

Enzymatic activity and post-harvest quality of 'Galia' melon under storage temperatures and modified atmosphere

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ABSTRACT: 'Galia' melon is a climacteric fruit with reduced shelf life, thus the goal of this work was to evaluate the post-harvest quality of fruits stored under normal and modified atmosphere at different temperatures. Fruits from cultivar Solarnet were stored under normal atmosphere (control) and modified atmosphere at 3, 7 and 11 °C and relative humidity of 90 ± 5%. Fruit evaluations were performed at 14, 21, 28 and 35 days of storage for pulp firmness, mass loss, internal and external appearance, chilling injury, total soluble phenolics, soluble sugars and enzymes polyphenoloxidase, peroxidase and catalase. Storage at 11 °C had the lowest pulp firmness and bigger mass loss in both atmospheres. Storage at 3 °C under modified atmosphere had the best postharvest conservation, but induced chilling injury, with development of superficial brown spots. 'Galia' melons showed higher peroxidase activity under storage at 3 °C in both storage atmospheres.

Key words: chilling injury; climateric fruits; *Cucumis melo* L.; storage conditions

Atividade enzimática e qualidade pós-colheita do melão 'Gália' sob temperaturas de armazenamento e atmosfera modificada

RESUMO: Melões climatéricos como o do tipo 'Gália' têm reduzida conservação pós-colheita, portanto o objetivo do trabalho foi avaliar a qualidade pós-colheita dos frutos sob atmosfera normal e modificada em função de diferentes temperaturas. Melões cv. Solarnet foram colhidos e armazenados em atmosfera normal (controle) e modificada a 3, 7 e 11 °C e umidade relativa de 90 ± 5%. Os frutos foram avaliados aos 14, 21, 28 e 35 dias após armazenamento quanto à firmeza da polpa, perda de massa, aparência externa e interna, injúria por frio, fenólicos e açúcares solúveis totais e atividade das enzimas peroxidase, polifenoloxidase e catalase. O armazenamento a 11°C propiciou as menores firmezas de polpa e maiores perdas de massa dos frutos, nas duas atmosferas. O armazenamento a 3 °C em atmosfera modificada teve melhor conservação pós-colheita do melão, porém, causou maior injúria por frio, com incidência de manchas escuras superficiais. Melões 'Gália' apresentaram maior atividade da peroxidase sob armazenamento a 3 °C em ambas as atmosferas de armazenamento.

Palavras-chave: injúria por frio; frutos climatéricos; *Cucumis melo* L.; condições de armazenamento

Introduction

Melon (*Cucumis melo* L.) is one of the most consumed fruit vegetables in the world, due to its organoleptic characteristics, nutritional and functional properties. Melon fruits are characterized by being rich in polyphenols, organic acids, lignans and other polar compounds, offering potential health benefits (Rodríguez-Pérez et al., 2013). Due to their productive and qualitative characteristics, nowadays the 'Galia' type melons are the most sought (Lima et al., 2017).

However, the quality of fruits and vegetables shows reduction after the harvest, especially in flavor, firmness (Amaro et al., 2013, Goulao et al., 2007), total soluble sugars and loss of fresh mass (Goulao et al., 2007). These aspects are influenced directly by time, temperature and relative humidity during the storage (Aroucha et al., 2018).

The 'Galia' type melons have a high rate of respiration and senescence post-harvest, requiring the use of technologies such as refrigeration in order to maintain the quality. They are typically climacteric fruits, thus, presenting an increase in metabolism and respiration, with consequent reduction in the useful life (Morgado et al., 2015; Verzera et al., 2014). Thereby, one of the strategies to increase its shelf life is through the refrigerated storage (Aung & Chang, 2014). However, one of the problems of 'Galia' type melon storage has been the physiological disturbance caused by exposure to low temperatures, characterized as dark spots on the fruit surface, developing symptoms characteristic from "chilling".

For the conservation of fruits and vegetables, other alternatives can be adopted, such as the use of modified atmosphere, being able to conserve the freshness and to increase the storage period. The main advantages of using modified atmosphere are the reduction in the O₂ content and the increase in CO₂ concentrations, creating an atmosphere responsible for the respiration rate retardation and inhibition of the synthesis and action of ethylene (Ward, 2016).

The control of temperature and relative humidity, allied with the use of modified atmosphere, in the storage of noble melons, are some of the physical processes used to delay the fruit ripening. For this, the knowledge of its physiology is fundamental, avoiding possible external and internal disorders and making it possible to send this fruit from the production centers to the most distant markets in the country or abroad.

It is known that the surface browning is from an oxidative enzymatic nature and that the use of temperatures in the storage and modified atmosphere can decrease the oxidative reactions in virtue of the reduction of the O₂ concentration from its inside, also due to the reduction in the aerobic processes, because the low availability of O₂ for the polyphenol oxidase activity (Dominguez et al., 2016, Mendoza et al., 2016).

However, studies are scarce for the 'Galia' type melons. In this sense, the objective of this work was to evaluate the post-harvest quality from 'Galia-type' melons kept under normal and modified atmosphere under different storage temperatures.

Material and Methods

The 'Galia' type cv. Solarnet melons from the Agroindustry Nolem Comercial Importadora e Exportadora Ltda., located in the Mossoró-Açu-RN Agropolo, were harvested when the region around the peduncle still showed no signals of opening, following the criteria for exportation to the European market (Lima et al., 2004). After harvest, the fruit were washed with chlorinated water (200 mg L⁻¹ of active chlorine) and brushed with fungicide solution around the peduncle (Imazalil, 2 mL L⁻¹) and transported afterwards to the Multidisciplinary Laboratory of Irrigated Agriculture from the Federal Rural University of the Semi-arid Region, Mossoró-RN.

The used experimental design was completely randomized, in a subdivided plots scheme, having a 3 x 2 factorial scheme in the plots (3 temperatures x 2 storage atmospheres), and having in the subplots the storage periods (5 weeks) with 4 replicates (with each unit experimental group consisting of two fruit).

The treatments consisted of three storage temperatures: 3, 7 and 11 °C, and relative humidity of 90 ± 5% and two storage atmospheres: normal (control) and modified atmosphere (microperforated polyethylene bags with nominal thickness of 20 mm and water vapor passage of 250 – 260 g m⁻² day⁻¹ at 20 °C and 50% of RH).

A total of 48 fruit were used for the initial analysis of quality characterization. Other fruit analyzes were carried out in four time intervals of 14, 21, 28 and 35 days of storage. And after two days prior to the analysis, the fruit were removed from storage and transferred to ambient conditions of 25.6 °C and 65.6% relative humidity, as suggested by King & Ludford (1983) and Wang & Qi (1997), in order to complete its maturation.

The evaluations were carried out for loss of mass and firmness determined by the pulp resistance to penetration, using a FT 327 model manual penetrometer, with an 8 mm probe in diameter. In order to determine the firmness, 4 readings per fruit were carried out at the pulp center in the fruit equatorial region, expressed in N. The incidence of chilling injury was determined by the following scale of scores from 5 to 1, where 5 = absent (0%), 4 = mild (1-10%), 3 = moderate (11.30%), 2 = severe (31-50%) and 1 = extremely severe, above (50%).

The external appearance of the peel and internal of the pulp were determined according to the scale adopted by Lima et al. (2004). The total soluble solids (TSS, °Brix) from the pulp were determined in an ATAGO PR 100 digital refractometer. Total soluble sugars (TSSu) were extracted in water and determined with Antrona reagent, according to Yemm & Willis (1954).

After the physical evaluations, the fruit were longitudinally sectioned and separated into four equidistant slices of each fruit, the pulp being extracted to the limiting region of the edible part with the peel.

The activity of the peroxidase (POD) and polyphenoloxidase (PPO) enzymes was determined according to Matsuno & Uritani (1972). The POD was determined by the addition of 50

μL of enzymatic extract in a total volume of 3 mL of reaction medium, which consisted of 50 mmol L^{-1} potassium phosphate buffer (6.50 pH), 500 μL guaiacol, 500 μL of H_2O_2 . The POD activity was determined by the change in absorbance at 470 nm, expressed in $\text{UAE min}^{-1} \text{g}^{-1}$ of MF.

The PPO activity was determined by the addition of 500 μL of enzymatic extract in a total volume of 3 mL, which consisted in 2000 μL of 50 mmol/L phosphate buffer (7.0 pH), 500 μL of catechol at 10 mmol L^{-1} and 500 μL of enzymatic extract, in a total volume of 3 mL. The results were expressed in UAE/min/g of MF.

Catalase (CAT) activity was determined by the addition of 500 μL of enzymatic extract, in a total volume of 3 mL, which consisted of 2 mL – 50 mmol/L phosphate buffer (7.0 pH), 500 μL of H_2O_2 at 340 mmol L^{-1} , and the activity expressed in $\text{UAE min}^{-1} \text{g}^{-1}$ of MF. The unit of enzymatic activity (UAE) represents the amount of enzyme that caused a reduction in absorbance at 270 nm of $0.001 \text{ unit min}^{-1}$.

The total phenolic content was determined in the epicarp after freezing in liquid nitrogen, according to the colorimetric method of Folin – Denis. The contents of total phenolic were determined in a spectrophotometer at 760 nm, using the standard – curve to express the results in $\text{mg } 100 \text{ mL}^{-1}$.

Data were subjected to analysis of variance and regression using the System for Statistical and Genetic Analysis (SAEG). The models were chosen based on the significance of the regression coefficients, using the t-Test adopting the level of 5% of probability, coefficient of determination (R^2) and biological phenomenon.

Results and Discussion

There was significant interaction at the 1% probability level among the analyzed variables. The firmness of the pulp from the 'Galia' melon fruits in the harvest was of 32.60 N, being slightly above the recommended for the harvest of this type of melon, which is of 30.00 N according to Filgueiras et al. (2000).

The loss of pulp firmness was very marked in the first 14 days, especially in the fruit stored at 11 °C, whose loss of pulp firmness reached in this period was of 88% in the normal atmosphere (Figure 1A) and 96% in the modified atmosphere (Figure 1B). For the fruits stored at 3 and 7 °C, the reduction was of 49 and 68% for the normal atmosphere and of 46 and 71% for the modified atmosphere, regarding the firmness at harvest.

At 21 days of storage, fruit kept at 3 °C had the highest pulp firmness at both storage atmospheres, with values above 12.00 N until the end of the experimental period, which was not observed for fruit stored at 7 and 11 °C, which values were of 9.48 and 3.05 N for the normal atmosphere (Figure 1A) and 8.29 and 0.20 N for the modified atmosphere (Figure 1B), respectively.

It is probable that the type of film employed did not provide an adequate CO_2 modification and O_2 reduction that would allow effective reduction of ethylene action and

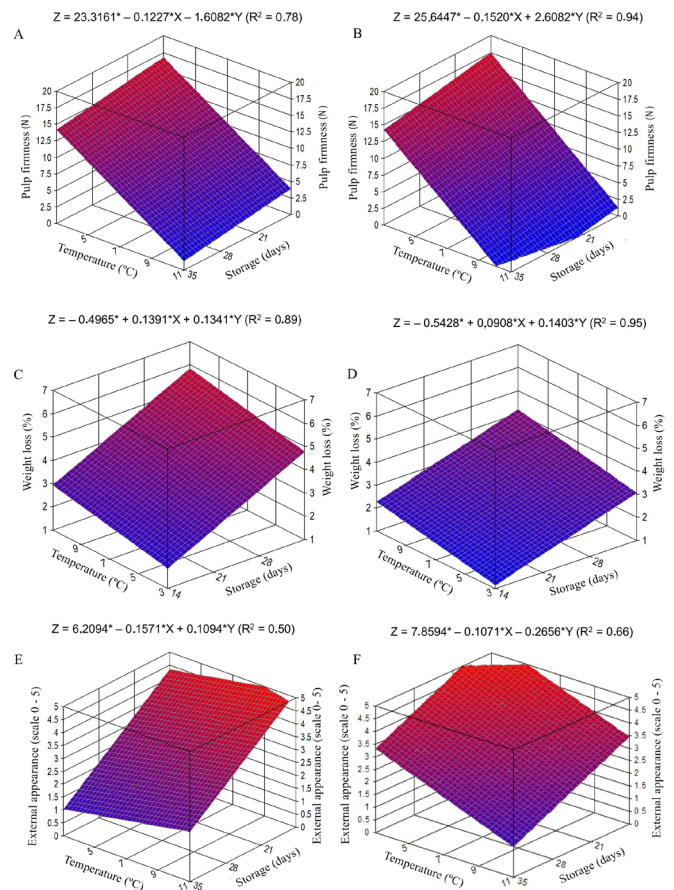


Figure 1. Curves and representative regression equation from the values of pulp firmness (A and B), mass loss (C and D) external appearance (E and F) from 'Galia' type cv. Solarnet melons in function of the storage time and temperature, kept under normal (A, C and E) and modified (B, D and F) atmospheres.

synthesis, therefore, causing degradation of the cell wall and consequent softening of fruit pulp. Loss of firmness is one of the most limiting factors in the useful life of 'Galia type' melons, because these fruits quickly lose water to the environment during storage, showing depressions that leave them looking like wilted fruit, thereby diminishing their appreciation by the consumer.

The loss of the fruit fresh mass was dependent on the used storage temperature, and although the modified atmosphere contributed to the retention of pulp firmness of the stored fruit (Figure 1B), this effect was not sufficient to maintain the fruit internal quality. Regardless of the storage temperature and atmosphere employed, there was an increase in mass loss, being higher in fruit kept under normal atmosphere (Figure 1C).

The accumulated mass loss was of 1.85, 2.39 and 2.92% at 14 days of storage, respectively, for fruit stored at 3, 7 and 11°C under normal atmosphere (Figure 1C). In the same storage period, the use of modified atmosphere associated with refrigeration resulted in lower fruit mass loss, with values of 1.14, 1.71 and 2.27% (Figure 1D), respectively, at 3, 7 and 11

°C. This can be explained by the fact that the use of modified atmosphere does not allow the fruit to lose excess water to the external environment, due to the upkeeping of high RH of the air around the fruit, with a value close to the RH of its interior, consequently, reducing mass loss.

The reduction in the firmness of 'Galia' melon fruit can be attributed to the decomposition of the cell wall components (Bu et al., 2013), caused by the activity of the degradative enzymes pectin methyl esterase and polygalacturonase (Amaro et al., 2013). Similar behavior was observed by Aroucha et al. (2018) in the yellow melon storage of the cvs. Goldex and Iracema, where the increased storage period caused a decrease in pulp firmness and mass loss in both cultivars.

The obtained results, in the present work, corroborate with those of Morgado et al. (2015), which noted a reduction in the firmness of the 'Louis' melon pulp stored for 25 days at 22 and 9 °C, observing higher firmness in the fruits stored at 6 °C. The authors point that the reduction in firmness may have been due to the increased activity of cell wall degradation enzymes by increasing storage temperature (Yuan et al., 2013; Chen et al., 2015).

Regarding the external appearance of the 'Galia' type melons, the most pronounced external quality loss was in fruit kept under normal atmosphere (Figure 1E), regardless of the storage temperature employed. Melons kept under normal atmosphere (Figure 1E) presented a conservation period of only 21 days (score = 3.0 - slight depressions and/or senescence spots), regardless of the storage temperature used, as well as those stored in a modified atmosphere at 7 °C. However, despite indices greater than 3.0 in external appearance for fruit kept under a modified atmosphere at 3 °C (Figure 1F), the incidence of chilling injury limited the post-harvest conservation period in less than 14 days. It is important to remember that in the evaluation of the external appearance, the dark spots caused by the chilling injury were not taken into account, thus not exerting influence on the attribution of the subjective notes of the external visual aspect.

The reduction in external quality was also observed by Aroucha et al. (2018) in yellow melons stored at 10 °C for 35 days after harvest and which obtained index 3. In the present work, the obtained index was 4 in melons stored at 3 °C in modified atmosphere, being then higher than the observed by the authors mentioned above. The visual quality of the melon is one of the most important aspects for the consumer at the time of purchase, and considering that in the present work the obtained index was 4, it is also considered that the fruit present quality for commercialization (Supapvanich & Tucker, 2013; Aroucha et al., 2018).

Regardless of the storage temperature used, the appearance of the pulp had a slight loss in quality, where the fruit remained with satisfactory commercial quality (index \geq 3.0) at the 35 days of storage (Figure 2A and 2B). This behavior was also observed by Aroucha et al. (2018) in the 'Goldex' and 'Iracema' melons cvs. During the storage at 10 °C for 35 days

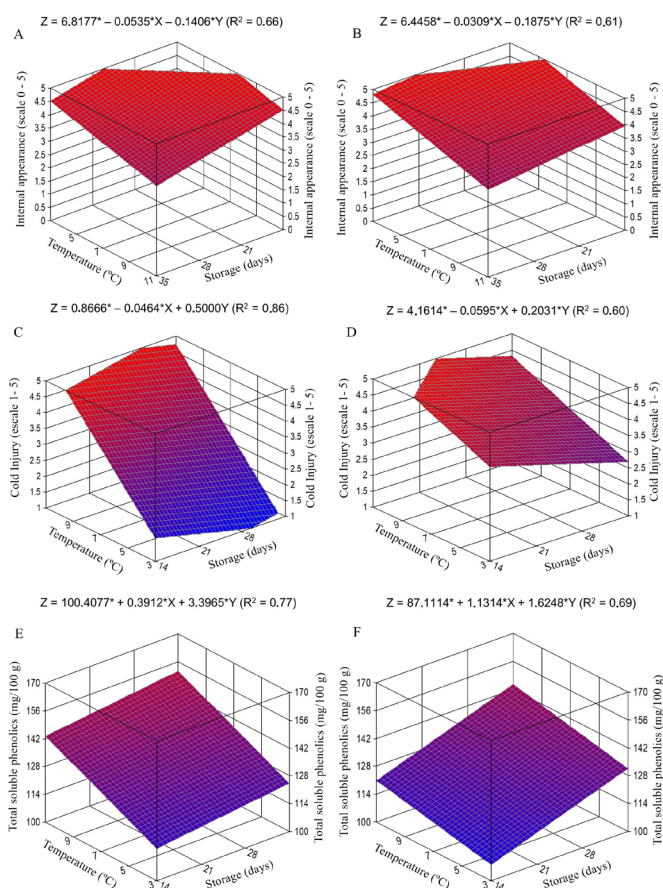


Figure 2. Curves and representative regression equation from the values of the internal appearance (A and B), injury by chilling (C and D) and total soluble phenolics (E and F) from 'Galia' type cv. Solarnet melons in function of the storage time and temperature, kept under normal (A, C and E) and modified (B, D and F) atmospheres.

and by Senho et al. (2008) by storing 'Frevo' hybrid yellow melon for 28 days at 7, 9, 11, and 13 °C.

In both storage atmospheres used (normal or modified), all fruit stored at 3 °C, with subsequent exposure to room temperature (25.6 °C and 65.6% RH) for 2 days, presented symptoms from chilling injury (Figure 2C and 2D), characterized by the incidence of dark spots on the fruit surface (Figure 3).

There was a positive effect of the modified atmosphere use (Figure 2D) in the first 14 days of storage at 3 °C for the chilling injury. In these conditions, the melons had an incidence of superficial spots above 50% (0.9 index) and 10% (3.9 index), for fruit kept under normal atmosphere (Figure 2C) and modified (Figure 2D), respectively.

For melons stored under a modified atmosphere, difference in chilling injury index was noted (Figure 2D), in which they presented indexes greater than 3.0 during the first 28 days of storage, compared to indexes of 0.9 in fruit kept in normal environment (Figure 2C).

However, melon fruit stored at 7 °C presented chilling injury indexes above 4, regardless of the storage atmosphere employed. Based on the incidence of this disorder and the

external visual aspect, it is possible to estimate the post-harvest conservation period of the fruit from 21 days to 7 °C, regardless of the storage atmosphere, which in turn might not be sufficient for exporting to the European external market.

Despite the increase in the post-harvest conservation period of fruit stored at 7 °C (21 days under normal and modified atmosphere), the appearance of superficial dark spots may be related to the cultivar susceptibility.

It was also observed that the more sensitive fruit to the appearance of superficial dark spots (lower indices of chilling injury) (Figure 2C and 2D) tended to show higher phenolic contents throughout the storage (Figure 2E and 2F).

The maximum total soluble phenolic contents were 133.82 and the minimum of 126.20 mg 100g⁻¹, for normal (Figure 2E) and modified (Figure 2F) atmosphere, respectively. During storage, the increase in the phenolic compost content, both in the normal and in the modified atmosphere, indicates that there was phenolic synthesis until 35 days of storage, maximum concentration, showing a tendency of concomitantly reducing to the decrease of temperature.

Under the statistical point of view, using only polynomial equations, it was not possible to fit a mathematical model that would explain the relatively constant behavior of the TSS and TSSu over the storage time, since the equations presented very low determination coefficients, allied to the non-significance of the t-test related to the equations parameters (data not shown).

Mean values of TSS in 'Galia' melons during storage were of 9.9 (normal atmosphere) and 10.2% (modified atmosphere). While the TSSu contents presented 8.1 (normal atmosphere) and 8.3% (modified atmosphere). This shows that the maturation stage of the 'Galia' melon fruit obeyed the minimum quality requirements (minimum TSS content of 9.0%) according to Filgueiras et al. (2000).

The POD activity varied linearly with storage time (Figure 4), being higher for fruit stored at 3 °C in normal atmosphere (Figure 4A). The lower storage temperatures had an influence on the higher POD activity, having correlation with the chilling injury indexes in fruit kept under normal atmosphere (Figure 4A), the same did not occur with those under a modified atmosphere (Figure 4B), in which no differences was noted in POD activity between the studied temperatures.

At the 35 days of storage at 3 °C, under normal and modified atmosphere, POD activity was maximal, with mean values of 9.45×10^6 e (Figure 3A) and 8.59×10^6 UAE min⁻¹ g⁻¹ of MF (Figure 4B), respectively.

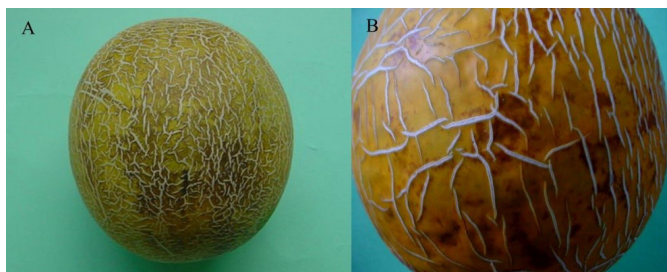


Figure 3. External appearance of the 'Galia' type melon fruit stored at 3 °C in normal (A) and modified (B) atmospheres.

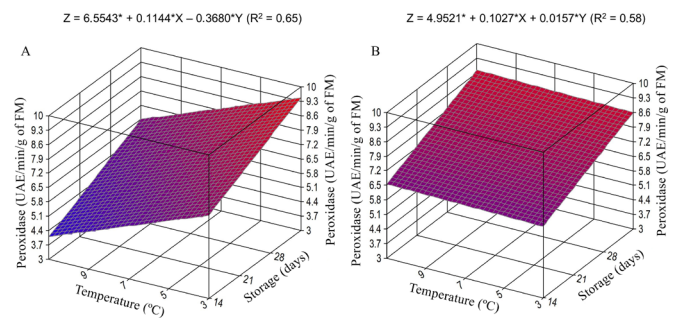


Figure 4. Curves and representative regression equation of the peroxidase values ($Z \times 10^6$) from 'Galia type' cv. Solarnet melons in function of the storage time and temperature, kept under normal (A) and modified (B) atmosphere.

This increase may be related to the low temperatures, which, possibly, could have caused chilling injury, with consequent greater activity of the antioxidative enzymes. Similar behavior was observed by Morgado et al. (2015), when they verified that the storage at 3 °C provided the highest POD activity in yellow melon.

Although it is one of the enzymes involved in the oxidation of phenolic compounds, no PPO and CAT activity was detected in 'Galia' melons, regardless of the atmosphere, temperature and storage time (data not shown).

Conclusion

The storage at 11 °C resulted in lower pulp firmness and greater mass loss of the 'Galia' melons, in both atmospheres.

The storage at 3 °C in modified atmosphere provided better post-harvest conservation of the 'Galia' melon cv. Solarnet, however, it also caused greater injury from the chilling, with incidence of superficial dark spots.

'Galia' melons showed higher peroxidase activity under storage at 3 °C in both storage atmospheres.

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