

RESEARCH ARTICLE

Quality of potato tubers according to the storage time

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ABSTRACT

The storage of potato tubers, even for short periods, is essential to stabilize the supply of the product on the market. However, it can affect several physicochemical and biochemical properties of the product, negatively impacting its quality. This study aimed to evaluate the effect of storage time on the physical and biochemical properties of two potato cultivars.

Potato tubers of Ágata and Atlantic cultivars were stored at room temperature for 35 days. At 0, 7, 21, and 35 days of storage, analyses of pulp firmness, total soluble sugar content (^oBrix), dry mass content (MS), and tuber mass loss were performed. Also, the activity of the enzymes, superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), the hydrogen peroxide content (H₂O₂), total soluble sugars (AT), and reducing sugars (AR) was monitored. The reduction in pulp firmness was linear for both cultivars and is related to the loss of tuber mass. The observed decrease in enzymatic activity is a negative factor as it reduces the ability to defend against oxidative stress. The reduction in the sugar content is interesting, as it maintains the quality of the tubers, especially for industrial processing.

Highlighted Conclusion

1. Potato tubers of cultivars Ágata and Atlantic stored under ambient conditions showed loss of fresh mass, reduced firmness, and increased soluble sugar content.
2. The cultivars Ágata and Atlantic can be stored for up to 35 days under ambient conditions without damaging their physicochemical quality significantly.

INTRODUCTION

In Brazil, potato production is carried out during all months of the year, with specific periods in different country regions. Seeking to meet the demand of the industry and fresh consumption, the production can be stored for a certain period, under variable conditions, thus maintaining a constant supply of tubers to the market, especially to the processing industry, which in this way can adapt production to the consumption (Bacarin et al. 2005).

Due to logistical factors, high costs, and the need for infrastructure, refrigerated storage is not used in Brazil for potatoes intended for consumption and industry (Bervald et al. 2010). In this way, the tubers are stored under ambient temperature conditions, which reduces the sanity and viability time of the tuber (Bisognin et al. 2008).

Storage conditions (cold room, room temperature, bags or in bulk) and storage period can modify several qualitative and physiological parameters of potato tubers due to the physiological aging of the tuber. Delaplace et al. (2009) verified, in tubers stored under refrigeration, the occurrence of changes in the content of antioxidant compounds, as well as in the activity of enzymes SOD, CAT, GR and APX (Superoxide Dismutase, Catalase, Glutathione Reductase, and Ascorbate Peroxidase), which are also associated with the antioxidant metabolism. In addition, a reduction in the healing capacity of the tuber tissues is reported according to the storage period (Kumar et al. 2010).

Comparing the storage of tubers at 4°C and 20°C, Bervald et al. (2010) found an accumulation of reducing sugars in several potato cultivars under low temperatures. For potatoes intended for industrial processing, the content of dry mass and reducing sugars are of fundamental importance as they affect the quality of the product. The dry mass content is related to the yield and texture of the potato after industrial processing. In contrast, reducing sugar content is associated with the quality of the tubers for processing, which may, in some cases, make

it unfeasible (Garcia et al. 2015). Reducing sugars react with the α -amino group of nitrogenous compounds; this non-enzymatic reaction (Maillard reaction) leads to the formation of a dark color and a bitter taste of the product after frying and the formation of acrylamide, a powerful neurotoxic with possible carcinogenic effect (Zhao et al. 2013).

Post-harvest losses during the storage period can be mechanical, physiological, and pathological. These are maximized the longer the storage period and the worse the storage conditions, causing an increase in the incidence of injuries that become gateways for pathogens and trigger physiological processes that lead to loss of tuber quality, directly affecting their commercial standard and causing lower remuneration to the producer (Ferreira and Netto 2007).

In temperate countries with a significant share in potato production, such as the United States, Canada, Germany, Italy, and England, tubers are stored for long periods due to the seasonality of production since, in general, it is only possible to harvest one crop per year (Owolabi et al. 2013). In this case, several technologies are used to prolong the shelf life of the stored product, among them the use of gamma radiation, sprouting inhibitors, and cold chain storage (Rezaee et al. 2013). The physiological behavior of tubers under such storage conditions is the subject of several studies (Bervald et al. 2010; Owolabi et al. 2013; Rezaee et al. 2013) and is already well understood, but little information is available in the literature about the physiological behavior of tubers stored under ambient conditions.

In this context, this study aimed to evaluate the effect of storage time on the physical and biochemical properties of two potato cultivars

MATERIAL AND METHODS

The research was conducted at the Department of Agronomy of the Universidade Estadual do Centro Oeste (UNICENTRO), Campus Cedeteg, in Guarapuava-PR.

A completely randomized design was used (DIC) in a 2 x 4 factorial scheme. Two cultivars (Ágata and Atlantic) and four evaluation periods were assessed. After harvesting, the tubers of each cultivar were washed and stored in a dark room under ambient conditions of humidity and temperature. Class II tubers (diameter between 40 and 85 mm) were used, following the classification according to Ordinance nº 69, of February 21, 1995, of the Ministry of Agriculture, Supply and Agrarian Reform. Plant material (tubers) was collected for analysis at 0, 7, 21, and 35 days of storage. The variables analyzed were pulp firmness, total soluble sugar ($^{\circ}$ Brix), dry mass (MS), tuber mass loss, reducing sugars (AR), total soluble sugar (AST), the activity of enzymes SOD (Superóxidos Dismutase, EC. 1.15.1.1), POX (Peroxidase, EC. 1.11.1.7) e CAT (Catalase, EC. 1.11.1.6), and hydrogen peroxide content in tuber tissue.

To evaluate the loss of fresh mass, three tubers per repetition had their mass variation monitored, and the results were presented as relative mass (%). Pulp firmness was determined using a benchtop digital texturometer model TDBC-200 (SoilControl, São Paulo). The tip used was 4 mm. The total soluble sugar content was determined with a portable digital refractometer (Model PAL-1, Atago). To quantify the dry mass, two tubers per repetition were weighed, cut into slices, and taken to a drying oven at 65 °C until reaching constant mass.

To determine the activity of SOD, CAT, and POX, the enzymatic extract was obtained by macerating 0.65 g of tuber with liquid nitrogen plus 4% PVPP (m/m) and then adding an extraction buffer to the proportion of 4 mL to 1 g of plant tissue.

The extraction buffer consisted of 100 mM potassium phosphate buffer pH 7.5, 1 mM EDTA, and 4 mM DTT. The macerate was centrifuged for 30 minutes at 12,000 x g in a refrigerated centrifuge at 4 °C. The precipitate was discarded, and the supernatant was stored at -20°C and used as an enzyme extract. The protein content was determined according to Bradford (1976).

To determine SOD activity, a reaction medium composed of potassium phosphate buffer (52.5 mM, pH 7.8), EDTA (0.1 mM), NBT (0.075 mM), methionine (13 mM), and riboflavin (2 μ M) was used. For the reaction, 25 μ L of the enzymatic extract were added to 3 ml of the reaction medium. Readings were performed in PP (polypropylene) cuvettes at 560 nm after exposure to light (15W lamp) for 10 minutes. One unit of SOD was considered the amount of enzyme capable of inhibiting 50% of NBT photoreduction under the conditions of the present study. Enzyme activity was expressed in units per milligram of protein (U mg⁻¹ prot.).

The determination of CAT activity was performed based on the methodology proposed by Shabala and Cuin (2012). Reaction medium concentrations were 50 mM phosphate buffer (pH 7.5) and 16 mM hydrogen peroxide. The consumption of H₂O₂ was monitored at 25°C for 60 seconds at a wavelength of 240 nm. To calculate the enzymatic activity, the molar extinction coefficient of hydrogen peroxide was used as 39.4 mM⁻¹ cm⁻¹, which was expressed in μ M min⁻¹ mg⁻¹ of protein.

POX had its activity determined according to the methodology proposed by Flurkey and Jen (1978). The reaction medium consisted of 25 mM potassium phosphate buffer (pH 6.8), 3.3 mM guaiacol, and 10 mM hydrogen peroxide. Tetraguaiacol formation was monitored at 470 nm for 60 seconds, and the molar extinction coefficient of $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ was used to calculate the enzymatic activity.

For quantification of hydrogen peroxide, approximately 0.5 g of potato tuber were macerated and homogenized in 5 mL of TCA (trichloroacetic acid) at 0.1% (m/v). After centrifugation at 12,000 x g, the precipitate was discarded, and the supernatant was used to quantify hydrogen peroxide. For quantification of hydrogen peroxide, an aliquot of 0.5 mL of the supernatant was added to 0.5 mL of potassium phosphate buffer (25 mM, pH 7.0) and 1 mL of potassium iodide (1 M). Readings were taken at 390 nm, and values were calculated based on a standard curve determined with H_2O_2 .

The extraction of total soluble sugar and reducing sugars was performed based on the alcoholic extraction methodology proposed by Trethewey et al. (1998). Approximately 0.3 g of tuber were immersed in 80% ethanol for 30 min, macerated, and centrifuged at 5,000 x g. Two more washes of the sample were performed, followed by centrifugation. The supernatant from the three centrifugations was pooled in a volumetric flask, and the volume was made up to 10 mL.

The quantification of total soluble sugar content was performed by the Phenol-Sulfuric method, according to Pulz (2007), with modifications. Readings were performed at 470 nm, and the total soluble sugar content was determined according to the sucrose standard curve.

The Somogyi-Nelson method (Nelson 1944) was used to quantify the content of reducing sugars. Readings were taken at 540 nm, and the content of reducing sugars was determined based on a glucose standard curve.

Data were subjected to analysis of variance (ANOVA), and when significant ($p \leq 0.05$), the means were compared using the Tukey test ($p \leq 0.05$). Equations were adjusted using R Studio software (Ferreira et al. 2018). Pearson linear correlation analysis was performed with the same program for the collected data. The graphs were made with the aid of Sigma Plot 11.0 software.

RESULTS AND DISCUSSION

The tuber fresh mass loss was affected by the interaction between cultivars x storage period. For both cultivars, in the experimental period, a simple linear adjustment was observed for the fresh mass loss according to the storage time (Figure 1A). The Atlantic cultivar presented daily losses of 0.08%, a value higher than the losses observed for the Ágata cultivar, which were 0.073% per day.

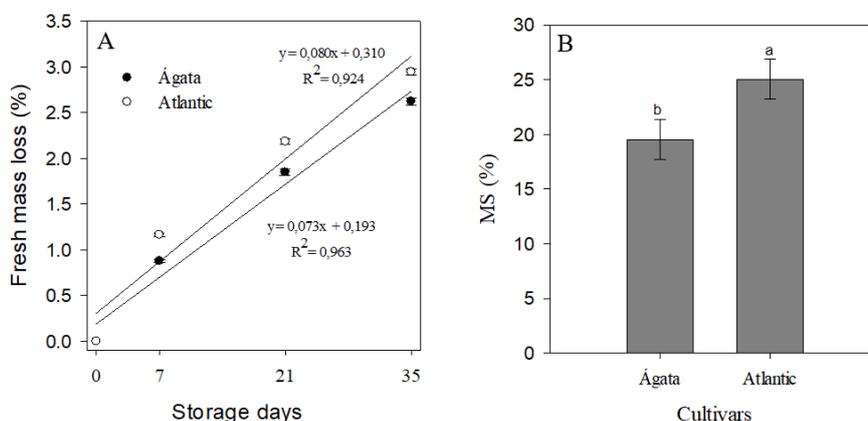


Figure 1. Fresh mass loss (A) and dry mass content (B) of potato tubers of two cultivars submitted to different storage times. Guarapuava-PR, 2021.

* Bars indicate the standard error of the mean. Columns followed by an equal capital letter do not differ from each other by Tukey's test ($p \leq 0.05$).

The contents of the dry mass of the tubers were not altered by the storage period; among the cultivars, Atlantic presented a value 5% higher than Ágata (Figure 1B). For potatoes intended for industrial processing, as is the case with 'Atlantic', the dry mass content of the tubers must be greater than 20% in order to obtain quality products. The values observed in this study meet the industry requirements concerning dry mass for the entire period studied. For both cultivars, the values of the present study are above those observed by Fernandez et al. (2010) (14.1% for Ágata and 19% for Atlantic), Quadros et al. (2009) (20.8% for Atlantic), and Evangelista (2011) (14.5% for Ágata and 21.6% for Atlantic). This is due to the effect of interaction between genotype x environment since the variation in dry mass content in cultivars produced in different environments is normal (Feltran et al. 2004).

In tuber firmness (Figure 2A), a decreasing linear regression was observed for both cultivars according to the storage time. The Atlantic cultivar showed a reduction of 0.99 N per day of storage, whereas, in the Ágata cultivar, this reduction was three times lower than in the Atlantic cultivar, with a value of 0.33 N per day.

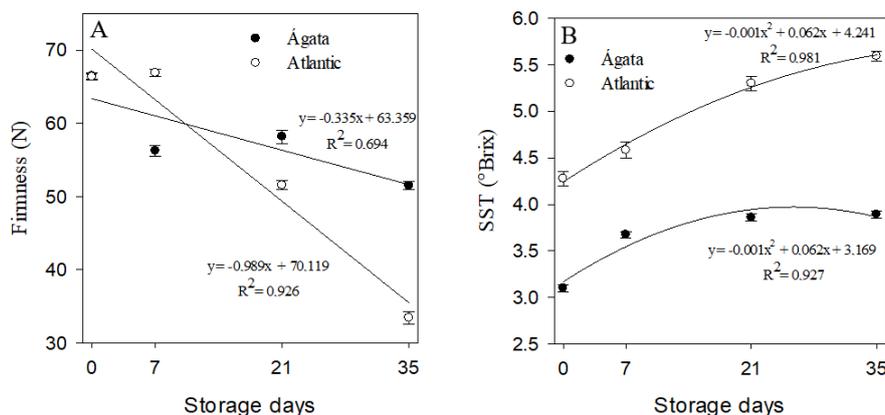


Figure 2. Firmness (A) and total soluble solids (B) of potato tubers of two cultivars submitted to different storage times. Guarapuava-PR, 2021.
* Bars indicate the standard error of the mean.

In terms of soluble sugar, for both cultivars, the variation followed a quadratic regression model (Figure 2B). At the beginning of the storage period, the Atlantic cultivar presented a soluble sugar content of 4.24 °Brix, while the Ágata cultivar presented 3.17 °Brix.

The higher values of soluble sugar observed for 'Atlantic' concerning 'Ágata' corroborate the pattern observed by Evangelista et al. (2011), who obtained 4.3° Brix for Ágata and 4.96° Brix for Atlantic. The differences observed between the cultivars and between different studies are due to the variation in the edaphic conditions of cultivation and intrinsic factors of each cultivar (Feltran et al. 2004).

Tuber fresh mass losses occur during storage due to water evaporation, sprouting, and consumption of substances in respiration (Ali et al. 2017). The positive correlation observed between the soluble sugar content and fresh mass loss ($r = 0.58$, $p < 0.0001$) suggests that the mass reduction observed in tubers according to the storage period is mainly related to water loss through the tubers, concentrating the solutes present in the tubers and increasing the soluble sugar content.

A negative correlation was observed between firmness and mass loss in storage ($r = -0.76$, $p < 0.001$), suggesting that the mass losses observed in tubers are mainly due to water loss, according to Imaizumi et al. (2015), much of the reduction in potato tuber firmness is due to the loss of cell turgor pressure, which is directly related to the degree of hydration of the tuber tissues.

For total soluble sugars (AT), isolated significance was observed for cultivars and evaluation periods. The Ágata cultivar had a 59% higher AT content than the Atlantic (Figure 3A). Concerning the storage period, a decreasing linear regression is observed for AT levels (Figure 3B).

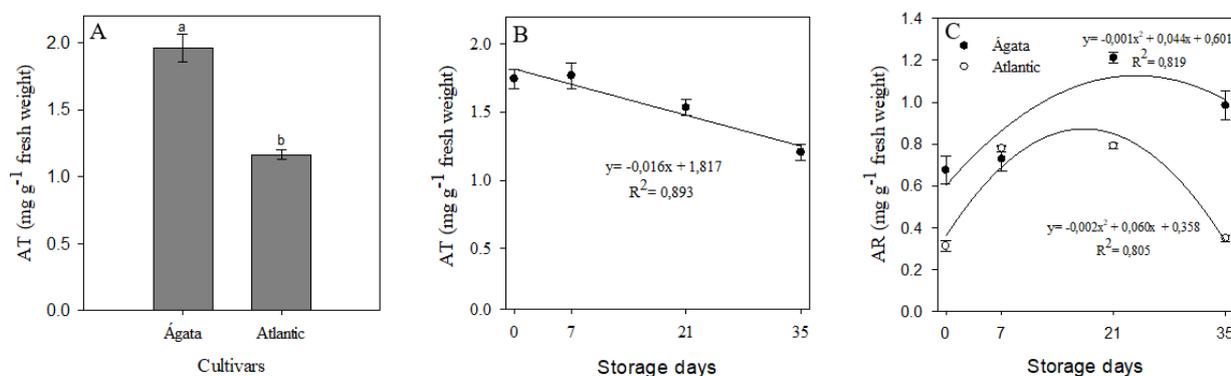


Figure 3. Total sugars in two cultivars (A) and at different storage times (B) and interaction of reducing sugars (C) of potato tubers of two cultivars submitted to different storage times. Guarapuava-PR, 2021.
* Bars indicate the standard error of the mean. Columns followed by an equal capital letter do not differ from each other by Tukey's test ($p \leq 0.05$).

In the interaction results for the content of reducing sugars (AR), both cultivars Ágata and Atlantic presented quadratic adjustment according to the storage period (Figure 3C). The Ágata cultivar had a higher reducing sugar content than the Atlantic during the entire experimental period.

Yamdeu et al. (2016), studying the behavior of carbohydrates in different cultivars, found that tubers stored at room temperature showed levels of reducing sugars tens of times lower than those presented by those stored at 4 °C and tubers stored under refrigeration showed a rapid increase in the RA contents. Soltys-Kalina et al. (2015) point out that the harvest period, storage conditions, and genotype affect the RA content.

The AR content is a trait of high importance for the Atlantic cultivar, intended for industrial processing. During the storage period, the maximum observed value of AR was lower than the maximum tolerated for industrial purposes, which varies from 1.5 to 5 mg g⁻¹ of fresh weight, depending on whether the objective is the production of chips or fries (French fries) (Yamdeu et al. 2016).

Although the cultivar Ágata has higher levels of AR and AT than Atlantic, it is considered that such traits do not affect the quality of this cultivar. According to Evangelista et al. (2011), this cultivar is intended for culinary use and puree preparation, where the AR and AT contents do not affect the quality of the tubers. According to Vreugdenhil et al. (2007), only AT contents higher than 10% lead potato tubers to have a sweet taste, an undesirable trait for consumers.

A negative correlation was observed between the tuber dry mass content and the reducing sugar content ($r = 0.50$, $p < 0.001$), corroborating the results of Ojeda et al. (2010).

The activity of the SOD enzyme was not affected by storage time. The cultivar 'Ágata' showed approximately twice as much activity (111%) as 'Atlantic' (Figure 4A).

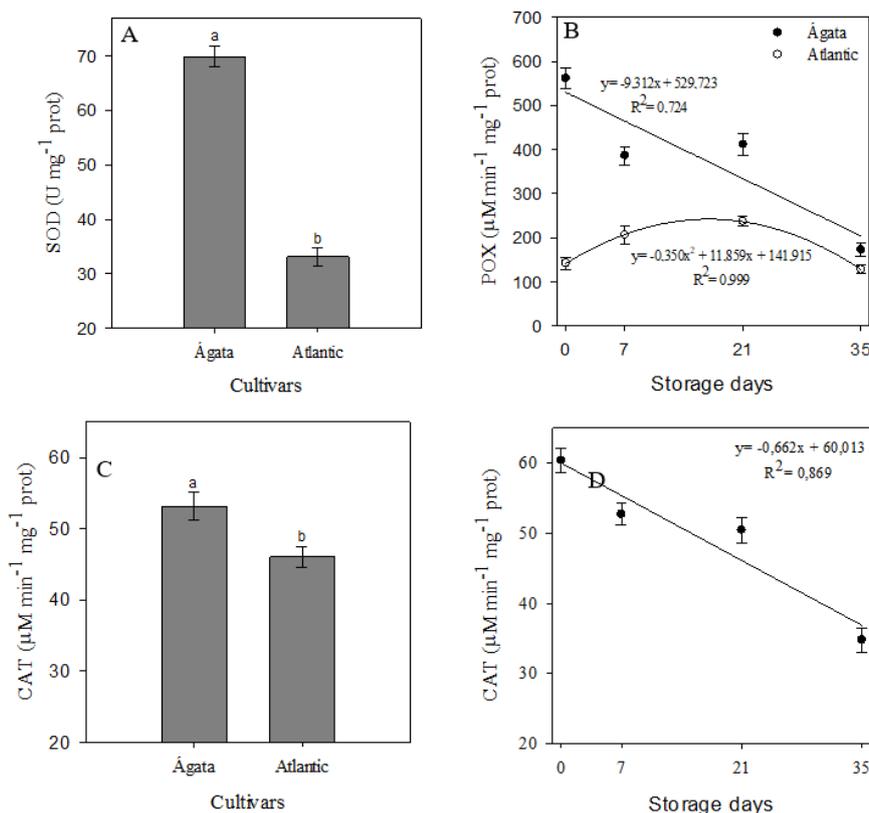


Figure 4. Enzyme activity, catalase in two cultivars (A) and at different storage times (B), interaction of peroxidase enzyme (C) and superoxide dismutase (D) activity of potato tubers of two cultivars submitted to different storage times. Guarapuava-PR, 2021.

* Bars indicate the standard error of the mean. Columns followed by an equal capital letter do not differ from each other by Tukey's test ($p \leq 0.05$).

A significant interaction was observed between the storage period and the evaluated cultivars for the POX enzyme activity. In the interaction results, the cultivar Ágata showed a simple decreasing linear regression, while for Atlantic, a quadratic regression was observed (Figure 4B).

In the Catalase enzyme activity, the cultivar Ágata showed an average activity of 15% higher than the Atlantic (Figure 4C). For the storage periods, a simple decreasing linear regression was adjusted (Figure 4D).

The concentration of hydrogen peroxide in the leaf tissue was affected only by the storage time, showing a quadratic regression, increasing its concentration at the beginning of the storage period and decreasing after 15 days (Figure 5).

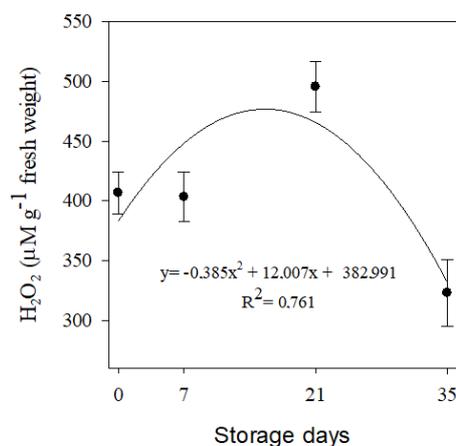


Figure 5. Level of hydrogen peroxide (H₂O₂) in potato tubers subjected to different storage times. Guarapuava-PR, 2021.
*Bars indicate the standard error of the mean.

SOD is the first line of enzymatic defense of plants against reactive oxygen species (ROS), catalyzing the dismutation reaction of the superoxide anion to hydrogen peroxide and water (Barbosa et al. 2014). The two main enzymes responsible for preventing harmful damage from H₂O₂ are catalase, which breaks hydrogen peroxide into water and molecular oxygen, and ascorbate peroxidase, which uses ascorbate to reduce hydrogen peroxide to water and monodehydroascorbate.

The SOD enzyme levels for both cultivars remained constant during the evaluation period. On the other hand, the activity of the CAT enzyme showed a reduction according to the storage period. As hydrogen peroxide levels do not follow the behavior of the CAT enzyme, other enzymes may be involved in protecting cells from the harmful effect of hydrogen peroxide produced by SOD.

Mizuno et al. (1998) reported an increase in the activity of the enzyme Ascorbate peroxidase in tubers, especially those stored under refrigeration, where there was a greater increase in the activity of the SOD enzyme. This suggests that there is possibly an increase in APX activity to compensate for the reduction in CAT activity. The increase in APX activity can impair the nutritional quality of potato tubers as, according to Blauer et al. (2013), one of the main factors responsible for the reduction of ascorbic acid (vitamin C) content in stored tubers and its consumption by APX for detoxification of reactive oxygen species.

Rojas-Beltran et al. (2000) observed that the cultivars Bintjé and Désirée do not present significant differences in gene expression and activity of the SOD enzyme according to the storage period. Storage conditions were similar to those of the present study, with a temperature of 20°C and 85% relative humidity. Regarding CAT, these authors found a reduction in its activity and gene expression. These data corroborate those of the present study, where there was no change in SOD enzyme activity and no decrease in CAT enzyme activity according to the storage time. Similar results are presented by Kawakami et al. (2000), who observed constant activity of SOD and reduction of CAT up to six weeks of storage at 20°C.

In tubers stored under refrigeration (4°C), Delaplace et al. (2009) observed increased activity of SOD, CAT, and APX. The increase in the activity of these enzymes is related to the cold stress that the tubers are subjected to when stored at very low temperatures. According to Cheng et al. (2013), low storage temperatures as a stress factor lead to several metabolic and defense changes in tubers, such as changes in carbohydrate metabolism, resulting in accumulation of reducing sugars, changes in respiration, oxidative stress and other functions not yet fully elucidated.

The CAT enzyme regulates the cellular level of H₂O₂ generated during the healing process (Bajji et al. 2007), because when H₂O₂ accumulates, the suberization process is partially inhibited. The loss of healing capacity according to the storage time may be related to the reduction in basal levels of the Catalase enzyme observed in the present study. The decrease in the Peroxidase enzyme activity observed for the Ágata cultivar for the 35th day of storage may also indicate the loss of healing capacity. In a study conducted by Kumar et al. (2010), a reduction in the healing capacity of potato tubers was observed with the increase in the storage period.

During storage under ambient conditions of potato cultivars Ágata and Atlantic, changes in the activity of POX and CAT enzymes reduce the biochemical capacity of tubers to defend against stress. The reduction in the total sugar content is a factor that can be considered positive because, at this specific point, it improves the quality of the tubers for processing. In general, the cultivars responded differently during the study period; however, the biochemical changes in potato tubers were not extreme; that is, both cultivars can be stored for up to 35 days at room temperature without major changes in tuber quality.

Finally, it is concluded that potato tubers of cultivars Ágata and Atlantic stored under ambient conditions showed loss of fresh mass, reduced firmness, and increased soluble sugar content. Storage under ambient conditions led to a reduction in total soluble sugar content for both cultivars, the desired trait for the Atlantic cultivar, destined for industrialization. The cultivars Ágata and Atlantic can be stored for up to 35 days under ambient conditions without damaging their physicochemical quality significantly.

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