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Comparative Response of Mango Fruit towards Pre- and Post-Storage Quarantine Heat Treatments

Saqib Javed [2,†], Huimin Fu [1,†], Amjad Ali [2], Atif Nadeem [2], Muhammad Amin [3], Kashif Razzaq [2], Sami Ullah [2], Ishtiaq A. Rajwana [2], Shafa Nayab [2], Vasileios Ziogas [4], Ping Liu [1,*] and Syed Bilal Hussain [5,*]

1 Guangxi Laboratory of Germplasm Innovation and Utilization of Specialty Commercial Crops in North Guangxi, Guangxi Academy of Specialty Crops, Guilin 541004, China; fuhuimin@126.com
2 Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture, Multan 600000, Pakistan; saqibbandesha4@gmail.com (S.J.); amjadali.pk121@gmail.com (A.A.); atifn062@gmail.com (A.N.); kashif.razzaq@mnsuam.edu.pk (K.R.); sami.ullah1@mnsuam.edu.pk (S.U.); ishtiaq.rajwana@mnsuam.edu.pk (I.A.R.); shafa.nayab@mnsuam.edu.pk (S.N.)
3 Department of Horticultural Sciences, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan; m.amin@iub.edu.pk
4 Institute of Olive Tree Subtropical Crops and Viticulture, ELGO-DIMITRA, 73134 Chania, Greece; ziogas@elgo.iosv.gr
5 Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd., Lake Alfred, FL 33850, USA
* Correspondence: liupingsmile@126.com (P.L.); syedbilalhussain@ufl.edu (S.B.H.)
† These authors contributed equally to this work.

Abstract: The present study investigates the comparative effect of pre- and post-storage quarantine heat treatments (hot water treatment (HWT) and vapor heat treatment (VHT)) on the post-harvest performance of the mango fruit cv. 'Chenab Gold'. The results indicate that the application of HWT at 48 °C for 60 min or VHT at 47 °C for 25 min after 21 days under cold storage enhanced the ethylene production and fruit weight loss, while decreasing fruit firmness and vitamin C content. Noticeably, the post-storage heat treatments ruptured the fruit and destroyed their market value. However, fruit treated with HWT or VHT at harvest exhibited slow weight loss, better skin color, and maintained biochemical attributes as compared to the control when kept under ambient storage conditions. Taken together, the application of hot water before storage has a positive influence on mango fruit quality, while post-storage heat treatment has a devastating impact upon fruit quality and shelf life, cancelling its potential commercial application.

Keywords: *Mangifera indica*; post-harvest quarantine heat treatments; cold storage; post-harvest quality; shelf life

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1. Introduction

Mangifera indica, commonly known as mango, belongs to the genus *Mangifera*, which includes a number of species ranging from 45 to 69 in the Anacardiaceae family [1]. Presently, it is grown in tropical countries being the sixth most widely grown fruit crop in the world. Mango production in the world is estimated to be about 46.51 million MT [2]. Pakistan ranks as the sixth major producer of mango in the world with a contribution of about 5% to the total world production [3]. Mango has second position in the Pakistan fruit industry and is cultivated on 0.17 million hectares with an annual production of 1.7 million tons [4]. Langra, Sindhri Anwar Ratol, Fajri Samar Bahisht, Zafran, Fazli, Dusheri, Saroli, Ghulab Khas, Bagan pali, Swarnarica, Neelum, and White and Black Chaunsa are just a few of the important commercial varieties of mango grown in Pakistan.

Fruit yield and quality are largely influenced by both pre- and post-harvest factors [5]. Among them, insect infestation has a devastating impact on mango yield and quality. Mango fruit flies are a serious threat to the mango fruit industry. Every year, the fruit fly

infestation causes a great loss worldwide [6]. In Pakistan, two fruit fly species (oriental and peach fruit fly) have been identified [7]. To avoid this problem, different insecticides are used to eliminate the fruit fly maggots, but this application harms human health [8]. Due to global awareness of consumer health, the use of chemicals has been banned by importing countries, such as Iran and China [8]. As an alternative, mango fruits are treated with non-chemical treatments to eliminate fruit fly maggots and larvae, and these treatments have received much attention in recent years due to their effectiveness [9]. Different heat treatments are used to kill the fruit fly larvae in mango, which include hot water treatment (HWT), vapor heat treatment (VHT), and irradiation [10].

The HWT treatment involves the transfer of heat from the water to the peel and flesh of the fruit [11]. A hot water quarantine protocol is used to kill the fruit fly larvae and eggs that exist upon the mango fruit [12]. Moreover, HWT is an effective approach for controlling the post-harvest decay of fruit and vegetables [13]. Additionally, HWT increases the activity of antioxidant enzymes, such as catalase and peroxidase, as well as the amount of total phenolic compounds in the fruit tissues [14]. The technology of VHT is also extensively used to quarantine various tropical fruit before export [15]. During VHT, the fresh fruits are heated with saturated hot air until the core pulp temperature reaches a specific value, then held for a while constant to eliminate target pests [11]. Previous studies on the heat treatment of mangos show variable results, which could be due to variations in variety, temperature, and duration of exposure. Organoleptic characters have also been found to be affected by heat treatments, such as sugars, soluble solids, acidity, and ascorbic acid contents in mango [16,17].

Normally, mangos are air-freighted to other countries after the application of heat treatments. However, the post-shipment quarantine heat treatment is desirable in case of refrigerated shipments by sea or by road due to two key reasons. Firstly, the treatment before low-temperature shipping causes severe quality deterioration in mango fruit; secondly, sometimes, the approved country-specific facilities are not available at the country of shipment origin. Hence, it is very important to explore the heat treatment potential for mango fruit at the country of destination. The USA is already practicing this for irradiation-based quarantine treatment of fruits and vegetables imported from different countries. To date, there is no information available about the impact of post-shipment heat treatments on the quality and shelf life of mango fruit. Hence, the scope of the present study is to compare the response of pre- and post-shipment heat treatments on the mango cv. Chenab Gold at 0 day and after 21 days of low-temperature storage, respectively.

2. Materials and Methods

A total of 216 healthy and disease-free mango fruit (cv. Chenab Gold) was harvested at the green mature stage from a mango grove located in Multan, Pakistan. After harvesting, the fruits were immediately dipped in Amistar solution (Azoxystrobin, 0.8 mL L^{-1}) for 3 min to avoid sap burn injury. Following air-drying, the fruits were packed in plastic bins and then divided into two equal lots of 108 fruit. Each lot was composed of three biological replications of 12 fruit each for individual treatment. One lot was subjected to heat treatments at harvest (referred as 0 d)—control (untreated), HWT (Chinese protocol; 48 °C for 60 min), and VHT (Japanese protocol; 47 °C for 25 min)—and transported to the Postharvest Science and Technology Lab, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan for post-harvest quality and shelf-life analysis under ambient conditions (25 °C ± 1; 60–65% RH). The second lot was subjected to post-storage heat treatments. Briefly, fruits were transported to Roomi Foods (Kabir Wala, District Khanewal, Pakistan) where the fruits were kept under cold storage (12 °C ± 1; 85–90% RH; [18]) for 21 days (referred as 21 d). After 21 d of cold storage, fruits were treated as the control (untreated), HWT (48 °C for 60 min), and VHT (47 °C for 25 min). Afterward, the samples were transferred to the Postharvest Science and Technology Lab for analysis under ambient conditions. The physiological parameters (ethylene production rate, respiration rate, fruit

weight loss, and fruit peel color) were recorded daily until the fruits started to decay, whereas destructive analyses were performed on the first day and last day of shelf storage.

2.1. Physiological Parameters

2.1.1. Respiration Rate and Ethylene Production

To record ethylene production and respiration rate, a three-gas analyzer (F-950, Felix Instruments, Camas, WA, USA) was used. At 25 °C, four fruits of known weight were put in a 2.25 L air-tight plastic jar, and the lid of the jar was tightly secured. Four fruits derived from each replicate per treatment were placed in a 2.25 L plastic container and sealed at room temperature. Incubation for 1 h was followed by measuring the respiration rate and ethylene production using the probe inserted in the headspace of the jars. A blank (jar without fruit) was used for taking blank readings. Respiration rate and ethylene production were expressed as mmol CO_2 kg^{-1} h^{-1} and μmol C_2H_4 kg^{-1} h^{-1}, respectively.

2.1.2. Fruit Weight Loss

Fruit weight loss was calculated from an initial weight on day 0 and the weight on the sampling day as follows:

Fruit weight loss (%) = [(weight on day 0 − weight on the sampling day))/(weight on day 0)]

2.1.3. Fruit Firmness

Fruit firmness was estimated using a digital handheld fruit penetrometer (FR-5120, Lutron Electronics Enterprises, Taipei City, Taiwan) with an 8 mm tip. Fruit firmness was expressed in Newton (N).

2.1.4. Peel Color

A color meter (CR-400, Konica Minolta, Tokyo, Japan) was used to estimate changes in fruit color on the polar side of each fruit. To classify the three-dimensional color space, the values of L, a, and b were used. The following is the description of positive and negative values. L designates the lightness of fruit, a (redness), $-a$ (greenness), b (yellowness), and $-b$ (blueness). The calculation of the hue angle (h° value) and chroma (C value) were conducted using the following equations: h° value = arctan (b/a) and C value = $(a^2 + b^2)^{1/2}$ [19].

2.2. Biochemical Analysis

For the biochemical analysis, the juice of the mango fruit pulp from each replication was extracted with a muslin cloth and filtered through filter paper (Whatman Grade 41). The filtered juice was used for the determination of the following biochemical attributes.

Juice pH, Total Soluble Solids, and Titratable Acidity

Juice pH was measured by a pH meter (Starter 3100 OHAUS Corporation, NJ, USA), whereas the content of total soluble solids (TSS) was measured by a digital refractometer (PAL-1, Atago, Tokyo, Japan) and expressed as a percentage. Titratable acidity (TA) was determined by following the protocol of Hortwitz [20]. A 10 mL juice sample was mixed with 40 mL distilled water and titrated against 0.1N NaOH solution, while phenolphthalein was used as an indicator.

2.3. Non-Enzymatic Antioxidants

2.3.1. Vitamin C

The ascorbic acid or vitamin C content of mango juice was determined by indophenol's method [21]. Mango juice in a 0.4% oxalic acid solution (1:9 v/v) was filtered and titrated against 2,6-dichlorophenolindophenol. The content was expressed as mg 100 mL^{-1} juice.

2.3.2. Total Phenolic Content and Antioxidant Activity

The antioxidant activity and phenolic contents were measured from mango pulp tissue by following the procedure of Razzaq et al. [22]. Briefly, 1 g of pulp sample was taken and homogenized with a 5 mL extraction mixture (Methanol: Acetone: HCl (90:8:2)). Afterward, samples were centrifuged and the supernatant of each sample was used for total phenolic content and antioxidant assays.

Antioxidant capacity was determined through a DPPH assay and expressed as % Inhibition. Briefly, 50 µL supernatant was mixed with 5 mL of DPPH solution (0.004% w/v), followed by dark incubation at room temperature for 30 min. After incubation, 200 µL of the mixture was taken in a microplate and run on a spectrophotometer at 517 nm wavelength.

To determine total phenolic content, 100 µL of the above-prepared supernatant was mixed with 200 µL Folin–Ciocalteu reagent (10% v/v) and vortexed thoroughly for 10 s. Afterward, 800 µL of sodium carbonate solution (0.7 M) was added and vortexed thoroughly for another 10 s. Then, the mixture was subjected to dark incubation at room temperature for 2 h. After incubation, 200 µL of the mixture was taken in a microplate, and samples were run on a spectrophotometer (Epoch Eliza reader, Winooski, VT, USA) at 765 nm wavelength. The content was expressed as GAE mg 100 g^{-1}.

2.4. Enzymatic Antioxidants

The enzymatic antioxidant activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were determined using the method of Ali et al. [23]. The enzyme extract was prepared by taking 1 g frozen pulp and homogenized by using a 2 mL phosphate-buffered solution (pH 7–7.8). Afterward, the samples were centrifuged and the supernatant was used for the antioxidative enzyme assay. The enzyme activities were expressed as Units mg^{-1} of proteins.

2.4.1. Catalase (CAT) Activity

The above-prepared enzyme extract (100 µL) was thoroughly mixed with a 100 µL freshly prepared H_2O_2 solution (5.9 mM). The samples were run on a spectrophotometer (Epoch Eliza reader, Winooski, USA) at a wavelength of 240 nm to determine CAT enzyme activity.

2.4.2. Superoxide Dismutase (SOD) Activity

For the SOD enzyme activity, 100 µL of enzyme extract were homogenized with 200 µL of methionine, 500 µL of phosphate buffer (50 mM, pH 5), 200 µL of Triton X, 100 µL of Nitro blue tetrazolium, and 800 µL of distilled H_2O. Then, the mixture was exposed to UV light for 15 min, followed by the addition of 100 µL of riboflavin and vortexed. The absorbance values of the samples were recorded at 560 nm wavelength by using a spectrophotometer (Epoch Eliza reader, Winooski, VT, USA).

2.4.3. Peroxidase (POD) Activity

For the POD enzyme activity, the reaction mixture was prepared by mixing 100 µL of H_2O_2 (40 mM), 100 µL of guaiacol, and 800 µL of phosphate buffer (50 mM, pH 5.0). Spectrophotometric samples were prepared by homogenizing 100 µL of the enzyme extract with 100 µL of the reaction mixture. The values of absorbance were recorded at 470 nm.

2.5. Statistical Analysis

The experiment was laid out by following a Completely Randomized Design with a two-factor factorial arrangement. The factors were heat treatments and storage intervals. The collected data were subjected to a two-way analysis of variance and least significant difference (LSD) means comparison test by using Statistix 8.1® software. All treatments were replicated three times. The graphs were prepared by using the data interaction effect (heat treatments x storage intervals). The differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Ethylene Production and Respiration Rate

Mango fruit exhibited changes in ethylene production due to heat treatments and storage intervals (Figure 1A,B). On day 21 of cold storage, the HWT- and VHT-subjected fruits had a higher ethylene production compared to the control, while on day 0 of cold storage, the fruits were alike (Figure 1A). During ambient storage, the 0-day cold-stored fruits had an increasing profile of ethylene production irrespective of heat treatments. Compared with the control, the HWT-subjected fruits had a lower ethylene production rate from 3 d to 6 d (Figure 1B). Moreover, the mango fruits did not exhibit changes in respiration rate (Figure 1C,D).

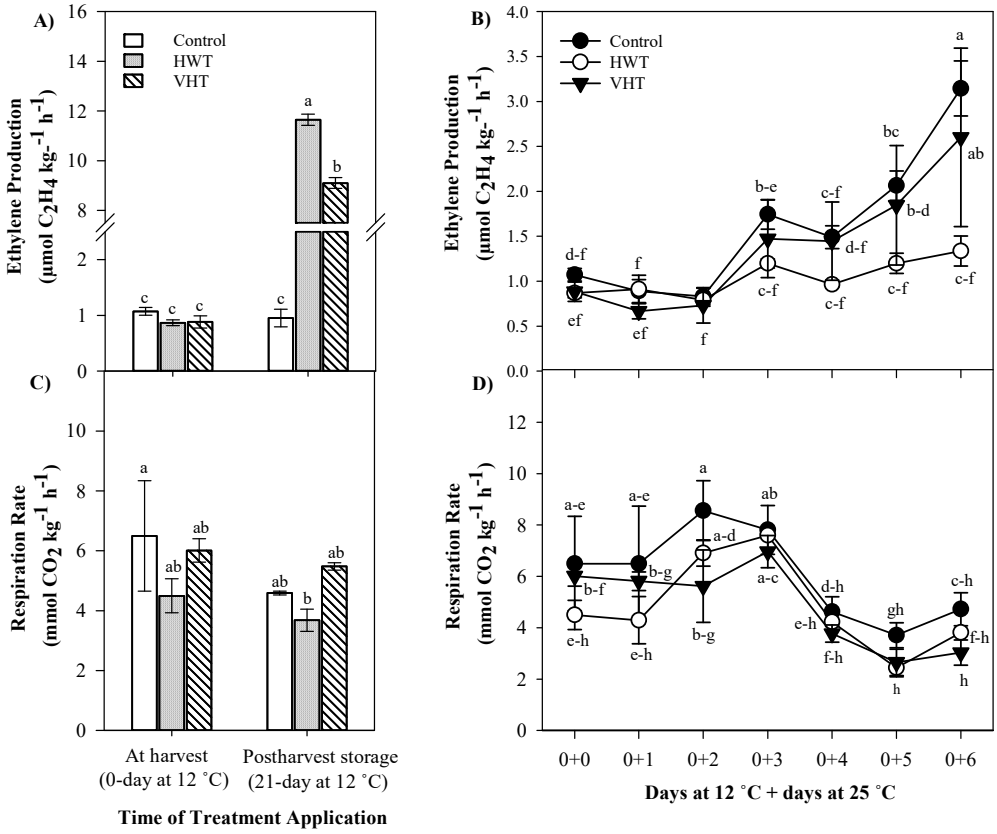

Figure 1. Effect of the heat treatments applied at harvest and post-harvest storage on the ethylene production (**A**,**B**) and respiration rate (**C**,**D**) of mango fruits. Error bars show standard errors of the means (n = 3). Different letters indicate significant difference according to LSD at $p \leq 0.05$.

3.2. Fruit Weight Loss

Irrespective of heat treatments, the mango fruits exhibited an increase in weight loss during cold storage as well as during ambient storage (Figure 2). On day 21 of cold storage, the post-storage HWT-subjected fruits had a lower (8.45%) fruit weight loss compared to the control and VHT-subjected fruits (Figure 2A). During ambient storage, compared with the control, the HWT- and VHT-subjected fruits exhibited a lower fruit weight loss from 3 d to 6 d; however, there were no differences between them (Figure 2B).

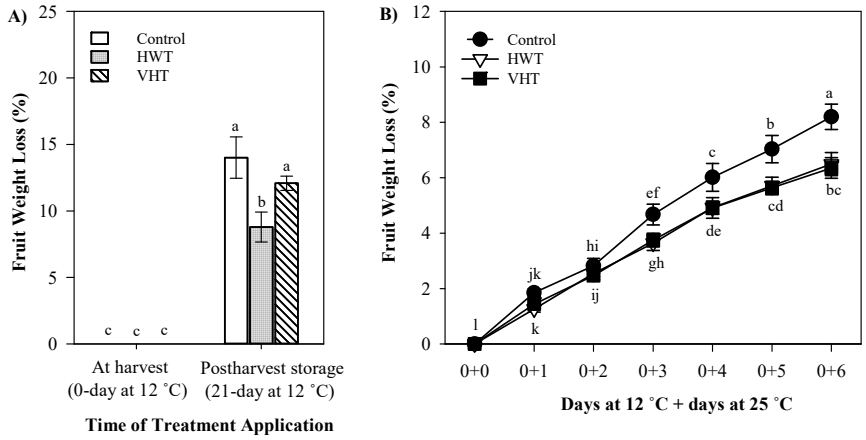

Figure 2. Effect of the heat treatments applied at harvest and post-harvest storage on the weight loss (**A**,**B**) of mango fruits. Error bars show standard errors of the means (n = 3). Different letters indicate significant difference according to LSD at $p \leq 0.05$.

3.3. Fruit Firmness

Irrespective of heat treatments and storage intervals, the mango fruit exhibited a decline in fruit firmness over time (Figure 3). At harvest, the HWT- and VHT-subjected fruits had a low firmness (114 N and 87 N, respectively) compared to the control fruits (141 N) and similar changes were also observed in the post-harvest-storage heat-treated fruits (Figure 3A). During ambient storage (0 d), the control fruits retained more fruit firmness than the HWT- and VHT-subjected fruit; however, no changes were observed on 6 d (Figure 3B).

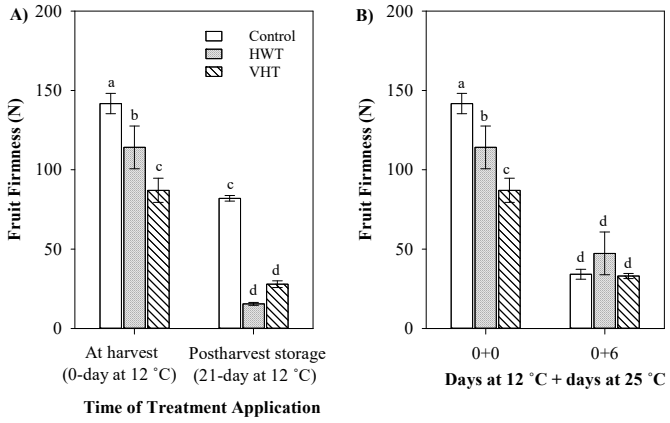

Figure 3. Effect of the heat treatments applied at harvest and post-harvest storage on the fruit firmness (**A**,**B**) of mango fruits. Error bars show standard errors of the means (n = 3). Different letters indicate significant difference according to LSD at $p \leq 0.05$.

3.4. Peel Color

The value of *L* did not change due to the heat treatments applied at harvest and postharvest storage (Figure 4A,B). In contrast to the value of *L*, the value of *a* showed changes by exhibiting higher values during post-harvest storage; however, the heat treatments had no effect (Figure 4C). During ambient storage, the value of *a* showed a progressive and increased profile, irrespective of heat treatments. However, the control and VHT-subjected fruits had higher values from 2 d to 6 d (Figure 4D). As for the value of *b*, the pre-storage VHT-subjected fruit had higher values, followed by the control and HWT-subjected fruits; moreover, similar changes were also observed among post-storage heat-treated fruits (Figure 4E). Under ambient storage, no changes in the value of *b* were found among at-harvest heat-treated fruits (Figure 4F). The mango fruits did not differ in the c value of peel

color, irrespective of heat treatments, at harvest and post-harvest storage (Figure 4G,H). h° values decreased during post-harvest storage; however, the heat treatments had no effect. During ambient storage, the value of h° showed a decreasing profile, irrespective of heat treatments. However, the HWT-subjected fruit had higher values from 2 d to 6 d (Figure 4I,J).

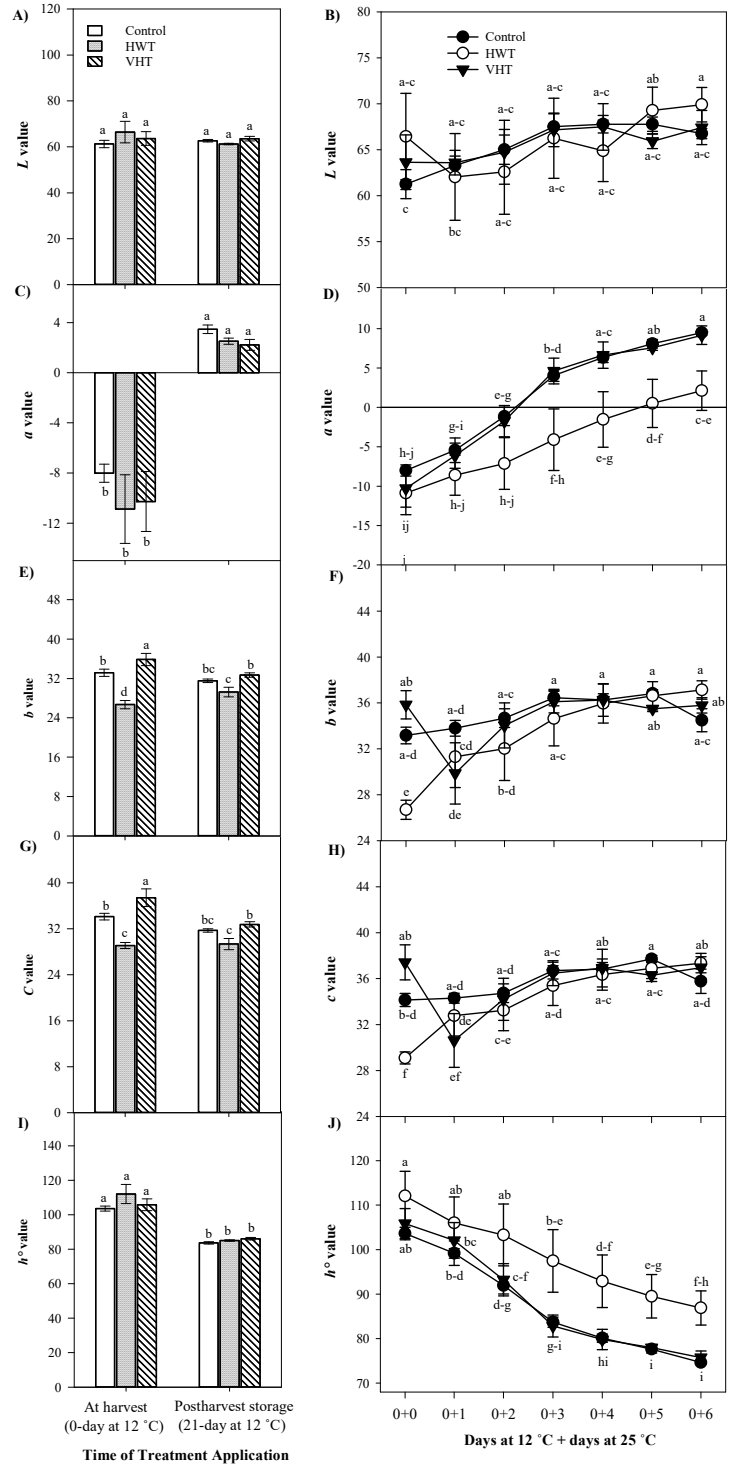

Figure 4. Effect of the heat treatments applied at harvest and post-harvest storage on the *L* value (**A**,**B**), *a* value (**C**,**D**), *b* value (**E**,**F**), and *C* value (**G**,**H**) and h° value (**I**,**J**) of mango fruits. Error bars show standard errors of the means (n = 3). Different letters indicate significant difference according to LSD at $p \leq 0.05$.

3.5. Total Soluble Solids, Titratable Acidity, and Juice pH

At harvest and post-harvest storage, the heat treatments and their subsequent ambient storage caused changes in TSS, TA, and juice pH (Table 1). On day 21 of cold storage, the HWT-subjected fruit had a higher TSS content than the VHT-subjected and the control fruits; however, no change was found on day 0 of the cold-storage heat-treated fruits (Table 1). During ambient storage, the 0-day cold-stored fruits had an increasing profile of fruit TSS, irrespective of heat treatments. Compared with the control, the HWT- and VHT-subjected fruits had a higher TSS content on days 1 and 7 of ambient storage (Table 1).

Table 1. Effect of the heat treatments applied at harvest and post-harvest storage on total soluble solids (TSS), titratable acidity (TA) and juice pH of mango fruits. Data are presented as mean ± SE (n = 3). Different letters indicate significant differences according to LSD at $p \leq 0.05$.

Treatments	Time of HT Application		Days at 12 °C + Days at 25 °C	
		TSS (%)		
	At Harvest (0 d at 12 °C)	**Post-harvest Storage (21 d at 12 °C)**	**0 d + 6 d**	**21 d + 1 d**
Control	12.53 ± 0.62 c	13.40 ± 0.52 cd	17.60 ± 0.20 b	D
HWT	14.34 ± 0.46 c	17.50 ± 0.23 b	20.40 ± 0.65 a	D
VHT	13.60 ± 0.66 c	12.10 ± 0.76 d	19.33 ± 0.68 a	D
		TA (%)		
Control	1.27 ± 0.33 a	0.35 ± 0.03 b	0.17 ± 0.02 b	D
HWT	0.98 ± 0.07 a	0.33 ± 0.00 b	0.22 ± 0.01 b	D
VHT	0.98 ± 0.11 a	0.33 ± 0.03 b	0.43 ± 0.09 b	D
		Juice pH		
Control	3.23 ± 0.01 b	3.87 ± 0.04 a	3.91 ± 0.02 c	D
HWT	3.32 ± 0.02 b	3.99 ± 0.02 a	4.68 ± 0.08 b	D
VHT	3.29 ± 0.03 b	3.91 ± 0.04 a	4.95 ± 0.04 a	D

D indicates that fruits were decayed and were not available for analysis.

At harvest and post-harvest storage, the heat treatments did not affect the TA content compared with their respective control. Overall, the TA content was reduced on 21 d of cold storage as compared to day 0 of cold storage (Table 1). During ambient storage, the 0-day cold-stored fruits had a lower TA content on day 6 as compared to day 0. Moreover, the VHT-subjected fruits exhibited a higher TA content (0.43%) than the control and HWT-subjected fruits on day 6 (Table 1). In contrast to the TA content, the juice pH had opposite changes on days 0 and 21 for the cold-stored heat-treated fruits (Table 1). During ambient storage, the 0-day cold-stored HWT- and VHT-subjected fruits exhibited an increase in juice pH value towards their respective control (Table 1).

3.6. Vitamin C, Antioxidant Capacity, and Total Phenolic Contents

At harvest and post-harvest storage, the heat treatments affected the contents of VC compared with their respective control; however, there was a reduced VC content on day 21 as compared with day 0 of cold storage (Table 2). During ambient storage, the at-harvest heat-treated fruit had a lower VC content on day 6 than day 0. Moreover, the HWT- and VHT-subjected fruit, compared with the control, had a higher vitamin C content on day 6 (Table 2). The at-harvest heat treatments did not change the AC of mango fruits; likewise, no changes were observed during the shelf storage of at-harvest heat-treated fruits (Table 2). However, the AC of the HWT- and VHT-subjected fruits was lower after 21 d of cold storage compared to the control, as indicated in Table 2. The mango fruits exhibited a higher TPC in the HWT- and VHT-subjected fruits as compared to the control on day 0 of cold storage. In contrast, the control fruit had a higher TPC as compared with the HWT- and VHT-subjected fruits on day 21 of cold storage (Table 2). During ambient storage, the 0-day cold-stored fruits had a decreasing profile of TPC from 0 d to 6 d, irrespective of heat

treatments. However, compared with the control, the VHT-subjected fruit had a higher TPC on day 6 (Table 2).

Table 2. Effect of the heat treatments applied at harvest and post-harvest storage on vitamin C content (VC), antioxidant capacity (AC), and total phenolic content (TPC) of mango fruits. Data are presented as mean ± SE (n = 3). Different letters indicate significant differences according to LSD at $p \leq 0.05$.

Treatments	Time of QHT Application		Days at 12 °C + Days at 25 °C	
		VC (mg 100 g^{-1})		
	At Harvest (0 d at 12 °C)	**Post-harvest Storage (21 d at 12 °C)**	**0 d + 6 d**	**21 d + 1 d**
Control	25.65 ± 1.18 c	3.90 ± 0.30 g	12.50 ± 0.44 f	D
HWT	37.59 ± 0.58 a	4.20 ± 0.30 g	14.32 ± 0.04 e	D
VHT	27.90 ± 0.00 b	2.40 ± 0.30 h	16.87 ± 0.39 d	D
		AC (% Inhibition)		
Control	86.18 ± 0.29 c–e	89.14 ± 0.08 a	87.83 ± 0.29 a–c	D
HWT	87.02 ± 0.57 b–d	84.72 ± 0.95 e	88.01 ± 0.49 ab	D
VHT	85.42 ± 00.49 de	86.14 ± 0.95 b–e	86.47 ± 0.21 b–d	D
		TPC (GAE mg 100 g^{-1})		
Control	49.31 ± 2.05 e	100.94 ± 3.69 a	43.57 ± 2.21 e	D
HWT	102.18 ± 1.35 a	88.15 ± 5.48 b	44.58 ± 4.52 e	D
VHT	77.76 ± 1.08 c	84.81 ± 2.24 bc	65.28 ± 0.84 d	D

D indicates that fruits were decayed and were not available for analysis.

3.7. Antioxidative Enzyme Activities

At harvest, the HWT- and VHT-subjected fruits had a reduced SOD activity as compared to the control fruits; however, on day 21 of cold storage, only the VHT-subjected fruit exhibited a decreased SOD enzymatic activity as compared to the control (Table 3). Under ambient storage conditions, when compared to day 0, the control fruit exhibited a decreasing profile of SOD activity and the VHT heat-treated fruits had an increasing profile of SOD activity, while no change in the HWT-subjected fruits was observed on day 6; moreover, no change was found among the heat-treated fruits on day 6 (Table 3).

Table 3. Effect of the heat treatments applied at harvest and post-harvest storage on the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzyme activities of mango fruits. Data are presented as mean ± SE (n = 3). Different letters indicate significant differences according to LSD at $p \leq 0.05$.

Treatments	Time of QHT Application		Days at 12 °C + Days at 25 °C	
		SOD (U mg^{-1} of Protein)		
	At Harvest (0 d at 12 °C)	**Post-harvest Storage (21 d at 12 °C)**	**0 d + 6 d**	**21 d + 1 d**
Control	49.60 ± 4.83 c	103.81 ± 6.24 a	32.80 ± 1.23 de	D
HWT	28.89 ± 0.55 de	89.71 ± 5.62 b	33.99 ± 0.93 d	D
VHT	21.39 ± 0.33 e	84.21 ± 5.07 b	36.65 ± 0.66 d	D
		POD (U mg^{-1} of protein)		
Control	65.95 ± 2.82 d	0.24 ± 0.05 e	88.74 ± 3.87 c	D
HWT	91.96 ± 1.91 c	0.43 ± 0.14 e	223.46 ± 1.80 a	D
VHT	103.96 ± 2.83 b	0.30 ± 0.03 e	94.23 ± 3.50 c	D
		CAT (U mg^{-1} of protein)		
Control	6.26 ± 0.06 ab	3.25 ± 0.14 de	6.48 ± 0.53 a	D
HWT	5.11 ± 0.06 bc	4.82 ± 1.11 c	2.32 ± 0.03 e	D
VHT	4.19 ± 0.06 cd	4.03 ± 0.03 cd	5.07 ± 0.27 c	D

D indicates that fruits were decayed and were not available for analysis.

The mango fruits exhibited changes in POD activity due to the heat treatments and storage intervals. At harvest, the HWT- and VHT-subjected fruits had a higher POD activity as compared to the control; however, no change in POD activity was observed in the post-harvest storage heat-treated fruits. Moreover, low POD activity was observed, irrespective of heat treatments, in the post-harvest storage heat-treated fruits, as compared to the 0-day cold-stored heat-treated fruits (Table 3). Compared to day 0 of ambient storage, there was an increase in POD activity in the control and HWT-subjected fruits, while a decreased POD activity was observed in the VHT-subjected fruit on day 6 of ambient storage (Table 3). The mango fruits exhibited no change in CAT activity due to heat treatments applied at harvest and post-harvest storage (Table 3). During ambient storage, CAT activity was decreased in the HWT-subjected fruits and increased in the VHT-subjected fruits, while there was no change in the control fruit on day 6 as compared to day 0 (Table 3).

4. Discussion

Post-harvest fruit quality can be affected by pre-harvest, during harvest, and post-harvest practices. Among them, the post-harvest heat treatments and cold storage have a prime role in the post-harvest fruit performance. Their application at the right time with the appropriate protocol determines the fate of the agricultural produce; otherwise, the fruits can develop negative and unacceptable traits due to the onset of physiological disorders [24]. According to Thomas and Joshi [25], the quality of ripe mangos remained good during cold storage for seven days and was acceptable until the 10th to 14th day with minimal changes in the internal and external fruit quality attributes. However, in the present study, the application of hot water treatments after post-harvest storage (21 d at 12 °C ± 1; 85–90% RH) ruptured most of the fruit within 24 h of treatment, resulting in poor shelf life, which might be due to the extended storage interval.

The amounts of ethylene production and respiration rate are the indicators of metabolic activity and indicate the potential shelf-life ability of the product [26]. A previous study reported that mango fruit exhibit lower ethylene production during the initial days of fruit ripening, followed by a climacteric peak [27]. In the present study, the mango fruits showed a rise in ethylene production after cold storage as well as during ambient storage and this change might be due to the activation of respirational climacteric activity [28]. Djioua et al. [29] found that the hot water dipping of mango fruit at 50 °C for 30 min reduced the respiration rate as compared to the control. Surprisingly, in the present study, the respiration rate of the heat-treated fruit showed a trend for reduction.

Fruit weight loss during post-harvest handling is a major constraint in the fresh market. Due to the quick production of ethylene during ripening, the mango fruits lose most of their weight at room temperature [30]. As a result, the fruits become soft and shriveled, which reduces its market value. In our study, an increase in weight loss of about 8–14 times was observed after 21 d of cold storage as compared to day 0 of cold storage, irrespective of heat treatments. Likewise, an increasing trend was found during the ripening of the fruits on the shelf. In addition, fruit firmness is one of the most widely used indicators of fruit quality and determines the ability of the fruit to handle long-distance travels and maltreatments. Firmness changes during ambient as well as cold storage conditions; however, the rate of firmness varies with storage conditions. The decline of firmness is due to the release of water and is also linked to the intense activity of softening-related enzymes, such as polygalacturonate, galactosidases, and pectin methylesterase [31]. In addition, the destruction of the cell wall by various enzymes affects fruit firmness [32]. In our study, a decreasing trend of firmness was observed between days 0 and 21 of storage and during ripening at the shelf, which might be due to the activation of softening-related enzymes. Similarly, in the Ataulfo mango, a reduction in fruit firmness was observed during cold storage at 5 °C for 15 d [33]. Visual appearance and vivid colors are the foremost indicators that influence the consumer's decision to buy fruit. The peel and pulp color of mango fruit are strongly correlated to ripeness, which can be measured as a sensual quality parameter [34]. Mainly three values of colors (L = fruit lightness), (a = value coordinate

from green to red), and (*b* = value coordinate from yellow to blue) are used to determine the color changes in mango. In the previous study of Djioua, Charles, Lopez-Lauri, Filgueiras, Coudret, Freire Jr, Ducamp-Collin, and Sallanon [29], the variable impact of heat treatment was found for *L*, *a*, and *b* in mango fruit. In our experiment, there was no change observed in the lightness of the peel color (*L*) of the pre-storage and post-storage fruits. The color value of *a* changed due to cold storage intervals; moreover, a gradual increase in the *a* value was observed during ambient storage, which suggests that with the advancement of maturity, the *a* value increases from the green stage to red-purple stage. A similar changing trend was also found during the shelf study and in cold storage [29]. The hue values of mango skin and flesh decrease during storage and ripening. The decrease in hue values during cold storage or after ripening implies the degradation of chlorophyll (greenness) and carotenoid biosynthesis (yellowing and redness) [35]. The samples with the lowest hue values are more ripened than those with the highest values. Thus, the high hue values observed on mangos treated with HWT during ambient storage indicate that the ripening process was delayed.

The total soluble solids play an important role in the fruit maturity process and determine the acceptance of rich nutrients as well as economic benefits in the fruit trade. The heat-treated fruits showed an increase in the TSS content at days 0 and 21 of cold storage and as well during the ambient storage of 0-day cold-stored fruits. Similar to our work, the heat-treated 'Chokanan' mango fruits showed a 15% increment in TSS contents [36]. This increment might be due to the hydrolysis of polysaccharides after heat treatment [37]. Furthermore, heat enhances the solubility of some materials, which might also contribute to the increase in TSS [36].

TA is responsible for the unique taste and flavors of most fruit and hence it is also a reliable parameter of fruit quality [38]. In general, fruit reduces the acid content due to the increased rate of metabolic activities and the conversion of organic compounds into sugars [39]. In the present study, a decrease in TA contents was observed during ambient and cold storage. Our findings are consistent with those of Rodríguez Pleguezuelo et al. [40], who found a decrease in titratable acidity (0.04–2.71%) in different mango cultivars stored at 18 to 34 °C. Maintaining the juice pH is quite effective in extending the fruit shelf life [38]. Changes in citric, malic, and ascorbic acid cause changes in TA and juice pH. Our results indicate that advancement in fruit maturity increased the juice pH by converting more organic acids into other metabolites. Similarly, Rodríguez Pleguezuelo, Durán Zuazo, Muriel Fernández, and Franco Tarifa [40] also reported an increase in juice pH (2.85–4.38%) in different mango cultivars stored at 18 to 34 °C.

Vitamin C is one of the non-nutritive bioactive phytochemicals in a variety of horticultural crops. It has a variety of beneficial biological properties in the human body [41]. Mango fruits have a sufficient amount of vitamin C content; however, this content varies among cultivars [42]. According to our results, the vitamin C content was decreased during ambient and cold storage conditions. Vitamin C is a water-soluble and temperature-sensitive vitamin [43], and it might be possible that high temperature during heat treatments and also room temperature under ambient conditions decreased the vitamin C content.

Phenolic compounds due to their biological properties can exhibit antioxidant, anti-inflammatory, antiviral, and anticancer activities [44]. Total phenols and flavonoids form an active oxygen scavenging system of defense and guarantee the normal activities of plant macromolecules [45]. In the present study, the HWT- and VHT-subjected fruit on day 0 of cold storage had a higher phenolic content compared to the control fruits. The enhanced total phenolic content in the heat-treated fruit might be due to the activation of the fruit's defense mechanisms, thus increasing the stress resistance of mangos and prolonging the storage life [26]. The initial heat treatment of the mango fruits could have acted as a priming factor, causing the orchestration of metabolic cascades that could enhance the ability of the fruit tissues to adapt to the secondary abiotic stress conditions of cold storage [46]. According to Mayani et al. [47], the highest phenolic content was recorded in mango fruit (cv. 'Kesar') treated with the hot water dip treatment for 20 min at 58 °C. Another study

reported a similar result, indicating that the total phenolic content was increased in the heat-treated mango fruits [26]. On the other hand, the post-storage heat-treated fruit had a lower TPC than the control, which indicates that cold storage conditions changed the fruit metabolic system. Most probably, the decrease in these phenols might be due to the enhanced activity of polyphenol oxidase, which led to the breakdown of the cell structure during the ripening and consumption of occurring polyphenols within the fruit tissues [48]. Mangos are a rich source of bioactive compounds (β-carotene, ascorbic acid, and total phenolics) and possess a high antioxidant capacity [49]. According to our results, the antioxidant capacity was significantly lowered in the HWT- and VHT-subjected fruit on day 21 of cold storage. The decrease in antioxidant activity during storage can be attributed to a decreased level of vitamin C, total phenolics, phenolic acids, and other compounds, such as flavonoids, carotenoids, and anthocyanins when the fruit and vegetables are stored [50].

Fruit and vegetables are highly sensitive to enzymatic browning during storage, thus reducing sensory quality, processing performance, and market price. Polyphenol oxidase (PPO) and POD are enzymes that are important in the oxidation of polyphenols, affecting fruit color and overall quality [26]. On the other hand, fruits have self-protection mechanisms that activate various antioxidant defense enzymes, such as SOD, CAT, and POD, to defend themselves from oxidative stress [51]. SOD is a key enzyme that protects fruit from oxidative damage by lowering the number of reactive oxygen species in the fruit [52]. In the present study, the SOD enzyme activity was higher in the post-storage heat-treated fruits. A higher SOD activity is related to stress tolerance because this enzyme neutralizes the reactivity of the superoxide radical, which is overproduced during stress conditions [53]. During fruit ripening, SOD levels vary among fruits, such as oranges, blackberries, and guavas [54]. In mangos, the SOD activity was increased considerably in the pulp tissue of the 'Alphonso' variety under ambient conditions [55]. In the present study, the SOD activity was only increased in the VHT-subjected fruit on the seventh day of ambient storage.

The potency of the antioxidative peroxidase enzyme system controls the peroxidative destruction of cell walls, and POD is a primary system for the enzymatic elimination and control of intercellular levels of H_2O_2. POD is found in many plant-based foods and plays a function in food quality, such as color and flavor deterioration [56]. According to our results, the pre-storage heat-treated fruits had higher values of POD activity as compared to the control fruits, while a very low POD activity was recorded in the case of the post-storage heat treatments (Table 3). These results indicate that metabolic activities largely change when cold-stored conditions are immediately applied to mango fruits. Moreover, the variation in CAT activity is thought to be the cause of the irregular behavior of enzyme activities [22]. Different fruits, such as blackberry, guava, and mango, have been observed to have a decreasing trend in CAT activity as they were ripe under ambient conditions [57].

5. Conclusions

The present study evaluated the effects of quarantine heat treatments (hot water at 48 °C for 60 min and VHT at 47 °C for 25 min) on the 'Chenab Gold' mango cultivar at harvest and post-harvest cold storage followed by ambient storage. Our results concluded that heat treatments after 21 d of cold storage triggered more ethylene production and fruit weight loss and decreased the fruit firmness and vitamin C content, resulting in poor fruit quality and shelf life turnout. On the other hand, the pre-storage heat treatments (HWT or VHT) had a positive impact on the fruits with improved color and slow fruit weight loss, and higher TSS and VC contents. Overall, the research presented in this paper lays a solid recommendation that the application of quarantine heat treatment after 21-day cold storage decays mango fruits in a short time.

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