



Research article

Eco-safe hot water dip alleviates antioxidant level and sensory quality of Indian jujube fruits

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ABSTRACT

Indian jujube (Ber) is highly perishable climacteric fruit owing to high decay index limiting its marketability and demands interventions to prolong shelf life. Fungicides are normally used to control rot during postharvest storage, however, residues left necessitate eco-safe alternatives like hot water dipping. Mature, pre-climacteric jujubes were dipped in 45, 50 or 55 °C water for 8, 6 or 4 min, respectively and then stored at 5 °C for periodic quality evaluation. Dipping fruits in 55 °C water resulted in 32.69 and 35.27, 64.21 and 58.57, 30.41 and 30.42, 38.50 and 52.20 % lower weight loss, decay index, malondialdehyde (MDA) and electrolyte leakage, whereas 15.40 and 16.77, 19.51 and 20.48 % greater antioxidant activity and ascorbic acid respectively for Umran and Pakwhite compared to 25 °C water dip. The highest glucose, fructose, malic, citric, and tartaric acids were 23.44 ± 1.04 and 29.9 ± 0.95 , 30.68 ± 1.72 and 41.17 ± 2.34 mg/100 g, 138.1 ± 6.45 and 112.97 ± 6.16 , 57.49 ± 1.71 and 53.78 ± 1.90 , 79.58 ± 5.1 and 65.3 ± 4.83 µg/100 g whereas lower sucrose 12.34 ± 0.94 and 16.33 ± 1.05 mg/100 g were respectively recorded in 55 °C water dipped Umran and Pakwhite fruits. High dip water temperature (55 °C) exhibited better quality with the lowest decay index and weight loss, greater membrane integrity, bioactives content and sensory acceptance scores. Hence, hot water dipping was shown to be an effective residue-free option to extend the marketable period of jujubes to capture distant markets.

1. Introduction

The fruit of Indian jujube (Ber) is climacteric in nature with regard to ripening and has limited shelf stability, which hinders value chain management and processing after harvest. The fruit spoils quickly in transit and storage due to numerous biochemical and physiological processes like rapid moisture loss, high respiration and ethylene biosynthesis rates, mechanical damage, fruit fly attack, and susceptibility to various postharvest diseases [1]. These problems limit marketability and pose serious threats to the development of a sustainable jujube process industry. Cold temperature is required to extend shelf life, however, Ber fruits are susceptible to chilling injury [2]. The best suited lowest temperature for safe cold storage of jujube fruits is 7.50 °C. However, fresh jujubes are reportedly susceptible to chilling injury expressed as sheet pitting at 5 °C or below if stored for longer than 2 weeks [3]. Climacteric fruits are bestowed by nature with the capacity to continue normal ripening when harvested at different maturity stages, which allows control of

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losses by regulating ripening and maintaining marketability [4]. Storing fresh fruits at appropriate cold temperature and humidity guarantees greater shelf life in subtropical and tropical environments. The distribution and market chains today require long shelf stability of fresh produce to withstand distribution neglects and exploit distant markets [5].

Jujube fruits may rot quickly in tropical regions due to high humidity and temperature conducive for microbial proliferation. Postharvest infection of Ber by decay organisms after harvest is a threatening challenge resulting in substandard fruits with short life and poor economic return [6]. Normally, chemical fungicides are used to control fruit rot, however, residual fungicide content is considered by some consumers to threaten human health and endanger the ecosystem [7]. These grave concerns have resulted in greater demand for fresh fruits and vegetables with no pesticide application [8]. Different packaging and non-chemical preservation methods have been employed to prolong shelf life and maintain quality attributes of jujubes [9]. Among these, heat treatments in the form of steam, vapor heat or dipping in hot water are valuable non-chemical techniques with no deleterious effect on environment or human health. Dipping fresh produce in heated water prior to storage is green, an ecofriendly, low cost, safe, nonpolluting, nontoxic, easily applied [10] and effective with brief treatment time as well as consistent in dip water and fruit temperature monitoring [4]. Hot water dipping (HWD) is a promising technique to control fungal decay and insect infestation [11], sanitize produce for safe commercial application [12], delay ripening, prolong shelf life and maintain quality, induce resistance to chilling injury by increasing ratio of membrane phospholipids [10], and inhibit water loss by promoting lignification (i.e., healing) of damaged tissues. These treatments also stimulate the production of anti-pathogenic and heat shock proteins [13] and provoke defense mechanism by synthesis of anti-oxidant enzymes and bioactive compounds [14]. Heat treatments are effective in extending the stability and marketability of several fruits by altering the pace of gaseous exchange through modification of the internal microenvironment and reduce the pace of different physiological processes leading to senescence as well [15].

Dipping fruits in hot water is low-cost technique as compared to refrigeration, commercially used to improve sensorial attributes [16], increase storage duration and marketable period of fruits [2]. Keeping in view the limited shelf life of Indian jujube (Ber) fruits, the current research was conducted to evaluate the impact of hot water dipping as a low-cost, nonchemical treatment alternative to fungicide application to extend the shelf stability of jujube fruits during storage.

2. Materials and methods

2.1. Plant materials

Fruits from two commercially grown *Z. mauritiana* varieties (Umran and Pakwhite) were harvested at the green mature stage characterized by color change from dark green to light green for Umran and yellow green in Pakwhite, 120 days after blossom/anthesis from 20-year-old trees planted at the experimental orchard of the Horticultural Research Station, Bahawalpur, Punjab (71.3866°E and 29.2272°N, 100.28-m altitude from sea). This harvest maturity corresponds to the fruits being physiologically mature but pre-climacteric and still hard at this stage. Umran has oblong shaped, medium sized fruits while Pakwhite fruits are large sized with round shape. The fruits were harvested based on skin color in the field while colorimetric analysis was subsequently carried out at the lab for L, a* and b* scores with respective values 64.43, -15.82, 73.77 and 63.28, -17.87, 44.99 respectively for Umran and Pakwhite fruits. After harvesting, Ber fruits were kept in a mobile blast cooling unit in ventilated cardboard boxes and transported within 4.50 h to the Physiology Laboratory of the Post Harvest Research Centre, Ayub Agricultural Research Institute, Faisalabad and stored overnight at 5 ± 1 °C. Ber fruits selected for uniform maturity that were free from scratches, insect and disease attack were used for HWD. The fresh fruits were sanitized by dipping in 20 g/L sodium hypochlorite solution for 2 min and then air dried at ambient temperature. All chemicals (reagent grade except sugar and organic acid where HPLC grade standards and solvents) used for analysis were purchased from the Fisher Scientific, Merk and Sigma-Aldrich suppliers locally available.

2.2. Experimental procedure

The water temperature in the HW treatment unit was adjusted to suit the various time-temperature combinations separately on the digital control panel of a locally made heating/circulating hot water tank model HCB-1/04 [Pakistan Counsel for Scientific and Industrial Research (PCSIR)] having 108 L capacity with 60 L × 40 W × 45 D cm³ dimensions. Fruits for individual treatments were dipped for 8, 6 or 4 min, respectively, in hot water at 45 (HWD₁), 50 (HWD₂) or 55 °C (HWD₃), whereas fruits dipped in water at 25 °C for 10 min served as the control (HWD₀). The fruits placed in a perforated stainless-steel bucket and immersed in the hot water after the predefined temperature was achieved. After dip for the specified time, the bucket containing the fruits was removed from the hot water tank. The heat treatment was carried out for various times and at different temperatures keeping in view the thermal sensitivity of bioactive nutritive components and to make comparison of different time temperature combinations on postharvest quality and nutrient retention of treated fruits during storage. Fruits after each dip treatment were dried at ambient conditions and kept in pre-sterilized UV resistant collapsible ventilated plastic (high density polyethylene) baskets/crates (600 × 400 × 180 mm) stacked on a separate stacking position of an upright movable stacking wrack placed in a cold chamber (Cold space, Modular cold storage manufacturers, Ireland) equipped with a temperature and humidity regulation panel (Atmospheric Products Limited, Manchester, UK) for 35 days. The environment inside the chamber was adjusted to 5 ± 1 °C temperature and 95 % RH. For each treatment 300 fruits per container were used for study with three replicates.

2.3. Quality evaluation during storage

Ber fruit were analyzed at 1-week interval for weight loss, decay index, ascorbic acid, malondialdehyde content, electrolyte leakage, sugars, organic acids, total polyphenols, antioxidant activity and sensory evaluation to ascertain fruit freshness.

2.3.1. Loss in weight

The HWD treated and control Ber fruits for weight loss estimation were packed in breathable red nylon mesh polypropylene net bags (20" L × 6" W). Fruits were weighed at day 0 and at each subsequent evaluation interval during storage using digital balance (Sartorius GM 1501; AG, Germany). The weight loss was calculated at all intervals following Yang et al. [17].

$$\text{Loss in weight (\%)} = \frac{W_0 - W_i}{W_0} \times 100 \quad (\text{i})$$

where W_i represents fruit weight at each interval during storage and W_0 is fresh weight at day 0.

2.3.2. Decay index

The number of fruits in each HWD treatment were counted at the beginning. At each testing interval, the number of decay infected fruits in all treatments were counted. The incidence of natural infection of fruits by rot was calculated according to Wang et al. [6].

$$\text{Decay index (\%)} = \frac{\text{No. of infected fruits}}{\text{Total No. of fruits}} \times 100 \quad (\text{ii})$$

2.3.3. Ascorbic acid

The de-stoned Ber fruits were blended to get homogenous pulp. Five (5 mL) homogenate was thoroughly mixed with 95 mL (0.40 %) oxalic acid solution and filtered. Ten (10 mL) filtrate or standard ascorbic acid solution (0.10 %) were titrated with 0.004 % 2, 6 dichlorophenol indophenol (DCPIP) dye solution till pink color persistent for 15 s. The ascorbic acid content was measured as mg DCPIP equivalents per 100 g fresh fruits following Chen et al. [18].

2.3.4. Quantification of sugars and organic acids

Sugars; glucose, fructose and sucrose and malic, citric, tartaric and succinic acids of HWD treated jujube fruit's juice were determined using HPLC (PerkinElmer 200 series, USA). All the reagents, solvents and standards used for quantification were HPLC grade. D-(−)-fructose, D-(+)-glucose, D-sucrose and malic acid were obtained from the Fisher Scientific (Fair Lawn, NJ, USA) while citric, tartaric and succinic acids were obtained from Sigma-Aldrich (St. Louis, MO, USA). One milliliter juice from each treatment was mixed with 9 mL HPLC grade water. Half milliliter diluted juice was homogenized with 3 mL HPLC water, filtered from 0.45 μm nylon filter paper and stored in glass HPLC vials. The HPLC was equipped with the quaternary solvent pump, thermostatted column and refractive index detector. The analytes were separated using a C-18 column and their quantification was done by injecting 10 μL extract following Shahzad et al. [19]. Sugars were expressed as mg/100 g while organic acids as μg/100 g.

2.3.5. Sample preparation for phytochemical analysis

Five gram jujube fruit pulp was blended with 15 mL methanol (1:3) and extracted by vortexing for 3 h on a reciprocating shaker (HS-501 D, JkiKA Labortechnik, Germany) at 12,000×g. The extract was centrifuged for 10 min at 10,000×g, 4 °C and concentrated on rotary evaporator (Rotavapor® R-300, Büchi Labortechnik AG, Switzerland) at 45 °C. The resulting extracts were then kept at −40 °C in 100 mL airtight polypropylene bottles till further analysis.

2.3.5.1. Antioxidant activity. Fruit extract (1 mL) was mixed by vortexing with 1 mL (50 μg/mL) 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent solution and kept for 30 min in dark place at ambient temperature. Ten μL from each extract-DPPH mixture was loaded on a separate hole of 96 well ELISA plate. Absorbance was read through BioTek ELISA reader (PMBL, HFC, PYI) against reagent blank in triplicate at 517 nm. The antioxidant activity was expressed as inhibition percent (iii) [20].

$$\text{Inhibition (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{blank}}} \times 100 \quad (\text{iii})$$

2.3.5.2. Polyphenol content. Fruit extract (125 μL) was blended with 500 μL distilled water (DW) and 125 μL 10 % Folin-Ciocalteu reagent. After 6 min, 1.25 mL sodium carbonate (7.50 %) solution was added, volume was made 3.00 mL with DW and mixed by vortexing. After incubation for 90 min, 10.00 μL from each extract was loaded on a separate cell of 96 well ELISA plate. Phenolic content was measured at 760 nm as gallic acid equivalent (mgGAE/100 g) based on method described by Hu et al. [20].

2.3.6. Ripening index

Jujube fruit's total soluble solids (TSS) were measured from strained juice at 20 °C using digital refractometer (HI 96801, Hanna Instruments Inc., Romania). Titratable acidity was measured using 0.30 mL filtered juice blended with 30 mL deionized water on a digital fruit acidity meter (GMK-835 N, G-Won, Hitech Co., Korea) adopting Reche et al. [1] method with slight modifications. Firmness was determined at two equidistant points on opposite sides at the equatorial axis using an 8 mm cylindrical stainless-steel

probe attached to a digital penetrometer (PCE-FM-200, PCE Instruments, Germany). The ripening index was calculated from dimensionless firmness (F_{WB}) = $|F_{WB}, i|$, TSS and TA [21].

$$RPI_{WB} = \ln \left(100 \times F_{WB} \times \frac{TA}{TSS} \right) \quad (iv)$$

2.3.7. Electrolyte leakage

Ten 0.05 cm thick Ber fruit discs were washed thrice and immersed in 40 mL deionized water. The electrical conductivity (C_1) of water was recorded with a handheld EC meter (model DiST 4, Hanna Instruments Inc., Romania). The water containing 0.50 g fruit discs was then boiled for 10 min, and volume was adjusted to 40 mL upon cooling. EC (C_2) was recorded again and the electrolyte leakage was calculated following Yang et al. [17].

$$\text{Electrolyte leakage (EC\%)} = \left(\frac{C_1}{C_2} \right) \times 100 \quad (v)$$

2.3.8. Malondialdehyde content

Fruit pulp (2 g) was thoroughly mixed in 10 mL thiobarbituric acid (0.25 % w/v), heated for 10 min, subsequently cooled and centrifuged at 25 °C, 10,000×g for 10 min. The absorbance of the supernatant was then recorded at 450, 532 and 600 nm in a UV spectrophotometer and malondialdehyde was reported as mmol/g [17].

$$\text{Total MDA} = \frac{4 \times \text{Extract volume (mL)} [6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}]}{\text{Supernatant volume (mL)} \times \text{amount of sample} \times 1000}$$

2.3.9. Sensory evaluation

The sensory evaluation for color, flavor and taste was performed using 9-point hedonic scale at different storage intervals to check the overall acceptability of HWD jujube fruits. Prior written consent was sought from 25 participants, 25–58 years age (15 males and 10 females) from which 15 panelists were selected based on preferences. All panelists were faculty members and trained for 1 h session thrice before sensory evaluation of jujube fruits [22]. Sensory tests were performed indoors at ambient temperature (25 °C) under normal white florescent light in a separate booth. Unsalted crackers and mineral water were used by the panelists to cleanse their palates after tasting each sample to overcome remnant effect of taste and flavor of previous testing on preceding samples. Fruits of similar color and size were selected and stored overnight at ambient temperature prior to sensory testing. On the evaluation day, jujube fruits were washed with tap water and dried with a paper towel. Each panelist was served 2 fruits per treatment in a separate odor-free, disposable cups covered by plastic plates and labelled with random 3-digit numbers. Each of the panelist analyzed all four treatments (4 cups) for each jujube variety in a single session. Every panel member tasted 2 jujube fruits from both varieties receiving each treatment and was asked to rate the appearance (color), jujube flavor, taste and overall acceptance of samples using 1–9 hedonic scales (i.e. 1 = extremely dislike, 2 = very much dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither like nor dislike, 6 = slightly like, 7 = moderately like, 8 = like very much, 9 = extremely like). For quantification of intensity of parameters, 0 (none) to 10 (extremely strong) numerical scale with 0.5 increment was used [23].

2.4. Statistical analysis

The experiment was laid out and conducted under completely randomized design (CRD) having split plot arrangements with hot water dip treatments as main plot factor while storage intervals as subplot factor [24]. The data obtained from all parameters were analyzed separately for each variety using the analysis of variance (ANOVA) to check the combined effect of HWD and storage using R version 4.2.0 (The R Foundation, 2020). Agricolae package was used for multiple means comparison following Tukey's honestly significant difference (HSD) test at significance level ($\alpha \leq 0.05$) [25]. The graphs were prepared in Excel 365 (The Microsoft Corporation, USA). The results are reported as mean \pm standard error of three replicates for each parameter. Pearson's correlation analysis

Table 1

Weight loss (%) of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

Varieties	Treatments	Storage days					Treatment means
		7	14	21	28	35	
Umran 3.19 \pm 1.59B	HWD ₀	2.49 \pm 0.14 ^h	3.12 \pm 0.26 ^{fg}	4.10 \pm 0.17 ^c	4.55 \pm 0.20 ^b	6.57 \pm 0.30 ^a	4.03 \pm 0.39A
	HWD ₁	1.73 \pm 0.17 ^j	2.08 \pm 0.10 ⁱ	3.19 \pm 0.14 ^{efg}	3.77 \pm 0.17 ^d	4.62 \pm 0.16 ^b	3.08 \pm 0.24B
	HWD ₂	1.63 \pm 0.17 ^{jk}	2.36 \pm 0.17 ^h	3.06 \pm 0.20 ^{fg}	3.35 \pm 0.16 ^e	4.17 \pm 0.15 ^c	2.91 \pm 0.32C
	HWD ₃	1.49 \pm 0.20 ^k	2.57 \pm 0.17 ^h	3.00 \pm 0.20 ^g	3.21 \pm 0.20 ^{ef}	3.74 \pm 0.17 ^d	2.80 \pm 0.30D
Days mean		1.84E	2.53D	3.34C	3.72B	4.77A	
Pakwhite 3.35 \pm 1.80A	HWD ₀	2.26 \pm 0.17 ^{jk}	3.05 \pm 0.20 ^h	4.36 \pm 0.20 ^{de}	6.09 \pm 0.20 ^b	8.34 \pm 0.33 ^a	4.82 \pm 0.44A
	HWD ₁	2.08 \pm 0.17 ^k	2.85 \pm 0.20 ^{hi}	4.48 \pm 0.20 ^{de}	5.67 \pm 0.22 ^c	6.38 \pm 0.24 ^b	4.49 \pm 0.48b
	HWD ₂	1.98 \pm 0.10 ^k	2.76 \pm 0.17 ⁿ	3.61 \pm 0.17 ^g	4.65 \pm 0.20 ^d	5.70 \pm 0.22 ^c	3.76 \pm 0.39C
	HWD ₃	1.87 \pm 0.20 ^k	2.53 \pm 0.17 ^{ij}	3.09 \pm 0.22 ^h	3.80 \pm 0.24 ^g	4.18 \pm 0.14 ^{ef}	3.12 \pm 0.41D
Days mean		2.08E	3.07D	3.89C	5.05B	6.15A	

was performed between responses from all parameters and was used to construct heat map clusters with a free online data visualization platform, SR plot [26].

3. Results and discussion

The ANOVA with varieties as the blocking factor showed that variety, storage duration and hot water treatments all significantly contributed to overall variability ($P < 0.05$) in Ber fruits at different evaluation intervals during storage. The analysis findings were therefore focused on the impact of HWD treatments for fruits from each variety and storage intervals.

3.1. Weight loss

The magnitude of loss in weight ($P < 0.05$) was remarkably higher in 25 °C water dipped fruits and differed significantly among varieties (Table 1). Weight loss increased linearly during storage ($y = 0.75x + 1.00$, $R^2 = 0.99$). HWD resulted in a substantial decline for loss in weight proportionate to temperature of hot water in fruits from both varieties. Substantially lower weight loss was noted for fruits treated with hot water at 55 °C for 4 min (HWD₃). Control fruits showed significant loss in fresh weight with visible shriveling at 3rd week evaluation. At the end of the 35-day storage, dipping Ber fruits in hot water resulted in 27.67, 31.31 and 32.52 % weight loss reductions for Umran and 27.17, 31.51 and 35.61 % lower weight loss in Pakwhite fruits receiving HWD treatments at 45, 50, and 55 °C for 8, 6, and 4 min, respectively, compared to the control fruits.

In postharvest storage, fresh fruits are liable to lose weight due to rise in rate of metabolic activities like respiration, evaporation and dehydration resulting in undesirable fruit quality due to water loss, shrinkage, wilting and diming [27]. It may also influence sensorial quality of jujube fruits, accompanied by loss of flavor, nutrition and marketability [28]. The loss in fresh weight of jujube fruits levitates linearly with length of storage [18] which is in concordance with results from the current study. The fundamental mechanism regulating the pace of water or weight loss in fresh commodities is a differential in vapor pressure between surrounding environment and fruit tissues in addition to respiration and storage temperature [29]. HWD redistribute pre-existing epicuticular wax which partly or solely seals cracks to maintain membrane integrity in papaya [13] which consequently slowed down respiration and evaporation rates resulting in lower weight loss. Lower weight loss for hot water treated fruits in previous research is in support of the present study where weight loss was proportionately reduced with a sequential increase in dip water temperature. Remarkably lower loss in weight was noted for HWT jujubes [2,30], and cantaloupe [31] as compared to control which are congruent with results from the current study. Likewise, in line with the current research, hot water treated jujube fruits revealed 54.30 % lower weight loss than control fruits on 75th day in storage [17].

3.2. Decay index

HWD treatments resulted in a considerable reduction in the decay index of treated fruits (Fig. 1). HWD-treated Ber fruits followed a declining trend for decay index inversely proportional to successive rises in HWD exposure temperature ($y = -0.86x + 4.79$, $R^2 = 0.96$). Conversely, the decay index was maximal in control jujubes at ($P < 0.05$) portraying inability of untreated fruits to escape the fungal attack. The decay index increased in successive testing with length of storage in both control and treated fruits, but the intensity was higher in untreated fruits ($y = 1.15x - 0.80$, $R^2 = 0.94$). The decay reduction response of the HWD treatments compared with the controls revealed 55.39, 64.68 and 73.23 % and 55.06, 63.18 and 70.27 % lower decay index, respectively, in Umran and Pakwhite fruits immersed in the HWD₁, HWD₂ and HWD₃ treatments.

Fresh produce is attacked by microflora at harvest, during handling and storage till consumption. Decay causing pathogens normally infiltrate through epidermal pores, scratches and wounds on fruit surface to cause rot [2] and result in diverse diseases which afterwards spoil substantial quantity. Residual content of antimycotic chemicals and their environmental threats compelled growers, researchers and consumers for alternatives to decrease or even replace fungicides with safe, ecofriendly and nontoxic substitutes to

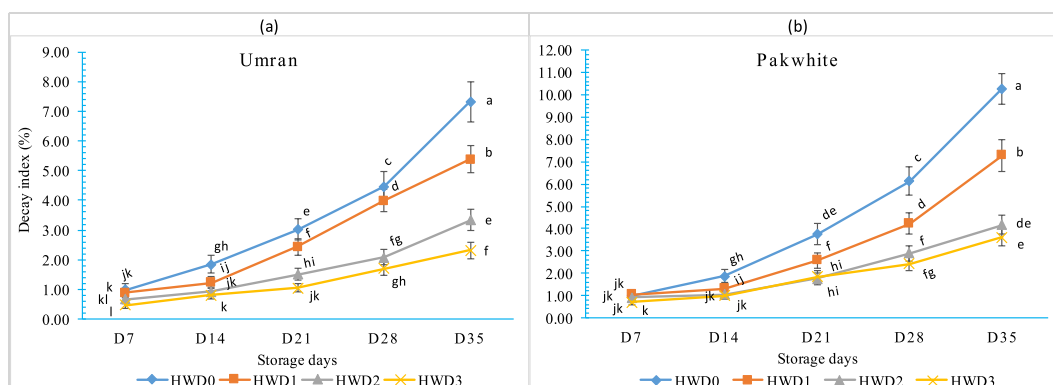


Fig. 1. Decay index of HWD jujube fruit at different points during storage at 5 °C and 95 % relative humidity.

avoid fruits decay. The variance in decay index in the current study may be attributed to variability in genetic makeup of Ber varieties depicting slightly higher decay resistance in Umran fruits during postharvest storage. The findings of the present study are similar to Chang et al. [2], Promyou et al. [30] and Yang et al. [17], Saad and El-Rab [31] and Alias et al. [13] who reported that decay was lower in hot water treated jujube, cantaloupe and papaya fruits respectively. HWD exhibit sterile effect due to annihilating inchoate contagions by dislodging and destroying some microbes and their spores [17] and thus decrease decay manifestation during storage. It was in confirmation with previous research reports who recorded inhibition of microbial growth and ultimately reduction in decay index in kumquat [32], peach [33] and apple [34] fruits. Secondly, heat treatment provokes defense system in external epicarp layers to prevent pathogen proliferation and induce heat shock protein synthesis [12] in response to external stress. Thirdly, HWD melts and redistributes pre-existing waxes on the fruit surface which also reduces microbial establishment. The highest decay in control Ber fruits is attributed to higher respiration, and ethylene synthesis leading to earlier and speedy ripening making it soft and more susceptible to invasion by decay microbes. In contrast, accrual of excess reactive oxygen species (ROS) and MDA in untreated fruits consequently arouses fruit decay and senescence [35] through the loss of membrane integrity by oxidative stress. The magnitude of decay rises with an increase in the length of storage which matches with findings by Saad and El-Rab [31].

3.3. Ripening index

The ripening index in general exhibited a linear increase over time ($y = 0.06x + 4.85$, $R^2 = 0.99$) regardless of HWD treatments, related primarily to increases in the sugar:acid ratio. RPI_{WB} differed among varieties as well as in response to HWD treatments, but proceeded in similar fashion in fruits from both varieties during storage. For 25 °C dipped fruits, a gradual rise in RPI_{WB} was observed while HWD slowed down the normal pace of ripening due to lower respiration and ethylene production, possibly less drop in acid content at cold storage temperature. The ripening index was likewise lower in HWD-treated fruits compared with control fruits because of delayed ripening. The lowest RPI_{WB} was recorded in fruits dipped in water at either 50 °C for 6 min or 55 °C for 4 min and those two treatments are significantly different in both Ber varieties (Fig. 2).

The ripening index, basically accounts the sugar-acid ratio and mesocarp firmness by incorporating the most basic physicochemical attributes to ascertain sensorial perception for ripeness [36]. It is the best objective criterion to gauge physiological ripeness suitable for eating of fruits and is used to compensate the limited scope of TSS/TA to define the desired quality at ripeness due to the possible role of other attributes like texture as somewhat softening of mesocarp is required in fruits consumed after ripening [21]. Ethylene synthesis from 1-aminocyclopropane-1-carboxylic acid (ACC) is temperature sensitive and high temperature delayed ripening by inhibiting ethylene activity [37]. In concordance with findings for RPI_{WB} of HWD jujube fruits in the current study, Jat and Lakhawat [38] previously reported a gradual increase in RPI_{WB} in untreated while delayed ripening and lower RPI_{WB} for γ -irradiated jujube fruits. HWD of jujube fruits at 50 °C for 4 min positively impacted in delaying red color development and ripening which is consistent with Yang et al. [17] who reported that full red color development was 15 days later in HW treated jujubes. Likewise, to present research, Rojas-Candelas et al. [21] noted the disparity in RPI_{WB} among apple cultivars while Cárdenas-Pérez et al. [36] reported a decline in the RPI_{WB} of mango during storage. The delayed ripening among HWD jujubes may be attributed to altered energy metabolism leading to lower sugar-acid ratio alongwith less drop in acidity and firmness which is consistent with findings by Huan et al. [39].

3.4. Ascorbic acid

Ber varieties significantly differed in ascorbic acid content of fruits at various evaluation points during storage. Ascorbic acid content of HWD-treated and control; ambient water-dipped fruits from both varieties gradually decreased with storage duration, however, the decline was substantially higher in control than HWD fruits (Fig. 3). The fruits immersed in hot water at 55 °C for 4 min exhibited better retention of ascorbic acid. Mean scores for ascorbic acid reduced in magnitude till the end of storage depicting

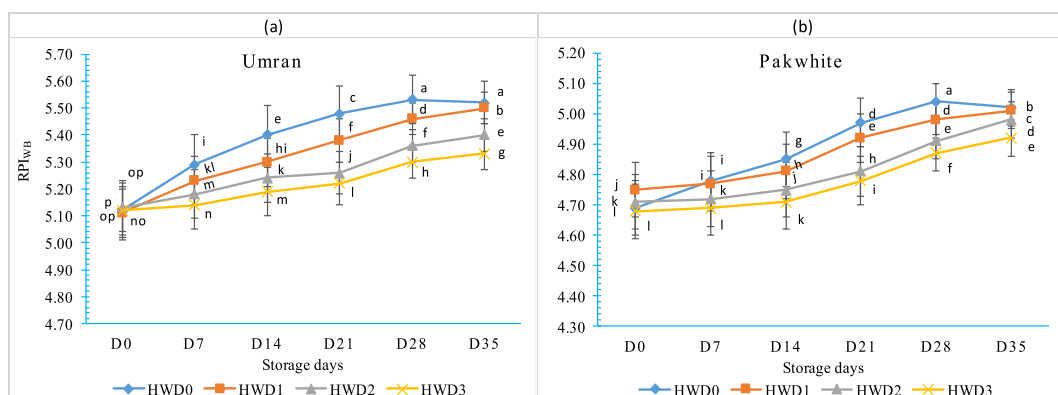


Fig. 2. Ripening index of HWD jujube fruit at different points during storage at 5 °C and 95 % relative humidity.

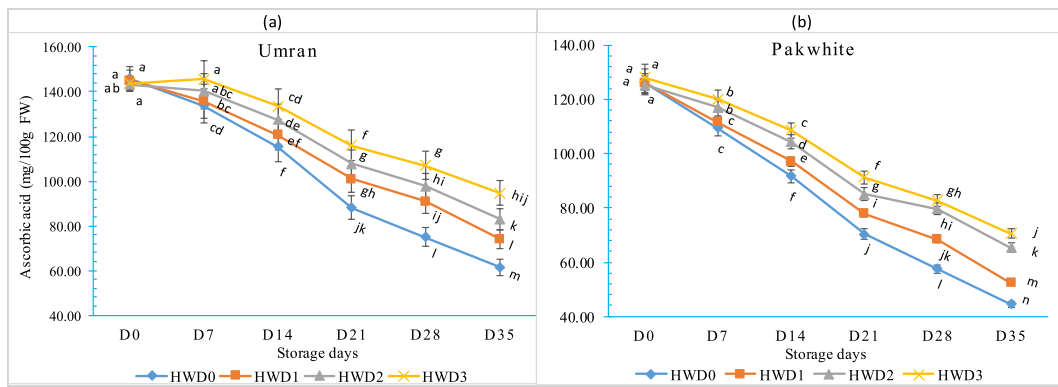


Fig. 3. Ascorbic acid retention in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

declining trend and inverse relation with storage duration ($y = -13.98x + 151.83, R^2 = 0.99$). The analysis displayed 2.75, 6.96 and 8.81 % and 3.75, 6.65 and 10.64 % higher ascorbic acid in fruits from Umran and Pakwhite varieties dipped respectively in water at 45, 50 and 55 °C for 8, 6 and 4 min as compared to fruits dipped in water at ambient temperature. The HWD treatments showed proportionate effects on retaining ascorbic acid which is directly related to exposure temperature (Fig. 3).

The higher ascorbic acid retention among HWD jujube fruits in the present research was presumably due to inactivation of enzymes by hot water resulting in lower ascorbic acid decline owing to its slower conversion into other organic acids in cold storage similar to previous research report by Jat et al. [40] for γ -irradiated Ber fruits. This result is somewhat surprising considering that ascorbic acid is known to be heat labile, however, the enzyme ascorbate oxidase responsible for its oxidation is inactivated at elevated temperatures in nectarines, plums, peaches, apricots and cherries except red bell peppers [41], and broccoli [42]. Conversely, dipping jujubes in hot water inhibit ascorbic acid enzymatic oxidation or degradation and treated fruits thus retained higher level than control with better quality and prolonged shelf life which is consistent with Yang et al. [17]. Ascorbic acid has an imperative role in upholding the ROS quenching system to reduce free radicle levels and thus greater quantity of ascorbic acid aids in delaying jujube fruit ripening [43]. Yang et al. [17] revealed a declining tendency of ascorbic acid during storage, however, the extent of drop was more pronounced in control jujubes whereas HW treated fruits retained 3.20-fold greater ascorbic acid than control after 45 days storage. Similarly, the higher magnitude of ascorbic acid in HWD Ber fruits in the present study is consistent with results from Promyou et al. [30] and Yang et al. [17] for jujubes dipped in hot water.

3.5. Sugars

Glucose and fructose quantity in fruits is enhanced during ripening owing to enzymatic breakdown of polysaccharides like starch and disaccharides sucrose. HWD treatments revealed remarkable variation for glucose and fructose among Ber varieties during storage. The more rapid surge in glucose and fructose content was recorded in Ber fruits dipped in 25 °C water with peak values attained in 2nd week. While HWD treatments significantly belated the rise in glucose and fructose content due to slow ripening on account of lower ethylene synthesis and one week later (21st day) emergence of peak based on dip water temperature and exposure time (Fig. 4 a and b). The monosaccharide content started decline after attaining peak value and its quantity was inversely related with dip water temperature on 35th day in HWD treatments due to better quality by slowing down the normal pace of ripening and respiration. Higher glucose and fructose content at pre-climacteric stage resulted from greater conversion of sucrose into

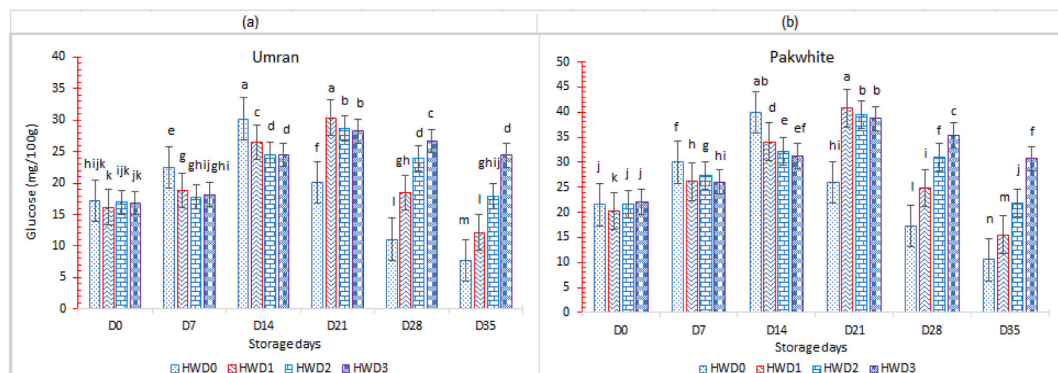


Fig. 4a. Retention of glucose content in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

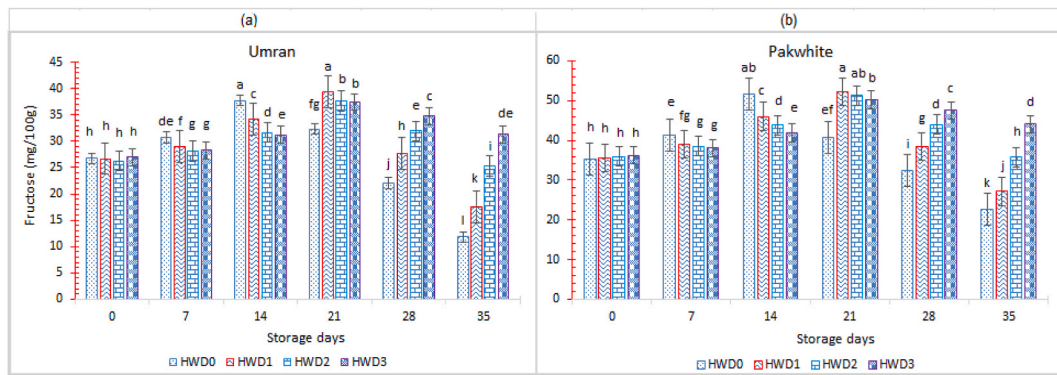


Fig. 4b. Retention of fructose content in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

monosaccharides than synthesis of sucrose from polysaccharide (starch) disintegration owing to lower pace of ripening.

Accrual of sugars in fruits is main determinant of sweetness and taste and is one of the most imperative quality attribute [44]. The mechanism underlying higher sugar retention in HWT fruits might be ascribed to activation of glucosidase, arabinase and galactosidase enzymes owing to enhanced catabolism [8]. Exposure of fruits to heat treatments modify the metabolic process of disaccharides and monosaccharides which spare sugars and resultantly use organic acids as substrate in respiration and results in higher retention of sugars at later storage intervals. Similar to the present research, hot water dipped banana [45] and peach fruits [39] retained greater quantity of total sugars as compared to control during later storage due to accelerated ripening and higher respiration rate in control whereas HWD restrained the pace of respiration and delayed ripening in treated fruits. Similar to Jat et al. [40] findings, faster rise of sugars in control jujube fruits may be ascribed to the greater synthesis of monosaccharides from polysaccharides degradation due to speedy ripening. Secondly, the oxidation process taking place in fruits during storage leads to transformation of acids in to sugars which aligns well with declining quantity of ascorbic and other organic acids (Figs. 3 and 5 a-d). Date fruits from all HWT treatments revealed substantially higher fructose, glucose and sucrose contents than control fruits [8] which contradicts the current research findings during early storage due to early ripening of untreated fruits, however, it is in conformity with the current research findings in late storage with higher sugars in HWD Ber fruits.

Sucrose content increases during ripening of jujube fruits [46]. Sucrose displayed incremental trend ($y = 5.59x + 1.08$, $R^2 = 1.00$) during storage but the quantitative rise was less pronounced in HWD treated fruits than control jujubes (Table 2) which is in concordance with findings by Yang et al. [47] who found that sucrose content rise during ripening of jujubes. The glucose and fructose contents were lower in fruits during pre-climacteric, attained peak at climacteric stage and then declined thereafter following 2nd order polynomial relation with storage ($y = -1.84x^2 + 12.32x + 7.60$, $R^2 = 0.91$ for glucose and $y = -2.02x^2 + 13.11x + 18.77$, $R^2 = 0.91$ for fructose) due to their continual use in respiration and other physiological reactions which is in similarity with Song et al. [48] Yan et al. [49] and Xu et al. [50]. During ripening, the energy (ATP) demand is higher leading to earlier reduction of sugars in control [39] while reduction in starch disintegration can be possible reason for retention of higher sugars at later evaluation intervals due to delayed ripening in HWD fruits, thus extending the shelf life of jujubes. HWD slow down ripening and senescence in jujubes and inhibit disintegration and use of saccharides during storage, thus retaining nutritive value of fruits which is similar to results reported by Xu et al. [50] for reducing sugars in seedless long jujube fruits.

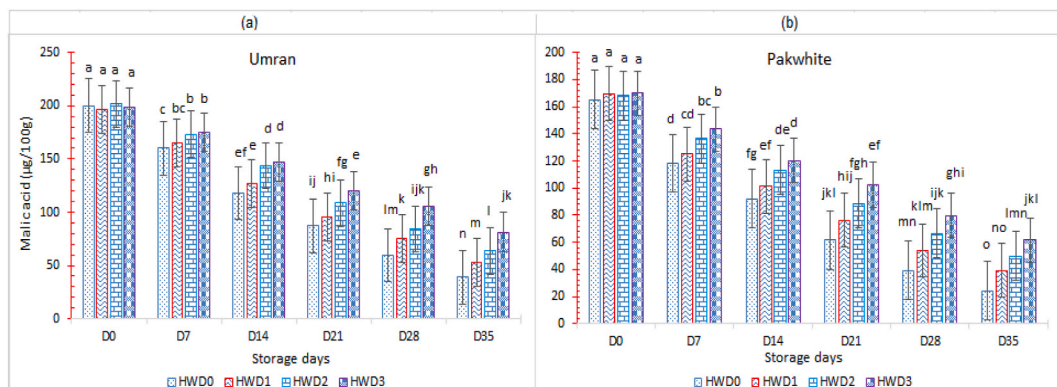


Fig. 5a. Malic acid retention in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

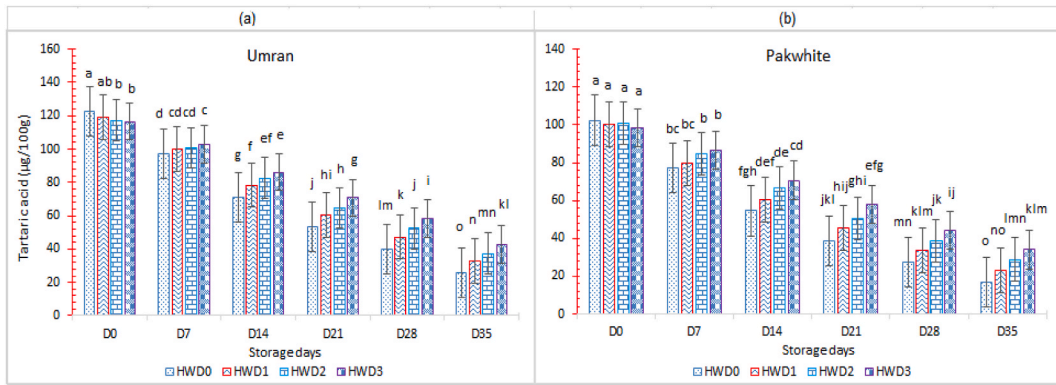


Fig. 5b. Tartaric acid retention in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

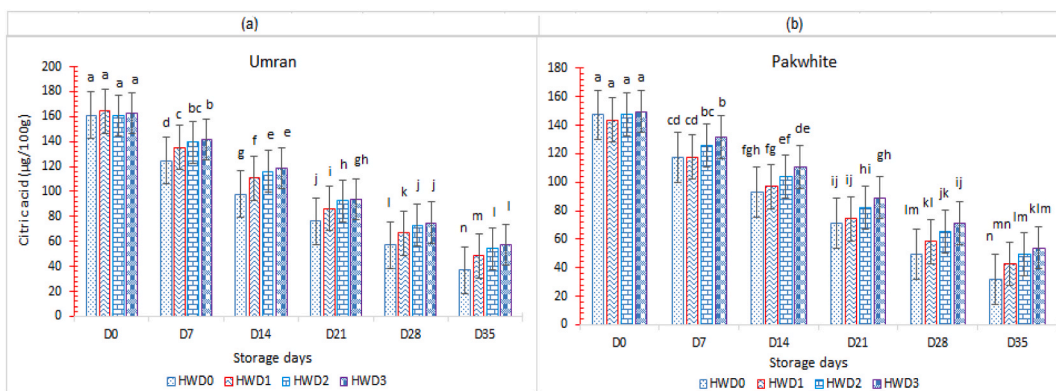


Fig. 5c. Citric acid retention in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

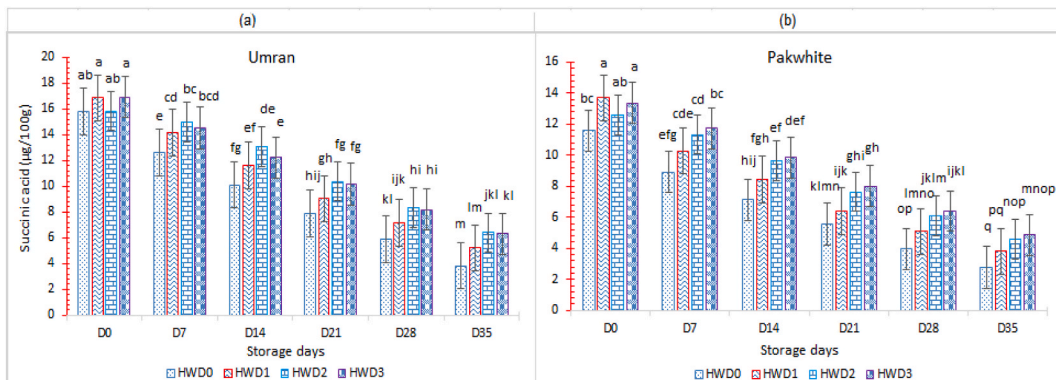


Fig. 5d. Succinic acid retention in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

3.6. Organic acids

The individual organic acid content of the fruits from both Ber varieties was evaluated at different time points during storage, and it was observed that there were significant differences. The magnitude of each acid in fruits with hot water dips (HWD) and those dipped in ambient water gradually decreased with storage duration. However, the content declined more in control fruits than HWD fruits (as shown in Fig. 5 a–d). The fruits immersed in hot water at 55 °C for 4 min exhibited better retention of organic acids. As the storage duration increased, the scores for different acids decreased quantitatively depicting a declining trend and an inverse relation with storage duration ($y = -15.95x + 122.57$, $R^2 = 0.99$ for tartaric, $y = -26.49x + 204.09$, $R^2 = 0.99$ for malic, $y = -21.56x + 172.80$, $R^2 = 1.00$ for citric and $y = -1.98x + 16.33$, $R^2 = 1.00$ for succinic acid). The trend was quantitatively measured for each acid, and it was

Table 2
 Sucrose content (mg/100 g) of HWD jujube fruit on different testing points during storage at 5 °C and 95 % relative humidity.

Varieties	Treatments	Storage days						Treatment means
		0	7	14	21	28	35	
Umran 17.73 ± 0.64B	HWD ₀	4.83 ± 0.62 ^k	14.16 ± 0.58 ^h	21.42 ± 0.67 ^e	30.06 ± 0.73 ^c	36.37 ± 0.62 ^b	39.88 ± 0.50 ^a	24.45 ± 1.45A
	HWD ₁	4.90 ± 0.67 ^k	11.32 ± 0.66 ⁱ	15.91 ± 0.49 ^g	21.15 ± 0.50 ^e	26.23 ± 0.71 ^d	30.78 ± 0.65 ^c	18.38 ± 1.22B
	HWD ₂	4.80 ± 0.39 ^k	10.13 ± 0.50 ⁱ	13.66 ± 0.49 ^h	17.22 ± 0.60 ^f	20.89 ± 0.51 ^e	25.48 ± 0.57 ^d	15.36 ± 1.08C
	HWD ₃	4.95 ± 0.55 ^k	8.55 ± 0.53 ^j	10.63 ± 0.58 ⁱ	14.09 ± 0.55 ^h	17.12 ± 0.54 ^g	21.10 ± 0.48 ^e	12.74 ± 0.96D
Days mean		4.87F	11.04E	15.41D	20.63C	25.15B	29.31A	
Pakwhite 24.12 ± 0.75A	HWD ₀	7.62 ± 0.75 ^f	20.35 ± 0.57 ⁱ	29.5 ± 0.53 ^h	39.52 ± 0.53 ^d	47.69 ± 0.50 ^b	55.96 ± 0.42 ^a	33.44 ± 1.67A
	HWD ₁	7.62 ± 0.62 ^f	16.37 ± 0.57 ⁿ	22.46 ± 0.52 ^k	30.70 ± 0.42 ^g	38.48 ± 0.42 ^e	44.63 ± 0.36 ^c	26.71 ± 1.48B
	HWD ₂	7.58 ± 0.66 ^f	12.55 ± 0.65 ^p	16.6 ± 0.47 ⁿ	22.02 ± 0.33 ^k	27.67 ± 0.45 ⁱ	33.02 ± 0.33 ^f	19.90 ± 1.22C
	HWD ₃	7.65 ± 0.52 ^f	10.44 ± 0.52 ^q	14.14 ± 0.51 ^o	18.19 ± 0.39 ^m	21.59 ± 0.51 ^k	26.55 ± 0.57 ^j	16.43 ± 1.05D
Days mean		7.62F	14.92E	20.67D	27.61C	33.85B	40.04A	

found that the concentration of organic acids decreased in the order of malic < citric < tartaric < succinic acid. The jujube fruits at the white mature stage had the highest concentration of organic acids and their content decreased gradually at subsequent storage intervals, more rapidly in control than HWD fruits. HWD treatments resulted substantial reduction in the rate of decline of acids depending on the temperature of dip water. The least decline in organic acid content was observed in Ber fruits dipped in water at 55 °C for 4 min, which slowed down the rate of respiration and delayed ripening due to lower ethylene synthesis.

In order to extend storage life of fruits, it is effective to inhibit ethylene synthesis and reduce the pace of respiration. As fruits ripen in postharvest storage or undergo senescence, the quantity of organic acids declines, however, HWD postpones the disintegration of these organic acids and helps to retain flavor quality [51]. Organic acids concentration increases during early stages of maturity, but declines during later stages of maturity and ripening. Tepe et al. [46] found that jujube fruits had the highest quantity of malic acid at fully mature stage. Our results are consistent with previous findings that suggest HWT banana fruits have greater sugar and acid contents [45], which is due to alterations in sugar and acid metabolism caused by the slower pace of respiration. During postharvest ripening and senescence, organic acids are used in metabolic processes such as the synthesis of phenols, sugars, and other compounds in respiration. Malic and citric acid are involved in the GABA metabolic pathway, glyoxylic acid and TCA cycles [47], and are intermediate products of the TCA cycle. These acids are directly consumed as substrate in respiration to yield water and CO₂ [52], or combine with metal ions to form weak acid or strong alkali salts [53]. The reason behind the higher organic acid levels is slower pace of ripening and senescent as well as reduced respiratory and other physiological metabolic processes sparing organic acids in HWD jujube fruits.

3.7. Malondialdehyde content

The fruits from the two Ber varieties that received different HWD treatments showed a considerable variability in MDA during storage. This means that Umran fruits had more stable membrane lipids compared to Pakwhite fruits. The fruits that were immersed in

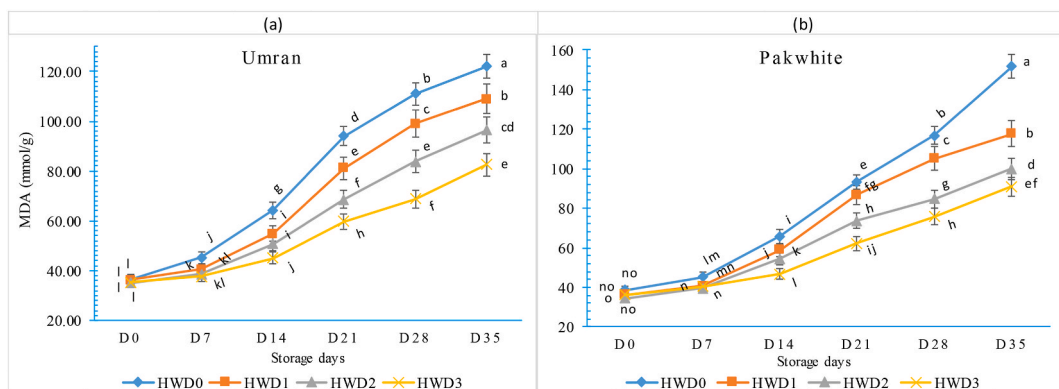


Fig. 6. Malondialdehyde content in HWD jujube fruit at different storage intervals at 5 °C and 95 % relative humidity.

HW evidenced a significant diminution in MDA content (Fig. 6); in contrast, markedly greater MDA was measured in control fruits of both varieties. However, response to HWD treatments showed a declining linear trend ($y = -8.43x + 90.15$, $R^2 = 0.99$) related to the dip water temperature. The maximum MDA reduction was recorded in the HWD₃ treatment.

The MDA content of Ber fruits showed an incremental trend at subsequent evaluations, and as the storage duration increased, there was a direct relationship to MDA production (Fig. 6). Significantly elevated values for MDA content were measured in fruits at the end of 35-days storage, which might be due to extended exposure time to storage stresses, resulting in higher degradation of membrane lipids. MDA content after harvest was directly associated with storage duration and increased in both untreated and HWD fruits with the passage of time, however, its magnitude was significantly higher in control fruits. Various aldehydes, including malondialdehyde, are produced as an ultimate product of membrane lipid oxidation, which can cause damage to the structural integrity and functionality of cell membranes. This can augment metabolic disorders, elevates fruit decay prevalence and accrual of excessive MDA content and reactive oxygen species (ROS) which consequently hastens the decay and senescence of fruits [35]. MDA accumulation is recognized as an indicator of oxidative stress in plants and can be used as an index to gauge the integrity of membrane structure in stored fresh fruits, especially under cold storage [54]. Studies have shown that heated water dip of jujubes is effective in reducing oxidation of membrane lipids and declining the rise of MDA content, compared to non-treated fruits [17; 30] which is in line with the current study findings. Thus, HWD helps to improve quality and shelf stability of jujube fruits by sustaining membrane integrity. Shadmani et al. [55] reported significantly increased accumulation of antioxidant enzymes in papaya fruits treated with HWD. Accrual of ROS is basic mechanism for the loss of structural integrity of membrane which could be decreased by elevated level of antioxidant enzymes and consequently result significant reduction in MDA content. Thus, HWD could maintain a delicate balance between oxidation and antioxidants in fruits through controlling oxidative stress [17]. During storage, MDA content tends to increase. However, fruits such as papaya [55], peach [39], and green mume [14] that were treated with hot water immersion exhibited considerably lower MDA content than control, which corresponds well with results from the current research.

3.8. Electrolyte leakage

During storage, HWD jujube fruits of both varieties displayed substantial disparity in the amount of electrolyte leakage. Regardless of treatments, electrolyte leakage increased with length of storage. However, HWD supervised a considerable decline in the amount of electrolyte leakage that was inversely proportional ($y = -0.57x + 4.92$, $R^2 = 0.89$) to the water temperature in fruits from both varieties. At the start of the storage period, the electrolyte leakage was minimal, but it progressively increased up to the 35th day. Among HWD fruits, the highest electrolyte leakage value was recorded for Ber fruits dipped in 45 °C water for 8 min (HWD₁) (Table 3). The membrane stability of Ber fruits, as indicated by electrolyte leakage, followed a steady descending trend ($y = 0.75x + 0.89$, $R^2 = 1.00$) with the progression of storage. HWD-treated Ber fruits revealed that the treatments were effective in producing 27.3, 38.4 and 46.6 % and 31.1, 37.4 and 44.7 % reductions in electrolyte leakage respectively in Umran and Pakwhite fruits after respective dip treatments for 8, 6, and 4 min at 45, 50, or 55 °C.

Electrolyte leakage is regarded as an index of membrane damage. External stresses during storage expose cell membranes to shocks by disturbing delicate balance between antioxidants and free radicals leading to oxidative stress which leads to disintegration of membrane constituents and result in leakage of electrolyte due to loss of membrane selectivity and increased porosity. However, fruits dipping in heated water can improve homeostasis of antioxidant enzymes and heat shock proteins transcripts, which helps to protect membrane proteins from disintegration and maintain cellular integrity [56]. Heat pre-conditioning could effectively retain membrane functionality and protect membrane constituting components of plant cells. When jujube fruits are treated with hot water, electrolyte leakage was delayed and quantum was reduced as compared to untreated fruits [17] which is effective in reducing the incidence of physiological disorders and helps in sustaining integrity of both cell walls and membranes. HWT stimulates the defense mechanism existing in the peripheral layers of jujube fruit's epicarp [30]. The variability in electrolyte leakage among Ber fruits from the current study may be attributed to differences in genetic makeup and peel characteristics of the jujube varieties. Ber fruits dipped in water at higher temperature exhibited better membrane stability, which resulted in lower ion leakage by keeping membrane constituents intact. This indicates that the membrane integrity was proportionately better in HWD treated fruits than untreated ones due to protective effect of water temperature in slowing down disintegration of membrane constituents. Studies conducted on chilling sensitive

Table 3
Electrolyte leakage ($\mu\text{S}/\text{cm}$) of HWD jujube fruit at different testing points during storage at 5 °C and 95 % relative humidity.

Varieties mean	Treatments	Storage days						Treatment mean
		0	7	14	21	28	35	
Umran 3.08 ± 1.13 B	HWD ₀	1.49 ± 0.20 ^q	2.85 ± 0.28 ^k	3.88 ± 0.33 ^f	4.84 ± 0.36 ^c	5.32 ± 0.37 ^b	6.00 ± 0.40 ^a	4.06 ± 1.26A
	HWD ₁	1.44 ± 0.20 ^q	2.00 ± 0.22 ^o	2.68 ± 0.26 ^l	3.35 ± 0.30 ^j	3.89 ± 0.33 ^f	4.71 ± 0.36 ^d	3.01 ± 1.07B
	HWD ₂	1.48 ± 0.20 ^q	1.77 ± 0.22 ^p	2.45 ± 0.26 ^m	3.06 ± 0.28 ^j	3.51 ± 0.32 ^h	4.03 ± 0.33 ^e	2.72 ± 0.97C
	HWD ₃	1.46 ± 0.20 ^q	1.90 ± 0.22 ^{op}	2.24 ± 0.24 ⁿ	2.64 ± 0.26 ^l	3.25 ± 0.30 ⁱ	3.69 ± 0.32 ^e	2.53 ± 0.89D
Days mean		1.47F	3.13E	2.81D	3.47C	3.99B	4.61A	
Pakwhite 3.93 ± 1.33 A	HWD ₀	1.74 ± 0.22 ^q	3.18 ± 0.30 ^{lm}	4.36 ± 0.35 ^h	5.54 ± 0.39 ^d	6.98 ± 0.44 ^b	9.08 ± 0.50 ^a	5.14 ± 1.58A
	HWD ₁	1.73 ± 0.22 ^q	2.78 ± 0.28 ^{no}	3.46 ± 0.30 ^k	3.97 ± 0.33 ^j	5.30 ± 0.37 ^e	6.11 ± 0.41 ^c	3.89 ± 1.23B
	HWD ₂	1.81 ± 0.22 ^q	2.65 ± 0.26 ^o	3.11 ± 0.28 ^m	4.18 ± 0.33 ^j	4.80 ± 0.36 ^e	5.12 ± 0.37 ^f	3.61 ± 1.10C
	HWD ₃	1.74 ± 0.22 ^q	2.31 ± 0.24 ^p	2.89 ± 0.28 ⁿ	3.28 ± 0.30 ^j	3.98 ± 0.33 ^j	4.34 ± 0.35 ^{hi}	3.09 ± 0.96D
Days mean		1.75F	2.73E	3.45D	4.24C	5.27B	6.16A	

papaya [55], peach [39], tomato [57] and mango [58] fruits have shown that heat treatments alone or in combination with other treatments, can effectively reduce electrolyte leakage and maintain integrity of cell membranes.

3.9. Antioxidant potential

The antioxidant capacity of fruits from the two Ber varieties was similar after HWD treatments at day 0. However, HWD fruits retained higher antioxidative capacity in the DPPH assay (Table 4) at subsequent testing intervals. HWD resulted in a considerable improvement in retention of antioxidant capacity, which was linearly proportional to HWD temperature ($y = 3.10x + 55.08$, $R^2 = 1.00$). The antioxidant potential was maximal at the beginning, and progressively declined during the 35-day storage. The highest DPPH inhibition value was found in fruits treated at 55 °C for 4 min. Overall, the highest HWD temperature with least exposure time was most effective in retaining greater antioxidant capacity. The previous study found that green jujube fruits have the highest DPPH free radicle quenching activity, which declines as the fruit ripens and is lowest in ripe red fruits [20] which is similar to results from present investigation.

In the present research, the higher level of antioxidants in HWD Ber fruits can be attributed to the fact that treated fruits exhibited significantly higher level of compounds with antioxidant properties. The reason why fruits immersed in hot water at higher temperatures have higher levels of antioxidants is due to the accumulation of greater amounts of polyphenols in response to the HWD. This leads to the activation of defense antioxidants, less accrual of free radicles and the retention of higher quantity of compounds with antioxidant activity. As a result, a lower quantity of antioxidants is required to quench free radicals. The antioxidant activity based on percent inhibition by DPPH radicles varied from 70.69 to 93.93 % in Iranian [59] and 64–90 % in Romanian jujubes [60], which is consistent with the results of current study. Jujube fruits are known to have exceptionally high phenol content, making them superior to grapefruits and cherries for antioxidant activity [61].

The fruits dipped in water at 55 °C for 4 min had a greater antioxidant value possibly due to less exposure time, as bioactive compounds (ascorbic acid, polyphenols and flavonoids) with antioxidative activity are mostly heat labile [62]. The previous study also found that HWD notably increased the level of antioxidants in dates from two cultivars than untreated fruits [9], which corresponds well with findings from present study. The study concludes that enhanced DPPH scavenging is linearly related with greater quantity of non-enzymatic defense antioxidants.

3.10. Total polyphenol contents

The amount of polyphenols found in Ber fruits varied significantly during storage, depending upon the type of dip treatment used. The fruits from both varieties exhibited noticeable variation at different evaluation points during storage. The total phenolic contents in the fruits declined over time, regardless of the HWD treatment applied. However, the extent of the decrease was less pronounced in treated fruits compared to the control fruits (Fig. 7). A linear association was observed between total phenolics and the HWD treatments in terms of water temperature ($y = 23.13x + 206.92$, $R^2 = 0.99$), which might be attributed to less exposure time at higher temperature.

Phenolics were the highest at the beginning, and the treated Ber fruits retained remarkably higher phenolic contents at all subsequent evaluation times than the control fruits that were dipped in ambient temperature (25 °C) water. The phenol content of jujube fruits declines during ripening [39] as these are likely to be hydrolyzed or converted into other constituents like organic acids, sugars and some other compounds. This may also be due to shift in solubility from soluble to insoluble form in harvested fruits [62]. However,

Table 4
Antioxidant activity (%) of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

Varieties mean	Treatments	Storage days						Treatment mean	
		0	7	14	21	28	35		
Umran 66.75 ± 2.59A	HWD ₀	102.29 ±	83.64 ±	72.52 ±	56.64 ±	32.54 ±	23.41 ± 1.06 ^P	61.84 ± 1.26D	
		1.18 ^a	0.84 ^d	1.18 ^g	0.98 ^j	1.05 ^{no}			
	HWD ₁	101.29 ±	88.42 ±	75.52 ±	60.50 ±	35.50 ±	29.63 ± 0.79 ^o	65.14 ± 1.25C	
		0.71 ^a	1.10 ^c	1.14 ^f	1.14 ⁱ	1.01 ^{lm}			
	HWD ₂	102.93 ±	95.69 ±	76.88 ±	65.53 ±	38.25 ± 1.05 ^l	32.69 ±	68.66 ± 1.24B	
		1.25 ^a	0.88 ^b	0.93 ⁱ	0.78 ^h		0.91 ^{mn}		
	HWD ₃	102.32 ±	98.32 ±	80.32 ±	69.87 ±	41.66 ± 0.87 ^k	35.66 ± 0.86 ^l	71.36 ± 1.22A	
		0.93 ^a	0.99 ^b	1.08 ^e	0.97 ^g				
	Days mean		102.21A	91.52B	76.31C	63.13D	36.99E	30.35F	
	Pakwhite 58.9 ± 2.58 B	HWD ₀	94.52 ± 1.16 ^a	77.57 ±	65.37 ±	43.68 ±	26.50 ±	19.38 ± 0.98 ^P	54.51 ± 1.23D
				1.00 ^e	1.12 ^g	1.03 ^j	1.10 ^{mn}		
		HWD ₁	95.31 ± 0.84 ^a	81.96 ±	67.39 ±	47.82 ±	29.57 ±	23.01 ± 0.47 ^o	57.51 ± 1.22C
			1.04 ^d	0.95 ^g	0.98 ⁱ	1.10 ^{lm}			
HWD ₂		94.52 ± 0.75 ^a	85.15 ±	71.36 ±	50.34 ±	32.67 ± 1.05 ^l	25.55 ±	59.93 ± 1.23B	
			1.10 ^c	0.82 ^f	0.92 ⁱ		0.91 ^{no}		
HWD ₃		95.38 ± 0.99 ^a	90.62 ±	75.46 ±	54.64 ±	37.47 ± 0.91 ^k	28.35 ±	63.65 ± 1.22A	
			0.82 ^b	1.14 ^e	1.01 ^h		0.82 ^{mn}		
Days mean			94.93A	83.82B	69.89C	49.12D	31.55E	24.07F	

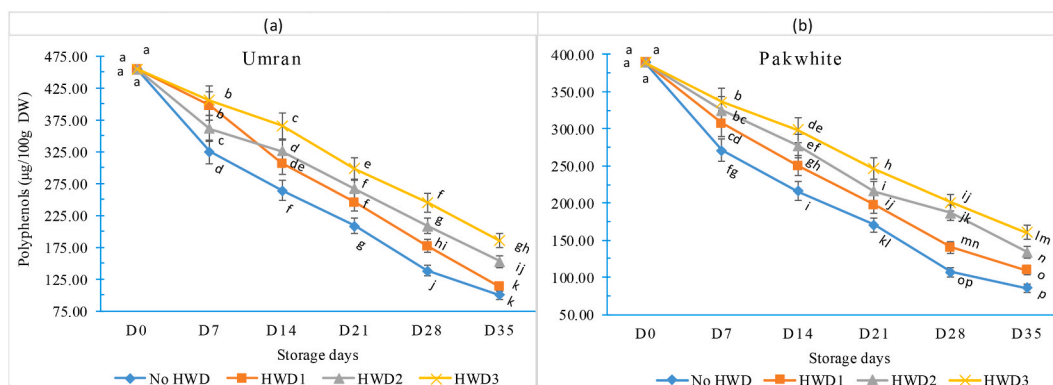


Fig. 7. Total polyphenol content of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

the polyphenol content in hot water treated jujube fruits was proportionately higher in quantity than in the control group and directly related to the dip water temperature. Fruits dipped in water at 55 °C for 4 min exhibited significantly greater phenolics owing to less treatment time, as phenols are heat sensitive. Higher temperature with long exposure time may decompose polyphenols. Shen et al. [63] reported higher phenolics in 50 °C water dipped Satsuma mandarin as compared to fruits treated for same time (3 min) in water at 52 °C or 54 °C which evidenced present research findings that less exposure time with high dip temperature is better in retaining polyphenols. Therefore, a higher polyphenol prevalence in HWD Ber fruits from this research is similar to HW treated date fruits [8], which is attributed to the inactivation of the phenolic metabolizing enzymes i.e. polyphenol oxidase. Similarly, Endo et al. [14] observed delayed reduction in phenol content of HW treated mature green mume fruits. Likewise, papaya fruits [13] immersed in hot water accumulated higher polyphenols.

3.11. Sensory evaluation

Both Umran and Pakwhite varieties exhibited distinct responses to the various hot water dip treatments and storage durations. However, the trend of the responses was similar, indicating that the factors studied affected the sensorial attributes of both types of Ber fruits in same way. The mean values represented the average sensorial values throughout the storage period. The data illustrated a noticeable influence of the various hot water dip treatments on the sensorial parameters, including color, flavor, taste and overall acceptance of the Ber fruits. Different treatments appeared to influence sensory perception scores differently at various storage durations, indicating variations in the response to these treatments over time (Fig. 8 a–c and Table 5). The duration of storage plays a significant role for changes in sensorial scores of the Ber fruits. As the storage duration increases, fluctuations were recorded in the sensory scores.

Peach fruits that were immersed in hot water at 50 °C for 3 min exhibited better sensory scores than other treatments, according to Shah et al. [64]. Medjool dates that were dipped for 10 min attained the highest taste scores, while the fruits that were immersed in hot water for 5 and 10 min received the maximum values for appearance, flavor and overall acceptance [65]. Similar response was noted in studies by researchers such as Li et al. [8] who reported that dry dates that were dipped in hot water for 5 min (cv. Khadrawi) and 3 min (cv. Hillawi) showed improved color and the greatest overall sensorial acceptance values. In addition, HWT significantly inhibited off taste and off odor development in fresh cut Chinese cabbage during storage, as reported in study by Grzegorzewska et al. [16]. Likewise, various studies reported higher sensory perception scores for HW treated mango [66], banana [67] and Satsuma mandarin fruits [68] with retention of better quality attributes which are consistent with results from current investigation. Moreover, HWT has pivotal role in volatile development, and treated Sonata strawberry fruits differed significantly for volatiles including acetaldehyde, methyl and ethyl esters [69], when compared to untreated ones. HWD fruits retained higher sensory scores in quince during storage as compared to 1-MCP treated ones owing to lack of aroma [11] after 1-MCP treatment.

3.12. Correlation analysis and heat map clustering among quality parameters of jujube fruits

The correlation among physiological and sensory attributes of jujube fruits following hot water dip treatments during cold storage was performed (Fig. 9 a, b). The trend of relationship among various parameters was almost similar in jujube fruits from both varieties, however, response was more pronounced in Pakwhite than Umran. Secondly, flavor don't show any relation with other parameters in Umran. The Pearson correlation confirmed strong positive association between physiological parameters like WL, DI, RPI, MDA, EC and sucrose in both varieties while all these parameters exhibited significant negative correlation with phytochemicals (Vit. C, phenols, DPPH), reducing sugars (glucose and fructose), organic acids (Tartaric, malic, citric and succinic) and sensory attributes (color, flavor, acceptance) except flavor and succinic acid in Umran. The increase of weight loss, RPI, EC and MDA indicated that the jujube fruits underwent accelerated ripening and senescent in control fruits and more susceptible to pathogen infection and decay. It is evidenced from literature reports that when fruits are under stress or invaded by pathogens, these activate their inbuilt defense mechanism mainly in the form of ROS which along with ascorbic acid improve resistance to disease or stress conditions. Enzymatic or

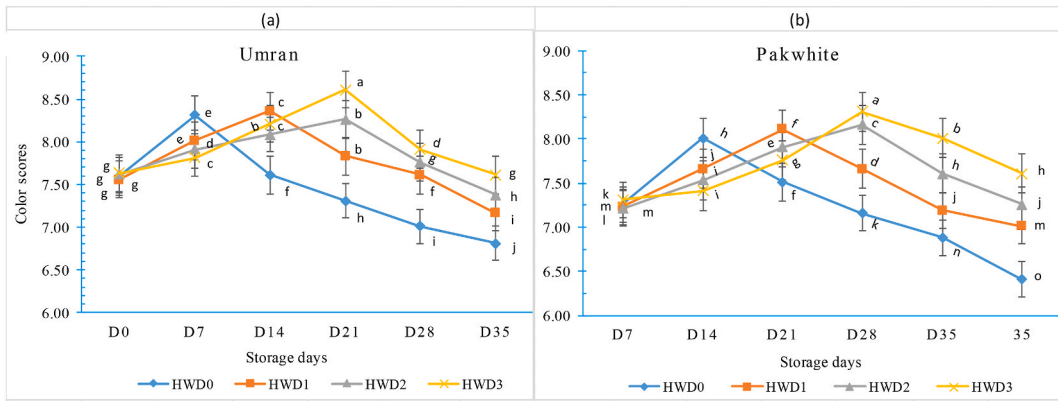


Fig. 8a. Sensory evaluation (color scores) of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

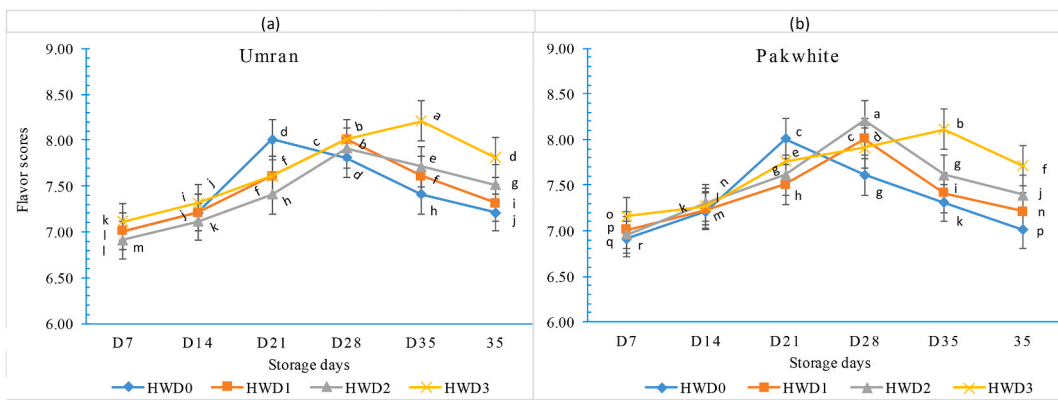


Fig. 8b. Sensory evaluation (flavor scores) of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

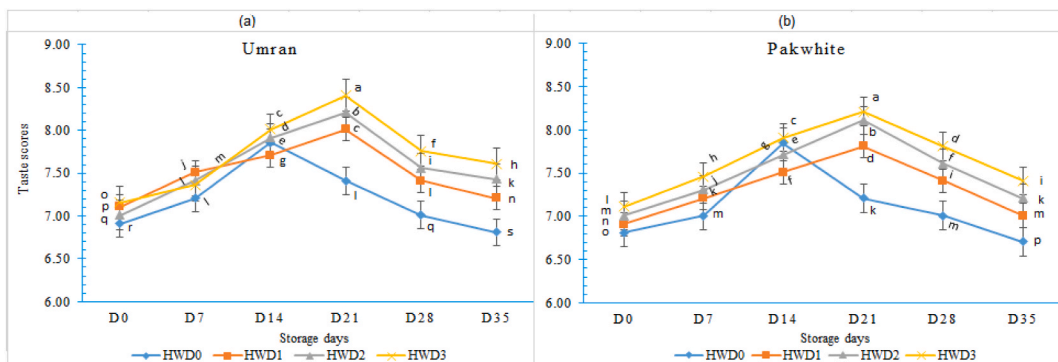


Fig. 8c. Sensory evaluation (taste scores) of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

non-enzymatic antioxidants already prevalent in fruits or synthesized in response of stress quench these ROS to decrease electrolyte leakage from membrane by delaying lipid oxidation [14]. These findings are well illustrated by correlation among attributes. In line with current study findings, Yang et al. [17]. reported same relationship trend among jujube fruits for decay incidence, weight loss, red index, REL and MDA content, which were inversely related with ascorbic acid content, firmness, CAT and SOD activities. Similarly, Wang et al. [70] revealed that crisp fine jujube fruits were positively correlated with ascorbic acid, firmness, and SOD activity, but inversely related with crisp rotten fruits, percent soft, percent rotten fruits, TSS content and weight loss.

To differentiate among jujube fruits from both varieties based on physiological and sensory parameters at various durations, heat map clustering was performed. The gradient change in color from blue to red denotes the variation in different quality attributes from

Table 5
Overall acceptance scores of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

Varieties mean	Treatments	Storage days						Treatment Mean
		0	7	14	21	28	35	
Umran 7.63A	HWD ₀	6.81 ± 0.21 ⁿ	7.31 ± 0.20 ^j	7.81 ± 0.23 ^f	7.60 ± 0.22 ^g	7.22 ± 0.20 ^k	7.00 ± 0.20 ^l	7.29 ± 0.24D
	HWD ₁	6.92 ± 0.22 ^m	7.40 ± 0.22 ⁱ	8.01 ± 0.24 ^e	8.12 ± 0.23 ^d	7.61 ± 0.22 ^g	7.20 ± 0.20 ^k	7.54 ± 0.26C
	HWD ₂	7.01 ± 0.20 ^l	7.52 ± 0.23 ^h	8.22 ± 0.24 ^c	8.61 ± 0.24 ^a	8.02 ± 0.23 ^e	7.42 ± 0.22 ^j	7.79 ± 0.30B
	HWD ₃	7.04 ± 0.20 ^l	7.63 ± 0.22 ^g	8.41 ± 0.22 ^b	8.66 ± 0.25 ^a	8.21 ± 0.24 ^c	7.64 ± 0.22 ^g	7.92 ± 0.30A
Days mean		6.93F	7.46D	8.11B	8.23A	7.76C	7.31E	
Pakwhite 7.33B	HWD ₀	6.61 ± 0.22 ^o	6.81 ± 0.23 ^m	7.51 ± 0.21 ^g	7.20 ± 0.21 ^j	6.90 ± 0.25 ⁱ	6.72 ± 0.20 ⁿ	6.96 ± 0.22D
	HWD ₁	6.71 ± 0.19 ⁿ	7.01 ± 0.22 ^k	7.60 ± 0.22 ^f	7.82 ± 0.22 ^e	7.30 ± 0.22 ⁱ	7.02 ± 0.20 ^k	7.24 ± 0.26C
	HWD ₂	6.62 ± 0.20 ^o	7.31 ± 0.21 ⁱ	7.82 ± 0.23 ^e	8.24 ± 0.24 ^b	7.61 ± 0.23 ^f	7.24 ± 0.20 ^l	7.46 ± 0.30B
	HWD ₃	6.70 ± 0.23 ⁿ	7.41 ± 0.22 ^h	7.96 ± 0.22 ^d	8.44 ± 0.25 ^a	8.01 ± 0.25 ^c	7.46 ± 0.22 ^h	7.66 ± 0.30A
Days mean		6.66F	7.13D	7.72B	7.91A	7.46C	7.08E	

Mean ± SE values are calculated as an average of three observations; means carrying different alphabets in rows or columns separately for each variety are significantly different from each other.

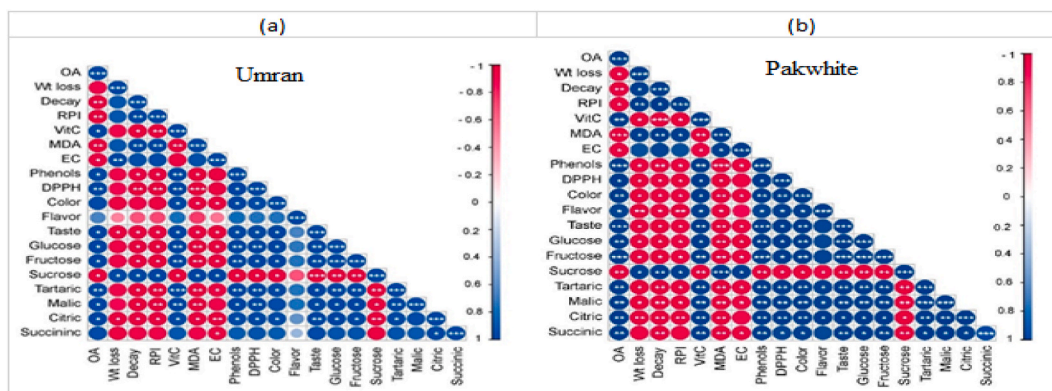


Fig. 9. (a & b) Pearson correlation among various quality parameters of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

low to high. All attributes are grouped into 2 major clusters in heat map along vertical axis. Cluster 1 comprises parameters responsible for the significant variability in jujube fruits negatively impacting quality and shelf stability, including EC, sucrose, RPI, decay, MDA and weight loss. Among dip treatments, HWD₀ with label F-1 representing a color composition dominated by dark blue dark red in cluster 1 depicts maximum variation in control fruits on 35th day whereas F-4 with light pink and white color in cluster 1 for Pakwhite while crimson and white for Umran, indicates a significantly lower quality changes. The parameters in cluster 2A, includes organic acids; malic acid, tartaric acid, citric acid, succinic acid and bioactive compounds like polyphenols, ascorbic acid and antioxidants. These are basic attributes responsible for the quality retention and better sensory perception. In Fig. 10 (a, b), both HWD₃ and HWD₂ predominantly displayed dark red color on day 0 with gradient change to blue or light blue on 35th day, suggesting the minimum

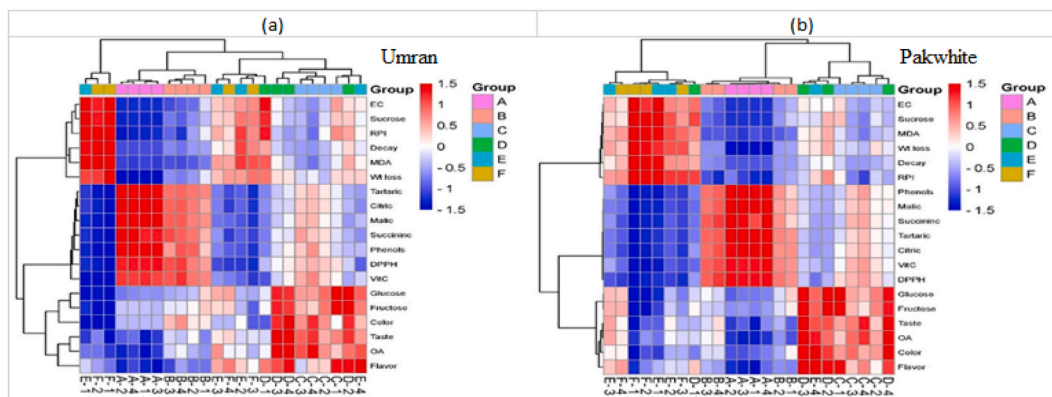


Fig. 10. (a & b) Heat map clusters of HWD jujube fruit at different evaluation points during storage at 5 °C and 95 % relative humidity.

decline. In contrast, HWD₁ (B2) begins with a dark pink at 7 day of storage, fading to light pink (C2) transiently changing to light blue (D2) and even dark blue (E2 and F2) as storage time extends. The cluster 2B1, comprised of glucose and fructose while 2B2 consists of sensory attributes (color, flavor, taste and acceptance) of jujube fruits. F-4 (HWD₃) exhibits lighter shades of blue, pink, red and white colors representing least quality changes whereas F-1 (control) presents dark blue color illustrating significant change in sugars and sensory quality. The color distribution implied lowest sugars with least sensory perception scores for both jujube varieties. Suffix 1 means the highest decline in quality attributes for control while 2 shows color range from dark red to dark blue but lower intensity than control. Meanwhile, suffix 3 consistently showed lighter color as compared to HWD₁, denoting higher content of sugars and better sensory scores with increasing dip temperature.

Variability in content of different quality parameters are evident from heat map analysis of fruits from both jujube varieties across various HWD treatments and storage durations. Compared with HWD₁ and HWD₂, HWD₃ better retained the physiological and sensory attributes of jujube fruits during the entire storage period, and the RPI, sucrose, were higher in HWD₂ and HWD₁ at 35th day. Glucose, fructose and flavor were higher on 14th day in control, whereas on 21st day in HWD₁. Color, taste and OA scores for HWD₃ and HWD₂ were highest on 21st while flavor on 28th day in HWD₃. Likewise to current research, Cui et al. [72] used heat map to depict variation of aroma volatiles in MAP jujube fruits with length of storage. Yang et al. [17] reported trend similar to current research by clustering of firmness, ascorbic acid, acidity, TSS, and enzymes with MDA, EC and maturity with extension in storage time, further confirming that HWD has potential to maintain quality of jujube fruits.

4. Conclusion

Being nonchemical, HWD is an eco-safe technique that can be used to prolong the shelf life of fruits. When jujube fruits were treated with hot water at different temperatures, they exhibited better quality with lower weight loss, decay index and MDA whereas these retained higher ascorbic acid and antioxidant value and delayed ripening with lower ripening index. Among HWD treatments, the fruits dipped in hot water at 55 °C for 4 min exhibited the highest retention of ascorbic acid, organic acids, glucose, fructose, polyphenols and antioxidant value. These also had the lowest disease index, weight loss, sucrose content, RPI, MDA content and leaked electrolyte as well as better sensorial perception scores on 35th day, which indicates greater shelf stability. The reason for the retention of better quality attributes in jujube fruits exposed to 55 °C was relatively less exposure time in hot water dip treatments. Most bioactive compounds are heat labile, and longer exposure time also accelerates the rate of ripening and senescent processes linked with greater decay index, weight loss, organic acid use and early rise in sugar level, which ultimately results in a short shelf life. Also high dip water temperature with less exposure time is conducive for retaining greater quantity of bioactive compounds, better nutritive quality and enhance shelf stability. Therefore, HWD can be used as a quarantine treatment, a cheap and eco-friendly alternative to fungicide application for shipping fruits to distant markets without danger of residual chemicals. However, further research is needed to quantify individual polyphenols and enzymes to pinpoint their contribution in conferring higher antioxidant capacity. Additional research work is also necessary to probe the impact of HWD in integration with other postharvest interventions to make it a promising solution to address the crucial issue of postharvest loss reduction.

Ethics statement

The informed prior written consent was sought from participants with voluntary participation. The confidentiality, privacy and safety of participants as well as respecting the rights and well-being of participants while ensuring the integrity and validity of the research findings. The training provided to panel members as per departmental guidelines for sensory evaluation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

CRediT authorship contribution statement

Zafar Iqbal: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Tahir Zahoor:** Writing – review & editing, Investigation, Conceptualization. **Imran Pasha:** Writing – review & editing, Validation, Supervision, Resources. **Muhammad Shahid:** Visualization, Software, Methodology, Formal analysis.

Declaration of competing interest

Authors declare no conflict of interest with any other researcher. Moreover, the data presented in current manuscript is part of Ph.D research which is not published yet neither it is under consideration for publication in any journal. All the authors have studied the manuscript and are willing to publish it.

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