

Ethylene management, controlled atmosphere and wax application on quality of 'Priscila' pepper after cold storage

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ABSTRACT

The objective of the present study was to verify the effect of ethylene management, controlled atmosphere and wax application on the quality of green bell peppers (cv. Priscila) after 30 days of storage. The study consisted of three experiments. After 30 days of storage no significant difference in the incidence of decay and mass loss was observed, although the application of AVG 7 days before harvest maintained higher levels of titratable acidity. In the second experiment, higher titratable acidity was obtained when green bell peppers were stored under controlled atmosphere (CA) as compared to cold storage. Finally, in the third experiment, the application of wax maintained higher titratable acidity and reduced the occurrence of decay. The application of AVG 7 days before harvest maintains higher titratable acidity and greener fruit skin as compared to the treatment of peppers with the ethylene inhibitor 1-MCP. In addition, the best CA condition to store green bell peppers is 3 kPa O₂ + 5 kPa of CO₂, because it reduces the mass loss and maintains higher titratable acidity. Wax application, in turn, reduces the incidence of decay, retards the appearance of yellow skin color, decreases the respiration rate and maintains higher titratable acidity.

Key words: *Capicum annuum*, decay incidence, mass loss, storage condition, titratable acidity

Manejo do etileno, atmosfera controlada e aplicação de cera na qualidade de pimentões 'Priscila' após o armazenamento refrigerado

RESUMO

O objetivo do presente estudo foi verificar o efeito do manejo do etileno, atmosfera controlada e aplicação de cera sobre a qualidade de pimentões (cv. Priscila) após 30 dias de armazenamento. O estudo consistiu de três experimentos. Após 30 dias de armazenamento não houve diferença significativa na incidência de podridões e perda de massa, embora a aplicação de AVG 7 dias antes da colheita manteve maiores teores de acidez titulável. No segundo experimento, maior acidez titulável foi obtida quando os pimentões foram armazenados em atmosfera controlada (AC) em relação à atmosfera refrigerada. Por último, no terceiro experimento, a aplicação de cera manteve maior acidez titulável e reduziu a incidência de podridões. A aplicação de AVG 7 antes da colheita mantém maior acidez titulável em comparação ao inibidor de etileno 1-MCP. A melhor condição de AC para o armazenamento de pimentões é 3 kPa O₂ + 5 kPa CO₂, pois reduz a perda de massa e mantém maior acidez titulável. Aplicação de cera reduz a incidência de podridões, retarda o aparecimento da cor amarela na epiderme, diminui a taxa respiratória e mantém maior acidez titulável.

Palavras-chave: *Capicum annuum*, incidência de podridões, perda de massa, condições de armazenamento, acidez titulável

Introduction

Pepper (*Capicum annum*) belongs to the solanaceae family. Pepper fruits have been used as condiment, either for *in natura* consumption or as part of industrialized products. Because of the elevated quality losses (Hojo et al., 2007), the commercialization of pepper fruits *in natura* must proceed immediately after harvest. Thus, the high perishability of pepper fruits makes it relevant to research new techniques for the storage of this product.

Perhaps one promising technique for maintaining the postharvest quality of peppers is the management of ethylene during storage. This plant hormone is responsible for triggering a series of biochemical reactions that culminate in fruit ripening (Chitarra & Chitarra, 2005; Ma et al., 2006). Ethylene management is widely used during apple storage, mainly due to its positive effects on quality maintenance during postharvest live (Brackmann et al., 2009). Since its use has not yet been studied in pepper fruits, it is not well understood at which extent ethylene affects the postharvest quality of these fruits.

Among the chemicals used to control ethylene production or action, the most widely used are Aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP), respectively. 1-MCP is a volatile compound that binds irreversibly to the ethylene receptors, thereby inhibiting ethylene action (Ma et al., 2006). Once the action of ethylene is inhibited, fruit ripening and senescence are delayed and, as a consequence, fruits maintain better quality during the postharvest shelf-life (Blankenship & Dole, 2003; Watkins, 2006). The chemical AVG, in turn, inhibits ethylene synthesis by binding to the active site of 1-aminocyclopropene-1-carboxylase synthase (ACC synthase), the enzyme responsible for the synthesis of the precursor of ethylene, 1-aminocyclopropene-1-carboxylase (ACC). The application of AVG has proven to be beneficial in apples (Steffens et al., 2010; Salas et al., 2011). However, the effects of AVG on pepper fruits are not yet described in literature, making it necessary to study its effects in this species.

In addition to ethylene management, also the use of controlled atmosphere (CA) during the storage of peppers is not yet well studied. The CA consists in reducing the O₂ partial pressure, while increasing those of CO₂ inside the storage environment. By manipulating the partial pressures of O₂ and CO₂ one is able to reduce the metabolic activity of fruits during the postharvest period. In fact, CA has been successfully used to maintain the postharvest quality of apples (Brackmann et al., 2009; Brackmann et al., 2010) and pears (Pedreschi et al., 2009), among other fruits. The control of O₂ and CO₂ is usually used in combination with reduced storage temperatures. However, it is noteworthy that the storage temperature cannot be reduced indiscriminately. In fact, some studies have demonstrated that pepper fruits are sensible to low temperatures and that the temperature limit for these fruits is between 9 to 13 °C at a relative humidity of 90 to 95% (Chitarra & Chitarra, 2005). However, other authors suggest that peppers can be even stored under 7 to 9 °C (Barros et al., 1994).

Besides the two storage methods described above, the postharvest quality of pepper could potentially be improved by the application of waxes on the fruit skin. The application of

wax has been shown to offer a barrier for mass loss, to reduce the respiration rate and to maintain titratable acidity of fruits (Beaulieu et al., 2009). However, more studies are necessary to determine the correct region of the fruit for receiving the wax application. Other researchers found that wrapping the fruit with plastic films (Barros et al., 1994) or PVC (Hojo et al., 2007) is also able to decrease substantially mass loss and to maintain fruit quality.

Thus, the objective of the present study was to assess the effect of ethylene management, controlled atmosphere and wax application on the quality of green bell pepper cv. Priscila after 30 days of storage at 9 °C.

Material and Methods

The study was carried out on the Post-harvest Research Center of the Federal University of Santa Maria (UFSM). The green bell peppers were obtained from a greenhouse localized in the experimental area of the Plant Science Department from the same university. Immediately after harvest, fruits were transported to the Post-harvest Research Center where a pre-selection was undertaken. All fruits exhibiting defects or injuries were eliminated from the experimental samples. Thereafter, samples were homogenized and located inside experimental chambers with 80L. Each treatment was composed by four replicates, containing five fruits each. The three experiments were conducted in a completely randomized statistical design. The storage temperature of all experiments was adjusted to 9 °C (± 0.2 °C) and the relative humidity was set at 96% (± 1.0 %).

In the first experiment the following treatments were assessed: [1] control (cold storage only); [2] application of AVG (150 g ha⁻¹) 14 days before harvest, followed by cold storage; [3] application of AVG (150 g ha⁻¹) 7 days before harvest, followed by cold storage; [4] ethylene absorption (with potassium permanganate) during cold storage; [5] application of 1-MCP (1 μ L L⁻¹) before cold storage; and [6] application and maintenance of ethylene (2 ppm) throughout the cold storage. The AVG was obtained from the commercial product Retain® (15% of active ingredient) and 1-MCP from Smartfresh® (0.43% of 1-MCP). The second experiment was composed by three treatments: [1] control (cold storage only); [2] controlled atmosphere with 3 kPa O₂ + 5 kPa CO₂; and [3] controlled atmosphere with 3 kPa O₂ + 10 kPa CO₂. To obtain the desired O₂ concentration, N₂ was injected into the storage chambers. The CO₂ concentrations were obtained by flushing the storage chamber with CO₂ from a high pressure cylinder. The third experiment was composed by the following treatments: [1] control (cold storage only); [2] wax application only over the fruit skin before cold storage; and [3] wax application only on the fruit peduncle before cold storage. In order to apply the wax, fruits were submerged in a solution containing the commercial product Garfresh N® diluted in water 1:3 (v/v).

After 30 days of storage, eight quality parameters were evaluated for each experiment: a) Decay incidence: was evaluated at the opening of the chambers and after 7 days at 20 °C. To obtain the level of decay, a scale (1 - 4) was used,

where 1= no incidence of decay and 4 = 100% of fruit skin with decay incidence; b) Mass loss: evaluated at the opening of the chambers and after 7 days at 20 °C. This parameter was estimated by weighing fruits before and after the storage and shelf-life periods. The data were expressed in percentage of the initial mass; c) Skin color: was evaluated right after removing pepper fruits from the storage chambers and after 7 days at 20 °C by using a colorimeter (Minolta, model CR 310) which uses the tridimensional color system and expresses the data in terms of a*b*; d) Ethylene production; e) Respiratory rate; f) Titratable acidity and g) Soluble solids (SS): evaluated according methodology proposed by Brackmann et al. (2010).

Data expressed in percentage were transformed with the formula $\text{arc.sin}(x/100)0.5$ prior to the variance analyses. After the variance analyses, means were compared by Tukey's test, with 5% of probability of error.

Results and Discussion

In order to gain an insight on the maturation stage of fruits before storage, an initial analysis was carried out. This analysis revealed that the mass loss of pepper fruits follows a positive linear equation with the postharvest days (Figure 1A). These results are in agreement with another report, which showed that the mass loss is explained by a linear equation (Hojo et al., 2007). Similar behavior was also observed for fruit respiration, even though in this case the equation was negative (Figure 1B). The titratable acidity, soluble solids and skin color were only analyzed after 7 days in shelf-life at 20°C. The results indicated that, at this stage, green bell peppers exhibit reduced acidity, low contents of soluble solids (Figure 1C) and green skin color (Figure 1D), thus showing similar values from those already reported literature (Hojo et al., 2007; Boas et al., 2012).

After 30 days of storage plus 7 days of shelf-life at 20 °C, no significant difference was observed in decay incidence and mass loss (Figure 2A and 2B). These results showed that ethylene management did not affect any of these two parameters. Possibly this result was found because fruits from plants of the genus *Capsicum* are considered as non-

climacteric (Biles et al., 1993; Lee et al., 2010), and thus most likely exhibit a distinct response to ethylene as compared to climacteric fruits.

Either at the opening of chambers or after 7 days of shelf-life at 20 °C the skin color of fruits from all treatments did not show significant difference from the fruits of the control treatment (Figure 2C and 2D). Nevertheless, fruits treated with AVG exhibited greener skin color than those treated with 1-MCP or with 2 ppm of ethylene (Figure 2C). The higher degradation of green color observed in 1-MCP-treated peppers was likely the consequence of the higher ethylene production of these fruits (Figure 2E). In fact, changes in skin color of fruits have been associated ethylene production and consequently with its management (Woolf et al., 2005; Watkins, 2006). Some studies have also shown that more significant changes in pepper color occur during the shelf-life period (Hojo et al., 2007; Boas et al., 2012).

At the opening of chambers, ethylene production was higher on fruits treated with ethylene or 1-MCP, but was not

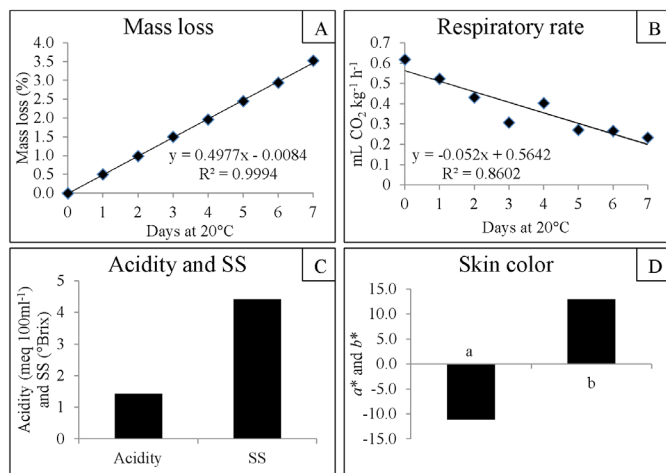


Figure 1. Mass loss, respiratory rate, titratable acidity, soluble solids (SS) and skin color from initial analysis of 'Priscila' pepper. Santa Maria, RS, Brazil, 2015. A and B: Regression analysis with 5% of probability error

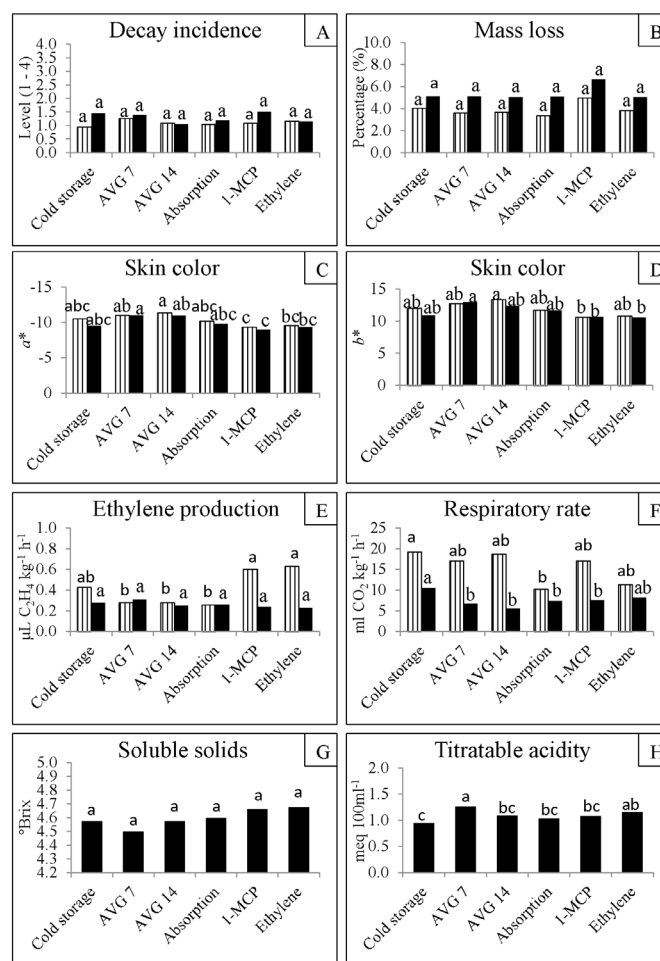


Figure 2. (experiment 1) Decay incidence (A), mass loss (B), skin color (C and D), ethylene production (E), respiratory rate (F), soluble solids (G) and titratable acidity (H) of 'Priscila' pepper with different ethylene management and cold storage during 30 days. Santa Maria, RS, Brazil, 2015. AVG: Application of AVG 14 days and 7 before harvested; Absorption: ethylene absorption with permanganate sachets; 1-MCP: 1 $\mu\text{L L}^{-1}$ of 1-MCP application; Ethylene: application and maintenance of two ppm of ethylene in storage chambers. Means followed by equal letters, among treatments, do not differ by Tukey test, at 5% of probability of error. \square : Opening of chambers; \blacksquare : 7 days at 20 °C

significantly different from fruits of the control treatment (Figure 2E). These results disagree with some reports that have demonstrated that 1-MCP application can reduce ethylene production (Blankenship & Dole, 2003; Woolf et al., 2005; Watkins, 2006). However, it is noteworthy that pepper fruits are non-climacteric (Biles et al., 1993; Lee et al., 2010), and thus the effect of 1-MCP on these fruits are different as compared to climacteric fruits. After seven days of shelf-life, no significant differences between the treatments were observed for ethylene production (Figure 2E).

In both times of evaluation (right after removing fruits from storage chambers or after 7 days at 20 °C) lower respiratory rate was detected in pepper fruits from the ethylene absorption treatment as compared to fruits in cold stored only (control) (Figure 2F). These results revealed that ethylene absorption had similar effect to the application of 1-MCP on green bell peppers. Silva et al. (2011) observed higher respiratory rate on pepper fruits treated with ethylene. This result explains the lower respiration rate of peppers stored with ethylene absorption. However, on this work no significant difference was observed between ethylene absorption and ethylene application. The non-significant difference is explained because the respiration rate of pepper responds more with the temperature than with maturation stages (Antoniali et al., 2006).

The treatments tested did not affect significantly the contents of soluble solids (Figure 2G). Steffens et al. (2010) also observed non-significant difference in soluble solids on apples treated with AVG. However, the application of AVG 7 days before harvest resulted in the highest titratable acidity in relation to the other treatments, except when compared to ethylene application (Figure 2H). Comparable results were also obtained in 'Golden Delicious' apples treated with AVG 4 weeks before harvest (Salas et al., 2011). In addition, Silva et al. (2011) have demonstrated that ethylene application keeps higher titratable acidity in pepper fruits in relation to the control treatment.

Right after the end of storage, it was observed that the three storage conditions tested in the second experiment did not affect significantly the incidence of decay in pepper fruits (Figure 3A) and that the average values were similar to those of the first experiment (Figure 2A). After seven days of shelf-life, decay incidence was significantly increased in fruits stored with 10 kPa CO₂. These results demonstrate that green bell peppers are sensitive to high CO₂ concentrations, which probably induced the development of physiological disorders and increased the incidence of decay. In CO₂-sensitive pears, elevated concentration of CO₂ induces some physiological disorders (Pedreschi et al., 2008). Regarding mass loss, it was observed that at the end of the storage period fruits of the control treatment had lost significantly more mass (Figure 3B). However, after 7 days of shelf-life mass loss was higher in fruits stored with the highest CO₂ concentration (10 kPa CO₂). This significantly increased mass loss can be mainly explained by the elevated incidence of decay on fruits of this treatment (Figure 3A).

Skin color is an important visual attribute, which makes this an important quality parameter. In this study, skin color after storage was similar to that recorded before storage (Figure 3C and 3D), and was not significantly affected by the

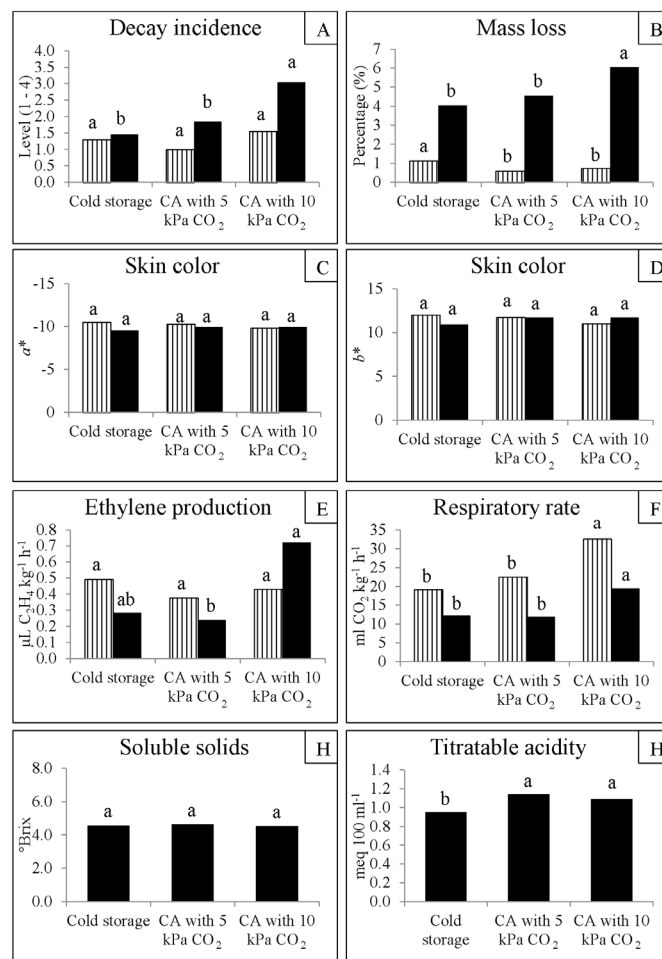


Figure 3. (experiment 2) Decay incidence (A), mass loss (B), skin color (C and D), ethylene production (E), respiratory rate (F), soluble solids (H) and titratable acidity (G) of 'Priscila' pepper in cold storage (CS) and controlled atmosphere (CA) at the temperature of 9 °C during 30 days. Santa Maria RS Brazil, 2015. Fruit of treatment of controlled atmosphere (CA) were stored with 3 kPa de O₂; Means followed by equal letters, among treatments, do not differ by Tukey test, at 5% of probability of error. ▨: Opening of chambers; ■: 7 days at 20°C

treatments. Manolopoulo et al. (2010) have reported that skin color of green peppers after storage at 5°C was maintained at similar pre-storage levels when modified atmosphere was used. However, the same authors found significant differences in skin color before and after storage when pepper fruits were stored at 10 °C, demonstrating that the temperature has more effect on color changes than the gas composition.

Ethylene production at the removal of fruits from storage was not significantly affected by the treatments (Figure 3E). However, after 7 days at 20 °C, higher ethylene synthesis was observed in fruits stored under 10 kPa CO₂ as compared to 5 kPa CO₂. The elevated ethylene production of these fruits also coincided with high respiration rate, both right after the end of storage and after 7 days of shelf-life at 20 °C (Figure 3F). The higher respiration rates exhibited by pepper fruits stored under 10 kPa CO₂ may well be explained by the elevated incidence of decay in these fruits (Figure 3A). In line with this, it has previously been shown that the respiration rate depends on the cultivar, and on the temperature and composition of the storage atmosphere (Steffens et al., 2007).

The storage conditions tested in this experiment did not affect significantly the levels of soluble solids (Figure 3G). Another study has also reported that the contents of soluble solids in peppers show only minor changes during storage, what has been related to the non-climacteric behavior of these fruits (Silva et al., 2011). However, when pepper fruits were stored in either of the controlled atmosphere conditions tested, higher levels of titratable acidity were obtained as compared to fruits in cold storage (Figure 3H). These results showed that controlled atmosphere with 5 kPa of CO₂ has a similar effect as 10 kPa CO₂, but it prevents the high levels of mass loss and decay incidence. In fact, it has already been reported that the use of controlled atmosphere in apples maintains higher titratable acidity after storage (Brackmann et al., 2009).

During storage and shelf-life, the incidence of decay represents a significant problem for fruits and vegetables. One can observe from Figure 4A that the wax application, over the whole fruit skin or only on the peduncle, reduced the incidence of decay in relation to pepper fruits of the control treatment. These results indicate that wax application is more efficient in preventing decay incidence in green bell peppers than

ethylene management (Figure 2A) or CA (Figure 3A). Barros et al. (1994) have demonstrated that wrapping fruits with a plastic film delayed the onset of decay in cv. 'Agrônômico 10G' peppers. Regarding mass loss, it was observed that the treatments had no significant effect on this parameter either at the removal of fruits from the storage chamber or after 7 days of shelf-life at 20 °C (Figure 4B). However, other researchers have found significant reduction of mass loss in pepper fruits treated with wax or with PVC coating (Hojo et al., 2007; Beaulieu et al., 2009).

No difference in skin color between treatments for variable a* (Figure 4C), which represents the green color, was observed neither at the end of storage or after 7 days of shelf-life. However, fruits from the control treatment had yellower skin color right after the removal from storage (Figure 4D). After 7 days of shelf-life at 20 °C, fruits that received wax application over the skin were less yellow than fruits of both other treatments. This observation indicates less chlorophyll degradation, since when chlorophylls are catabolized the yellow pigments, such as carotenes, become visible.

Ethylene production right after removing fruits from storage was higher in pepper fruits of control treatment as compared to fruits that received wax application on the peduncle (Figure 4E). However, both treatments did not differ from fruits treated with wax over the whole fruit skin. After 7 days of shelf-life, no significant differences in terms of ethylene production were detected between treatments. Lee et al. (2010) verified that pepper is a non-climacteric fruit and therefore ethylene production is very low during the postharvest period. Similar results were also obtained in the first experiment when the management of ethylene did not result in significant reduction of ethylene production in relation to control fruits (Figure 2E). This higher ethylene production in control fruits culminated with elevated respiration rates, both at the removal from store and after 7 days of shelf-life (Figure 4F). This was most likely responsible for the elevated incidence of decay in these fruits (Figure 4A). In addition, both elevated respiration rate and decay incidence can be explained by the high levels of internal ethylene, since ethylene is able to initiate the ripening process of pepper fruits (Biles et al., 1993).

Similar to the other two experiments, the soluble solids did not differ among fruits from different treatments (Figure 4G). Nevertheless, the titratable acidity showed significant difference between treatments (Figure 4H). Higher titratable acidity, as compared to the control, was verified when wax was applied on the fruit peduncle. However, there was no significant difference between wax being applied over the fruit skin or being restricted only to the fruit peduncle. Previous studies have also indicated that wax application can reduce the respiration of fruits and, consequently, decrease the consumption of organic acids (Beaulieu et al., 2009).

Conclusion

Ethylene management via the application of AVG 7 days before harvest maintains higher titratable acidity and greener skin color in green bell peppers than the treatment with 1-MCP. The application of ethylene, 1-MCP or ethylene absorption

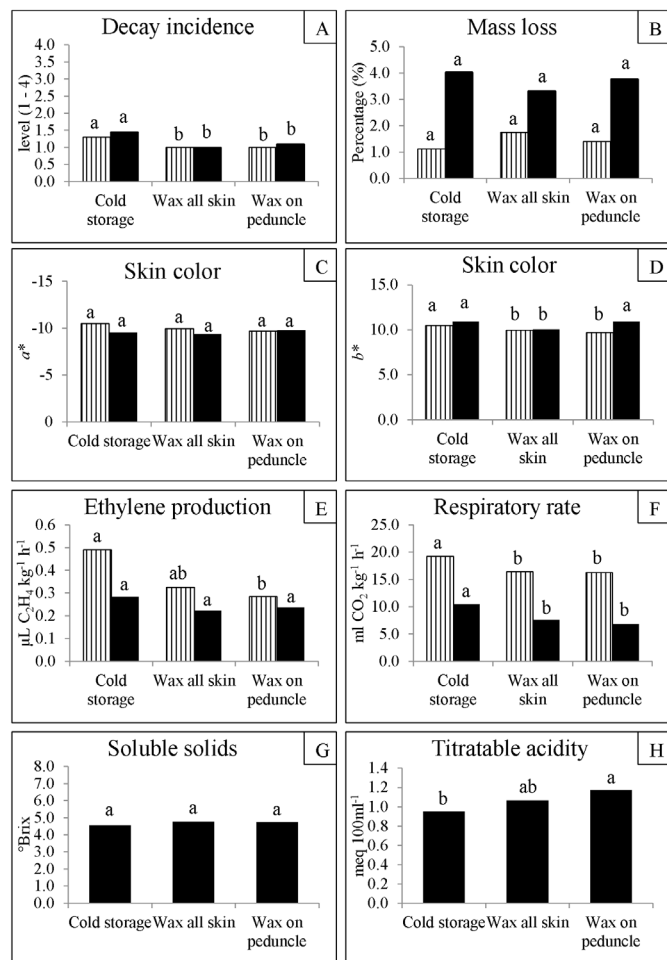


Figure 4. (experiment 3) Decay incidence (A), mass loss (B), skin color (C and D), ethylene production (E), respiratory rate (F), soluble solids (G) and titratable acidity (H) of 'Priscila' pepper in cold storage (CS) and with wax application, at the temperature of 9 °C during 30 days. Santa Maria, RS, Brazil, 2015. Means followed by equal letters, among treatments, do not differ by Tukey test, at 5% of probability of error. □ : Opening of chambers; ■ : 7 days at 20 °C

does not result in expressive benefits in maintaining the quality of green bell peppers.

The best controlled atmosphere to store green bell peppers is 3 kPa O₂ + 5 kPa CO₂, because it maintains higher titratable acidity and reduces mass loss. Controlled atmosphere with 3 kPa O₂ + 10 kPa of CO₂ increases the incidence of decay, mass loss and the respiration rate. Thus, CA conditions with high CO₂ are not indicated for storing 'Priscila' green bell peppers.

The application of wax to pepper fruits reduces the incidence of decay, delays the appearance of yellow skin color, decreases the respiration rates and maintains higher titratable acidity.

The levels of soluble solids in pepper fruits do not vary with the storage conditions.

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