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POSTHARVEST QUARANTINE HEAT TREATMENTS ATTENUATE FRUIT PHYSIOLOGICAL LOSSES, ADVANCE FRUIT RIPENING AND IMPROVE ANTI-OXIDATIVE QUALITY OF TWO NOVEL MANGO (*MANGIFERA INDICA* L.) CULTIVARS DURING SIMULATED AIR CONDITIONS

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Abstract

Mango fruit export is significantly hindered by quarantine pests. Fruit fly, being a devastating quarantine pest need disinfectant treatments such as postharvest quarantine heat treatments (PQHT); hot water treatment (HWT) and vapour heat treatment (VHT) for export to high value markets. We reported for the first time, effects of HWT and VHT on fruit quality of two novel and emerging Pakistani mango cultivars 'Azeem Chaunsa' and 'Chenab Gold' after fruit harvest. For this purpose, an integrated study was executed to investigate the effects of HWT & VHT treatment on fruit quality of 'Chenab Gold' and 'Azeem Chaunsa' under simulated air conditions (Temp. 28±2 ºC; RH 60-65%) till fruit ripening in two independent experiments. Irrespective to days at shelf, VHT-treated mango fruit exhibited significant lower fruit weight loss, respiration rate and retained significantly higher fruit vitamin-c contents and sensory attributes in both commercial mango cultivars as compared to untreated and HWT-treated fruit. Moreover, significant improved fruit total phenolic contents (TPC), anti-oxidative activity and activity of catalase (CAT) enzyme were observed in VHT-treated fruit for both 'Azeem Chaunsa' and 'Chenab Gold' mango fruit during the entire fruit ripening at shelf. However, with progress in days at shelf, both VHT and HWT enhanced the fruit ripening as evident by higher fruit total soluble solid (TSS), sugar acid ratio, lowest fruit firmness and developed more peel colour development as compared to untreated fruit. So, it can be concluded that VHT treatment is more efficient PQHT for 'Chenab Gold' and 'Azeem Chaunsa' than HWT and can be employed

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as disinfectant treatment without compromising fruit physiological, anti-oxidative and sensory quality losses during simulated air shipments.

Keywords: 'Azeem Chuansa', 'Chenab Gold', Hot Water Treatment, Fruit Qulaity, Vapour Heat Treatment, Fruit Quality, Phytosanitary.

1. INTRODUCTION

Diets high in fruit and vegetable are widely recommended for their health-promoting properties. Mango (*Mangiferae indica* L.) is known as 'King of Fruits' owing to its high nutritional quality, rich taste and appealing colour [7]. This fruit crop is widely cultivated in tropical and subtropical regions, spreading up-to 87 countries worldwide. According to statistical data, over 59 MT of mango, guava and mangosteens were produced globally during 2022, Pakistan ranking at 4th place with approximately 2.78 MT. Furthermore, Pakistan has a significant share in global fresh mango export, over 0.11 MT of fresh mango worth approximately value of 110 million US$ were exported worldwide in the fiscal year 2022 [8].

'Sindhri', 'Sufaid Chaunsa' and 'Samar Bahist Chaunsa' are commercial and export quality mango cultivars of the country and mostly exported to Gulf countries, Europe, Japan, China, Japan and China. Recently, two mango cultivars 'Azeem Chaunsa' and 'Chenab Gold' have joined the export quality mango club of the country due to their excellent fruit quality. Moreover, these two cultivars also have extended the mango harvest window of the country. For export of fresh fruit, quarantine pests such as fruit flies are major problem for any country, especially peach fruit fly (*Bactrocera zonata* S.) and oriental fruit fly (*Bactrocera dorsalis)* are serious quarantine pests [18], [10]. These obnoxious pests, being polyphagous, attack on several fruit crops such as peach, mango, guava, citrus, fig and apricot fruit plants.

Although some chemical treatments had been being used as disinfectant treatment against these quarantine pests in the past including chemical fumigation for fresh fruit but now have been replaced by chemical-free disinfectant treatments. Physical heat treatments, also known as postharvest quarantine heat treatments (PQHT) had been reported to be used as and commercially employed as phytosanitary measure for export of many tropical and sub-tropical fresh produce including mango. Generally, these PQHT not only kill the eggs and larval stages of fruit flies in the fruit flesh, also had been reported to control incidence of diseases after harvest in grapes, papaya and mango without compromising on fruit quality.

Moreover, these PQHT are accepted by quarantine authority of many international markets including China, Iran, EU and Japan [13]. These PQHT involve fruit heating to a specific flesh core temperature for a specific period of time. Globally there are three PQHT reported for mango currently being employed. They are vapour heat treatments (VHT), hot water immersion treatment (HWT) and forced-hot-air treatment (FHAT) [13]. VHT, also known as humid air heating, involves conductive energy transfer by passing the water vapour saturated air streams on fruit surface and hence diverting the heat towards

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fruit flesh core [13]. This VHT as PQHT is indispensable for mango exports to high-value markets in Japan and currently being employed to mango imports from Australia, Thailand and Pakistan [13], [25]. Similarly, HWT is hot immersion PQHT involving transfer of heat from hot water to fruit skin and ultimately to fruit flesh core. This PQHT also being employed for mango export from Pakistan to Iran, Europe and China [3], [2]. The effects of both HWT and VHT have been reported as disinfectant treatment on commercial mango cultivars of Pakistan including 'Sindhri', 'Samar Bahisht Chaunsa' and 'Sufaid Chaunsa' [3], [12], [25].

These studies have recommended HWT (48ºC for 60 min) and VHT (47°C for 25 min) for commercial and exporting mango of Pakistan. Additionally these are also used at commercial level in the country for exporting mango as well. However, by the introduction and registration of two new mango cultivars, 'Chenab Gold' and 'Azeem Chaunsa', as commercial mango cultivars, these two mango cultivars have become very famous among the mango growers of the country. Additionally, no information is available about effects of both the PQHT treatments on these new mango cultivars. So, there is dire need to explore the effects of already optimized HWT and VHT as disinfectant treatment for 'Chenab Gold' and 'Azeem Chaunsa' mango. So, keeping in view above all facts, an integrated study was executed to investigate the effects of HWT and VHT as PQHT on fruit physiological, biochemical, anti-oxidative and organoleptic attributes of 'Chenab Gold' and 'Azeem Chaunsa' mango at ambient conditions.

2. MATERIALS AND METHODS

2.1 Fruit Source

Physiologically mature mango (*Mangifera indica* L.) fruit of two cultibars 'Azeem Chaunsa' and 'Chenab Gold' of uniform size, free from any diseases, insect damage and visual blemishes were harvested at commercial maturity (Table 1) from a commercial mango orchard, 'Chenab Mango Farm', Jalapur Pirwala, Multan, Pakistan (29°36'48.7"N,71°08'54.9"E).

Table 1: Maturity Indices of Mango Cultivars at Fruit Harvest

Mango Cultivar	Fruit Firmness (N)	TSS (°Brix)	Fruit shape (Fruit cheeks)	Fruit skin colour
'Azeem Chaunsa'	140±5.612	8.7±0.211	Fully developed	Dark green to light green
'Chenab Gold'	135±6.141	7.1±0.193	Fully developed	Dark green to light green

Value followed by ± is standard deviation (SD). n=10 (10 fruit)

2.2 Fruit Processing and Postharvest Quarantine Physical Heat Treatments

After harvesting, fruits were physically de-sapped, washed, treated with Scholar® fungicide 0.5ml/liter, air dried and readily transferred to physical heat treatments facility at Roomi Foods (Pvt.) Ltd., Kabirwala, Multan, Pakistan. The harvested fruits were divided into three lots, 1st lot was subjected to vapour heat treatment [(VHT): 47ºC for 25

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min)], 2nd lot was subjected to hot water treatment [(HWT): 48ºC for 60 min)], while, 3rd lot was served as control with no heat treatment (Untreated). After treatments, fruits were packed in 2.5 kg corrugated cardboard boxes and readily transported to Postharvest Science and Technology Lab, MNS-University of Agriculture, Multan. These fruits were kept at ambient conditions (Temp. 28±2 ºC; RH 60-65%) to ripen and were studied for various physiological attributes (data recording at 1-day interval), biochemical and anti-oxidative attributes (data recording at 2-days interval). The experiment was laid as per completely randomized design (CRD) under factorial arrangement having three replications (3 fruit per replication). Various PQHT and storage duration at shelf were served as factor-I and –II, respectively.

Data Collection

2.3 Physiological and physical parameters

Fruit weight was measured on daily basis by weighing the fruit with digital weighing balance (RC31P30, Ohaus Corporation, Parsippany, USA) and the weight loss was calculated as % weight loss on daily basis. Fruit ethylene and respiration rate was determined by incubating 2 mango fruit per replication in an air tight plastic jar of 4 L capacity at room temperature for 1 hour and taking the reading with the help of already calibrated handheld three gas analyzer (F-950, Felix Instruments, Camas, USA) on daily basis. The final calculated ethylene and respiration rate were expressed in µL kg h^{-1} and ml CO_2 kg h^{-1}, respectively.

Fruit colour of mango fruit on both cheeks was determined by using hand held chroma meter (CR-400, Konica Minolta Inc, Tokyo, Japan). The value was determined in colour coordinates *L*, *a** and *b** defining fruit lightness, redness and yellowness, respectively. The fruit firmness was determined by using force gauge (PCE-FM 200, PCE instruments, UK) fitted on stand equipped with 8 mm probe from both cheek of fruit and expressed in Newton (N).

2.4 Biochemical Attributes

For quantification of biochemical characteristics, juice of 3 fruit were extracted with the help of electric juicer/blender (MJ-CB800STN, Panasonic Co., Tokyo, Japan), juice was used to quantify total soluble solids (TSS), titratable acidity (TA), sugar acid ratio and Vitamin C contents. A digital refractometer (PAL-1, Atago, Japan) was used to measure TSS and final values expressed in °Brix. Fruit juice TA was quantified by the procedure based upon titration method with 0.1 *N* NaOH using phenolphthalein as indicator proposed by Hortwitz [11] with some modification as suggested by [23].

The final value was calculated as % of citric acid. The sugar acid ratio was calculated by dividing the value of TSS with respective TA value for each sample. Vitamin-C content was measured by titrating supernatant with dye (2, 6-dichlorophenol Indophenol) until light pink colour attained [24] modified by [23].

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The final value was calculated against the standard of ascorbic acid and given as mg $100g^{-1}$. Juice pH was calculated by handheld pH meter (HI98107, Hanna Instruments, Rhode Island, USA).

2.5 Anti-oxidative Characteristics

Non-enzymatic anti-oxidative [total phenolic contents (TPC), total anti-oxidative activity, carotenoids) and enzymatic anti-oxidative [activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes] attributes were quantified from mango fruit pulp. For non-enzymatic anti-oxidative parameters quantification, 1 g mango pulp was ground in a pre-chilled pastor and mortar with 10 ml of extraction mixture consisting of methanol: acetone: HCl in 90:8:2 ratio, respectively.

The homogenate mixture was centrifuged at 7000 rpm for 5 minutes in a refrigerated centrifuge (Z216 MK, Hermle Labortechnik GmbH, Wehingen, Germany) to get supernatant. This supernatant was used to quantify TPC, total anti-oxidative activity and carotenoids. The procedure given by [1] was adopted to quantify TPC using Folin Ciocalteu (FC) reagent and gallic acid was used as standard.

Briefly, reaction mixture consisting of 200 µL FC reagent (10%) was mixed with similar amount of supernatant followed by mixing with the help of vortex for 60 s, then 800 µL Na_2CO_3 (700 m*M*) was added with supernatant and put into 96-well plate to get absorbance reading at 760nm by using microplate spectrophotometer (Epoch, BioTek® Instruments, Inc., Winooski, USA) equipped with computer operated Gen5 software. The final value was expressed as mg gallic acid equivalent (GAE) per 100 g. For estimation of total anti-oxidative activity same supernatant was mixed with methanol based solution of free radical 2, 2-diphenyl 1-picrylhydrazyl (DPPH) as described by [29]. The final reading was expressed as % of DPPH scavenging.

The carotenoids were estimated by following the protocol outlined by [14]. The same supernatant was used for estimation of carotenoids and absorbance of supernatant was taken at 470, 645 and 662nm using microplate spectrophotometer (Epoch, BioTek® Instruments, Inc., Winooski, USA). The final reading was expressed as µg $g^{-1}$of fruit weight. For quantification of enzymatic anti-oxidative parameter, the supernatant was extracted from mango pulp as per method described by [23] with some modifications [20].

Briefly, 1 g mango pulp was homogenized in a pre-chilled pastor and mortar with 5 mL saline phosphate buffer (pH 7.2) followed by centrifugation at 12000 rpm at 4°C to get supernatant for quantification of activity of SOD, POD and CAT enzymes. Firstly 100 µL supernatant was used to quantify for protein contents using 5 mL Bradford reagent (Bradford, 1976).

The mixture was thoroughly mixed absorbance was taken at 595 nm. Standard curve of bovine serum albumin (BSA) was used as reference and final reading was calculated as mg g^{-1} of fruit weight (FW). Activity of SOD enzyme was measured by sample ability to inhibit photo reduction of nitro blue-tetrazolium (NBT) as described by [26].

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Concisely, the master reaction mixture contained phosphate buffer (pH 5), methionine (22 µ*M*), Triton-X (01 µ*M*), NBT (22 µ*M*) and supernatant in a test tube. These test tubes exposed to UV light for 15 minutes to initiate reaction and finally riboflavin (0.6 *M*) was added. Then the absorbance was notes at 560 nm with the help of microplate spectrophotometer. One unit of SOD was taken as the amount of supernatant to inhibit 50% NBT.

Final value was calculated as U mg^{-1} protein. Activities of POD and CAT enzymes were measured by the procedure given by Liu et al. (2009). The reaction mixture consisted on freshly prepared H_2O_2, guaiacol for POD, while, for CAT only on H_2O_2. The reaction was initiated by adding supernatant and taking the absorbance at 470 nm and 240 nm for POD and CAT enzymes, respectively. The final reading was expressed as U mg^{-1} protein for both enzymes.

2.6 Sensory Attributes and Shelf Life Estimation

Mango sensory attributes were quantified at fruit ripening only. A panel of 10 judges were provided with mango pulp samples to evaluate sensory attributes based on hedonic scale ranging from 1-9 (Peryam and Pilgrim,1957) with some modification suggested by [2]. Where, 9= Like extremely, 5= neither like nor dislike, 1= Dislike extremely. The recorded sensory attributes were fruit taste, aroma, texture and pulp colour.

The final reading of these attributes were given as score. Mango fruit shelf life was assessed based on visual quality and fruit textural softness. The fruit were monitored to stage of fruit ripening until it became overripe and cosmetic quality become poor and unmarketable (fruit skin wrinkled excessively and extreme lenticel dis-colouration) and counted in number of days.

2.7 Statistical Analysis

The experiments were laid out as per completely randomized design (CRD) with factorial arrangement, where, various postharvest physical heat treatments and shelf period served as factors. The statistical analysis for mango cv. ‘Chenab Gold’ and ‘Azeem Chaunsa’ were performed separately using two-way ANOVA. Means comparison was LSD test at $P \leq 0.05$ by using computer software STATISTIX 8.1®. Pearson’s correlation was also computed through this software and graph was drawn by using Origin2024b® software.

3. RESULTS

3.1 Fruit Weight Loss, Ethylene and Respiration Rate

Mango fruit weight loss increased with days at shelf in all treatments, however, VHT-treated fruit showed significant lower fruit weight loss as compared to HWT- or untreated mango fruit in both mango cultivars (Fig 1 A&B). Irrespective to days at shelf, VHT-treated mango fruit exhibited about 49% and 38% lower fruit weight loss for ‘Azeem Chaunsa’ and ‘Chenab Gold’ mango as compared to untreated fruit, respectively (Table 2). With

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advancement of fruit ripening, an increment in mango fruit ethylene gas evolution was noted in all type of treated mango fruit of both cultivars. A rise in ethylene gas was noted on day-4 and day-3 of shelf in 'Azeem Chaunsa and 'Chenab Gold' mango, respectively (Fig. 1 C&D). On average, HWT-treated fruit exhibited significant higher ethylene as compared to VHT- and untreated fruit (Table 2).

Similarly, mango fruit showed an increased respiration rate upto day-4 and day-5 of shelf in 'Azeem Chaunsa' and 'Chenab Gold' mango followed by a decrease in respiration rate. Afterwards, VHT-treated fruit exhibited significant lower respiration rate in both mango cultivars at shelf compared with HWT- and untreated fruit (Fig. 1 E&F). Irrespective to days at shelf, VHT-treated fruit showed lower respiration rate as compared to HWT-treated fruit in both mango cultivars (Table 2).

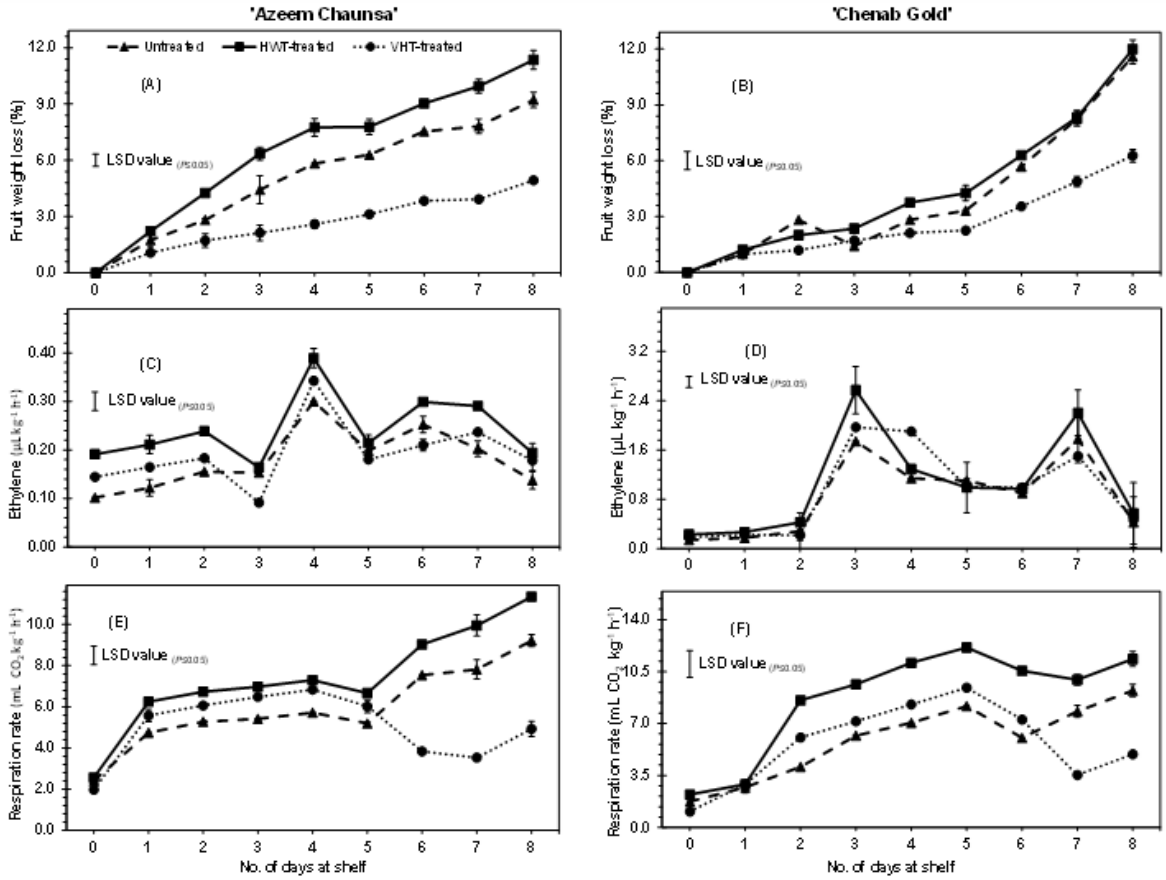

Figure 1: Effects of postharvest quarantine heat treatments on fruit weight loss (A & B), ethylene production (C & D) and respiration rate (E & F) of 'Azeem Chaunsa' and 'Chenab Gold' mango fruit at shelf conditions (28±2 ºC). Vertical bars represents ± SE of means. n= 9 (3 fruit × 3 replications)

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Table 2: Main effect of postharvest quarantine heat treatment (PQHT) on 'Azeem Chaunsa' and 'Chenab Gold' mango fruit physiological, biochemical and anti-oxidative attributes

Fruit Parameters	'Azeem Chaunsa'			LSD (P≤ 0.05)	'Chenab Gold'			LSD (P≤ 0.05)
	Untreated	HWT-treated	VHT-treated		Untreated	HWT-treated	VHT-treated	
Weight loss (%)	5.07 **b**	6.52 **a**	2.59 **c**	1.204	4.10 **b**	4.46 **a**	2.55 **c**	0.406
Ethylene (µL kg-1 h-1)	0.180 **c**	0.243 **a**	0.192 **b**	0.074	0.854 **c**	1.057 **a**	0.939 **b**	0.014
Respiration rate (mL kg-1 h-1)	5.93 **b**	7.42 **a**	5.03 **b**	1.214	5.89 **b**	8.71 **a**	5.62 **b**	1.871
TSS (°Brix)	14.36 **c**	21.70 **a**	16.82 **b**	2.047	11.03 **c**	17.99 **a**	13.63 **b**	0.781
TA (%)	0.57 **a**	0.35 **c**	0.44 **b**	0.085	0.74 **a**	0.37 **c**	0.55 **b**	0.097
Sugar Acid (Ratio)	25.19 **c**	62.00 **a**	38.23 **b**	6.103	14.09 **c**	48.62 **a**	24.78 **b**	5.091
Fruit Firmness (N)	137.64 **a**	98.47 **c**	122.08 **b**	10.411	103.22 **a**	67.87 **c**	76.69 **b**	6.24
Juice pH	3.91 **a**	4.24 **a**	4.13 **a**	NS	2.45 **c**	3.78 **a**	3.03 **b**	0.367
Carotenoids (µg g-1)	0.033 **a**	0.048 **a**	0.040 **a**	NS	0.043 **c**	0.058 **a**	0.051 **b**	0.0087
Vitamin C (mg 100g-1)	110.15 **c**	125.07 **b**	142.29 **a**	13.142	126.82 **c**	156.95 **b**	172.02 **a**	10.247
TPC (mg GAE 100g-1)	94.70 **c**	109.24 **b**	125.38 **a**	14.157	114.77 **c**	131.11 **b**	140.37 **a**	17.231
Anti-oxidative Activity (% Inhibition)	58.08 **c**	66.06 **b**	72.33 **a**	5.002	66.81 **c**	79.43 **b**	85.24 **a**	7.122
SOD Activity (U mg-1 Protein)	37.61 **c**	40.39 **b**	43.35 **a**	2.147	49.95 **a**	52.12 **a**	54.57 **a**	NS
POD Activity (U mg-1 Protein)	2.48 **a**	3.01 **a**	2.76 **a**	NS	2.11 **c**	3.95 **a**	3.13 **b**	0.907
CAT Activity (U mg-1 Protein)	3.65 **c**	4.26 **b**	5.79 **a**	0.917	3.95 **c**	4.82 **b**	6.04 **a**	1.024

Means sharing similar letter in a row is non-significant at $P \leq 0.05$. HWT=Hot Water Treatment, VHT= Vapour Heat Treatment, TSS= Total Soluble Solids, TPC= Total phenolic Contents, SOD= Superoxide Dismutase Enzyme, POD= Peroxidase Enzyme, CAT= Catalase Enzyme, NS= Non-Significant ($P \leq 0.05$)

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3.2 Fruit Peel Colour

Irrespective to storage period at shelf, mango peel colour *L* was significant lower in VHT-treated fruit, about 17% and 12% lower value for 'Azeem Chaunsa' and 'Chenab Gold' mango fruit, respectively than untreated fruit, as compared to HWT- and untreated fruit.

Significant difference were exhibited by the PQHT mango with days at shelf for both mango cultivars, the value of *L* increased as the days of shelf advanced (Table 3).

Similarly, when the average of PQHT were compared VHT-treated mango fruit exhibited lower values of peel colour *b** in both mango cultivars, about 23% and 18% lower value than untreated fruit mango peel colour *b** for 'Azeem Chaunsa' and 'Chenab Gold' mango fruit (Table 3).

However, in case of peel colour *a**, irrespective to shelf days, VHT-treated fruit exhibited significant lower value as compared to HWT-treated fruit only, peel colour a* of untreated fruit were statistically same with VHT-treated mango fruit of both cultivars (Table 3).

3.3 Total Soluble Solids (TSS), Titratable Acidity (TA) and Sugar Acid Ratio

All the treated mango fruit exhibited an increasing TSS with advancement of shelf period for both mango cultivars (Fig. 2 A&B).

Irrespective to shelf days, HWT-treated fruit exhibited significantly higher TSS as compared to VHT- and untreated fruit for 'Azeem Chaunsa' and 'Chenab Gold' mango fruit (Table 2). In contrast, fruit TA of both mango cultivars treated with PQHT decreased with advancement of shelf days in all the treatments (Fig. 2 C&D).

As for as the treatment means concerned, untreated fruit retained higher fruit TA in both the mango cultivars compared with HWT- and VHT-treated mango fruit (Table 2).

With advancement of shelf days, sugar acid ratio of all treated mango fruit exhibited an increasing trend in both mango cultivars. On day-8 of shelf, HWT-treated fruit exhibited significant higher sugar acid ratio as compared to other treatments in both mango cultivars (Fig. 2 E&F).

Irrespective to shelf days, highest sugar acid ratio exhibited by HWT-treated 'Azeem Chaunsa' and 'Chenab Gold' mango fruit (Table 2).

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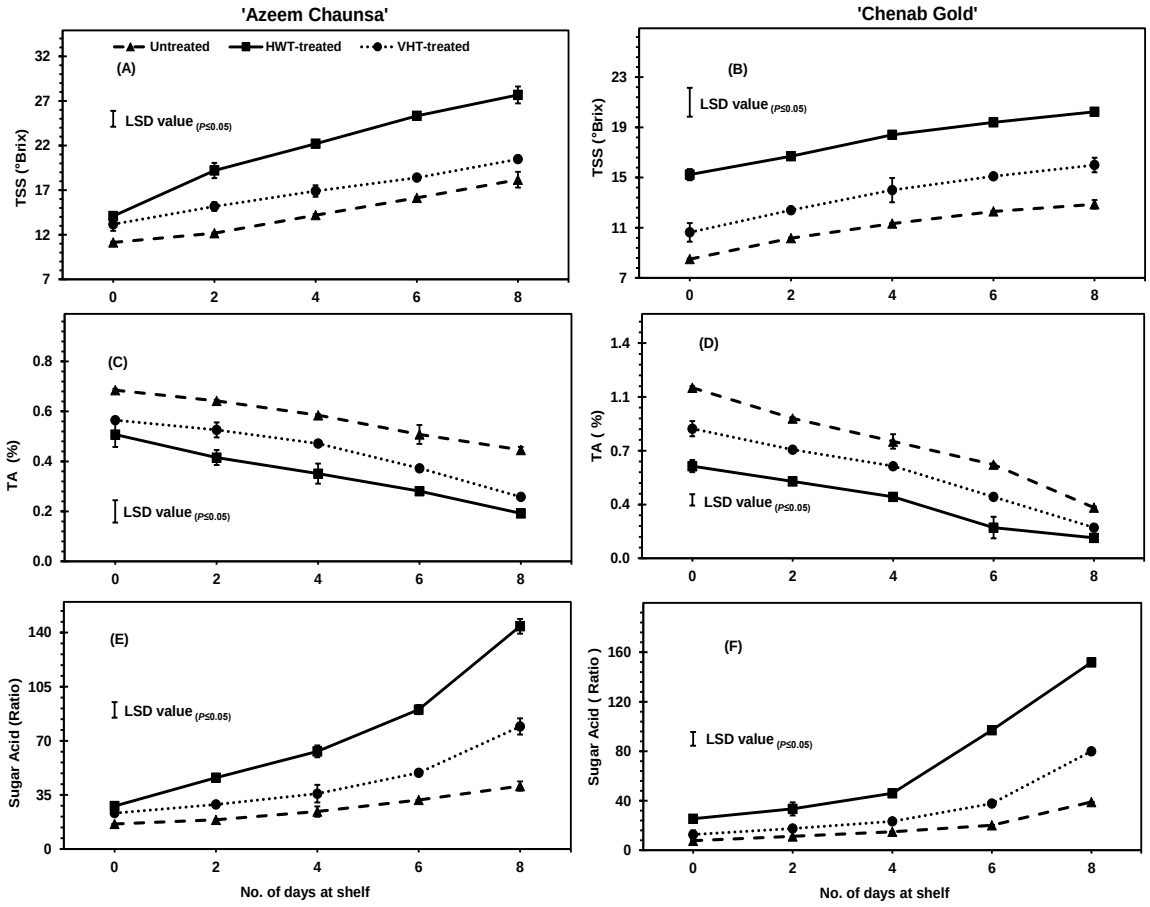

Figure 2: Effects of postharvest quarantine heat treatments on fruit TSS (A & B), TA (C & D) and sugar acid (E & F) of 'Azeem Chaunsa' and 'Chenab Gold' mango fruit at shelf conditions (28±2 ºC). Vertical bars represents ± SE of means. n= 9 (3 fruit × 3 replications)

3.4 Fruit Firmness, Juice pH and Carotenoids

With the advancement of shelf days, mango fruit of both cultivars exhibited a decreasing fruit firmness in all the treatments. The lowest fruit firmness was noted in HWT-treated 'Azeem Chaunsa' mango, while, VHT-treated and HWT-treated 'Chenab Gold' mango fruit on day-8 of shelf as compared to untreated fruit (Fig. 3 A&B). Overall, untreated fruit retained higher fruit firmness than VHT- and HWT-treated fruit during entire period of fruit ripening at shelf in both mango cultivars (Table 2). Irrespective to PQHT treatments, juice pH of Chenab Gold mango exhibited significant increasing trend as the shelf period advanced, however, non-significant differences were noted for 'Azeem Chaunsa' mango (Fig. 3 C&D). Similarly, fruit carotenoids of 'Chenab Gold' mango exhibited a significant increasing pattern as the shelf period progressed in all the treatments. However, non-significant differences in fruit carotenoids were found for 'Azeem Chaunsa' mango (Fig.

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3 E&F). Irrespective to shelf days, HWT-treated 'Chenab Gold' fruit exhibited significant higher fruit juice pH and carotenoids as compared to VHT- and untreated mango (Table 2).

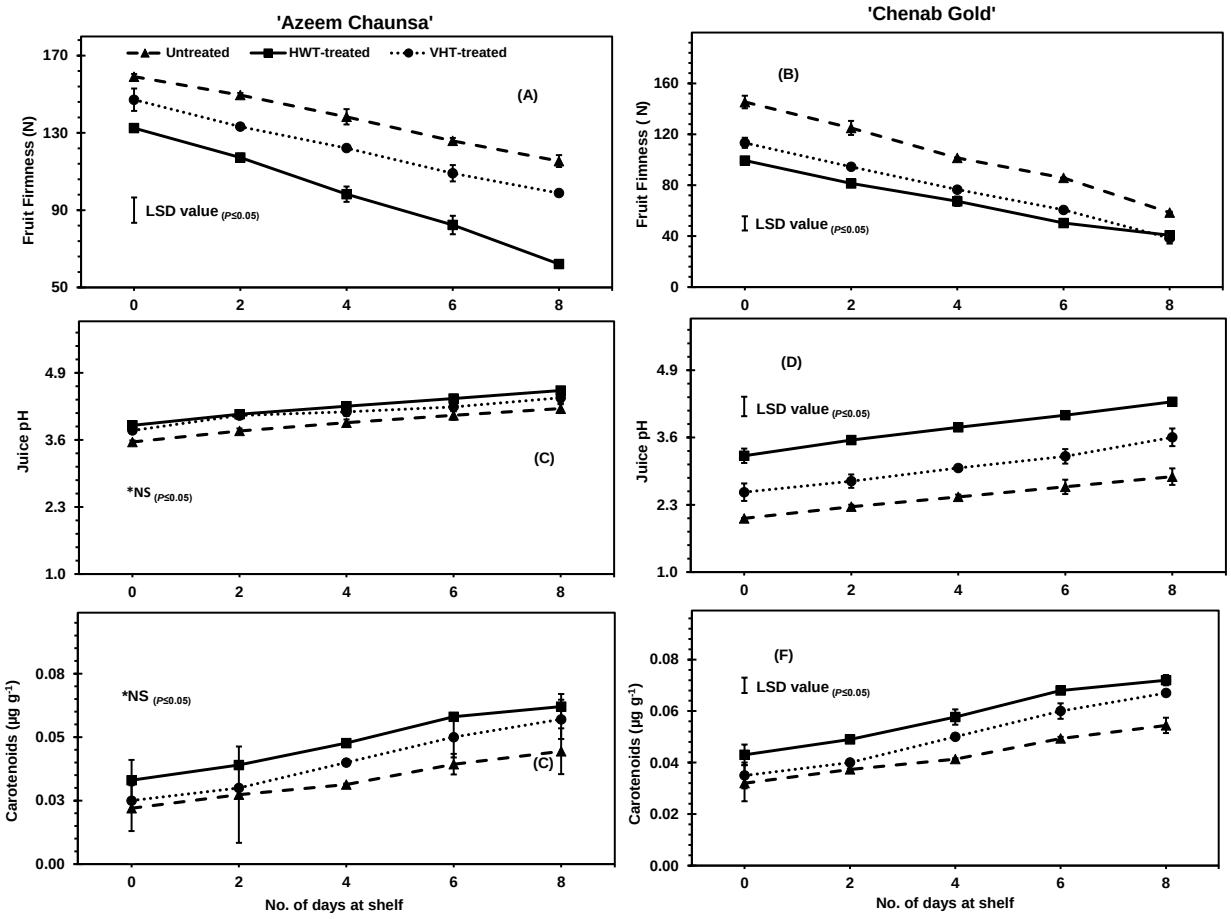

Figure 3: Effects of postharvest quarantine heat treatments on fruit firmness (A & B), juice pH (C & D) and carotenoids (E & F) of 'Azeem Chaunsa' and 'Chenab Gold' mango fruit at shelf conditions (28±2 ºC). Vertical bars represents ± SE of means. n= 9 (3 fruit × 3 replications)

3.5 Vitamin-C, Total Phenolic Contents (TPC) and Anti-Oxidative Activity

Significant losses in fruit vitamin-c were observed with the passage of fruit ripening at shelf in all the PQHT treated mango fruit in both the cultivars. However, VHT-treated 'Azeem Chanusa' and 'Chenab Gold' mango fruit retained significant higher vitamin-c contents during the entire ripening period as compared to HWT- and untreated fruit (Fig. 4 A&B). Overall, VHT-treated fruit retained 30% and 36% higher vitamin-c in 'Azeem Chaunsa' and 'Chenab Gold' mango fruit, respectively, as compared to untreated fruit during fruit ripening at shelf (Table 2). With the advancement of shelf days, TPC and anti-oxidative activity of mango fruit exhibited a significant increasing trend in both the mango

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
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Vol: 67 Issue 09 | 2024
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cultivars irrespective to PQHT treatments (Fig. 4 C-F). VHT-treated fruit retained significant higher TPC and anti-oxidative activity as compared to HWT- and untreated fruit during the entire storage period. Overall, VHT-treated 'Azeem Chaunsa' exhibited about 33% and 24% significant higher TPC and anti-oxidative activity, respectively as compared to untreated fruit. Similarly, 'Chenab Gold' mango fruit treated with VHT exhibited about 23% and 30% higher TPC and anti-oxidative activity, respectively as compared to untreated fruit (Table 2).

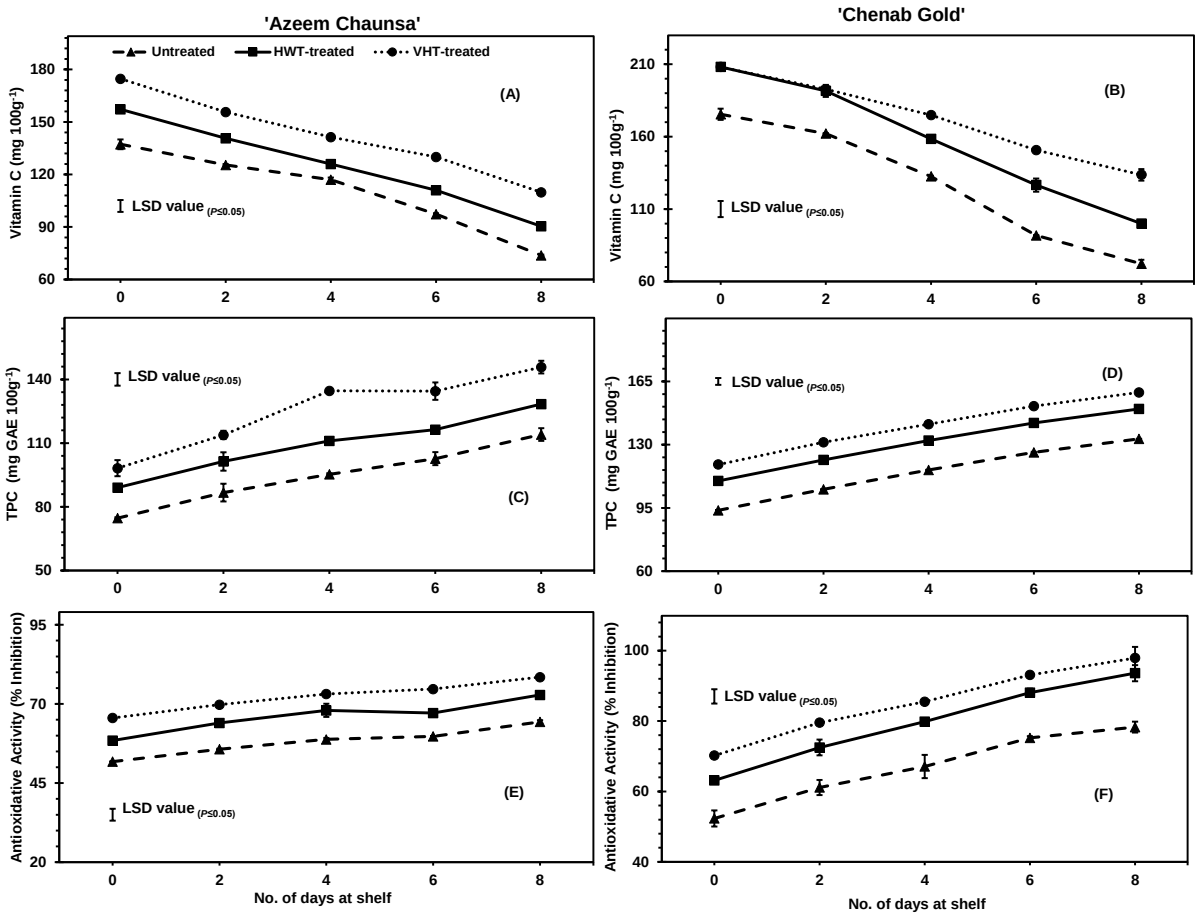

Figure 4: Effects of postharvest quarantine heat treatments on vitamin C (A & B), TPC (C & D) and anti-oxidative activity (E & F) of 'Azeem Chaunsa' and 'Chenab Gold' mango fruit at shelf conditions (28±2 ºC). Vertical bars represents ± SE of means. n= 9 (3 fruit × 3 replications)

3.6 Activities of Superoxide Dismutase (SOD), Peroxidase (POD) and Catalase (CAT) Enzymes

All the treated mango fruit exhibited a significant increasing activities of SOD, POD and CAT enzymes as the fruit ripening at shelf progressed in both 'Azeem Chaunsa' and 'Chenab Gold' mango cultivars (Fig 5 A-F). Irrespective shelf days, VHT-treated fruit

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13828597

exhibited significant higher CAT enzyme activity in both the mango cultivars, while, significant higher SOD enzyme activity in Chenab Gold as compared to HWT- and untreated fruit. However, for 'Chenab Gold' mango significant higher activity of POD was exhibited by HWT-treated fruit as compared to other treatments (Table 2).

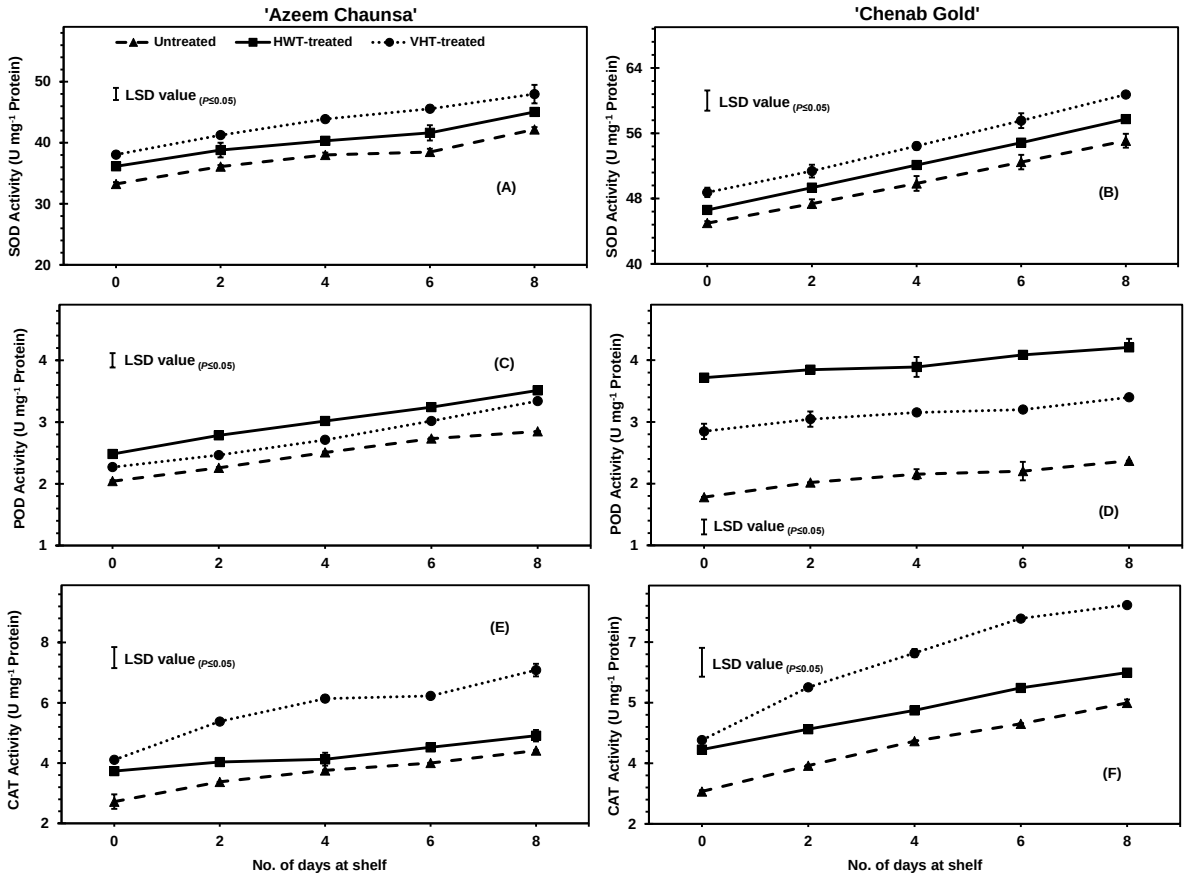

Figure 5: Effects of postharvest quarantine heat treatments on activities SOD (A & B), POD (C & D) and CAT enzymes (E & F) of 'Azeem Chaunsa' and 'Chenab Gold' mango fruit at shelf conditions (28±2 ºC). Vertical bars represents ± SE of means. n= 9 (3 fruit × 3 replications)

3.7 Sensory Attributes and Shelf-life

'Azeem Chaunsa' and 'Chenab Gold' mango fruit sensory attributes were evaluated at eating soft stage of fruit ripening. These sensory attributes were fruit taste, pulp colour, flavor, taste and aroma. VHT-treated fruit exhibited higher scores for mango fruit taste, flavor and texture for both 'Azeem Chaunsa' and 'Chenab Gold' mango cultivars as compared to HWT- and untreated fruit (Fig. 6 A&B). Similarly, VHT-treated fruit showed higher shelf life of both 'Azeem Chuansa' (≈ 9 days) and 'Chenab Gold' (≈10 days) mango cultivars as compared to HWT- and untreated fruit.

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Vol: 67 Issue 09 | 2024
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However, HWT-treated mango fruit showed higher pulp colour and aroma scores for ‘Azeem Chaunsa’ and ‘Chenab Gold’ mango as compared to VHT- and untreated fruit (Fig. 6 A&B).

4. DISCUSSION

Response of two novel mango commercial mango cultivars ‘Azeem Chaunsa’ and ‘Chenab Gold’ were investigated for HWT and VHT under simulated air conditions. With the advancement of fruit ripening in both the mango cultivars, fruit physiological attributes (fruit weight loss, ethylene and respiration rate) thereby increased with increasing time period at shelf. Mango fruit ripening is relatively complex process characterized by rise in ethylene production and respiration rate [27].

Similarly, as fruit ripen, excessive water loss in the form of transpiration and evaporation lead to decrease in weight of fruit. As evident from our results of ethylene production and respiration rate (Fig 1). PQHT promoted the fruit ripening of both mango cultivars by increasing the fruit gaseous exchange (ethylene and CO_2) as compared to untreated fruit. However, in contrary to ethylene and respiration rate, significant lower fruit weight was observed in VHT-treated fruit as compared to untreated fruit (Fig. 1).

Postharvest heat treatments have been reported to have variable effect on fruit ripening either promoting, inhibiting or have also been reported to cause disruption in normal fruit ripening [16], [17]. Among PQHT, HWT- treated fruit exhibited higher respiration rate and ethylene production. Likewise, in ‘Sultana’ grapes, VHT-treated fruit did not exhibit significant higher weight losses as compared to untreated fruit [17]. These results are in contrary to findings of [6] reporting HWT- treated blue berry fruit exhibited lower weight loss as compared to other treatments.

Variable ripening responses to heat treatments can be attributed to fruit crop differences and even cultivars differences within a fruit species [25]. Generally, fruit ripening involves fruit outer surface colour change and texture change due to break down of chlorophyll accompanied by accumulation of carotenoids and fruit pulp softening due to pectin and cell wall components breakdown, respectively. Mango commercially harvested hard and mature green, as the fruit ripening advanced it become yellow and soft. Heat treatments have been reported to accelerate these changes in mango [13].

The both mango cultivars treated with PQHT exhibited significant higher fruit softening and fruit skin colour development as compared to untreated fruit (Table 3; Fig. 3). Similarly, similar findings have been reported in ‘Carabao’, ‘Keningston’, ‘Sindhri’, ‘Samar Bahisht Chaunsa’ and ‘Sufaid Chaunsa’ mango [5], [19], [25]. During fruit ripening, total phenolic contents and anti-oxidants have variable responses may be increasing or decreasing influenced by type of fruit crop, ripening conditions and postharvest treatments. Our results revealed a significant higher Vitamin C, TPC and antioxidants by both HWT- and VHT-treated mango fruit as compared to untreated fruit in both the mango cultivars (Fig. 5).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 09 | 2024
DOI: 10.5281/zenodo.13828597

Heat treatment might have accelerated changes at cellular and enzymatic level leading to increased TPC and anti-oxidative activity. Similar, findings have been in 'Sindhri', 'Samar Bahisht Chaunsa' and 'Sufaid Chaunsa' and 'Ivory' mango, where VHT- and HWT-treated fruit exhibited significant higher TPC and anti-oxidative activity [30], [9], [25]. As fruit ripen, higher respiration rate produces more and more free radicals, reactive oxygen species (ROS), leading to oxidative stress.

In response to this fruit, activates anti-oxidative system in the form of accelerated activities of enzymes; superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) acting as 1st line of defense [28]. Our results revealed significant increased SOD activity in VHT- and HWT-treated fruit in 'Chenab Gold' mango, whilst CAT activity in both mango cultivars as compared to untreated fruit (Fig. 6, Table 2).

No reports are available reporting effect of phytosanitary treatments on mango fruit SOD, CAT and POD enzymes activity. However, some studies on loquat fruit and sweet potato inoculated with fungi and subsequently exposed to hot air treatments have reported significant higher SOD and POD enzymes activity in heat treated fruit [31], [30].

Sensory attributes and shelf life are key attributes of fruit quality influencing consumer satisfaction and marketability. VHT- and HWT-treated fruit exhibited higher score of various sensory characteristics including fruit taste, flavor, pulp colour, aroma and shelf life as compared to untreated fruit (Fig. 6).

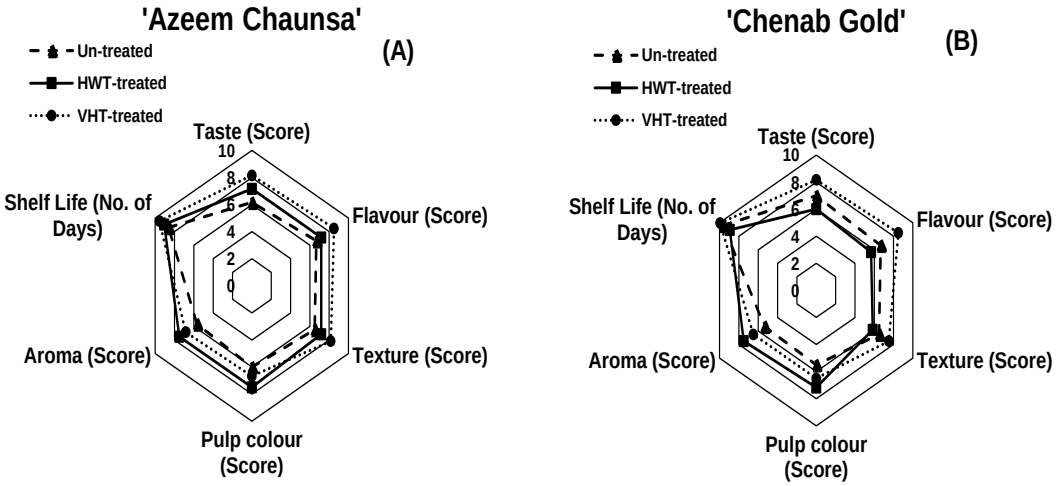

Figure 6: Effects of postharvest quarantine heat treatments on fruit sensory attributes; taste, flavor, texture, pulp colour, aroma and shelf life of 'Azeem Chaunsa' (A) and 'Chenab Gold' (B) mango fruit at shelf conditions (28±2 ºC). n= 10

During mango ripening, starch substantially converted into soluble sugars leading to more sweetness and flavor and taste [22]. Heat treatments have been reported to increase conversion of starch to sugars, similar results have been reported by [25] in 'Samar Bahisht Chaunsa' and 'Sufaid Chaunsa' mango. Our results revealed comparatively

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Vol: 67 Issue 09 | 2024
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higher shelf life displayed by VHT- and HWT-treated fruit in both the mango cultivars. Fruit shelf life and anti-oxidative activity are closely related, as antioxidants play a crucial role in maintaining fruit quality. Fruit contains antioxidants in the form of vitamins, phenolic compound and carotenoids, protecting the fruit from oxidative stress and ultimately increasing shelf life.

Our results revealed a significant positive correlation of 'Azeem Chaunsa' and 'Chenab Gold' mango's shelf life with TPC, carotenoids, vitamin C contents, activities of SOD, POD and CAT enzymes (Fig. 7). So, the higher shelf life of VHT- and HWT-treated 'Azeem Chaunsa' and 'Chenab Gold' mango's shelf life can be attributed to relative higher amount of anti-oxidative compound as compared to untreated fruit.

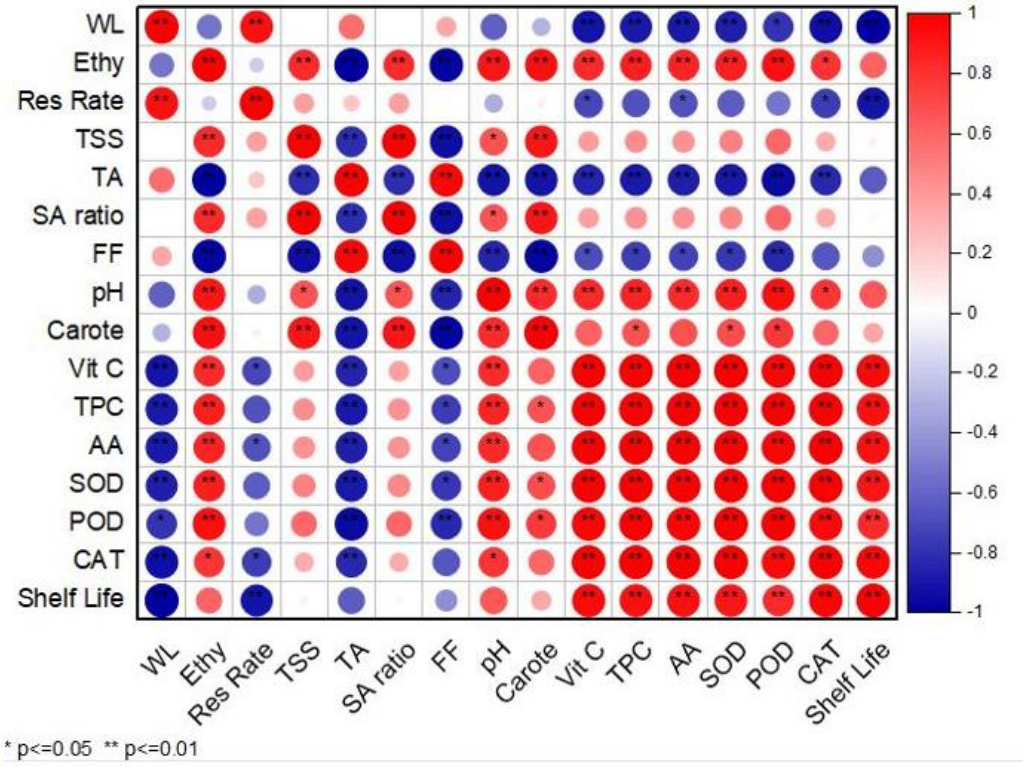

Figure 7: Correlation between fruit physiological, biochemical and anti-oxidative attributes of mango fruits. *WL* weight loss, *Ethy* ethylene production, *Res Rate* fruit CO_2 evolved, *TSS* total soluble solids, *TA* Titratable acidity, *SA ratio* Sugar acid ratio, *FF* fruit firmness, *Carote* Carotenoids, *Vit C* vitamin-c contents, *TPC* Total phenolic contents, *AA* Anti-oxidative activity, *SOD* superoxide dismutase enzyme activity, *POD* peroxidase enzyme activity, *CAT* catalase enzyme actiity

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
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Table 3: Effect of Postharvest Quarantine Heat Treatment (PHQ) on 'Azeem Chaunsa' and 'Chenab Gold' Mango Fruit Peel Colour

Mango Cultivar	Treatments	Days at shelf					Means
		Day-0	Day-2	Day-4	Day-6	Day-8	
Fruit peel colour *L*							
'Azeem Chaunsa'	Untreated	44.95±0.715	50.96±0.290	55.44±0.242	55.04±0.733	58.77±0.367	53.03 **B**
	HWT	51.06±0.338	55.24±0.048	59.05±0.028	60.35±0.009	65.21±0.009	58.18 **A**
	VHT	41.32±0.722	45.99±0.737	46.23±0.384	52.25±0.202	55.52±0.698	48.26 **C**
'Chenab Gold'	Untreated	60.86±0.594	66.87±0.511	71.28±0.209	75.54±0.413	83.02±0.657	71.51 **B**
	HWT	66.97±0.603	70.82±0.620	76.26±0.323	81.12±0.292	89.30±0.551	76.89 **A**
	VHT	58.23±0.356	61.90±0.731	67.82±0.403	71.09±0.598	74.80±0.706	66.77 **C**
Fruit peel colour *a**							
'Azeem Chaunsa'	Untreated	-11.42±0.009	-10.08±0.009	-9.13±0.004	-5.72±0.060	-3.14±0.009	-7.90 **B**
	HWT	-9.31±0.006	-7.83±0.006	-7.11±0.006	-3.41±0.006	-0.94±0.006	-5.72 **A**
	VHT	-14.37±0.065	-11.77±0.006	-10.31±0.009	-7.66±0.003	-4.97±0.006	-9.82 **B**
'Chenab Gold'	Untreated	-11.76±0.020	-9.88±0.035	3.08±0.243	5.98±0.169	7.00±0.007	-1.12 **B**
	HWT	-8.98±0.650	-5.71±0.042	6.07±0.143	8.93±0.133	11.14±0.087	2.29 **A**
	VHT	-11.96±0.081	-9.12±0.210	-5.00±0.058	3.63±0.030	6.47±0.018	-3.20 **B**
Fruit peel colour *b**							
'Azeem Chaunsa'	Untreated	28.47±0.028	33.48±0.035	36.29±0.045	38.57±0.038	43.50±0.028	36.06 **A**
	HWT	29.82±0.036	34.82±0.028	38.42±0.033	39.66±0.033	44.53±0.265	37.45 **A**
	VHT	24.84±0.024	28.72±0.028	30.83±0.031	32.52±0.025	35.47±0.344	30.48 **B**
'Chenab Gold'	Untreated	30.79±0.107	33.83±0.052	36.28±0.106	40.30±0.519	36.81±0.419	35.60 **A**
	HWT	33.52±0.166	36.83±0.051	39.28±0.106	43.30±0.522	39.92±0.466	38.57 **A**
	VHT	24.52±0.166	27.83±0.053	29.95±0.407	33.66±0.327	30.33±0.237	29.26 **B**
LSD Values (*P*≤ 0.05)			**Treatments**	**Treatments × Days at Shelf**			
For 'Azeem Chaunsa' mango		*L*	2.821**	2.104*			
		*a**	1.909*	NS			
		*b**	2.412*	NS			
For 'Chenab Gold' mango		*L*	3.017**	3.391**			
		*a**	2.174*	NS			
		*b**	3.112*	NS			

Treatment means sharing different letters in row are significant at P ≤ 0.05. Values followed by means are SE of means. NS, * and ** represent non-significant, significant and highly significant P ≤ 0.05, respectively.

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5. CONCLUSION

During fruit ripening at ambient conditions, PQHT promoted the fruit ripening in both mango cultivars as evident by higher physiological, textural and biochemical attributes in both VHT- and HWT-treated mango fruit. However, these heat treatments retained higher overall fruit anti-oxidative attributes leading to improved sensory attributes and shelf life of both mango cultivars.

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Author Contributions

M.A. and I.A.R. conceived the idea and supervised the trials; A.R. conducted the trials, data collection and analysis, initial drafting; S.U. validated data, developed graphs and tables, finished the initial draft; A.H. and B.A.S. helped reviewing and finalizing the manuscript.

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