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Chitosan Coating: A Postharvest Treatment to Delay Oxidative Stress in Loquat Fruits during Cold Storage

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Received: 14 March 2018; Accepted: 17 April 2018; Published: 19 April 2018

Abstract: Loquat is a non-climacteric fruit consumed fresh for its essential nutrients and phytochemical compounds. In this study, the effects of chitosan coating (1% *w*/*v*) on changes in the enzymatic antioxidant and membrane damage in three loquat selections (CREAFRC-S18; CREAFRC-S35 and CREAFRC-S36) and three loquat cultivars (Golden Nugget, Algerie and Nespolone rosso di Trabia) stored at 7 °C over 21 days were evaluated. Chitosan treatment enhanced the activities of superoxide dismutase, catalase and ascorbate peroxidase. Moreover, this treatment inhibited polyphenol oxidase and guaiacol peroxidase activities, extending the storage life of loquat. Chitosan also preserved membrane integrity by inhibiting lipoxygenase activity and malondialdehyde accumulation. Principal component analysis provided a global view of the responses of both loquat selections and cultivars to the postharvest chitosan coating and storage temperature. These findings suggest that chitosan treatment could be a valid tool for improving the activity of antioxidant enzymes, preserving the enzymatic browning of loquat fruits.

Keywords: chitosan; antioxidant enzymes; membrane damage; principal component analysis

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a non-climacteric fruit that is exclusively consumed fresh for its essential nutrients and phytochemical compounds, which provide significant health benefits. This fruit is highly perishable, has a short postharvest life, and is susceptible to browning, chilling injury, purple spot and microbial decay [1–3].

Cold storage is the postharvest practice applied commercially in loquat fruit, but loquat is very sensitive to low temperatures from 0 to 10 °C, exhibiting cultivar-dependent chilling injury (CI) after a long cold-storage period [1–3].

Several studies have demonstrated that CI in fruits is the result of postharvest oxidative stress due to an imbalance between the production and scavenging of reactive oxygen species (ROS) by antioxidant compounds and enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Previous studies have shown a positive relationship between antioxidant enzyme activity and chilling tolerance in several fruits [4].

Different postharvest treatments, including 1-methylcyclopropene (1-MCP), hot air, low-temperature conditioning, modified-atmosphere packaging, methyl jasmonate, and coating in combination with cold storage, have been applied to reduce deterioration and CI incidence, prolong shelf life and retain the nutritional value of loquat and other fruits [1,2,5–10]. Edible coatings

act as physical barriers on fruit surfaces and decrease permeability to O_2, CO_2 and water vapour, leading to a reduction in respiration rates, fruit ripening and senescence [11]. Chitosan-based coatings have been considered an environmentally friendly coating with antimicrobial action that can extend the shelf life of several fruits [2,11].

The effectiveness of edible coatings on fruit organoleptic quality, based on chitosan and sucrose ester fatty acids, has already been tested on loquats [2,12]. A few studies have been conducted on the effects of chitosan coatings on the behaviour of the enzymatic antioxidant system in some fruit crops, such as sweet cherry and strawberry [13–15].

The effects of chitosan coatings combined with cold storage on postharvest oxidative stress in loquat fruit were investigated for the first time in this paper. The aims of this study were to analyse the effect of chitosan coatings on the enzymatic antioxidants and membrane integrity maintenance of three loquat cultivars and three loquat selections.

2. Materials and Methods

2.1. Fruit Samples

Three loquat cultivars ('Algerie', 'Nespolone Rosso di Trabia' and 'Golden Nugget') and three advanced selections resulting from the CREA-OFA (Caserta, Italy) breeding program (CREAFRC-S18; CREAFRC-S35 and CREAFRC-S36) were selected for this study. The fruits were hand-harvested at the commercial ripening stage in 2014 from trees grown using standard commercial practices and trained in the same experimental orchard located in Caserta, Southern Italy (41°09′ N, 14°08′ E), and owned by the CREA-OFA (Caserta, Italy). At harvest, all analysed cultivar/advanced selections showed a minimum total soluble solid value, often required for commercialisation, ranging from 9 to 10° Brix. The fruits were then randomly distributed into two groups prior to treatment. Chitosan (Iko Hydro, Rutigliano, Italy) with 90% deacetylation and a molecular weight of 360 kDa was prepared at 1% (w/v) as described by Petriccione et al. [16]. The chosen chitosan concentration, adopted in this study emerged from our preliminary analysis, which tested three different chitosan concentrations (0.5, 1 and 2%), finding that the chitosan coating at 1% gave the best results for this fruit crop. Loquat fruits were dipped in the chitosan solution for 60 s to allow chitosan to adhere to the whole fruit surface, forming a uniform coating; the same was done to control fruits dipped in distilled water. Samples were dried at 25 °C for 1 h and stored in a controlled chamber at 7 °C and 95% relative humidity. Fruits were removed after 7, 14 and 21 days of cold storage. The chosen temperature adopted in this study emerged from our preliminary analysis verifying that a 7 °C cold storage regime avoided the appearance of chilling injury in these genotypes. For each sampling date, three biological replicates per treatment and per cultivar/selection were prepared. Three lots of ten fruits per cultivar/advanced selection per timing were prepared. The same number of lots was prepared for the control fruits dipped in distillate water. All analyses were performed at each sampling date during cold storage on two technical replicates for each biological replicate.

2.2. Enzyme Extraction and Activity Assays

Total soluble proteins were extracted by resuspending 1 g of frozen loquat fruit tissue powder, including skin and pulp, in 3 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM sodium-ethylenediaminetetraacetic acid (EDTA) (pH 7) and 5% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 18,000$\times$ g for 10 min at 4 °C. The resulting supernatant was used for catalase (CAT; EC 1.11.1.6), ascorbate peroxidise (APX; EC 1.11.1.11), guaiacol peroxidase (POD; EC 1.11.1.7) and superoxide dismutase (SOD; EC 1.15.1.1) activity determinations.

The protein content for all examined crude enzyme extracts was estimated by the Bradford assay [17] using bovine serum albumin as a standard.

CAT, APX, POX and SOD activities were assayed according to the method described by Pasquariello et al. [15]. The specific activities were expressed as µmol H_2O_2 per kg per sec, µmol ascorbate per kg per sec, µmol tetraguaiacol per kg per sec and U per kg, respectively.

Polyphenoloxidase (PPO) activity was determined following the method described by Pasquariello et al. [15] with some modifications. A total of 2 g of whole fruit was homogenized in 5 mL of 200 mM sodium phosphate buffer (pH 6.5) containing 5% (*w*/*w*) PVPP. The homogenate was centrifuged at 12,500× *g* for 10 min at 4 °C and was used for PPO activity determination. The specific activity was expressed as µmol catechol per kg per sec.

Lipoxygenase (LOX) activity was quantified following the method described by Pasquariello et al. [15], and the activity was expressed as nmol hydroperoxides per kg per sec.

2.3. Malondialdehyde Content Determination

The level of lipid peroxidation was also determined by measuring malondialdehyde (MDA) accumulation in the fruit tissue. The malondialdehyde content was evaluated following the method of Pasquariello et al. [15]. The level of lipid peroxidation was expressed as nmol of MDA formed per kg fresh weight (FW) and calculated in agreement with Bao et al. [18].

2.4. Statistical Analysis

All of the data are expressed as the mean ± standard deviation (S.D). Differences between means in uncoated and chitosan-coated fruits were assessed by ANOVA and Duncan's test, with differences being considered significant at $p \leq 0.05$. Correlations among the evaluated parameters were analysed using Pearson's correlations ($p < 0.05$ and $p < 0.01$). Principal component analysis (PCA) was applied to describe the relationship between the different enzymes and to identify the principal components that accounted for the majority of the variation within the dataset. The factors were orthogonally rotated following the Varimax method with Kaiser normalisation, thus maximising the sum of the variances of the squared loadings. All analyses were performed using the SPSS software package, version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Effect of Chitosan Coating on Antioxidant Enzyme

Chitosan coating combined with cold storage was able to preserve fruit quality, delaying physiological and biochemical changes that occur during postharvest life. Furthermore, chitosan coating may prevent oxidation reactions, playing an important role in strengthening the antioxidant system of fruit [19]. Oxidative stress occurs when the generation of ROS exceeds the capacity of a cell to maintain cellular redox homeostasis. In postharvest, fruits can rarely avoid oxidative stress, but some treatments can help reduce ROS over-production, therefore delaying oxidative damage.

To counteract oxidative stress caused by an imbalance of ROS, plants have evolved an efficient antioxidative defence system, including different types of enzymatic and non-enzymatic systems that scavenge/detoxify ROS [19]. Enzymatic antioxidants include SOD, CAT and APX, which serve as frontline defence antioxidants. SOD catalyses the dismutation of O_2^- to H_2O_2, CAT dismutates H_2O_2 to oxygen and water, and APX reduces H_2O_2 to water by utilising ascorbate as an electron donor. The balance between the SOD and APX or CAT activities in cells is crucial for determining the steady-state level of O_2^- and H_2O_2 [19].

CAT and APX are key enzymes in modulating the level of H_2O_2. They both catalyse the decomposition of hydrogen peroxide to form oxygen and water, but use different mechanisms. Specifically, APX, contrary to CAT, uses ascorbate as an electron donor, which requires an ascorbate regeneration system—the ascorbate-glutathione cycle [20].

The activities of the CAT and APX enzymes in loquat fruit showed continuous decreasing trends throughout the storage period with significantly ($p \leq 0.05$) higher values in chitosan-coated fruit

(Table 1). In coated fruit at the end of storage, the higher values of CAT activity were registered in Algerie and CREAFRC-S36, while for APX activity they were registered in Nespolone rosso di Trabia and CREAFRC-S36.

Table 1. Activity of catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD), on the three loquat cultivars/selections at harvest (0) and after 7, 14 and 21 days of cold storage for chitosan-coated (Chitosan) and uncoated fruits (Control).

Cultivar/Selection	Storage Time (Days)	Treatment	CAT (μmol kg^{-1} s^{-1})	APX (μmol kg^{-1} s^{-1})	SOD (U 10^3 kg^{-1})
Algerie	0	Control	157.80 ± 9.81 e	59.52 ± 0.17 g	6.18 ± 0.38 a
		Chitosan	157.80 ± 9.81 e	59.52 ± 0.17 g	6.18 ± 0.38 a
	7	Control	124.63 ± 10.14 c	44.77 ± 0.21 d	8.78 ± 0.23 e
		Chitosan	147.97 ± 9.95d e	55.44 ± 0.51 f	6.76 ± 0.22 b
	14	Control	104.60 ± 9.81 b	35.15 ± 0.10 c	9.51 ± 0.17 f
		Chitosan	139.07 ± 9.67 cd	48.80 ± 0.31 e	7.50 ± 0.16 c
	21	Control	90.40 ± 11.00 a	25.50 ± 0.15 a	10.21 ± 0.31 g
		Chitosan	125.73 ± 9.80 c	33.00 ± 0.18 b	8.21 ± 0.33 d
Golden Nugget	0	Control	137.77 ± 10.16 e	70.77 ± 0.31 g	5.02 ± 0.12 a
		Chitosan	137.77 ± 10.16 e	70.77 ± 0.31 g	5.02 ± 0.12 a
	7	Control	103.37 ± 9.55 c	52.37 ± 0.17 d	8.96 ± 0.18 d
		Chitosan	124.37 ± 10.19 de	64.33 ± 0.22 f	6.29 ± 0.22 b
	14	Control	83.30 ± 10.16 b	38.94 ± 0.17 a	11.16 ± 0.27 e
		Chitosan	111.07 ± 9.96 cd	55.11 ± 0.10 e	7.84 ± 0.17 c
	21	Control	57.83 ± 10.30 a	28.91 ± 0.23 a	11.63 ± 0.13 f
		Chitosan	85.70 ± 10.26 b	36.23 ± 0.21 b	9.02 ± 0.20 d
Nespolone Rosso di Trabia	0	Control	162.17 ± 9.45 e	63.59 ± 0.39 f	5.20 ± 0.13 a
		Chitosan	162.17 ± 9.45 e	63.59 ± 0.39 f	5.20 ± 0.13 a
	7	Control	114.77 ± 10.16 c	50.44 ± 0.21 d	8.10 ± 0.12 d
		Chitosan	140.17 ± 10.16 d	57.22 ± 0.11 e	5.70 ± 0.41 b
	14	Control	73.97 ± 9.70 ab	38.30 ± 0.22 b	9.01 ± 0.11 e
		Chitosan	113.73 ± 9.81 c	50.80 ± 0.32 d	6.85 ± 0.26 c
	21	Control	57.13 ± 9.97 a	34.61 ± 0.34 a	10.31 ± 0.16 f
		Chitosan	79.63 ± 9.50 b	44.80 ± 0.17 c	8.04 ± 0.18 d
CREAFRC-S18	0	Control	211.77 ± 10.12 e	60.28 ± 0.39 g	11.69 ± 0.32 a
		Chitosan	211.77 ± 10.12 e	60.28 ± 0.39 g	11.69 ± 0.32 a
	7	Control	139.20 ± 10.00 c	50.33 ± 0.38 e	17.72 ± 0.33 e
		Chitosan	191.50 ± 9.65 d	57.97 ± 0.81 f	14.84 ± 0.24 b
	14	Control	114.77 ± 9.90 b	29.88 ± 0.22 c	18.84 ± 0.24 f
		Chitosan	154.57 ± 9.60 c	43.36 ± 0.45 d	16.21 ± 0.13 c
	21	Control	85.80 ± 10.35 a	16.92 ± 0.17 a	19.19 ± 0.16 f
		Chitosan	110.40 ± 10.50 b	25.88 ± 0.22 b	16.71 ± 0.34 d
CREAFRC-S35	0	Control	188.67 ± 9.95 e	57.08 ± 0.23 e	10.38 ± 0.42 a
		Chitosan	188.67 ± 9.95 e	57.08 ± 0.23 e	10.38 ± 0.42 a
	7	Control	141.73 ± 9.55 c	50.24 ± 0.19 d	13.59 ± 0.34 d
		Chitosan	165.83 ± 10.40 d	60.29 ± 0.39 f	11.15 ± 0.17 b
	14	Control	113.47 ± 10.20 b	38.23 ± 0.28 c	15.00 ± 0.19 e
		Chitosan	146.47 ± 10.10 c	50.62 ± 0.39 d	12.16 ± 0.17 c
	21	Control	91.33 ± 10.11 a	15.08 ± 0.12 a	15.59 ± 0.34 f
		Chitosan	111.33 ± 9.75 b	30.22 ± 0.20 b	13.65 ± 0.38 d
CREAFRC-S36	0	Control	158.97 ± 10.30 e	51.75 ± 0.46 f	10.51 ± 0.16 a
		Chitosan	158.97 ± 10.30 e	51.75 ± 0.46 f	10.51 ± 0.16 a
	7	Control	131.43 ± 10.05 cd	37.05 ± 0.16 d	14.31 ± 0.33 e
		Chitosan	152.80 ± 9.80 e	51.23 ± 0.11 f	11.44 ± 0.30 b
	14	Control	110.53 ± 10.10 b	29.92 ± 0.13 b	15.59 ± 0.34 f
		Chitosan	144.67 ± 10.20 de	40.90 ± 0.11 e	12.74 ± 0.17 c
	21	Control	83.10 ± 9.75 a	26.68 ± 0.33 a	16.48 ± 0.28 g
		Chitosan	120.73 ± 9.95 bc	34.25 ± 0.25 c	13.66 ± 0.38 d

Values followed by the same letter within the same column were not significantly different according to Duncan's test ($p \leq 0.05$).

Chitosan postharvest treatment combined with low-temperature storage improved the CAT and APX activities, avoiding the development of chilling injury due to the over-production of ROS. This trend is confirmed by previous studies on other commodities [11]. The higher APX activity observed during storage in coated fruit could be due to the high availability of ascorbic acid, as previously demonstrated in these cultivars/selections by Petriccione et al. [12].

The effective removal of H_2O_2 occurred via cooperative mediation between CAT and APX enzymes. CAT has a high catalytic rate, but a low affinity towards the substrate [21], whereas APX has a higher affinity, but requires a sufficient amount of reducing power [22,23].

In all loquat cultivars/selections, the SOD activity increased with time in cold storage. Chitosan-coated fruit showed a significantly ($p \leq 0.05$) lower level of SOD activity than uncoated fruit. The highest SOD activity was found in Golden Nugget and CREAFRC-S38 among the analysed cultivars and selections, respectively. (Table 1).

The increase in SOD could be from the dismutation of O_2^- generated in response to postharvest oxidative stress, adjusting the levels of O_2^- and H_2O_2.

The effect of chitosan coating on SOD activity is comparable to 1-MCP or methyl-jasmonate postharvest treatment on loquat fruits and on other fruit crops, such as peach, plum, pear and sweet cherry [24,25].

Our results are in agreement with Song et al. [26], who showed that chitosan/nano-silica treatments induced the highest activities of antioxidant enzymes (SOD, CAT and APX) in cold-stored loquat fruits.

3.2. Effect of Chitosan Coating on Membrane Damage

In addition to investigating the antioxidant enzyme activities, we also examined the effects of postharvest chitosan treatment on membrane damage resulting from lipid peroxidation. LOX plays an important role in generating peroxidative damage in membrane lipids of plant tissues because is it partly responsible for the formation of O_2^- and singlet oxygen [27].

LOX activity increased gradually as the storage period progressed, but chitosan coating significantly ($p \leq 0.05$) delayed this increase in all analysed loquat cultivars/selections (Figure 1a). Our results suggest that in uncoated fruit, the increase of LOX activity could be due to the accumulation of H_2O_2, which accelerates the destruction of the cell membrane structure and accelerates the development of pericarp browning in loquat cultivars/selections, as previously demonstrated by Petriccione et al. [12]. Furthermore, chitosan postharvest treatment prevents membrane damage.

The MDA content further indicated membrane damage and it is has been evaluated [28]. MDA is one of the intermediate products of lipid peroxidation and is a good indicator of membrane integrity [29].

The MDA content in all analysed loquat fruit increased with storage time, but chitosan-coated fruit showed a significantly lower MDA content than uncoated fruit in all analysed loquat cultivars/selections (Figure 1b). The lowest values for MDA content and LOX activity were registered in Golden Nugget at the end of cold storage in coated fruits.

The reduced accumulation of MDA content and low LOX activity in chitosan-coated loquat fruits suggest that chitosan could be an effective system for preventing oxidative damage during cold storage. Our results confirmed the results of previous studies that demonstrated chitosan postharvest treatment preserves the cellular membrane structure integrity in different commodities [14,15].

Furthermore, Xi et al. [30] demonstrated that a combined chitosan and nano-SiOx coating maintained a higher content of sodium carbonate-soluble pectin, and inhibited pectin chain degradation in Chinese cherry. Postharvest treatment improved the cellular membrane structure integrity, reducing the activity of pectin degrading enzymes [30] or the integrity of SSP via interactions between SSP and calcium ions or protonated chitosan groups [31].

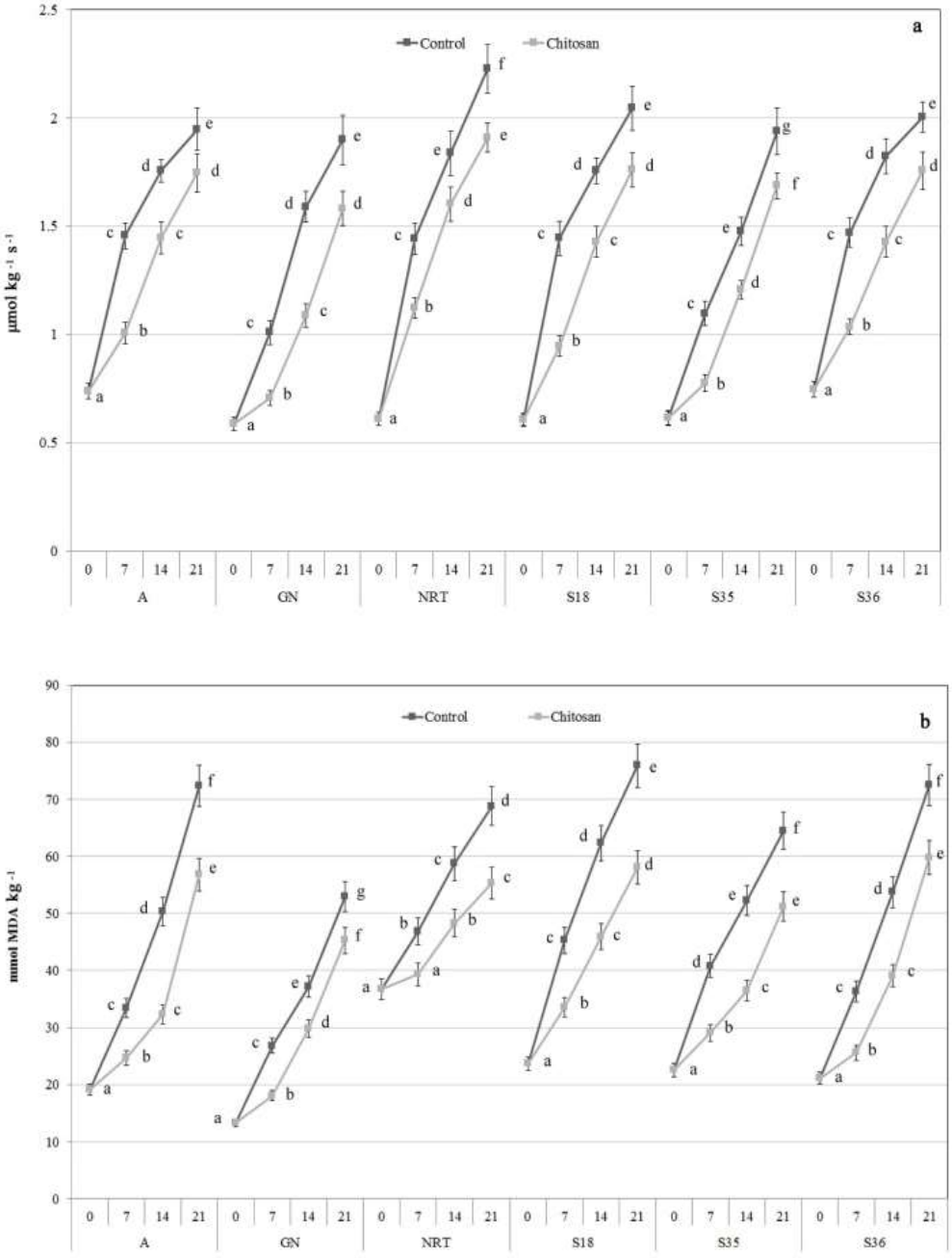

Figure 1. Evaluation of lipoxygenase (LOX) activity (**a**) and malondialdehyde content (MDA) (**b**) on the three loquat cultivars/selections ("Algerie" (A), "Golden Nugget" (GN), "Nespolone Rosso di Trabia" (NRT),"CREAFRC-S18" (S18), "CREAFRC-S35 (S35) and "CREAFRC-S36" (S36)) at harvest (0) and after 7, 14 and 21 days of cold storage for chitosan-coated (Chitosan) and uncoated fruits (Control). Error bars indicate standard deviation.

3.3. Effect of Chitosan Coating on Enzymatic Browning

Flesh browning reduces the commercial value and is considered to be a serious problem for postharvest storage and processing of loquat fruit [3].

Browning is mainly caused by enzymatic oxidation, involving the oxidation and polymerisation of phenolic compounds by PPO and POD. The first enzyme catalyses the oxidation of phenols to quinones that in turn polymerize into undesirable brown pigments. PPO activity increases when cells are damaged, intracellular compartmentation is disrupted and phenolic substrates are released from

vacuoles [32]. POD oxidises several antioxidant compounds in the presence of hydrogen peroxide as an electron acceptor, and its involvement in the browning of various fruits and vegetables has been reported [33].

Our results demonstrated that POD and PPO activity in all loquat cultivars and advanced selections increased during storage time, with lower values in coated fruits. In loquat fruits, the postharvest chitosan treatment significantly ($p \leq 0.05$) reduced the PPO (Figure 2a) and POD activities (Figure 2b), with an average decrease of 18.9% and 17.1% at the end of cold storage, respectively. The lowest activity was detected in Nespolone rosso di Trabia for POD activity and in Nespolone rosso di Trabia and Golden Nugget for PPO activity.

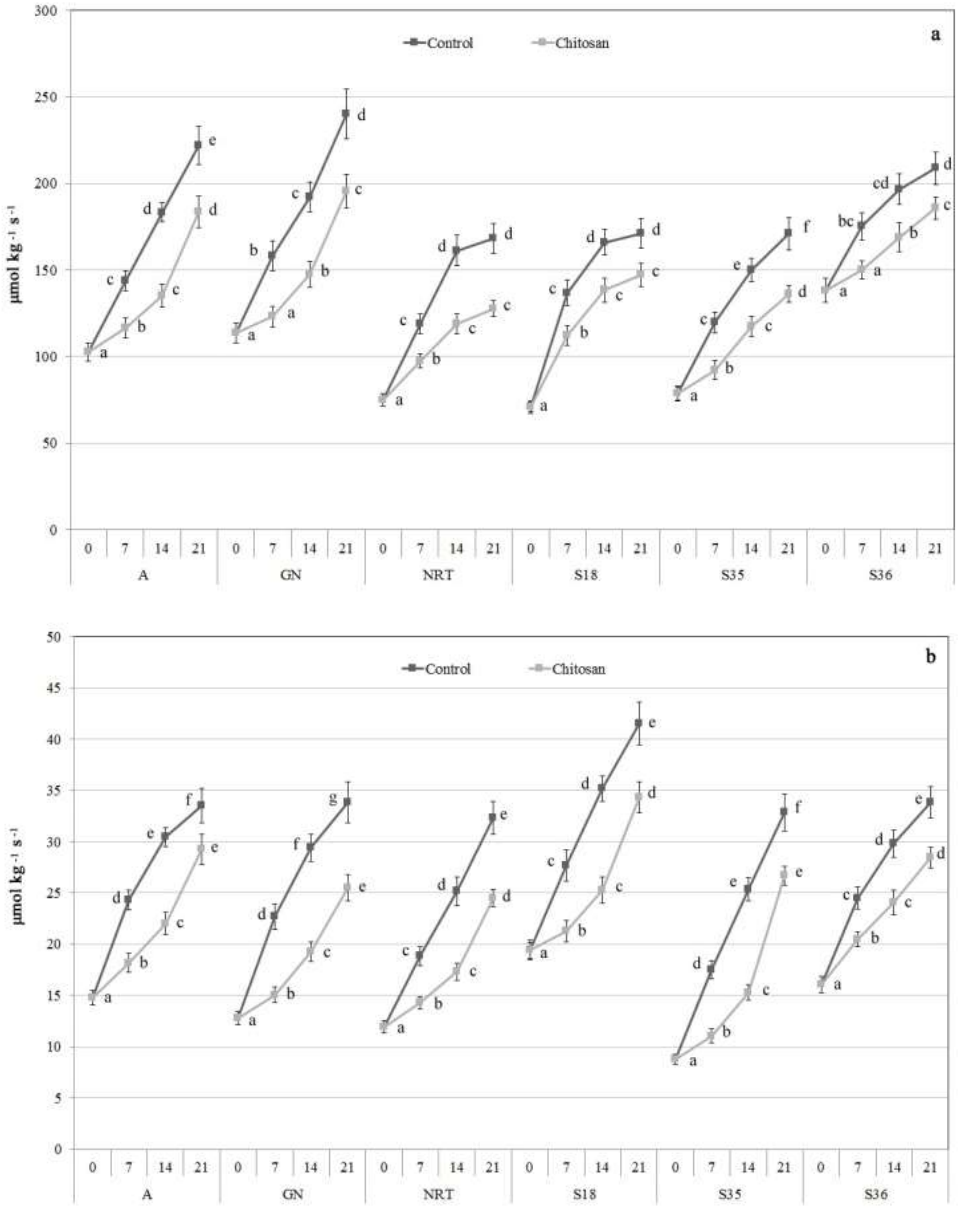

Figure 2. Evaluation of guaiacol peroxidase (POD) (**a**) and polyphenol oxidase (PPO) (**b**) activity on the three loquat cultivars/selections ("Algerie" (A), "Golden Nugget" (GN), "Nespolone Rosso di Trabia" (NRT),"CREAFRC-S18" (S18), "CREAFRC-S35 (S35) and "CREAFRC-S36" (S36)) at harvest (0) and after 7, 14 and 21 days of cold storage for chitosan-coated (Chitosan) and uncoated fruits (Control). Error bars indicate standard deviation.

The increase in the PPO and POD activities observed in loquat fruits throughout the entire cold storage period explains the decrease in total phenolic compounds observed in our previous study carried out on the same loquat cultivars and advanced selections [12]. Previous studies have demonstrated that the increase in PPO activity was associated with higher tissue browning and a significant reduction in total phenolic compounds in loquat, sweet cherry and pear fruits [12,16,34]. Chitosan coating effectively delayed tissue browning in loquat fruit [12] due to a lower PPO and POD activity.

The lower activities of the PPO and POD enzymes could be explained by the lower rates of oxygen permeability into chitosan-coated loquat fruits [35]. Furthermore, chitosan-coated fruits showed a lower LOX activity and MDA content, suggesting that chitosan treatment helps to prevent the loss of cellular compartmentalisation, which delays the browning and separation of PPO and POD enzymes from their substrates. Similarly, inhibition of PPO and POD activities due to several postharvest treatments, such as 1-MCP, acetylsalicylic acid and methyl-jasmonate, on loquat and other fruits has been demonstrated [1,13,36–39].

3.4. PCA and Pearson Correlation after Cold Storage

A PCA was applied to assess the effectiveness of chitosan coating and cold storage on three loquat cultivars and three advanced selections by analysing the activity of enzymes correlated with oxidative stress and membrane damage. Analysing the eigenvalues of the covariance matrix, we observed that the two principal components (PCs) were able to account for 88.4% of the total variance in the dataset. PC1 explained 68.16% of the variance in the dataset, whereas PC2 explained an additional 20.25% of the variance. PC1 had high positive loading for the LOX, PPO and POD activities and MDA content, as well as high negative loading for the CAT and APX activities. PC2 was only positively correlated with the SOD activity. The PCs allowed a clear separation between these variables, identifying their relationships (Figures 3 and 4).

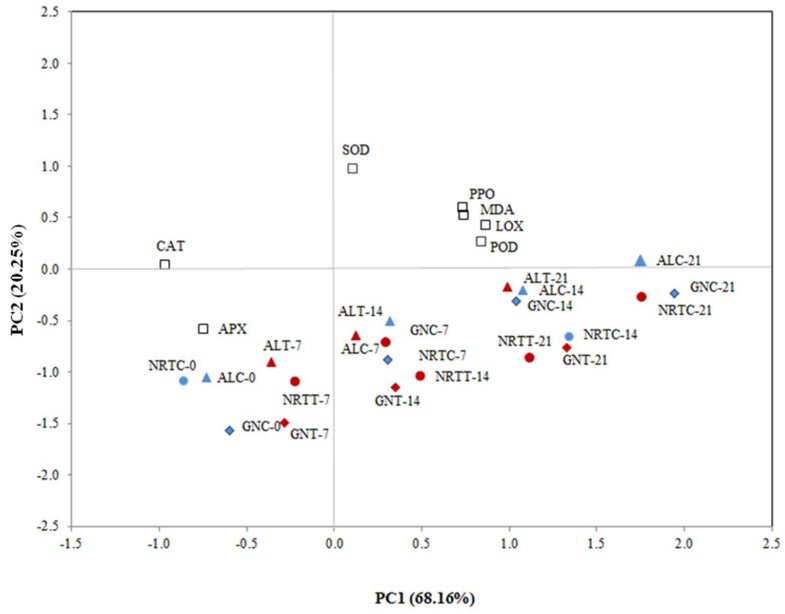

Figure 3. Two-dimensional principal component analysis (PC1: 68.16%, PC2: 20.25%) of the antioxidant enzyme activities and MDA content in the three loquat cultivars ("Golden Nugget" (GN), "Algerie" (AL), and "Nespolone Rosso di Trabia" (NRT)) during cold storage, at harvest (C0), after 7 days (C-7), 14 days (C-14), and 21 days (C-21) for uncoated fruit, and at harvest (T-0), after 7 days (T-7), after 14 days (T-14), and after 21 days (T-21) for chitosan-coated fruit. (APX = ascorbate peroxidise; CAT = catalase; LOX = lipoxygenase; MDA = malondialdehyde content; POD = guaiacol peroxidase; PPO = polyphenol oxidase; SOD = superoxide dismutase).

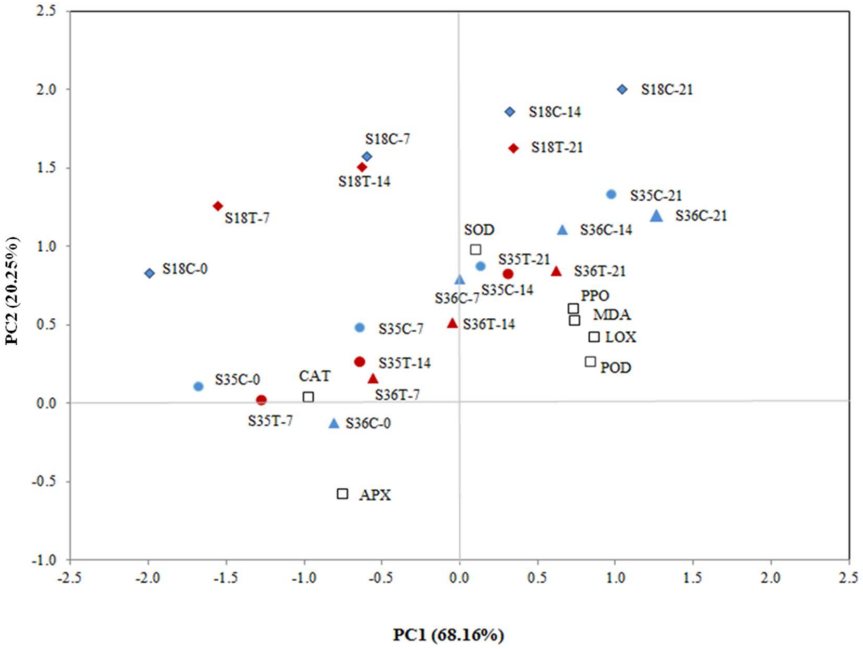

Figure 4. Two-dimensional principal component analysis (PC1: 68.16%, PC2: 20.25%) of the antioxidant enzyme activities and MDA content in the three loquat selections "CREAFRC-S18" (S18), "CREAFRC-S35 (S35) and "CREAFRC-S36" (S36)) during cold storage, at harvest (C0), after 7 days (C-7), 14 days (C-14), and 21 days (C-21) for uncoated fruit, and at harvest (T-0), after 7 days (T-7), 14 days (T-14), and 21 days (T-21) for chitosan-coated fruit. (APX = ascorbate peroxidise; CAT = catalase; LOX = lipoxygenase; MDA = malondialdehyde content; POD = guaiacol peroxidase; PPO = polyphenol oxidase; SOD = superoxide dismutase).

Furthermore, the multivariate space of the first two PCs shows that the enzymatic activities were differently influenced by chitosan treatment during cold storage in loquat fruits. These results are in agreement with our previous studies carried out on sweet cherry and strawberry cultivars [14,15].

The score plot highlights the similarities and differences induced by the chitosan coating in several loquat cultivars and advanced selections during cold storage. Shifts in the average PC scores from negative to positive were observed for all analysed loquat fruits. Loquat cultivars showed a higher shift in score values compared to loquat advanced selections, but both showed lower average PC scores in chitosan-coated fruits than uncoated ones. This suggests that as the time in cold storage increased, loquat cultivars exhibited higher oxidative stress than the advanced selections. Furthermore, the rate of oxidative damage of chitosan-coated loquat fruits after 21 days of cold storage was comparable to that of uncoated fruits at 14 days.

Chitosan treatment and cold storage improve the storability of loquat fruits in a cultivar-dependent manner, delaying the appearance of oxidative stress damage. As demonstrated by previous studies on several fruits, chitosan treatment could increase the activities of oxygen-scavenging enzymes, decreasing ROS production, which is responsible for oxidative stress and alters the autocatalytic lipid degradation pathway [14,15].

A correlation-based approach using the Pearson coefficient was adopted to evaluate the positive and negative relationships between the MDA content and enzyme activities, such as CAT, SOD, APX, LOX, PPO and POD, for coated and uncoated loquat fruits during cold storage. Significant positive correlations (in blue) and negative correlations (in red) are displayed in Figure 5.

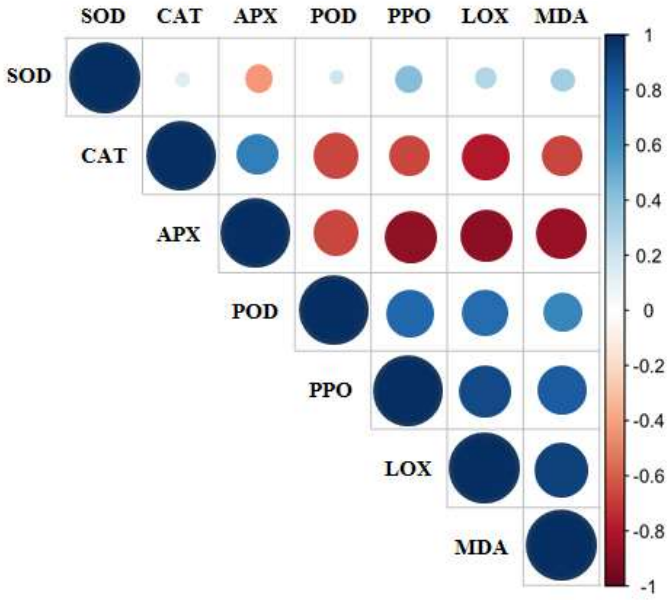

Figure 5. Correlation matrix based on Pearson's correlation coefficients between APX (ascorbate peroxidase), CAT (catalase), SOD (superoxide dismutase), LOX (lipoxygenase), MDA (malondialdehyde content), POD (guaiacol peroxidase), and PPO (polyphenol oxidase). The circle size and colour intensity are proportional to the correlation coefficients. Positive correlations are displayed in blue and negative correlations in red.

The colour intensity and circle size are proportional to the Pearson correlation coefficients. The correlation coefficients indicate that in loquat fruits, the enzymes involved in different metabolic pathways are differentially regulated by postharvest treatments and cold storage, as previously demonstrated in other fruit crops [40–42].

CAT was negatively correlated with APX ($r = -0.430$; $p \leq 0.01$). These results indicate that the CAT and APX enzymes, both of which are responsible for H_2O_2 degradation, together with SOD make up a defence system to scavenge ROS. Furthermore, SOD was positively correlated with POD ($r = 0.200$; $p \leq 0.05$), PPO ($r = -0.427$; $p \leq 0.01$), LOX ($r = 0.313$; $p \leq 0.01$) and the MDA content ($r = 0.360$; $p \leq 0.01$), suggesting that oxidative stress is probably partially responsible for membrane damage during low-temperature storage.

The results show a significant positive correlation between LOX and MDA ($r = 0.910$; $p \leq 0.01$), highlighting that LOX is responsible for oxidative damage to lipids, which forms toxic products, such as MDA. Furthermore, a significant positive correlation between POD and PPO activity ($r = 0.794$; $p \leq 0.01$) was registered. Both enzymes are considered to be directly responsible for enzymatic browning, which in loquat fruit occurs from the core area and is accompanied by lignification of the flesh as the time in cold storage increases [3]. Changes in texture during postharvest can be due to the loss of neutral sugars, as well as cell wall polysaccharide solubilisation and depolymerisation [43]. In loquat fruits, the increase of firmness during postharvest life is also due to an activation of lignin synthesis pathways [3,12].

LOX was also positively correlated with PPO ($r = 0.879$; $p \leq 0.01$) and POD ($r = 0.781$; $p \leq 0.01$); increases in the PPO, POD and LOX activities during cold storage can cause deterioration of the loquat fruit, changing its characteristics [10].

4. Conclusions

Chitosan treatment is a valid and sustainable tool for delaying postharvest oxidative stress, which is responsible for the qualitative decay in these loquat fruits. This treatment can effectively lead to an increased antioxidant enzyme system and reduced loss of cellular compartmentalisation.

Furthermore, chitosan treatment plays a positive role in maintaining higher bioactive compounds content in all analysed cultivars and advanced selections, as demonstrated in our previous study. These findings suggest that chitosan treatment can be used for commercial purposes during the postharvest life of loquat fruit.

Author Contributions: M.S.P., G.A. and M.P. conceived and designed the experiments; L.Z., F.M. performed the experiments; M.S.P., G.A. and M.P. analysed the data; M.P. and M.S. contributed reagents/materials/analysis tools; M.S.P., G.A., M.S. and M.P. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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