

## Effect of storage temperature on the quality of marolo fruit (*Annona crassiflora* Mart) “*in natura*”

Efeito da temperatura de armazenamento na qualidade do marolo (*Annona crassiflora* Mart) “*in natura*”

Efecto de la temperatura de almacenamiento en la calidad de la fruta marolo (*Annona crassiflora* Mart) “*in natura*”

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### Abstract

The adequacy of the best storage temperature for fruits and vegetables is an essential measure that helps in maintaining quality and extending shelf life. In this sense, this work aimed to study the influence of temperature on the quality of the marolo (*Annona crassiflora* Mart) “*in natura*”, where the fruits were washed, sanitized with 1,216  $\mu\text{M}$  hypochlorite solution, and stored at 4 different temperatures (0, 6, 12, and 20 ° C) controlling the relative humidity (80 to 90%). The parameters color  $L^*a^*b^*$ , pH, titratable acidity, soluble solids, firmness, total and soluble pectins, enzymes (pectinamethylesterase and polygalacturonase), and vitamin C were analyzed at different storage times. The maximum storage period was 8 days for fruits kept at 0 and 6 ° C. The use of higher temperatures (12 and 20 ° C) resulted in a shorter storage time (6 and 4 days, respectively). We found that the color parameter  $L^*a^*b^*$  was not influenced by time and temperature during the process, while firmness and soluble solids were affected only by temperature. On the other hand, the parameters pH, titratable acidity, total and soluble pectin, enzymes (pectinamethylesterase and polygalacturonase), and vitamin C were influenced by both temperature and storage time ( $p < 0.05$ ). Thus, we prove that to increase shelf life and maintain the best characteristics for consumption, the marolo must be stored between 0 and 6 ° C.

**Keywords:** Shelf life; Postharvest; Temperature; Savannah fruits; Enzymes modification.

### Resumo

A adequação da melhor temperatura de armazenamento para frutas e hortaliças é uma medida fundamental que auxilia na manutenção da qualidade e no prolongamento do prazo de validade. Nesse sentido, este trabalho teve como objetivo estudar a influência da temperatura na qualidade do marolo (*Annona crassiflora* Mart) “*in natura*”, onde os frutos foram lavados, higienizados com solução de hipoclorito 1.216 µM e armazenados em 4 temperaturas diferentes (0, 6, 12 e 20 ° C) controlando a umidade relativa (80 a 90%). Os parâmetros cor L \* a \* b \*, pH, acidez titulável, sólidos solúveis, firmeza, pectinas totais e solúveis, enzimas (pectinametilesterase e poligalacturonase) e vitamina C foram analisados em diferentes tempos de armazenamento. O período máximo de armazenamento foi de 8 dias para os frutos mantidos a 0 e 6 ° C. O uso de temperaturas mais elevadas (12 e 20 ° C) resultou em um menor tempo de armazenamento (6 e 4 dias, respectivamente). O parâmetro de cor L \* a \* b \* não foi influenciado pelo tempo e temperatura durante o processo, enquanto a firmeza e os sólidos solúveis foram afetados apenas pela temperatura. Por outro lado, os parâmetros pH, acidez titulável, pectina total e solúvel, enzimas (pectinametilesterase e poligalacturonase) e vitamina C foram influenciados tanto pela temperatura quanto pelo tempo de armazenamento (p <0,05). Assim, comprovamos que para aumentar a vida útil e manter as melhores características para consumo, o marolo deve ser armazenado entre 0 e 6 ° C.

**Palavras-chave:** Vida de prateleira; Pós-colheita; Temperatura; Frutos de savana; Modificação de enzimas.

### Resumen

La adecuación de la mejor temperatura de almacenamiento para frutas y verduras es una medida esencial que ayuda a mantener la calidad y prolongar la vida útil. En este sentido, este trabajo tuvo como objetivo estudiar la influencia de la temperatura en la calidad del marolo (*Annona crassiflora* Mart) “*in natura*”, donde los frutos fueron lavados, higienizados con solución de hipoclorito 1.216 µM, y almacenados a 4 temperaturas diferentes (0°C). , 6, 12 y 20 ° C) controlando la humedad relativa (80 a 90%). Los parámetros color L \* a \* b \*, pH, acidez titulable, sólidos solubles, firmeza, pectinas totales y solubles, enzimas (pectinametilesterasa y poligalacturonasa) y vitamina C se analizaron en diferentes tiempos de almacenamiento. El período máximo de almacenamiento fue de 8 días para frutas mantenidas a 0 y 6 ° C. El uso de temperaturas más altas (12 y 20 ° C) resultó en un tiempo de almacenamiento más corto (6 y 4 días, respectivamente). Encontramos que el parámetro de color L \* a \* b \* no se vio influenciado por el tiempo y la temperatura durante el proceso, mientras que la firmeza y los sólidos solubles se vieron afectados solo por la temperatura. Por otro lado, los parámetros pH, acidez titulable, pectina total y soluble, enzimas (pectinametilesterasa y poligalacturonasa) y vitamina C fueron influenciados tanto por la temperatura como por el tiempo de almacenamiento (p <0.05). Así, comprobamos que para alargar la vida útil y mantener las mejores características de consumo, el marolo debe almacenarse entre 0 y 6 ° C.

**Palabras clave:** Vida útil; Postcosecha; Temperatura; Frutos de la sabana; Modificación de enzimas.

## 1. Introduction

The preservation of biodiversity in agricultural systems is a crucial objective for agencies focused on environmental protection and sustainability, and for the global bioeconomy (Silva et al., 2020). In this context, preservation has become necessary to guarantee biodiversity and the development of the Cerrado biome. The marolo (*Annona crassiflora* Mart) fruit is a fruit native to this biome and has significant values for beta-carotene, carotenoids, phenolics, minerals and fatty acids, which are higher than those found in many commonly consumed fruit species (Silva et al., 2013; Silva et al., 2020). According to Silva et al (2013), although marolo fruit has good sensory characteristics and great commercialization potential, its sale is still limited to farmers markets during the harvest season, and their harvest through extraction, it is also important to highlight the metabolism of this fruit, which shows signs of climacteric fruit and high perishability, and the conservation study is a great alternative in extending the useful life of this fruit.

Epidemiological studies show that frequent consumption of foods rich in bioactive compounds, such as beta-carotenes and polyphenolic compounds, is associated with a low incidence of degenerative diseases (He et al., 2007). Due to the healthy implications, there is a growing interest in foods (fruits and vegetables) that they feed, but also supply these compounds in sufficient quantities to prevent the incidence of some metabolic diseases (Kähkönen et al., 1999).

Fruits are perishable vegetable products due to their high-water content. The quality of a fruit includes important attributes such as appearance, texture, flavor, among other parameters, which can be directly affected by temperature and storage time. (Herremans et al., 2015). Finger (2002) reported that fruits and vegetables start an accelerated process of

deterioration in the harvest. However, the rate of deterioration can be determined by the combination of factors internal and external to plant organs. According to Cia et al. (2007), the storage of blackberries at room temperature results in high loss of mass and incidence of rot, because of the high perishability. To reduce these problems, post-harvest conservation techniques are applied to decrease metabolic activity, especially those related to respiration rate, in fruits and vegetables (Wills & Golding, 2016).

Cold storage is one of the most used post-harvest conservation methods, and it can increase the period of conservation of fruits, increasing their useful life (Silva et al., 2013). According to Vilas Boas (2000), the quality of a fruit or vegetable never or rarely improves during the post-harvest period. Thus, refrigerated storage at the optimum temperature to extend the shelf life and preserve the quality of fresh fruits and vegetables is probably the most common way to reduce losses in the post-harvest environment. This method slows down the microbiological development and physiological processes – such as breathing – conserving aroma, flavor, color, texture, and other quality attributes of the stored product. Hence, this technique can be a viable alternative for the conservation of perishable exotic species with high nutritional value (Chitarra & Chitarra 2005; Tournas & Katsoudas 2005).

Therefore, this study aimed to measure the temperature influence on the quality of the fruit and find the appropriate temperature for storing the marolo (*Annona crassiflora* Mart).

## 2. Methodology

The research is explanatory and experimental, with part conducted in the field where the fruits were collected and in the Laboratories of Food Analysis and Food Chemistry and Biochemistry, of the Federal University of Lavras, being of a quantitative and qualitative nature (Pereira et al., 2018).

### 2.1 Obtaining the fruits and setting up the experiment

The fruits were harvested from July to December 2013 from a native Cerrado area where marolo (*Annona crassiflora* Mart) was the predominant specie. The Cerrado area was located 15 km from Paraguaçu, in Southern Minas Gerais state (Brazil). The fruits were harvested early in the morning after 48 days of anthesis, placed in plastic pouches, and taken to the Fruits and Vegetables Laboratory of the Food Science Department of Federal University of Lavras. Then, the fruits were washed with tap water for dust removal and immersed in 1.216 µM sodium hypochlorite solution for 15 min. Good hygienic practices were applied during fruit handling and washing. After removing the excess of solution, 1.150 grams of fruits (each fruit ≈ 1.5kg) were placed in trays and stored in cold chambers with relative humidity of 85 ± 5% at different temperatures (0, 6, 12, and 20 °C) for up to 12 days. The fruits were analyzed every 2 days in relation to the physical and chemical characteristics, where the darkened fruits or wrinkled skin were discarded and not considered for further analysis.

The temperature of the processing room was maintained at around 20 °C throughout the process. The utensils used were disinfected and the processing room was previously washed and sanitized with 200 ppm sodium hypochlorite and ethanol 70% (v/v). Throughout the process, sterile gloves, masks, caps, and aprons were used.

### Experimental design

The experiment was conducted in a Fully Randomized Design (CRD) with a 4 x 6 factorial, with 4 storage temperatures (0, 6, 12 and 20 °C) and 6 times (0, 2, 4, 6, 8, 10 days) with three repetitions. Each experimental plot consisted of three trays with two fruits each.

## 2.2 Physical and chemical analysis

### Color

The fruit's color was determined at five different points of the peel using a Minolta CR-400 colorimeter with the determination in CIE mode  $L^*a^*b^*$ , where the  $L^*$  coordinate represents how much lighter or darker is the sample - values varying from 0 (completely black) to 100 (completely white),  $a^*$  coordinate corresponds to green and red - values from -80 to +100, respectively, and the  $b^*$  coordinate is related to the intensity of the blue to yellow - ranging from -50 (all blue) to +70 (all yellow).

### Firmness

This parameter was determined individually in the whole fruit in the equatorial region, after removing a small portion of the peel using a Magness - Taylor penetrometer with a 5/6-inch diameter probe. The results were expressed in Newtons (N).

### pH and titratable acidity

Five grams of fruits were homogenized with 45 mL of distilled water in a T18 Ultra Turrax (Wilmington, NC, USA) at 22,000 rpm for 1 min at 20 °C. Then, the samples were filtered through a screen filter plate and the filtrate. The pH of the filtrate was measured directly using a pHmeter Schott Handylab (London, UK), following the AOAC official method (ASSOCIATION..., 2016). 5 mL of filtrate and 45 mL of distilled water were used for measuring total titratable acidity (TA) using hydroxide.

### Soluble solids

The soluble solids (SS) were also quantified in the filtrate by refractive index using a digital refractometer ATAGO PR-100 (Tokyo, Japan) with automatic temperature adjustment, and the results were expressed in Brix degree, as described by AOAC Methods (AOAC, 2016).

### Total sugars

Total sugars were determined by the anthrone method using a Beckman 640B spectrophotometer (Colorado, US). Three grams of the fruit were homogenized with 100 mL of ethanol 80% using an Ultra Turrax T18 (Wilmington, NC, USA) at 22,000 rpm for 1 min at 20 °C, and the homogenate could stand undisturbed for 12 h for sugar extraction. Afterwards, the sample was filtered and washed three more times with 80% ethanol for complete sugar extraction. All the alcoholic fractions were pooled together and concentrated using a heater block timer until only 5 to 10 mL of alcohol were left. The concentrated sample was diluted to 100 mL in distilled water. An aliquot of 0.5 mL of the diluted sample was diluted again up to 10 mL in distilled water. An aliquot of 1 mL was mixed with 2 mL of anthrone and heated in a boiling water bath for 8 min. The sample was then cooled down in an ice bath, and absorbance was read at a 620 nm with a Beckman 640B spectrophotometer. The results were expressed as glucose grams per 100 g tissue.

### Vitamina C

Vitamin C was determined with dinitrophenil hydrazine following the method described by Strohecker and Henning (1967). Absorbance was read at 520 nm carried out using a Beckman 640B spectrophotometer, and the result was expressed as milligrams per 100 g pulp.

### **Total and soluble pectins**

The pectins were extracted according to the technique of McCready & McColomb (1952), and determined spectrophotometrically at 520 nm, according to the technique of Blumenkrantz & Asboe-Hansen (1973). The results were expressed in mg of galacturonic acid per 100 g of pulp.

### **Pectinamethylesterase (PME)**

The extraction of the PME enzyme was made by mixing the techniques of Buecher & Furmanski (1978) and Vilas Boas (1995) with minor modifications. The determination of the activity of the SME followed the techniques of Hultin et al. (1966) and Ratner (1969), with modifications (Vilas Boas, 1995). One unit of PME was defined as the amount of enzyme capable of catalyzing the demethylation of pectin corresponding to the consumption of 1 mol of NaOH per gram of fresh pulp.min.

### **Polygalacturonase (PG)**

For the extraction of the PG enzyme, a methodology applied by Buescher & Furmanski (1978) was used, with modifications by Vilas Boas (1995). Dosing was performed according to Markovic et al. (1975), with modifications by Vilas Boas (1995). The enzymatic activity was expressed in 1 mol of galacturonic acid per gram of pulp per minute.

## **2.3 Statistical analysis**

To obtain statistical data from physical and chemical variables, it was performed used statistical package R, where all tests performed were considered at 5% significance (F test in ANOVA, Scott-Knott test in multiple comparisons, and T test in regression).

## **3. Results and Discussion**

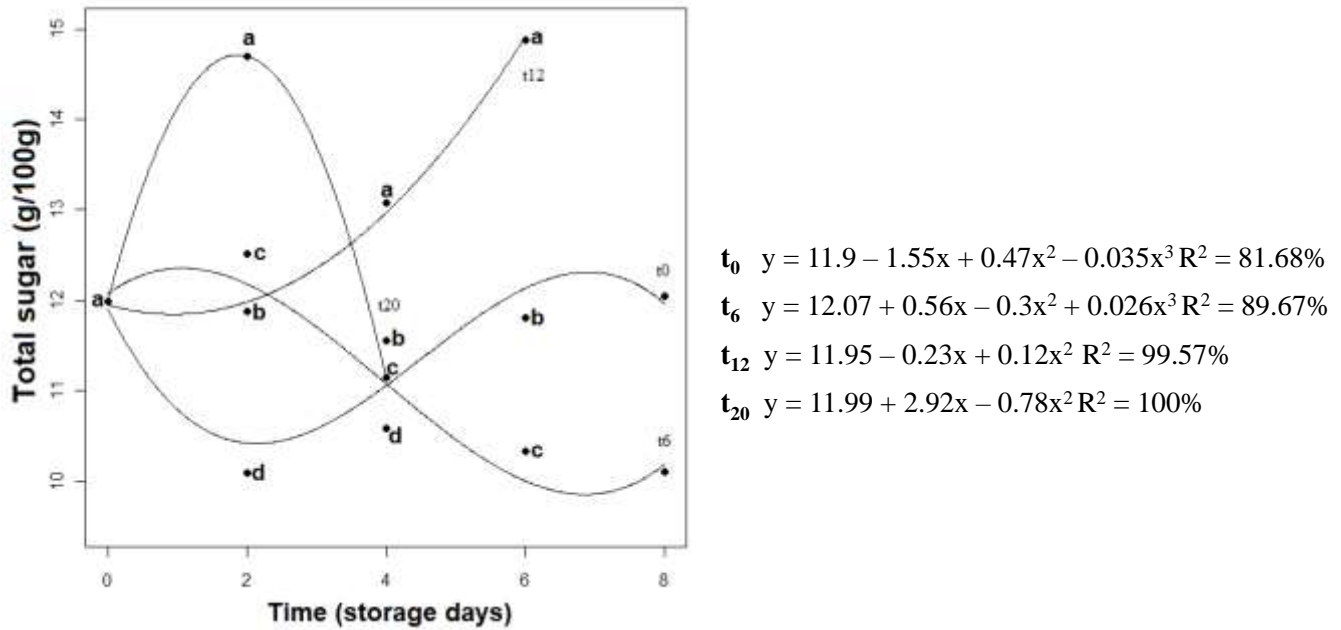
The use of storage temperature for extending the useful life of fruits is a methodology widely applied and which brings significant benefits to humanity over the years, especially in highly perishable fruits, slowing metabolic processes. As marolo is a highly perishable fruit (Silva et al, 2013), we varied the storage temperature in order to extend the useful life of the fruits, reaching a maximum period of 8 days of storage at 0 and 6 °C. The fruits submitted to temperatures of 12 and 20 °C, reached respectively 6 and 4 days of storage, confirming that temperature is an essential factor in the shelf life of this fruit.

Throughout the experiment, significant reductions were observed in the levels of vitamin C and total pectin, as well as in firmness. The storage temperature did not influence the color of the fruits, being, therefore, insignificant ( $p > 0.05$ ). The average values for this experiment throughout temperature's storages were  $L^* = 69.63$ ,  $a^* = 4.45$  and  $b^* = 32.97$ .

The content of soluble solids is one of the attributes responsible for the pleasant taste of the fruits and can be influenced by several environment factors, culture management, as the stage of maturation in the harvest, as well as by intrinsic factors of the fruit, mainly its drainage capacity, i.e., in importing photoassimilates (Chitarra & Chitarra, 2005). The fruits stored at 20 °C presented the highest averages for the content of soluble solids (around 19.47 °Brix), corroborating the high content of total sugars (Figure 1). The storage at 0, 6, and 12 °C presented average values of 17.52 °Brix, while at 20 °C was found the highest level related to a higher metabolism due to the higher temperature: similar correlations are observed between temperature and soluble solids, total sugars, and soluble pectin. Fruits kept at 6 and 20 °C showed a reduction in total sugars during storage (Figure 1). The reduction in Total Soluble Sugars (AST) can be associated with their consumption in respiratory metabolism since this assumes great importance as an energy source. Because physiological processes such as respiration that occur during post-harvest are related to changes in nutrient compounds at three levels: hydrolysis or breakdown

of polysaccharides into sugars, oxidation of sugars to pyruvic acid (glycolytic cycle), and aerobic transformation of pyruvic acid and other organic acids in CO<sub>2</sub> and water – Krebs cycle (Taiz and Zeiger, 2002). Thus, this metabolic process may be responsible for the variation of sugars with the storage time.

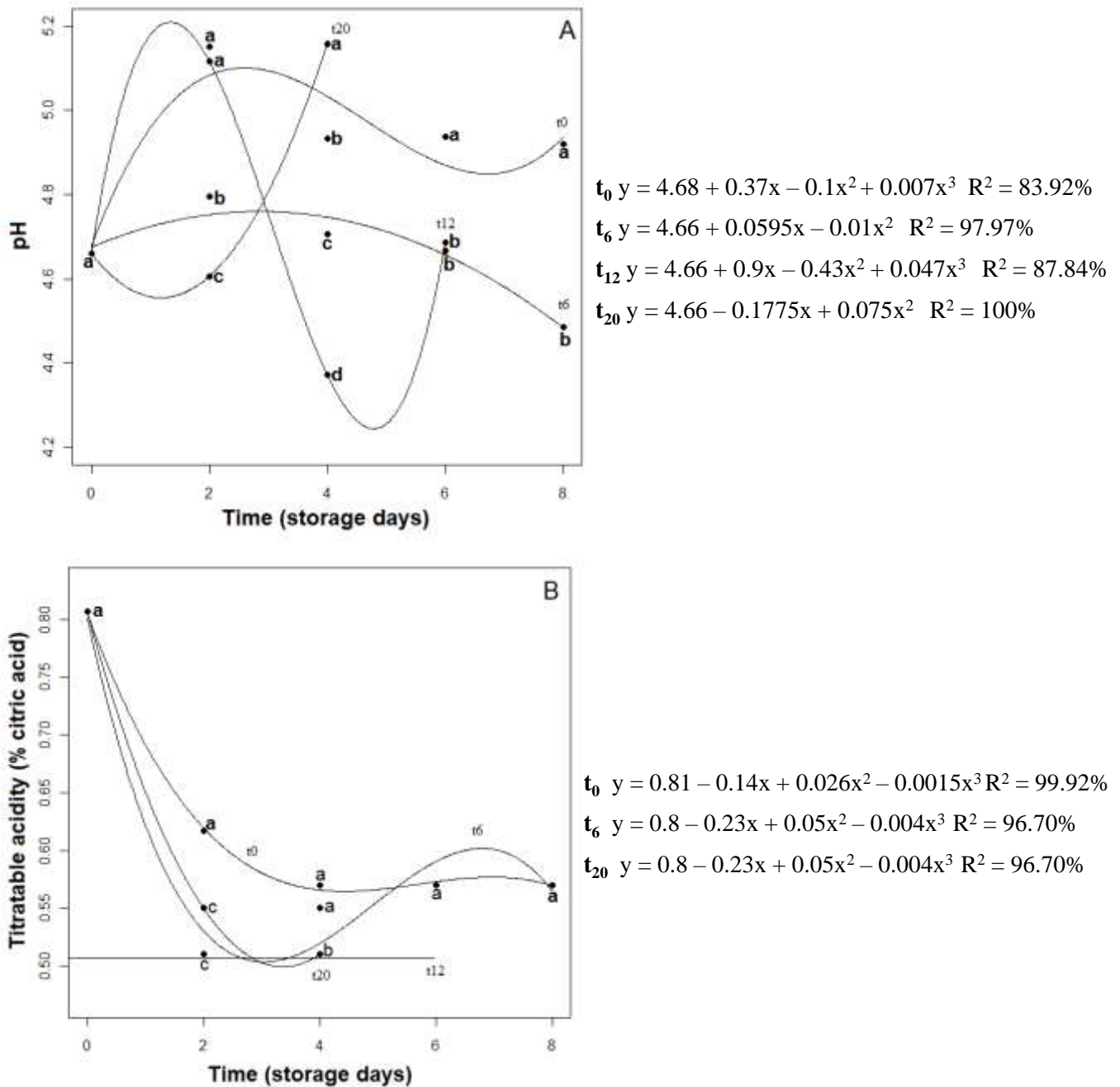
**Figure 1.** Average values of the total sugar content in "in natura" marolo stored at different temperatures (0, 6, 12, and 20 °C) and the regression equations for each temperature. Mean values with the same letter between the regression equations represent statistical similarities between temperatures, at 5% probability by the Scott-Knott test.



Source: Own source (2021).

Regarding to pH and titratable acidity variables, we observed a great influence on temperature and storage time (Figure 2). When comparing the pH of fruits stored at different temperatures, fruits kept at 0 and 20 °C showed an increase in pH over the storage days, while at 6 and 12 °C there was a reduction (Figure 2a).

**Figure 2.** Average values of a) pH and b) titratable acidity in marolo “*in natura*” stored at 0, 6, 12, and 20 °C and respective regression equations. Mean values with the same letter between the regression equations represent statistical similarities between temperatures, at 5% probability by the Scott-Knott test.



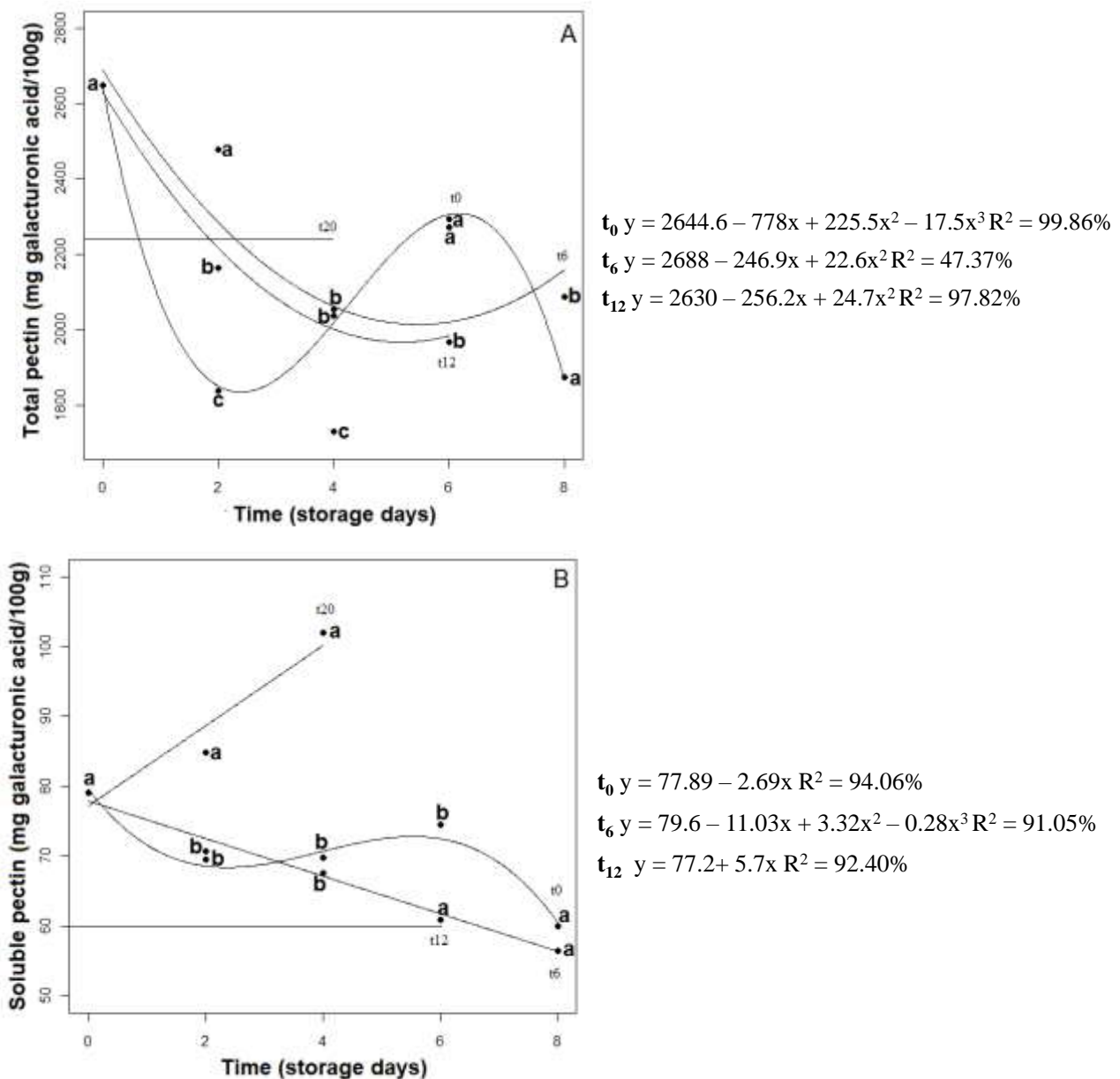
Source: Own source (2021).

The same behavior was observed for the titratable acidity (Figure 2b), regardless of the storage temperature. According to Brody (1996), the organic acid content tends to decrease during the oxidation process of tricarboxylic acids due to the respiration process, being essential in the synthesis of phenolic, lipid and volatile aroma compounds. With few exceptions, this content decreases with the maturation of fruits due to its use as a substrate in the respiratory process or its conversion into sugars, which can be influenced by the storage conditions to extend their useful life (Chitarra & Chitarra, 2005).

Pectins are a group of structural polysaccharides present in the primary cell wall and in the intercellular layers of

plants. They contribute to the adhesion between cells, consequently, they provide mechanical resistance to the cell wall of vegetables (Zielinski et al., 2014). In this study, we observed a considerable reduction in the total pectin contents in the marbles by increasing the storage days to 0, 6, and 12 °C (Figure 3a). At 20 °C, this parameter showed a differentiated behavior, remaining constant until the 4th day of storage, which coincides with the maximum period reached at this temperature. Regarding the soluble pectin content (Fig. 3b), we also observed a reduction in this parameter in fruits stored at 0 and 6 °C. This fact may be related to the fruit's physiology since it is a compound fruit. The variables total pectin and soluble pectin were affected by the interaction between temperature and storage time ( $p < 0.05$ ). The properties of pectin and the firmness of the cell wall are influenced not only in number, but also by the distributions of carboxy groups of galacturonic acid along the homogalacturan chain (Giovane et al., 2004).

**Figure 3.** Average values of a) total pectin and b) soluble pectin (mg galacturonic acid/100g) in marolo “*in natura*” stored at 0, 6, 12, and 20 °C and respective regression equations. Mean values with the same letter between the regression equations represent statistical similarities between temperatures, at 5% probability by the Scott-Knott test.



Source: Own source (2021).



Firmness is an important quality attribute for fruits. It is related to post-harvest life and, consequently, to the acceptance of the product by the consumers. In this experiment, storage at 0 °C showed a greater influence on this parameter, where the fruits showed the highest levels for firmness. This behavior can be correlated with a lower enzyme activity (Fig. 4a and 4b), as well as with the less exacerbated metabolic process (Table 1). Firmness is considered a good parameter to assess the influence of storage temperature on fruit ripeness since inadequate temperatures will be ineffective in physiological retardation (Salomão et al., 2016). Temperature is the main aspect to be considered, because at sufficiently low levels, it tends to inhibit the processes that cause quality reduction, since there were no significant differences in the interaction between time and temperature. Chitarra & Chitarra (2005), states that the firmness of the fruits is strictly related to the solubilization of some pectic substances, converting insoluble pectin into soluble pectin during ripening, which leads to softening, consequently, decreasing the resistance of the fruits.

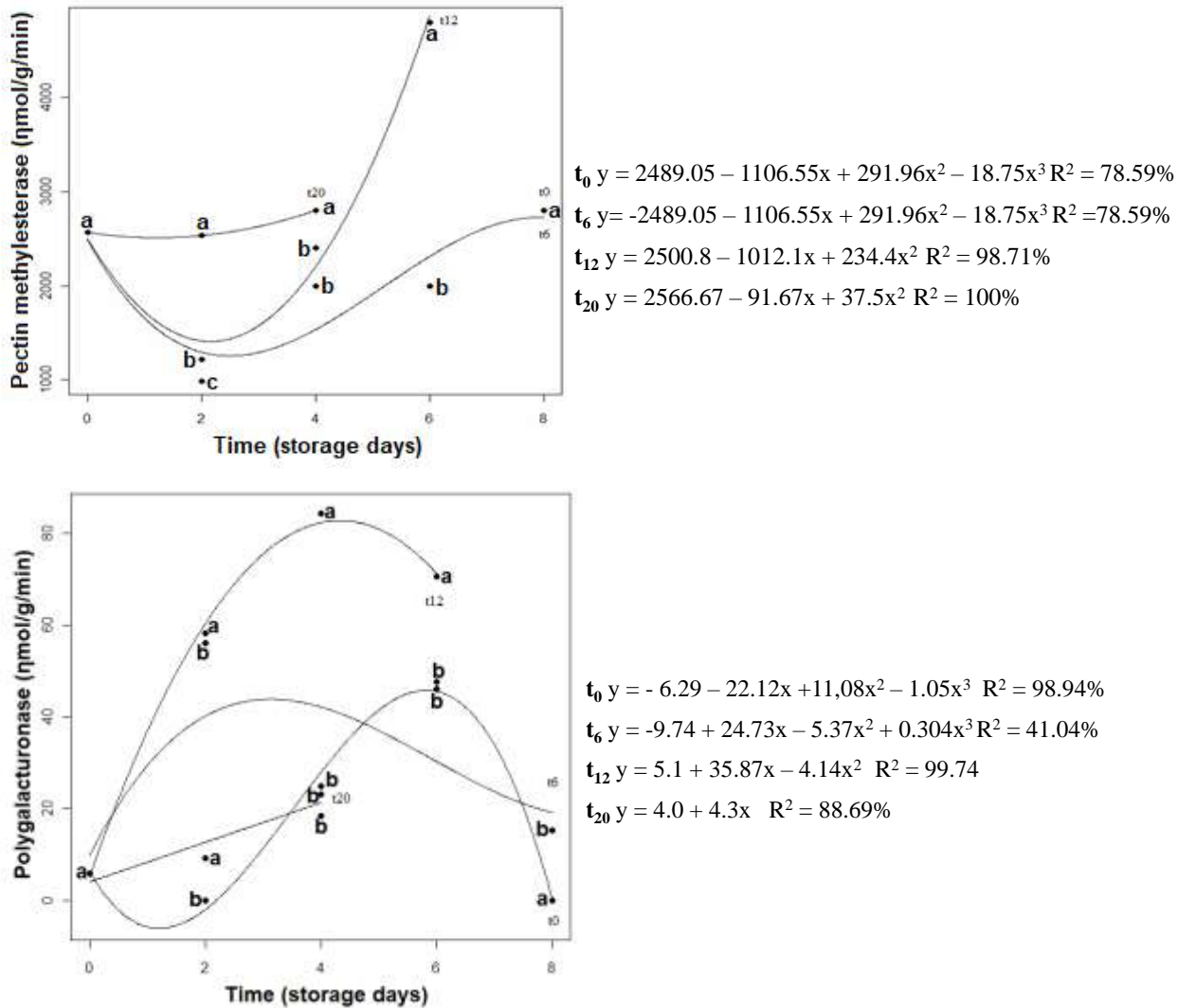
**Table 1.** Effect of temperature storage on the firmness of the marolo.

Temperature (°C)	Firmness (N)*
0	5.83 <sup>a</sup>
6	4.30 <sup>ab</sup>
12	4.19 <sup>ab</sup>
20	3.80 <sup>b</sup>

\*Mean values followed by the same letter do not differ from each other, at 5% probability by the Scott-Knott test. Source: Own source (2021).

Hydrolytic enzymes such as pectinamethylesterase, polygalacturonase, cellulase, glucanahydrolases and cell wall transglycosidases attack structural carbohydrates and are largely responsible for the loss of tissue firmness (Chitarra & Chitarra, 2005). In this study, we detected a substantial increase in the activity of the pectinamethylesterase - PME (Fig. 4a) and polygalacturonase - PG (Figure 4b) enzymes during the storage of the fruits, mainly at the highest temperatures (12 and 20 °C). This fact may be related to an optimum temperature of action of these enzymes, combined with a greater process of demethoxylation through the activity of the PME. In fruits stored at 0 °C, a peak was observed on the 6th day of storage with a tendency to fall. Fruits stored at 6 and 20 °C showed similar values at the end of storage (8 days). Silva et al (2013), studying the physiological development of marolo, detected mean values for PME activities of 3,166.73  $\eta\text{mol.g}^{-1}\text{.min}^{-1}$ , higher than those found in this study for fruits stored at temperatures 0, 6 and 20 °C; for PG levels, they detected mean values of 18.52  $\eta\text{mol.g}^{-1}\text{.min}^{-1}$ , lower than the levels found in this experiment. Gross and Wallner (1979) reported that, in most fruits, the soluble fraction of pectic substances increases during ripening, in a process attributed to the action of pectolytic enzymes, contributing to the fruit softening process, a behavior evidenced in this work.

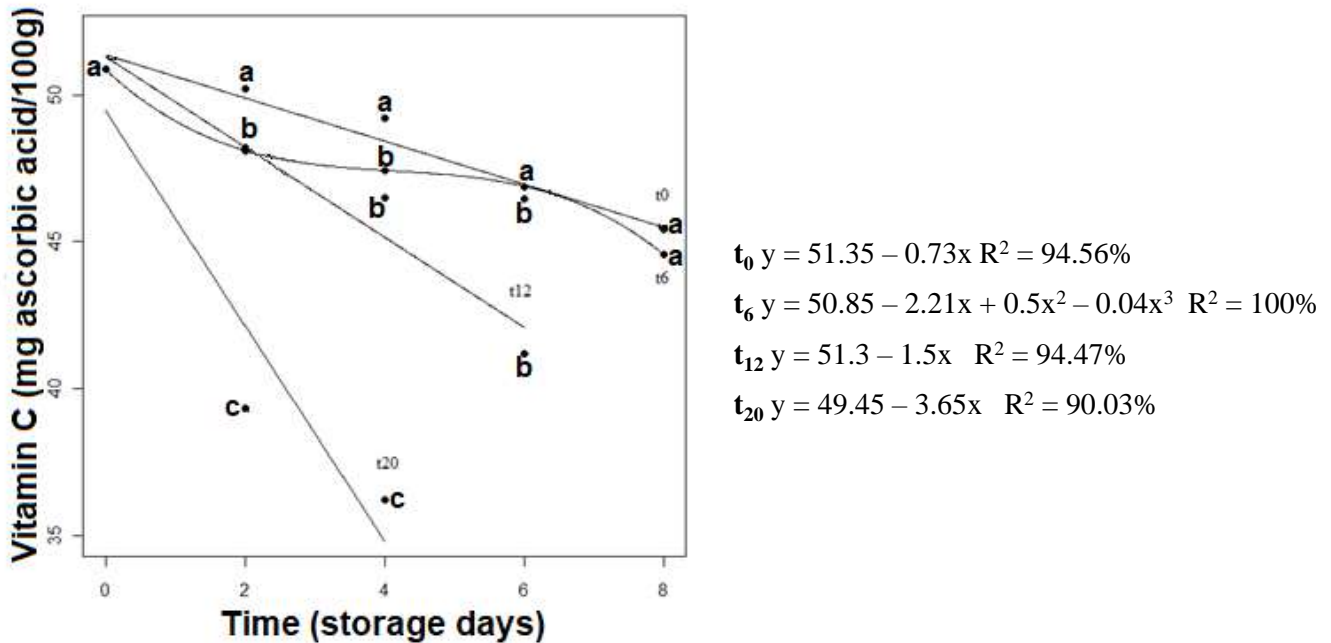
**Figure 4.** Average values of a) pectinamethylesterase and b) polygalacturonase ( $\eta\text{mol/g/min}$ ) in marolo “*in natura*” stored at 0, 6, 12, and 20 °C and respective regression equations. Mean values with the same letter between the regression equations represent statistical similarities between temperatures, at 5% probability by the Scott-Knott test.



Source: Own source (2021).

In this work, the effect of the storage temperature on vitamin C was also observed, where we observed a significant influence of the interaction between temperature and storage time ( $p < 0.05$ ) (Figure 5). The reduction in the levels of vitamin C was detected at all storage temperatures throughout the experiment. However, fruits kept at lower temperatures (i.e., 0 and 6 °C) showed the best results: around 40 mg / 100g of fruit. Silva et al (2013), studying the physiology of the marolo, detected values similar to those found in our study. According to Vilas Boas (1999), vitamin C is more easily degradable in the presence of light, oxygen, and heat. Thus, the ascorbic acid content can be used as an index of food quality because it varies in the product according to the conditions of cultivation, storage, and processing (Chitarra & Chitarra, 2005).

**Figure 5.** Average values of vitamin C (mg ascorbic acid/100g) in marolo stored at 0, 6, 12, and 20 °C and respective regression equations. Mean values with the same letter between the regression equations represent statistical similarities between temperatures, at 5% probability by the Scott-Knott test.



Source: Own source (2021).

#### 4. Conclusion

Based on the analyzes carried out the temperatures of 0 and 6 °C were considered optimal for the storage of marolo fruits, maintaining their best characteristics for consumption, firmness, and nutritional value for up to eight days. However, it is possible to choose the temperature at 6 °C, which will require less expenditure of electricity. These results shed light on the changes that occurred in this fruit during its storage, as well as making it possible to extend its useful life.

#### Acknowledgments

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