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EFFECT OF HEAT TREATMENTS ON 'AZEEM CHAUNSA' MANGO FRUIT QUALITY AND ANTI-OXIDATIVE ATTRIBUTES AT AMBIENT CONDITIONS

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Abstract

Mango is an important tropical fruit crop around the world. Mango fruit is liked because of its charismatic taste, appealing color and high nutritious value. Among the mango producers of the world, Pakistan ranks 6th after India, China, Thailand, Indonesia, and Mexico. Another problem was shorter export window due to un-availability of late season cultivar. Export window (September to late October) extension was recently achieved by development of late season cultivar Azeem Chaunsa. Farmers and exporters had readily adopted Cv. 'Azeem Chaunsa' to increase the net income. However, distant markets export could only be made possible through adoption of quarantine measures heat treatments viz., vapor heat treatment and hot water treatment. In this study, impact of heat treatments on fruit quality, fruit respiration, shelf life and firmness were determined. Fruit biochemical characters (total soluble solids, juice pH, titratable acidity and ascorbic acid content), phytochemical characters (pigments, and different enzymes activities) and organoleptic analysis were evaluated in Vapor Heat Treatment (VHT), Hot Water Treatment (HWT) and control under ambient conditions. The experiment was laid out in a completely randomized design. Among physiological parameters ethylene production, respiration and weight loss were higher in HWT treated mangoes compared to vapor treatment. Better ripening color was obtained through VHT compared to HWT. TSS and pH were higher in HWT treated mangoes compared to VHT and control. Higher titratable acidity was observed in control compared to treated mangoes. On day-6 of fruit ripening, mango fruit treated with HWT and ethylene ripening exhibited the highest respiration rate during 2020 & 2021 season. Our study concluded that VHT can be adopted to export Azeem Chaunsa without compromising fruit physiological, biochemical and phytochemical parameters during simulated air shipments.

Key words: Organoleptic parameters, Protein content, Ascorbic acid, Fruit shelf life, Ripening.

Introduction

Mango (*Mangifera indica* L.) is well known worldwide for its excellent eating quality, high nutritional content, and importance as a food source (Kim *et al*., 2009). It is a good source of dietary antioxidants and phenolic compounds (Ma *et al*., 2011). The majority of fruit quality studies focus on phenolic content and antioxidants because they prevent free radical reactions and ultimately defend the human body from damage brought on by reactive oxygen species (ROS) as consumers become more aware of the fruit's health-promoting qualities (Kaur & Kapoor, 2001b). Bioactive chemicals included in various fruits, especially mango, protect against malignancies and cardiovascular illnesses like atherosclerosis (Hu, 2003; Yahia *et al*., 2017). Thus, feeding mango regularly could provide the essential amounts of bioactive compounds with good antioxidant activity. Cultivar, postharvest manipulation, agronomic practices, and different ripening stages affect the antioxidant capacity of mango (Kevers *et al*., 2007).

Mango's short shelf-life as a climacteric fruit is an inhibiting factor to increasing its supply period in international trade. The Pakistani mango industry faces challenges in overcoming fruit quality and limited shelf-life issues to deliver mangoes to international markets (Hafeez *et al*., 2012). Mango fruit reaches its climacteric peak during fruit ripening at ambient circumstances in 3 to 4 days (Narayana *et al*., 1996), while the other varieties, for example, 'Baneshan', 'Tomy Atkins' and 'Keitt' have a shelf-life of 4 days, whereas Alphanso has a shelf-life of 8–9 days, 'Kensington Pride' has a shelf-life of 9–12 days, and 'Haden' has a shelf-life of 12–14 days. In addition to widely known exportable cultivars of mango, 'Azeem Chaunsa' (late- season) has also gained popularity due to its export potential and extension of the mango supply chain till the end of October (Iqbal *et al*., 2021). The international trade mango industry faces numerous postharvest constraints, including fruit fly infestation, lenticel development, disease incidence, uneven ripening, and chilling injury during low-temperature storage.

Various fruit fly disinfestation treatments are being used in the world for mango export as per country's phytosanitary requirement (Jacobi *et al*., 2001) such as hot water treatment (HWT) for China and Iran (Jabbar *et al*., 2012), vapor heat treatment (VHT) for Australia, New Zealand and Japan and irradiation for USA (Sivakumar *et al*., 2011). The fruit quality of heat-treated mangoes depends on different factors, including variety, fruit size, harvesting maturity, temperature, pre-treatment conditions, and post-harvest environmental factors (Jacobi *et al*., 2001).

Hot water treatment (HWT) is usually employed as an appropriate phytosanitary non-chemical technique with higher efficiency and lower cost operation to disinfest mangoes from eggs and larvae of fruit flies (Paull & Chen, 2000). In this technique, the fruit was submerged in heated water at prescribed temperature depending on the agreed protocol. HWT is mandatory phytosanitary treatment for exporting mangoes from Pakistan to Iran and China (Jabbar *et al*., 2011). According to the findings of Lurie (1998), HWT kills insects, prevents fungus, and maximizes resistance to chilling injury in fresh horticultural commodities. HWT promotes softening and ripening,

improving the postharvest quality of many fruit including citrus (Porat *et al*., 2000) and mangoes . HWT is adopted at large scale due to its high efficacy in minimizing postharvest diseases and low expense. HWT inhibits ripening, minimizes deterioration, and reduces fights against chilling injury and pathogens in numerous products.

Vapor heat treatment (VHT) is another postharvest phytosanitary approach to export tropical fruit (Malik *et al*., 2021) and particularly used to kill fruit fly eggs and larvae before shipment (Ragan, 2002; Thi-Nghiem *et al*., 2009). In this technique, the fruits were warmed with air-soaked water vapor at about 40-50°C temperatures to dispose of eggs and larva as phytosanitary treatment before shipment to market (Ragan, 2002). In carabao mangoes, VHT decreased the occurrence of anthracnose and stem-end rot (Esquerra and Lizada, 1990). VHT effectively disinfested papaya fruit against fruit fly species, making it a viable phytosanitary treatment before shipment (Hsu *et al*., 2018). Organoleptic characteristics have also been found to be affected by heat treatment, such as sugars, soluble solid, acidity and ascorbic acid contents in mango.

Latest ripening technique is another concern to look. Because climacteric fruit has a short shelf-life, the current practice of using CaC_2 for mango fruit ripening must be replaced because it is banned in most importing countries due to high health hazards (Siddiqui & Dhua, 2010). Several attempts have been made in the past to investigate a CaC_2 substitute for mango fruit ripening. It is thought that ethylene-based ripening is the most suitable and preferable strategy. Commercially and in industry, ethylene ripener is employed as a ripening agent. Nevertheless, details about this product's ripening behaviour on active research cultivars like 'Azeem Chaunsa' are limited. There is currently no information on how the post-harvest fruit quality of 'Azeem Chaunsa' mango fruit changes as it ripens. So, this industry-driven scientific study aimed to determine how VHT and HWT affected the fruit quality and shelf-life of the mango cultivar 'Azeem Chaunsa' and to take advantage of the fruit's potential for long-distance air transportation under ambient circumstances.

This study provides the first detailed evaluation of the ripening behavior and post-harvest quality of 'Azeem Chaunsa' under VHT and HWT, addressing the potential of these treatments to enhance shelf-life and maintain fruit quality during long-distance air transportation. The study aims to determine how VHT and HWT affect the ripening process, fruit quality, and shelf-life of the 'Azeem Chaunsa' mango cultivar. It is hypothesized that VHT and HWT will positively influence the post-harvest quality and shelf-life of the 'Azeem Chaunsa' mango, enabling the fruit to retain marketable quality for long-distance air transportation under ambient conditions.

Material and Methods

Fruit source: Fruit of 'Azeem Chausnsa'mango were harvested from 'Akram Chawan farm'at green mature stage along with 4-6 inches long pedicels, physically de-sapped, washed, treated with Scholar fungicide 0.6 ml/liter. Fruits were sort out of uniform size, free from diseases and blemishes and placed in plastic containers. Fruits were divided into three lots (for VHT, HWT, and control). The first lot was subjected to VHT (47 ± 2ºC for 25 min), followed by Japanese protocol during the processing.

The total duration of vapor heat treatment was 3.30 hours and the core pulp temperature were maintained at 47 ± 2ºC for 25 minutes. The second lot was subjected to HWT (48 ± 2ºC for 60 min) and the fruit core temperature was confirmed by using a digital thermometer with an electric probe while 3rd was considered a control with no treatment. After phytosanitary treatments, fruits were packed in 2.5 kg corrugated boxes and transported to PST Lab, MNS-University of Agriculture, Multan for simulated study (air-freight) at ambient condition 25 ± 2ºC with relative humidity 60-65%.

Each lot was further subdivided into two sub-lots. One was being treated with an ethylene sachet (3 gm) while the 2nd sublot was not treated with fruit ripening dose. All the fruits were kept at ambient condition and was analyzed for various physiological, physical, biochemical and antioxidative parameters on alternate days. The experimental scheme is provided in Fig. S1.

Measurement of Ethylene (µ mol /kg /h) and CO_2 (m mol /kg /h): The ethylene and CO_2 were measured by taking two fruits from each replication in airtight box. The displayed values on screen were noted and expressed in ($\mu L\ kg^{-1}\ h^{-1}$) and ($mL\ CO_2\ kg^{-1}\ h^{-1}$) respectively at the peak point.

Fruit weight loss: The percent fruit weight loss was measured by selecting two fruit from each replication to measure weight loss on alternate day. Weight loss was calculated with help of given formula and expressed in (%).

$$\text{Weight loss} = \left(\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right) * 100$$

Physical parameters: Among physical parameters, fruit peel color change during fruit ripening period, fruit firmness was studied.

Fruit peel colour (*L*, a* and b):** The change in fruit colour from both sides of mango was determined using chroma meter. The Chroma meter gave *L**, *a**, and *b** values.

The data was noted at every shelf interval (0, 2, 4, 6, and 8 day).

Fruit firmness (Newton): The fruit firmness was determined by using digital penetrometer and expressed in Newton.

Biochemical parameters: Among biochemical parameters total soluble acids, juice PH, titratable acidity, ascorbic acid content was measured. The TSS (Total Soluble Solid) was determined by using digital handheld refractometer and expressed in °Brix. Juice pH was determined by dipping the digital pH meter probe (Starter 3100 OHASU, USA) for 1-2 minutes. Titratable acidity (%) was determined by the method of (Hortwitz, 1960).

Titratable acidity was expressed in % and calculated by the following formula:

$$\text{TA (\%)} = \left(\frac{0.0067 \times Vol.of\ NaOH \times 30}{5 \times 10}\right) \times 100$$

Ascorbic acid (mg/100 mL) was calculated by following the formula outlined by Ruck (1961).

$$Ascorbic\ acid\ (mg/100\mathrm{m}L) = \frac{1 \times R1 \times V}{R \times W \times V1}\ 100$$

Phytochemical parameters: Total antioxidants were estimated by the protocol of Mimica-Dukić *et al*., (2003), while total phenoilcs were estimated by using the procedure adopted by Ainsworth & Rogers (2007) with few modifications.

The total antioxidant contents: 2,2-diphenyl-1-picrylhydrazyl DPPH (%) inhibition were measured by taking 50 µL of supernatant in a test tube and 5mL of 0.004% DPPH was added in it. It was incubated in dark condition at room temperature for 30 minutes and the absorbance was noted at 517nm using Epoch, Eliza Microplate Reader (Bio-Tek Instruments, Inc. Vermont, USA). Total antioxidants were calculated by using the following formula.

The absorbance value of a blank represents the absorbance value of a control reaction including all reagents except samples.

$$Total\ antioxidant\ (\%\ Inhibition) = \frac{(A\ blank\ X\ 100)}{A\ blank}\ 100$$

The total phenolic contents (GAE mg/100 g): The total phenolic contents were determined by taking out 100 µL supernatant in Eppendorf tube and 10% FC-reagent (200 µL) was added in it and it was vortexed for mixing it thoroughly; then 800 µL sodium carbonate 700 mM) added in it and again vortexed. This prepared sample was incubated at room temperature for 2 hours. After incubation, the reading was noted at 765nm by using Epoch, Eliza Micro plate reader (Bio-Tek instruments, Inc. Vermont, USA). A standard curve of gallic acid at values of 0.02-0.1 mg mL-1 was used to calculate the total phenolic content. Each sample's total phenolic content was determined and addressed as mg GAE mg/100 g FW.

Fruit pigments viz., anthocyanin and carotenoids were also determined.

Anthocynain: The anthocyanin was measured by using the protocol of Proctor Proctor, (1974). The supernatant collected absorbance noted at 530, 620, and 650nm using Epoch, Eliza Microplate Reader (Bio-Tek Instruments, Inc. Vermont, USA). Following formula was used for anthocyanin calculations:

$$\Delta A\ g^{-1}\ FW = (A530 - A620) - 0.1\ (A650 - A620)$$

Carotenoids (µg/g FW) were estimated by following the protocol outlined by Lichtenthaler, (1987). The supernatant was obtained after centrifugation for estimation of carotenoids. The absorbance of supernatant was taken at 470, 645 and 662nm in Epoch, Eliza Microplate Reader (Bio- Tek Instruments, Inc. Vermont, USA. The carotenoids were expressed as µg per gram fresh weight (µg/g F.W).

Antioxidant enzyme activity: Superoxide dismutase (SOD), Catalase (CAT), and peroxidase (POD), were determined by using the Lin *et al*., (2007) method.

SOD enzyme (U/mg of protein): The method outlined by Lin *et al*., (2007) was followed for the determination of SOD enzyme activity. 100µL of enzyme extract, 200µLof methionine (22 M), 500µL of phosphate buffer (pH 5), 200µL of Triton X (0.1 M), 100µL of NBT (20 M) and 800µL of distilled water were exposed to UV light for 15 minutes in laminar air flow cabinet. A 100 µL riboflavin (0.6 M) was added in it after exposure of UV light. The absorbance was taken at 560nm and expressed in units/mg of protein.

CAT enzyme activity (U/mg of protein): The catalase enzyme activity was estimated by following the protocol of Lin *et al*., (2007). Enzyme extract (100 µL) was taken in a test tube and 100µL H2O2 (5.9 mM) added in it. The aborbance was taken at 240nm in Epoch, Eliza Microplate Reader (Bio- Tek Instruments, Inc. Vermont, USA. CAT activity was expressed in units/mg of protein.

POX activity (U/mg of protein): The peroxidase enzyme activity was measured by following the protocol of Liu *et al*., (2009). Enzyme extract (100 µL) was mixed reaction mixture containing 50mM 800µL phosphate buffer (pH 5) and 100µL guaiacol (20mM) and 100µL H_2O_2 (40mM). The absorbance of sample was noted at 470nm using an Epoch, Eliza micro reader (Bio-Tek Instruments, Inc. Vermont, USA). The activity of POX was expressed in units /mg of protein.

Organoleptic parameters: The sensory evaluation of mango fruit was carried out at fruit ripening. The general appearance and organoleptic qualities i.e., colour, taste, texture, flavour and aroma were performed at 8th day of shelf.

Fruit shriveling (%) and disease incidence (%): The fruit from each replication were visually observed for fruit shrivelling and estimating disease incidence.

Fruit shelf-life (No. of days): The fruit were kept at shelf (ambient condition) until the commencement of senescence and the number of days were recorded to determine the shelf-life.

Statistical analysis

The experiment was conducted according to the completely randomized design (CRD) with three factor factorial (physical heat treatments, ripening treatments, days at shelf) arrangement and replicated thrice. The collected data were subjected to statistical analysis (ANOVA and means comparison test) by using Statistix 8.1® software (Steel *et al*., 1997).

Results

Physiological parameters

Measurement of Ethylene (µ mol /kg /h) and CO2 (m mol /kg /h): During both season 2020 & 2021, irrespective to ripening treatment and days at shelf, HWT- treated

mango fruit exhibited significant higher ethylene production as compared to other treatments (Fig. 1A-B). When compared to ripening treatments only, the fruit treated with-ethylene had significant higher rate of ethylene production as compared to without-ethylene treated fruit (Fig. 1C-D). In both growing season 2020 & 2021, a gradual increase in ethylene production till 6th day and then a slight decline on 8th day was found (Fig. 1E-F). On day-6 of fruit ripening, the HWT- treated fruit, ripened with- ethylene exhibited higher ethylene production in both seasons (Fig. 1G-H).

The fruit treated with HWT had a significantly higher respiration rate than VHT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective of ripening treatment and days at shelf (Fig. 2A-B). If the only ripening treatments were compared, mango fruit with-ethylene exhibited a significantly higher respiration rate than without-ethylene treated fruit during both the growing seasons (Fig. 2C-D). Mango fruit showed an increasing trend in respiration rate till 6th day and then it slightly decreased on 8th day in all postharvest phytosanitary treated mango as the shelf period progressed in both the seasons (Fig. 2E-F). On day-6 of fruit ripening, mango fruit treated with HWT and ethylene ripening exhibited the highest respiration rate during 2020 & 2021 season (Fig. 2G-H).

Fruit weight loss: Irrespective of ripening treatment and days at shelf, mango fruit treated with HWT exhibited significantly higher weight loss than VHT-treated and untreated mango fruit in both the growing season 2020 & 2021 (Fig. 3A-B). As far as the ripening treatments were concerned, ethylene treated fruit showed significantly higher weight loss than untreated fruit during both the growing seasons (Fig. 3C-D). As the shelf period progressed the mango fruit showed an increasing trend for weight loss in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. 3E-F). On day-8 of fruit ripening, mango fruit treated with HWT with-ethylene ripening exhibited the highest weight loss during 2020 & 2021 season (Fig. 3G-H).

Physical parameters

Fruit peel colour (*L, *a** and *b**):** Significantly higher value of *L** was shown by the fruit treated with VHT compared to HWT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective of ripening treatment and days at shelf (Fig. S1 A-B). If the only ripening treatments were compared, mango fruit with-ethylene exhibited significantly higher L* value than without-ethylene treated fruit during both the growing seasons (Fig. S1 C-D). Mango fruit showed an increasing trend for *L** value in all postharvest phytosanitary treated mango fruit till 8th day (Fig. S1 E-F). On day-8 of fruit ripening, mango fruit treated with VHT with-ethylene ripening exhibited the highest *L** value during 2020 and 2021 season (Fig. S1 G-H).

Irrespective of ripening treatment and days at shelf, mango fruit treated with VHT treatment exhibited significantly higher value of a* compared to HWT-treated and untreated mango fruit in both the growing season 2020 & 2021 (Fig. S2 A-B). As far as the ripening treatments were concerned, ethylene treated fruit showed significantly higher value of a* compared to untreated fruit during both the growing seasons (Fig. S2 C-D). As the shelf period progressed the mango fruit showed an increasing trend for *a** value in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. S2 E-F). On day-8 of fruit ripening, mango fruit treated with VHT with-ethylene ripening exhibited the highest *a** value during 2020 and 2021 season (Fig. S2 G-H).

During both season 2020 & 2021, irrespective of ripening treatment and day at shelf, VHT- treated mango fruit exhibited significantly higher value of b* compared to other treatments (Fig. S3 A-B). When compared only ripening treatments, the fruit treated with- ethylene had a significantly higher b* value than without-ethylene treated fruit (Fig. S3 C-D). In both growing seasons 2020 & 2021, a gradual increase in *b** value was found with the advancement of shelf intervals (Fig. S3 E-F). On day-8 of fruit ripening, the VHT- treated fruit ripened with-ethylene exhibited the higher *b** value in both seasons (Fig. S3 G-H).

Fruit firmness (Newton): Irrespective to ripening treatment and days at shelf, untreated mango fruit exhibited significantly higher fruit firmness as compared to VHT and HWT-treated mango fruit in both the growing season 2020 & 2021 (Fig. S4 A-B). As for as the ripening treatments were concerned, fruit without ethylene treatment showed significant higher fruit firmness as compared to ethylene treated fruit during both the growing seasons (Fig. S4 C-D). As the shelf period progressed the mango fruit showed a decreasing trend for fruit firmness in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. S4 E-F). On day-8 of fruit ripening, untreated mango fruit without-ethylene ripening exhibited the highest fruit firmness during 2020 & 2021 season (Fig. S4 G-H).

Biochemical parameters: During both season 2020 & 2021, irrespective of ripening treatment and days at shelf, HWT- treated mango fruit exhibited significantly higher total soluble solids than other treatments (Fig. 4 A-B). When compared only ripening treatments, the fruit treated with-ethylene had significantly higher total soluble solids than without-ethylene treated fruit (Fig. 4 C-D). In both growing season 2020 & 2021, a gradual increase in total soluble solids was found with the advancement of shelf intervals (Fig. 4 E-F). On day-8 of fruit ripening, the HWT-treated fruit ripened with-ethylene exhibited the higher juice TSS in both seasons (Fig. 4 G-H).

Significantly higher pH was shown by the fruit treated with HWT compared to VHT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective of ripening treatment and days at shelf (Fig. 5 A-B). If the only ripening treatments were compared, mango fruit with-ethylene exhibited significantly higher pH than fruit without-ethylene during both the growing seasons (Fig. 5 C-D). Mango fruit showed an increasing trend for pH in all postharvest phytosanitary treated mango as the shelf period progressed in both the seasons (Fig. 5 E-F). On day-8 of fruit ripening, HWT-treated mango fruit with-ethylene ripening exhibited the highest pH during 2020 & 2021 season (Fig. 5 G-H).

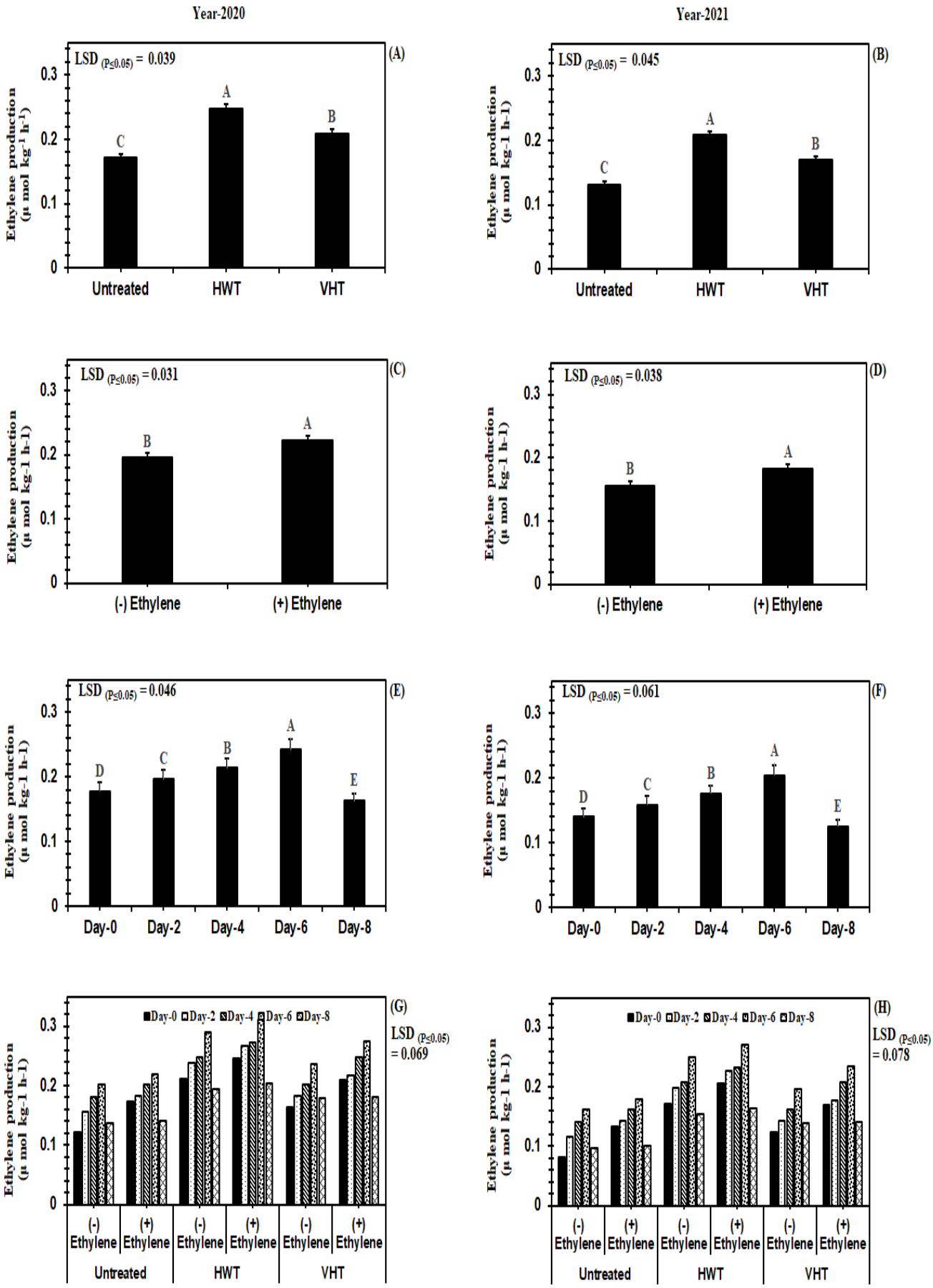

Fig. 1. Effect of postharvest phytosanitary heat treat ments, ripening treatments and days at shelf on ethylene production of 'Azeem Chanusa' mango during 2020 (A, C, E, G) and 2021 (B, D, F, H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

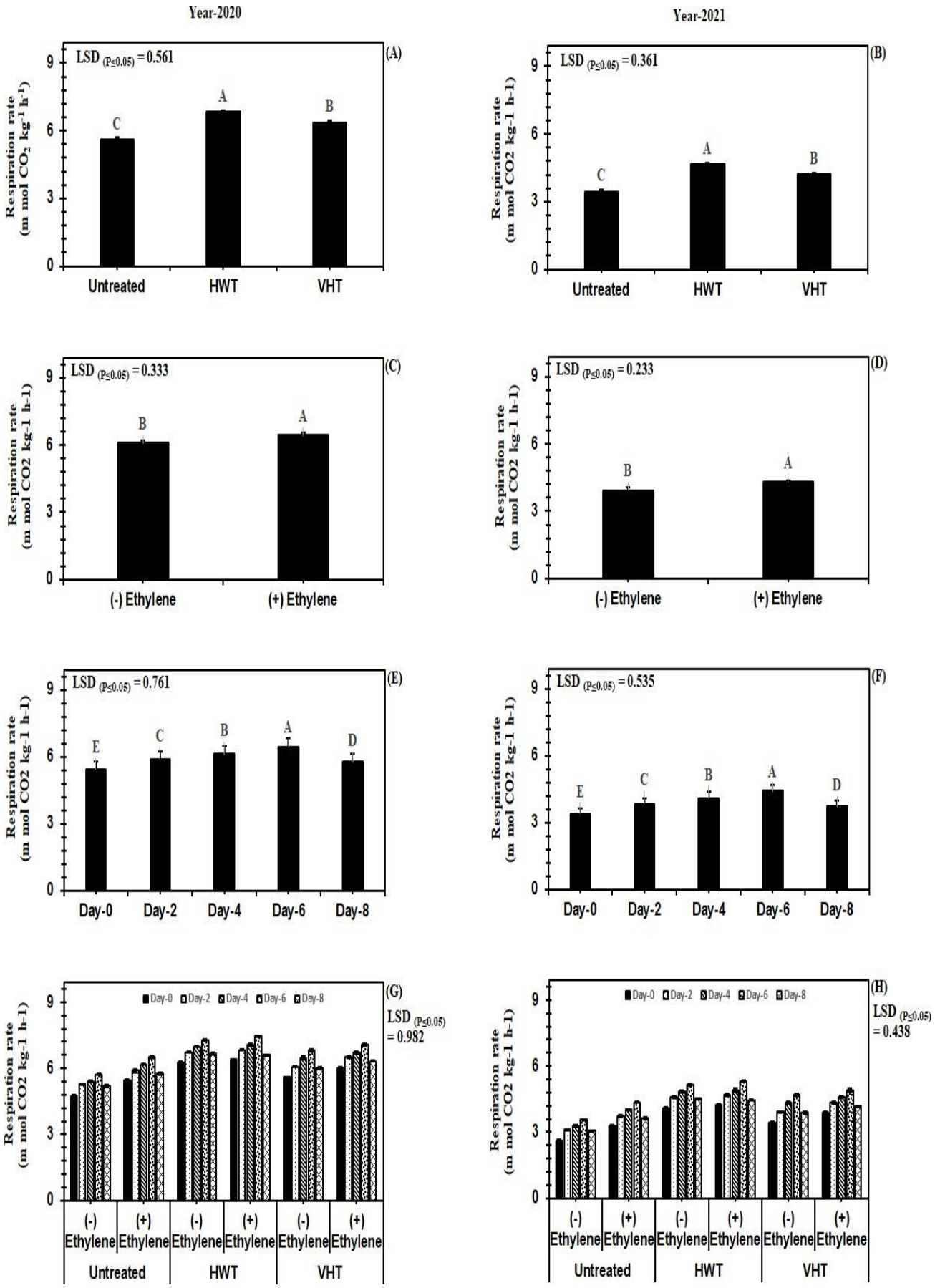

Fig. 2. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on respiration rate of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

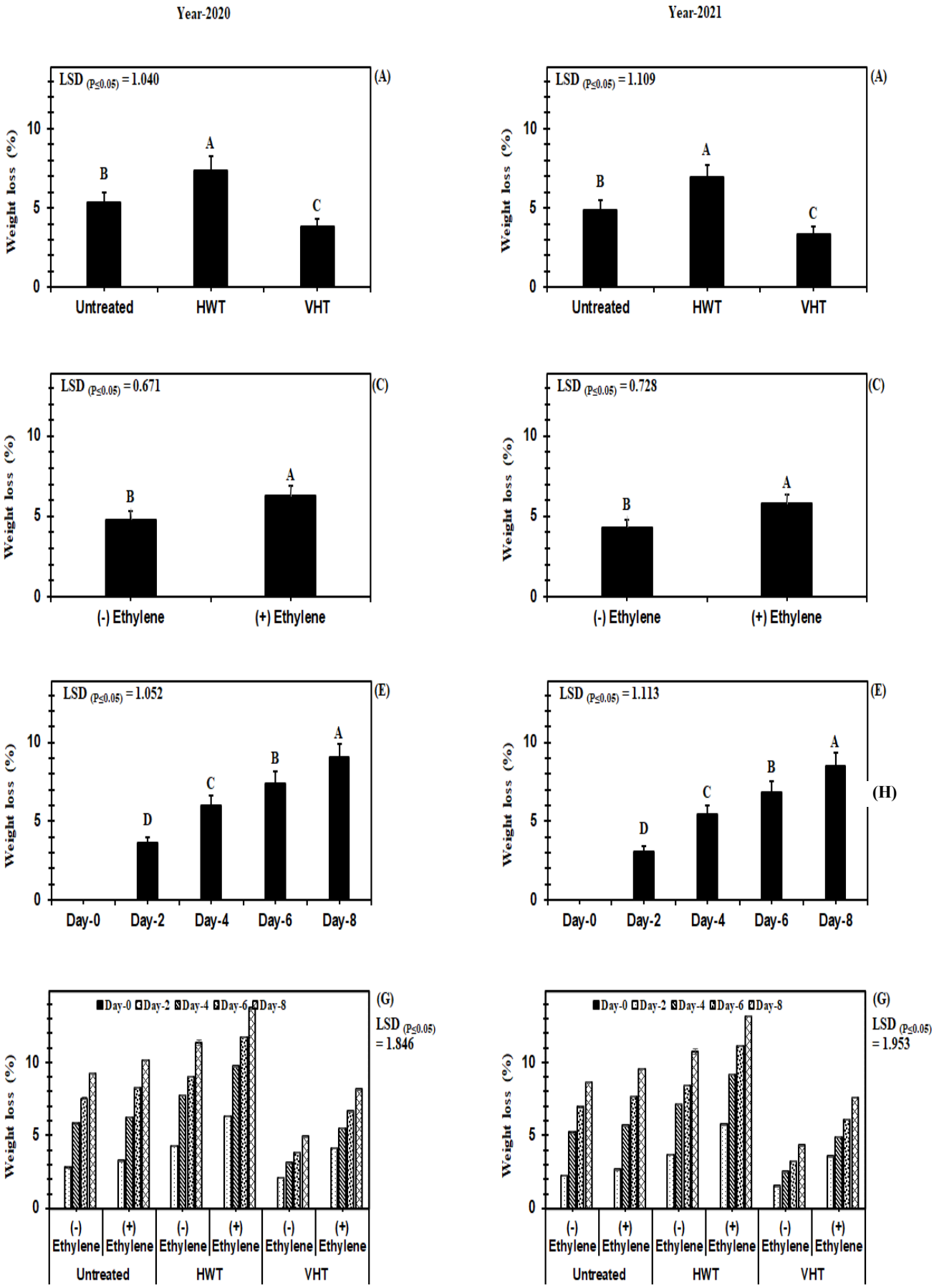

Fig. 3. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on weight loss of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

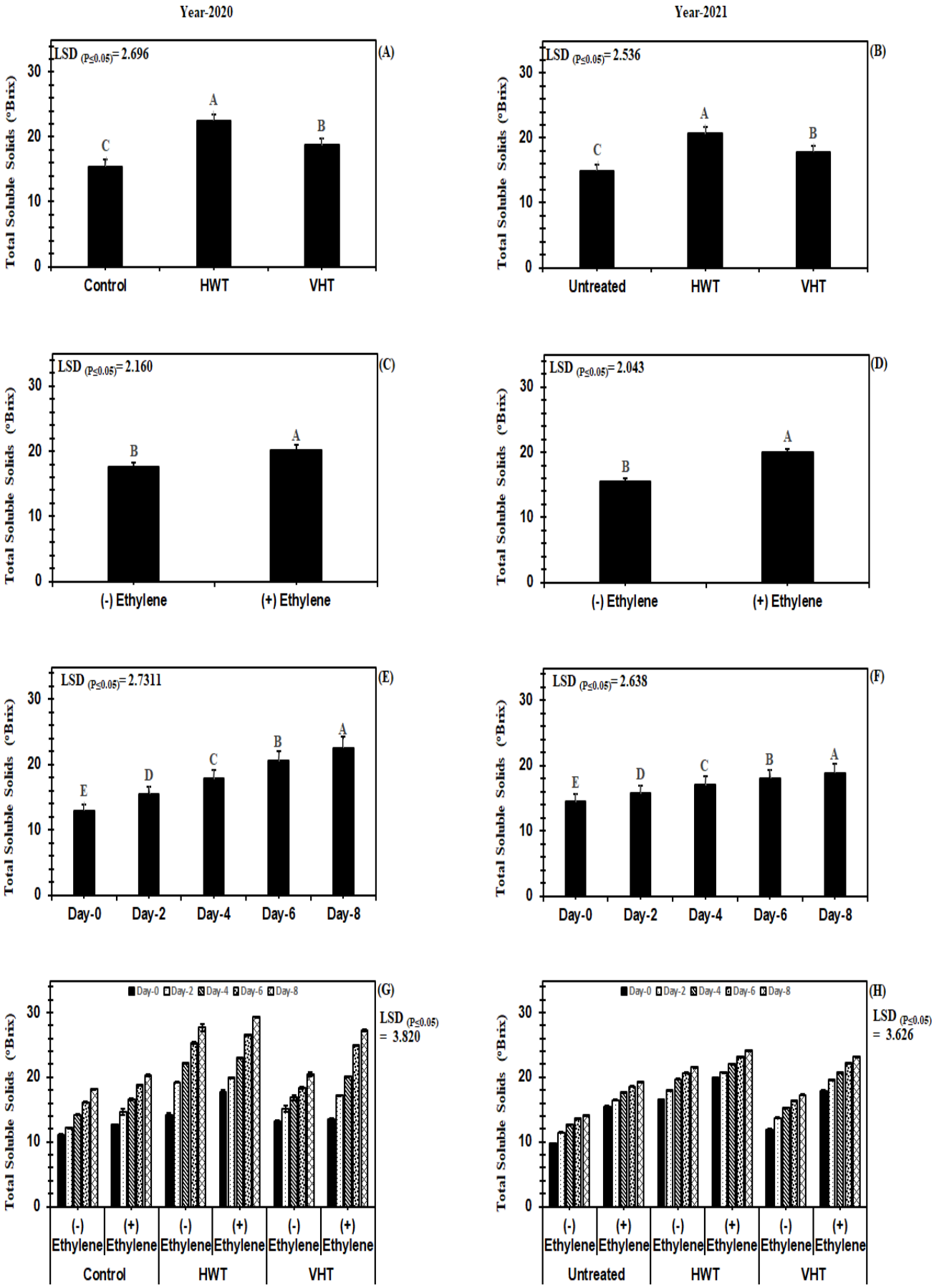

Fig. 4. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on total soluble solids of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

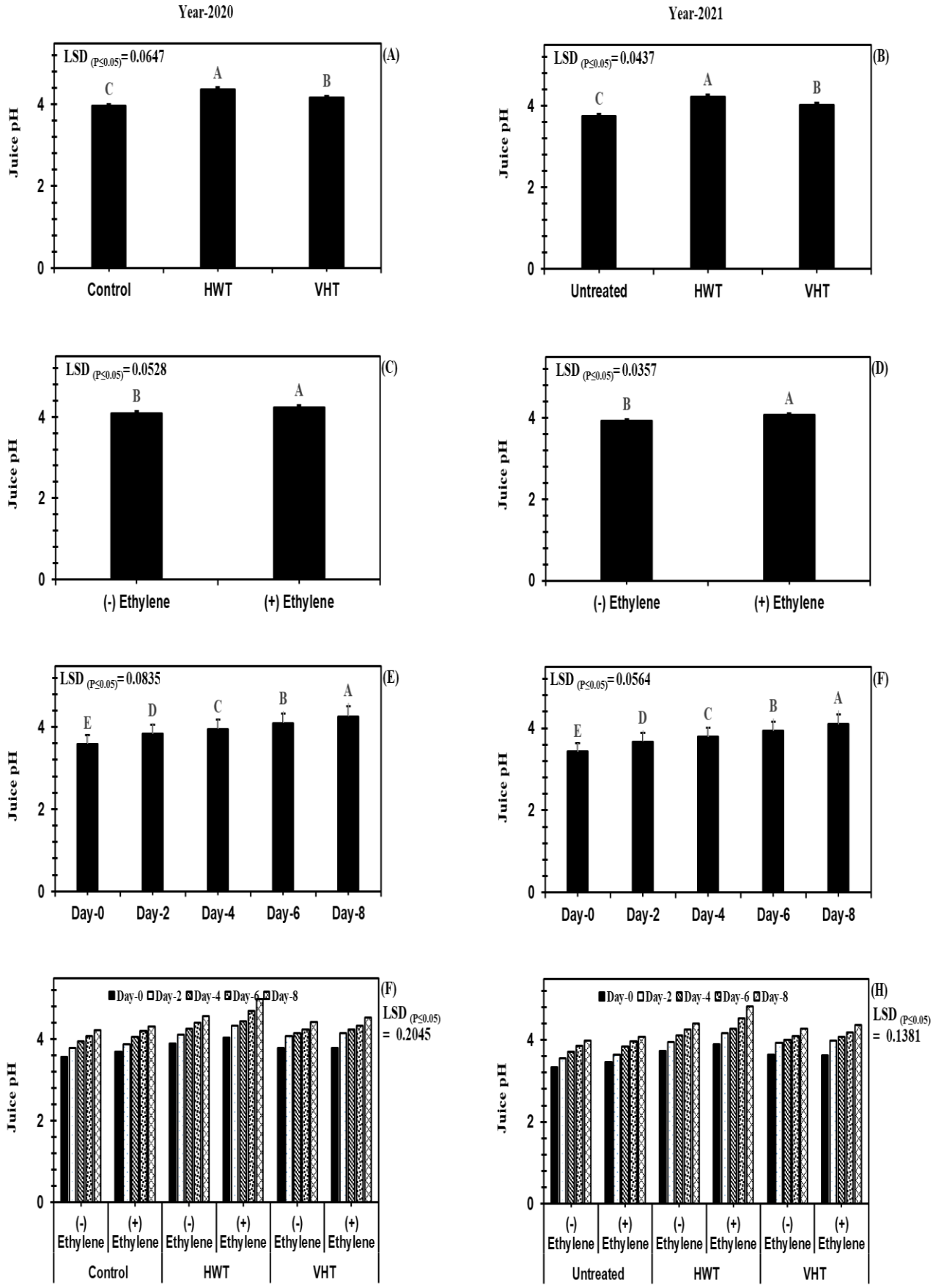

Fig. 5. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on juice pH of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

Irrespective of ripening treatment and days at shelf, untreated mango fruit exhibited significantly higher titratable acidity than HWT and VHT-treated mango fruit in both the growing season 2020 & 2021 (Fig. 6 A-B). As for the ripening treatments, fruit without ethylene treatment showed significantly higher titratable acidity than ethylene treated fruit during both growing seasons (Fig. 6 C-D). As the shelf period progressed the mango fruit showed decreasing trend for titratable acidity in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. 6 E-F). On day-8 of fruit ripening, untreated mango fruit without ethylene ripening exhibited the highest titratable acidity value during 2020 & 2021 season (Fig. 6 G-H).

Irrespective to ripening treatment and days at shelf, mango fruit treated with VHT treatment exhibited significantly higher ascorbic acid as compared to HWT-treated and untreated mango fruit in both the growing season 2020 & 2021 (Fig. 7 A-B). As for the ripening treatments, fruit with ethylene ripening treatment showed significantly higher ascorbic acid than fruit without-ethylene during both the growing season (Fig. 7 C-D). As the shelf period progressed the mango fruit showed a decreasing trend for ascorbic acid in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. 7 E-F). On day-8 of fruit ripening, VHT- treated mango fruit without-ethylene ripening exhibited the highest ascorbic acid value during 2020 & 2021 season (Fig. 7 G-H).

Phytochemical parameters

Total Antioxidant contents: During both season 2020 & 2021, irrespective to ripening treatment and days at shelf, VHT- treated mango fruit exhibited significant higher total antioxidants as compared to other treatment (Fig. 8 A-B). When compared ripening treatments only, the fruit treated with-ethylene had significant higher total antioxidants as compared to without- ethylene treated fruit (Fig. 8 C-D). In both growing season 2020 & 2021, a gradual increase in total antioxidants was found till 8th day (Fig. 8 E-F). On day-8 of fruit ripening, the VHT- treated fruit ripened with-ethylene exhibited the higher total antioxidants in both seasons (Fig. 8 G-H).

Significantly higher total phenolic contents were shown by the fruit treated with VHT as compared to HWT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective to ripening treatment and days at shelf (Fig. 9 A-B). If the only ripening treatments were compared, mango fruit with-ethylene exhibited significant higher total phenolic contents as compared to without-ethylene treated fruit during both the growing seasons (Fig. 9 C-D). Mango fruit showed an increasing trend for total phenolic contents till 8th day in all postharvest phytosanitary treatments (Fig. 9 E-F). On day-8 of fruit ripening, VHT-treated mango fruit with-ethylene ripening exhibited the highest total phenolic contents during 2020 and 2021 season (Fig. 9 G-H).

Antioxidative enzymes activity: Significantly higher SOD enzyme activity was shown by the fruit treated with VHT as compared to HWT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective to ripening treatment and days at shelf (Fig. 10 A-B). If the only ripening treatments were compared, mango fruit without-ethylene exhibited significant higher SOD enzyme activity as compared to ethylene treated fruit during both the growing seasons (Fig. 10 C-D). Mango fruit showed an increasing trend for SOD enzyme activity in all postharvest phytosanitary treated mango as the shelf period progressed in both the seasons (Fig. 10 E-F). On day-8 of fruit ripening, mango fruit treated with VHT and without ethylene ripening exhibited the highest SOD enzyme activity during 2020 and 2021 season (Fig. 10G-H).

During both season 2020 & 2021, irrespective of ripening treatment and days at shelf, VHT-treated mango fruit exhibited significantly higher CAT enzyme activity than other treatment (Fig. 11 A-B). When compared only ripening treatments, the fruit treated with-ethylene had significantly higher CAT enzyme activity than without-ethylene treated fruit (Fig. 11 C-D). In both growing season 2020 & 2021, a gradual increase in CAT enzyme activity was found till 8th day with progress of shelf interval (Fig. 11 E-F). On day-8 of fruit ripening, the VHT- treated fruit ripened with- ethylene exhibited the higher CAT enzyme activity in both seasons (Fig. 11 G-H).

Irrespective to ripening treatment and days at shelf, mango fruit treated with HWT exhibited significantly higher POX enzyme activity as compared to VHT-treated and untreated mango fruit in both the growing season 2020 & 2021 (Fig. 12 A-B). As for as the ripening treatments were concerned, ethylene treated fruit showed significant higher POX enzyme activity as compared to untreated fruit during both the growing seasons (Fig. 12 C-D). As the shelf period progressed the mango fruit showed an increasing trend for POX enzyme activity in all postharvest phytosanitary treated mango fruit subjected to ripening in both the seasons (Fig. 12 E-F). On day-8 of fruit ripening, mango fruit treated with HWT with-ethylene ripening exhibited the highest POX enzyme activity during 2020 & 2021 season (Fig. 12 G-H).

Organoleptic parameters

Fruit shrivelling, disease incidence and shelf-life: Significantly higher fruit shrivelling was shown by the fruit treated with HWT compared to VHT-treated and untreated mango fruit in both the growing season 2020 & 2021, irrespective of ripening treatment (Fig. 13 A-B). If the only ripening treatments were compared, mango fruit with-ethylene exhibited significantly higher fruit shriveling than fruit ripened without-ethylene during both the growing seasons (Fig. 13 C-D).

During both season 2020 & 2021, regardless of ripening treatment, untreated mango fruit exhibited higher disease incidence than VHT and HWT treated fruit (Fig. 14 A-B). When compared only ripening treatments, the fruit treated with-ethylene had significantly higher disease incidence than without-ethylene treated fruit in both growing seasons (Fig. 14 C-D).

The VHT- treated mango fruit had significantly higher shelf-life than HWT and untreated fruit in both season 2020 & 2021, irrespective of ripening treatment (Fig. 15 A-B). If the only ripening treatments were compared, mango fruit without- ethylene exhibited more shelf-life as compared to fruit without-ethylene during both the growing seasons (Fig. 15 C-D). Maximum shelf-life was observed in fruit that were exposed to VHT and without ethylene treated fruit (not received any ripening treatments) during 2020 & 2021 season (Fig. 15 E-F).

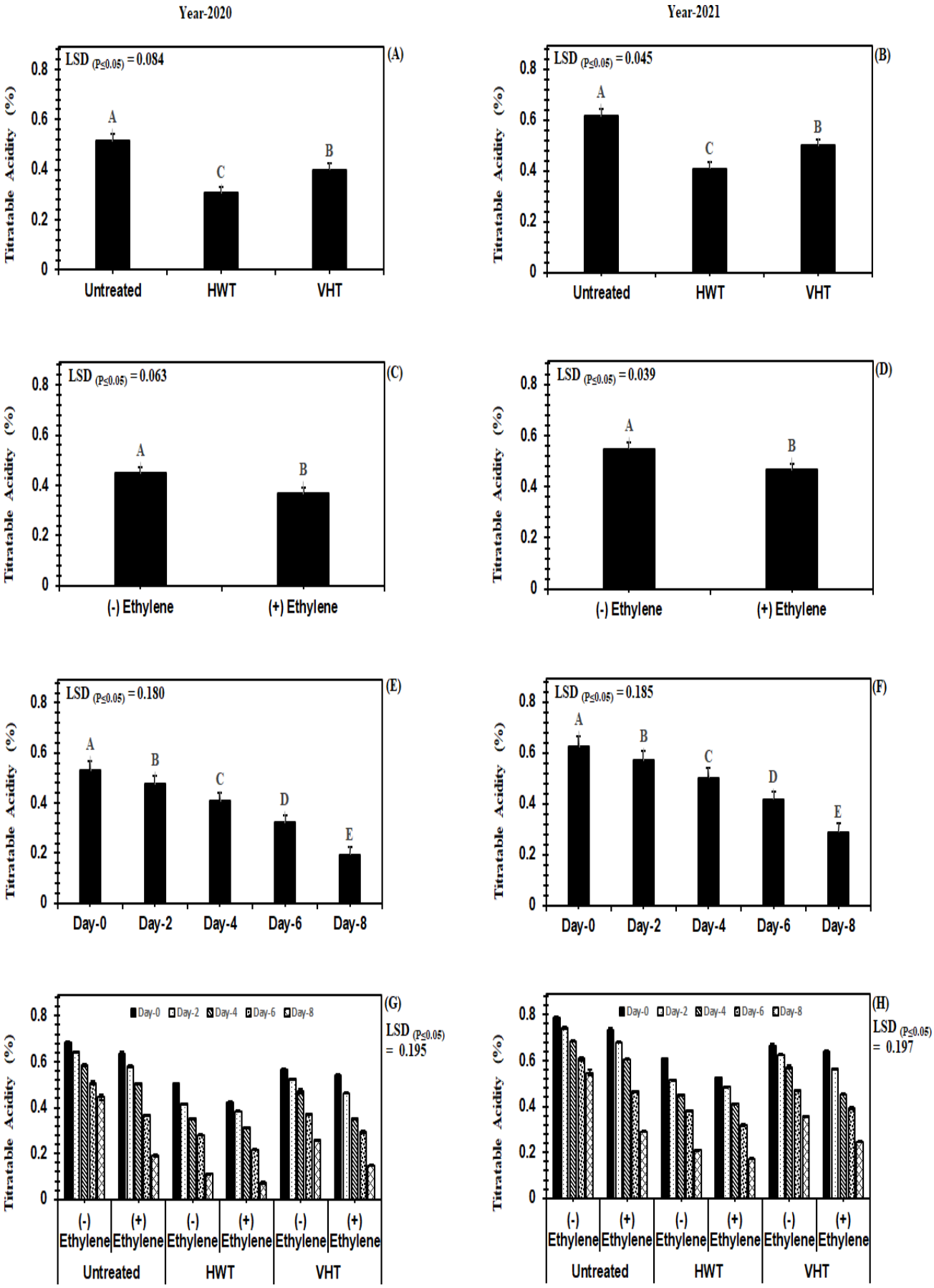

Fig. 6. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on titratable acidity of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

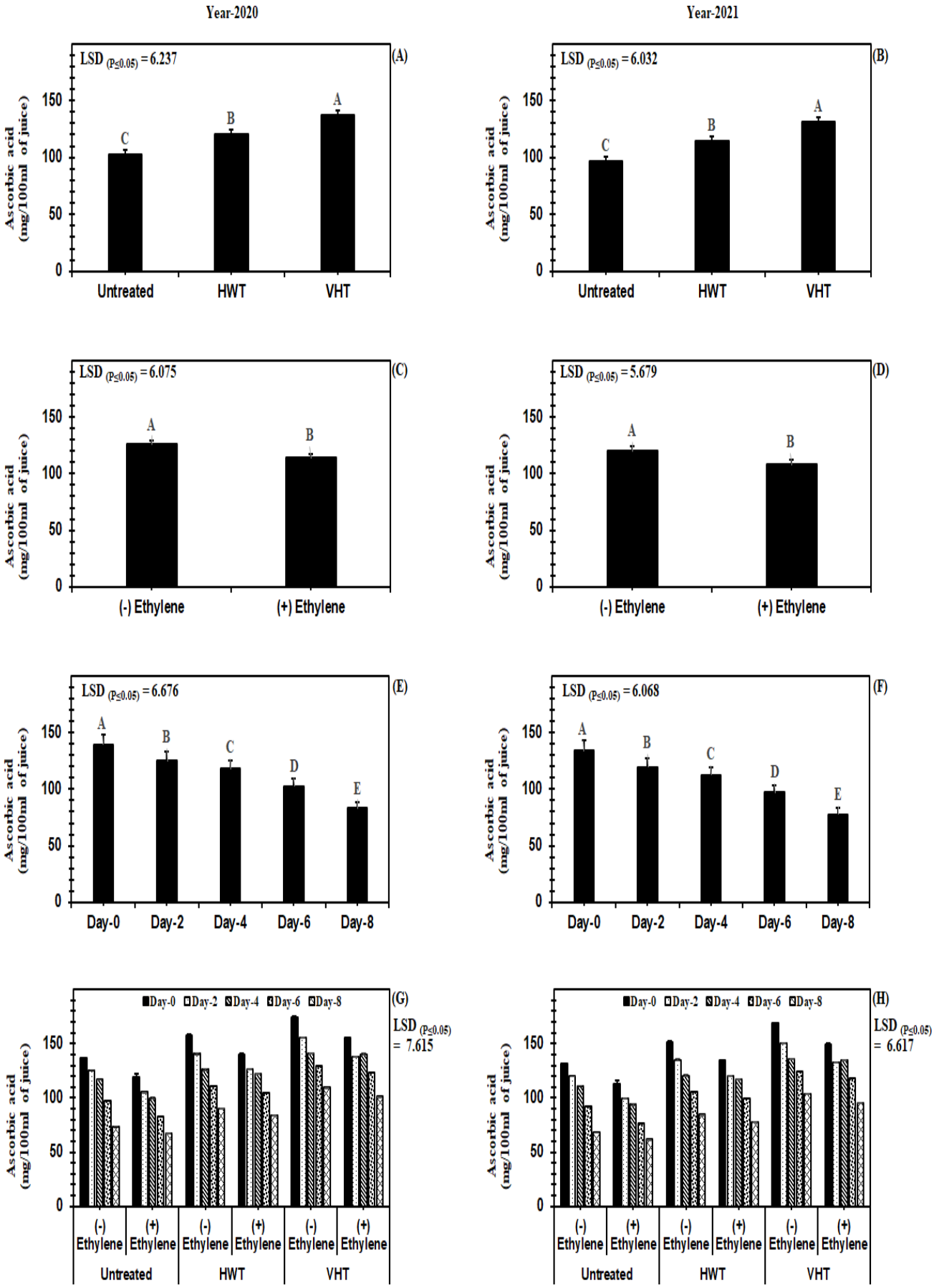

Fig. 7. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on ascorbic acid of 'Azeem Chanusa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

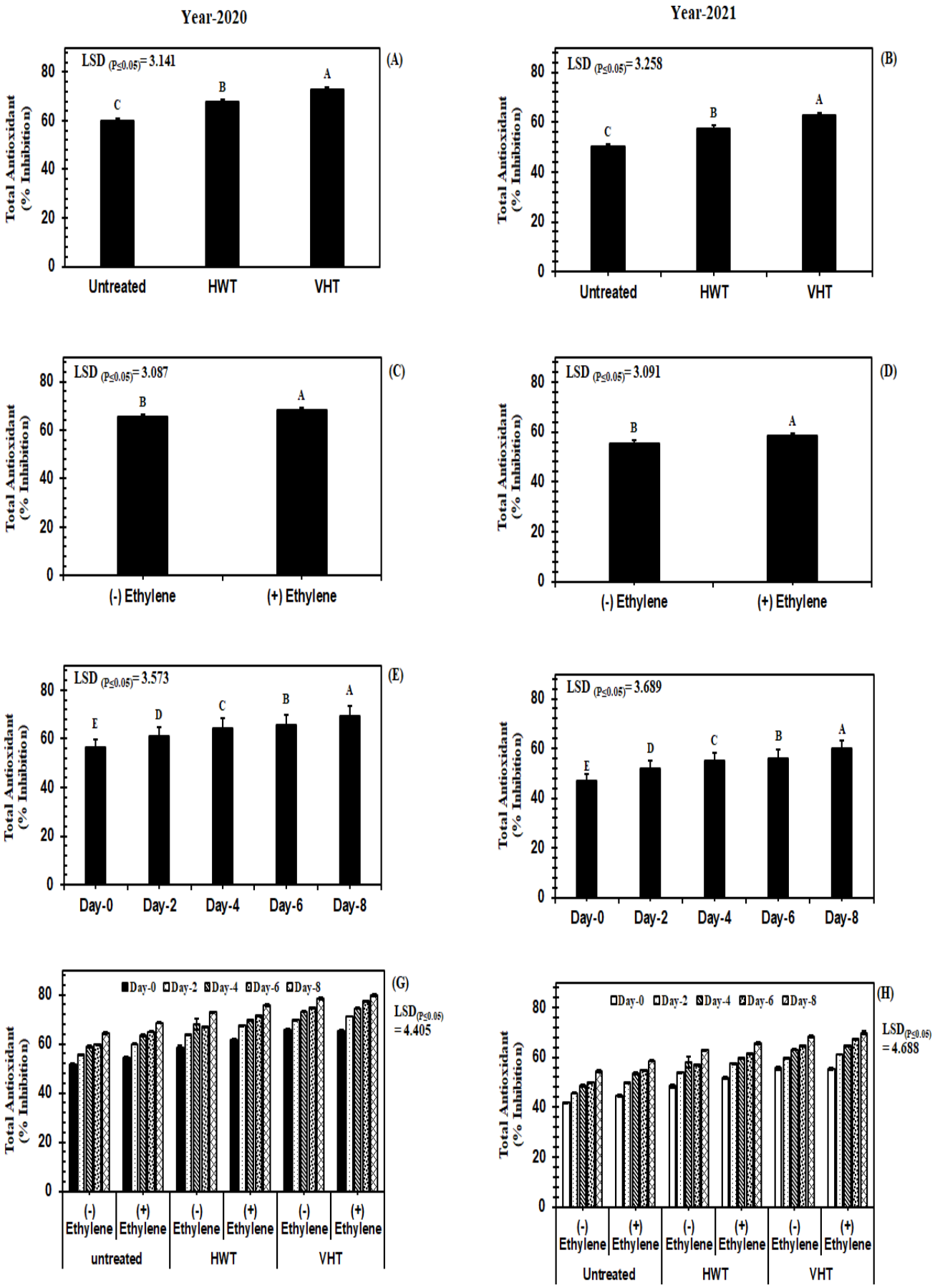

Fig. 8. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on total antioxidants of 'Azeem Chaunsa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

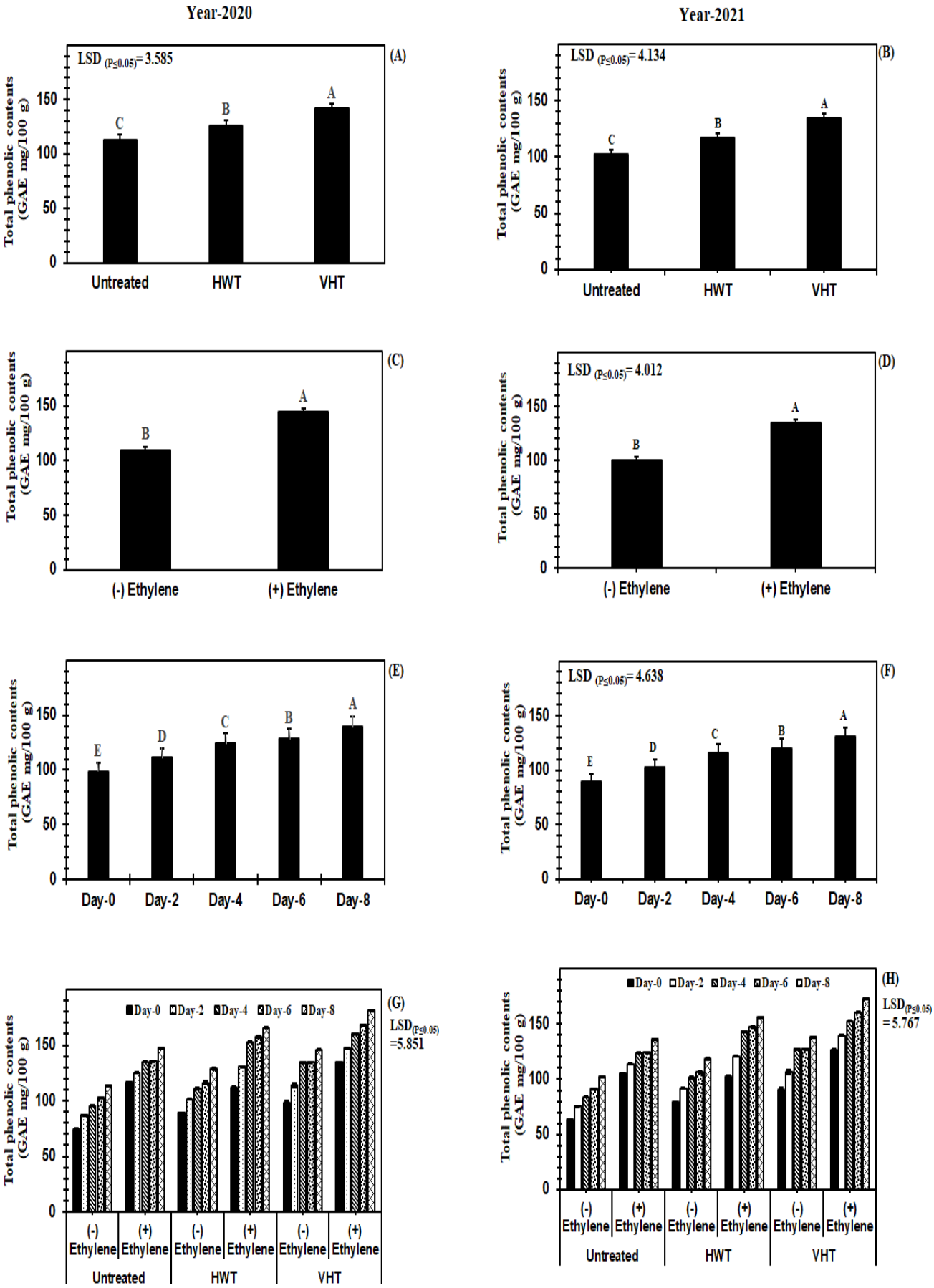

Fig. 9. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on total phenolic contents of 'Azeem Chaunsa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

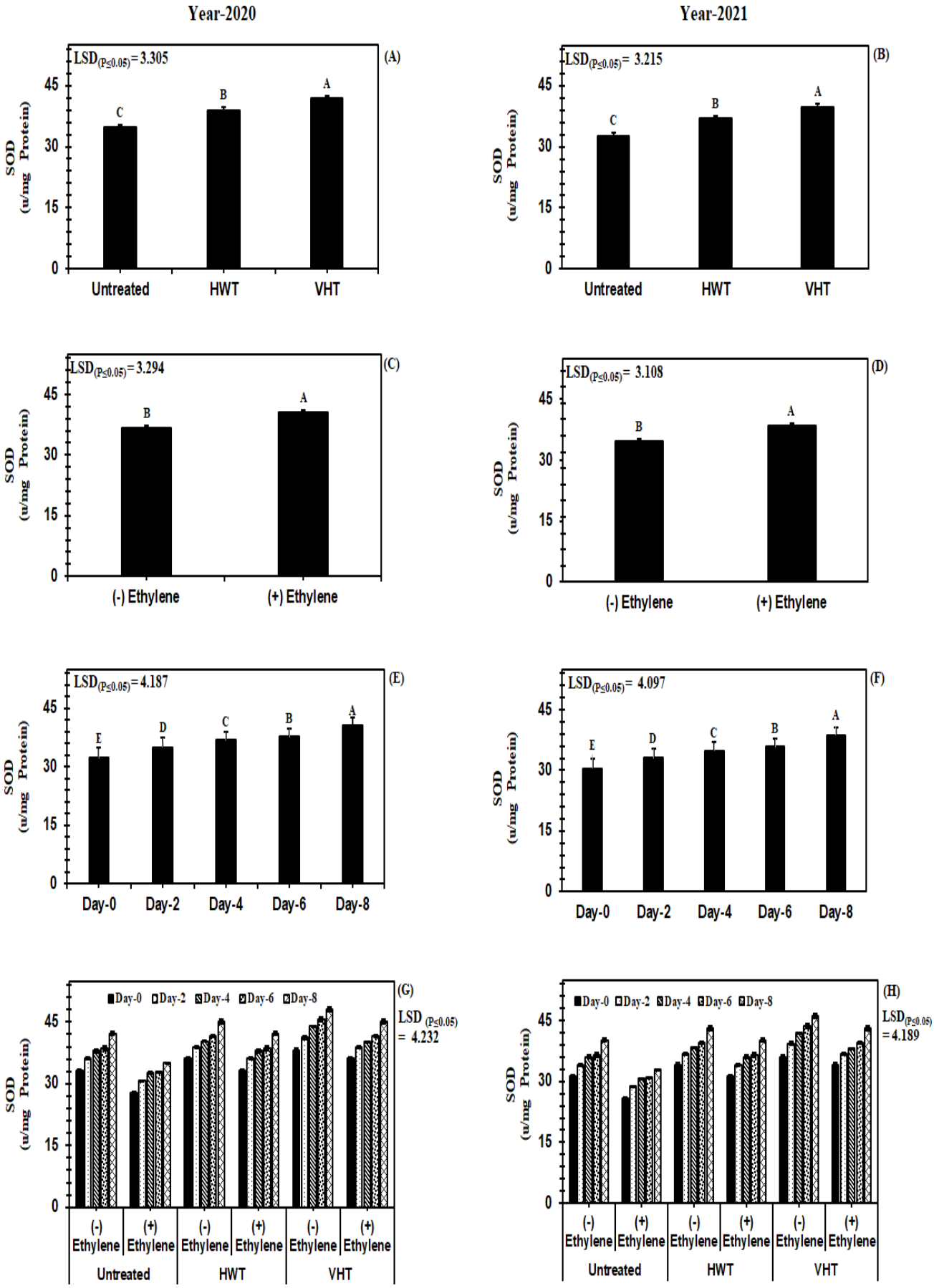

Fig. 10. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on superoxide dismutase of 'Azeem Chaunsa' mango during 2020 (A,C,E,G) and 2021 (B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

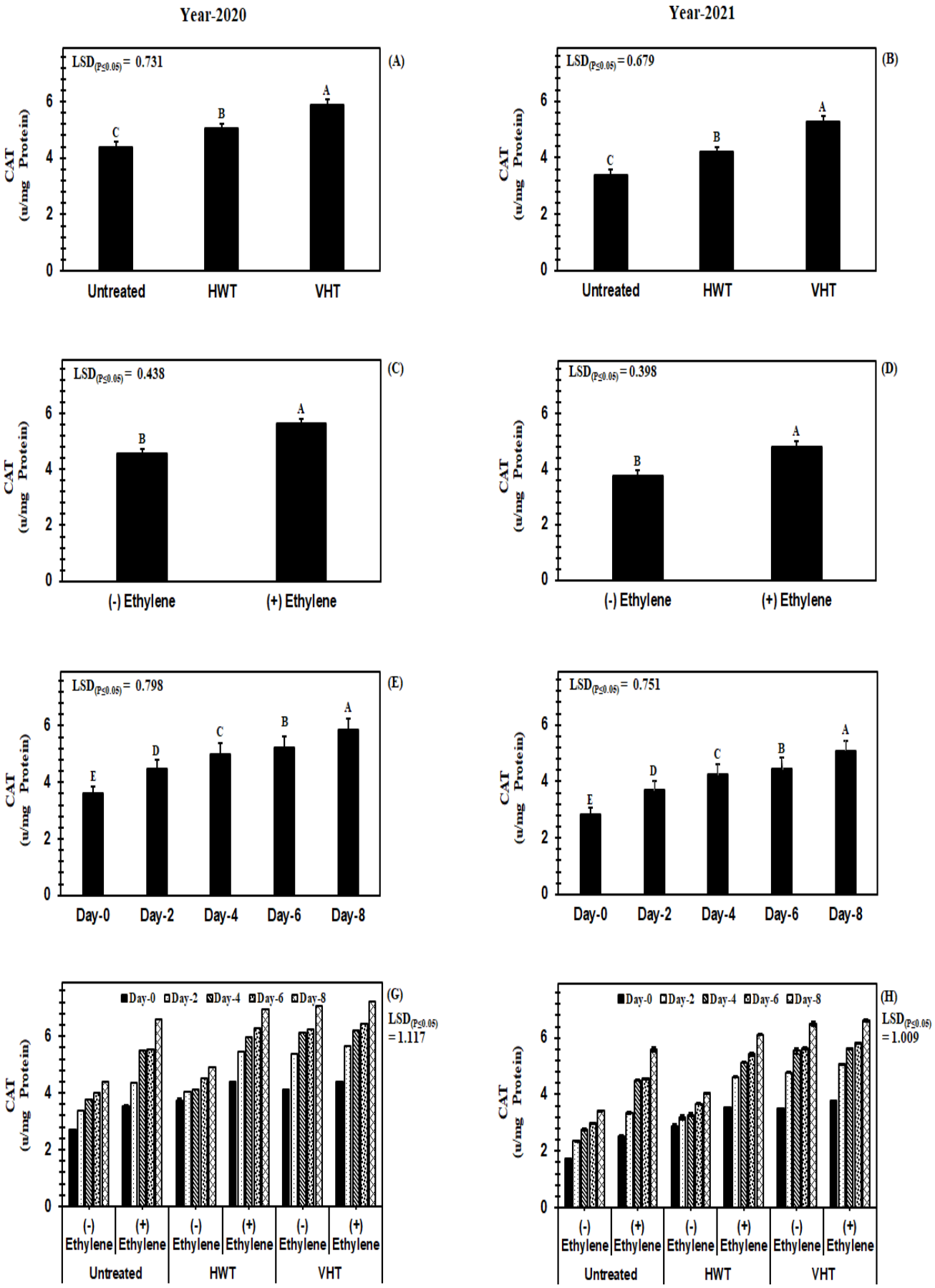

Fig. 11. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on catalase of 'Azeem Chaunsa' mango during 2020 (A, C,E,G) and 2021 B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

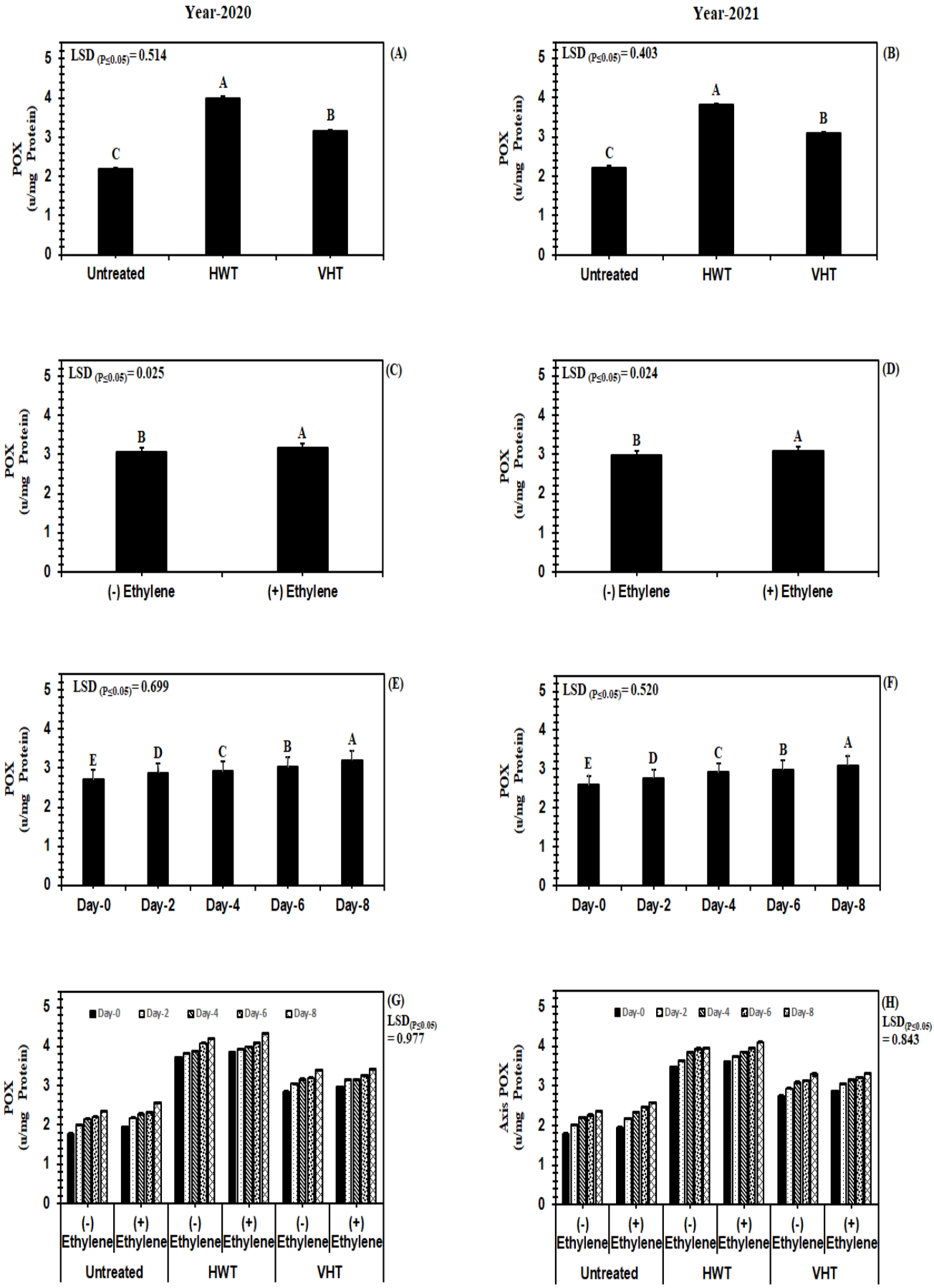

Fig. 12. Effect of postharvest phytosanitary heat treatments, ripening treatments and days at shelf on Peroxidase of 'Azeem Chaunsa' mango during 2020 (A,C,E,G) and 2021. B,D,F,H). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

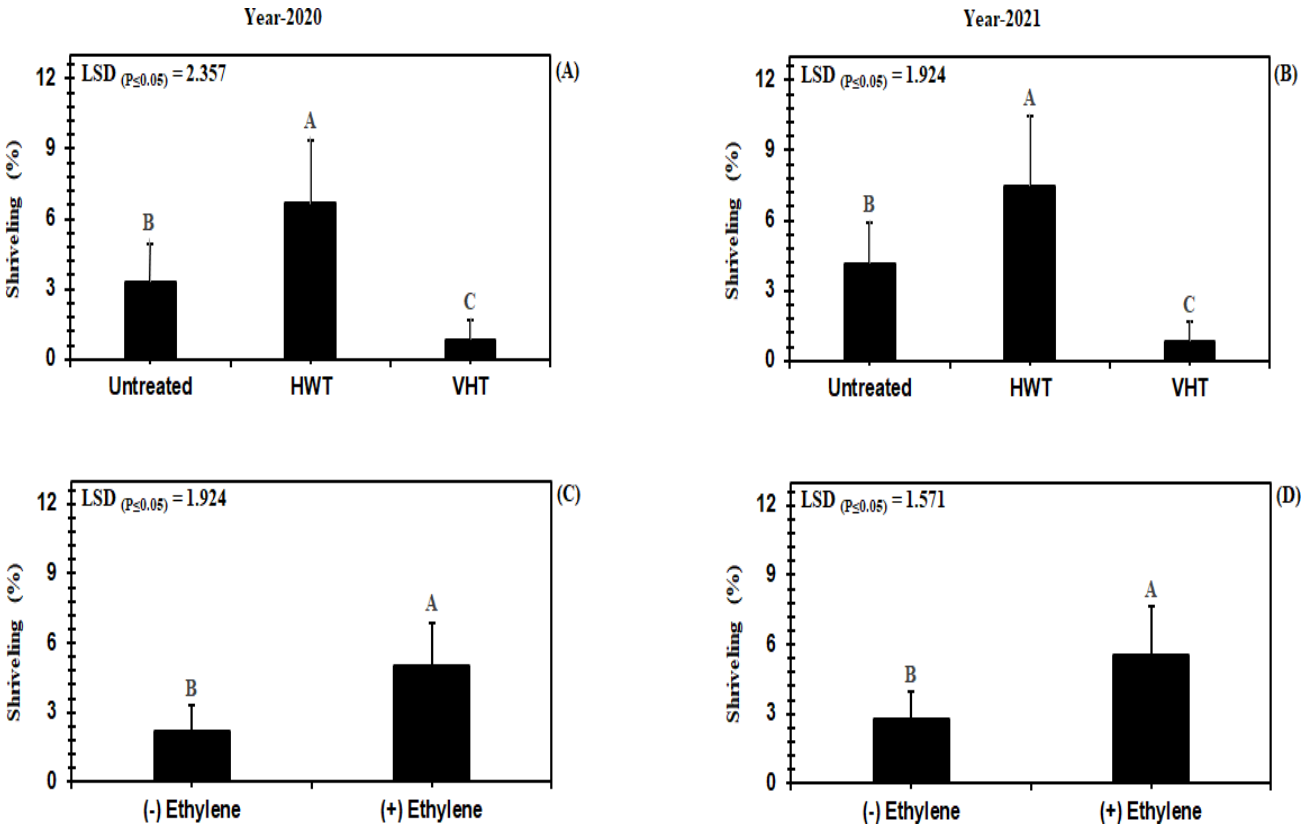

Fig. 13. Effect of postharvest phytosanitary heat treatments and ripening treatments on fruit shrivelling of mango cv. Azeem Chanusa during 2020 (A,C) and 2021 (B,D,). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

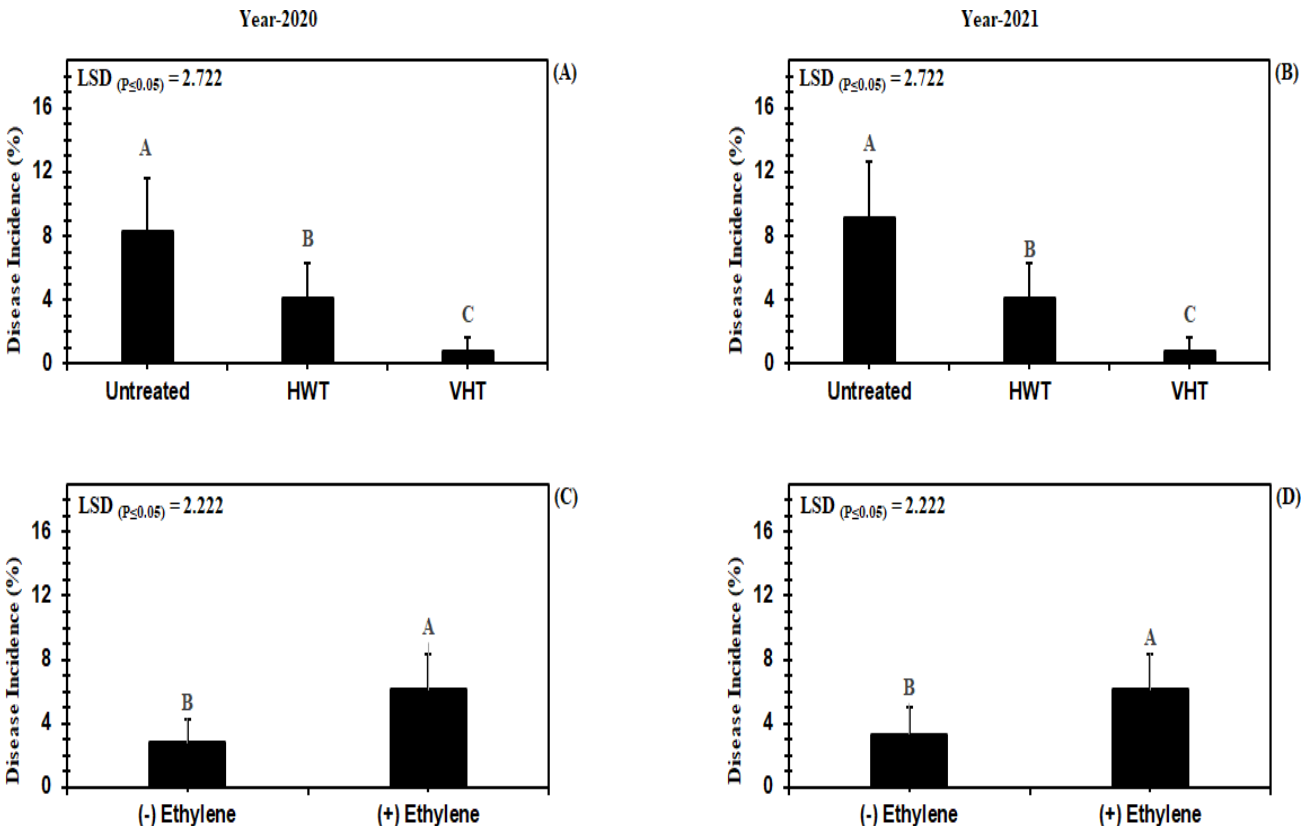

Fig. 14. Effect of postharvest phytosanitary heat treatments and ripening treatments on fruit disease incidence of mango cv. Azeem Chanusa during 2020 (A,C) and 2021 (B,D,). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

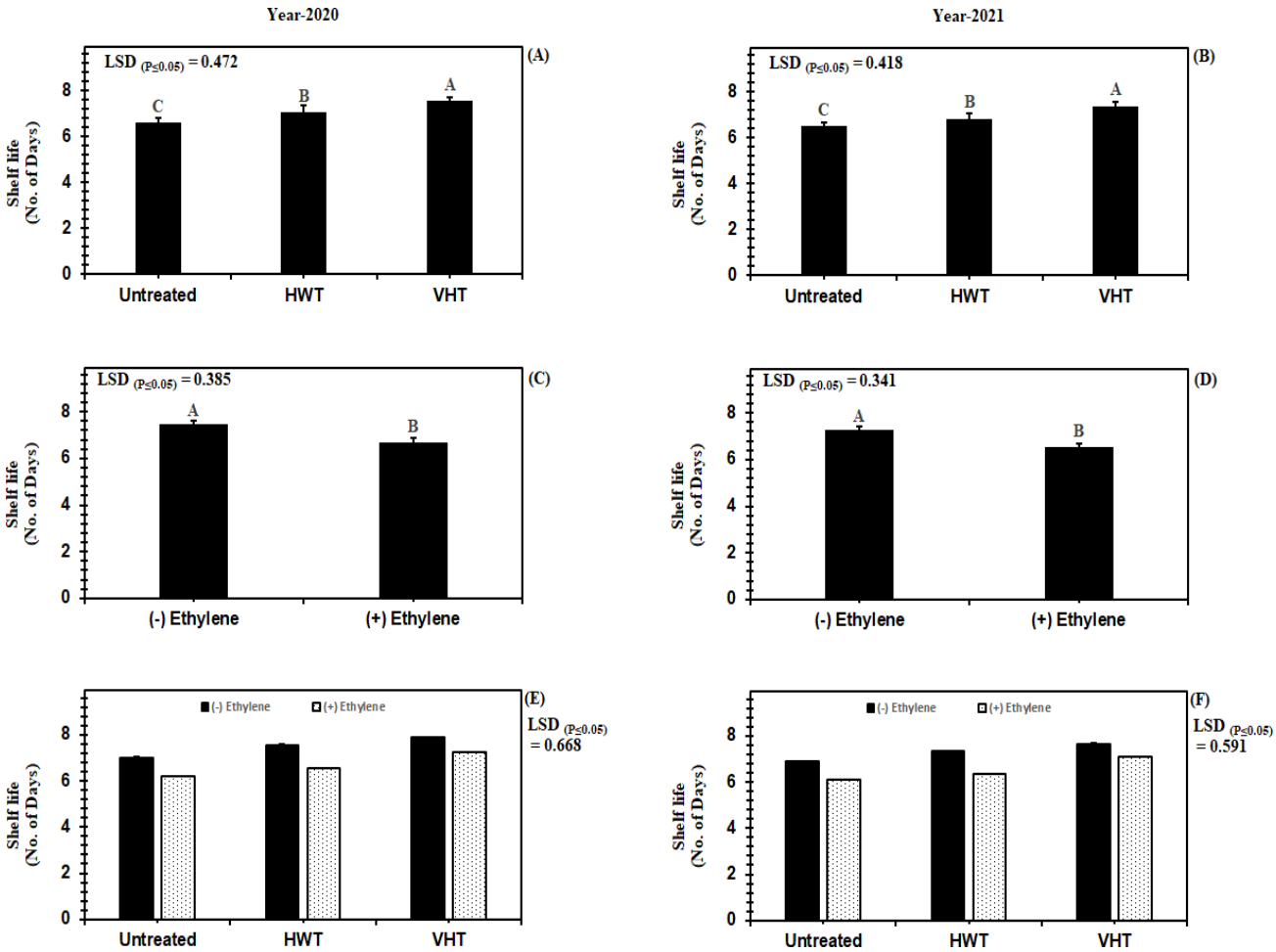

Fig. 15. Effect of postharvest phytosanitary heat treatments and ripening treatments on shelf-life of 'Azeem Chaunsa' mango during 2020 (A,C,E) and 2021 (B,D,F). The vertical bars denote means standard error (n=3). HWT= hot water treatment, VHT= vapor heat treatment. The mean bar showing different letters are significant at $p \leq 0.05$.

Discussion

With advancement of fruit ripening period, an increased ethylene production was observed, irrespective of shelf intervals and phytosanitary heat treatments (PHT). Due to the climacteric nature of mango fruit, an increasing trend in respiration rate was also discovered. Lower levels of respiration rate were found during the early days of fruit ripening (pre-climacteric), followed by a rapid increase in respiration rate (climacteric peak), and then a sharp decline was seen at the fully ripe stage. Throughout the entire trial, a growing weight loss was seen as the shelf-life increased. These weight losses in the fruit are mostly related to the physiological weight loss that occurs during ripening as a result of the fruit's increased respiration rate, fast water loss through transpiration and other internal biological changes (Narayana *et al*., 1996). The 'Ataulfo' mango fruit had the highest rate of ethylene production prior to reaching the respiratory climacteric peak and afterwords dropped as the fruit ripening period advanced, according to Montalvo *et al*., (2007). The genetically intricate process of mango fruit ripening is brought on by an increase in ethylene production and respiration rate (Venkatesan & Tamilmani, 2010). PHT treatments have been shown to dramatically reduce postharvest disease incidence in addition to its phytosanitary function. However, depending on the type of treatment, the temperature, and the length of the PHT treatment, different fruit crops showed varying responses. Our study results showed VHT and HWT-treated mango fruit demonstrated increased ethylene gas evolution, higher respiration rate, and weight loss. These results are in agreement with findings in case of Blood Red fruit, where time of VHT extended increased weight loss during storage (Hussain & Rab, 2015).

With increasing shelf days and regardless of PHT, fruit firmness reduced over the course of the entire shelf-life in addition to the ripening treatment. Also, a correlation between longer shelf-life and fruit shrivelling and disease incidence was discovered. However, PHT greatly decreased mango fruit disease and fruit shrivelling. Fruit generally loses firmness and shrivels more when weight loss decreases, which may be caused by moisture loss in the fruit. Moreover, ageing may be a factor in the rise in disease incidence (Shah *et al*., 2021). As previously reported by Hasan *et al*., (2020) in mango fruit, PHT has been shown to reduce these losses in fruit quality.

This study also depicted a pattern of decreasing TA, ascorbic acid and increasing TSS with the advancement of shelf intervals in mango fruit in all PHT treated mango fruit. Similar to our findings, TSS increased with passage of time in 'Smar Bahisht Chaunsa' (Razzaq *et al*., 2013), 'Kensington Pride' (O'Hare, 1995), 'Keitt' (Padda *et al*.,

2011), and 'Viringe' (Othman and Mbogo, 2009). It was discovered that the acidity decreased over the course of the time. As fruit nears senescence, biological components may degrade, contributing to the decrease in acidity. Dates have also been observed to have less titratable acidity (Mostafa & El Akkad, 2011; Soliman *et al*., 2011). Ascorbic acid levels in mango fruit decreased over time, and postharvest temperature control is crucial for preserving ascorbic acid contents (Lee & Kader, 2000). The majority of the time, postharvest heat treatments did not influence ascorbic acid levels despite decreases during storage, according to the literature (Jabbar *et al*., 2011).

The fruit's total phenolic content and presence of all antioxidant components account for the total antioxidant activity. In the current study, the antioxidant levels rose over time. Our findings support those of Palafox-Carlos *et al*., (2012), who found that the total antioxidant content of the Ataulfo mango increased as ripening progressed but decreased at a completely ripe stage. Our investigation found that mango fruit exposed to PHT had higher TPC levels. Many factors, such as the degree of ripeness of the produce after harvest, the kind of cultivar, and the length of storage, can cause an increase or decrease in the TP of fruit and vegetables during postharvest storage (Kalt, 2005). The number of phenolic compounds in cooked vegetables, such as green pepper and onion, was observed to rise with heat treatment. Previously, it was discovered that hot water treatment of 'Tommy Atkins' mango fruit had no discernible effect on their soluble phenolic levels (Kim *et al*., 2009). Carotenoid is the primary pigment in mature mango fruit and is what gives the peel its characteristic yellow or orange hue (Rathore *et al*., 2007). As the shelf period progressed carotenoids increased in mango fruit with fruit ripening. As a climacteric fruit, an increase in respiration caused by ethylene caused chlorophyll degradation and an increase in carotenoids biosynthesis, resulting in a significant change in mango fruit colour (Saltveit, 1999). The PHT catalyze this chlorophyll conversions into carotenoids, resulting in better fruit colour development at fruit ripening. Similar findings have also reported in mango, hot water treated fruit exhibited better fruit colour (Hasan *et al*., 2020). As for as the result of activity of enzymes is concerned. The findings of our study revealed that PHT mango fruit exhibited a gradual increase in CAT and SOD enzyme activity. Similar to our study, a continual increase found in CAT and SOD enzyme activity throughout ripening period (Huang *et al*., 2007). In contrary to our results, with advancements of fruit storage, many fruit had been reported to exhibit decreasing activities of SOD in mango and orange (Huang *et al*., 2007; Singh & Saini, 2014). Such type of variation in the behavior of antioxidant enzymes can be attributed to the differences in the activities of isoenzymes. POX enzyme activity increased during the study period, which could be attributable to slower AOS generation due to slower metabolism, which results in a weaker reaction of POX. It was previously observed that the activity of antioxidative enzymes such as SOD, CAT, and POX was higher in 'Amber Jewel' Japanese plums were refrigerated at 5°C rather than 0°C (Singh & Singh, 2013). Heat treatments have been previously observed to limit the burst of ROX and boost the antioxidant capacity of fruit, as previously described for peach cv. Xiahui-5 fruit, which protects against oxidative damage.

Conclusion

In this study, effect of hat treatments on physical, biochemical, phytochemical and organoleptic parameters on cultivar Azeem chaunsa was determined. Overall, it is concluded from this study that at ambient conditions the treated fruits of 'Azeem Chaunsa' retained quality upto day-8, on shelf during both years. The vapor heat treated mango fruit showed significantly reduced disease incidence and losses for the physiological traits, while significantly maintaining higher biochemical attributes in mangoes as compared to hot water and untreated fruits. However, significantly higher TSS, pH, carotenoids, and POX were observed in HWT-treated fruit. The treated mango fruit irrespective of PQHT treatments, ethylene treatments accelerated the fruit ripening as compared to non-ethylene treated fruit. It can be concluded that VHT can be successfully applied for the export under ambient conditions to meet the requirements of international markets for export.

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Author's Contribution

Conceptualization, AUR and IR.; methodology, AUR.; software, AUR; formal analysis, AUR; investigation, AUR; resources, AUR.; data curation, AUR.; writing-original draft preparation, AUR; writing-review and editing, IAR; visualization, AUR; supervision, IAR; M.A, SU; project administration, IAR.; funding acquisition, AUR.

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