Formation of damage periderm in Markies and Challenger potato tubers under the influence of temperature[1]

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ABSTRACT

The curing before storing the tubers is necessary for the formation of damage periderm, promoting greater post-harvest conservation. In which, the rate curing and the maintenance of the quality of the tubers depends on the temperature. This way, the search aimed to determine the effect of the curing temperature on the regeneration of the damage periderm and on the carbohydrate metabolism of cultivar Markies and Challenger potatoes tubers destined for industry. For this, the curing was carried out for 14 days on tubers with excoriation injury and tubers without excoriation (control) at temperatures of 8, 14 and 20 °C (RH ± 90%). The fresh mass loss rate (FMLR) daily was higher in the excoriation tubers. The excoriation injury and the temperature of 8 °C increased the total soluble sugar (TSS), reducing sugars (RS) and non-reducing sugars (NRS) of the tubers of 'Markies' and TSS of 'Challenger'. The excoriation injury tuber the process of cell division was induced in the periclinal plane, forming phellogen, with few layers of collapsed cortical cells. In both cultivars, at 14 °C the new phellogen became more evident and at 20 °C some layers of a new cork were formed. In 'Markies' the development of new periderm was earlier than in 'Challenger', even at 8 °C. It is concluded that the temperature of 14 ºC provided better curing and regeneration of the excoriation periderm tubers maintain post-fry quality of potatoes destination by industry processing.

Keywords: curing; phellogen; reducing sugars; plant anatomy; plant histology.

INTRODUCTION

Currently, the consumption of pre-fried potatoes is increasing due to the search for foods that are easy to prepare (Pereira & Suinaga, 2015). This has led to increased yields and tuber storage for year-round supply of potatoes to processing industries (Bisognin *et al.*, 2008).

However, excoriation injury during harvesting and transport reduces storage potential by increasing water loss, respiration and the incidence of microorganisms (Silva & Pilon, 2015).

To reduce the physiological changes caused by excoriation injury, it is necessary that the tubers curing before storage. In curing, the phellogen ceases its cell division and thickening and suberization of the cell wall occurs, making the tubercles more resistant to damage(Sabba & Lulai, 2005; Lulai, 2007b). And in tubers with excoriation injury, there is the formation of a traumatic phellogen that gives rise the periderm of damage that acts in mechanical protection (Jin *et al.*, 2018).

Cell division and suberization processes are temperature sensitive, with the periderms curing rate of damage being faster at higher temperatures (Wang *et al.*, 2015), about 20 °C. However, high temperatures reduce the quality of the tubers (Zommick *et al.*, 2014).

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While lower temperatures, as 8 °C can induce the accumulation of reducing sugars that react with the amino acid asparagine, forming pigments called melanoidins, which is directly related to the browning of sticks potato during frying, due to the occurrence of the Maillard reaction (Shibao & Bastos, 2011), which is considered a serious problem in the pre-fry potato processing industry.

Thus, the choice of curing temperature depends on the cultivar used (Pereira *et al.*, 2022), and should provide adequate regeneration of the damaged periderm without compromising the quality of the tubers.

That way, this study aimed to determine the effect of the curing temperature on the regeneration of the damage periderm and on the carbohydrate metabolism of cultivar Markies and Challenger potato tubers destined for industry.

MATERIAL AND METHODS

Plant material

Markies and Challenger cultivars potato tubers (*Solanum tuberosum* L.) from the producing region of Perdizes - MG were used. The harvest was carried out 10 days after the complete drying of the aerial part of the plant. The tubers were selected and sent to the Postharvest Laboratory of the Plant Science Department at the Universidade Federal de Viçosa in Minas Gerais.

Excoriation injury tuber

The tubers were abraded with sandpaper until the periderms was removed. The control consisted of intact tubers, without damage from excoriation. Afterwards, the tubers were stored in acclimation chambers at temperatures of 8, 14 and 20 °C (RH ± 90%) for 14 days to the curing.

Fresh mass loss rate

The tuber fresh mass loss rate was evaluated by the difference between the initial fresh mass of the tubers, before entering the acclimation chambers, and the final fresh mass expressed daily until reaching a constant point, with the results expressed in %, as if follows: , where FMLR - Fresh mass loss rate (%); IFM - Initial fresh mass (g) and FFM - Final fresh mass (g).

Quantification of total soluble sugar (TSS) and reducing sugars (RS) and non-reducing sugars (NRS) content

The quantification of sugars was obtained with 5 g of fresh mass from the tubers for each sample and 80% boiling ethanol. The samples were crushed and centrifuged for 10 min at 2000 rpm, followed by filtering on paper filter. The centrifugation of the material and filtration was repeated twice more, and the final volume of the extract was equally completed in all samples.

TSS quantification was performed according to the Sulfuric Phenol method (Dubois *et al.*, 1956), using 1% sucrose solution as standard. The reaction consisted of 250 µL of the samples, 250 µL of 5% phenol and 1.25 mL of 95-97% sulfuric acid. The solution was remained in a thermostatic bath for 20 min at 30 °C. Readings were taken at 490 µm in a spectrophotometer.

The RS were determined using the dinitrosalicylic acid (DA) methodology described by Gonçalves *et al.* (2010) with modifications, using 0.2% fructose as standard. To prepare the DA reagent, 5 g of dinitrosalicylic acid dissolved in 250 mL of distilled water at 80 °C was used. Added to 100 ml of 2N NaOH and 150 g of sodium potassium tartrate 4-hydrate, the volume was completed to 500 ml with distilled water. The reaction consisted of 500 µL of the DA reagent and 500 µL of the sample followed by a thermostatic bath for 5 min and 4 mL of water. Reading was taken at 540 µm in a spectrophotometer.

The NRS content was determined by the difference between the TSS and RS contents and expressed in %.

Color classification after frying

The tubers were cut into sticks and fried in an electric fryer with a capacity of 3 L (Model: Ford®) for 3 min at 180 °C. The color of French fries was determined visually based on the color scale of the "United States Standards for Grades of Frozen French Fried Potatoes" (USDA, 1967) used by the Brazilian potato processing industry.

Damage periderm formation

Samples of 1 cm^3 were taken from the tubers areas where excoriation and from the control tubers. The samples were fixed in FAE (Formaldehyde – Acetic Acid – Ethanol) for 48 h and then kept in 70% ethanol (Ruzin, 1999). The samples were reduced to 0.25 x 0.25 x 0.25 mm and then the tissues were dehydrated in ethanol at 70, 80, and 95% for 2 h each, and embedded in Epon resin plus ethanol (1:1), for one week, then pure Epon at 60 °C, for 48 h. After the resin had hardened, with the aid of a Leica microtome, Spencer model, cuts were made in cross sections measuring 5µm thick. Slides mounted with synthetic resin were

stained in 0.05% Toluidine Blue, 0.1 M sodium phosphate buffer pH 6.5 (O'Brien *et al.*, 1964) and 1% Neutral Red and sodium buffer 0.1 M pH 6.5 (Kirk, 1970). The samples were examined microscopically to determine the structural characteristics of the cell layers of the intact tubers and after curing.

Statistical analysis

A completely randomized design in split plots was used, with the plot at temperatures of 8, 14 and 20 ºC, and the subplot the control (no excoriation) and tubers with excoriation injury, with each treatment consisting of 8 replications, consisting of two tubers. Data were subjected to analysis of variance and regression analysis was performed for FMLR and Tukey's test ($p \geq 0.05$) for TSS, RS and NRS using the Statistical and Genetic Analysis System (UFV, 2007).

RESULTS AND DISCUSSION

Fresh mass loss rate

The FMLR did not vary over the 14 days of curing in the tubers at 8 and 20 °C, but the FMLR was higher in the excoriation tubers (Figure 1), indicating that the periderm acts as a barrier to water loss and that the damage increases respiration. At 8 °C, the FMLR of damaged tubers was 3.5 and 3.65 times greater than that of the control, for Markies and Challenger cultivars, respectively (Figure 1A and 1B). While at 20 °C, FMLR was 2.15 and 1.9 times greater than the loss of control, for cultivars Markies and Challenger, respectively (Figure 1E and 1F). The difference in FMLR between the tubers with excoriation damage and the control was smaller at 20 °C, indicating that higher temperatures accelerate water loss and respiration, regardless of the occurrence of mechanical damage.

In curing at 14 °C, initially the loss water from the injured tissue was high because evaporation is higher than that of intact tissue. This leads to a constant decrease in fresh mass in potato tubers with increasing curing time (Bacarin *et al.*, 2005; Freitas *et al.*, 2006)Pérola, Asterix e C-1786-6-94, armazenados nas temperaturas de 4; 12 e 20ºC, por 30 e 60 dias e, após 30 dias de armazenamento, recondicionados. Antes de cada coleta, os tubérculos foram processados e divididos em sub amostras e logo depois liofilizados. Do material liofilizado determinaram-se os teores de carboidratos solúveis totais e de açúcares redutores. Antes do armazenamento, o clone C-1786-6-94 apresentava a maior quantidade de carboidratos solúveis totais. Quando armazenados por 30 dias a 4ºC, todos os genótipos aumentaram os teores de carboidratos solúveis totais e açúcares redutores, havendo diferenças nas taxas de incremento. O armazenamento por mais 30 dias a 4ºC provocou respostas distintas entre os genótipos. O armazenamento a 12ºC por um período de 30 dias induziu aumento nos teores de açúcares; aos 60 dias de armazenamento houve uma tendência a diminuir açúcares redutores. No recondicionamento de 4 para 20ºC houve redução acentuada nos teores de açúcares redutores e carboidratos solúveis totais. O recondicionamento de 12 para 20ºC teve efeito expressivo na redução dos teores de açúcares redutores e carboidratos solúveis totais. No armazenamento a 20ºC pequenas flutuações ocasionais foram percebidas nos teores de açúcares com tendência a diminuir ao longo do armazenamento.The levels of total soluble carbohydrates and reducing sugars were quantified in tubers of potato genotypes (Atlantic, Pérola, Asterix and C-1786-6-94, reaching a minimum at 10.8 days for the Markies cultivar (Figure 1C) and 10.46 days for the Challenger cultivar (Figure 1D). This reduction is relation with the maturation of the periderm, in which suberization occurs (Daniels-Lake *et al.*, 2014)and simulated harvest injury on the loss of fresh weight (FW, indication that this period occurred adequate formation the damage periderm, what not is observed at 8 and 20 °C temperature (Figura 3B, 3D e 3F e Figura 4B, 4D e 4F).

At 20 °C, the 14 day curing was not enough to promote the regeneration of the damage periderm (Figure 1E and 1F), different from what was expected, as cell division and suberization are temperature sensitive, with a higher rate of damage curing at higher temperatures (Khanal & Uprety, 2014). According to these authors, the curing process of potatoes stored at 5 °C is three times slower than at 10 °C, which is also three times slower than at 20 °C.

In the Innovator cultivar, temperatures between 14/20 °C favored the formation of damage periderm (Pereira *et al.*, 2022). Indicating that the effect of curing temperature varies between cultivars.

Total soluble sugar (TSS), reducing sugars (RS) and non-reducing sugars (NRS) and post-frying color classification

In tubers with damage by excoriation of the Markies cultivar, the temperature of 8 °C increased the levels of TSS, RS and NRS, while in the 'Challenger' there was an increase only in TSS (Table 1). The temperature of 8 °C

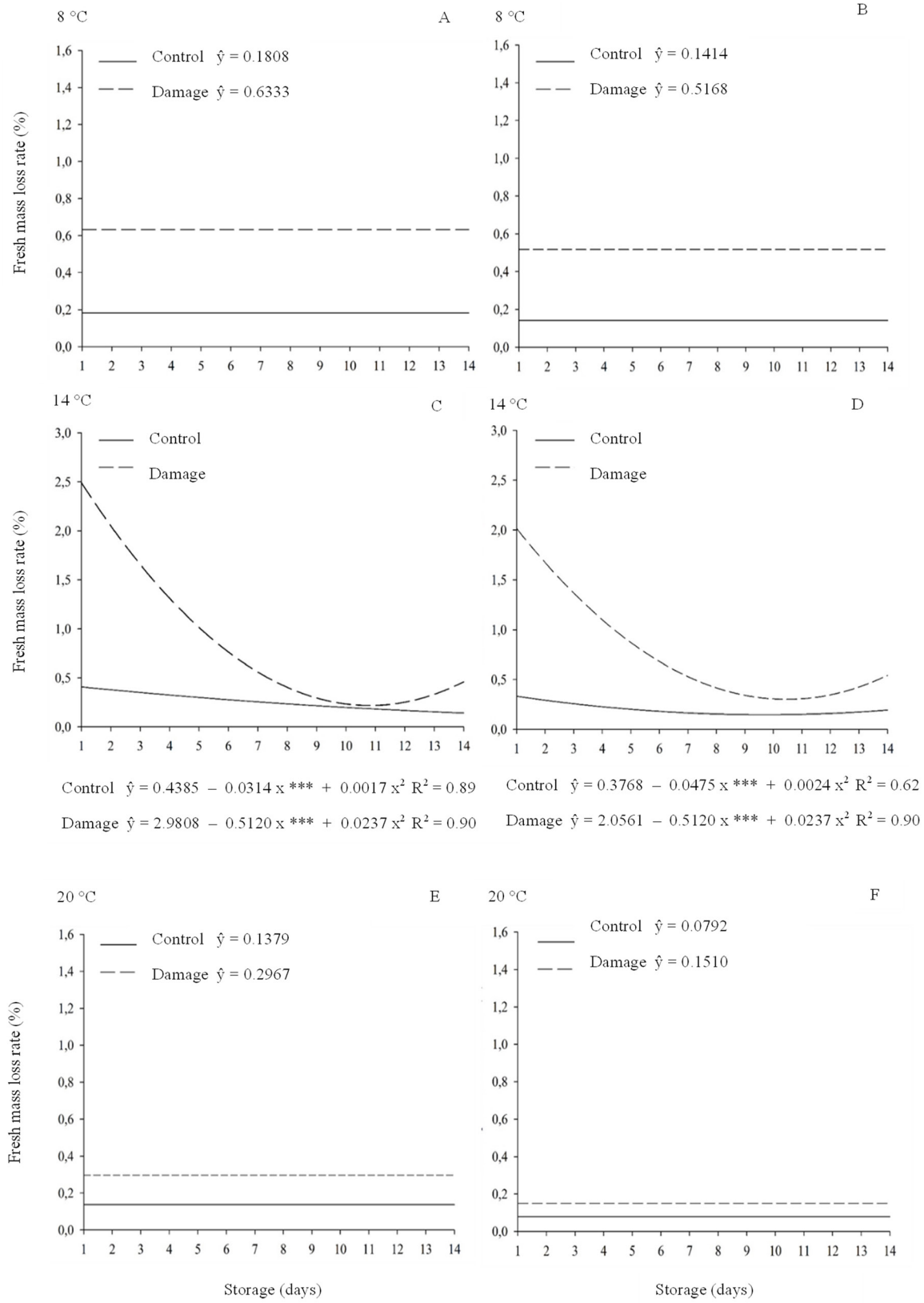

Figure 1: Fresh mass loss rate (FMLP) (%) of potato tubers from Markies (A, C, E) and Challenger (B, D, F) cultivars evaluated daily for 14 days at 8, 14 and 20 °C, RH ± 90%.

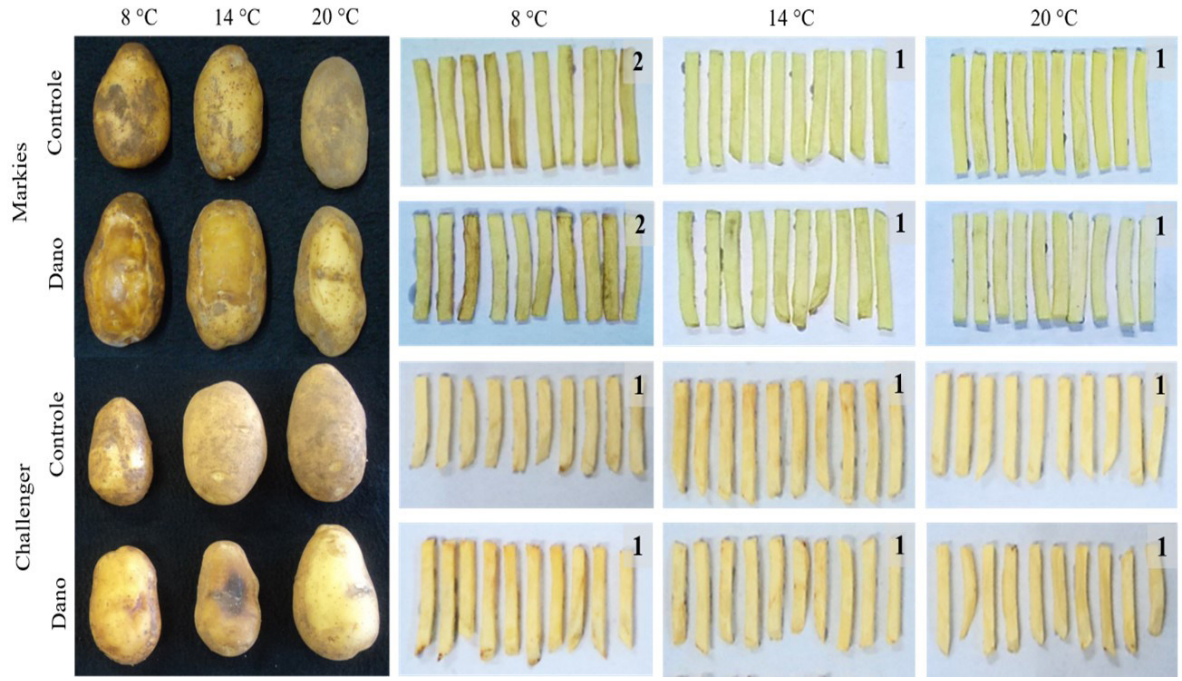

Figure 2: Visual appearance from Markies and Challenger cultivars potato tubers and potato sticks after frying at 180 °C for 3 min and 14 days of curing at 8, 14 and 20 °C (RH ± 90%).

induced the accumulation of sugars in these cultivars, which is a mechanism of resistance to the cold of potato tubers (Bervald *et al.*, 2010), in addition, low temperatures promote reduced respiration, leading to lower consumption of TSS.

In both cultivars, the tubers with excoriation damage at 8 °C had sugar contents above 0.2%, which is considered a high value (Pádua *et al.*, 2012). However, in the 'Challenger', the elevation only of the TSS did not cause the browning during frying, with the sticks classified in category 1, while the increase in RS contents in the Markies cultivar promoted the browning of the sticks during frying, being classified in category 2 (Figure 2).

The browning occurs due to the Maillard reaction, where the condensation of the RS carbonyl group with the amino group free of amino acids, peptides or proteins, forming the Schiff base which undergoes rearrangements producing Amadori (aldose sugar) or Heyns (ketosis sugar) which it undergoes dehydration, enolization and retroaldolization, forming dicarbonyls, reductones and Strecker degradation products. These compounds polymerize with lysine or arginine residues into proteins, resulting in dark pigments known as melanoidins (Francisquini *et al.*, 2017).

Category 1 and 2 are considered suitable for the processing industry, with a small concentration of melanoidins. The low occurrence of the Mailard reaction in the Markies and Challenger cultivars is an important aspect of visual quality and food safety, because in a lateral reaction, the Maillard reaction causes the formation of acrylamide, a carcinogen product (Pelucchi *et al.*, 2011)but data on humans are inconclusive. We thus carried out a critical review and meta-analysis of studies of exposure to acrylamide and cancer. Methods: We identified 586 publications, 25 presented relevant results. We conducted meta-analyses of studies of dietary intake based on random-effects models by calculating pooled relative risks (RR.

Damage periderm formation

The periderm of potato tubers of the Markies (Figure 3) and Challenger cultivars (Figure 4) is constituted by phellogen and only a few cork layers, with no evident phelloderm.

In the tubers with excoriation injury, there were conspicuous anatomical changes at all curing temperatures, with induction of the cell division process in an organized manner, mainly in the periclinal plane, forming a band similar to phellogen formed from the dedifferentiation of corti-

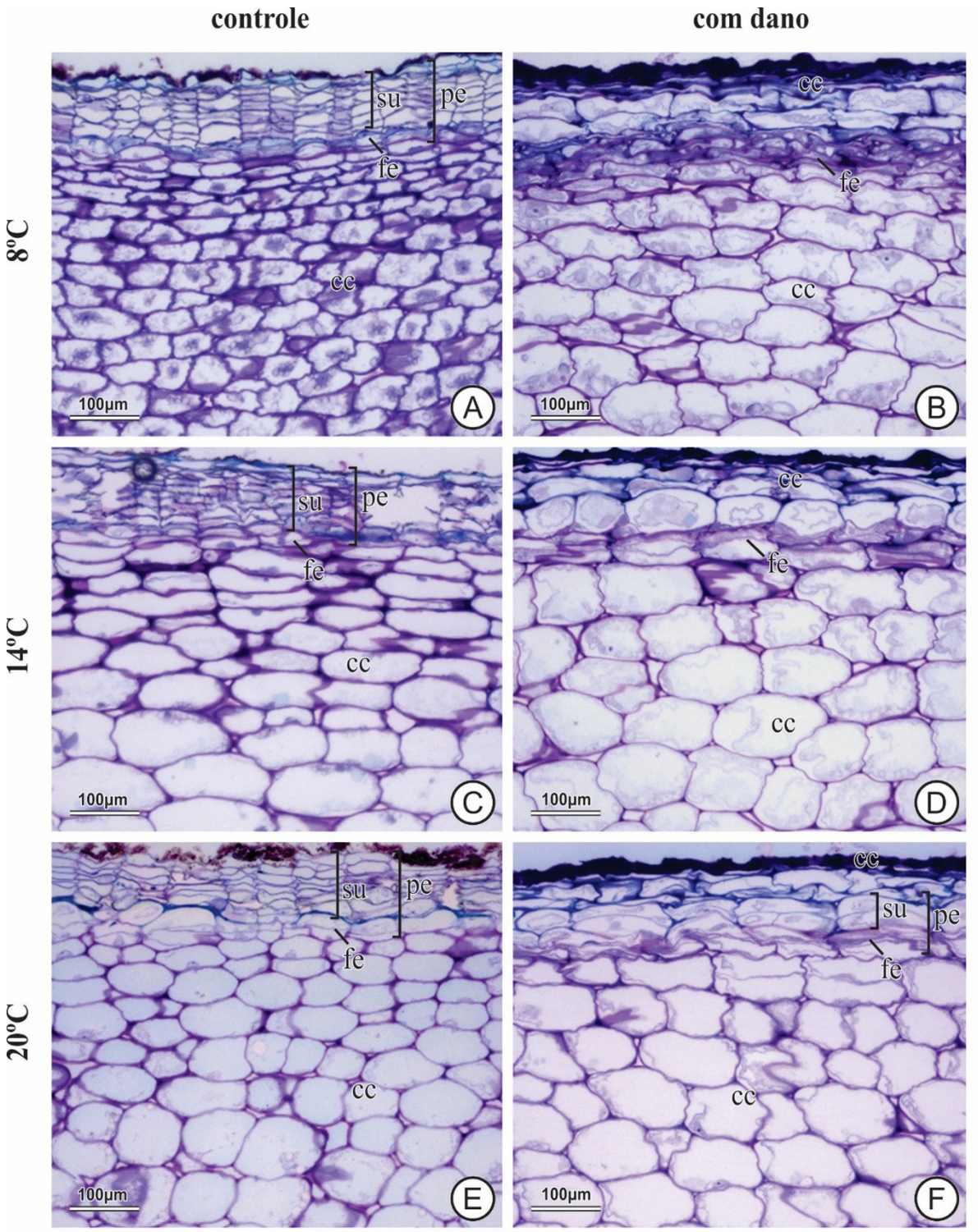

Figure 3: Markies cultivar potato tubers stored for 14 days at 8 (A-B), 14 (C-D) and 20 °C (E-F), without (A, C, E) and with (B, D, F) excoriation periderms. The tubers surface is oriented towards the top of each micrograph. Cross sections stained with Toluidian blue. cc, cortical cells; fe, phellogen; eg, periderm; su, cork.

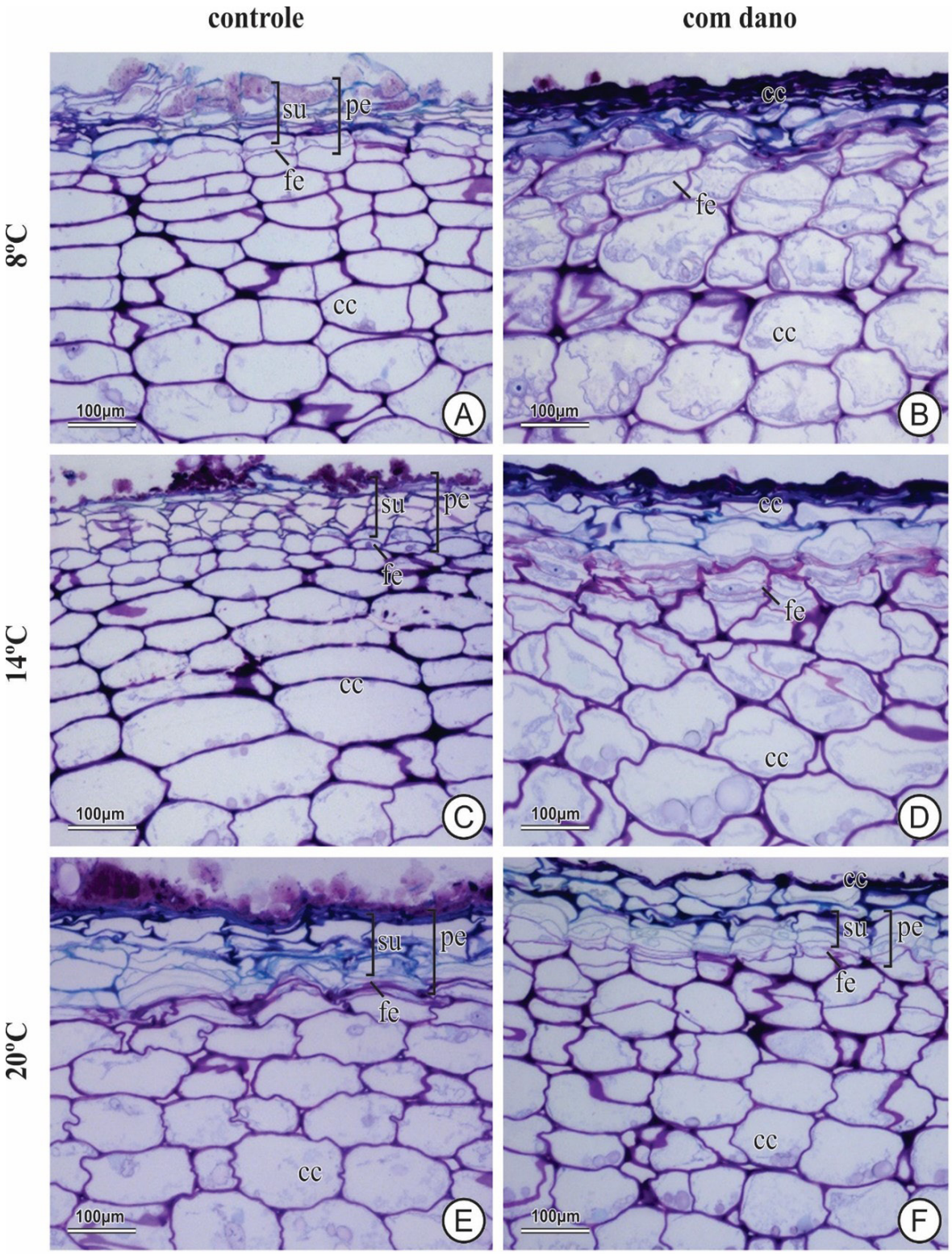

Figure 4: Challenger cultivar potato tubers stored for 14 days at 8 (A-B), 14 (C-D) and 20 °C (E-F), without (A, C, E) and with (B, D, F) excoriation damage. The tubers surface is oriented towards the top of each micrograph. Cross sections stained with Toluidian blue. cc, cortical cells; fe, phellogen; eg, periderm; su, cork.

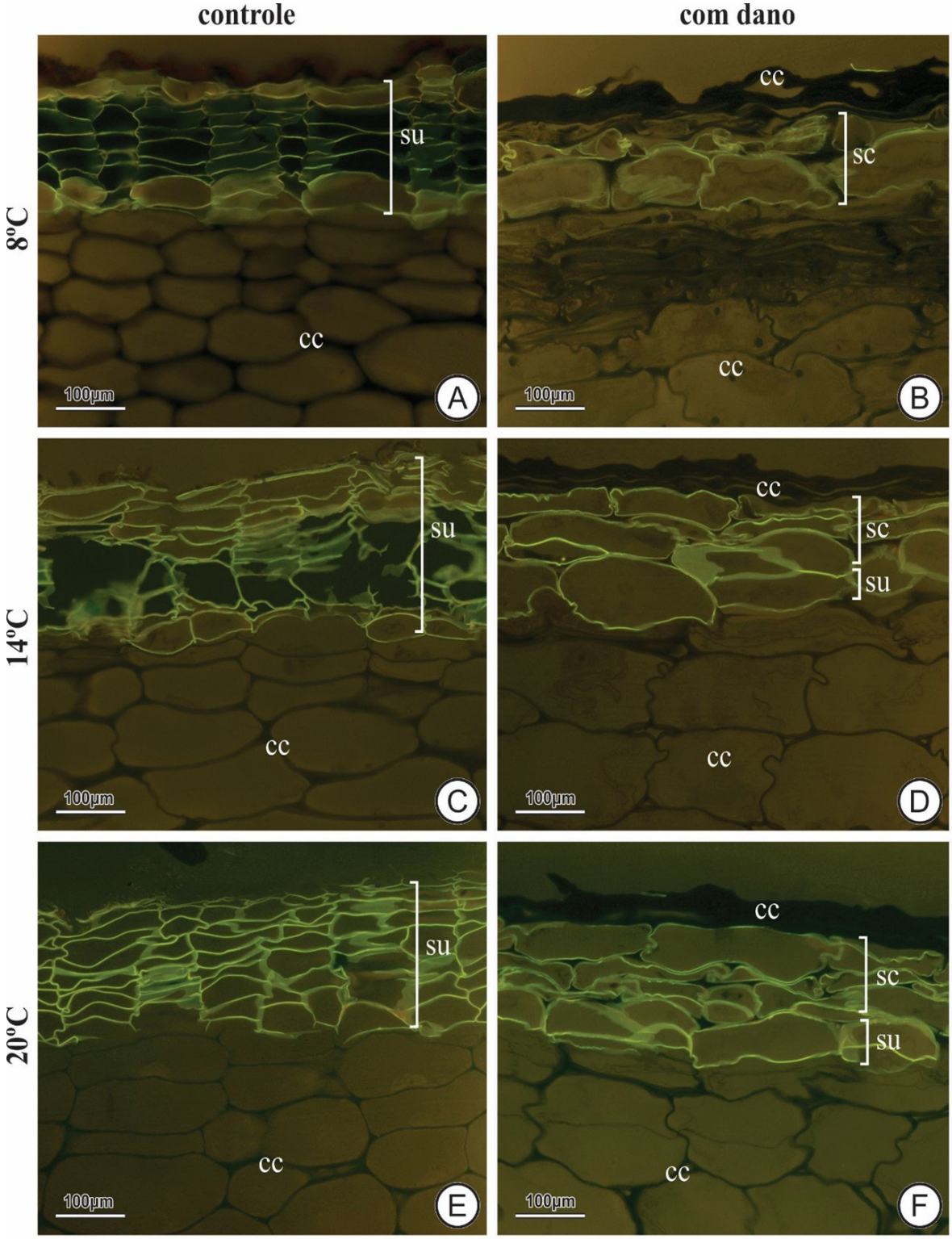

Figure 5: Markies cultivar potato tubers stored for 14 days at 8 (A-B), 14 (C-D) and 20 °C (E-F), without (A, C, E) and with (B, D, F) excoriation damage. The tubers surface is oriented towards the top of each micrograph. Cross sections stained with neutral red under UV light. Yellow-green secondary fluorescence indicates lipids (suberin). cc, cortical cells; sc, suberified cells; su, cork.

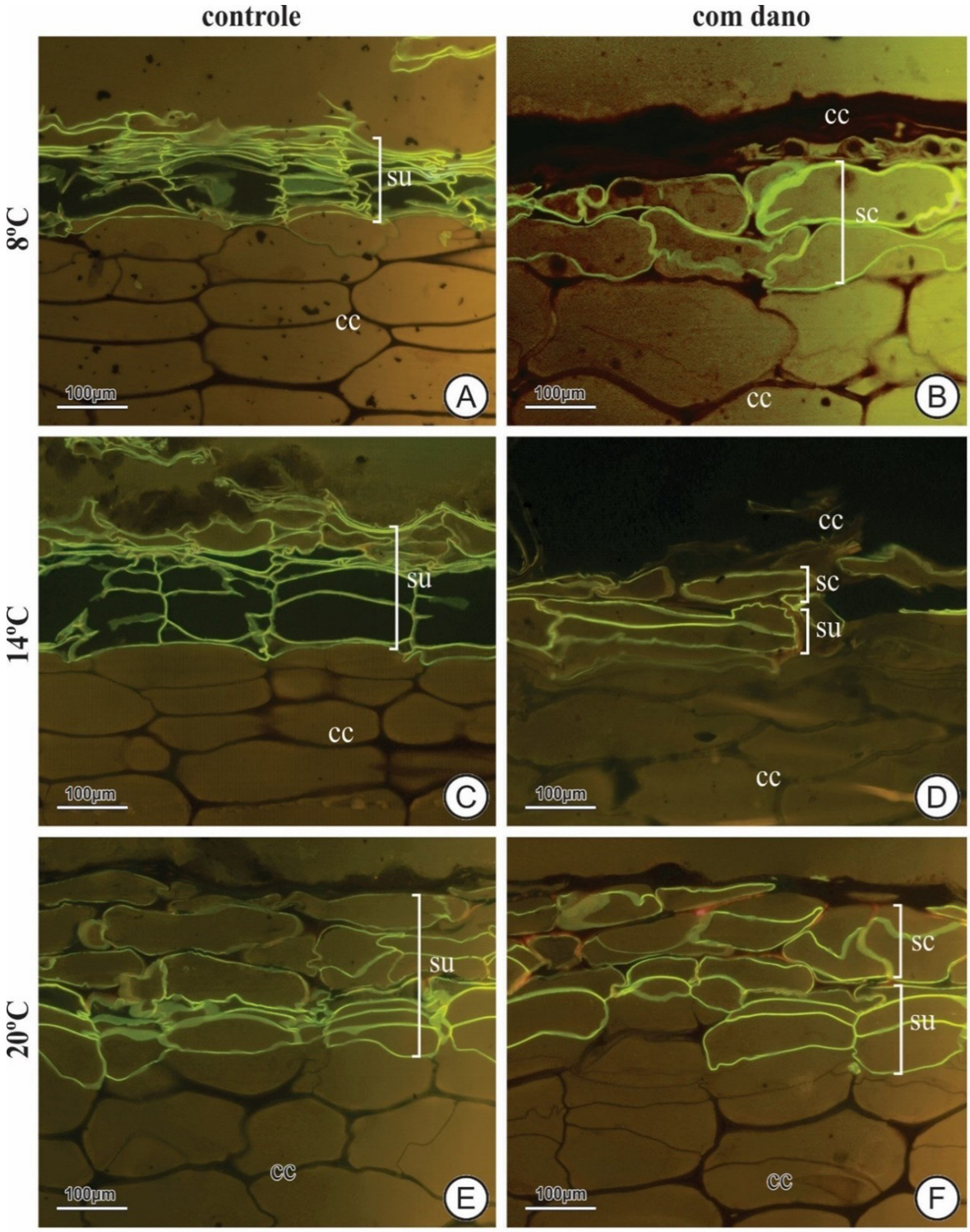

Figure 6: Markies cultivar potato tubers stored for 14 days at 8 (A-B), 14 (C-D) and 20 °C (E-F), without (A, C, E) and with (B, D, F) excoriation damage. The tubers surface is oriented towards the top of each micrograph. Cross sections stained with neutral red under UV light. Yellow-green secondary fluorescence indicates lipids (suberin). cc, cortical cells; sc, suberified cells; su, cork.

Table 1: Mean values of total soluble sugar (TSS), reducing sugar (RS) and non-reducing sugar (NRS) for the respective combinations of temperature and control treatment and tubers with damage by excoriation of Markies and Challenger cultivars after 14 days of curing at 8, 14 and 20 °C, RH ± 90%

		TSS (%)		RS (%)		NRS (%)	
		Control	Damage	Control	Damage	Control	Damage
Markies	8 °C	0.4084 Ba	0.6211 Aa	0.1489 Ba	0.2142 Aa	0.2595 Ba	0.4070 Aa
	14 °C	0.1197 Ab	0.1792 Ab	0.0307 Ab	0.0324 Ab	0.0890 Ab	0.1468 Ab
	20 °C	0.1488 Ab	0.1488 Ab	0.0362 Ab	0.0352 Ab	0.1126 Ab	0.1125 Ab
Challenger	8 °C	0.2095 Ba	0.3092 Aa	0.0798 Aa	0.1139 Aa	0.1297 Aa	0.1953 Aa
	14 °C	0.1220 Ba	0.2057 Ab	0.0362 Aa	0.0603 Ab	0.0858 Aa	0.1454 Aab
	20 °C	0.1383 Aa	0.1206 Ab	0.0635 Aa	0.0388 Ab	0.0748 Aa	0.0818 Ab

The means followed by the same uppercase letter in the row and lowercase letter in the column for each variable do not differ at 5% probability by Tukey's Test.

cal cells tubers, located a few layers below the excoriation boundary (Figure 3B, 3D and 3F and Figure 4B, 4D and 4F). The most superficial cortical cells became collapsed and only one or two layers of cortical cells remained above this new phellogen. At 14 °C, the installation of this new phellogen in both cultivars becomes more evident (Figure 3D and Figure 4D), and at 20 °C some layers of a new cork were formed (Figure 3F and Figure 4F).

The only difference between cultivars is that in Markies cultivar the development of new periderm is earlier than in Challenger cultivar, even at 8 °C (Figure 3B and Figure 4B), as the number of cell layers depends on the genotype, conditions of the tubercle's environment and growth stage (Pringle *et al.,* 2009).

The storage time of only 14 days provided the formation of cork layers, suggesting that as the storage time goes by, the number of cork layers can be increased and, consequently, the mechanical strength of this periderm. The number of cork and phellogen cell layers was generally greater in the native periderm than in the damage periderm (Figure 3 and 4), similar to that found by Sabba & Lulai (2002).

In periderm fluorescence microscopy, using neutral red under UV light (Figure 5 and 6) for both cultivars the yellow-green color indicates the presence of lipids (suberin). According to Lulai (2007a), the tuber cell wall of the native periderms has a waxy component that protects against cell desiccation, protecting against bacterial and fungal infections. These waxes are integrated into the suberized cell wall and provide a reduction in water loss associated with cell death by desiccation. These same authors claim that rapid accumulation of wax on cell walls at the wound site is essential to prevent cell desiccation and death.

The visual appearance of the tubers after the excoriation demonstrates the apparent regeneration of the damaged periderms (Figure 2). According to (Lulai, 2007a), the color of the natural surface of the excoriated areas is not restored after curing, as verified in the present study (Figure 2).

CONCLUSIONS

The temperature of 14 ºC provided better curing and regeneration of the excoriation periderm tubers maintain post-fry quality of potatoes destination by industry processing.

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