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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2020 17(4): 4021-4033.

OPEN ACCESS

Quality traits and storability life of peach (*Prunus persica* L.) Fruit as effected by hot water dipping treatment

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Due perishable nature of peach, it does not maintain its quality after harvest for an extended period. In order to increase or enhance the shelf life of peach to meet consumer's demand, a number of techniques such as treatment of fruit with hot water play key role in influencing fruit ripening processes. To study hot water treatment effect fruit quality and storability of peach fruit cv. 'Early Grand', an experiment was conducted at Post-harvest laboratory, Department of Horticulture, The University of Agriculture Peshawar using experimental design Completely Randomized Design (CRD) with two factors having and three replications. Healthy and disease free peach fruits were dipped in hot water at various temperatures (30, 40 and 50 °C) for five minutes and stored for various storage durations (0, 10, 20 and 30 days). The results revealed that hot water treatment (10 to 50 °C) significantly increased total soluble solid, TSS/TA ratio, total sugar, and decreased titratable acidity and ascorbic acid content of peach fruit. While increasing hot water treatment up to 40°C, significantly increased fruit firmness and decreased weight loss and percent disease incidence of fruit. Similarly, total soluble solid, total sugar (%), TSS/TA ratio, weight loss increased, while fruit firmness, titratable acidity and ascorbic acid decreased in freshly harvested peach fruit to fruit stored for 30 days. It could be concluded that the hot water treatments at 40 °C and storage period for 10 days induce best results on postharvest life of peach.

Keywords: Biotic and Abiotic stress, Perishability, Hot water dipping treatment, Quality, Ripening

INTRODUCTION

Peach (*Prunus persica* L) belongs to the family Rosaceae, is a widely grown fruit in temperate regions throughout the world. Around 2000 B.C, peach was originated in China as in a wild form. At the time of Holy Christ, Romans were cultivating peach and later on it was disseminated in all over the world after The Romans spread it in their entire empire of Europe

(Ferguson et al.1987). In Pakistan, it is grown in Khyber Pakhtunkhwa Province and other areas of Pakistan like South Waziristan, Gilgit, Chitral and Hunza valley. According to Pakistan Agricultural Statistics in 2010-2011, In Pakistan, the peach cultivation area is 13.819 thousand hectares, and the total annual roduction is 70.75 thousand tons. The most commonly used peach cultivars in Pakistan are Early Grand, Florida King, Shireen,

Shah Pasand, Golden Early, 6th A, 8th A, etc. In KP, peaches occupy 19% of the total fruit land. In Malakand Division, the average production of peach is 12.53 tons/ha (Sajid et al. 2020). Peach cover an area of 100 hectares in Punjab, 5600 in Khyber Pakhtunkhwa and 9500 hectares in Baluchistan with production of 500, 57800 and 25400 tons respectively. Due to various biotic and abiotic stresses like disease attack, insects and most importantly lack of proper preservation, the yield of peach in Khyber Pakhtunkhwa province is very low (Khattak, 2002).

Peach fruits are highly perishable leading to many pre- and post-harvest problems, which adds to the reduction in the potential yield and productivity (Sajid et al. 2020). Due to its perishable nature, it does not maintain its quality after harvest for an extended period. In order to increase or enhance the shelf life of peach to meet consumer's demand, a number of techniques such as fumigation and pre-harvest spraying of nutrients are used to overcome the postharvest losses of fruit commodities (Neo and Saikia, 2010). During marketing or shipping, peach fruits suffer from high susceptibility to flesh softening that makes it more sensitive for pathogen attack and deterioration leading to a shorter handling period and limited marketability. Therefore, post-harvest practices for maintaining fruit characters of improved marketing capability and extended shelf life are seriously considered. It would be achieved by reducing the quality losses due to the physiological and biochemical changes that fruits undergo after harvesting. Physiological weight loss of about 20–30% (Ullah et al. 2018) is determined by both water loss, due to transpiration of the living fruit tissues, and by dry matter loss due to respiration. Also, a wide range of post-harvest fruit losses is caused by several post-harvest diseases. In this regard, efforts are being made to find effective and safe techniques to control fruit post-harvest diseases, reduce quality losses, and increase the production and quality of fruits, as an alternative to the use of synthetic fungicides (Mohamed and Akladios, 2017; Mohamed et al. 2018).

The fresh products play an important role in the market competition and its value is more in local and international market. Due to the nature of their perishability, convenience and customer preferences, the conservation of product quality demands constant attention (Loius et al. 2001) Shelf life of a fruit can be increased by giving proper post-harvest treatments. It also reduces packaging house losses. There are a very limited

number of registered products in post-harvest regulations. Heat treatment given before storage is a very relevant strategy which provides fruits with less damage and better quality (Lurie, 1998). A high temperature application to the fruits is important physical treatments given in post-harvest in order to delay fruit ripening, control pest, reduce disease incidence, improve the fruits resistance against chilling injuries, and extend their shelf life (Wang, 1998).

Many other processes in fruit ripening are influenced by heat treatments, i.e. color, cell wall metabolism, respiration, ethylene production, fruit softening and volatile compounds production (Ketsa et al.1999, Lurie and Nussinovich, 1996; McDonald et al., 1999). Cell wall degrading enzymes are also triggered due to protein synthesis and alteration in gene expression (Paull and Chen, 2000). Heat application followed by cold storage can decrease chilling injuries, pathogen incidence and development in many fruits (McDonald et al.1999).The objective of the study was to evaluate the response of peach fruit to hot water treatment as well as on quality and storability.

MATERIALS AND METHODS

Experimental site and procedure

An experiment Hot water treatment effect quality and storability of peach was conducted at Post-harvest Research laboratory, Department of Horticulture, The University of Agriculture Peshawar-Pakistan. Peach fruits were harvested at physiological mature stage from Peach orchard already established at Horticultural Research Farm, The University of Agriculture Peshawar, Pakistan. The research farm is located at 34.01° N latitude, 71.35° E longitude at an altitude of 350m above sea level in Peshawar valley with a sub-tropical climate (Ahmad et al. 2019). Peshawar is located approximately 1600 km north of the Indian Ocean. The research farm is irrigated by the Warsak canal from river Kabul (Alam et al. 2020). Both the summer and winter weathers are extreme (Basit et al. 2019a), characterized by severe winter and hot prolonged summer where the average minimum temperature during winter is 50 °C while during summer, the average maximum temperature reaches up to 45 °C. The wettest month (with the highest rainfall) is March (78 mm) and driest month (with the lowest rainfall) is June (7 mm) approximately. Peach cv. Early Grand of age approximately 10 years were selected to be pruned in the months of November

to December on a regular basis to avoid alternating bearings. Peach trees were planted in a square system with a plant-to-plant and row-to-row distance of 6 m. Cultural practices such as weeding, irrigation, fertilizer application, and pruning have been carried out on a regular basis. Uniform-sized disease-free trees were selected for the experiment (Sajid et al. 2020).

An experimental design Completely Randomized Design (CRD) with two factors factorial arrangement having three repetition were used during experimental study. Freshly unripe, sound and healthy peaches were selected and dipped in hot water for constant time period of five minutes at different temperature (30°C, 40°C and 50°C) and after cooling of selected fruit they were stored for 30 days with an interval of 10 days.

Physio-chemical attributes studied

Data were recorded on following quality attributes

The fruit weight loss (%) was measured by difference of weight of fresh fruit and weight of fruit after storage. The following formula was used to determine the weight loss (%).

$$\text{Weight loss (\%)} = \frac{\text{weight of fresh fruit (g)} - \text{weight after interval (g)}}{\text{Weight of fresh fruit (g)}} \times 100$$

The fruit firmness (kg cm⁻²) of the fruit was determined through fruit firmness tester/analyzer (Wanger, FT-327 Model) with capacity of 28 lb (Basit et al. 2020), equipped with an 8 mm plunger tip, using sample of 3 fruits from each treatment² (Pocharski et al. 2000). Total soluble solid (°Brix) content of fruits was measured with using hand refractometer (Kernco, Instruments Co. Texas) (Basit et al. 2019b). Juice obtained from the selected fruits was mixed carefully and placed a drop of the juice on the prism of the refractometer, and enclosed with a transparent led. Rotation of the sample was noted through the eye piece of the refractometer with a procedure followed by (Sajid et al. 2019).

Titration of the Sample

In 100ml volumetric flask (10ml) grapefruit juice were taken and diluted up to the mark. In a titration flask 10ml of these diluted samples were taken and as an indicator 2-3 drops of phenolphthalein were added and then titrated against 0.1 N NaOH solutions until the light pink color appeared. Consecutive three readings were taken by the use of following formula:

$$\text{Titrateable Acidity(\%)} = \frac{N \times T \times F \times 100}{D \times S} \times 100$$

N= NaOH Normality

T= in (ml) NaOH used.

F= constant acid factor 0.0064 (citric acid)

D= In ml Citrus Sample taken for dilution

S= Diluted sample taken for titration in ml

Dye method was used for determination of ascorbic acid (mg. 100g⁻¹) as described by (Rangana, 1977). With help of pipette 10 ml of juice were taken from the extracted fruit and was added to graduated cylinder. With the help of oxalic acid solution the volume was raised up to 100 ml to make 10% solution. 10% solution were titrated from the burette containing dye (50 mg of 2-6 dichloro-phenol indo phenol + 42mg baking soda) until pink color was attained. Each sample reading was noted. By using the following formula, Vitamin C content were calculated.

$$\text{Ascorbic acid content (mg/100g)} = \frac{F \times T \times 100}{D \times S} \times 100$$

F = Dye factor

T = ml of dye used for sample titration

D = ml of sample taken for dilution

S = ml of diluted juice taken for titration

By using the following formula the total soluble solids and acid ratio was calculated.

$$\text{TSS/Acid} = \frac{\text{Total soluble solid}}{\text{titrateable acidity}}$$

Total sugar of peach fruit was determined with the method as described by Lane Eynon (AOAC, 1984). Percent Disease incidence per treatments was calculated after 15 days of interval by using following formula.

$$\text{Percent Disease incidence} = \frac{\text{No. of diseased fruits}}{\text{Total No. of fruits}} \times 100$$

Statistical Analysis

The data collected was subjected to Analysis of Variance (ANOVA) by using Complete Randomized design (CRD) for different variables suggested by (Basit et al. 2018) and analyzed statistically according to the procedure reported in Steel and Torrie (1980) using MStatC package. Least significant difference (LSD) test was used for any significant difference among the treatments at 5% level of probability.

RESULTS AND DISCUSSION

Weight loss (%)

Data presented in Table 1 showed that hot water treatment, storage duration and their interaction had significantly affected weight loss of peach fruit. Fruits dipped in water having

temperature of 50 °C had highest value of weight loss (7.63%) as compared to other treatments. With prolonging storage duration of fresh fruit up to 30 days, weight loss of peach fruit increases from 0.76 to 10.41%. As regard to the mean values of interaction, maximum weight loss (16.90%) was observed in the fruits dipped in water having temperature of 50°C and stored for 30 days, while the minimum was recorded in control and fresh fruit (Fig 1). Weight loss of peach fruit at 50°C as compared to other heat groups might be due to higher evaporation from the fruits surface (more porous or rough surface of fruits). This might be because of the reason that during the fruit ripening, cell wall degradation and membrane permeability caused the evaporation from the fruit surface. Also, with the phenomenon of water moment from inner cells to the outside atmosphere during transpiration in the form of water vapors (Shah et al.2020). The improvement in fruit weight may be due to increase in the metabolic activity of some important enzymes (protease, nitrate reductase and glutamine synthetase) and increased photosynthesis which enhanced the plant growth and development (Mondal et al. 2012). Similar effects were also observed by Candir et al. (2009), who stated that peach fruits treated at 40-45°C had lower weight loss. During storage duration of peach, fruit weight decreases due to loss of moisture as a result fruit turgidity decrease and fruits become soften (Vander, 1981). Similar results were also observed by, Tareen et al. (2012) and Ozmindar et al. (2009) in grapes during storage intervals. Khan et al. (2007) also observed increase in the weight loss of the fruit with the increase of heat treatment duration.

Fruit firmness (kg cm⁻²)

Table 1 shows the results of fruit firmness measured with manual penetrometer affected significantly by hot water treatment and storage duration. There was significantly an increase in value of fruit firmness (1.30 to 1.73 kg cm⁻²) with hot water treatment up to 40°C after that a decline was observed in fruit firmness (1.29 kg cm⁻²) in hot water treatment of fruit at 50°C. Similarly fruit firmness of peach decreases from 2.43 to 0.41 kg cm⁻² in freshly harvested fruit to fruits stored for 30 days. Firmness is one of the most important characteristics that consumers are most interested in, and therefore economically important in overall products is very high (Sajid et al. 2020). Fruit softening may be cause either by the hydrolysis of starch or by the breakdown of

insoluble proto-pectins into soluble pectin or by increased membrane penetration due cellular breakdown (Brummell and Harpster, 2001). In the ripening process, the loss of pectic substances in the middle lamellae of the cell wall is the key step leading to the loss of cell integrity or firmness (Mercado et al. 2011). The similar results were also observed by Lurie et al. (1998), that peach fruits, when treated at 38°C or 40°C softened slower than control. During storage of fruit, firmness of fruits decreases as result of disassembly of primary cell wall and middle lamella structures due to enzymatic activities and pectin solubalization (Chang-Hai et al. 2006). Similar results were also observed by Zhou et al. (2002), that the firmness of fruit decreases as the storage duration of fruit increases.

Total soluble solid (°Brix)

Significant increase in total soluble content of fruit (8.63 to 11.49 °Brix) was noted with increasing hot water treatment up to 50°C. With increasing storage duration of peach fruit up to 30 days, a significant increase in total soluble solid (9.67 to 9.86 °Brix) was observed (Table 1). Figure 2 shows that maximum total soluble solid content (12.24 °Brix) was observed in the fruits dipped in water having temperature of 50°C and stored for 30 days as compared to fresh fruit of control treatment. Conversion of starch into sugar and hydrolysis of polysaccharides in cell wall cause an increase in storage duration which increases the TSS of fruits. Rojas-Grau et al. (2007) quoted the similar findings where they stated that by extending fruit ripening, postharvest respiration is reduced; in addition, it also reduces the phenomenon of starch transformation to sugars that is needed for sustaining the fruits' total soluble solid. TSS of fruits increases with high respiration and other metabolic activities and this may be because of proto-pectin's breakdown into pectic-substances, disaccharides and fructose into monosaccharides (Sharma et al. 2012). Increased percentages of total soluble solids throughout the storage period are likely due to increase enzymatic activities which are responsible for the hydrolysis conversion of starch and insoluble sugars into soluble sugars. This conversion may result in the degeneration in the amount of carbohydrates, pectin, and partial hydrolysis of protein and decomposition of glycosides into subunits during respiration (Aranzana et al. 2011). These results are in harmony with Ozdemir and Dundar (2006), who reported that total soluble solid contents of orange

fruit had increased during storage. Similarly, Kinh et al. (2001) observed rise in value of TSS of apple pulp with increased storage duration.

Titrateable acidity (%)

Hot water treatment and storage duration except their interaction significantly affected titrateable acidity of peach fruit. Titrateable acidity of peach fruit significantly decrease from 0.45 to 0.30% with increasing hot water treatment temperature up to 50°C. Regarding storage duration, a decline was observed in titrateable acidity (0.40 to 0.30%) with prolonging storage duration up to 30 days. These results are in line with the results of Rapisarda et al. (2001) who noticed a decrease in percent acidity of orange fruit with increasing storage duration. The decrease in titrateable acidity indicated the maturity of the fruits. The decrease of titrateable acidity might be due to the use of organic acid as source of energy for the breakdown of pectin in to pectenic acid. Kaseem et al. (2010), Sarrwy et al. (2012) and Bhat et al. (2012) also recorded decrease in the titrateable acidity of persimmon, peach, date palm and pear fruits respectively when calcium was applied as foliar spray at the pre-harvest stage. Workneh et al. (2012) observed that the maximum decrease in titrateable acidity of tomatoes was due to the higher temperature in storage. Hot water dip treatments are applied only for some moment of time at temperatures higher than those used for vapor heat or hot air. Since many years non-chemical methods i.e hot water dip are widely used for control of post-harvest decay in various fruits and vegetables (Lurie, 1998). These results are in line with the results of Rapisarda et al. (2001), as well as with Ozdemir and Dundar (2006) who observed an increase in the proportion of TSS/Acid of orange. This increase is due to lowering of percent acidity and an increase in the TSS which specifies the ripeness of the fruits. Comparable results were also observed by Khalil et al. (2002).

TSS/Acid ratio

Table 2 indicated that hot water treatment and storage intervals had a significant effect on sugar-acid ratio, while their interaction had a non-significant effect on TSS/Acid ratio. Increasing hot water treatment of peach fruit highest value of TSS/Acid ratio (38.06) in the fruits dipped in water having temperature of 50 °C, followed by the TSS/Acid ratio (30.44 and 38.06) noted in fruits dipped in water having temperature of 40 °C and 30 °C respectively. Whereas minimum TSS/Acid

ratio (19.50) was observed in control fruits. Similarly increasing storage durations of freshly harvested fruit up to 30 days of storage, a significant increase in TSS/acid ratio (25.52 to 30.14) was observed. Hussain et al. (2008) reported that increase in TSS might be due to the changes in pectins and starch into simplest form of sugars during ripening when action of different enzymes occurred i.e. pectinase, methyl esterase and polygalacturonase. When duration of storage increases, titrateable acidity reduces, because by prolonging the storage duration, the fruits organic acids are converted to soluble sugars and decomposed. As a result, acidity decreases while TSS and sugar increases (Singleton et al., 1999). During storage the fruit utilizes the acids so the acid in fruit is decreased (Bhattarai and Gautam, 2006).

Ascorbic acid (mg 100g⁻¹)

Hot water treatment and storage duration had a significant effect on ascorbic acid content of peach fruit, while their interaction had a non-significant effect on ascorbic acid content (Table 2). The ascorbic acid content decrease from 6.03 to 4.30 mg 100g⁻¹ with increasing hot water treatment up to 50°C. Regarding storage intervals, the ascorbic acid content (5.45 to 5.02 mg 100g⁻¹) of peach fruit decrease with increasing storage duration up to 30 days. Fruits are natural sources of ascorbic acids (vitamin C) and it is known that the ascorbic acid of fruits decreases during ripening and processing. Ascorbic acid has direct relationship to acidity while it is inverse to pH level. The level of ascorbic acid (vitamin C) tends to decrease as the fruit ripens due to a direct action of ascorbic acid oxidase enzyme (ascorbinase), oxidation and subsequent change of ascorbic acid into 2, 3-dicetogulonic acid (Chitarra, 2005). Han et al. (2004) reported delayed degradation of vitamin C in chitosan-based luffa fruits (*Luffa cylindrical* L.). These results are in line with the results of Rapisarda et al. (2001) who observed a decrease in ascorbic acid contents during storage of different fruits. Similarly, Kinh et al. (2001) reported that ascorbic acid contents of apple decreased during storage. Yahia et al. (2007), also reported that level of ascorbic acid content was higher in control fruits as compared to the fruits of tomato which were treated with hot water.

Table 1: Weight loss, Fruit firmness, total soluble solid and titratable acidity of peach fruit as affected by hot water dipping and storage intervals
Each number is an average of five fruits in each treatment combination

Hot water treatment	Weight loss	Fruit firmness	Total soluble solid	Titratable acidity
Dipping (°C)	(%)	(kg.cm ⁻²)	(°Brix)	(%)
Control	5.04b	1.30bc	8.63d	0.45a
30°C	2.94c	1.57ab	8.89c	0.40b
40°C	2.57c	1.73a	10.08b	0.33c
50°C	7.63a	1.29c	11.49a	0.30d
LSD≤0.05	1.24	1.70	0.15	0.27
Storage duration (days)				
0	0.76d	2.43a	9.67	0.40a
10	2.36c	1.89b	9.70b	0.38ab
20	4.63b	1.16c	9.86a	0.37bc
30	10.41a	0.41d	9.86a	0.34c
LSD≤0.05	1.24	1.70	0.15	0.27
Interaction				
Treatment xStorage	Fig 1	----	Fig 2	----
Level of significance	*	NS	*	NS

Numbers followed by different letter is significantly different from each other in the same parameter at $p \leq 0.05$.

Table 2: TSS/Acid ratio, ascorbic acid, total sugar and percent disease incidence of peach fruits as affected by hot water dipping and storage intervals
Each number is an average of five fruits in each treatment combination

Hot water treatment	TSS/Acid ratio	Ascorbic acid	Total sugar	Percent disease incidence
Dipping (°C)	(%)	(mg.100g ⁻¹)	(%)	
Control	19.50d	6.03a	7.60a	25.00b
30°C	22.69c	5.50b	6.17b	20.00bc
40°C	30.44b	5.07c	5.88c	14.17c
50°C	38.06a	4.30d	5.60c	49.17a
LSD≤0.05	1.70	0.34	0.07	1.70
Storage duration (days)				
0	25.52c	5.45a	6.53a	0.00d
10	26.94bc	5.34ab	6.39	25.00c
20	28.09b	5.10b	6.23c	35.84b
30	30.14a	5.02b	6.11d	47.50a
LSD≤0.05	1.70	0.34	0.07	1.70
Interaction				
Treatment xStorage	----	----	Fig 3	Fig 4
Level of significance	NS	NS	*	*

Numbers followed by different letter is significantly different from each other in the same parameter at $p \leq 0.05$.

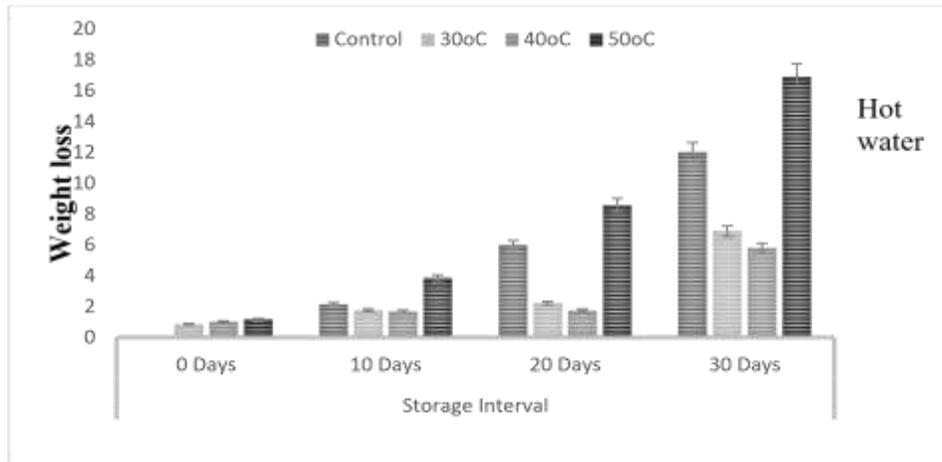


Figure1: Effect of hot water treatments and storage durations on weight loss (%) of peach

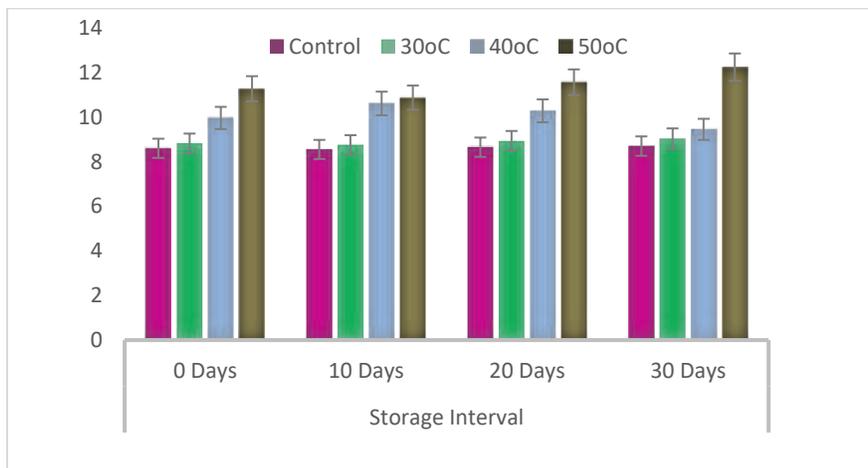


Figure 2: Effect of hot water treatments and storage durations on Total Soluble Solids (Brix°) of peach

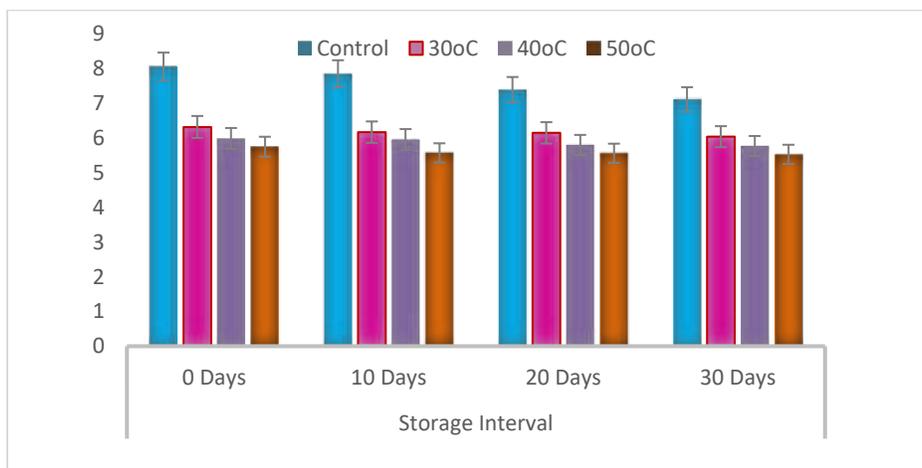


Figure 3: Effect of hot water treatments and storage durations on Total Sugars (%) of peach

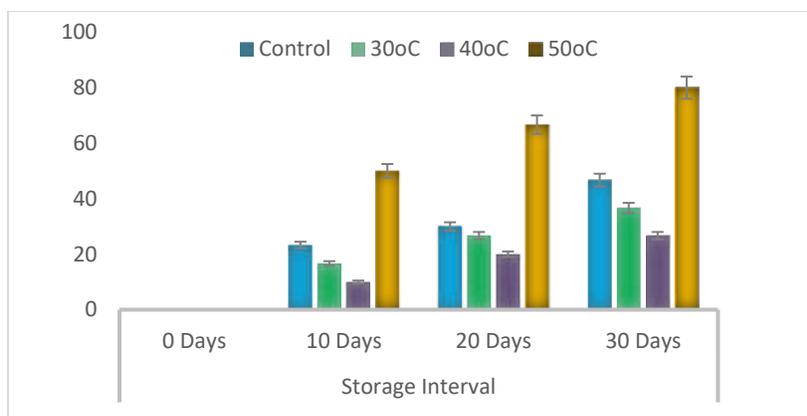


Figure 4: Effect of hot water treatments and storage durations on percent disease incidence of peach

These results are in correspondence with Liu et al. (2012) in peach fruits, that, when peach fruits treated with 40 °C gave better result as compared to other treatments. Aung et al. (1998) reported that total sugars were significantly higher in control in citrus fruit.

Total sugar (%)

Significant decrease in total sugar content from 5.60 to 7.60% was recorded in fruits with prolonging hot water treatment temperature up to 50°C. Total sugar value of peach fruit decrease from (6.53 to 6.11 %) in zero days of storage to the fruit stored for 30 days (Table 2). Regarding interaction of hot water treatment and storage duration, maximum total sugar value (8.06 %) was observed in control and fresh fruits as compared to the fruits that were dipped in hot water having temperature of 50°C and stored at 30 days interval (Fig 3). Similarly, a decreased in total sugar was noted in sweet oranges fruit with increasing the duration of storage (Moazong et al., 1997). At the early stages of maturation the starch is accumulated which is hydrolyzed to sugars at edible maturity (Magein and Leurquin, 1998) during storage (Beaudry et al. 1989), resulted in increased total sugar with increased storage duration (Crouch, 2003). The increase and the subsequent decrease in these biochemical attributes may possibly be attributed to the numerous catabolic processes taking place in the fruits preparing for senescence. Hulme (1958) stated that in apple, starch, hemicellulose and other polysaccharides acting as a source of sugars get hydrolyzed into mono and disaccharides during ripening which in turn lead to an increase in TSS and sugars during storage.

Percent disease incidence

It is obvious from Table 2 that hot water treatment, storage duration and their interaction significantly affected percent disease incidence of peach fruit. Percent disease incidence decrease (25.00 to 14.17%) in control fruits to fruits dipped in hot water at 40°C, afterward an abrupt increase in percent disease incidence (49.17%) was observed. Regarding different storage duration, an increase in percent disease incidence of peach freshly harvested fruits to fruit stored for 30 days (0 to 47.50%). The interaction of hot water treatment and storage duration showed that the maximum disease incidence (80.00%) was observed in the fruits dipped in water having temperature of 50 °C water and stored for 30 days as compared to freshly harvested fruits of control treatment (Fig 4). Ghasemnezhad et al. (2008) reported that temperature above than 47.5°C for 2 and 5 min, fruits were susceptible to heat damage resulted in rind browning. Basal level of skin damage was observed in all heat treatments. The hot water treatments also cleaned the fruit surface, melted the waxes, and sealed the open stomata (Yaun et al. 2013). According to Fallik et al. (2004), to avoid the fruit damage, duration of the fruits should be used accordingly, i.e. fruits treated with high temperature should kept for short duration and fruits treated with low temperature should kept for long duration.

CONCLUSION

Hot water treatment significantly affected all the qualitative parameters. Among the hot water treatments, the treatment of peaches with water at 40°C reduced the disease incidence and maintained fruit firmness. Storing the peach fruits for 10 days was found effective in minimizing the

weight loss and disease incidence and maintaining the ascorbic acid content, titratable acidity and total sugar content. Peaches should be dipped in hot water with the temperature of 40°C to enhance its storability up to 10 days.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: IA and ST Shah, Performed the experiments: IA, Nasrullah K and O Khan. Analyzed the data: I Ahmad and MA Khalid. Contributed materials/analysis/ tools: S Aman, N Ain, M Abbas, I Ullah and MA Khalid. Wrote the paper: ST Shah & AB. Reviewed the manuscript: A Basit. All authors read and approved the final version.

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