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Assessment of Quality and Biochemical Changes in Indian Jujube Fruits (*Ziziphus mauritiana* Lamk.): Effect of Chitosan Coating and Putrescine Treatments

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ABSTRACT

Indian jujubes are perishable fruits with a short storage life after harvest. In this study, Indian jujube fruits were stored at 5 °C and 90% RH for 30 days after treating the fruits with 1% chitosan (CHS), 1 mM putrescine (PUT), and a combination of 1% CHS and 1 mM PUT (CHS+PUT). Physicochemical characteristics and cell wall degrading enzymes were measured at 10-day intervals. The results indicated that the efficiency of the CHS+PUT was better than CHS or PUT as individual treatments. The CHS+PUT treatment substantially decreased weight loss, decay incidence, and malondialdehyde content in Indian jujube fruits. The CHS+PUT treatment minimized losses of bioactive compounds (ascorbic acid and phenols), total antioxidant activity, and titratable acidity. In addition, CHS+PUT treatment delayed fruit softening by suppressing polygalacturonase activity and pectin methylesterase enzymes. Soluble solids content significantly increased through storage time, but applying CHS and PUT individually or in combination inhibited this increase. These results confirmed that the 1% CHS and 1 mM PUT treatments maintained the postharvest quality of Indian jujube fruits in cold storage.

Abbreviations

Chitosan (CHS), Malondialdehyde (MDA), Pectin methylesterase (PME), Polyamines (PAs), Polygalacturonase (PG), Polyvinylpolypyrrolidone (PVPP), Putrescine (PUT), Soluble solids content (SSC), Titratable acidity (TA), Total antioxidant activity (TAA), Total phenolic content (TPC)

Introduction

Indian jujube (*Ziziphus mauritiana* Lamk.) is a member of the Rhamnaceae family and is native to tropical and subtropical areas of South Asia. Indian jujube is a rich source of carbohydrates, protein, fat, organic acids, minerals, and vitamins. This fruit is usually used for both food (fresh, dried, candied, pickled forms, juice, flour, meal, and butter) and traditional medicine in Asia (Wang et al., 2023; Aldhanhani et al., 2022). It is a climacteric and highly perishable fruit with poor shelf life at ambient temperature (Jat et al., 2022; Qiuping and Wenshui, 2007). In harvested fruits, increasing the respiration rate and ethylene production leads to accelerated ripening, softening, color change, and rotting, which elevates postharvest losses and limits the shelf life and quality of fruits. Therefore, appropriate

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postharvest treatments or methods are necessary to prolong storage life and prevent adverse changes in perishable fruits. In Indian jujube, the effects of gamma irradiation (Jat et al., 2022), edible coating (Chen et al., 2019), and calcium (Jain et al., 2019) have reportedly received research focus.

The edible coatings are used as a thin layer on the surface of foods to prevent mechanical damage, microbial infestation, tissue dehydration, softening, and enzymatic browning (Saleem et al., 2021; Zhou et al., 2022). In addition, edible coatings maintain postharvest quality and improve the appearance of coated fruits and vegetables (Saleem et al., 2021). Chitosan (CHS), a deacetylated derivative of chitin, is a non-toxic, transparent, biocompatible, and biodegradable polysaccharide obtained from the exoskeleton of crustaceans (Zhou et al., 2022; Sharma et al., 2022). CHS coating has applications in the postharvest storage of various horticultural crops, e.g., papayas (Zhou et al., 2022), strawberries (Saleem et al., 2021), peppers (Sharma et al., 2022), and Indian jujubes (Qiuping and Wenshui, 2007). Edible coating based on CHS can improve the freshness of products by reducing microbial infection, softness, and oxidative stress (Saleem et al., 2021; Ghosh et al., 2021). CHS coating delayed ripening and senescence, suppressed respiration rate, ethylene biosynthesis, weight loss, decay incidence, and malondialdehyde accumulation in these fruits. It inhibited fruit softening by suppressing cell wall degrading enzymes, improved antioxidant capacity, and maintained sensory qualities by conserving higher total soluble solids, titratable acidity, and ascorbic acid content.

Polyamines (PAs), low molecular weight aliphatic amines, are widely present in all living organisms. Putrescine (PUT), spermidine, and spermine are biologically the main PAs (Mishra et al., 2022). Many growth and developmental processes, including flowering, fruit set, fruit maturation, ripening, senescence, and stress response, are controllable by PAs (Mishra et al., 2022; Fawole et al., 2020). Plants can biosynthesize PAs and ethylene via a precursor, S-adenosyl methionine (SAM), which delays fruit ripening and senescence (Fawole et al., 2020). Therefore, balancing these two antagonistic plant hormones is crucial for delaying or accelerating these processes (Razzaq et al., 2014).

Exogenous application of PUT reportedly prolonged the shelf life of fruits by maintaining postharvest quality and firmness, enhancing antioxidant capacity, suppressing decay incidence and cell wall-degrading enzymes, and lowering respiration rate, ethylene production, weight loss, electrolyte leakage, and malondialdehyde accumulation (Mishra et al., 2022; Fawole et al., 2020; Ahmad and Ali, 2019; Hanif et al., 2020). Nevertheless, previous studies showed that the postharvest application of CHS coating enriched with PAs is more effective for extending the shelf life and maintaining the quality of various fruits and vegetables, such as bell peppers (Sharma et al., 2022), strawberries (Bal and Urun, 2020), and banana (Hosseini et al., 2018) compared to individual applications.

However, little information is available in the literature about the combined effects of CHS coatings and PAs on the postharvest storage of Indian jujube fruits. Thus, this study aimed to evaluate the impacts of CHS coating and PUT on the quality, bioactive compounds, and cell wall-degrading enzymes in Indian jujube fruits during cold storage.

Materials and Methods

Plant materials, treatments, and storage conditions

Indian jujube fruits were harvested at the mature-green stage from 6-year-old trees grown in the tropical fruit research station of Minab (Hormozgan Province, Iran). Immediately after harvest, the fruits were transferred to a laboratory for postharvest evaluations. The fruits were screened for uniform maturity, color, and size, without any diseases, damage, or spots. They were divided randomly into four groups as follows:

1) PUT treatment: fruits were dipped into a 1 mM PUT (Sigma Aldrich) solution for 2 min.

2) CHS coating treatment: CHS solution (1% w/v) was prepared by dissolving CHS powder (Sigma Aldrich) in distilled water containing 0.5% (v/v) of glacial acetic acid and 0.1 mL of Tween-80 as an emulsifier. The solution was stirred continuously. The pH of the solution was adjusted to 5.2 using 1 N sodium hydroxide (Meighani et al., 2015). Indian jujube fruits were dipped for 2 min in this solution.

3) CHS-PUT treatment: 1 mM PUT was added to the prepared CHS solution, and the fruits were immersed for 2 min.

4) Control: the fruits were dipped in distilled water for 2 min.

All fruits were air-dried at room temperature for 2 h. Treated fruits were packed in perforated polyethylene boxes for 30 days at 5 °C and 90% relative humidity. Immediately after harvest (before treatment, day 0), at 10-day intervals, we evaluated all fruit characteristics.

Firmness, weight loss, and decay incidence

A texture analyzer determined fruit firmness (Santam, STM-5, Iran). The analyzer was equipped with a flat probe (8 mm) and had a penetration depth of 10 mm, with a fixed speed of 10 mm min^{-1}. Fruit firmness was expressed as Newton (N) (Liu et al., 2014). Weight loss was calculated as a percentage of initial weight based on the following equation:

$$Weight\ loss\ (\%) = \frac{(W1 - W2)}{W1} \times 100$$

Where W1 is the fruit weight at the beginning of the experiment, immediately after treatment, and W2 is the fruit weight after storage on each sampling date (Hosseini et al., 2018).

The number of decayed fruits was counted regardless of the severity of the decay during the cold storage. Decay incidence was calculated based on the following equation (Liu et al., 2014):

$$Decay\ incidence\ (\%) = \left(\frac{number\ of\ decayed\ fruits}{total\ number\ of\ fruits}\right) \times 100$$

Titratable acidity, soluble solids content, and ascorbic acid content

Titratable acidity (TA) was measured by titrating 10 mL fruit juice and 40 mL distilled water, containing phenolphthalein as an indicator against 0.1 N NaOH (Saleem et al., 2021). A digital handheld refractometer determined the SSC (Euromex, RD. 5635) and expressed it as °Brix. Titration methods had 2,6-Dichlorophenolindophenol to determine the ascorbic acid content, and the results were expressed as mg per 100 g fresh weight (Li et al., 2009).

Sample extraction for antioxidant and phenol analyses

Indian jujube fruit extract was prepared for the analyses of phenols and antioxidant activity using a method outlined by Sabir et al. (2020) with minor modifications. Fruit samples (5 g) were homogenized in 30 mL methanol (80%) and then centrifuged at 10000× g for 20 min at 4 °C. The supernatant was collected and stored at −20 °C until further analysis.

Total phenolic content and antioxidant activity

Total phenolic content (TPC) in fruit tissue was determined via a Folin–Ciocalteu reagent according to Sabir et al. (2020) at 760 nm by a spectrophotometer (PG Instruments Ltd. T80+ UV/VIS). Gallic acid was used as a standard for the calibration curve (0–100 mg L^{-1}). TPC concentrations were expressed as grams of gallic acid equivalent per 100 g of fruit fresh weight. Total antioxidant activity (TAA) in fruit tissue was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method at 517 nm according to Hanif et al. (2020). The inhibition percentage of DPPH was calculated by the following equation:

$$DPPHsc\ (\%) = \left(1 - \frac{absorbance\ of\ sample}{absorbance\ of\ control}\right) \times 100$$

Malondialdehyde (MDA) content

MDA content in Indian jujube fruit was determined according to Thapa et al. (2023), with a slight modification. Fresh fruit pulp (1 g) was homogenized in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged at 10,000 g for 20 min at 4 °C. Then, 2 mL of the supernatant was added to 2 mL TCA containing 0.6% thiobarbituric acid (TBA) and centrifuged at 12,000 g for 15 min. The mixture absorbance was read at 450, 532, and 600 nm. The MDA content was expressed as µmol g^{-1} FW.

Polygalacturonase and pectin methylesterase assay

Polygalacturonase (PG) enzyme activity was measured according to a method by Chen et al. (2019), with minor modifications. Fresh fruit tissue (2 g) homogenized in a 4 mL sodium acetate buffer (40 mM, pH5.5) containing 1 M NaCl and 0.5 g polyvinylpolypyrrolidone (PVPP) for three h and then centrifuged for 20 min at 12000 g at 4 °C. The supernatants were used for assaying. The reaction mixture contained 0.2 mL of sodium acetate buffer (40 mM, pH 4.6), 0.3 mL of 1% (w/v) polygalacturonic acid, 0.1 mL of the enzyme extract, and 0.4 mL of distilled water. The mixture was incubated for 1 h at 37 °C, and the reaction was terminated by adding 1 mL of 3,5-dinitrosalicylic acid and boiling the solution for 5 min. After cooling, the reaction mixture was diluted to 25 mL with distilled water. The absorbance was measured at 540 nm. One unit of enzyme was defined as the production of 1 µmol galacturonic acid per minute.

Pectin methylesterase (PME) was extracted by homogenizing the fresh fruit tissue in 8.8% (w/v) NaCl and 2.5% (w/v) PVPP at 4 °C. The homogenate was centrifuged at 12000 g for 20 min. The supernatant was collected, adjusted to pH 7.5, and used for the assay. The reaction mixture contained 2 mL of pectin (0.5%), 0.15 mL

of bromothymol blue (0.01%), 0.75 mL of water, and 0.1 mL of enzymatic extract. All solutions (pectin, indicator dye, and water) were adjusted to pH 7.5 with 2 mol L^{-1} of NaOH just before each trial was started. After adding the prepared enzyme to a cuvette, the solution was gently shaken. Absorbance values were measured immediately at 620 nm and measured again after three min. The different values in absorbance between zero and three min measured PME activity. One unit was the enzyme required to liberate 1 mol of methyl ester per minute (Liu et al., 2018).

Statistical analysis

In an experimental design, there were two sources of variation, regarded as the treatments, i.e., chemical treatment (CHS and PUT) and storage time (0, 10, 20, and 30 days). Three replicates were used in each treatment at each sampling date. Data were analyzed using a one-way analysis of variance (ANOVA) with SAS statistical software (version 9.1). Comparison of mean values involved the least significant difference (LSD) ($p \leq 0.05$).

Results

Firmness, weight loss, and decay incidence

Initial firmness in Indian jujube fruit was 64.26 N, which gradually decreased in treated and control samples during cold storage. Control samples lost about 47.78% of their firmness within 30 days. Meanwhile, it decreased at a slower rate in response to CHS, PUT, and CHS+PUT treatment. On day 30, CHS+PUT-treated fruits had a significantly greater firmness value (45.69 N) compared to the control (33.53 N) and PUT-treated samples (42.14 N) (Table 1).

Table 1. Effect of pretreatment using putrescine (PUT, 1 mM) and chitosan (CHS, 1%) on firmness, weight loss, decay incidence, and soluble solids content of Indian jujube fruit during storage at 5 °C.

Storage time (days)	Treatments	Firmness (N)	Weight loss (%)	Decay incidence (%)	Soluble solids content (°Brix)
0 (at harvest)		64.21± 1.45^{a}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	13.43 ± 0.21^{e}
10	**Control**	59.36 ±1.00b^{c}	0.62 ± 0.06^{g}	0.00 ± 0.00^{e}	14.12 ± 0.21^{cd}
	CHS	62.46 ± 1.36^{ab}	0.21 ± 0.01^{g}	0.00 ± 0.00^{e}	13.86 ± 0.15^{cde}
	PUT	63.13 ± 1.12^{a}	0.25 ± 0.04^{fg}	0.00 ± 0.00^{e}	13.64 ±0.18^{cde}
	CHS+PUT	63.87 ± 1.76^{a}	0.12 ± 0.03^{g}	0.00 ± 0.00^{e}	13.83 ± 0.16^{cde}
20	**Control**	43.57 ± 1.31^{fg}	3.43 ± 0.30^{b}	7.33 ± 1.33^{b}	14.93 ± 0.25^{b}
	CHS	56.43 ± 1.23^{c}	1.62 ± 0.03^{e}	1.33 ± 0.67^{de}	14.23 ± 0.19^{cd}
	PUT	48.46 ± 0.85^{de}	2.06 ± 0.13^{d}	3.33 ± .067^{cd}	14.17 ± 0.19^{cd}
	CHS+PUT	51.13 ± 0.81^{d}	1.31 ± 0.09^{e}	1.33 ± 0.67^{de}	14.01 ± 0.18^{cde}
30	**Control**	33.53 ± 0.53^{i}	6.12 ± 0.22^{a}	14.67 ± 1.33^{a}	15.87 ± 0.13^{a}
	CHS	39.85 ± 1.24^{h}	2.96 ± 0.17^{c}	5.33 ± 1.33^{bc}	14.84 ± 0.28^{b}
	PUT	42.14 ± 0.51^{gh}	2.86 ± 0.15^{c}	7.33 ± 0.67^{b}	14.38 ± 0.29^{bc}
	CHS+PUT	45.69 ± 0.52^{ef}	2.03 ± 0.02^{d}	4.67 ± 0.67^{c}	14.11 ± 0.21^{cd}

Data are mean values ± SE of three replicates. Different superscript letters indicate significant differences in the same column using the LSD test ($p \leq 0.05$).

An increasing trend in weight loss of the control and treated fruits were found through storage time (Table 1). CHS and PUT treatment limited the weight loss in comparison with the control samples. However, on day 10, there was no significant difference between the treated and control fruits. On the final day of storage, CHS+PUT treatment showed a minimum value (2.03%) while the control samples showed a maximum value (6.12%) of weight loss. As a result of this study, the weight loss in the treated fruits was lower than the 4%–6% limit defined for commercially available fresh fruits (Chen et al., 2019).

As can be seen in Table 1, decay incidence in Indian jujube fruits gradually increased in all the treatments through storage time. No significant differences were recorded on day 10 of storage among the treated and control samples. However, the decay incidence in the treated fruits was significantly lower than in the control samples after 20 and 30 days of cold storage. At the end of

storage, the highest decay incidence was recorded in the control sample at 19.33%, while CHS+PUT treatment showed the lowest decay incidence (6.67%), followed by CHS (8.67%) and PUT (12.67%) treatments.

Titratable acidity and soluble solids content

In evaluating fruit quality, the most important parameters are SSC and TA. In this study, SSC increased linearly over time in Indian jujube fruit, particularly in the control samples. On day 10 of storage, no significant difference was observed in SSC between the treated and control fruits. By the end of storage, SSC values had increased by 17%, 11%, 7%, and 5% in the control, PUT, CHS, and PUT+CHS treatments, respectively, when compared to the condition before storage (day 0). The maximum SSC value (15.78 °Brix) was recorded in control samples on the final day of storage (Table 1).

Interactions between treatment and storage time on the TA content of Indian jujube fruits were not significant. During storage time, TA content significantly decreased from 0.73 mg 100g^{-1} at harvest (day 0) to 0.49 mg 100 g^{-1} at the end of storage (Fig. 1A). As shown in Figure 1B, TA content in the control fruits (0.57 mg 100g^{-1}) was markedly lower than in fruits treated with CHS (0.64), PUT (0.61), and CHS+PUT (0.67) treatments.

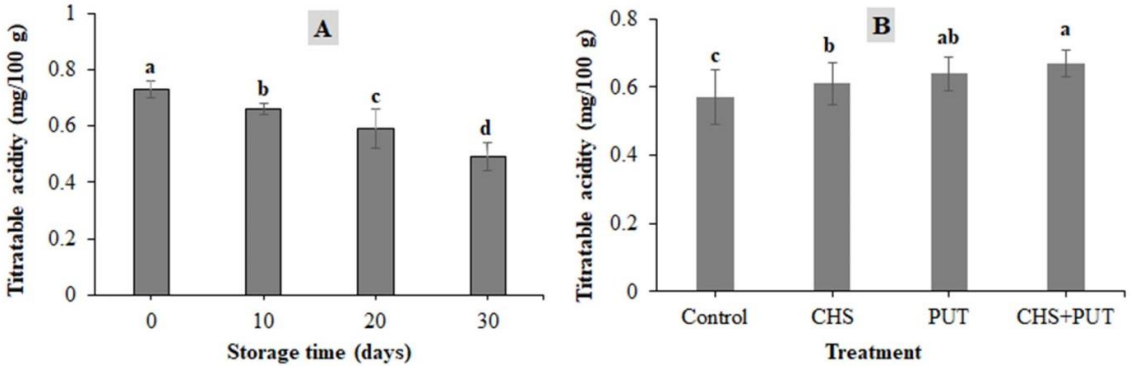

Fig. 1. Effect of storage time (A) and treatment (B) on titratable acidity (TA) of Indian jujube fruit. Data are mean values ± SE of three replicates. Different letters indicate significant differences using LSD test ($p \leq 0.05$).

Ascorbic acid content

As shown in Figure 2A, the ascorbic acid content of the control and treated Indian jujube fruits continuously decreased during cold storage. This decrease was effectively inhibited by CHS, PUT, and CHS+PUT treatments. The initial ascorbic acid content was 71.66 mg 100 g^{-1} FW. A maximal decrease in ascorbic acid content was recorded in the control sample (42%) when compared to the condition before storage. On the final day of storage, the highest levels of ascorbic acid content occurred in response to CHS+PUT (58.11 mg 100 g^{-1} FW), followed by the PUT treatment (55.81 mg 100 g^{-1} FW).

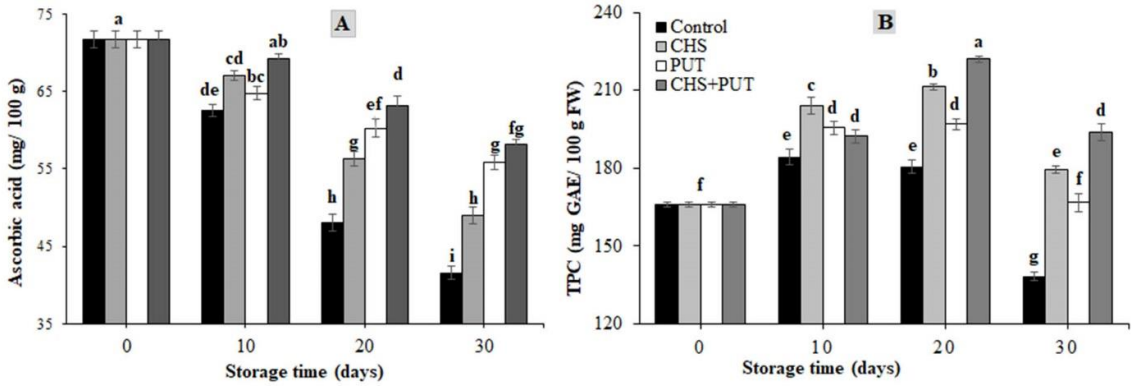

Fig. 2. Ascorbic acid content (A), and total phenolic content (TPC, B) in Indian jujube fruits treated with putrescine (PUT, 1 mM) and chitosan (CHS, 1%) during cold storage. Data are mean values ± SE of three replicates. Different letters indicate significant differences using the LSD test ($p \leq 0.05$).

Total phenolic content and antioxidant activity

The TPC of Indian jujube fruit significantly increased up to 10 days of cold storage, irrespective of treatments. On day 20 of storage, the increase of TPC continued in the treated fruits and reached the maximum, whereas a slow decrease without significant difference was recorded in the control samples. Thereafter, the TPC of Indian jujube fruits decreased significantly with the progression of storage time up to 30 days in control and treated fruits. The maximum concentration of TPC was recorded in the PUT+CHS treatment (222.04 mg GAE 100 g^{-1} FW) on the 20th day of storage with a 42% increase compared to before treatment. On the final day of storage, control samples (138.32 mg GAE 100 g^{-1} FW) had the lowest TPC (Fig. 2B).

As shown in Figure 3A, the initial (day 0) total antioxidant activity (TAA) of Indian jujube fruits was 65.14%. An increase in TAA of the control samples was observed during the first 10 days of storage, while in treated fruits it increased until day 20 of cold storage and afterward, it decreased in all samples until day 30 of cold storage. On days 20 and 30 of storage, PUT and/or CHS-treated Indian jujube fruits had significantly higher TAA compared with the control sample. The highest TAA was recorded in fruits treated with CHS+PUT (81.44%) on day 20 of storage, followed by fruits treated with CHS (77.25%) and PUT (79.47%), while the least TAA (42.86%) was recorded in the control samples on the last day of storage.

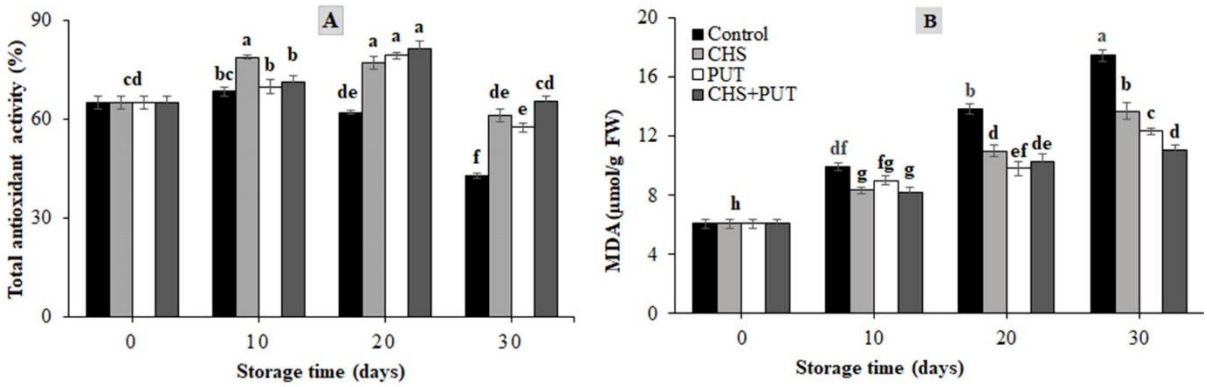

Fig. 3. Total antioxidant activity (A), and malondialdehyde content (MDA, B) in Indian jujube fruits treated with putrescine (PUT, 1 mM) and chitosan (CHS, 1%) during cold storage. Data are mean values ± SE of three replicates. Different letters indicate significant differences using the LSD test ($p \leq 0.05$).

Malondialdehyde content

Changes in the MDA of Indian jujube fruits during cold storage are illustrated in Figure 3B. MDA content gradually increased with the progression of storage in control and all treatments. However, all treatments significantly suppressed MDA accumulation compared to the control samples. On the final day of storage, MDA content in CHS, PUT, and CHS+PUT treated fruits were 13.69, 12.34, and 11.08 µmol g^{-1} FW, respectively compared to 17.45 µmol g^{-1} FW in the control sample. Our results showed 2.89, 2.25, 2.02, and 1.82 fold more MDA content, respectively in control, CHS, PUT, and CHS+PUT treatments than the initial content before treatment (at harvest).

Polygalacturonase and pectin methylesterase activity

Activities of cell wall-modifying enzymes such as PG and PME increased steadily in Indian jujube fruits during cold storage regardless of the treatments. PUT and/or CHS treatments significantly suppressed PG and PME activities during cold storage. However, no significant differences in PG activity occurred among the fruits treated with CHS, PUT, and CHS+PUT at different sampling intervals. A maximum PG (14.11 U g^{-1} FW min^{-1}) and PME (8.73 U g^{-1} FW min^{-1}) activity recorded in control samples on the final day of storage was 3.2 and 3.7 fold, respectively as compared to harvest time. About 27%, 38%, and 43% less PG activity were found in CHS, PUT, and CHS+PUT treatments compared to the control samples on the 30th day of storage (Fig. 4A). A minimum PME activity at the end of storage occurred in fruits treated with CHS+PUT (8.2 U g^{-1} FW min^{-1}) followed by PUT-treated (8.71 U g^{-1} FW min^{-1}) fruits (Fig. 4B).

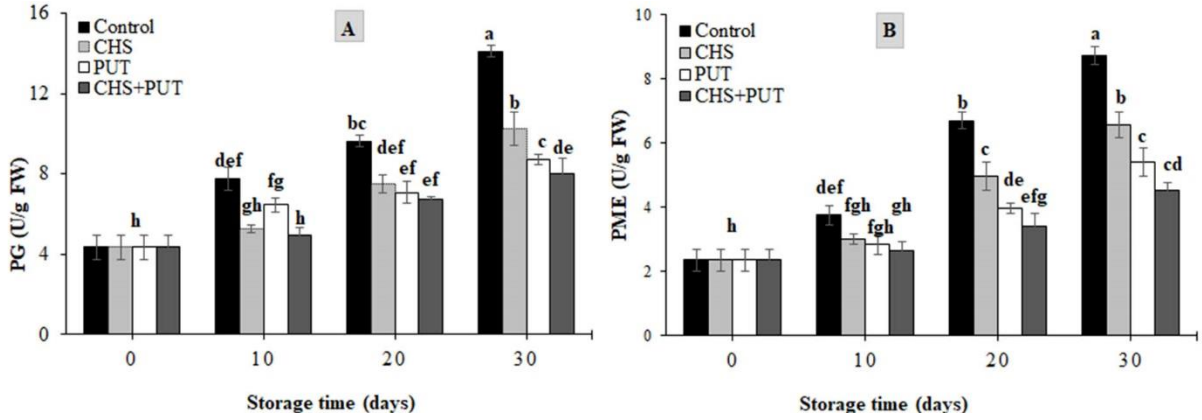

Fig. 4. Polygalacturonase (PG, A) and pectin methylesterase (PME, B) enzyme activities in Indian jujube fruit treated with putrescine (PUT, 1 mM) and chitosan (CHS, 1%) during cold storage. Data are mean values ± SE of three replicates. Different letters indicate significant differences using the LSD test ($p \leq 0.05$).

Discussion

Indian jujube is one of the most commercialized jujube species in the world (Wang et al., 2023). The fruits are perishable and have a short storage life. Application of PUT and CTS coating treatment showed beneficial effects on the physicochemical characteristics of Indian jujube fruit during cold storage. Firmness is an important parameter for determining shelf life and judging the quality of fresh fruit by consumers (Liu et al., 2014). The decrease in fruit firmness is usually caused by cell wall-degrading enzymes that hydrolyze cell wall components during fruit ripening or postharvest (Chen et al., 2019). Fruit softening is related to increased depolymerization and decomposition of cell wall components. In climacteric fruits, cell wall-hydrolyzing enzymes increased due to CO_2 production (Liu et al., 2014). As a result of decreased CO_2 production and other ripening and senescence processes, firmness remains stable in fruits treated with CHS and PUT (Razzaq et al., 2014). In cold storage, fruit firmness correlated negatively with the activity of cell wall-degrading enzymes (Razzaq et al., 2014). In the present study, fruits treated with CHS and PUT had firmer tissue than the control samples during cold storage. Previous studies reported similar results when CHS coating was applied to strawberries (Saleem et al., 2021), plums (Liu et al., 2014), and chili fruit (Ghosh et al., 2021).

During postharvest, weight loss mostly occurs due to the loss of moisture from the peel, an outcome of transpiration, and is caused by a deficit in vapor pressure between the internal and external atmospheres of the fruit (Chen et al., 2019). According to Bal and Urun (2020), the beneficial effect of CHS coatings is due to a semipermeable layer on the surface of the fruits against moisture and gases. CHS coating effectively prevented weight loss in mango (Razzaq et al., 2014), banana (Hosseini et al., 2018), and chili fruit (Ghosh et al., 2021). PUT, as a polyamine, probably limits weight loss by slowing respiration rate, delaying ripening and senescence processes (Bal and Urun, 2020), maintaining membrane integrity, and delaying the removal of epicuticular waxes, which play a main role in moisture loss through the fruit peel (Ahmad and Ali, 2019; Shiri et al., 2013). Therefore, the combination of CHS and PUT synergistically prevented moisture loss and gas exchange, thus reducing the weight loss of Indian jujube fruits during cold storage. Our results concurred with those reported by Bal and Urun (2020) and Ghosh et al. (2021), indicating that CHS+PUT treatment was more effective in reducing weight loss during cold storage.

CHS coating creates a protective and stable layer on the surface of fruits, which can prevent or delay the decay of treated fruits by suppressing the proliferation of pathogens (Saleem et al., 2021). Qiuping and Wenshui (2007) observed that CHS coating significantly reduced the stem-end rots of Indian jujube when stored at ambient temperature. Similarly, postharvest applications of CHS coating decreased infections with gray mold and rhizopus rot in the strawberries after four days of storage at 20 °C (Romanazzi et al., 2013). Two mechanisms of action in controlling decay by CHS coating are known, i.e., reducing the proliferation of decay-causing fungi and inducing resistance in host tissues (Romanazzi et al., 2013). Less decay incidence in PUT-treated fruits may be due to the anti-pathogenic properties of polyamines (Valero et al., 2002). In papaya fruit, decay incidence was significantly lower in PUT-treated (2 mM) samples than in control during storage (Hanif et al., 2020). According to the

present study, the combination of CHS and PUT treatment acted synergistically and substantially prevented the postharvest decay in Indian jujube fruits similar to those that have been reported in strawberries (Bal and Urun, 2020) and table grapes (Shiri et al., 2013).

An increase in SSC value during storage can be due to the hydrolysis of starch and its conversion to sugars (Hanif et al., 2020; Jain et al., 2019), a decrease in respiration rate (Qiuping and Wenshui, 2007), loss of moisture, and an increase in dry matter of fruits (Hosseini et al., 2018; Kumari et al., 2015). A lower concentration of SSC in the PUT-treated fruit compared to the control can be due to the delay in the senescence and ripening process, along with the suppression of amylase and phosphorylase enzyme activity (Hanif et al., 2020). These findings indicate that a combination of CHS and PUT synergistically affected the SSC concentration of fruits during cold storage. Our results are consistent with the previous report by Chen et al. (2019) that indicated increases in SSC of 'Cuimi' Indian jujube during storage time.

Due to the fruit ripening process, the metabolism of acids continues during storage by converting acids to sugars through the respiration process, thus reducing the TA content in fruits (Jongsri et al., 2017). A higher TA content in fruits treated with CHS, PUT, and both, compared to the control, may result from delayed senescence and lower respiration rates in these fruits (Kumari et al., 2015). Similar to our findings, higher TA content has been reported in mango (Jongsri et al., 2017) and grape (Shiri et al., 2013) fruits by postharvest applications of CHS and/or polyamines. In contrast, Hosseini et al. (2018) reported a higher TA content in banana (cv. 'Cavendish') fruit treated with CHS and PUT during cold storage. However, CHS and PUT treatments had no significant impact on the TA content of strawberry fruits under cold storage (Bal and Uran, 2020).

According to Sharma et al. (2017), oxidative conversion of dehydroascorbic acid to diketogluconic acid in the storage conditions of fruits leads to a lower ascorbic acid content. Previously, a decrease in ascorbic acid content in Indian jujube fruits during storage was reported (Qiuping and Wenshui, 2007). This study demonstrated that CHS+PUT treatment had a beneficial effect on maintaining ascorbic acid content. A similar result was obtained by using CHS+PUT treatment on strawberries (Bal and Urun, 2020) and chili fruit (Ghosh et al., 2021) or CHS coating on Indian jujube fruits (Qiuping and Wenshui, 2007). A less sharp decrease in ascorbic acid content in CHS+PUT treatment could be due to the suppression of ascorbate oxidase activity and anti-senescence properties of PAs (Hanif et al., 2020) or inhibition of ascorbic acid oxidation due to gas permeability by CHS coating (Bal and Urun, 2020).

A similar trend to our results was reported in the TPC of strawberry (Bal and Urun, 2020), pomegranate (Fawole et al., 2020), and papaya (Zhou et al., 2022) fruits by postharvest application of PUT and/or CHS during storage. The initial increase in the TPC may be due to the continued biosynthesis of phenolic compounds in postharvest conditions due to ripening processes (Meighani et al., 2015). In addition, the reduction of TPC could be due to the breakdown of cell structure and phenolic compounds as a result of enzymatic activities (Fawole et al., 2020; Sharma et al., 2017) and the polymerization of tannins (Hosseini et al., 2018). Kumari et al. (2015) reported that lower activities of PPO and POD were associated with higher TPC retention in salicylic acid and CHS-treated fruits. In the current study, TPC appeared at significantly higher levels in the PUT+CHS treatment compared to the control samples. These were consistent with previous studies on strawberries (Bal and Urun, 2020) and banana cv. 'Native' (Hosseini et al., 2018).

Bioactive compounds such as phenols, flavonoids, anthocyanin, and ascorbic acid are responsible for antioxidant activity in fruits (Kumari et al., 2015). Higher TAA occurred in PAs+CHS-treated bell peppers in research by Sharma et al. (2022). Hanif et al. (2020) obtained higher TAA in PUT-treated papaya. Our results showed that changes in TAA correlated with changes in TPC during cold storage. Earlier studies found a good correlation between TAA and TPC in fruits (Hanif et al., 2020; Ghosh et al., 2020).

MDA is the end-product of the oxidation of unsaturated fatty acids and can be an indirect, feasible marker of senescence and oxidative stress (Sharma et al., 2022). Reactive oxygen species primarily cause lipid peroxidation (Mishra et al., 2022). Therefore, CHS coating can act as a physical barrier to oxygen that does oxidative damage and lipid peroxidation in fruits during cold storage (Saleem et al., 2021). In addition, PAs are cationic compounds that bind to anionic macromolecules in the cell membranes, thus altering membrane fluidity and the activities of membrane-bound enzymes (Valero et al., 2002). A lower MDA content reportedly occurred in PUT and CHS-treated chili fruits in research by Ghosh et al. (2021). Sharma et al. (2022) obtained similar findings in PAs+CHS-treated bell peppers, and Mishra et al. (2022) in PAs-treated black plums.

The decomposition of cell walls results from cell wall degrading enzymes, including PG, PME, and cellulase, so their increasing activities reduce the fruit firmness and integrity of the cell wall structure (Saleem et al., 2021). In the current study, the inhibition of PG and PME activity by PUT, CHS, and CHT+PUT treatments was associated with maintaining fruit firmness. A decrease in cell wall degrading enzyme activity and encoding gene transcript levels of cellulase, PG, PME, and β-galactosidase by ascorbic acid+CHS treatments inhibited fruit softening while extending the shelf life of papaya fruits (Zhou et al., 2022). An edible coating limits oxygen supply and inhibits the activity of cell wall-degrading enzymes (Saleem et al., 2021). Inhibition activities of cell wall degrading enzymes have been reported previously by Chen et al. (2019) in carnauba wax (CW) and CW-containing glycerol monolaurate-treated Indian jujube. Saleem et al. (2021) obtained similar results in ascorbic acid- and CHS-treated strawberries. Ahmad and Ali (2019) reached similar conclusions in PA-treated carambola. Similarly, Razzaq et al. (2014) reported that mango fruit treated with PUT significantly suppressed the activity of cell wall enzymes (exo-, endo-PG, and EGase) during ripening and cold storage.

Conclusions

Exogenous applications of CHS individually and in combination with PUT have beneficial effects on the quality maintenance of Indian jujube fruits during cold storage (5 °C). CHS (1% w/v) and PUT (1 mM) significantly decreased the decay incidence and controlled weight loss in fruits. Also, they lowered the degradation rate of bioactive compounds (e.g., phenols and ascorbic acid), partially maintained fruit firmness, and suppressed PME and PG enzyme activities. In addition, lipid peroxidation (MDA) was significantly inhibited by a synergistic interplay between CHS and PUT, thus resulting in membrane integrity. We recommend an optimal combination of CHS and PUS for quality maintenance in Indian jujubes in postharvest storage. These treatments do not have the side effects of chemicals and leave no harmful residues on fruits.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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