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Importance of 1,4-dimethylnaphthalene in maintaining the quality of stored tubers of Asterix and Challenger cultivars

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Abstract

This work aimed to analyze 1,4-DMN efficiency in the suppression of sprouting and the consequent maintenance of quality in potato tubers of Asterix and Challenger cultivars stored during different periods under varying temperatures: eight treatments with and without 1,4-DMN under temperatures of 8 °C and 20 °C. Treatments effect were evaluated after 0, 45, 90, 135 and 180 days after application. The evaluated parameters were the loss of fresh mass, length of the sprouts and activity of the enzymes polyphenoloxidase and peroxidase. The results indicate that the application of 1,4-DMN reduces the loss of fresh mass, this effect was more intense for the cultivar Challenger, at 8 °C. There were also significant effects in reducing the average length of the sprouts for both cultivars evaluated, after 180 days, at 20 °C. There was little or no difference in the level of oxidative stress caused in the tubers. Hence, the effects of 1,4-DMN on the quality control of potatoes included reduced loss of fresh mass and a shorter average length of sprouts for both cultivars evaluated compared to control, for both temperatures. These effects intensify in the cultivar Challenger and treatments with temperatures of 20 °C.

Keywords: quality maintenance; sprout suppressor; storage temperature.

Practical Application: This work provides insights into the efficiency of 1,4-Dimethylnaphthalene (1,4-DMN) in suppressing sprout, thus maintaining the quality of potato tubers of Asterix and Challenger cultivars stored under different temperatures during different periods.

1 Introduction

Potato is the most relevant tuber in the world (Zhang et al., 2019). Brazilian harvest reached 3.67 million tons in 2021. The Minas Gerais state alone produced 1.21 million tons of potatoes tubers (Instituto Brasileiro de Geografia e Estatística, 2021). Most of these tubers are sold in natura. However, society eating habits are changing towards a stronger demand for processed food. This demand for industrial products creates pressure for uniform, high-quality raw materials in Brazil (Freitas et al., 2006).

Potato tubers destinate for industrial processing must have a high dry matter content, low sugar contents, be free of diseases, damages and physiological disorders (Silva et al., 2019). Sprouting, the breaking dormancy of sprouts, is a major quality problem. Sprouting increases the respiratory rates and causes water loss due to transpiration. Also, it affects the starch content by converting it into reducing sugars, glucose and fructose (Singh & Kaur, 2016). Besides, sprouts can cause mechanical damage to the adjacent potatoes. These damages favour the contamination by pathogens that depreciates the raw material and causes considerable losses during storage (Mani & Hannachi, 2015).

Adequate temperature and humidity conditions can reduce the damage coming from these factors. Generally, the optimal storage temperature of potatoes destined for industrial processing is 8 °C (Voss et al., 2001) since it prolongs dormancy. Along with temperature and humidity control, sprout suppressors are useful in maintaining the quality and extending the durability of stored products.

The chloropropane (CIPC), widely used in the United States and Europa, is among the most efficient sprouting inhibitors. However, the use of CIPC is restricted because of its potential toxic effect on humans and the environment (Paul et al., 2016). In Brazil, the use of CIPC is illegal. Therefore, several studies have investigated alternatives to CIPC which are equally efficient in suppressing sprouting while being safe.

From the characterization of several compounds of the naphthalene family, the 1,4 and 1,6-Dimethylnaphthalene (1,4-DMN) isomers were identified as the most potent sprout suppressors. These suppressors are considered chemicals of low risk for environmental and human health. Hence, they are adequate to suppress sprouting in stored potatoes (Weerd et al., 2010).

These characteristics make 1,4-DMN a candidate product for potato conservation, being useful to study the quality

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maintenance and storage of these tubers. Therefore, the present study aim was to determine the efficiency of 1,4-DMN in suppress sprouting and, consequently, maintain the quality of potato tubers of Asterix and Challenger cultivars stored under different temperatures during different periods (days).

2 Materials and methods

2.1 Experimental design and area characterization

The experiment was conducted in split-plot design, with the treatments in the plots and the evaluation time in the subplots. A completely randomized design was used, with with four replicates. Each experimental unit was composed of two tubers.

Tubers of the Asterix and Challenger cultivars were planted in March 2017 in Perdizes, 19°21'10" S and 47°17'34" W and climate classified as Cwb according to Köppen and Geiger, characterized by rainy summer and dry winter. Perdizes has an average temperature of 20.1 °C and the average annual rainfall is 1603 mm. The selection was made regarding mass uniformity, with a variation between 90 and 250 g. The Asterix and Challenger cultivars were chosen because they have quite high yield, numerous tubers and desirable characteristics in the industrial process: tubers with acceptable external appearance, shallow buds, suitable size for obtaining of large toothpicks and oval-elongated shape.

2.2 Experimental procedure and analyses

The treatment was applied to the tubers by heating Petri dishes containing filter paper soaked with 1,4-DMN diluted in alcohol until complete volatilization inside the hermetically sealed buckets. The fumigation time was 30 minutes and the treatments were: T1 - Asterix 1,4-DMN 8 °C, T2 - Asterix Control 8 °C, T3 - Asterix 1,4-DMN 20 °C, T4 - Asterix Control 20 °C, T5 - Challenger 1, 4-DMN 8 °C, T6 - Challenger Control 8 °C, T7 - Challenger 1,4-DMN 20 °C, T8 - Challenger Control 20 °C. 20 µL of 1,4-DMN (sigma) per kg of potato were used, diluted in 15 mL of 95% alcohol, and the controls contained only 15 mL of 95% alcohol (Weerd et al., 2010).

After applying the treatments, the tubers were removed from the buckets and stored in incubators (BOD's), under temperatures of 8 °C and 20 °C, relative humidity of 85-90% and absence of light. The temperature of 8 °C was used because it is suitable for storing potatoes for industrial processing. Already, the temperature of 20 °C was used because it is the average ambient temperature of the place where the experiment was conducted

Five evaluation periods were performed for each treatment: 0, 45, 90, 135 and 180 days after application, with day 0 (zero) being evaluated on the day of treatment application. The evaluations were, as follow: loss of fresh mass, length of sprouts, activity of polyphenoloxidase (PPO) and peroxidase (POD) enzymes. All analyzes were performed at the Post-Harvest Technology Technology Laboratory at the Federal University of Viçosa (UFV), in Viçosa-MG.

2.3 Loss of fresh mass

The loss of fresh mass was determined by weighing the tubers in each evaluation period, using a semi-analytical balance, taking into account the initial mass, the percentage was obtained by difference during the storage period. The results were expressed as a percentage of fresh mass loss (%).

2.4 Sprouts length

The length of the sprouts was measured in mm, with the aid of a digital caliper (Stainless Hardened). To obtain the sum of the average length of sprouts, the lengths of all the shoots present in each tuber were added and this number was divided by the total number of shoots in the tuber.

2.5 Polyphenoloxidase (PPO) activity

Five grams of frozen potatoes from each potato cultivar were immersed in 15 mL of extraction buffer (0.1 M potassium phosphate buffer, pH 6.5). This mixture was ground, filtered through gauze and centrifuged at 12.000 g for 30 minutes at 4 °C.

The polyphenoloxidase (PPO) activity was determined by adding an enzymatic extract aliquot (100 µL) to the reaction medium containing 1.5 mL of 0.1 M phosphate buffer (pH 7.0), 0.5 mL of catechol (1.68%) and 3.0 mL of water. The PPO activity was determined in a spectrophotometer (UV-1601) at λ= 420 ηm at 25 °C. Results were expressed in $EU/min^{-1}/mg^{-1}$ protein (Kavrayan & Aydemir, 2001). The protein of the enzymatic extract was determined with the method of Bradford (1976) using BSA (bovine serum albumin) as standard. The results were expressed in mg of protein in the enzyme extract.

2.6 Peroxidase (POD) activity

Five grams of frozen potatoes from each potato cultivar were immersed in 15 mL of extraction buffer (0.1 M potassium phosphate buffer, pH 6.5). This mixture was ground, filtered through gauze and centrifuged at 12.000 g for 30 minutes at 4 °C.

The activity of peroxidase (POD) activity was determined by adding an enzymatic extract aliquot (200 µL) to the reaction medium containing 1.5 mL of 0.1 M phosphate buffer (pH 7.0), 0.5 mL of guaiacol (1.68%), 0.5 mL of H_2O_2 (1.8%) and 3 mL of water. The POD activity was determined in a spectrophotometer (UV-1601) at λ= 470 ηm, at 25 °C. Results were expressed in $EU/min^{-1}/mg^{-1}$ protein (Lagrimini et al., 1997). The protein of the enzymatic extract was determined with the method of Bradford (1976) using BSA (bovine serum albumin) as standard. The results were expressed in mg of protein in the enzyme extract.

2.7 Data analysis

The data were submitted to analysis of variance and regression using the SAEG 9.1 Statistical Analysis System (Universidade Federal de Viçosa, 2007) and the means compared by the Tukey test ($p \leq 0.05$). The choice of the regression model was based on the significance of the regression coefficients using

the t test at a 5% probability level and on the determination coeficiente (R2= SQReg/SQtrat).

3 Results and discussion

When assessing the loss of fresh mass over the entire storage period (Table 1), we observed a difference in the fresh mass loss within Asterix cultivar with 1,4-DMN due to the different storage temperatures (8 and 20 °C), with higher losses at 20 °C. That same effect occurred between treatments 2 and 4 after 90, 135 and 180 days of storage of the Asterix cultivar without 1,4-DMN (Table 1). The loss of fresh mass determines, in part, the longevity of storage and also the maintenance of quality (Gupta et al., 2015).

Storage temperature had the same effects on the fresh weight loss in Challenger and 'Asterix' cultivars. Higher losses were observed at 20 °C at 90, 135 and 180 days of storage with 1,4-DMN. Similarly, for Challenger with 1,4-DMN at 8 °C and (treatment 5) and at 20 °C (treatment 7), high losses occurred at 135 and 180 days without the use of 1,4-DMN (Table 1). Therefore, regardless of cultivars and 1,4-DMN application, the loss of fresh mass during storage was associated with higher temperatures.

The effect of storage temperature on the loss of fresh mass is due to increased metabolic activity (Caldiz et al., 1996). Higher respiratory rates lead to intensified mobilization of reserves and water loss via transpiration (Bisognin et al., 2008). Bacarin et al. (2005) found similar results while studying the storage of potato tubers from several cultivars. Asterix cultivar, for example, presented a higher loss of fresh matter when stored under 20 °C.

Interestingly, the Challenger cultivars kept under temperatures of 8 °C with 1,4-DMN application had a significant reduction in loss of fresh weight (treatment 5 vs 6) at 45 days evaluation. Generally, sprouting correlates positively to mass loss. Thus, the use of sprout suppressors reduces those losses (Azad et al., 2017; Nyankanga et al., 2018). However, the 1,4-DMN effect on sprout inhibition appears to be transient, explaining why there is no significant effect of 1,4-DMN in subsequent evaluation periods. The levels of 1,4-DMN residues within the tubers decrease with increasing storage time (Weerd et al., 2010).

The cultivars under the same storage conditions after 180 days, there was a higher loss of fresh mass in the Asterix cultivar with 1,4-DMN under temperatures of 8 °C. However, for the same temperature without 1,4-DMN the cultivar Challenger showed higher fresh mass loss only at 45 days of evaluation. In the other evaluation periods, the differences were not significant.

Lower losses were observed for the 'Challenger' in the periods of 90, 135 and 180 days of storage under 20 °C with the use of 1,4-DMN. Also, considering the same storage temperature of 20 °C but without the use of 1,4-DMN, the 'Challenger' showed fewer losses. This effect was significant in the periods of 135 and 180 days. Nyankanga et al. (2018) observed a significant decrease in mass loss when comparing Kenya Mpya tubers treated with 1,4-DMN and untreated ones.

The linear models adjusted for fresh weight loss shows increased fresh weight loss along time in both cultivars under all treatments (Figure 1). Also, we can observe a greater slope of the line for treatments that considered a higher storage temperature. Proportionally, the temperature factor seems to be a more important contribution to varying fresh weight loss values than the 1,4-DMN factor (Figure 1).

Except for the control treatment at 8 °C in the Challenger cultivar (1B), determination coefficients (R^2) above 0.97 were found, indicating that more than 97% of the variation in fresh weight loss is due to the variation in the storage time considered, according to the adjusted linear models. The loss of tuber mass over the storage period is because of reserve consumption by breathing, the conversion of polysaccharides into sugars and water loss (Finger et al., 2018).

Comparing the effects of storage temperature on the length of sprouts in the Asterix cultivar (Table 2), we found that the use of 1,4-DMN leads to higher sprouts length average values for the storage at 20 °C in almost all evaluation periods. Exceptionally, at 135 days, there was a greater average length of the sprouts for tubers storage at 8 °C. Although significant, this difference contrasts the pattern observed in the same storage conditions in the other evaluation periods. There is no apparent reason leading to the sprouts damage during this specific evaluation period. Extending dormancy in storage provides potatoes of higher quality for industrial processing

Table 1. Average values for loss of fresh mass (%) in pottato tubers of Asterix (Ast) and Challenger (Cha) cultivars.

	Loss of Fresh Mass (%)				
Treatment	0 days	45 days	90 days	135 days	180 days
1-Ast/1.4-DMN/8 °C	0.00 A	2.02 B	3.40 CD	5.37 C	6.95 C
2-Ast/Control/8 °C	0.00 A	2.11 B	3.21 CD	4.38 C	5.35 CD
3-Ast/1.4-DMN/20 °C	0.00 A	4.48 A	7.96 A	15.36 A	18.35 A
4-Ast/Control/20 °C	0.00 A	3.55 AB	6.40 AB	13.75 A	16.44 A
5-Cha/1.4-DMN/8 °C	0.00 A	1.74 B	2.76 D	3.75 C	4.50 D
6 -Cha/Control/8 °C	0.00 A	4.57 A	4.04 CD	4.95 C	5.92 CD
7-Cha/1.4-DMN/20 °C	0.00 A	2.90 AB	5.43 BC	8.95 B	11.43 B
8-Cha/Control/20 °C	0.00 A	2.89 AB	4.89 BCD	7.75 B	11.36 B

Averages followed by the same letter, in the same column, do not differ at 5% probability by the Tukey test.

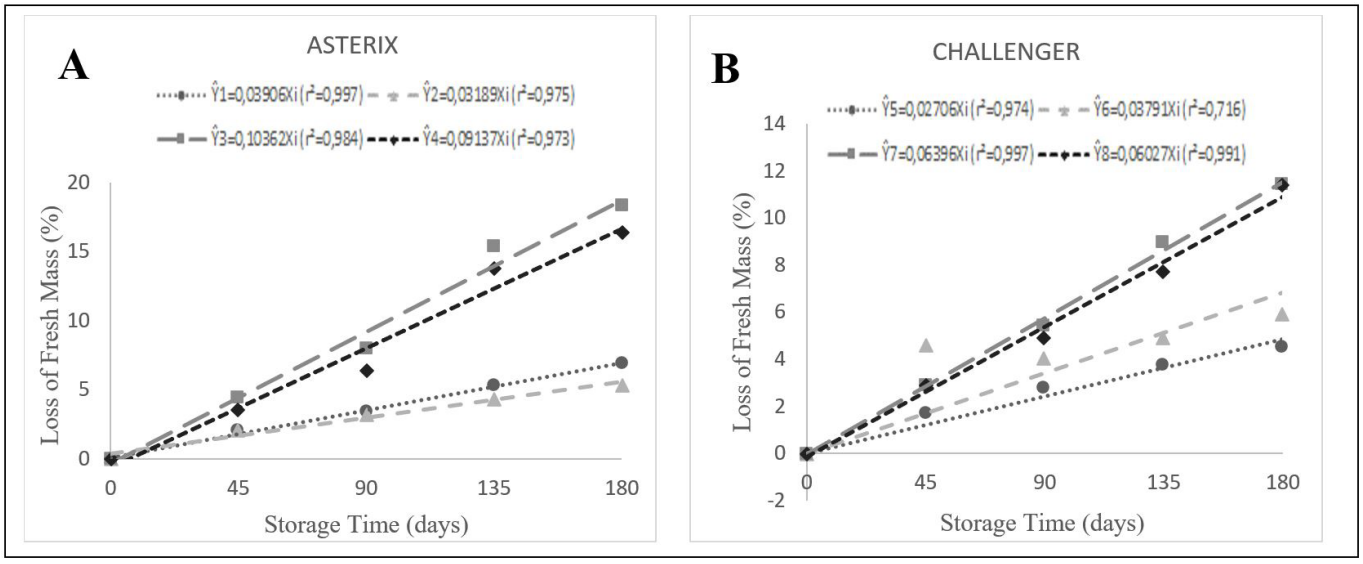

Figure 1. Loss of Fresh Mass (%) of Asterix (A) and Challenger (B) cultivars, as a function of storage time. ● = 1,4-DMN/8 °C, ▲ = Control/8 °C, ■ = 1,4-DMN/20 °C, ◆ = Control/20 °C.

Table 2. Sum of the average length of sprouts in potato tubers of Asterix (Ast) and Challenger (Cha) cultivars.

	Sum of average sprout length (mm)				
Treatment	0 days	45 days	90 days	135 days	180 days
1-Ast/1.4-DMN/8 °C	0.00 A	0.00 A	20.85 AB	273.52 A	83.34 DE
2-Ast/Control/8 °C	0.00 A	0.00 A	18.83 B	180.97 B	80.00 DE
3-Ast/1.4-DMN/20 °C	0.00 A	6.42 A	56.38 AB	107.68 C	113.90 D
4-Ast/Control/20 °C	0.00 A	3.24 A	64.04 A	87.67 CD	217.51 B
5-Cha/1.4-DMN/8 °C	0.00 A	0.00 A	13.11 B	66.39 CDE	61.87 E
6-Cha/Control/8 °C	0.00 A	0.00 A	12.91 B	49.78 DE	78.56 DE
7-Cha/1.4-DMN/20 °C	0.00 A	0.00 A	29.85 AB	44.09 DE	160.54 C
8-Cha/Control/20 °C	0.00 A	0.79 A	50.51 AB	39.62 E	410.24 A

Averages followed by the same letter, in the same column, do not differ at 5% probability by the Tukey test.

(Freitas et al., 2012). The mode of action of 1,4-DMN is not yet clearly understood, but it has been suggested that the natural dormancy period is extended through the regulation of phytohormones (Campbell et al., 2010).

The Asterix cultivar without 1,4-DMN application showed a greater average length of sprouts under 20 °C at 90 days of storage and under 8 °C in the period of 135 days. After 135 days, the length of the sprouts was equal (Table 2). For the Challenger cultivar, with and without 1,4-DMN, the higher storage temperature resulted in a significant higher length of sprouts, but only in the period of 180 days. The effects of 1,4-DMN in reducing the average length of the sprouts were significant for both cultivars, but only after a longer storage period (180 days) under 20 °C.

Nyankanga et al. (2018), when testing the use of 1,4-DMN in different potato cultivars, found that the use of DMN did not prevent the beginning of sprouting in the Shangi and Asante cultivars, but significantly reduced the development of sprouting during storage compared to untreated tubers. They also observed that the use of 1,4-DMN effectively suppressed the growth of sprouts compared to untreated tubers.

The best models adjusted for average sprouts length were linear and increasing (Figure 2). Thus, an increasing linear relationship between storage time and this characteristic is expected. The equations adjusted for the control treatments at 20 °C showed a greater inclination in both cultivars for sprouts length (Figure 2). That was mainly due to the higher averages observed for these characteristics after a long period of storage. Different cultivars require different levels of suppressors in the tubers to suppress sprouting during storage (Nyankanga et al., 2018).

For the Challenger cultivar, there was no effect of the storage temperature on the average levels of polyphenoloxidase (PPO), both in the presence and in the absence of 1,4-DMN (Table 3). Whereas, the Asterix cultivar with 1,4-DMN presented a higher average of PPO under 20 °C at 45 days and a lower average without 1,4-DMN under 20 °C at 35 days. There was no effect of 1,4-DMN on the average of PPO in any storage condition for both cultivars evaluated.

Comparison between cultivars under the same storage conditions showed a significant difference only in the presence of 1,4-DMN at 45 days (Table 3), with a higher mean of

polyphenoloxidase for the Asterix cultivar. The increase in PPO activity may be related to the tuber defence mechanisms generated by physiological changes at the beginning of sprouting (Afify et al., 2012).

Peroxidase activity (POD) is associated with enzymatic browning through oxidation of phenolic compounds, which causes deterioration and loss in product quality due to changes in colour and flavour (Terefe et al., 2014). It is possible to observe a trend of lower POD activity for tubers stored under 20 °C. However, there was no significant effect of any of the factors studied (cultivar, temperature, 1,4-DMN and storage time) on POD levels when comparing Challenger and Asterix cultivars under the same storage condition (Table 4).

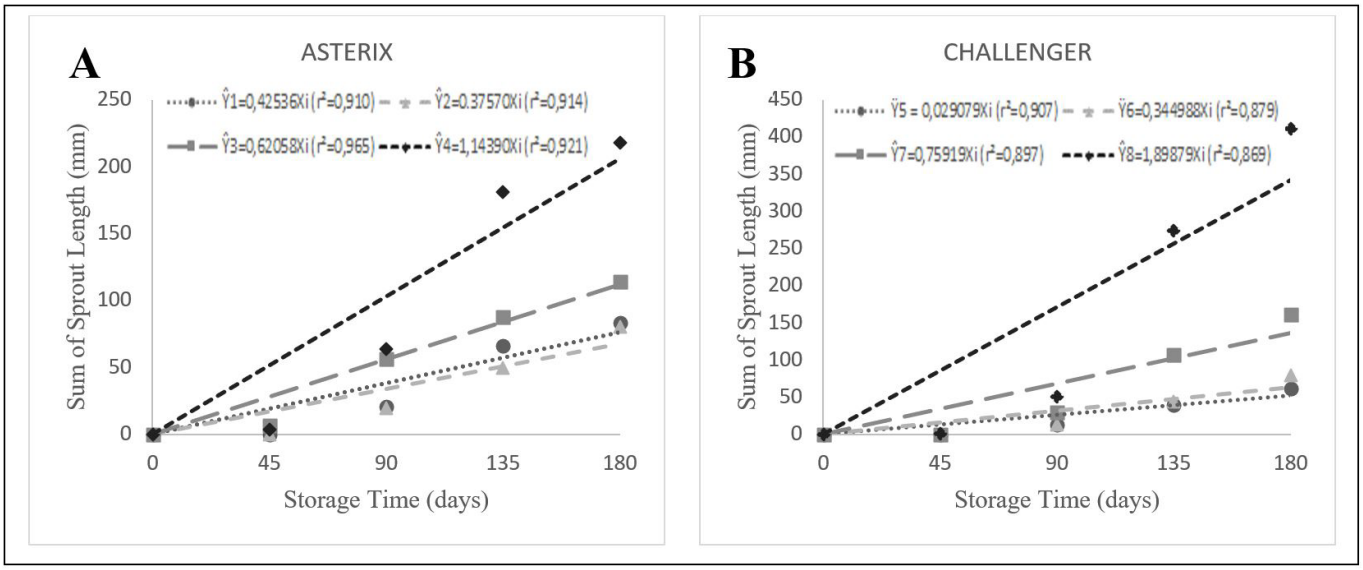

Figure 2. Sum of the average length of the sprouts of Asterix (A) and Challenger (B) cultivars, as a function of storage time. ● = 1,4-DMN/8 °C, ▲ = Control/8 °C, ▪ = 1,4-DMN/20 °C, ◆ = Control/20 °C.

Table 3. Average values for polyphenoloxidase enzyme activity in potato tubers of Asterix (Ast) and Challenger (Cha) cultivars.

	Polyphenoloxidase enzyme (UA/min/mg of protein)				
Treatment	0 days	45 days	90 days	135 days	180 days
1-Ast/1.4-DMN/8 °C	0.600 A	0.235 B	1.055 A	1.156 AB	0.339 A
2-Ast/Control/8 °C	0.369 A	0.177 B	1.055 A	1.847 A	1.102 A
3-Ast/1.4-DMN/20 °C	0.592 A	1.878 A	1.844 A	0.170 B	0.257 A
4-Ast/Control/20 °C	0.364 A	1.496 AB	0.939 A	0.255 B	0.402 A
5-Cha/1.4-DMN/8 °C	0.323 A	0.859 AB	1.673 A	0.249 B	0.258 A
6-Cha/Control/8 °C	0.238 A	0.273 B	1.542 A	0.569 AB	0.333 A
7-Cha/1.4-DMN/20 °C	0.322 A	0.282 B	1.222 A	0.660 AB	0.181 A
8-Cha/Control/20 °C	0.238 A	0.482 AB	1.289 A	0.145 B	0.319 A

Averages followed by the same letter, in the same column, do not differ at 5% probability by the Tukey test.

Table 4. Average values for peroxidase enzyme activity in potato tubers of Asterix (Ast) and Challenger (Cha) cultivars.

	Peroxidase enzyme (UA/min/mg of protein)				
Treatment	0 days	45 days	90 days	135 days	180 days
1-Ast/1.4-DMN/8 °C	1.036 A	0.128 A	0.708 A	0.370 A	0.352 A
2-Ast/Control/8 °C	0.489 A	0.175 A	0.540 A	0.592 A	0.790 A
3-Ast/1.4-DMN/20 °C	1.027 A	0.161 A	0.468 A	0.082 A	0.031 A
4-Ast/Control/20 °C	0.493 A	0.167 A	0.427 A	0.111 A	0.042 A
5-Cha/1.4-DMN/8 °C	0.160 A	0.000 A	0.299 A	0.172 A	0.018 A
6-Cha/Control/8 °C	0.093 A	0.148 A	0.298 A	0.166 A	0.303 A
7-Cha/1.4-DMN/20 °C	0.156 A	0.253 A	0.204 A	0.374 A	0.012 A
8-Cha/Control/20 °C	0.092 A	0.125 A	0.187 A	0.061 A	0.049 A

Averages followed by the same letter, in the same column, do not differ at 5% probability by the Tukey test.

Polyphenoloxidase and peroxidase are enzymes related to the oxidative stress of the plant. Higher stress levels lead to higher production of these enzymes. These enzymes reduce the levels of reactive oxygen species (ROS) (Brito et al., 2005), which could compromise several cellular structures, such as the plasma membrane, cell organelles, nucleic acids, lipids and proteins. These results indicate that there is little or no difference regarding the level of oxidative stress in both cultivars studied here.

4 Conclusion

The effects of 1,4-DMN in the quality control of potatoes resulted in less loss of fresh mass and reduction in the average length of the sprouts for the cultivars Asterix and Challenger. These effects were more effective for the cultivar Challenger and at a temperature of 20 °C.

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