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Conditioning of sweet potato roots at low temperature, carbohydrate metabolism and chips quality

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ABSTRACT: This study aimed to investigate the impact of low-temperature conditioning on carbohydrate metabolism and the quality of fried sweet potato chips. The experiment was conducted in a completely randomized design, in a split-plot scheme. The parcel was constituted by cultivars. sweet potato (BRS Cuia and BRS Rubissol) and storage conditions (6 °C, 13 °C and conditioning), while the subplot consisted of storage periods (0, 15, 30, 45, and 60 days). Six replications were used, each repetition consisting of two roots. Fresh weight loss, total soluble sugars, reducing sugars, non-reducing sugars, alcohol-insoluble solids, and chips color were evaluated. Conditioning combined with storage at 6 ºC led to a higher accumulation of reducing and non-reducing sugars, as well as intensified chips non-enzymatic darkening. This study demonstrated that conditioning was not effective in reducing cold-induced sweetening in Brazilian sweet potato cultivars.

Key words: chilling injury; *Ipomoea batatas*; storage; sugars

Condicionamento de raízes de batata-doce em baixa temperatura, metabolismo de carboidratos e qualidade de chips

RESUMO: Este estudo teve como objetivo investigar o impacto do condicionamento em baixa temperatura no metabolismo de carboidratos e na qualidade de chips de batata-doce frita. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema de parcelas subdivididas. A parcela foi constituída pelas cultivares de batata-doce (BRS Cuia e BRS Rubissol) e condições de armazenamento (6 °C, 13 °C e condicionamento), enquanto a subparcela foi composta por períodos de armazenamento (0, 15, 30, 45 e 60 dias). Foram utilizadas seis repetições, cada repetição consistindo de duas raízes. Foram avaliados a perda de massa fresca, açúcares solúveis totais, açúcares redutores, açúcares não redutores, sólidos insolúveis em álcool e cor dos chips. O condicionamento combinado com o armazenamento a 6 ºC levou ao maior acúmulo de açúcares redutores e não redutores, além de intensificar o escurecimento não enzimático dos chips. Este estudo demonstrou que o condicionamento não foi eficaz na redução da edulcoração induzida pelo frio em cultivares brasileiras de batata-doce.

Palavras-chave: lesão por frio; *Ipomoea batatas*; armazenamento; açúcares

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Introduction

Finding out the suitable storage condition is one of the key steps to preserve overall quality of sweet potato roots along their supply chain and management (Ji et al., 2017). Use of low temperatures has been efficient in reducing metabolism activity, increasing sweet potato roots shelf life. At 13 °C, sprouting incidence and root deterioration are delayed, providing a continuous processing industry supplying (Wolfe, 1992). However, exposure of sweet potato roots to temperatures lower than 13 °C, may induce the occurrence of chilling injury and, consequently, a cold-induced sweetening (CIS). CIS is a physiological disorder, causes an increase in reducing sugars (Sakamoto et al., 2014; Ji et al., 2017; Araújo et al., 2020). The processing of roots affected by CIS at elevated temperature favours the reaction of reducing sugars with free amino acids via non-enzymatic Maillard reaction, forming acrylamide. In addition to formation of this harmful carcinogenic agent to humans, there is also the development of darkened compounds (melanoidins) that affect the quality and color of sweet potato chips (McKenzie et al., 2013; Kumar et al., 2018; Araújo et al., 2020).

Use of conditioning is one of the strategies to reduce chilling injury and cold-induced sweetening. This method consists in storing the roots under mild temperatures prior to storage at lower temperatures, triggering higher tissue resistance to the cold (Li et al., 2018). Its effectiveness is proven in different fruits, such as mango, pear, and peach, in which the accumulation of proline and reduced membrane lipid peroxidation appear to be associated with cold stress tolerance. However, conditioning efficiency depends on the combination of temperature, exposure time, and by the species and cultivars studied (Li et al., 2017; Wang et al., 2017; Zhang et al., 2017a).

The effectiveness of conditioning in increasing tolerance to cold injury in fruits has been widely reported in the literature; however, little is known about its effects on the sweetening of tuberous roots and its influence on the accumulation of sugars. Li et al. (2018), for instance, studied the effects of conditioning at low temperatures on sweet potato roots and demonstrated that the conditioned and cold-stored roots presented higher levels of sugar during storage. However, the use of a single one cultivar ("Xinxiang") restricts it to extrapolate desirable and undesirable effects of conditioning to Brazilian cultivars.

In Brazil, although local clones of sweet potato is still the major genetic material cultivated, practices of cultivation have expanded with improved varieties (Lima et al., 2019; Araújo et al., 2020) with high yield potentials, such as BRS Rubissol and BRS Cuia. Lastly, these two Brazilian cultivars have been described as sensitive to cold, being rapidly affected by CIS at 6 °C (Araújo et al., 2020). Since the use of refrigerated storage as well as demand for fried sweet potato products has increased, it seems reasonable to evaluate the use of techniques that seek to mitigate cold-induced negative responses. This study investigated the effect of conditioning on carbohydrate metabolism in sweet potato roots and its influence on the quality of fried chips.

Materials and Methods

This study was performed at the Experimental Facilities of the Department of Crop Science, Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil (20º45'20''S and 42°52'40'' W, 651 m altitude), from September 2017 to February 2018 timeframe, namely as spring crop season.

Seedlings of sweet potato cv. BRS Cuia and BRS Rubissol (Frutplan LTDA) were set out in a spacing of 1.0 m (between ridges) × 0.4 m (between plants). The cultivation ridges were arranged in 3.0 m long by 0.30 m high. Soil management was performed conventionally by using disc plow and harrow. The fertilization was carried out according to the soil chemical analysis and technical recommendations as follows: liming for planting with 100 g m^{-2} of limestone; planting fertilization with 100 g m^{-2} of NPK 8-28-16; growth fertilization with 50 g m^{-2} of NPK 8-28-16 every 30 days. The irrigation was carried out by a sprinkler system under continuous activity.

The harvest was performed at 130 days after planting when roots presenting 300 to 600 g - free of diseases and damage - were selected and transported in plastic boxes. At the laboratory, the roots were submitted to the curing process according to described by Amoah et al. (2016), by storing them under 30ºC and relative humidity (RH) 90%, for 7 days in manufactured BOD incubator (Thermolab Scientific Equipments). After the curing process, roots were stored at 6 °C, 13 °C (control) or conditioned at 10 °C for 7 days, followed by storage at 6 °C and 92% RH.

For each treatment, samples were collected in five periods: 0, 15, 30, 45, and 60 days of storage and evaluated for fresh weight loss, total soluble sugars, reducing sugars, non-reducing sugars, alcohol-insoluble solids, and chips color were assessed.

During storage, sweet potato roots were weighed, and the results were expressed as a percentage of fresh weight loss (Equation 1).

$$\mathrm{FWL} = \frac{\mathrm{W_f} - \mathrm{W_0}}{\mathrm{W_0}} \cdot 100 \qquad (1)$$

where: FWL = loss of fresh mass (%); W_f = final fresh matter weight (g); and W_0 = initial fresh matter weight (g). Approximately 5 g of fresh pulp samples were macerated and homogenized in 80% ethanol heated to 85 °C. Subsequently, the extract was centrifuged at 13,000 g for 10 min to separate the supernatant. This step was repeated twice with 80% ethanol.

Total soluble sugars (TSS) were quantified by the phenol-sulfuric method (Dubois et al., 1956). It was collected 0.25 ml of the supernatant, 0.25 ml of 5% phenol solution, and 1.25 ml of sulfuric acid (H_2SO_4). The mixer was then incubated at 30 °C for 20 min. After cooling, the samples were read at 490 nm.

The observed optical density was fitted in a standard curve of sucrose (0-50 μg) and the TSS content was expressed as %TSS on a fresh weight basis.

Reducing sugars (RS) were quantified by dinitrosalicylic acid (DNS) method (Gonçalves et al., 2010). A 0.5 mL aliquot of supernatant and 0.5 mL DNS were added to tubes and incubated in a water bath until boiling. After cooling, 4 mL of water was added to the tubes, being followed by reading at 540 nm. The RS content was expressed in %RS, on a fresh weight basis, assessed by a standard curve of fructose (0-1.0 mg). NRS content was determined by the difference between TSS and RS, being expressed in % NRS on a fresh weight basis.

The alcohol-insoluble solids (AIS), jointly represented by proteins, crude fiber and mainly starch, were determined as described by Bonte et al. (2000). After the extraction of alcohol-soluble sugars, the insoluble residue (pellet) was dried at 65 °C in an oven for 72 h. The dried samples were weighed, and the result was expressed in %AIS on a fresh weight basis. To assess the color of the chips, sweet potato roots were peeled, cleaned, sliced into chips and fried in refined soybean oil for 2 minutes at 180 °C (Caetano et al., 2018) in a monitored fryer (Ford, Michigan, USA). Chips were classified into color patterns according to Véras et al. (2021), in which: 1, chips with a lighter surface; 2, chips with slightly darkened edges; 3, chips with more than 50% blackened surface; and 4, chips with more than 75% darkened surface (Figure 1).

The experiment was conducted in a completely randomized design, in a split-plot scheme. The parcel was constituted by cultivars sweet potato (BRS Cuia and BRS Rubissol) and storage conditions (6 °C, 13 °C and conditioning), while the subplot consisted of storage periods (0, 15, 30, 45, and 60 days). Six replications were used, considering two roots as an experimental unit.

The data were examined by analysis of variance using SAS software (Cody, 2015), with the means compared by Tukey test, at 5% probability. The data were submitted to descriptive statistics of means with error deviation. The plots were accomplished by using software Sigma Plot 10.0. Pearson's correlations between variables were performed using the R 'corrplot' package (Wei & Simko, 2017).

Figure 1. Color standards for analysis of fried sweet potato chips.

Results and Discussion

Regardless of temperatures of storage, there was a linear increase in fresh weight loss along the storage period. After 60 days at storage, cv. BRS Cuia roots previously conditioned presented a fresh weight loss of 1.6 and 2.0 times lower than observed in roots at 6 and 13 °C, respectively (Figure 2A). On the other hand, fresh weight loss observed in cv. BRS Rubissol under conditioning combined with storage at 6 °C was similar to each other, however, 1.25 times lower than from obtained at 13 °C at the end of storage (Figure 2B). The highest daily percentages of fresh weight loss were 0.72% for cv. BRS Cuia, and 0.52% for cv. BRS Rubissol stored at 13 °C, which can be associated with increased respiratory activity and transpiration (Madonna et al., 2018).

In both cultivars, there was a linear increase in the percentage of total soluble sugars (TSS) during storage for all treatments. After 60 days of storage, the percentages of TSS in cv. BRS Cuia were similar in the roots stored at 6 and 13 °C; however, TSS were lower when compared to the roots previously conditioned (Figure 3A). For cultivar BRS Rubissol, significant difference was observed, with conditioned roots presenting a higher percentage of TSS (Figure 3B). These results demonstrate that both cultivars are very susceptible to cold-induced sweetening, being under close agreement with reported by Araújo et al. (2020), which evidenned that both cultivars develop physiological disturbances caused by cold, such as CIS and chilling injury, when subjected to 6 °C.

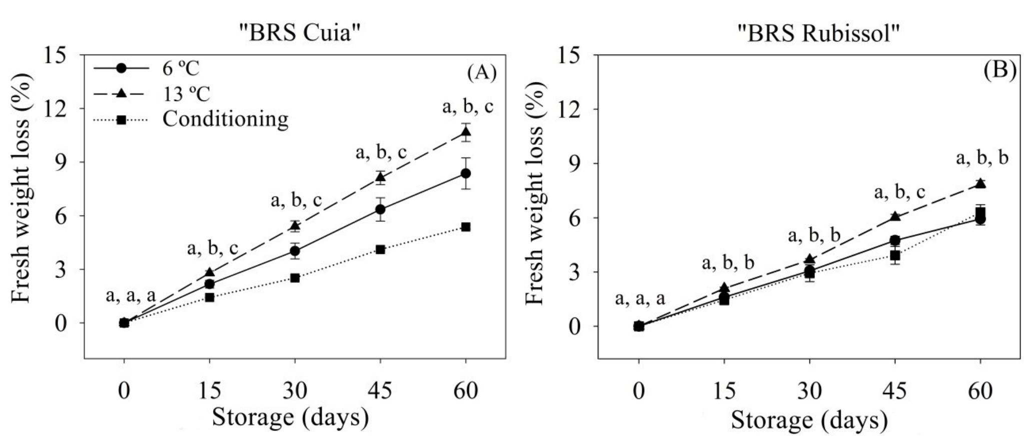

Figure 2. Fresh weight loss in sweet potato roots cv. BRS Cuia (A) and BRS Rubissol (B) submitted to conditioning and different temperatures during storage. The vertical bars represent the mean standard error (n = 6). Storage treatments followed by the same lowercase letter do not differ by Tukey's test at 5% probability.

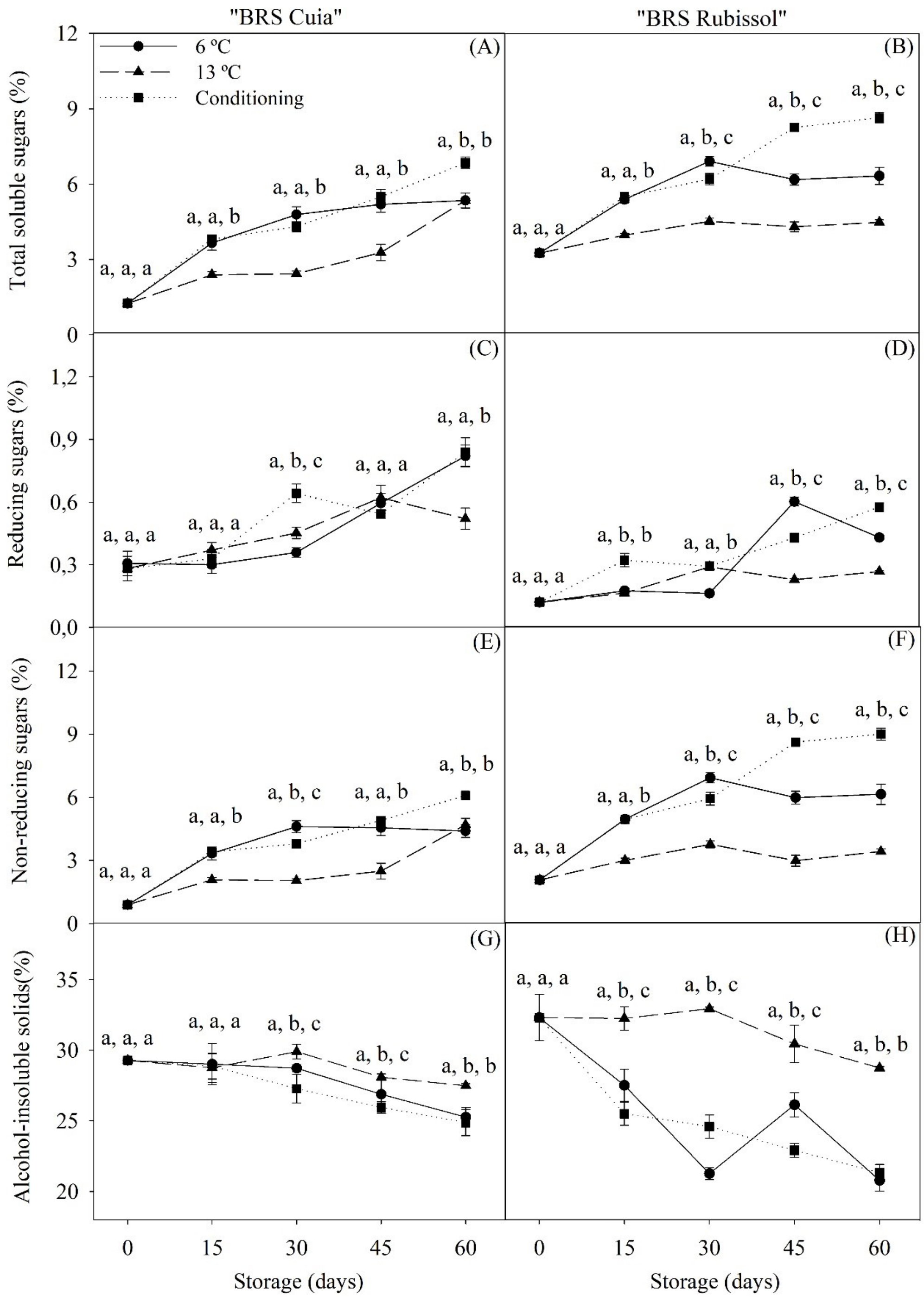

Figure 3. Total soluble sugars, reducing sugars, non-reducing sugars, and alcohol-insoluble solids in sweet potato roots cultivars BRS Cuia (A, C, E, and G) and BRS Rubissol (B, D, F, and H) submitted to conditioning and different temperatures during storage. The vertical bars represent the mean standard error (n = 6).Storage treatments followed by the same lowercase letter do not differ by Tukey's test at 5% probability.

The imbalance between sugar biosynthesis and starch degradation is one of the key events associated with cold-induced sweetening. It is likely that starch hydrolysis - as a direct response of amylolytic enzymes to the cold – is the primary cause of higher TSS accumulation in conditioned and stored sweet potato roots at 6 °C (Zhang et al., 2017b; Li et al., 2018). The increase in sugar content was also reported by Li et al. (2018) in study with sweet potato roots cultivar Xinxiang conditioned at 4 °C.

The percentage of reducing sugars (RS) increased over the storage period in both cultivars. At 60th day, the roots of cultivar BRS Cuia at 6 °C and previously conditioned showed the highest percentages of RS, with an increase of 275 and 300%, respectively (Figure 3C). Similarly, cultivar BRS Rubissol also accumulated more RS in the conditioned and storage roots at 6 °C and, with an increase of 2.143,9 and 7.000%, respectively, during the 60 days of storage (Figure 3D). Possibly, the highest accumulation of RS in the conditioned and stored roots at

6 °C was due to increased activity of enzymes that degrade sucrose, especially the soluble acid invertase. According to Araújo et al. (2020), soluble acid invertase is the main limiting enzyme concerning reducing sugars accumulation rate in sweet potato roots. Therefore, these results corroborate the hypothesis that the practice of conditioning is not effective in alleviating cold stress for these Brazilian studied cultivars.

Conditioned and stored roots of both cultivars displayed the highest percentages of non-reducing sugars (NRS) during storage. BRS Cuia presented a value of 6.08% in NRS on 60th day. In the same storage period, the roots of cultivar BRS Cuia at 6 °C and 13 °C did not differ in the percentage of NRS, with 4.39% and 4.69%, respectively (Figure 3E). The roots of cultivar BRS Rubissol from treatments 6 °C and conditioned also showed NRS accumulation during storage, which values reached the percentages of 5.25 and 7.67% of NRS, respectively, at 60 days. These values were up to 2.6 times higher than the percentage obtained in the roots stored at 13 °C for 60 days (Figure 3F).

Likely, the accumulation of NRS in sweet potato roots under low temperatures can be attributed to low tolerance to cold (Araújo et al., 2020). This evidence indicates that RS is transported to the cytosol and converted to NRS (Krause et al., 1998; Li et al., 2018), via sucrose phosphate synthase. Interestingly, NRS levels in cultivar BRS Cuia previously conditioned at 6 °C were 7.25 and 5.35 times (Figure 3E) higher as compared to RS content (Figure 3C). For cultivar BRS Rubissol, the percentages of NRS were 17.91 and 17.92 times (Figure 3F) higher than RS (Figure 3D).

For alcohol insoluble solids (AIS), there was a significant difference between treatments after 30 days of storage. For cv. BRS Cuia, a reduction in AIS levels in roots conditioned and stored at 6 °C was observed, indicating that the reduction in alcohol insoluble solids may have occurred due to starch degradation and its conversion into soluble sugars (Figure 3G). For cv. BRS Rubissol, the behavior was similar, in which the conditioned and stored roots at 6 °C showed a marked degradation during storage. For both cultivars, roots stored at 13 °C presented the highest percentage, displaying a higher starch content at the end of the evaluation period (Figure 3H).

The AIS represents a way of estimating the amount of apparent amide in sweet potato, because AIS contains mainly starch (Bonte et al., 2000; Sato et al., 2017). It seems that the reduction in AIS contents in conditioned and stored roots at 6 °C may have occurred due to starch degradation, probably via hydrolytic or phosphorolytic in hexose phosphates, glucose or maltose.

Regardless of cultivars, fried chips color was similar at the beginning of storage. For both cultivars, the chips showed a darker color in storage at 6 °C and conditioning treatment after 60 days of storage (Figure 4 and 5). These results indicate that the conditioning of both cultivars roots also decreased the processed product's overall quality, which was not observed in sweet potato chips stored at 13 °C. A different answer was reported by Araújo et al. (2020), which evidenced that darkening degree of cultivar BRS Rubissol increases over the storage period, but did not differ between temperatures of 6 and 13 °C.

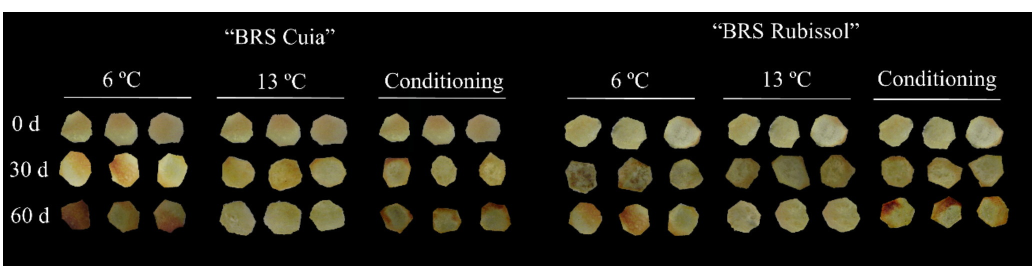

Figure 4. Color visual analysis of sweet potato chips cultivars BRS Cuia and BRS Rubissol submitted to different temperatures during storage.

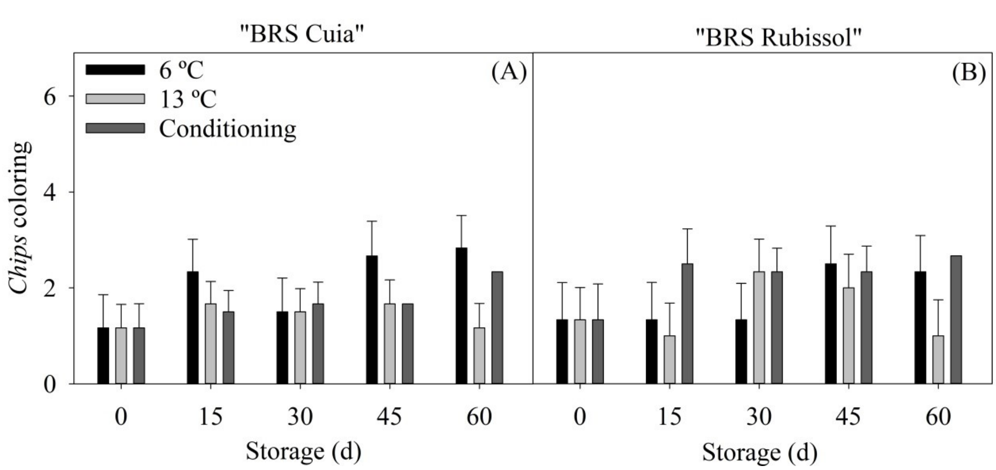

Figure 5. Color average based on darkening scale in sweet potato chips cultivar BRS Cuia (A) and BRS Rubissol (B) submitted to different temperatures during storage. The vertical bars represent the standard deviation of the mean.

Although conditioning combined with storage at 6ºC reduce the fresh weight loss, this may have increased the activity of enzymes related to sucrose conversion into reducing sugars, resulting in the darkening process in sweet potato chips via Maillard reaction. In this reaction, reducing sugars react with free amino acids, promoting the formation of dark compounds with an unpleasant taste, which reduces the overall quality of the product (McKenzie et al., 2013; Araújo et al., 2020). This response was also observed by Araújo et al. (2020), with sweet potato roots cultivar BRS Cuia, BRS Rubissol, and Beauregard stored at 6 °C for 60 days. In addition, it is under close agreement with described by Freitas et al. (2012), who studied potato tubers (*Solanum tuberosum* L.) stored at 4 °C for 180 days.

Pearson's correlation coefficients are shown in the following heatmap (Figure 6). For cultivar BRS Cuia, NRS showed a high positive correlation with TSS (r = 0.98; P = 0.01%). For cultivar BRS Rubissol, AIS correlated negatively with most of variables. However, TSS and NRS displayed high negative correlation, with r = -0.59; P = 0.01% and r = -0.59; P = 0.01%, respectively. Likewise, there was also a strong correlation between TSS and NRS (r = 0.99; P = 0.01%) for cultivar BRS Rubissol.

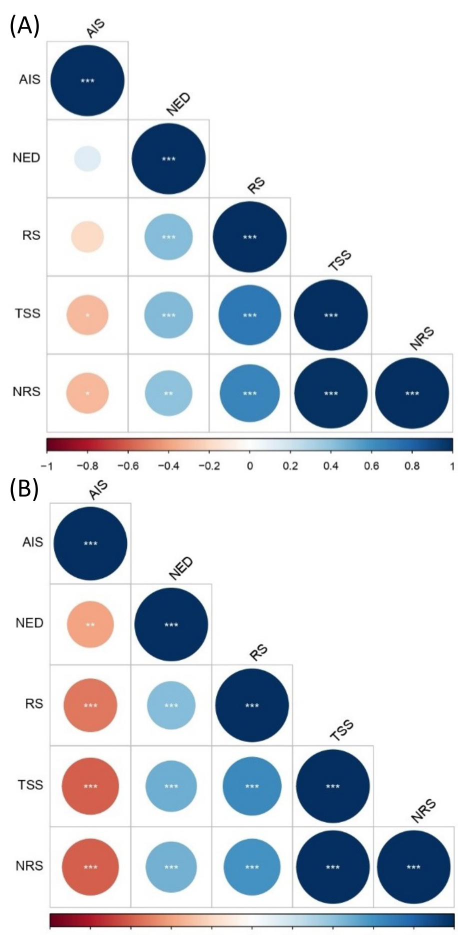

Figure 6. Pearson's correlation heatmap for a dataset containing total soluble sugars (TSS), reducing sugars (RS), non-reducing sugars (NRS), alcohol-insoluble solids (AIS) and non-enzymatic darkening (NED) of sweet potato BRS Cuia (A) and BRS Rubissol (B) cultivars. The bar color indicates the coefficient of correlation, in which dark blue consists of positive correlation (1), while dark red means a negative correlation (-1). Strong correlations are indicated by large circles, while weak correlations are indicated by small circles. Asterisks within the circle are P values. ***, **, and * indicate significance at 0.001; 0.01 and 0.005, respectively.

These results corroborate the hypothesis that the increase in non-reducing sugars in sweet potato roots is a consequence of reduction in total soluble sugars, since the percentages of NRS were higher than the percentages of RS, mainly in cultivar BRS Rubissol. This suggests the conversion of monosaccharides to sucrose (Figure 3F). Besides, the strong correlation between AIS and TSS, and NRS support the hypothesis that starch degradation cues the accumulation of soluble sugars (Figure 3), as reported by Bonte et al. (2000) and Sato et al. (2017).

These findings indicate that the exposure of cultivars BRS Cuia and BRS Rubissol at low temperatures (6 °C and conditioning) may induce the accumulation of sugars, as previously reported by Araújo et al. (2020). These cultivars possibly present intrinsic physiological and biochemical characteristics that led to non-responding to conditioning, which was evidenced by the similar behavior in roots stored at 6 °C. This result is similar to earlier observed by Li et al. (2018), who demonstrated that conditioned sweetpotato roots of cultivar Xinxiang increased sugar content and sweetness index during storage. Also, increases in sugar levels caused by low temperatures during storage may have induced changes in the metabolism of sweet potato roots. The characterization of these metabolic differences can assist the selection of Brazilian sweet potato cultivars that are resistant or tolerant to cold, allowing storage at low temperatures, extending their shelf life, and promoting continuous market and processing industries supply.

Conclusions

Conditioning combined with storage at 6 °C led to highest accumulation of reducing and non-reducing sugars, and triggered non-enzymatic darkening in the chips. This study demonstrated that conditioning was not effective in reducing cold-induced sweetening in studied Brazilian sweet potato cultivars.

Compliance with Ethical Standards

Author contributions: Conceptualization: MLMV, FLF; Data curation: MLMV, NOA, JPJT; Funding acquisition: FLF; Investigation: MLMV, NOA, MNSS; Methodology: MLMV, NOA, JPJT, MNSS; Methodology: MLMV, NOA, JPJT; Project administration: FLF; Resources: MLMV, FFA; Software: MLMV, NOA, JPJT; Supervision: FLF; Validation: MLMV, MNSS, FFA; Writing - original draft: MLMV, NOA, JPJT; Writing - review & editing: MLMV, NOA, JPJT, FLF.

Conflict of interest: The authors declared that there is no possible conflict of interest (professional or financial) that may influence the article.

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