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An approach of using different curing temperature based on potato cv. Innovator periderm differentiation, sugar metabolism, and industrial quality

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ABSTRACT. Excoriation damage is among the major causes of postharvest potato losses. Curing is known to increase the resistance of tubers to excoriation injury, with the temperature influencing the traumatic phellogen and periderm regeneration, as well as tuber processing quality. This study aimed to evaluate the effects of excoriation procedure and curing temperature on the industrial quality, histological characteristics, carbohydrate metabolism, and oxidative enzyme activity of potato tuber. Tubers with or without excoriation were cured at 8, 14, and 20°C for 15 days. Excoriation did not influence the levels of reducing sugars (RS), as well as polyphenoloxidase (PPO) and peroxidase (POD) activities. The concentrations of total soluble sugars (TSS) and non-reducing sugars (NRS) were higher in injured tubers, while reduction in curing temperature increased the concentrations of TSS, NRS, and RS. However, the sugar content was adequate as per the criteria of the pre-fried potato industry and potatoes classified in category 2 (USDA/fast-food industry color grading). The reduction in curing temperature also increased the activity of POD, despite the activity of PPO remaining unchanged. Formation of a closure layer from the outer parenchyma cells of the tuber that prevents desiccation and death of superficial cells, was observed. This protection was completed with the formation of the damage periderm. Periderm regeneration is faster at higher temperatures, 15 and 20°C, leading to a lower fresh mass loss and no enzymatic or non-enzymatic browning. The excoriation carried out at 14/20°C for 15 days was sufficient for curing, and for maintaining suitable post-fry quality of potatoes.

Keywords: plant histology; peroxidase activity; plant anatomy; reducing sugars; traumatic phellogen; periderm.

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Introduction

Potato is one of the most important food commodities worldwide. The estimated impact of root and tuber disease-related losses on Brazilian imports is 25% of the production, with the largest losses occurring due to postharvest handling and storage (Costa, Guilhoto, & Burnquist, 2015). Excoriation is the major cause of postharvest losses (Gao, Geng, Rao, & Ying, 2016); however, the effect of damage in potato tubers can be reduced through postharvest curing.

As periderm matures, the phellogen ceases cell division, and its cell walls thicken and become suberized, making the tubers more resistant to excoriation injury (Sabba & Lulai, 2005; Lulai, 2007). Tubers subjected to curing have a traumatic phellogen. This phellogen gives rise to a wound periderm that provides protection against excoriation and microorganisms and resistance to water loss (Jin et al., 2018).

Low curing temperatures inhibit sprouting, water loss, and microorganism infection, which prolongs postharvest storage (Chen et al., 2012). However, they may result in sugar accumulation, causing darkening of the tissue after frying, which reduces product quality and slows down cell division and suberization processes, as wounds heal at a faster rate at high temperatures (Wang et al., 2015).

Additionally, excoriation damage and low temperatures may increase the activities of oxidative enzymes (Barbosa, Silva, Willadino, Ulisses, & Camara, 2014), further contributing to darkening before frying. Histological,

metabolic, and enzymatic approaches of tubers subjected to curing allow us to follow the concurrent outcomes associated with a higher resistance to excoriation and a suitable tuber classification for consumption.

This study aimed to evaluate the industrial quality, carbohydrate metabolism, oxidative enzyme activity, and cytological and histological responses of potato tubers with or without excoriation after the curing process at different temperatures.

Material and methods

Plant material and experimental design

Potato tubers (*Solanum tuberosum* L.) cultivar Innovator were obtained from the commercial production area of Perdizes, state of Minas Gerais (19º21'10" S, 47º17'34" W and 1,000 m above sea level). Tubers were planted in May and harvested manually in September 2017. The experiment was conducted using a completely randomized design and split-plots. Curing temperatures (8, 14, and 20°C) were assigned to the plots and the excoriation treatments, tubers with or without excoriation damage, in the sub-plots. Each treatment consisted of eight replications, and for each repetition, two tubers were evaluated.

Curing treatment

The treatments consisted of tubers without excoriation damage (control) and with excoriation damage, created with the aid of coarse sandpaper (manual excoriation on one area of about 12 cm^3). Tubers subjected to both treatments were placed in different chambers at 8, 14, and 20°C (RH 90% ± 3) for 15 days to cure.

Samples of 1 cm^3 were taken from both treatment groups. The samples were fixed in FAA (Formaldehyde - Acetic Acid - Ethanol, 1:1: 9 v: v: v) for 48h and then stored in 70% ethanol (Ruzin, 1999). The samples were reduced to 0.25 mm^3, dehydrated in 70, 80, and 95% ethanol for 2h each, and soaked in methacrylate resin (Leica) plus ethanol (1:1) for one week, and in pure resin for 48h. Resin-infiltrated samples were transferred to histomolds with resin solution plus polymerizer and kept in an oven for seven days, according to the manufacturer's recommendations.

The 5-µm thick cross cuts were performed with the help of a microtome (Leica, Spencer model). The slides were mounted with 0.05% toluidine blue in 0.1 M sodium phosphate buffer pH-6.5 (O'Brien, Feder, & McCully, 1964). Tests for the histolocation of starch, proteins, lignin, and lipids in the peripheral cell layers of the tubers were performed. Histological tests with lugol (Johansen, 1940), acid floroglucin (Johansen, 1940), Xilidine Ponceau (Vidal, 1970), neutral red (Kirk, 1970), and Sudan Red (Brundrett, Kendrick, & Peterson, 1991) were performed to detect starch, lignin, protein, and lipids, respectively.

The photographic documentation of the histological sections was performed using a microscope (Olympus AX70) equipped with a U-Photo system. The images were analyzed for distinctive and structural characteristics of the periderm and storage parenchyma displayed by the samples of tubers subjected to the above-mentioned treatments.

Fresh matter loss rate (FMLR) of the tubers was obtained through the daily difference in tubers weight, wherein the result was expressed as a percentage.

The extraction of total soluble sugars (TSS), reducing sugars (RS), and non-reducing sugars (NRS) was performed as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). TSS reaction was accomplished according to the method of Dubois et al. (1956), while RS content was determined using the dinitrosalicylic acid (DNS) method (Gonçalves, Rodrigues-Jassom, Gomes, Teixeira, & Belo, 2010). NRS was calculated as the difference between TSS and RS, and expressed as a percentage.

Fresh cut experiment

To evaluate the post-fry color, the tubers were cut into French fries using a manual cutter and fried in an electric fryer, with a capacity of 3 L (Model: Ford®) for 3 min. at 180°C. The color of the post-fry potatoes was visually determined based on the grading scale recommended by the 'United States Standards for Grades of Frozen French-Fried Potatoes' (U.S. Department of Agriculture [USDA], 1967) and the fast-food industry color grading chart from 1 to 5.

The POD and PPO extracts were obtained according to Lagrimini, Gingas, Finger, Rothstein, and Liu (1997). POD activity was determined according to Lagrimini et al. (1997), with PPO activity determined according to Kavrayan and Aydemir (2001). Total protein content was determined (Bradford, 1976) using bovine serum albumin as the standard and activity of enzymes was expressed in units of absorbance (UA) $min.^{-1}$ mg^{-1} protein.

Statistical analysis

Data were subjected to analysis of variance and regression (ANOVA). P-values ($p < 0.05$) were considered to be significant. All statistical analyses were performed using the statistical software SAEG 9.1 - Statistical and Genetic Analysis System (SAEG, 2007). Data are presented as mean ± standard error (SEM).

Results and discussion

The phellogen differentiation of potato tubers from cultivar Innovator subjected to mechanical damage treatment was influenced by curing temperature (Figure 1). Histological outcomes were most evident on phellogen activity, although there were anatomical cell features common to tubers from the control and mechanical damage (excoriation) treatments (Figure 2). Small differences regarding protein presence in peripheral parenchyma cells of tubers were observed between tubers of both treatment groups (Figure 3). The relationship between these histological differences influenced the fresh matter loss ratio (Figure 4) and carbohydrate metabolism (Figure 5), although they provided similar outcomes in terms of the industrial quality of potato (Figure 6).

Tubers collected before the beginning of the curing treatment had the original periderm cell layers removed. Some sections showed that excoriation removed the cork layers, reaching the phelloderm and even more peripheral layers of the reserve parenchyma (Figure 1A and B). The phellogen from the tuber samples of control treatments had four to seven layers of cells with a smaller lumen and thinner walls that were frequently ruptured (Figure 1C, E, and G). The cells that made up the phellogen had purplish-colored walls, indicating a higher proportion of pectin (Figure 1). The next and innermost cell layers, approximately three layers, had characteristics of parenchyma cells that tended to be rectangular, starch-free, and smaller than those of the reserve parenchyma, identified as phelloderm (Figure 1C, E, and G). In damaged treatments, phellogen was fully active and had an average of 1 to 9 cell layers, while phelloderm was in formation and was similar to the reserve parenchyma, which makes its precise identification difficult. This difference in the number of the cell layers was partially attributed to the curing temperature, which, within the tested range, favored cell division and differentiation as it increased.

Pereira et al. (2020) observed that regardless of the temperature, ranging from 8 to 20°C, phellogen was formed after 15-day curing of potato tubers. Nevertheless, the number of periderm cell layers and thickness increased significantly with curing temperature. Although significant differences were observed between control and mechanically damaged treated plants, these might be attributed to the differences in phellogen activity as a more active meristem is expected from injured potato tubers.

Phelloderm and phellogen were observed while the cork had an irregular shape, with a variable number of layers, including the cork and periderm, present and observed in the control tubers regardless of the temperature (Figure 1A, C, E, and G). The traumatic phellogen displayed a purple color associated with a pectin-rich cell wall, while the bluish cellulosic wall was observed for the closure layer (Figure 1B, D, F, and H). The vascular tissue, although scarce, was observed in both treatments (Figure 1). Lulai and Suttle (2009) stressed that metachromatic staining of the walls facilitated the identification of the purplish phellogen. Furthermore, they reported that native periderm maturation and development of skinning resistance are followed by an increase in non-esterified pectins in the cell wall of the phellogen.

Despite our expectations, suberin deposition was not observed in cork cells or in the closure layer (data not shown). This is an unusual result as a periderm with suberin deposition that acts on wound healing and acts as a barrier against dehydration, disease, and insects (Lulai & Suttle, 2009). This lack of suberin deposition may have a significant influence on the postharvest conservation and quality of the tubers for this potato variety in particular. Occasionally, greenish-blue coloration was observed in the cork, but the presence of lignin was discarded because of the negative result from the acid floroglucin test (data not shown).

At 15 days of curing, a collapsed outer layer of parenchyma enclosing starch grains and with slightly thickened portions, attributed to the walls and precipitated cellular content, was observed at the periphery of the tuber. Inside this collapsed layer, was a peripheral tissue zone with non-collapsed cells, without starch content and with a cellulosic wall, denominated closure layer (Lulai & Suttle, 2009). The absence of starch in these cell layers indicates that these cells can be recruited and de-differentiated to form the traumatic phellogen. The lack of starch or the presence of smaller stach grains in this region supports this inference (Figures 1 and 2). The presence of starch grains in the collapsed layer was indicative that the metabolism in

this layer was impaired, avoiding the consumption of carbohydrates and probably other metabolites. The positive reaction for protein within the remaining debris was in accordance with this impaired metabolism (Figure 3C and D).

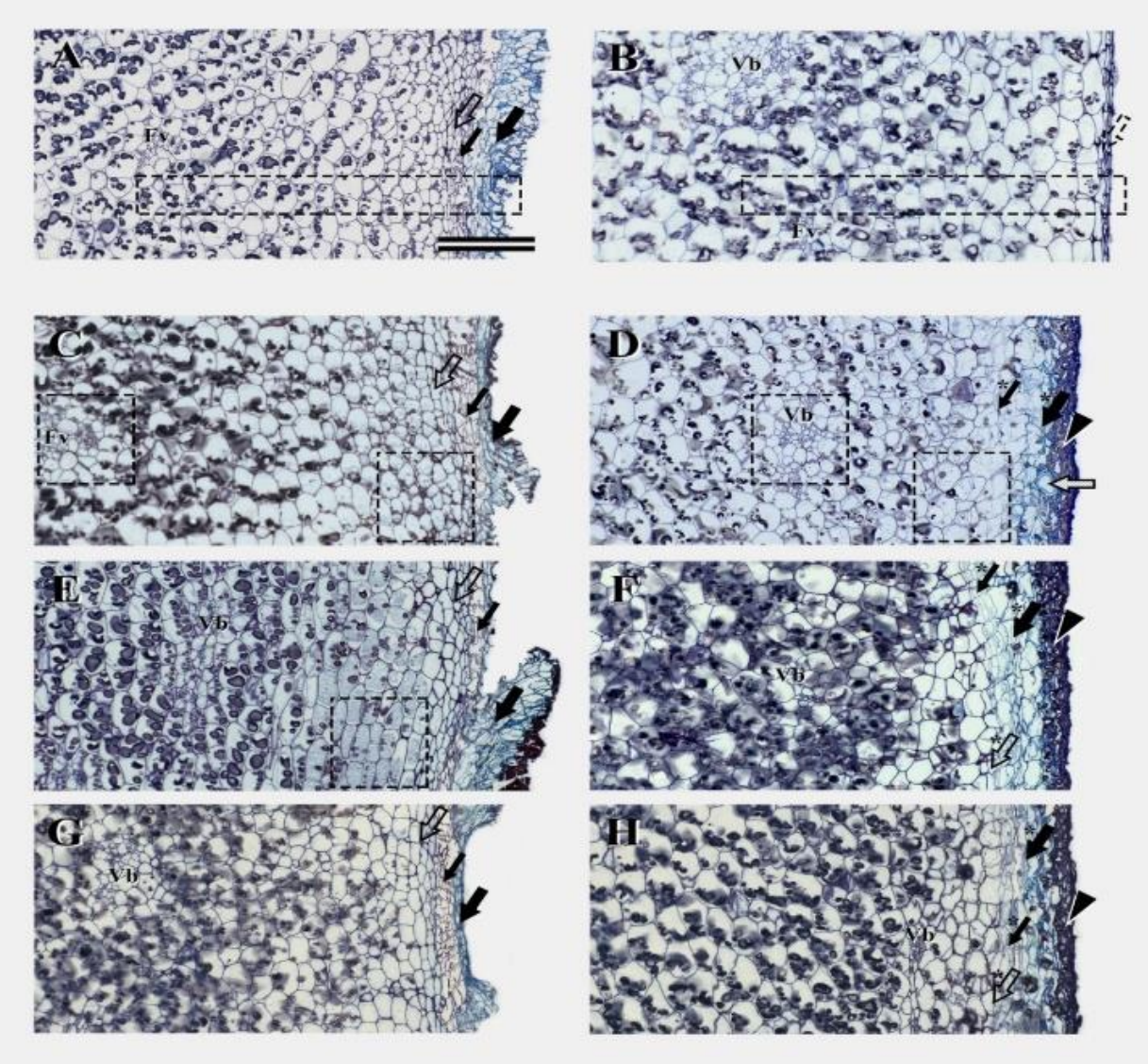

Figure 1. Histological sections of potato cv. Innovator showing the periderm and the reserve parenchyma. Time zero for control (A) and damage treatment before healing (B). Periderm after 15 days of curing at 8 (C), 14 (E) and 20°C (G). Periderm after 15 days of curing at 8 (D), 14 (F) and 20°C (H). Arrowhead indicates the collapsed region. Vb - vascular bundle; larger and full arrow - cork; thin arrow - phellogen; bigger and empty arrow - phelloderm; arrow with dashed borders - tissue layer after mechanical injury and arrowhead - collapsed parenchymatic layer. Arrows with an asterisk show phelloderm, phellogen and suber of traumatic origin. Details of regions with dashed borders show the periderm and details of the peripheral reserve parenchyma to be explored in Figure 2. Bar is equivalent to 400 µm, all sections are at the same magnification.

Traumatic phellogen was formed in the reserve parenchyma, near the tenth layer from the periderm (Figure 1C, E, and G). A cytological reference of this location was the presence of thickened secondary wall idioblasts of cellulosic nature observed near the fourth to the sixth cell layer of this reserve parenchyma. Although the possible function of idioblasts could not be identified, they were found near the collapsed cell layer in some samples (Figure 1D). However, the exact location of phellogen formation was inaccurate given the variation in the number of cell layers that are part of the collapsed peripheral parenchyma. This variation was attributed to differences in the intensity of excoriation injury at the time of excarification, which is yet to be determined. Lulai and Suttle (2009) raised questions as to what would be the first changes after injury, where changes in the osmotic potential of cells at the injury site are highlighted. However, these responses are related to suberization and report that the signals and mechanisms acting to induce suberization in potato tubers have not been identified yet.

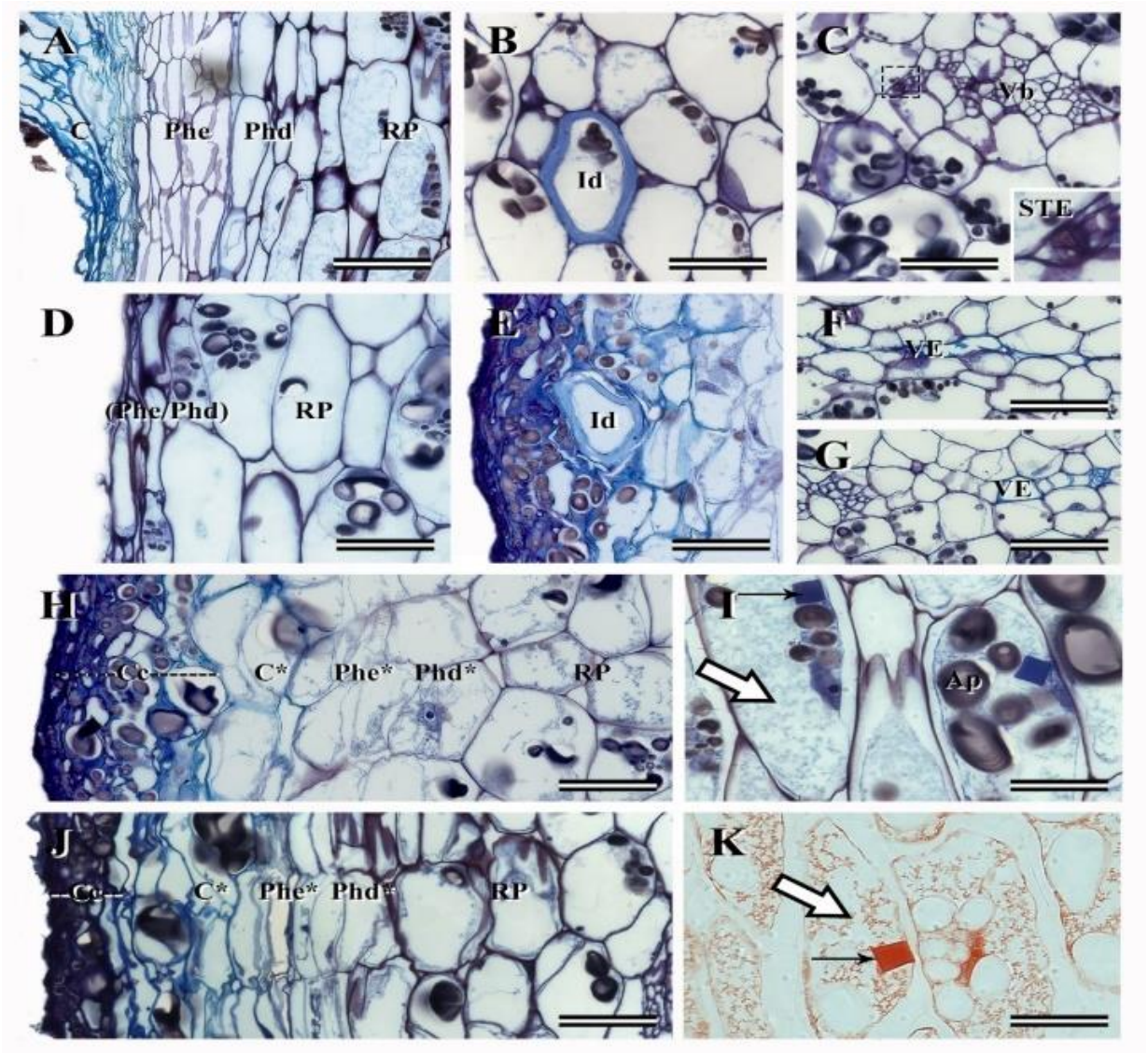

Figure 2. Periderm and parenchyma reserve tissue in the periphery of tuber from potato cv. Innovator A, B, C, D, E, F, G, H, I, and J, stained with toluidine blue and Lugol; K, reaction with Xilidine Ponceau. A - control and D - injury at time zero; B, C, E, F, G, H, I, J, and K treatments after 15 days of curing. B - 20°C control; C – excoriation damage 20°C; E - excoriation damage 8°C; F, G, I, and K - control 14°C; H - excoriation damage 8°C; and J - excoriation damage 20°C. C - Cork; Phe - phellogen; Phd - phelloderm; RP - reserve parenchyma; Vb - vascular bundle, Id - idioblast; STE – sieve tube element; VE - vessel element; Cc - collapsed parenchyma layer; Ap - amyloplast. Letters with an asterisk indicate structures of traumatic origin. Large arrow - dense cytoplasmic protein content; thin arrow - solid protein inclusion. Bars at A, B, C, D, E, F, G, H, and J equals 100 µm, bars at I and K equals 50 µm. Detail in C presents an additional 300% increase.

The activity of this newly formed phellogen, and the consequent new periderm, was dependent on the curing temperature. Tuber excoriation stimulated the formation of a traumatic phellogen, in which differentiation was faster as temperature was increased (Figure 1D, F, and G). The formation of a new layer of phellogen is one of the first responses to injury (Sabba & Lulai, 2004). At 14 and 20°C, more layers of cork were formed after 15 days of curing (Figure 1F and H). As the temperature increased, more prominent cell layers were formed, and the (re)differentiation process occurred to originate the new periderm. Pereira et al. (2020) observed that, after curing potato tubers at 8°C for a 15 day period, only the phelloderm was formed, whereas a newly formed periderm was observed from its activity after curing at 14 and 20°C.

The outer cell layers are recruited and their metabolism reactivated as starch consumption, cell division, and (de)differentiation are observed to originate from the traumatic phellogen. Regardless of treatment and curing temperature, it was observed that the size and quantity of starch grains reduced and decreased, respectively, from the inside towards the outside of the tuber (Figure 1). However, in the damage treatments, the transition is more abrupt.

A variable region was found between 10 and 20 cell layers of this reserve parenchyma, which covers the layer that undergoes cellular de-differentiation and gives rise to phellogen, which has a dense cytoplasmic

content (Figures 1C, 1E, and 3A). This content was less apparent (Figure 1D) or absent in mechanically damaged tubers (Figure 3B), being consumed or eliminated with the cell layers external to the formation of phellogen (Figure 1F and G). It is implied that this protein resource is also recruited for cell dedifferentiation and traumatic phellogen formation (Figures 1C to 1H, 2A, 2H to 2K, and 3). Tuber vascularization was observed just below these layers with dense cytoplasmic content (Figure 2C). The consumption of this content is in accordance with the recruitment of the outer cell layers involved in phellogen differentiation and activity.

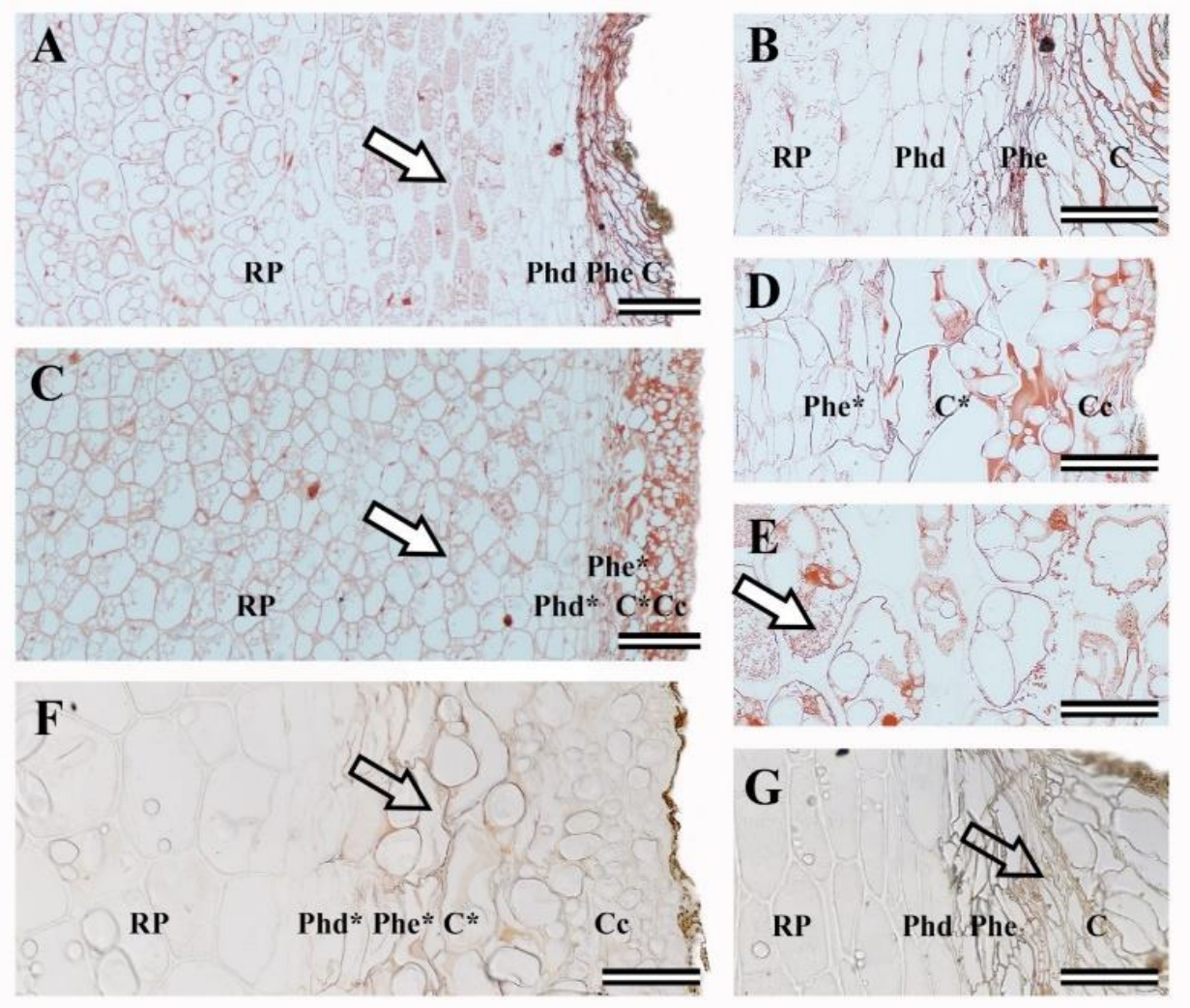

Figure 3. Histochemical tests of Xilidine Ponceau (A to E) and Sudan Red (F and G) in cross sections of potato cv. Innovator, after curing for 15 days at 14°C. A, B, E, and G, control treatment and C, D and F, excoriation damage treatment. C - Cork; Phe - phellogen; Phd - phelloderm; RP - parenchyma reserve; Cc - collapsed parenchyma layer. Letters with an asterisk indicate structures of traumatic origin. Large (white) arrow - dense cytoplasmic content of protein nature; large (transparent) arrow - positive reaction for lipids. A and C bars equals 200 µm; B, D, E, F, and G; bars = 100 µm.

In addition to cytoplasm composition, differences in the cell wall composition of the cell layers were observed in this region where phellogen was formed (Figures 2 and 3). Notably, a light purplish color of phellogen cell walls was more evident, whereas parenchyma cells had a darker-purplish to purple-bluish color (Figure 2).

The constitution of the wall where cellulose and pectins predominate in the cork and phelloderm cells, respectively, is highlighted (Figure 2A). The idioblasts that occur in the peripheral portion of the reserve parenchyma had a secondary, thickened cellulosic wall that contrasts with a thin wall of the cells of this reserve parenchyma. This reinforced wall has a stronger structure to resist excoriation than other cells, although its real function is yet to be determined. The parenchyma close to vascular tissues had fewer and smaller plastids in all treatments (Figure 2). The absence of starch in these cells is related to their consumption and carbohydrate transport to maintain metabolism and increased respiration (Daniels-Lake et al., 2014; Lulai, Campbell, Fugate, & Mccue, 2016), albeit at lower rates.

The phloem sieve elements and xylem vessel elements could be identified despite the reduced size of the sieve tube and sieve plate elements (Figure 2C) and the irregular shape and smaller secondary wall deposition in the vessel elements (Figure 2F and G). Nevertheless, the vascular tissue was scarce (Figure 1) and disproportional (Figure 2C, F, and G) compared to the amount of the reserve parenchyma tissue in the potato tubers. Considering the ongoing processes and coordination of phellogen formation, this may account for the symplastic transport via plasmodesmata in these outer cell layers. Additional studies must be performed to test this hypothesis.

Excoriation damage reached the phelloderm layers to the periphery of the reserve parenchyma (Figure 2D), where inner layers were eliminated as traumatic phellogen was formed (Figure 2E). The presence of thick-walled idioblasts was consistent with the elimination of cell layers. It could be seen in this collapsed layer that starch reserves were present, which summed with the formation of the closure layer, suggested the isolation and or absence of an active protoplast in the eliminated cells. The denser content in the wall region that fills the intercellular spaces contributed to the isolation of these cell layers (Figure 2E, H, and J).

A protein-rich and denser cytoplasm (Figure 2I and J) was acquainted with the reserve parenchyma just below the periderm. Soluble proteins found in potato tubers were mainly protease inhibitor proteins (50%) and patatin (40%) (Pouvreau et al., 2001). The protein denominated Cys protease inhibitor, CPI (cystatin), with eight similar-sized domains and sequences, was found in the layers below the phellogen in potato tubers (Nissen et al., 2009). The protein content of cytoplasm was present; however, it was less dense in treatments subjected to excoriation. The shape of protein inclusions resembled prismatic bodies found in peroxisomes (Figures 2 I and K), and were identified as Cys protease inhibitor proteins (cystatin) (Nissen et al., 2009).

Peroxisomes are dynamic organelles in relation to morphology and metabolism, involved in various processes such as carbon metabolism, secondary metabolism, development, stress response, and defense against pathogens (Hu et al., 2012). A feature shared by peroxisomes is their ability to metabolize hydrogen peroxide, thereby protecting the rest of the cell from this toxic byproduct (Johnson & Olsen, 2001). The identification of cystatin proteins (Nissen et al., 2009) and stress defense activity (Johnson & Olsen, 2001; Hu et al., 2012) support the hypothesis that these layers play a role in the viability and maintenance of the integrity of tubers challenged by biotic or abiotic stresses.

The reduction in the amount and size of starch grains and the amount of protein in the peripheral parenchyma was attributed to the consumption of these reserves, supporting the formation and activity of traumatic phellogen (Figure 2H and J). The activity of this traumatic phellogen was lower in the curing treatment at 8°C (Figure 2H) and stimulated at 20°C, where newly formed and distinct cork and phelloderm cells were observed (Figure 2J). These observations are consistent with the increased respiratory activity (Daniels-Lake et al., 2014) and starch consumption for phellogen formation (Lulai et al., 2016) and activity (Pereira et al., 2020).

Regardless of the curing process, the reserve parenchyma cytoplasm on the periphery of the tuber reacted positively to proteins (Figure 3A to E). Independently from the curing temperature, there was also protein occupying the wall region and intercellular spaces in the collapsed outer cell layers (Figure 3C and D). The concentration of these proteins, considering their nature (Pouvreau et al., 2001; Nissen et al., 2009), seems to reinforce or stick the collapsed cells together, contributing to a smaller FMLR.

This is particularly relevant considering the absence or small lipid deposition in cork, found with Sudan Red reagent (Figure 3F and G). The subber is a region composed of dead cells, and lipid deposition (Company-Arumí et al., 2016). Such a suberin is observed, acting in the protection and reduction of water loss by the tuber (Saba & Lulai, 2005; Lulai, 2007; Jin et al., 2018). The amount of suberin and wax produced in the damage periderm is 50%–60% lower than that in the native periderm (Schreiber, Franke, & Hartmann, 2005). The small deposition of lipids in cork is attributed to a characteristic inherent to the variety used or to environmental factors that, in the absence of specific reports for the variety used in this work, may explain these observations. The negative fluorescence response of lipids in sections treated with Neutral Red corroborates the absence or lower proportion of lipids in the cork cell walls (data not shown).

The faster establishment of the periderm was linked to the lower FMLR in higher temperature treatments (Figure 4) and the consumption of starch, TSS, and RS reserves (Figure 5). For healing of the excoriation region, the closure layer was formed, followed by the damaged periderm (Lulai et al., 2016, Pereira et al., 2020). This layer is formed before or concomitantly with the traumatic phellogen. The damage periderm is formed by multiple layers of suberized cells of the cork, and the phelloderm consumes starch from the

underlying cells to provide energy and carbon for the phellogen (Lulai et al., 2016). The formation of the closure layer prevents desiccation and death of superficial cells, a protection that is completed with the formation of the damage periderm (Lulai et al., 2016). Again, the increased thickness and cell layers of the potato periderm with curing temperature (Pereira et al., 2020) contributed to the lower FMLR observed.

The faster establishment of a functional periderm (Pereira et al., 2020) contributed to an earlier protection against fresh mass loss that was noticed at the 11th day after excoriation (Figure 4). Nevertheless, the curve of fresh mass loss of the injured and non-injured treatments were similar for tubers submitted to curing at 20ºC.

The FMLR was higher in the damaged tubers than in the control treatment for up to 15, 9, and 7 days of curing at 8, 14, and 20°C, respectively (Figure 4). The highest FMLR in damaged tubers is caused by water loss through transpiration and consumption of reserves through respiration. Damage increases respiratory activity due to increased vapor permeability in injured tissue (Daniels-Lake et al., 2014). Water loss is high, and it is estimated that the initial rate of water loss can be increased by 250 to 1,000 times in injuries such as cutting, bruising, and skinning (Schreiber et al., 2005).

The FMLR decreased over the curing period for all treatments, and was higher in tubers at 8°C than at temperatures of 14 and 20°C (Figure 4). A faster differentiation of traumatic phellogen and damage periderm occurs at higher temperatures, which reduces water loss from excoriation-damaged tubers to 14 and 20°C and consequently FMLR. The FMLR of the control tubers showed less variation in the curing temperatures under study.

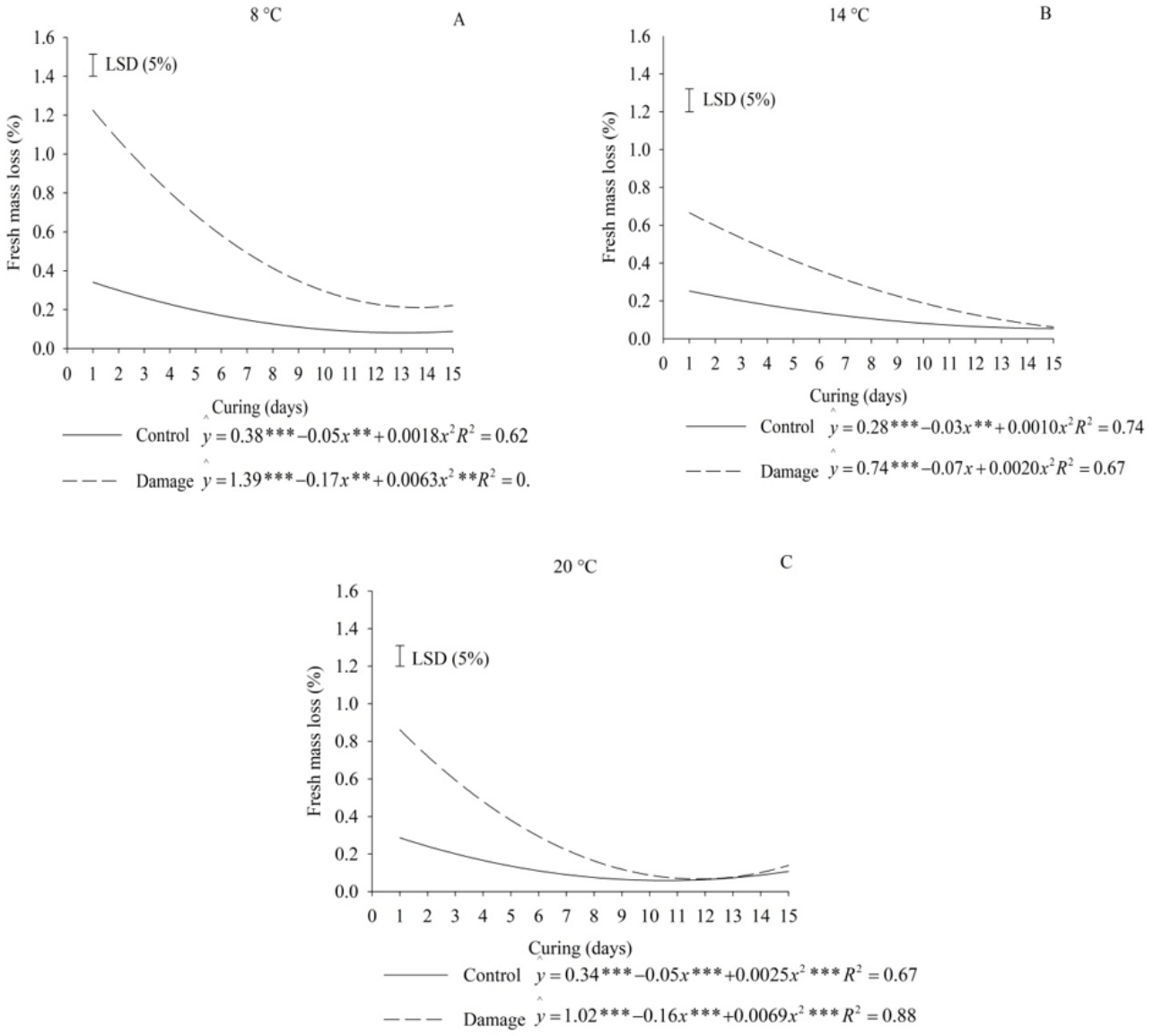

Figure 4. Fresh mass loss of potato cv. Innovator during the 15-day curing at 8°C (A), 14°C (B), and 20°C (C). LSD - significant minimum difference at 5% level by t-test.

The FMLR of the damaged tubers was 0.26, 0.14, 0.17%, and 0.035 for the control, and 0.055% and 0.15% for curing at 8, 14, and 20°C (RH 90% ± 3) at 15 days. When assessing fresh weight loss of tubers at 13°C (95% RH) for 7 days, Daniels-Lake et al. (2014) observed a loss of 1.2; 1.7, and 2.1% in intact, worn or cut tubers, respectively. These values increased at 37 days to 2.7, 3.4, and 4.0%, respectively, and no effect was observed in the assessed cultivars Crisping, Andover, and NorValley, indicative of the importance of the traumatic phellogen and periderm formation in reducing tuber mass loss.

Increasing the temperature from 8 to 20°C reduced TSS and NRS concentrations in the excoriation-damaged and undamaged tubers (Figure 5A and B), although this reduction was smaller for control tubers. RS was similar among tuber treatments and temperature of curing (Figure 5C). Although TSS and NRS were initially higher for tubers subjected to curing at 8°C, both tuber treatments showed similar TSS and NRS concentrations at 20°C (Figure 5A and C). However, at lower curing temperatures and higher stress conditions, greater sugars accumulated can function as cryoprotectants (Stitt & Hurry, 2002).

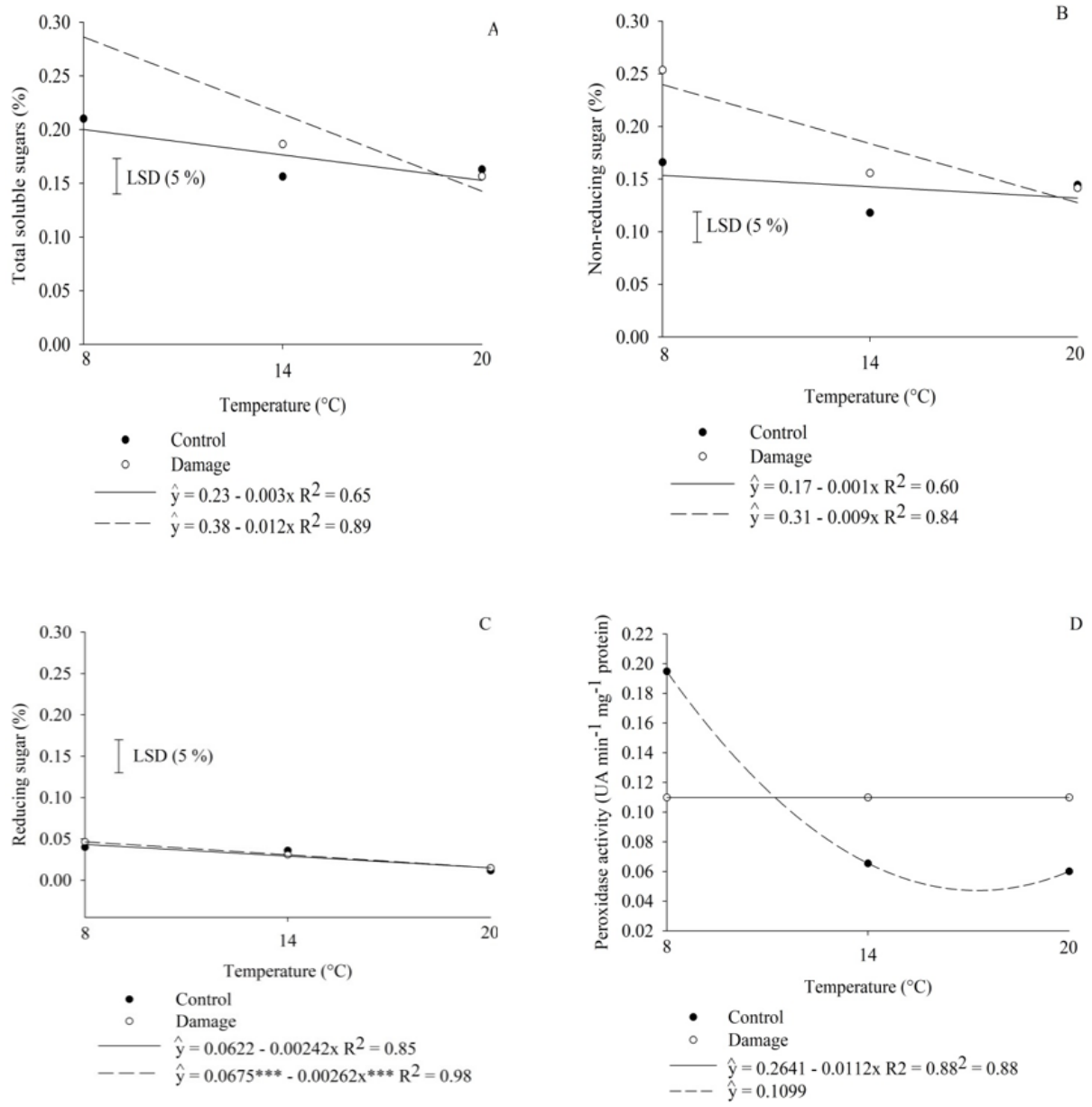

Figure 5. Total soluble sugars (A), non-reducing sugar (B), and reducing sugar (C) from potato tubers cv. Innovator control (without damage) and excoriation damage after 15 days of curing at 8, 14, and 20°C. Peroxidase activity (D) of potato cv. Innovator control after 15 days of curing at 8, 14, and 20°C. LSD - significant minimum difference at 5% level by t-test.

The occurrence of excoriation damage caused an increase in respiratory activity, thereby increasing the concentration of NRS (sucrose) to act as a respiratory substrate. Damage may increase respiratory activity 25 times

after 24h of damage when compared to intact tubers (Lulai et al., 2007). The increase in TSS concentration results from starch breakdown in NRS (Zommick, Knowles, & Knowles, 2014). Considering the differentiation processes that occur during the reestablishment of the periderm, higher curing temperatures may have stimulated the carbohydrate metabolism, therefore contributing to the observed industrial quality of the potato (Figure 6). In tubers with excoriation damage, the reserves are mobilized in the histological sections of the parenchymatic region where phellogen is formed. These carbohydrates are used to differentiate traumatic periderm. The absence of significant changes in RS concentration suggested that there was no conversion of NRS to RSA (glucose and fructose), or RS was consumed as fast as it was produced as a control, compared to mechanical injury.

At 20°C, TSS and NRS levels were similar between tubers with and without excoriation damage (Figure 5A and B). This may be due to an increase in the respiratory activity of control tubers with a higher curing temperature and faster carbohydrate consumption in damaged tubers for formation of the traumatic periderm.

Even at 8°C, with the highest sugar concentrations, the values are adequate for the processing industry. At 8°C in the control and excoriation-damaged tubers, the NRS values were 0.16% and 0.24%, and the RS were 0.042 and 0.046%, respectively (Figure 5B and C). For the French fries processing industry, NRS levels should be less than 0.33% fresh mass (Chapper, Bacarin, Pereira, & Terrible, 2002) and RS below 0.12% fresh mass (Stark, Olsen, Kleink, Ge, & Love, 2003).

Additionally, there was no variation in POD activity ($\hat{y}$ = 1.099) with curing temperature for the control treatment. However, POD activity was higher for excoriated tubers and curing at 8°C, although it decreased with temperature (Figure 5D). It is worth highlighting the considerable change in metabolism regarding POD activity in excoriated tubers cured at 20°C, as it is even lower than control treatments, indicating a lower stress level at this temperature. There was no treatment or curing temperature effect on POD activity (data not shown). This result is interpreted as the provided treatments (curing temperature or excoriation) failed to increase the level of stress in the potato tubers.

Regardless of the curing temperature and occurrence of excoriation damage, the chips were classified in category 2 after frying (Figure 6), which is adequate with proper RS and NRS. Darkening after frying is caused by the non-enzymatic Maillard reaction between RS carbonyl clusters and amino acids such as asparagine, resulting in the formation of melanoidins (Wiberley-Bradford, Busseb, & Bethke, 2016), which darken the chips after frying, making them unsuitable for industry, which requires products classified in Categories 1 and 2.

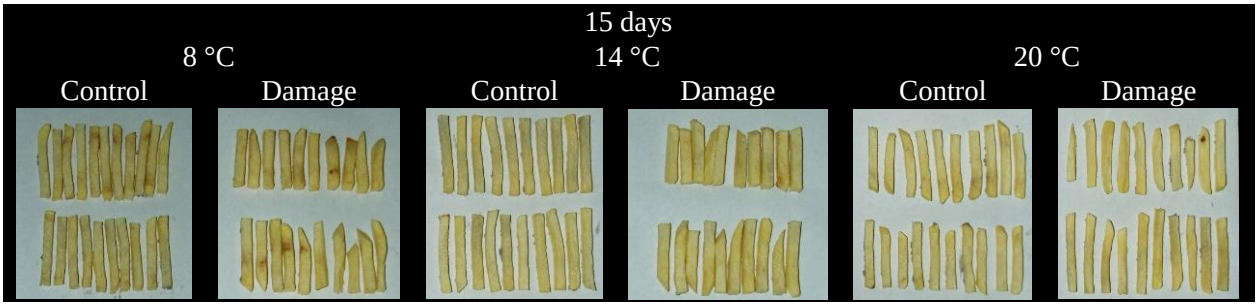

Figure 6. Post-frying coloring of potato sticks cv. Innovator control (without damage) and excoriation damage after 15 days of curing at 8, 14 and 20°C. The numbers below each photo represent the classification according to USDA and the fast food industry color grading chart, ranging from 1 to 5.

The low temperature is a condition of stress to the tubers, which leads to the activation of the plant's defense mechanism as the production of reactive oxygen species. Among these mechanisms is the activation of oxidative enzymes such as peroxidase (Barbosa et al., 2014). The POD activity and formation of H_2O_2 are necessary for suberization, so they are induced after damage (Bernards & Razem, 2001). However, in this study, no effect of excoriation damage on POD and PPO activity was found at the end of the 15 days of curing, even considering the mechanical stress inflicted by the excoriation procedure, and its possible effects on enzyme activity. It seems that stress, as observed for POD and PPO activity, reached a milder level, which also accounts for a better post-frying coloring of potatoes after curing at 14 or 20°C.

Conclusion

The potato tubers subjected to excoriation, including carbohydrate metabolism and (re)establishment of a functional periderm, share some anatomical and histological features. Differentiation processes and

periderm regeneration after excoriation are faster at higher curing temperatures of 15 and 20°C. The faster phellogen activity, and the maintenance of an outer collapsed layer, contributing to the prompt differentiation of a new periderm leads to a lower fresh mass loss, lower stress levels, and absence of enzymatic or non-enzymatic browning. The excoriation conducted at 14/20ºC for 15 days was sufficient for curing and maintaining suitable post-fry quality of potatoes.

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