept in controlled temperature of 25ºC. It is known that between the environmental conditions which affect the germinative process, the temperature is one of the most influent factors (Mayer and Poljakoff-Mayber, 1989).

For the most part of species adapted to tropical climate, the optimum temperature is around 20 to 30°C (Marcos, 2005). According this same author, there are species for which the alternate temperature have more significant effects than the constant temperature. This behavior, associated to species that have dormant seeds, like example of habanero pepper seeds, could favor the germination.

It is important to emphasize that the emergence was evaluated at 21 days, because at 14 days after sowing, none plant had emerged. Many authors have been observed in habanero pepper seeds that the seedlings emergence is slow and irregular, even in favorable conditions (Lakshmanan and Berke, 1998).

Based on data presented in Tables 5 and 6, is possible to observe a values reduction of electric conductivity for seeds in their different stages of development and drying methods. Higher values of conductivity in seeds harvested at 49 DAA was also observed, as well as what can be justified for the incomplete formation of the membranes of these seeds. With the advance of maturation process occurs the development and the structural organization of cellular membrane systems, along with an explanation on the reduction in values of electric

Table 4. Percentage of seedlings emergence in function of development stages (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

Development stages	Drying			
	Without resting		With resting	
	Natural	Controlled	Natural	Controlled
49	0^{bA}	0^{bA}	7^{dA}	6^{dA}
56	5^{bA}	5^{bA}	22^{bB}	30^{bA}
63	0^{bB}	4^{bA}	14^{cA}	16^{cA}
70	32^{aA}	22^{aB}	39^{aB}	63^{aA}
CV(%)	25.79			

Means followed by the same lower case letter in the column and capital letter in line do not statistically differ by the Scott-Knott test at 5% significance.

Table 5. Values of electric conductivity in habanero pepper seeds in function of development stages (49, 56, 63 and 70 days after anthesis) and drying methods (natural and controlled).

Development stages	Drying	
	Natural	Controlled
49	1207.1^{aA}	1268.3^{aA}
56	919.9^{bA}	1055.8^{bA}
63	944.2^{bA}	729.0^{cB}
70	791.5^{bA}	725.3^{cA}
CV (%)	10.50	

Means followed by the same lower case letter in the column and capital letter in line do not statistically differ by the Scott-Knott test at 5% significance.

conductivity. As seeds harvested in maturation stage became more advanced, the protection mechanisms of membranes to tolerate the desiccation are presented, promoting a decrease of solutes leaching after the drying, indicating a better structuring of membranes, which can be favored by the synthesis of heat-resistant proteins (Sun and Leopold, 1993).

These results are in agreement with those presented by the germination test and clearly demonstrate that the drying promotes damages to the seeds cellular membrane systems, which are differentiated in relation to the development stage of seeds, being in the stage of 49 DAA, seeds are more susceptible to these damages than in the stages of 56, 63 and 70 DAA, successively indicating that the membrane protection mechanism is developed during the seed development. A lot of seeds suffer a quick transition of one intolerance phase, approximately in the half of their period of development, predating or coinciding with their reserves deposition (Hong and Ellis, 1992).

The enzymes superoxide dismutase (SOD) and catalase constitute an efficient mechanism for detoxification, acting in the removal of free radicals. Analyzing the activity of these enzymes was possible to observe that there was alteration in the superoxide dismutase electrophoretic pattern over the time (Figure 1). The stress caused in seeds when not submitted to the resting can be the cause of oxidative process and free radicals production, being observed higher activity of SOD in treatments in this condition. For the treatments where the seeds were submitted to resting, it was possible to observe lower activity of this enzyme. However, it is important to highlight that the seeds which were dried in controlled conditions, presented pattern for this enzyme lower than the others treatments.

The catalase enzyme has the function of to eliminate the H_2O_2 produced in the photorespiration and in the β-oxidation of fatty acids. During the oxidative stress the peroxides increases in quantity and thus higher quantity of CAT is available to combat the increase in reactive oxygen species production (Mittler, 2002). It is possible to observe that seeds from fruits which were in resting for seven days, have a reduction in the activity of the CAT enzyme. This can be directly related to the condition of rest, which allows a restructuration of seeds enzymatic complex.

When the drying pattern was observed, it was possible to observe a lower activity of catalase when seeds were dried in controlled conditions (Figure 2), which is a possible implication that the process of natural drying

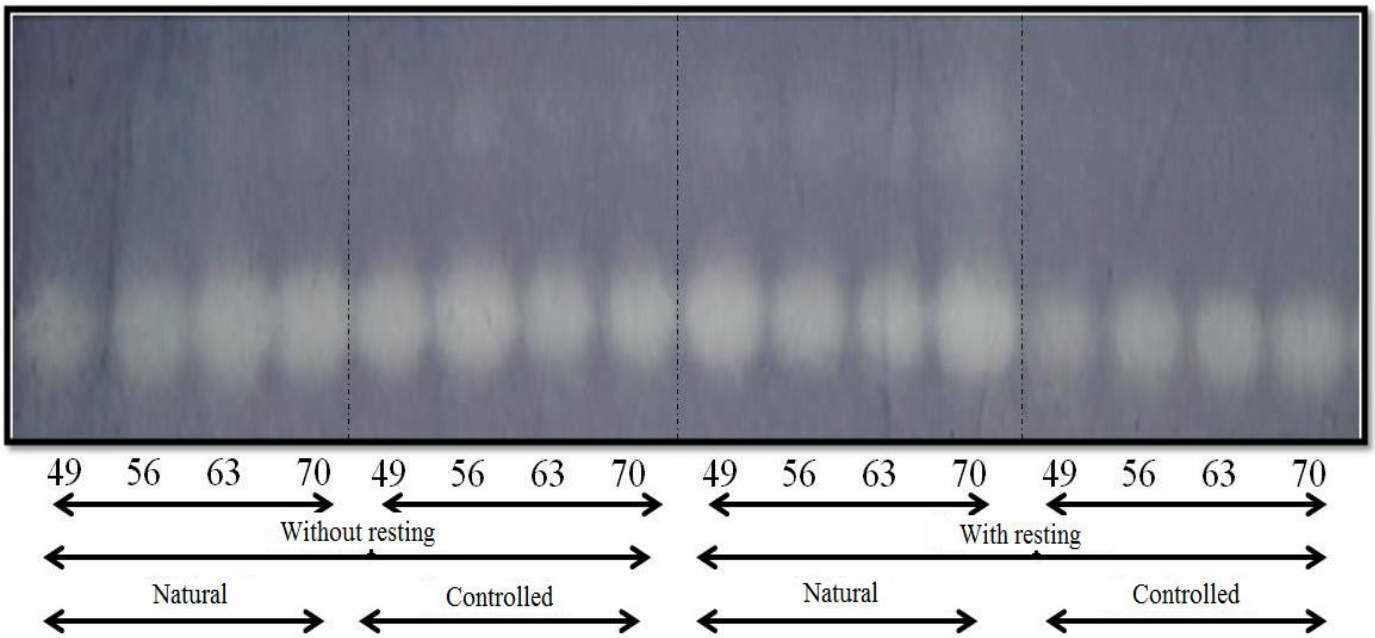

Figure 1. Electrophoretic pattern of superoxide dismutase (SOD) extracted of habanero pepper seeds in different stages of development (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

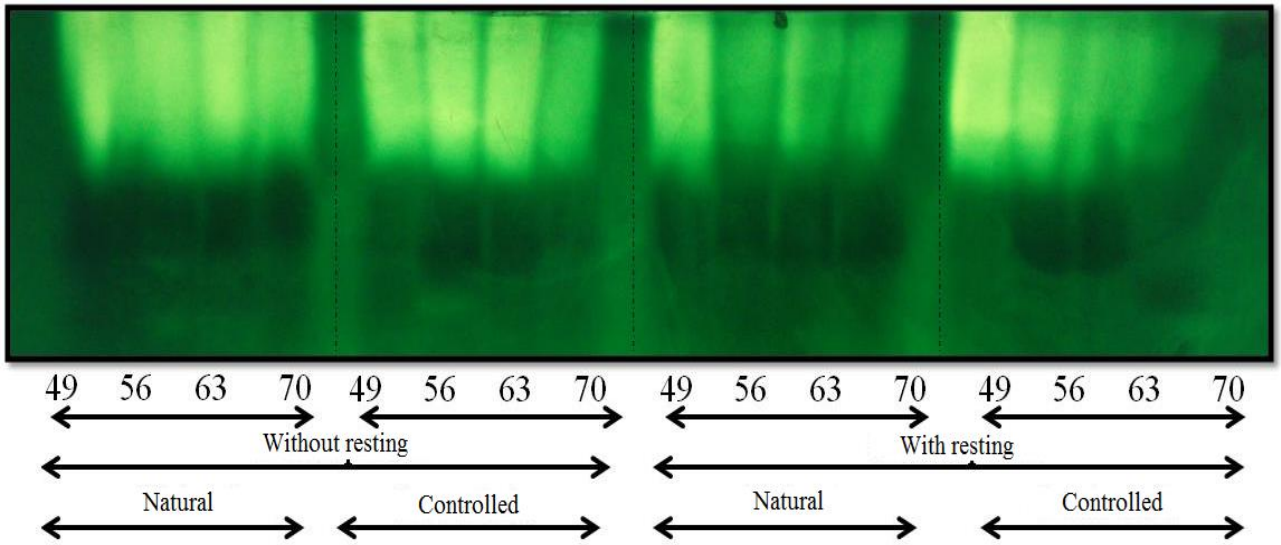

Figure 2. Electrophoretic pattern of catalase enzyme (CAT) extracted of habanero pepper seeds in different stages of development (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

infers in the enzymatic arrangement of seeds for the production of enzymes capable to remove fatty acids, accelerating the production of these enzymes to keep the quality.

The esterase (Figure 3) had behavior similar to those seeds dried at environmental temperature and at 35ºC. It is known that esterase, besides characterizing seeds in deterioration, could assist in the germinative process. The esterases are the most important group of enzymes in germination of oleaginous seeds. This big group of hydrolytic enzymes releases fatty acids of lipids, which are used in the β-oxidation, like energy source for the germinative process. Being this, the higher activity of this enzyme in the initial stages is due to the physiological

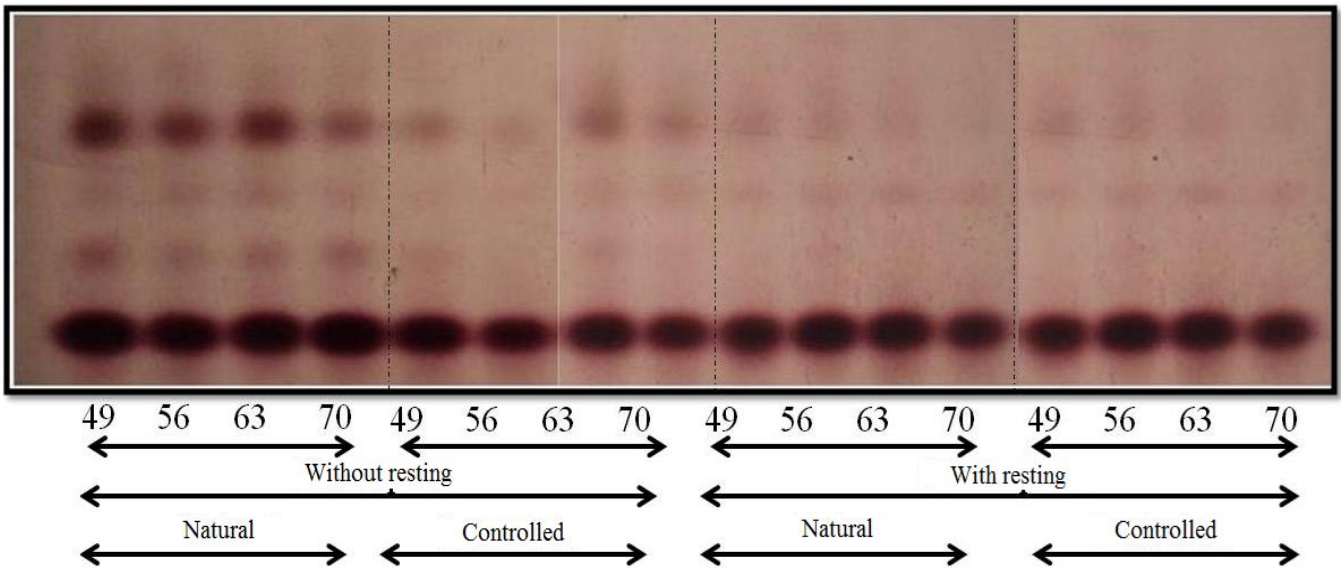

Figure 3. Electrophoretic pattern of esterase enzyme (EST) extracted of habanero pepper seeds in different stages of development (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

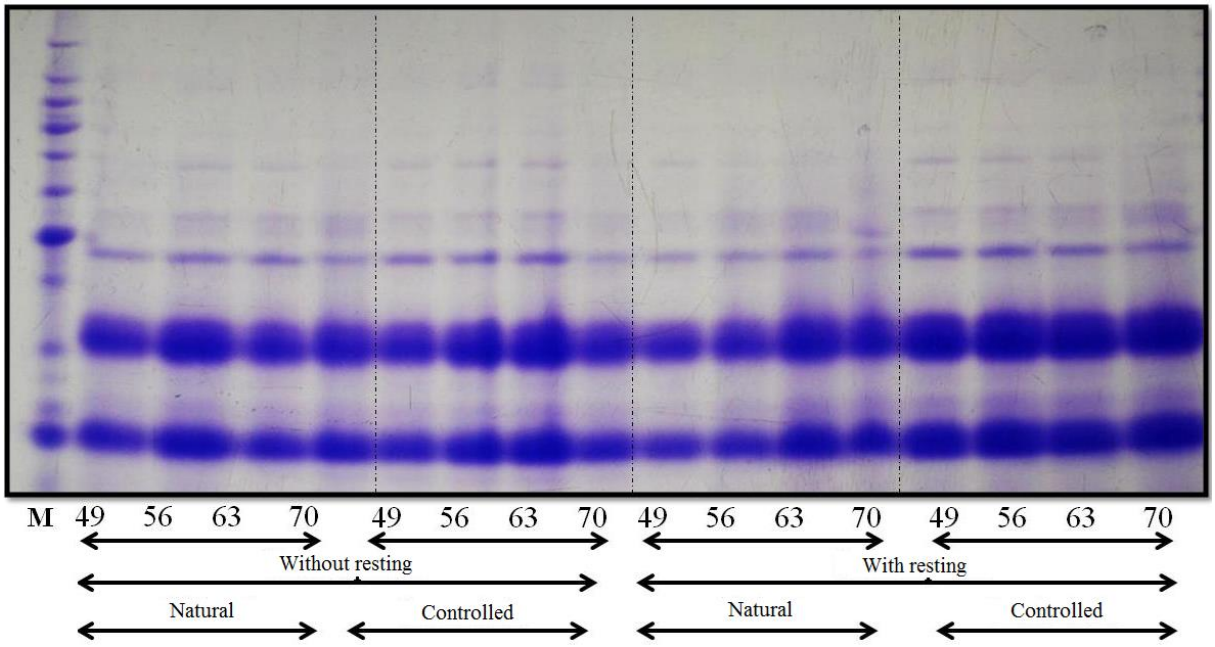

Figure 4. Electrophoretic pattern of heat resistant proteins (LEA) extracted of habanero pepper seeds in different stages of development (49, 56, 63 and 70 days after anthesis), conditions of resting (with and without resting) and drying methods (natural and controlled).

immaturity of these seeds, also as the damages caused by the drying inside the fruits.

The electrophoretic pattern of the heat-resistant proteins presented in Figure 4, revels the presence of heat-resistant proteins in all development stages evaluated, independently of the drying method and the resting condition of fruits. However, there was evidence of the absence of some bands in seeds without drying. These results are in agreement with the tendency observed in the others studies, which were verified a behavior of desiccation tolerance, by the maintenance of the quality demonstrated in the most part of evaluations of seeds

submitted to drying. These results are in agreement with the tendency observed in the others studies, which were verified a behavior of desiccation tolerance, by the maintenance of the quality demonstrated in the most part of evaluations of seeds submitted to drying (Vidigal et al., 2009).

Blackman et al. (1991) point out that these proteins are accumulated during the drying in the maturation phase and their stability, hydrophilicity and abundance in organisms tolerant to desiccation, and suggests a paper associated with tolerance to drying.

Conclusions

Habanero pepper seeds from fruits harvested and submitted to resting have high physiological quality and lower dormancy. Habanero pepper seeds, when harvested near to the maturity point and dried in controlled conditions (35ºC) in constant air flow, induces to the synthesis of heat resistant proteins.

Conflict of Interests

The authors have not declared any conflict of interests.

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