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Cold shock treatment increases chilling injuries in 'Douradão' peaches

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ABSTRACT: This study was carried out to evaluate the cold shock, before cold storage, influence on quality of cold stored 'Douradão' peaches, with the objective to reduce the chilling injuries. For this, the fruits were exposed at -2 or -4 °C for 1, 2, or 3 hours. After treatments the fruits were stored at 0.5 ± 1 °C and 85-90% RH for 30 days. The variables evaluated suggests that the treatment with cold shock does not influence the physical and chemical fruit characteristics but intensifies and accelerates the onset of symptoms characteristic of chilling injuries. This may be associated with changes in enzymes of endo-PG, PME, PL and β-Gal amount and activity that affect the dynamics of cell wall degradation, in the case of woolliness and changes in enzymes PAL, POD and PPO activity and the increased membrane permeability in the case of internal browning. The anticipation of the chilling injuries and decay in fruits subjected to cold shock suggests that this practice should not be used in order to maintain the fruit quality.

Key words: cell wall enzymes; internal browning; postharvest; *Prunus persica*; woolliness

Choque a frio aumenta as injúrias de frio em pêssegos 'Douradão'

RESUMO: Com o objetivo de avaliar se o estresse inicial por baixa temperatura, antes do armazenamento refrigerado, influencia a qualidade e o desenvolvimento de injúrias de frio em pêssegos 'Douradão', os frutos foram mantidos a -2 ou -4 °C por 1, 2 ou 3 horas. Após os tratamentos os frutos foram armazenados a 1 ± 0,5 °C e 85-90% UR, durante 30 dias. As variáveis avaliadas sugerem que o tratamento com choque a frio não influencia as características físicas e químicas dos frutos, porém intensifica e acelera o aparecimento de sintomas característicos de injúrias de frio. Isso pode estar associado à alteração na quantidade e atividade das enzimas endo-PG, PME, PL e β-Gal que afetam a dinâmica de degradação da parede celular, no caso da lanosidade, e alteração na atividade das enzimas PAL, POD e PPO e o aumento da permeabilidade de membranas no caso do escurecimento da polpa. A antecipação no aparecimento de injúrias de frio e de podridões nos frutos submetidos ao choque a frio sugere que esta prática não deve ser utilizada visando a manutenção da qualidade dos frutos.

Palavras-chave: enzimas de parede; escurecimento interno; pós-colheita; *Prunus persica*; lanosidade

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Introduction

The peach (*Prunus persica* L. Batsch) is a fruit much appreciated around the world for its taste, appearance, and economic value. The peach is a fruit with a short postharvest shelf-life, and it is recommended to store it between -1 and 1 °C, but it cannot withstand more than three weeks at these temperatures. (Kluge et al., 1997; Crisosto & Kader, 2016). When stored for long periods at low temperatures it is common for chilling injury to appear, such as woolliness, pulp with a leathery texture, and internal browning/reddening of the pulp (Lurie, 2021). These injuries develop faster and more intensely in fruit stored at 2 to 7 °C, compared to those stored at 0 °C or below (Crisosto & Kader, 2016). Chilling injury symptoms occur internally, when the fruit is exposed to ambient temperature after cold storage (Fruk et al., 2014).

The reduction in temperature during peach storage alters the activity of enzymes involved in cell wall degradation, favoring the continuous deesterification of pectins promoted by pectinamethylesterase (PME), without a synchronized depolymerization of these pectins due to the reduction in polygalacturonase (PG) activity. Consequently there is accumulation of low methoxylation and high molecular weight pectic compounds that bind to the existing calcium, remaining insoluble and causing woolliness by the formation of a gel with free water in the apoplast (Fruk et al., 2014).

In chilling-sensitive fruits, temperature reduction promotes changes in the integrity and permeability of cell membranes, affecting cell metabolism and the normal activity of membrane-associated enzyme complexes. The change in pulp coloration (internal browning/reddening), as a result of loss of cell compartmentation, is due to damage caused to cells by toxic intermediates, accumulated during low temperature exposure, and the oxidation of phenolic compounds, caused mainly due to increased activity of the enzyme polyphenoloxidase (PPO) (Lurie & Crisosto, 2005).

In an attempt to maintain the quality of the fruit during the refrigerated storage period, complementary techniques such as cold shock can be used. Cold shock treatment is a physical method of preserving fruits and vegetables, and can usually be accomplished with cold air or ice water. This technique involves keeping the fruit, for a short period of time, at temperatures slightly above those at which chilling injury occurs, to induce chilling tolerance (Zhang et al., 2020; Zhang et al., 2021).

Studies have shown that cold shock can maintain the quality of kiwi (Yang et al., 2019), papaya (Nian et al., 2022), nectarine (Zhao et al., 2019a), and cherry (Zhao et al., 2019b). Cold shock treatment can increase plant resistance by inducing increased cold shock proteins (Zhao et al., 2019a), which are related to plant tolerance to cold (Gu et al., 2020). Cold stress, like other types of moderate stress, can trigger physiological and molecular responses that culminate in increased tolerance of fruit to low temperature, either through induction of heat shock proteins or activation of the antioxidant defense system (Nian et al., 2022). However, there are no studies in the literature concerning the influence of applying an initial low temperature stress on peach quality maintenance during and after refrigeration. Thus, the present work aimed to evaluate the influence of cold shock on the quality of 'Douradão' peaches stored under refrigeration, focusing on the reduction of chilling injury.

Materials and Methods

'Douradão' peaches were harvested at the physiological maturity stage (breaking of the green background coloration) in a commercial farm located in the municipality of Itupeva, SP, Brazil. The harvest was done early in the morning and the fruits were immediately transported to the laboratory, where they were selected and fruits with mechanical damage and rottenness were removed, forming a homogeneous lot.

Cold shock treatments were performed by placing the fruits in B.O.D. type chambers in the following treatments: fruits kept at -2 °C, for 1h (-2 °C/1h); fruits kept at -2 °C, for 2h (-2 °C/2h); fruits kept at -2 °C, for 3h (-2 °C/3h); fruits kept at -4 °C, for 1h (-4 °C/1h); fruits kept at -4 °C, for 2h (-4 °C/2h); fruits kept at -4 °C for 3h (-4°C/3h). Untreated fruit and stored at 1 °C were considered as control and storage standard. After treatments the fruits were cold stored at 1 ± 0.5 °C and 85-90% RH for 30 days.

The analyses were performed in the following periods: lot characterization (Day 0); at 10 days of refrigerated storage + 3 days at room temperature (22 °C) (to simulate the time of commercialization and manifestation of the injury symptoms - 10+3); at 20 days of refrigerated storage + 3 days at room temperature (20+3); and, at 30 days of refrigerated storage + 3 days at room temperature (30+3). Biochemical analyses were also performed immediately after the fruits were removed from cold storage at: 10, 20, and 30 days; and after the 3-day simulated trading period, at: 10+3, 20+3, and 30+3 days.

The determinations performed were: Flesh firmness: determined with the aid of a digital penetrometer, with a flat tip of 8 mm in diameter and expressed in newtons (N); Soluble solids content: determined using a digital refractometer and expressed in °Brix; Titratable acidity: determined by potentiometric titration with NaOH 0.1 N up to pH 8.10 and expressed in percentage (%); Ascorbic acid content: determined by titration, through the reduction of the indicator 2,6-dichlorophenol indolfenol-sodium (DCFI) by ascorbic acid and expressed as mg ascorbic acid per 100 g pulp (mg 100^{-1}); Skin color: determined with the aid of a digital colorimeter, with illuminant D65, taking two readings on opposite sides of the equatorial region of the fruit and the results expressed in color angle (°h); Percentage of rot: evaluated by counting fruits with lesions larger than 0.5 cm in diameter; Polyphenoloxidase enzyme activity - PPO (EC 1.10.3.1): for enzyme extraction was used methodology described by Zheng et al. (2007), where 5 g of frozen pulp were macerated with 0.3 g of PVPP and 30 mL of sodium phosphate buffer (100 mM, pH 7.8), after maceration the samples were centrifuged at 10,000 rpm for 10 minutes at 4 °C, and quantification

was performed by spectrophotometry by the methodology adapted from Wang et al. (2004), monitoring the change in absorbance at 398 nm in a spectrophotometer for 10 seconds, the specific activity was expressed by Δ_{398} min^{-1} mg^{-1} protein; Peroxidase enzyme activity - POD (EC 1.11.1.7): the extraction was performed in the same way as for PPO enzyme, using the methodology described by Zheng et al. (2007). However, quantification was performed by spectrophotometry using the methodology described by Jiang et al. (2002) where 0.5 mL of the enzyme extract was incubated in 2 mL of buffer with substrate (100 mM sodium phosphate buffer, pH 6.4 and 8 mM guaiacol) for 5 minutes at 30 °C and the increase in absorbance at 460 nm was measured for 120 seconds after the addition of 1 mL of H_2O_2 (24 mM), the specific activity was expressed as Δ_{460} min^{-1} mg^{-1} protein; Phenylalanine ammonia lyase enzyme activity - PAL (EC 4.3.1.25): was determined by methodology adapted from Gao et al. (2018), where 0.1 g of frozen pulp was macerated in 10 mL of 0.1 M sodium borate buffer pH 8.8 containing PVP (5% w/v). The samples were then centrifuged at 12,000 rpm for 20 minutes at 4 °C. For quantification, a 1 mL aliquot of the filtered extract was taken plus 1 mL of sodium borate buffer (0.2 M) and 1 mL of phenylalanine. The samples were incubated for 60 minutes in a water bath at 36 °C, after which 0.1 mL of 6 N HCl was added to stop the enzymatic reaction. The determination was performed in a spectrophotometer with readings at 290 nm, the specific activity was expressed in mmoles min^{-1} mg^{-1} protein; Woolliness: a methodology adapted from Ju et al. (2000) was adopted, based on the appearance and amount of juice extracted the fruits were divided into five categories: 1 = firm fruit, dry looking fruit with no juice extraction; 2 = firm fruit, dry looking fruit with little juice extraction; 3 = soft fruit, with a dry appearance and no juice extraction; 4 = soft fruit, with a dry appearance and little juice extraction; 5 = soft fruit, with a moist appearance and with much juice extraction, and the results were expressed as percentage of fruit in each category; Enzymes: Endo-Polygalacturonase - endo-PG (EC 3.2.1.15); Pectin methylesterase - PME (EC 3.1.1.11); Pectin lyase - PL (EC 4.2.2.10); Expansin – Exp and β-Galactosidase – β-Gal (EC 3.2.1.23): were determined by means of the ELISA technique for relative quantification of proteins corresponding to enzymes involved in parietal metabolism. A sample containing 10 pg of the recombinant protein was assigned a value of 100. This means that if the value is 100, in that sample there were 10 pg of the protein under study. The analyses were performed only on the treatments considered most relevant; Total and soluble pectin content: the total and soluble pectin contents were determined according to the methodology described by McCready & McComb (1952), the results were expressed as mg galacturonic acid per 100g sample; Pectin solubilization: The percentage of solubilization of pectins was obtained by the following Equation 1:

$$\% \text{ solubilization} = \left(\frac{\text{soluble pectin content}}{\text{total pectin content}} \right) \times 100 \quad (1)$$

Internal browning index: was determined by the methodology adapted from Ben-Arie & Sonego (1980), by means of an observation grid, being: no browning (0% of fruit surface), slightly browning (< 25% of the fruit surface), moderate browning (25 - 50%), and severe browning (> 50% of the fruit). The index was calculated as (Equation 2):

$$\begin{aligned} ID &= \left[(0 \times \% \text{ of fruits without browning}) + \right. \\ &+ (1 \times \% \text{ of fruits little browning}) + \\ &+ (2 \times \% \text{ of fruits with moderate browning}) + \\ &+ \left. (4 \times \% \text{ of fruits with severe browning}) \right] / 4 \end{aligned} \quad (2)$$

Electrolyte leakage: was determined using the methodology of Yang et al. (2019) in which conductivity was measured before (Li) and after (Lf) the samples were boiled in a water bath. The following formula was used for the calculations (Equation 3):

$$E = \frac{(Lf - Li)}{Lf} \times 100 \quad (3)$$

The results were expressed as % electrolyte extravasation.

The experimental design was completely randomized, with seven treatments and four repetitions of three fruits for physical and chemical analysis and for chilling injury (woolliness and internal browning). For the biochemical and enzymatic analyses three replicates of three fruits per treatment were used, with triplicates of each replicate. The results obtained were submitted to variance analysis and the means were compared using the Scott-Knott test ($p < 0.05$).

Results and Discussion

The cold shock treatments at the tested temperatures caused an earlier onset of woolliness symptoms. It can be observed that on day 10+3 the fruits from the -2°C/1h, -4°C/1h, -4°C/2h, and -4°C/3h treatments presented percentages of fruits in category 3, indicating that they were woolliness, while the fruits from the control were 100% in category 5 indicating absence of this chilling injury (Table 1).

Cold shock treatment caused changes in the amount of cell wall enzymes (Table 2), in the amount of total and soluble pectins, and in the solubilization of pectic substances (Figure 1), causing the anticipation in the appearance of woolliness symptoms.

Regarding the amount of cell wall enzymes, on day 10 + 3, it was observed that fruits from the -4°C/3h treatment (which showed higher percentages of woolliness fruits) showed a reduction in the values of endo-PG, PME, PL and β-Gal enzymes, compared to the control treatment (Table 2).

An increase in total pectin contents was observed throughout the refrigerated storage period, with highest values on day 20+3 (Figure 1A). On day 10+3 the fruits in

Table 1. Woolliness (%) of 'Douradão' peaches submitted to cold shock treatment, stored under refrigeration and evaluated after three days at room temperature (22 °C).

Treatments	Categories*				
	1	2	3	4	5
			Day 10+3**		
Control	0.00 a	0.00 a	0.00 a	0.00 a	100.00 f
-2 °C/1h	0.00 a	0.00 a	8.33 b	16.67 b	75.00 c
-2 °C/2h	0.00 a	0.00 a	0.00 a	8.33 b	91.67 e
-2 °C/3h	0.00 a	0.00 a	0.00 a	16.67 b	83.33 d
-4 °C/1h	0.00 a	0.00 a	8.33 b	33.33 c	58.33 b
-4 °C/2h	0.00 a	0.00 a	8.33 b	33.33 c	58.33 b
-4 °C/3h	0.00 a	0.00 a	16.67 b	41.67 d	41.67 a
			Day 20+3**		
Control	0.00 a	0.00 a	8.33 a	25.00	66.67
-2 °C/1h	0.00 a	8.33 b	33.33 c	33.33 b	25.00 b
-2 °C/2h	0.00 a	0.00 a	8.33 a	25.00 a	66.67 d
-2 °C/3h	0.00 a	0.00 a	25.00 b	41.67 c	33.33 c
-4 °C/1h	25.00 c	0.00 a	33.33 c	16.67 a	25.00 b
-4 °C/2h	16.67 b	8.33 b	8.33 a	33.33 b	33.33 c
-4 °C/3h	0.00 a	0.00 a	41.67 d	41.67 c	16.67 a
			Day 30+3**		
Control	0.00 a	8.33 a	58.33 c	25.00 b	8.33 b
-2 °C/1h	0.00 a	41.67 c	50.00 b	8.33 a	0.00 a
-2 °C/2hs	0.00 a	25.00 b	33.33 a	25.00 b	16.67 b
-2 °C/3hs	0.00 a	8.33 a	58.33	33.33 c	0.00 a
-4 °C/1h	25.00 c	25.00 b	33.33 a	16.67 a	0.00 a
-4 °C/2hs	16.67 b	16.67 a	33.33 a	33.33 c	0.00 a
-4 °C/3hs	0.00 a	16.67 a	66.67 c	16.67 a	0.00 a

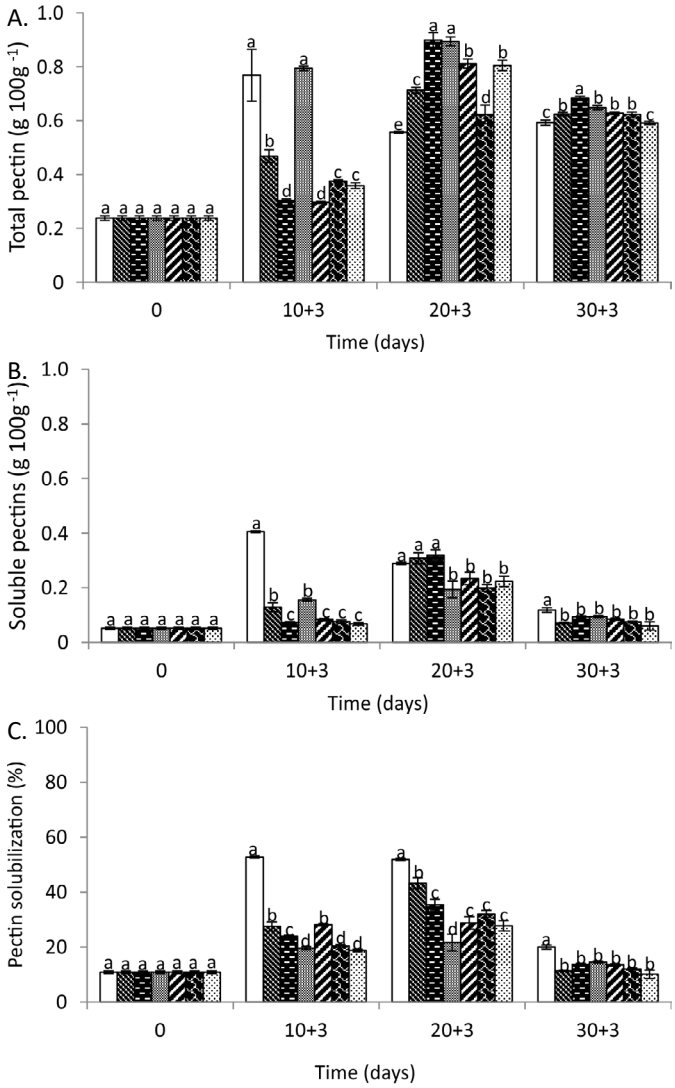
[1] Averages notes followed by equal letters in the column do not differ by the Scott-Knott test at 5% significance level.
* Categories: 1 = firm fruit, with a dry appearance and no juice extraction; 2 = firm fruit, with a dry appearance and some juice extraction; 3 = soft fruit, with a dry appearance and no juice extraction; 4 = soft fruit, with a dry appearance and some juice extraction; 5 = soft fruit, with a wet appearance and a lot of juice extraction.
** Analysis days: 10 days at 1°C and 3 days of commercialization (10+3); 20 days at 1°C and 3 days of commercialization (20+3); and, 30 days at 1°C and 3 days of commercialization (30+3).

Table 2. Content of the enzymes endo-polygalacturonase (endo-PG), pectinamethylesterase (PME), pectin lyase (PL), expasin (Exp.), and β-galactosidase (β-Gal) in 'Douradão' peaches submitted to cold shock treatment, stored under refrigeration and evaluated after three days at room temperature (22 °C).

Treatments	Enzymes*					
	Endo-PG	PME	PL	Exp	β-Gal	IR*
Characterization	40	25	40	30	32.5	89
			Day 10+3**			
Control	45	40	55	15	35	91
-4 °C/3h	20	2.5	20	15	12.5	99
			Day 30+3**			
Control	5	45	17.5	0	5	94.5
-4 °C/3h	10	7.5	5	5	5	99.5

* A value of 100 was assigned to the sample with the recombinant protein corresponding to an aliquot containing 10 pg. Meaning that the value 100, in the deposited sample has 10 pg of the protein under study. RR = enzyme recovery rate.
** Analysis days: 10 days at 1°C and 3 days of commercialization (10+3); and, 30 days at 1°C and 3 days of commercialization (30+3).

the control treatment showed higher soluble pectin content compared to the cold shock treatments (Figure 1B). In fruits in which cold shock was applied, higher values of total pectins were observed on day 20+3, reducing again on day



Vertical bars represent the standard error of the average (n = 4). [1] Averages followed by equal letters within each evaluation date do not differ by the Scott-Knott test at 5% significance level.

Figure 1. Total pectin (A), soluble pectin (B) and solubilized pectic substances (C) contents in 'Douradão' peaches submitted to cold shock treatment before refrigerated storage at 1 °C and exposure to room temperature 22 °C (10+3, 20+3 and 30+3).

30+3. The control treatment showed a gradual reduction in values throughout the entire storage period (Figure 1B). The application of cold shock reduced the solubilization of pectic substances (Figure 1C). It was observed that on day 10+3 the fruits from the control treatment, which did not show woolliness symptoms, showed significantly higher ($p < 0.05$) solubilization of pectic substances compared to the cold shock treatments, which already showed woolliness symptoms.

Several authors state that woolliness is the result of the unbalance in the activity of pectinolytic enzymes and cell wall, when there is an increase in the activity of PME, causing accumulation of pectic substances with low degree of esterification and reduction or inhibition of the activity

of PG, not allowing these substances to be totally degraded and solubilized, promoting reduction in the content of water soluble pectins and increase in the content of insoluble pectins (e.g.: prototectin and deesterified pectates), favoring the formation of pectin gels, responsible for the woolliness symptoms (Fruk et al. , 2014; Lurie, 2021). Although in the present work, in the treatment with cold shock at -4 °C/3h, the imbalance between endo-PG and PME enzymes was observed, this imbalance occurred in an inverse way, being observed a higher content of endo-PG and a lower content of PME in fruits with woolliness (Table 2), but with reduced solubilization of pectic substances (Figure 1C).

In addition to the change in the content of endo-PG and PME enzymes, fruits that were subjected to cold shock treatment at -4 °C/3h and showed early wolliness symptoms also had a reduction in the content of PL, Exp. and β-Gal enzymes (Table 2), indicating that these enzymes may also be related to this chilling injury.

According to Obenland et al. (2003), in addition to involving changes in PGs and PME activity, woolliness growth is accompanied by a decline in gene expression and expansin accumulation. In addition, several enzymes also, involved in the process of cell wall degradation, show low activity in woolliness fruits, such as endo-1,4-β-glucanases, endo-1,4-β-mannanases, β-galactosidases and α-arabinosities (Lurie, 2021). However, the participation of these enzymes in the process of woolliness growth has not yet been fully elucidated.

Internal browning symptoms of the pulp were observed on the second day of analysis (20+3). On this day, fruits treated with cold shock showed higher browning indices compared to the control (Table 3), indicating that cold stress also anticipates the development of this injuries.

There was an increase in PPO enzyme activity in all treatments both during refrigerated storage (Figure 2A) and after three days of commercialization (Figure 2B). Similar result can be observed for the enzyme POD during refrigerated storage (Figure 2C). When fruits were removed from cold storage and left at room temperature for commercialization, fruits from the -2 °C/3h, -4° C/1h, and -4° C/2h treatments showed a peak in POD activity on day 20+3 (Figure 2D). During the refrigerated storage the PAL enzyme activity remained constant until day 20 for all treatments, on day 30 there was a significant increase in activity, being more evident in the treatments -2 °C/1h, -2 °C/2h, and -2 °C/3h (Figure 2E), this activity remained high after the removal of the fruits from the refrigerated storage (Figure 2F).

Table 3. Internal browning index of 'Douradão' peaches submitted to cold shock treatment, stored under refrigeration and evaluated after three days at room temperature (22 °C).

Treatments	Days*		
	10+3	20+3	30+3
Control	0.00	2.08	17.19
-2 °C/1h	0.00	16.67	34.38
-2 °C/2h	0.00	8.33	14.06
-2 °C/3h	0.00	8.33	18.75
-4 °C/1h	0.00	16.67	17.19
-4 °C/2h	0.00	12.50	18.75
-4 °C/3h	0.00	12.50	31.25

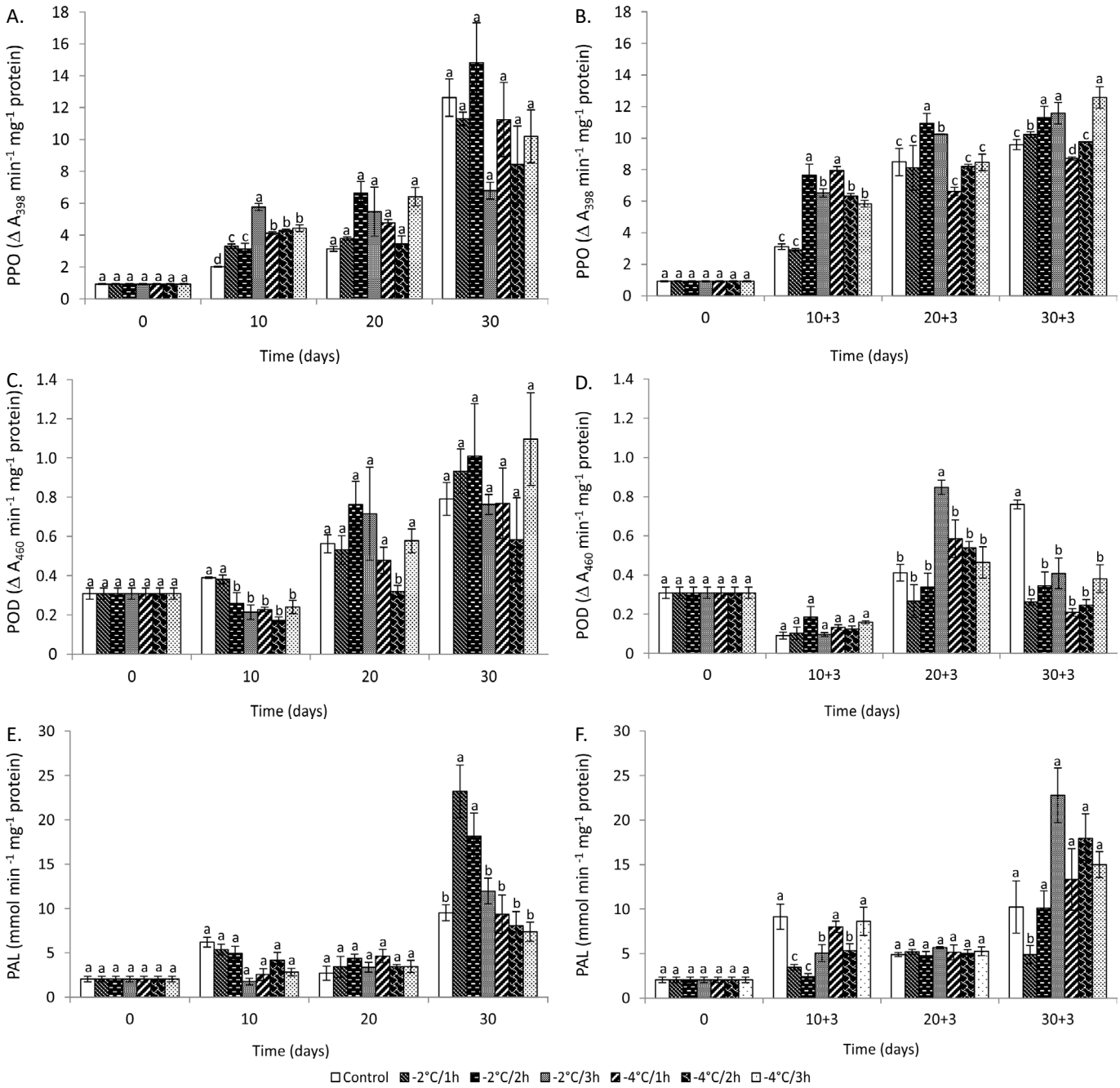
* Analysis days: 10 days at 1°C and 3 days of commercialization (10+3); 20 days at 1°C and 3 days of commercialization (20+3); and, 30 days at 1°C and 3 days of commercialization (30+3).

The amount of phenolic compounds present in the fruit and the activity of PPOs are determinants of the browning potential of the fruit of a given cultivar. This relationship occurs because, unlike wilt, internal browning is caused by the change in permeability of plasma membranes, which gradually increases during storage, allowing PPOs to contact their substrates (Gao et al., 2018). In the present study, an increase in electrolyte leakage was observed throughout the storage period (Figure 3A), indicating an increase in membrane permeability and coinciding with an increase in the browning index (Table 3), which was more evident in the treatments with cold shock at -2 °C/1h and -4 °C/1h, which provided greater contact between the browning enzymes and the substrates, indicating loss of membrane integrity. In this work it was also observed that the increase in electrolyte leakage (loss of membrane integrity) caused by cold shock is more related to internal browning of the pulp than the activity of the enzymes PPO, POD, and PAL.

The gradual increase in electrolyte leakage in kiwifruit subjected to heat shock, was also observed by Yang et al. (2019), however unlike in the present work, the authors observed that fruits that were subjected to cold shock, showed less electrolyte extravasation and consequently fewer symptoms of chilling injury.

Cold shock caused acceleration in the development of rots, which was evident in the -2 °C/1h, -2 °C/3h, and -4 °C/2h treatments, which had 16.67, 8.34, and 8.34% of rotten fruit on day 10+3 (Figure 3B).

In higher plants, POD is associated, among other functions, with disease resistance by forming bonds between phenolic compounds (Prasannath, 2017). In addition to pulp browning the enzymes PAL, POD and PPO are considered key enzymes of disease resistance and control (Kumari & Vengadaramana, 2017). For this reason, probably the -2 °C/1h, -2 °C/2h, -2 °C/3h, and -4°C/2h treatments that showed lower PAL activities on day 10+3 (Figure 2F), were the treatments that manifested rot symptoms. PAL is the key enzyme in the activation of phenylpropanoid metabolism and an increase in its activity is associated with the biosynthesis of active metabolites such as phytoalexins, phenols, lignins, and salicylic acid that are involved in plant defense metabolism (Prasannath, 2017). According to the same author, POD participates in the processes of building and maintaining cell walls by oxidizing phenols, aiding in the suberization and lignification of attacked cells during reaction against pathogens, and PPO is involved in the oxidation of polyphenols to quinones (antimicrobial compounds) and lignification of plant cells during microbial invasion.



Vertical bars represent the standard error of the average (n = 3). [1] Averages followed by equal letters within each evaluation date do not differ by the Scott-Knott test at 5% significance level.

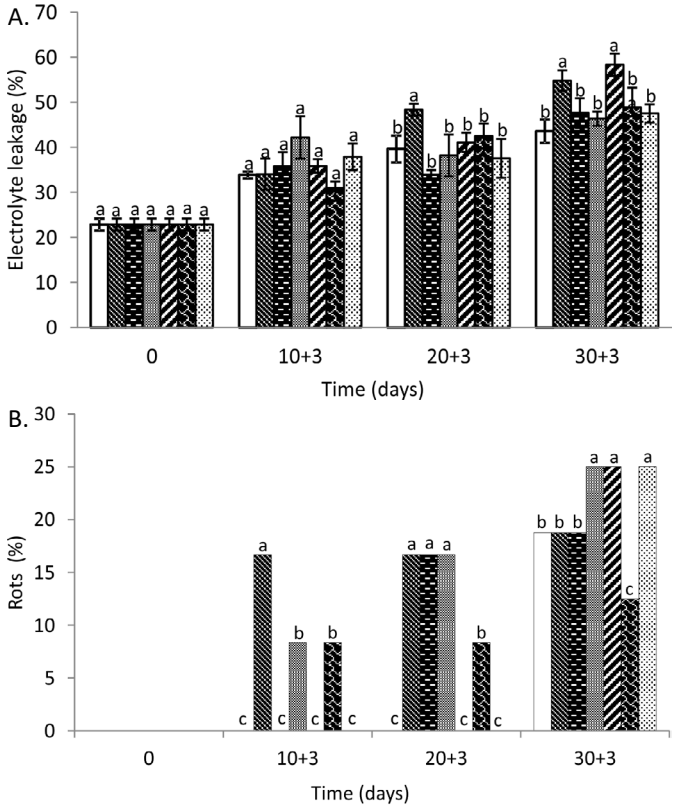
Figure 2. Activity of the enzymes polyphenoloxidase (PPO), peroxidase (POD), and phenylalanineammonia-lyase (PAL) in 'Douradão' peaches submitted to cold shock before refrigerated storage. (A), (C), and (E) are values right after removing the fruit from cold storage and (B), (D), and (F) are values after 3 days at room temperature (22 °C).

The physical and chemical variables were influenced only by the time of refrigerated storage and the averages for each day of evaluation are presented in Table 4.

A significant reduction in firmness and increase in soluble solids content was observed from day 0 to day 10+3. Titratable acidity, ascorbic acid, and rind color values, represented by the color angle (Hue) were decreasing for all treatments throughout the storage period (Table 4).

The variations observed in the physical and chemical characteristics, probably, are due to the ripening of the fruits by exposing them to the three-day period at room temperature. The reduction in observed Hue values (Table 4) also evidences that the fruits matured after the commercialization period, turning from green to yellow.

In contrast to what was observed in the present work, some research revealed that nectarine and cherry fruits can preserve their physiological and commercial qualities without showing cold injury symptoms when they underwent cold stress of -1.4 and -1.9 °C, respectively (Zhao et al., 2019a; Zhao et al., 2019b). Thus, the hypothesis that cold shock at the temperatures studied increases the shelf life of 'Douradão' peaches was denied.



Vertical bars represent the standard error of the average (n = 4). [1] Averages followed by equal letters within each evaluation date do not differ by the Scott-Knott test at 5% significance level.

Figure 3. Flesh electrolyte leakage (A) and percentage of rots (B) in 'Douradão' peaches subjected to cold shock treatment before refrigerated storage at 1 °C and exposure to room temperature 22 °C (10+3, 20+3 and 30+3).

Table 4. Average of physical and chemical variables of 'Douradão' peaches submitted to cold shock treatment, stored under refrigeration and evaluated after three days at room temperature (22 °C).

Variables	Days**				CV (%)
	0	10+3	20+3	30+3	
Firmness (N)	44.34 c	6.17 b	4.12 a	3.75 a	27.90
Soluble solids (°Brix)	9.77 a	12.07 b	11.78 b	11.60 b	7.08
Titratable acidity (%)	0.37 c	0.26 b	0.26 b	0.14 a	8.44
Ascorbic acid (mg $100g^{-1}$)	1.34 c	0.84 b	0.67 a	0.64 a	16.89
Hue angle (°h)	107.94 b	85.53 a	87.01 a	79.35 a	17.96

[1] Averages followed by equal letters in the row do not differ by the Scott-Knott test at 5% significance level.
* Analysis days: 10 days at 1°C and 3 days of commercialization (10+3); 20 days at 1°C and 3 days of commercialization (20+3); and, 30 days at 1°C and 3 days of commercialization (30+3).

Conclusions

The application of cold shock treatment anticipates the appearance of chilling injury and rot symptoms in 'Douradão' peaches.

Cold shock before refrigerated storage reduces the amount of pectinolytic and cell wall degradation enzymes endo-polygalacturonase, pectinamethylesterase, pectin lyase, expansin and β-galactosidase in 'Douradão' peaches and anticipates the appearance of woolliness symptoms.

Due to the negative influence on chilling injury cold shock is not recommended for increasing the shelf life and maintaining the quality of 'Douradão' peaches.

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Compliance with Ethical Standards

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