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PME and $CaCl_2$ vacuum infusion maintains the firmness and physicochemical characteristics of tomato fruits

Infusão a vácuo de PME e $CaCl_2$ matem a firmeza e as características físico-químicas dos frutos de tomate

La infusión al vacío de PME y $CaCl_2$ mantiene la firmeza y las características fisicoquímicas de los frutos de tomate

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Abstract
Tomato is a fruit of great commercial importance and highly cultivated. However, postharvest losses represent one of the main problems of this crop and can be minimized as alternative techniques. Therefore, the objective of the present work was to maintain tomato firmness by applying calcium chloride-associated pectin-methylesterase (PME) by the vacuum infusion method. Tomatoes of cultivar IAP-6 were submitted to vacuum infusion with water, vacuum infusion with 5% calcium chloride and vacuum infusion with PME associated with 5% calcium chloride, fruits without infusion were used as control. Fresh mass loss, fruit firmness, peel color, soluble solids content, pH, total acidity, PME activity and calcium activity were evaluated. The experiment was carried out in a completely randomized design in a 4x5 factorial scheme with three replications for 12 days, evaluated every 3 days. The means were compared using the Tukey test ($p < 0.05$). Data were analyzed graphically with confidence interval (CI $p < 0.05$). Regarding the loss of fresh mass there was an increase over time in all treatments. The PME + $CaCl_2$ 5% treatment was the most suitable for reducing firmness loss, as well as presenting the smallest variation of PME activity, as well as low levels of organic acids. Therefore, vacuum infusion with PME + $CaCl_2$ in tomatoes maintains acceptable firmness and physicochemical characteristics as well as $CaCl_2$ infusion.
Keywords: Calcium chloride; Pectinamethylesterase; *Solanum Lycopersicon.*

Resumo

O tomate é uma fruta de grande importância comercial e muito cultivada. No entanto, as perdas pós-colheita representam um dos principais problemas desta cultura e podem ser minimizadas como técnicas alternativas. Portanto, o objetivo do presente trabalho foi manter a firmeza do tomate por meio da aplicação de pectina-metilesterase (PME) associada ao cloreto de cálcio pelo método de infusão a vácuo. Tomates da cultivar IAP-6 foram submetidos à infusão a vácuo com água, infusão a vácuo com cloreto de cálcio a 5% e infusão a vácuo com PME associada a cloreto de cálcio a 5%, frutos sem infusão foram utilizados como controle. Foram avaliadas a perda de massa fresca, firmeza do fruto, cor da casca, teor de sólidos solúveis, pH, acidez total, atividade de PME e atividade de cálcio. O experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial 4x5 com três repetições por 12 dias, avaliadas a cada 3 dias. As médias foram comparadas pelo teste de Tukey ($p < 0,05$). Os dados foram analisados graficamente com intervalo de confiança (IC $p < 0,05$). Em relação à perda de massa fresca, houve aumento ao longo do tempo em todos os tratamentos. O tratamento PME + $CaCl_2$ 5% foi o mais adequado para reduzir a perda de firmeza, além de apresentar a menor variação de atividade da PME, além de baixos teores de ácidos orgânicos. Portanto, a infusão a vácuo com PME + $CaCl_2$ em tomates mantém a firmeza e as características físico-químicas aceitáveis, assim como a infusão de $CaCl_2$.

Palavras-chave: Cloreto de cálcio; Pectinametilesterase; *Solanum lycopersicon.*

Resumen

El tomate es un fruto de gran importancia comercial y muy cultivado. Sin embargo, las pérdidas poscosecha representan uno de los principales problemas de este cultivo y pueden minimizarse como técnicas alternativas. Por tanto, el objetivo del presente trabajo fue mantener la firmeza del tomate mediante la aplicación de pectina-metilesterasa (PME) asociada a cloruro de calcio por el método de infusión al vacío. Los tomates del cultivar IAP-6 se sometieron a infusión al vacío con agua, infusión al vacío con cloruro cálcico al 5% e infusión al vacío con PME asociado con cloruro cálcico al 5%, se utilizaron frutos sin infusión como control. Se evaluó la pérdida de masa fresca, la firmeza del fruto, el color de la piel, el contenido de sólidos solubles, el pH, la acidez total, la actividad PME y la actividad del calcio. El experimento se llevó a cabo en un diseño completamente al azar en un esquema factorial 4x5 con tres repeticiones durante 12 días, evaluadas cada 3 días. Las medias se compararon mediante la prueba de Tukey ($p < 0,05$). Los datos se analizaron gráficamente con intervalo de confianza (IC $p < 0,05$). En cuanto a la pérdida de masa fresca, se observó un aumento con el tiempo en todos los tratamientos. El tratamiento PME + $CaCl_2$ 5% fue el más adecuado para reducir la pérdida de firmeza, además de presentar la menor variación de actividad PME, así como bajos niveles de ácidos orgánicos. Por lo tanto, la infusión al vacío con PME + $CaCl_2$ en tomates mantiene una firmeza y características fisicoquímicas aceptables, así como la infusión de $CaCl_2$.

Palabras clave: Cloruro de calcio; Pectinametilesterasa; *Solanum lycopersicon.*

1. Introduction

Tomato (*Lycopersicon esculentum*) is a climacteric fruit that has a marked increase in ethylene production at the beginning of ripeness and results in high perishability, due to the increased acceleration of color changes, firmness and soluble solids content, as most of climacteric fruits (Mansourbahmani et al., 2017). Biochemical, chemical and physical treatments can be used to maintain the physical and nutritional integrity of postharvest fruits (Mahajan et al., 2014). Several studies have been conducted to reduce postharvest losses and increase shelf life of tomato fruits, such as those performed with the application of sodium selenite and calcium chloride (Zhu et al. 2016; Mansourbahmani et al., 2017).

Tomato firmness depends on the structural integrity of the cell wall and the middle lamella, whose responsible enzymes are pectinamethylesterase (PME) (EC 3.1.1.11) and polygalacturonase (PG) (EC 3.2.1.15) that are involved in pectin degradation and other cell wall materials (Jolie et al., 2010). 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). PME catalyses the hydrolysis of pectin methyl ester groups, releasing methanol and converting pectin to pectate. However, when exogenously applied and associated with calcium, SME interacts successively with calcium, forming calcium pectates that leads to a reduction in fruit cell wall degradation (Martín-Diana et al., 2006; Degraeve et al., 2003). This increase in firmness stems from the bond between divalent ions (Ca^{++}) and the group of free carboxylic acids in different pectin chains, resulting in a pectin chain network and gel formation (Durvetter et al., 2005), allowing as soon as such association (exogenous pectinamethyl esterase with calcium) prolongs fruit firmness (Galleto et al., 2010).

Thus, the use of calcium salts by vacuum infusion or by dipping associated or not with exogenous PME is a promising technique for improving firmness, since studies in strawberries (Fraeye et al., 2009), mango (Taain et al., 2011) and guava (Werner et al., 2009) demonstrated positive effect, making them firmer during storage.

Given the above, the objective of this study was to maintain the firmness of the tomato by applying calcium chloride-associated pectin-methylesterase (PME) by the vacuum infusion method.

2. Methodology

The tomatoes of cultivar IAP-6 were purchased from Itabaiana / SE region, located at latitude 10º41'06 "S, longitude 37º25'31" W and altitude 188 m, at green maturity stage, with average weight of 110 g and average length. 7 to 8 cm. These were collected according to their appearance, color, size and later transported to the Ecophysiology and Postharvest laboratory (ECOPOC) located in the Department of Agronomic Engineering of the Federal University of Sergipe, São Cristóvão / SE.

The tomatoes were washed in running water for 1 min, followed by washing in distilled water and kept on benches for drying with the aid of paper towels, after which the experiment was set up.

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

The infusion process was performed according to Sirijariyawat et al. (2012). As a control, uninfused tomatoes were used. The infusion solution consisted of: H_2O infusion; 5 g L^{-1} infusion of $CaCl_2$; infusion in 5 g L^{-1} $CaCl_2$ + PME 1mL kg^{-1} of fruit. The concentration of PME and $CaCl_2$ was used based on preliminary studies.

Whole fruits were immersed in a 600 mL glass becker containing 375 mL of aqueous solution. The vacuum used was 500 mmHg (66.75 kPa) for 10 minutes so that no air bubbles would come out of either the solution or the fruit. For infusion under vacuum conditions, the containers were placed in a pressure gauge desiccator coupled to a vacuum pump (model 8300, Diagtech, Sao Paulo, Brazil) and the vacuum level adjusted accordingly. After 10 minutes the vacuum was released to reach atmospheric pressure (within 1 minute) with subsequent elimination of the solution. Preliminary experiments at different infusion times were performed to determine the infusion time used in this study.

After preparation, the fruits were kept on drying benches for 2 minutes and stored in B.O.D. with temperature control (20 ºC ± 1 ºC) and relative humidity (85 % ± 5 %). Every three days the fruits were analyzed during a 12-day storage period after the beginning of the treatments (DAT), and the time zero was performed after the treatments.

At each sampling the fruits were evaluated for fresh weight loss (FWM), peel color (C), fruit firmness (FF), soluble solids (SS), titratable acidity (TA), PME activity and calcium determination. total.

To determine the MPF, the fruits were weighed using an analytical balance (BG 8000 Max model, GEHAKA, São Paulo, Brazil) at the beginning of the experiment and in each sampling period (0, 3, 6, 9 and 12 days) with the results expressed as a percentage of fresh mass.

For color determination (C) a portable colorimeter (Chroma Meter, model, CR-400 Konica Minolta, Osaka, Japan) was used at 2 equidistant sites in the equatorial region of the fruit. Brightness (L *), hue (ho), chromaticity (C *) and brightness (L) were recorded.

Fruit firmness (F) was measured using the digital penetrometer (TR Turoni, model 53205, Forli, Italia), with an 8 mm diameter tip, at 2 equidistant sites, in the equatorial region of the fruit. The results were expressed in Newton (N).

For the determination of total acidity (TA), pH and soluble solids (SS), tomato juice was obtained from the pulp extract. The SS was determined by direct refractory reading in Brix degrees, in two samples in each fruit, using digital banking refractometer (model RTD-45, Instrutherm, São Paulo, Brazil). The pH was measured using a benchtop pH meter (model pHS-

3E, LabMeter, Sao Paulo, Brazil) and the AT was determined by titration with 0.01 N NaOH solution following the AOAC method (2002) with the results expressed. in percentage of citric acid.

In the activity analysis of the PME 25 g of the pulp were homogenized with 50 mL of 0.2 N NaCl. The homogenate was filtered through gauze, the pH was adjusted to 6.0 with 0.1 N NaOH and the new homogenate incubated at 4 °C for 1 hour with shaking. The material was centrifuged at 25,000 g for 15 minutes at 4 °C. For determination of activity a 6 mL aliquot of extract was used and 30 mL of 1 % citric pectin in 0.2 N NaCl, pH 7.0 was added thereto. 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 enzyme activity (U) of pectin methyl esterase was defined as the amount of enzyme capable of catalyzing pectin demethylation corresponding to 1 nmol NaOH consumption for 10 minutes. Results were expressed in U per gram of fresh mass per minute (Jen and Robinson 1984).

For the determination of total calcium, approximately 20 g of tomato peel and pulp samples were used. Samples were dried at 60 °C to constant weight in a circulating oven. They were then digested in HNO_3: $H2SO_4$: $HClO_4$ solution (5: 1: 1, v / v / v). Calcium concentration was measured using an atomic absorption spectrophotometer (Perkin-Elmer, Model AAS 3110, Palo Alto, California, USA), and results were expressed as a percentage.

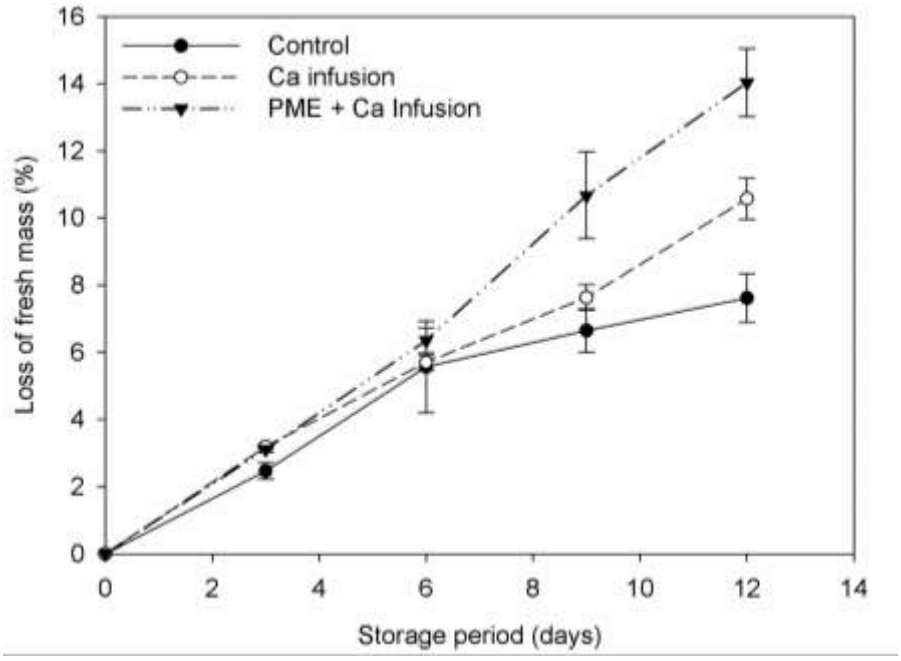
The shelf life of the fruits was estimated as the number of days required to reach the ripe red stage of the shell. Peel coloration was visually assessed using a self-prepared scale ranging from 0 to 6 recommended by Pratt & Worman (1962). The color development scale for ripening was: 0 - ripe fruits with green coloration; 1 - fruits with green breakage; 2 - fruits with equal coloration between green and pink; 3 - entirely pink fruits; 4 - totally red fruits; 5 - intensely red and firm fruits and 6 - fruits with noticeable softening.

The experimental design was completely randomized in a factorial scheme 3 (treatments) x 5 (storage times), with four replications, with the experimental unit consisting of a tomato. Statistical analysis was performed by analysis of variance (ANOVA), in which the means were compared using the Tukey test ($p < 0.05$). Data were analyzed graphically with confidence interval (CI $p < 0.05$).

3. Results and Discussion

Loss of firmness of tomato fruits is one of the most important characteristics during ripening, directly influencing postharvest storage and commercial value of the fruit. Thus, after the application of the treatments by the vacuum infusion method it was possible to notice that the tomatoes presented continuous and significant fresh mass loss during the storage period in all treatments (Figure. 1). This is linked to perspiration and respiration of the fruits, which are responsible for water loss (Khaliq et al., 2015).

Figure 1: Percentage of fresh mass loss in tomatoes subjected to treatments (Control, calcium chloride infusion and PME + CaCl2 infusion) stored over twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.

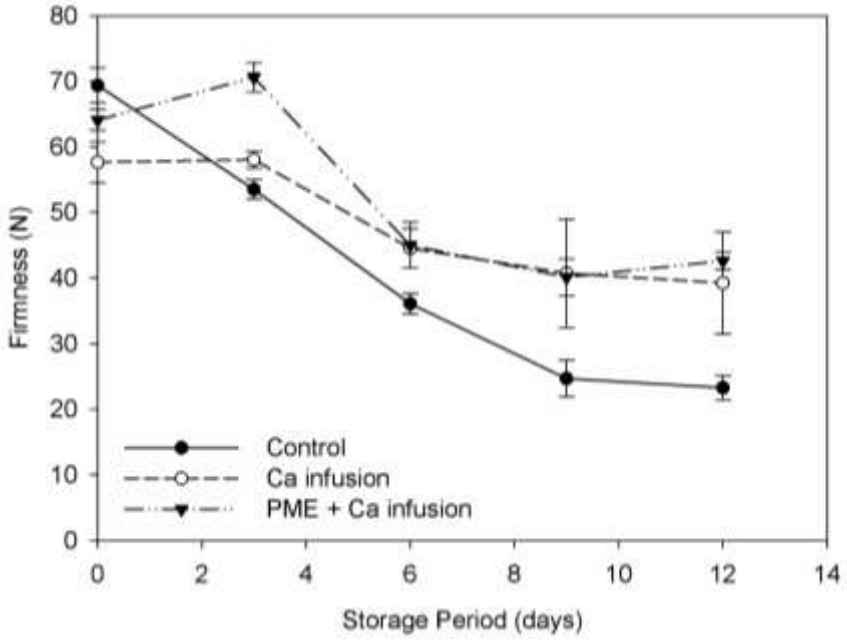


Source: Authors.

These results also showed that PMF was significantly influenced ($p \leq 0.05$) by the application of PME + $CaCl_2$ and $CaCl_2$ solutions with 14 % loss; 10 % and 7 % for PME + $CaCl_2$, $CaCl_2$ and control, respectively, over 12 days of storage. Probably, the highest PMF in the treatments (PME + $CaCl_2$ and $CaCl_2$) would be due to some factors such as: the accumulation of the solution in the peduncle insertion region, when infused, and that over time, the evaporation of this exposed solution would contribute to the PMF. Vacuum infusion may also be another factor which in turn may promote undesirable cellular changes. Excess calcium salts of the solution applied to the fruit could also be another factor as it would cause dehydration (Silva et al., 2015). Werner et al. (2009) observed that in guavas cv. Cortibel, which PMF increased as calcium was added. Similar results were also obtained by Carnelossi et al. (2018) when evaluating vacuum infusion of Pectin methyl esterase and calcium in minimally processed strawberries.

Firmness is another extremely important component for determining fruit quality and the results showed that it was significantly influenced by time and treatment application ($p < 0.05$). This parameter is highly demanded by consumers, which significantly influences the option at the time of purchase (Andreuccetti et al., 2005). The PME + $CaCl_2$ treatment (Figure 2) showed a positive influence over time, with firmness increase in the initial periods of 6 N on the third day. The positive response of PME + $CaCl_2$ treatment was due to successive interactions of PME with calcium, forming calcium pectates that led to a reduction in fruit cell wall degradation (Martín-Diana et al., 2006) and even a Improvement in structure, arising from the bonding between divalent ions (Ca^{++}) and the group of free carboxylic acids in different pectin chains, resulting in a pectin chain network and gel formation (Duvetter et al., 2005). Thus allowing such an association (exogenous pectinamethyl esterase with calcium) to prolong fruit firmness (Galleto et al., 2010) as seen during the 12 days of storage.

Figure 2: Tomato firmness (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.

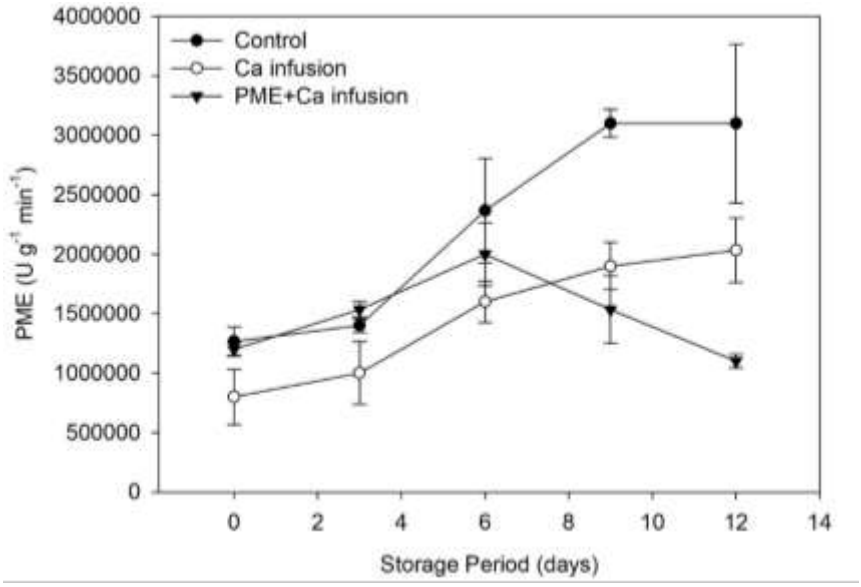


Source: Authors.

Calcium-only treatment maintained it's original firmness until the third day without fall, as exogenous calcium application inhibits endogenous PME. This response occurs due to pectin demethylation, which increases the number of carboxylic acid groups, providing Ca_2 binding with carboxyl groups of negatively charged pectin structure, increasing fruit firmness (Aghdam et al., 2012; Carnelossi et al., 2018). After the sixth day of storage, there was no difference between the treatments PME + $CaCl_2$ and $CaCl_2$, remaining constant until the end of the storage period. Postharvest calcium treatment has been effective in delaying firmness loss such as: guava (Werner et al., 2009), strawberry (Carnelossi et al., 2018), tomato (Senevirathna and Daundasekera, 2010) and apple (Ortiz, Graell and Lara, 2011). As for the control presented linear decrease and values always inferior to the other treatments.

PME activity tends, in most cases, to increase during ripening, as it is related to cell wall degradation and pulp softening, as well as the fruits of the control of this work (Figure 3) that showed linear decrease until ninth day and stabilization on day 12, inverse relationship to loss of firmness (Figure 2). These results clearly demonstrate that firmness is inversely related to SME activity, as the larger SME activity results in pectin degradation which is the main component of the fruit cell wall in which it maintains stiffness (Wen et al., 2013; Pinto et al., 2011).

Figure 3: Determination of PME in tomatoes submitted to the treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.



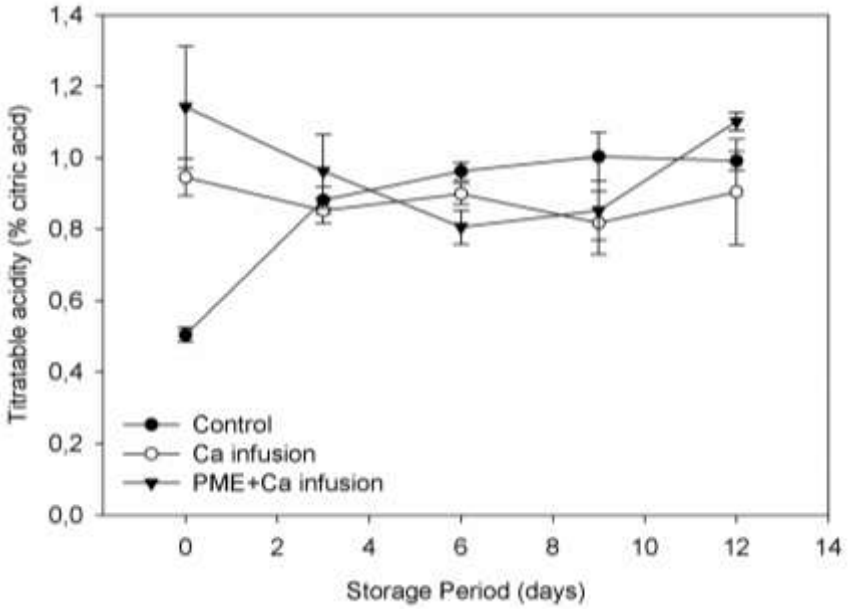
Source: Authors.

As for fruits submitted to the infusion with PME + $CaCl_2$ initially presented higher levels of PME activity (Figure 3), due to the infusion of exogenous PME and its action on the pectin methyl radical, increasing the internal concentration and promoting the binding. between the divalent ions (Ca^{++}) and the group of free carboxylic acids in different pectin chains (Duvetter et al., 2005), thus increasing firmness in the early days. From the sixth day on, the activity of the PME decreased drastically due to the scarcity of the substrate that led to stabilization of firmness (Figure 2).

With the application of $CaCl_2$ only in the fruits, the treatment promoted the inhibition of endogenous PME activity, causing it not to vary statistically in the initial period and to be inferior to the control due to the formation of calcium pectate, a compound that decreases the action of PME. providing greater stiffness of the middle lamella and cell wall (Xisto et al., 2004). Even without $CaCl_2$ statistical variation over time, the activity of the PME was statistically lower than the control, since the control without calcium allows greater activity of the PME and consequently greater loss of firmness and increase in the concentration of organic acids.

The titratable acidity varied as a function of time between treatments verifying significant difference ($p \leq 0.05$). The control showed an increase in acidity throughout the storage period, being more pronounced from the third day, as well as the greater loss of firmness and greater activity of the PME (Figures 2 and 3) because they are linked as product, result and agent. respectively. The PME + $CaCl_2$ treatment as opposed to the control and $CaCl_2$ showed a decrease in TA in the early periods, such decrease is related to the maintenance of firmness (Figure 2), since the loss of firmness is related to pectin degradation by PME activity (Figure 4) which has as its product the formation of organic acids (Yamamoto et al., 2011). Thus, if there was no decrease in firmness (in the first three days) there was no increase in TA in the initial periods. After this initial period, the TA increase for PME + $CaCl_2$ began well after the control and $CaCl_2$, indicating less firmness loss and delayed maturation. The $CaCl_2$ treatment presented intermediate behavior indicating the influence of calcium on the firmness and the amount of organic acids of the fruit. Mahmud et al. (2008) also observed that the lower amount of organic acids is related to calcium addition, which suggests that it results from reduced respiration, which would delay the ripening process of the treated fruits.

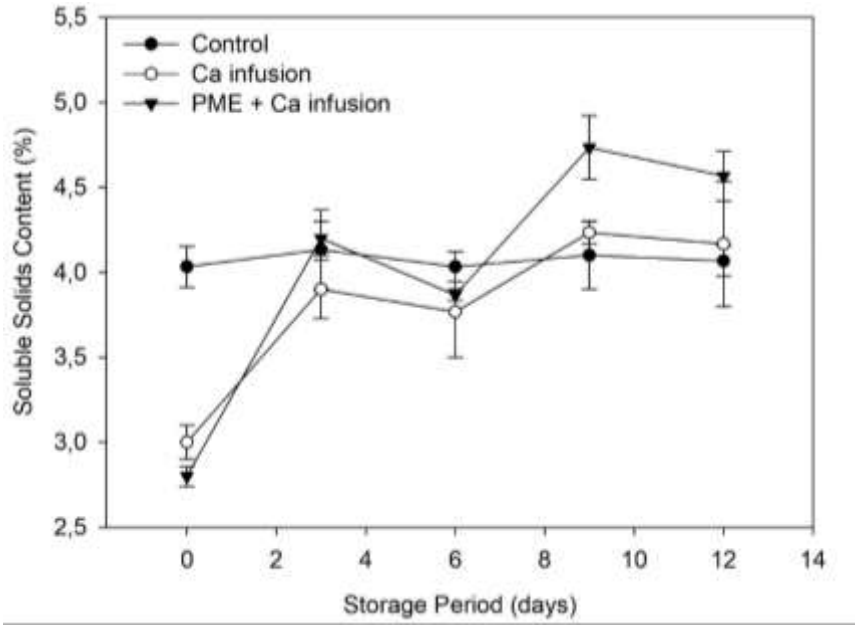
Figure 4: Titratable acidity of tomatoes subjected to the treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.



Source: Authors.

The soluble solids content was constant over time for the control (Figure 5) because green tomatoes do not yet have biochemical and physiological maturation to complete ripening and turn sugar into acids, and starch into sugar (Chitarra and Chitarra, 2005). For treatments PME + $CaCl_2$ and $CaCl_2$ it was found that the SS concentrations on the first day of analysis was much lower when compared to the control (2.8; 3.0; 4.03 respectively), this difference is probably due to the process. infiltration that solubilizes and reduces SS concentration. After the third day of storage the SS content was not influenced ($p \leq 0.05$) by the vacuum infiltration treatments, nor did it differ over time in the fact that the tomato is a fruit with minimum starch content. Slight variation may occur due to cell wall degradation, which increases the release of soluble pectin in intercellular spaces, contributing to a slight increase in SS content (Yao et al., 2014).

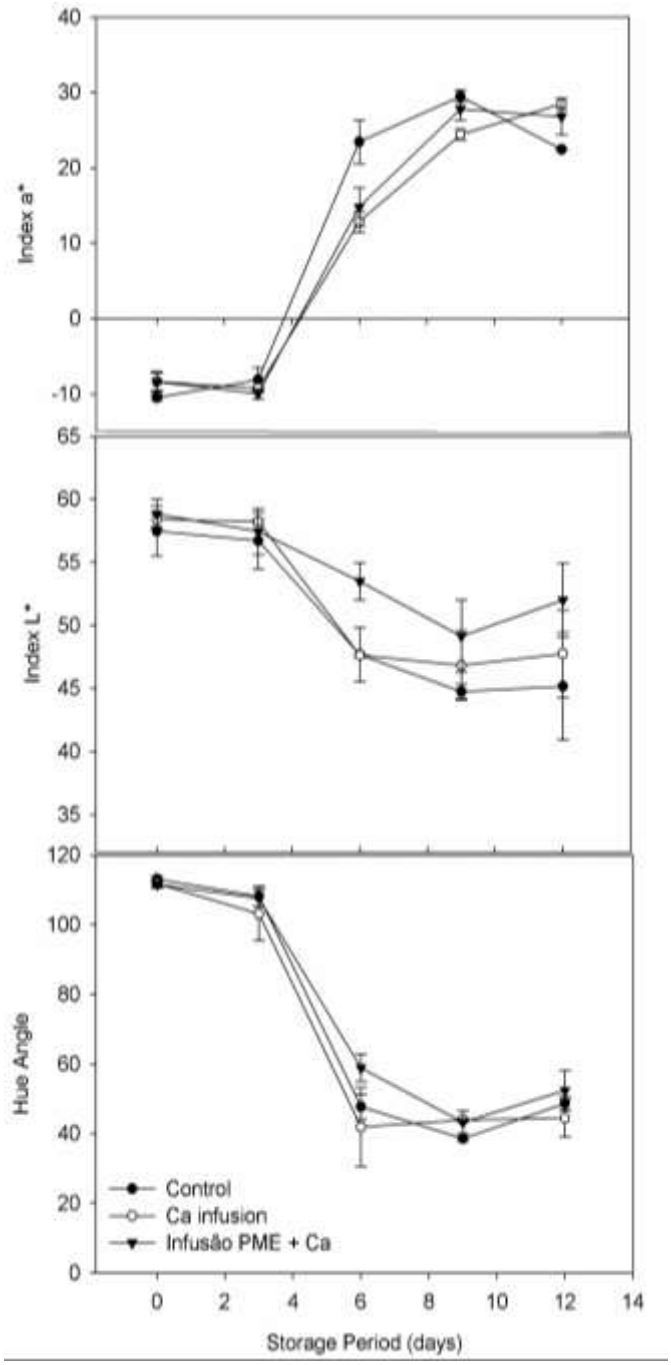
Figure 5: Soluble solids content of tomatoes subjected to the treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.



Source: Authors.

During storage, hue angle values (Figure 6) tended to decrease, reflecting the fruit's green color loss, with significant difference ($p \leq 0.05$) between treatments. The largest decrease was between the third and sixth day, when the tomato lost almost all green color (index a). Maturation involves a change in color from green when immature to red when mature, as this change is related to the synthesis and degradation of total pigments such as chlorophylls a, b and carotenoids (Rugkong et al., 2010). Thus, the change from green to red in this study was smaller in fruits treated with PME + $CaCl_2$ and $CaCl_2$.

Figure 6: Peel color of tomatoes subjected to treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.



Source: Authors.

The variation in color development compared to the control showed that PME + $CaCl_2$ and $CaCl_2$ treatments retarded the ripening process, probably inhibiting maturation metabolic systems. $CaCl_2$ may have an effect on the ethylene cycle that affects lycopene pigment synthesis during the ripening process (Brackmann et al., 2010). Results have shown that application of calcium chloride delays the ripening of developing fruits such as papaya (Silva et al., 2015) and that application of calcium has caused retardation of apple color development (Pizato et al., 2013). These results are consistent with those found in the literature where fruit color development was significantly retarded using calcium chloride treatment compared to control.

Table 1 and Painting I show the values and images of tomatoes throughout ripening and submitted to treatments. For all treatments there was an increase in fruit coloration during storage, and for control fruits, there was a great increase in coloration from the sixth day of storage. The color changes for the fruits of the other treatments were less intense. Influence of calcium infusion and PME + Ca on delayed color development.

Table 1: Staining of tomatoes subjected to the treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D.

Variables	Storage (Days)	Treatments		
		Control	Ca	PME+$CaCl_2$
Coloring	0	0	0	0
	03	1	0	0
	06	4	3	3
	09	5	4	4
	12	6	5	5

Source: Authors.

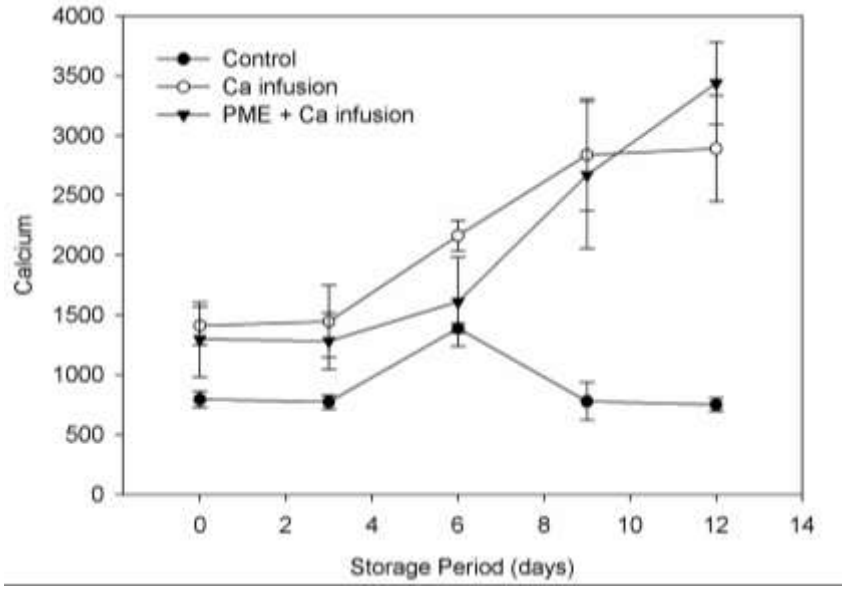
Painting 1: Images of tomatoes subjected to the treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D.

Treatments	Storage Days				
	0	03	06	09	12
Control					
Calcium chloride					
PME+$CaCl_2$					

Source: Authors.

The calcium content inside the fruits treated with PME + $CaCl_2$ and $CaCl_2$ were significantly higher than the control ($p > 0.05$) during the storage period (Figure 7) and did not differ between the PME + $CaCl_2$ and $CaCl_2$ treatments. During the storage period, calcium content increased for PME + $CaCl_2$ and $CaCl_2$ treatments. This increase was due to the solution entry through the peduncle insertion scar and the removal of the material for analysis was in the equatorial median region of the fruit, therefore, the solution migration was not immediate to the place of analysis but gradually. As seen by Senevirathna and Daundasekera, (2010) where they applied a solution containing $CaCl_2$ and black dye by vacuum infusion in tomato and found that the solution penetrates the peduncle scar and migrates into the pericarp.

Figure 7: Calcium content in tomatoes subjected to treatments (Control, calcium chloride infusion and PME + $CaCl_2$ infusion) stored for twelve days at 20 ° C ± 1 ° C in B.O.D. The bars in the graph refer to the standard error of the mean.



Source: Authors.

It was also found that during storage the calcium concentration between treatments and control varied from 2 times in the initial periods to 3 times in the final periods so that these concentrations were sufficient to produce the perceived delay in tomato ripening, also verified. by Senevirathna and Daundasekera (2010), by Balic et al. (2014) on grapes and by Kou et al. (2015) in pear.

4. Conclusion

5% PME + $CaCl_2$ vacuum infusion is effective in controlling ripening by reducing firmness loss.

The treatments PME + $CaCl_2$ and $CaCl_2$ were more efficient than the control in delaying the maturation and maintenance of the physicochemical and organoleptic characteristics.

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