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RESEARCH ARTICLE

Post-harvest behavior of green peppers after pectin methyl esterase and calcium chloride application

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ABSTRACT

The green pepper (*Capsicum annuum L.*) is an important Brazilian vegetable and it is very much appreciated, besides being a source of vitamins, minerals and fibers. However, it has serious post-harvest shelf-life problems, such as tissue softening. The exogenous application of pectin methyl esterase and calcium has been shown to be efficient in maintaining the firmness of several fruits. Thus, the present study had as objective to evaluate the effects of the application of exogenous pectin methyl esterase (PME) and calcium in post-harvest conservation of the cv. Yolo Wander. For this, the green peppers were submitted to vacuum infusion with water, vacuum infusion with 7% of calcium chloride and vacuum infusion of PME associated to 7% calcium chloride, fruits without infusion were used as control. Loss of fresh mass, fruit firmness, peel color, soluble solids content, pH, total acidity and PME activity were evaluated. In relation to the loss of fresh mass there was a significant increase over time in all treatments. Also, according to the results, the fruits not immersed or those immersed in calcium chloride showed greater maintenance of the firmness, as well as smaller variations in the activity of the SME and low levels of organic acids. The vacuum infusion with 7% calcium chloride maintained the firmness and the physicochemical characteristics of the green pepper. However, the application of PME + $CaCl_2$ did not promote the maintaining desirable firmness characteristics for the green pepper.

Keywords: *Capsicum annuum* L; Vacuum infusion; Firmness

INTRODUCTION

The pepper (*Capsicum annum L.*) is an important agricultural crop, as it has a rich content of micronutrients and antioxidants that makes a high nutritional value, such as vitamin C and A. However, there are some limitations in the post-harvest of this fruit such as loss of firmness and short shelf-life (Ghasemnezhad et al., 2011).

During the postharvest of the pepper, alterations in the cellular wall occur that cause excessive softening of the fruits. This softening is due to the loosening of the cell wall as a consequence of the degradation of pectin and hemicellulose components. During this softening, there is an increase in soluble pectin and a decrease in insoluble pectin, causing a reduction in firmness (Song et al., 2016), due to the action of the enzymes pectin methyl esterase (PME) (EC 3.1.1.11) and polygalacturonase (PG) (EC 3.2.1.15) (Jolie et al., 2010).

In the case of calcium-pectates, the calcium-pectates, which exert a negative effect on the calcium content of calcium and calcium salts, they are responsible for the degradation of the cell wall (Martín-Diana et al., 2006). This enzyme catalyzes the methyl ester hydrolysis by releasing methanol and the free carboxylic acids in the pectin, that when in the presence of divalent ions, such as calcium, the free carboxylic acid group can be retained, resulting in a network of pectin chains and formation of gel, promoting a better firmness of the vegetables (Duvetter et al., 2005).

It has been shown that this association (exogenous pectin methyl esterase with calcium) maintains firmness in the fruit (Galleto et al., 2010). It is known that the use of calcium in the post-harvest proves to be more effective than when applied during the pre-harvest, since its application is directly on the surface of the fruit (Silva et al., 2015). For calcium application, three techniques can be used: vacuum

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infiltration, immersion or hydro cooling. In the case of vacuum infusion, it basically consists of the penetration of the solution containing calcium into the intercellular spaces and expulsion of air from the air spaces of the tissue. This occurs due to the capillary effect and the pressure gradient generated by the air out (Valero; Serrano, 2010; Silveira et al., 2011).

The use of calcium salts by vacuum infusion or immersion, associated or not with exogenous SMEs, is a promising technique that can improve the post-harvest quality of the fruits, especially those related to maintaining the firmness and integrity of the cell wall. Experiments conducted with tomatoes (Gomes et al., 2005), strawberries (Fraeye et al. (Wainer et al., 2009), mango (Taain et al., 2011) and guava (Werner et al., 2009) demonstrated positive effects of the use of the PME enzyme associated with calcium infusion, making them firmer during storage.

Thus, the objective of this experiment was to evaluate the effects of the application of pectin methyl esterase (PME) associated with calcium chloride on postharvest conservation of cv. Yolo Wander.

MATERIAL AND METHODS

The Yolo Wander (cv.) green peppers were purchased from the city of Itabaiana, state of Sergipe, Brazil, at a completely green maturation stage, with an average weight of 220 g and an average length of 9 to 10 cm. They were collected according to their appearance, color and size, and later transported to the Ecophysiology and Post-harvest Laboratory (ECOPOC) of the Department of Agronomy of the Federal University of Sergipe, São Cristóvão, Sergipe. They were washed in running water for 1 minute followed by washing in distilled water. Then they were kept on benches for drying with paper towel aid and assembly of the experiment.

For infusion treatments, a stock solution of pectin methyl esterase (commercial PME from Aspergillus aculeatus, Novoshape, Novozymes, Bagsvaerd, Denmark) was used with an activity of 11.53 U mL $^{-1}$.

The infusion procedure was performed according to Sirijariyawat et al. (2012), so the whole fruits were immersed in a 600 mL glass Becker containing 375 mL of aqueous solution. The used vacuum was 200 mmHg (26.7 kPa) for 5 minutes so there was no more air bubble outlet, in both solution and fruit. For infusion under vacuum conditions, the containers were placed in a desiccator with manometer coupled to a vacuum pump (Model 8300, Diagtech, Sao Paulo, Brazil) and the vacuum level was adjusted accordingly. After 5 minutes, the vacuum was released to reach atmospheric pressure within 1 minute, with subsequent removal of the solution. Preliminary experiments at different infusion times were performed to determine the infusion time used in this study. As a control, green peppers were used without infusion. The infusion solution consisted in: infusion in H2O; infusion into 7 g L^{-1} of $CaCl_2$; infusion into 7 g L^{-1} of $CaCl_2$ + 1mL PME/kg fruit. The concentrations of PME and $CaCl_2$ were used based on the preliminary studies.

After the preparation, the fruits were kept on benches drying for 2 minutes and packed in B.O.D. with temperature control (20 ° C ± 1 ° C) and relative humidity (85% ± 5%).

Each experimental plot was composed of 3 fruits that were weighed using an analytical balance model BG 8000 Max (GEHAKA, São Paulo, Brazil) at the beginning of the experiment, and later in each sampling period (3, 6, 9 and 12 days) weighing was performed in order to quantify the accumulated mass loss, which was expressed as a percentage of fresh mass lost.

The color of the green pepper color (C) was determined with the Chroma Meter colorimeter model CR-400 (Konica Minolta, Osaka, Japan). Measurements were performed at 2 locations in the median region of each fruit. Lightness (L*), hue (oh), chromaticity (C*) and brightness (L) were recorded. Firmness (F) was measured using the TR model digital penetrometer (Turoni, Forli, Italy), with an 8 mm diameter tip. The results obtained were expressed in Newton (N).

For the determination of the total acidity (AT), pH and soluble solids (SS), the juice of the sweet green pepper was obtained from the same extract of the pulp. The SS were determined by means of direct refractometric reading in °Brix, the evaluations were performed in two samples in each fruit, using a digital bench refractometer model RTD-45 (Instrutherm, São Paulo, Brazil). PH was measured using a pHS-3E benchtop pH meter (LabMeter, São Paulo, Brazil) and TA was determined by titration with 0.01 N NaOH solution and the results were expressed as percent citric acid.

For the analysis of PME activity, 25 g of the pulp were homogenized with 50 mL of 0.2 N NaCl, the homogenate was gassed, the pH adjusted to 6.0 with 0.1 N NaOH and the new homogenate was incubated in chamber at 4° C for 1 hour under agitation by means of a magnetic stirrer. The material was centrifuged at 800 RPM for 15 minutes at 4° C. To determine the activity, a 6 mL aliquot of extract was added and 30 mL of 1% citric pectin in 0.2 N NaCl pH 7.0 was also added to this. The demethylation rate of the extract was measured by titration with 0.01 N NaOH, maintaining the pH at 7.0 for 10 minutes. A unit of enzymatic activity (UAE) of pectin methyl esterase was defined as the amount of enzyme able to catalyze the pectin demethylation corresponding to the consumption of 1 nmol NaOH for 10 minutes. The results were expressed in UAE per gram of fresh mass per minute (Jen & Robinson 1984).

The treatments were arranged in a completely randomized design in a 4x5 factorial arrangement, and the first factor consisted of four forms of application (without infusion (SI), with infusion in water (I), infusion with calcium chloride (Ca) and associated PME infusion to the calcium chloride (PME + Ca)) and the second factor consisted of five evaluation periods (0, 3, 6, 9, 12 days after the application of the treatments), with three replicates. Data were submitted to analysis of variance through the statistical program SISVAR (Ferreira, 2011). The comparisons of means between the forms of application were made by the Tukey test ($P \leq 0.05$). For the evaluation periods, response curve fitting was performed for each characteristic.

RESULTS AND DISCUSSION

It was found that the mass loss increased significantly over time for all treatments (Fig. 1). It was observed that during the first six days of storage, all treatments showed loss of mass around 13% (Fig. 1). On the ninth day the losses were more expressive, with lower values for fruits not immersed 29.5% and the other treatments, Ca, PME + Ca and immersed in water, with 53, 5 and 61% respectively, with no significant differences between them.

Possibly, the greatest loss of fresh mass in green peppers was where ocurred an infusion, occurred due to the occurrence of unwanted cellular changes resulting from the applied tension. This result may be associated with water loss due to fruit transpiration, resulting in softening during storage (Khaliq et al., 2015). The increase in mass loss, especially in the first eight days of storage, was also observed in green peppers by Hojo et al. (2007), and the water loss reached 16.1%.

Firmness has been drastically reduced over time for all treatments, as shown in Fig. 2. The fruits immersed in calcium and not immersed, maintained higher values of firmness after nine and twelve days of storage, respectively (Fig. 2). In the case of fruits those were treated with calcium, when compared to fruits with infusion, the greater firmness, possibly due to bridges formed between pectic

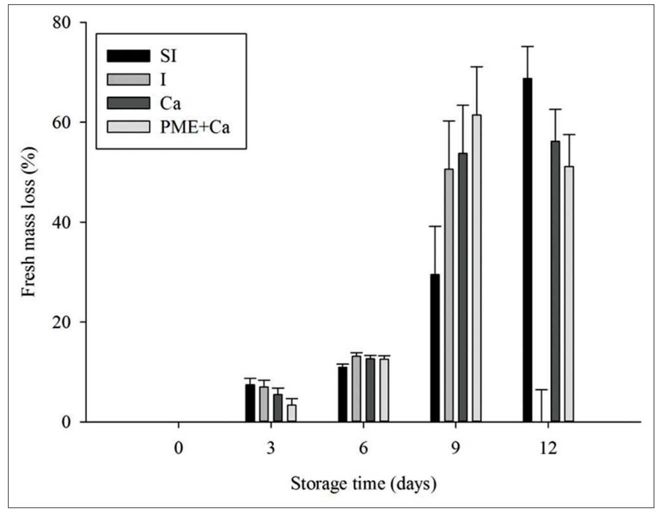

Fig 1. Loss of fresh mass in green peppers fruits stored over twelve days at 20 °C ± 1 °C in B.O.D. The bars indicate the standard error of the mean.

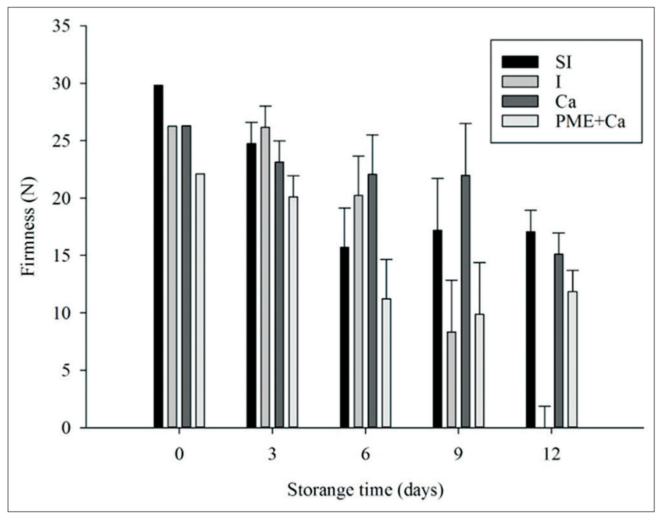

Fig 2. Firmness of pulp in green peppers fruits stored over twelve days at 20 °C ± 1 °C in B.O.D. The bars indicate the standard error of the mean.

acids and polysaccharides, complexing the cell wall and median lamella of residues of galacturonic acids attributing an improvement in structural integrity of the fruit (Mota et al., 2002). Unlike fruits without infusion that did not suffer negative pressure.

However, different responses to the use of these treatments were obtained in other experiments, as can be verified by Chen et al. (2011). The authors reported that the firmness of strawberry fruits treated with 4% calcium chloride remained constant until the tenth day of storage, different from what was reported by Suutarinen et al. (2002), as there was no difference in the firmness of strawberry fruits subjected to CaCl2 infusion and the control without infusion.

The addition of exogenous PME associated with calcium did not contribute to maintaining the firmness in green peppers (Fig. 2), and it presented a loss of firmness of the order of 58% until the ninth day of storage. Similar responses have been reported by Galleto et al. (2010) who verified loss of firmness in strawberries immersed in Calcium chloride + PME. Probably, the vacuum infusion caused loss of the tissue integrity that was compensated for by the infused active solute (Saurel, 2002), in this case calcium was the cementing agent, and not the association with exogenous PME.

It was also verified that the green pepper showed no change in color, they remained green throughout the storage, being this an intrinsic characteristic of this fruit. There was no variation in the values between treatments in relation to the hue angle (h), except for a drop on the third day for treatment with calcium chloride, and the value of 89.9 ° was obtained (Table 1). Regarding to the color intensity (C), the PME + Ca treatment on the first day showed greater intensity, while on the third day the treatment without infusion was the one with the highest value of this characteristic (Table 1). Similar results were found by Mahmud et al. (2008), as they demonstrated that the application of solutions of vacuum $CaCl_2$ in papaya promoted the maintenance of the green color of the fruit peel during 21 days of storage.

The fruits those were not immersed or immersed with calcium chloride presented the lowest variations of soluble solids (SS) contents. The non-immersed fruits did not differ statistically over time with mean values of 4.6 °Brix (Table 2). Whereas in the treatment with calcium the average value was 4.3 °Brix with small variations, that is, a small effect on the soluble solids content, as verified by Wickramasinghe et al. (2013) that found little effect on the content of solids soluble in calcium treated tomatoes. In the other treatments, different tendencies were observed, the infusion treatment in water increased from the sixth to the ninth day, from 3.1 to 5.0 °Brix, when it was discarded because it was not fit for consumption, also related to the increase of the fresh mass loss (Fig. 1) leading to the greater accumulation of soluble solids.

Fruits submitted to PME + Ca presented a peak of 6.0 °Brix on the third day and reducing to 2.53 °Brix on the last day, probably this variation is related to PME and senescence of the fruit, once according to Ghasemnezhad et al. (2011), the PME enzyme can result in SS increase in the fruit due to degradation or biosynthesis of polysaccharides, accumulation of sugars and reduction due to the increase of the respiratory rate, promoting greater degradation of SS contents, reducing it over the time. It was also verified by Vicentini (1999) that the green peppers presented an increase of soluble solids until the sixth day, followed by a decrease in the following days.

All treatments showed increases in TA up to the sixth day, with decreases after that period (Table 2). During storage,

Table 1: Peel color of green peppers fruits submitted to treatments without infusion (SI), infusion in water (I), infusion in calcium chloride (Ca) and infusion in pectin methyl esterase + CaCl2 (PME + Ca) stored over of twelve days at 20 °C±1 °C in BOD

Tratments	Angle of hue (*h*)				
	Storange (days)				
	0	3	6	9	12
SI	127,09^{Aa}	122,84^{Aa}	129,14^{Aa}	123,51^{Aa}	125,51^{Aa}
I	126,05^{Aa}	129,22^{Aa}	120,48^{Aa}	-	-
Ca	126,99^{Aa}	89,86^{Bb}	114,79^{Aa}	121,11^{Aa}	108,16^{Aa}
PME+Ca	126,76^{Aa}	121,46^{Aa}	121,99^{Aa}	109,12^{Aa}	113,70^{Aa}
Chromaticity a of shell					
SI	12,69^{ABa}	19,49^{Aa}	10,58^{ABb}	13,40^{ABb}	12,17^{ABb}
I	11,47^{Aa}	8,38^{Bb}	12,42^{Aab}	-	-
Ca	11,18^{Aa}	17,97^{Aa}	18,33^{Aa}	20,96^{Aa}	23,21^{Aa}
PME+Ca	12,24^{ABa}	21,33^{Aa}	17,05^{Aa}	21,94^{Aa}	15,55^{Aa}
Chromaticity b of a shell					
SI	29,92^{Ab}	38,03^{Aa}	34,41^{Aa}	39,85^{Aa}	37,88^{Aa}
I	34,37^{Aa}	36,14^{Aa}	33,52^{Aa}	-	-
Ca	35,12^{Aa}	38,12^{Aa}	38,74^{Aa}	42,40^{Aa}	36,79^{Aa}
PME+Ca	35,14^{Aa}	42,55^{Aa}	38,24^{Aa}	35,28^{Aa}	37,54^{Aa}
Peel color intensity (C)					
SI	15,60^{ABa}	23,16^{Aa}	16,75^{Aa}	16,52^{Aa}	14,79^{Bb}
I	14,00^{Aa}	14,12^{Aa}	14,48^{Aa}	-	-
Ca	13,94^{Ba}	21,40^{Aa}	20,23^{Aa}	27,14^{Aa}	28,02^{Aa}
PME+Ca	15,27^{Ba}	24,93^{Aa}	16,52^{ABa}	23,59^{Aa}	18,65^{Aa}

The averages followed by the same letter on the line and capitalized in the column do not differ significantly from each other by the Tukey test ($p \leq 0.05$).
(-) Samples that did not remain adequate for analysis

Table 2: Total soluble solids content (%),pH and total acidity (%), in treatments without infusion (SI), injected in water (I), in calcium (Ca) and powdered pectin methyl esterase with calcium bath (PME + Ca) at the start of the 20 day cycle at 20 ° C±° C in BOD and 75% RH

Variáveis	Storange (days)	Tratments			
		SI	I	Ca	PME+Ca
Soluble solids (ºBRIX)	0	4,63±0,1Aa	4,40±0,2Ba	3,96±0,3Bb	3,73±0,2Bc
	03	4,30±0,3Aa	4,00±0,1Bb	3,46±0,1Cc	6,00±0,8Aa
	06	4,60±0,1Aa	3,10±0,2Cb	4,20±0,6Ba	4,56±0,8Aa
	09	4,53±0,3Aa	5,03±0,9Aa	5,10±0,7Aa	2,65±0,2Cb
	12	4,96±0,3Aa	-	4,80±0,0Aa	2,53±0,6Cb
pH	0	6,27±0,4Aa	6,05±0,02Aa	5,80±0,1Aa	6,04±0,5Aa
	03	5,87±0,1Aa	5,84±0,2Aa	6,05±0,1Aa	5,89±0,8Aa
	06	6,49±0,1Aa	5,50±0,5Ab	5,40±0,6Ab	6,22±0,4Aa
	09	6,05±0,3Aa	5,81±0,5Aa	6,01±0,1Aa	4,96±0,4Bb
	12	6,21±0,4Aa	-	6,28±0,2Aa	6,73±0,2Aa
Total acidity (%citric acid)	0	0,92±0,1Aa	0,96±0,2Ba	0,79±0,3Bb	0,87±0.2Bab
	03	0,85±0,3Aa	0,70±0,1Ba	0,77±0,1Ba	0,86±0.08Ba
	06	1,02±0,1Ab	3,13±0,2Aa	1,52±0,6Ab	1,44±0.08Ab
	09	0,23±0,3Ba	0,23±0,9Ca	0,17±0,7Cb	0,39±0.2Ca
	12	0,23±0,2Ba	-	0,15±0,0Cb	0,20±0.06Ca

The averages followed by the same lowercase letter in the row and upper case in the column do not differ significantly from each other by the Tukey test ($p \leq 0.05$). The means are presented with their standard deviations. (-) Samples that were not adequate for analysis

the green peppers showed a great loss of fresh mass mainly from the sixth day (Fig. 1), reducing the cell wall turgidity and loss of firmness, as it can be seen in Fig. 2, this reduction was probably due to the senescence process.

It is known that there is a relationship between loss of firmness and TA, as this is commanded by the cell's turgidity and the integrity of pectin, the main component of the cell wall (Taiz and Zeiger 2015). With the loss of the firmness, the degradation of pectin occurs and the end products of this action are organic acids (Ghasemnezhad et al., 2011). As in this study, Silva et. al. (2011) also found an increase in the total acidity in peppers throughout the storage period.

The pH remained constant over time for all treatments and practically did not differ between them (Table 2). Similar behavior was observed by Chitravathi et al. (2014), who also found little variation of the pH of green peppers stored at 26 ± 2 ºC for 12 days. The pH of the solution ranged from 5.8 to 6.7, showing that it was in the range of non-acidic fruits (Table 2).

PME activity increased during the maturation and throughout storage time (Fig. 3), as it is directly related to cell wall degradation. For the fruits that were not submitted to infusion, the activity of PME was less marked, increasing only at the end of the storage period (Fig. 3), while those submitted to infusion had higher levels of PME activity (Fig. 3). In addition, it was verified that in the immersion with PME + Ca, the highest levels

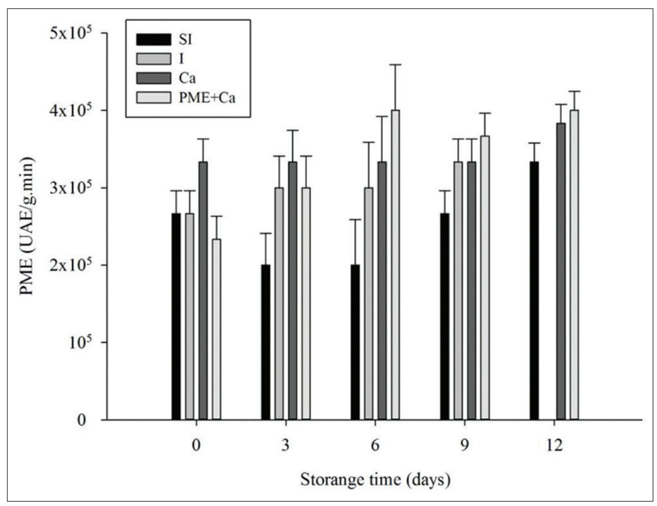

Fig 3. Activity of pectin methyl esterase (ATPME) in green peppers stored over twelve days at 20 °C ± 1 °C in B.O.D. The bars indicate the standard error of the mean.

of PME were possibly due to the sum of exogenous and endogenous PME activity, increasing the internal concentration and promoting the demethylation and complexation of Ca^{2+}. The higher activity of PME directly reflected the greater loss of firmness (Fig. 2), as the pectin solubilization occurred due to the increase in pectin methyl esterase activity (EC 3.1.1.11) and it was responsible for the softening and associated with maturation.

In the case of infusion with water, the increase in PME activity was greater from the sixth day of storage, coinciding with the loss of firmness (Fig. 2) and with increase of total acidity (Table 2). With the activity of the PME the degradation of the pectin that resulted in the production of organic acids occurred. This behavior may be due to changes in the solubilization of pectin and wall compounds during storage (Martín-Diana et al., 2006).

The calcium applied in the fruits provided a smaller increase in the activity of the PME so that it did not vary statistically over time, however, it was verified that the enzyme remained active over time. This result may have occurred due to the formation of calcium pectate, which causes a decrease in the action of this enzyme, providing greater rigidity of the middle lamella and the cell wall (Xisto et al., 2004).

The treatment with calcium and without infusion were the most adequate to maintain firmness and postharvest preservation, since they presented the smallest variations of PME activity, which resulted in the lower firmness losses and organic acid concentrations resulting from the solubilization of pectin by the loss of firmness. They were also more suitable for providing lower fresh weight loss and stability to staining.

CONCLUSION

The fruits not immersed or those immersed in calcium chloride presented a greater maintenance of the firmness, as well as smaller variations in the activity of the PME and low levels of organic acids. Vacuum infusion with 7% calcium chloride maintained the firmness and physicochemical characteristics of the green peppers. The application of PME + $CaCl_2$ did not promote the maintenance of the desirable firmness characteristics for the green pepper.

Contribution of the author

In this work all authors contributed in an effective way to the elaboration of the research and the manuscript. Airles Regina da Costa Paixão and Luiz Fernando Ganassali de Oliveira Júnior designed, conducted the research and wrote the manuscript. Pryanka Thuyra Nascimento Fontes and Alexandre Passos Oliveira performed the interpretation of the data and revised the manuscript. Marcelo Augusto Gutierrez Carnelossi and Adriano do Nascimento Simões supervised the project and reviewed the manuscript.

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APPENDIX

Appendix 1. Vaccum infusion method. A – Green pepper immersed in 375 ml of solution. B – Desiccador with pressure gauge. C - Green peppers in vacuum infusion process.