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POST HARVEST APPLICATION OF ALOE VERA GEL COUPLED WITH NEEM (*AZADIRACHTA INDICA*) LEAF EXTRACT MAINTAINS FRESH-CUT PEACH FRUIT QUALITY AND SHELF STORAGE STABILITY

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Abstract

Recent advances in edible coatings (EC) are helpful in extending shelf life, resolving fruit safety issues, and maintaining quality of fresh fruits. There is dire need to evaluate the cost effective and environmental friendly natural plant extracts for extending the shelf life of fresh-cut fruits. Aloe vera gel (AVG) is one of the best edibles and biologically safe preservative coatings for various foods. However, the effect of Aloe vera gel coating coupled with neem (*Azadirachta indica*) leaf extract (NLE) on post-harvest qualitative attributes of peach fruit during shelf storage remains unknown. For this purpose, peach fruits (cv. Indian blood) were coated with different treatments of AVG and NLE alone and in combination to evaluated for post-harvest shelf-life extension and changes in physiological and biochemical attributes when stored at ambient conditions (25 ◦C ± 2) for nine days. The results revealed that peach fruits when treated with AVG (100 ml L^{-1}) coupled with NLE (100 ml L^{-1}) maintained good status of total soluble solids (TSS) concentrations while kept a high level of titratable acidity (TA) as compared with the control treatment on final day of storage. Moreover, same treatment inhibited the loss of total phenolic content (TPC) of peach fruit and induced a significant increase in the activities of peroxidase dismutase (POD), superoxide dismutase (SOD), and catalase (CAT) enzymes on final day of storage. It is concluded that, AVG (100 ml L^{-1}) coupled with NLE (100 ml L^{-1}) could be a promising approach for enhancing peach fruits quality at shelf.

Keywords: Chemical-free edible coating; Aloe vera; Neem leaf extract; Secondary metabolites; Anti-oxidative activity; Storage period; Peach

Introduction

Peach *(Prunus persica* L.*)* belongs to the family of Rosaceae is one of the most important stone fruit crops, mostly grown in the world's temperate zones (Mir and Patel 2018). It is a well-liked amongst the stone fruits in Pakistan, contains considerable amount of minerals, vitamins and carbohydrates (Pakbin et al. 2014). It is rich in fiber content and is considered amongst the most nutritive fruits of the world (Mir and Patel 2018). It contains

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substantial number of vitamins (A, B1, B2 and B3) and minerals, including phosphorous, calcium, potassium, and iron (Awad, 2013). The higher antioxidant level makes this fruit helpful in minimizing blood pressure, cardiovascular problems, and cancer (Willats et al. 2001).

In Pakistan, the total area under peach cultivation was 16.3 thousand hectares for the year 2014-15 with the production of 66.7 thousand tons, while in Khyber Pakhtunkhwa (KP) province, it is cultivated on an area of 7.7 thousand hectares with a total production of 48.4 thousand tons (GOP, 2014-15). Like other climacteric fruits, Peach is highly perishable and soon after harvest quality losses occurs (Barman et al. 2014). Fruit softening at ambient temperature results in concise shelf life (Khaliq et al. 2019). Primary factors involved in reducing in post-harvest life include poor production technology, nutritional imbalance, insect pest or disease infestation, harvesting time, and improper transportation to distant markets (Cano-Salazar et al*.* 2013).

Peach fruit, if stored for a more extended period, develop chilling injury symptoms reduces the consumer acceptance. While different methods like wax coating, heat treatment, use of fungicides and acid dipping have been used to control post-harvest losses of fruit, including chilling injury (Sohail et al. 2013). Furthermore, different cultivars of peaches have shown variation in antioxidative enzyme activities (Agarwal et al. 2001) and significant qualitative traits such as sweetness, juice contents and aroma (Cano-Salazar et al.2013). Consequently, fruit should be handled individually, during ripening because fruit ripening pattern in one cultivar may not be appropriate toward other varieties even within the same species (Goulao and Oliveira, 2008).

As remarkable progress is being made in enhancing food production at the global level, half of the population in the third world does not have access to satisfactory food supplies (Sohail et al. 2013). Many reasons are causing this problem, but the main reasons are food losses due to post-harvest and marketing system (Khan et al. 2008). As this fruit is a source of foreign exchange these losses are causing lower production in the whole country (Khaliq et al.2019). The post-harvest losses of peach fruit in Pakistan were recorded 30 to 40% (Zeb and Khan 2008).

Edible coatings are a new technique used to increase the shelf life of whole or fresh-cut fruits and vegetables, applied directly on the food surface by brushing, spraying or dipping (Misir et al. 2014). Edible coatings are thin layers that improve fruit quality which covers the surface of the fruit and it can be safely eaten as a part of the whole fruit (Kokoszka and Lenart 2007). Edible coating is applied to the fruit surface in addition is also a replacement for natural protective waxy coatings and provides barrier to moisture, oxygen and solute movement in fruits and vegetables (F&V) (Zhou et al.2008). Edible coatings have various favorable effects on F&V such as a glossy appearance and better color, retracing weight loss or prolonging storage (Vargas et al. 2006).It creates a modified atmosphere and reduces weight loss during transport and storage (Turhan, 2009). Researchers recently used aloe vera extract as an edible coating on a large scale to

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increase the shelf-life of fruits and vegetables as this product is safe and environment friendly (Serrano et al. 2006, Ahmed et al. 2009 and Ali et al. 2019). Aloe vera extract is composed of “Aloin and Aloe-emodin” (Ahmed et al. 2009) used as antifungal, anti-bacterial and anti-inflammatory (Misir et al. 2014). Different scientists reported that the coating of aloe vera gel improves the shelf life of fruits without affecting the original taste of fruits and poses no risk to human health (Khaliq et al. 2019).

Aloe vera gel is applied to fruits as edible coating which has been widely used and prolongs the conservation of fresh fruits and vegetables (Misir et al. 2014). Aloe Vera leaves are rich in bioactive compounds; some are antioxidants broadly used in food engineering preservatives such as mannans (Eshun., 2004). The liquid extracts of aloe vera gel, and Neem, can be applied on most similarly commercial peach varieties (cv. Indian blood).

Neem (*Azadirachta indica*) extract has been used for centuries in Asia as insecticides and fungicides (EL-Eryan et al., 2017) and are residue free and safe from consumption point of view as compared to fungicides that are highly toxic to living beings. Neem leaves extract has a number of active ingredients like azadirachtin and Nimbidin that help in checking decay losses in fruits that are caused by the fungal infection (Raghav et al. 2016). Several research studies confirmed that plant extracts reduce decay incidence and maintained post-harvest fruits quality during storage (Hajilou and Fakhimrezaei 2011, Yanng et al. 2014 and Khaliq et al. 2019). In reality, it is a critical issue in Pakistan that has received little attention.

District Swat (North Pakistan) is the Peach basket for the rest of the country, yet its cultivation has been suffering from various problems, such as insects attack, diseases, low yield, storage problems (Ullah et al. 2018) and lack of scientific knowledge to overcome these problems (Khaliq et al. 2019). Majority of fruit farmers used chemicals to control pathogen and prolong the shelf life of fruits, which encourage the development of resistant pathogenic strains, increase toxic residues on fruit surface, and have a horrible effect on the environment and consumers. Therefore, the present research study was planned with the main hypothesis that the application of aloe vera gel and neem leaves extract can be used as an edible coating for enhancing the shelf life of Peach fruit cv. Indian Blood in addition their effect on different biochemical parameters were also studied. The specific objective of this study was to see how Aloe vera gel and Neem leaf extract affected the post-harvest physicochemical, biochemical characteristics, and antioxidant capacity of Peach fruit (cv. Indian blood) during storage.

Materials and Methods

Fruit collection

Peach fruit cv. Indian blood) were collected at physiological maturity from a commercial orchard located at Matta, District Swat, Khyber Pakhtun-Khwa (34° 56' 54" N, 72° 31' 56" E) Pakistan). A total of sixty (60) healthy, physically undamaged, and uniform-sized fruits

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were harvested randomly. The fruits were then shifted to laboratory after appropriate packaging. The fruits were washed with distilled water, graded, and dried at ambient temperature for further experimentation.

Preparation of Aloe vera gel and neem leaves extract

Aloe vera plants (*Aloe barbadensis miller*) were collected from a nursery, and gel was collected from its leaves by peeling it off and this colorless hydro parenchyma was blended in a blender. Fresh leaves of neem (*Azadirachta indica)* were collected, dried in shade and then grinded in an electric blender. The grinded powdered was extracted with a methanol solvent (260 ml) by using a maceration method for 3 days. After extraction, the sample was filtered by using a Bruckner funnel. The methanol solvent was evaporated by using a rotary evaporator under reduced pressure at 20 °C for 1 h. The crude extract became a semi solid mass.

Coating formulations and storage

For the preparation of the edible coating, three treatments were tested in 100 ml L^{-1} of distilled water:

(i) EC_0: Control (without edible coating),

(ii) NLE: Neem pure leaves extract (100ml L^{-1}),

(iii) AVG: *Aloe vera* gel extracts (100ml L^{-1}),

(iv) AVG+NLE: *Aloe vera* gel extracts (100 ml L^{-1}), and Neem pure leaves extract (100ml L^{-1}).

All of the treatments were applied by dipping fruits in different concentrations of Aloe vera gel and Neem plant extract for fifteen (15) minutes. The fruits were then air-dried and kept at ambient temperature (25 °C± 1). Quality analyses were carried out on an interval of three days up to nine days.

Physicochemical parameters

The stored fruits were analyzed as per schedule for physicochemical parameters (pH, total soluble solids, total and reducing sugar, non-reducing sugar, titratable acidity, and ash contents).

pH of fruit juice

The pH value of fruit juice was measured with a digital pH meter (WTW Inolab pH-L1, Germany).

Total soluble solids

The total soluble solid content (°Brix) was estimated by a digital optical refractometer (Atago Model PR-1, Tokyo).

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Total sugar and reducing sugar

The concentration of total sugars (TS, %) and reducing sugar (%) was determined by the phenol-sulfuric method as described earlier by (AOAC 2000).

*Ash content*s

Ash content (%) was determined according to the AOAC (2000) method No. 940.26. Two grams' moisture free fruit pulp samples were taken in to the pre-weighed crucibles. The crucibles were directly incinerated in the muffle furnace at 550 ^{o}C for about 5 hours till greyish white residue was obtained. The crucibles were then shifted to desiccator to cool it. Following formula was adopted to calculate the total ash content:

$$Ash(\%) = \frac{Weight\ of\ crucible\ with\ ash - Weight\ of\ empty\ crucible}{Weight\ of\ sample} \times 100 \qquad [1]$$

Titratable acidity

The titratable acidity (TA, %) of peach juice was determined by taking 10ml of juice with 500ml distilled water then 2-3 drops of phenolphthalein was added and titrated with 0.1N sodium hydroxide with continuous stirring till the color of sample change to pink color. TA was calculated by using the following formula.

$$TA\ (\%) = \frac{1}{10} \times \frac{ml\ NaOH\ used \times 0.1N\ NaOH \times mill\ equivalent\ factor}{Total\ sample} \times 100 \qquad [2]$$

Phytochemical screening

The qualitative phytochemistry tests were performed using aqueous extract to identify various constituents:

Test for tannins

About 2 ml of the liquid extract was mixed with water and heated on a water bath. The Mixture was filtered and ferric chloride was added to the filtrate. The deep black color was indication of presence of tannins.

Test for flavonoids

Extracts were treated with few drops of lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for terpenoids

About 5 mg of the extract was mixed with two ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of reddish-brown color in the inner face was indicating the presence of terpenoids

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Test for saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

Test for steroids

About 2 ml of acetic anhydride was added to five mg of the extracts, each with two ml of H_2SO_4. The color was changed from violet to blue or green in some samples indicated the presence of steroids.

Test for carbohydrates

About 0.5mg extracts were dissolved individually in 5 ml distilled water and filtered. The Filtrate was used to test the presence of carbohydrates.

Test for alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids. Filtrates were treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Test for proteins

About 0.5 mg of extract was taken and two drops of freshly prepared0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple color indicates that the presence of proteins, peptides or amino acids.

Determination of total phenolic content

Total Phenolic Content (TPC, mg g^{-1}) was determined by following the method of (Solomon et al. 2006).One ml of diluted extracts with 2.5 ml with 10% of Folin–Ciocalteau's reagent followed by neutralization with 2 ml sodium carbonate added (7.5 %). The mixture was then kept in dark place for 45 min. Absorbance measured at 765 nm wavelength using spectrophotometer (UV light -9200Biotech Engineering Management Co., UK). TPC was expressed as mg gallic acid equivalents (GAE) (Singleton and Rossi 1965).

Determination of total carotenoid content

Total carotenoids (mg g^{-1}) were extracted essentially by following the procedures established by Schiedt and Liaaen-Jensen. Fruits were peeled and cut into small pieces, and then homogenized in a food processor. The carotenoids of ten grams of the homogenate were extracted several times with acetone until no color was obtained and passed through a mixture of ether: n-hexane (1:1). A sample of this solution was dried out under an atmosphere of nitrogen, dissolved in n-hexane and the absorbance was read at 450 nm.

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Determination of total flavonoids content

Total Flavonoids Content (TFC, mg g^{-1}) by (Zhishen, Mengcheng, and Jianming 1999). One ml of diluted sample extracts was mixed with 0.3 ml of sodium nitrite (5 %). After an interval of 5 minutes 0.6 ml of aluminum chloride (10 %) was added and mixed. This was followed by addition of 2 ml of 1 M sodium hydroxide after an interval of 5 minutes. Finally, the absorbance was measured at 510 nm wavelength using spectrophotometer (UV-9200, Biotech Engineering Management Co., UK). The total flavonoid was calculated using quercetin as standard (Kiranmai et al. 2011).

Determination of DPPH free radical scavenging activity

The stable radical 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH, %) was used for determination of free radical scavenger activity of the extracts (Brands-Williams et al.1995). Extracts were added at an equal volume, to methanol solution of DPPH (0.2 mm). After 30 min at room temperature, the absorbance will be record at 517 nm. The percent of scavenging activity was calculated as the ratio of the absorption of the sample reactive to the control (0.1 Mm DPPH solutions without the extract). Radical scavenging activity will be expressed as the inhibition percentage calculated by the following formula.

$$DDPH\ scavenging\ activity\ (\%) = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100 \quad [4]$$

Peroxides dismutase, Catalase and Superoxide dismutase

Frozen juice was used to estimate the enzymatic activities of peroxides dismutase (POD), carotenoids (CAT) and superoxide dismutase (SOD) after homogenization using phosphate buffer. The enzyme extracts were prepared and readings were recorded at spectrophotometer at 240 nm. The activities of all these enzymes were measured in µg g^{-1}.

Statistical analysis

Results were subjected to statistical analysis by considering storage and treatment application as sources of variation, using two-way analysis of variance (ANOVA). Statistical differences with P-values under 0.05 were deemed significant, and a comparison of mean values was carried out by applying LSD method using Statistic 8.1 software (Steel et al. 1997).

RESULTS

pH

The data pertaining to changes in pH value of peach juice are presented in Fig. 1A. The data showed an increasing trend from 0 day to day 9 of storage, ranged from a minimum of 4.4 at 0 days of storage to a maximum of 4.9 at 9 days of storage. Results of interaction between different treatments and storage days proposed maximum pH value of 4.97 at

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control treatment (EC0) followed by values of 4.87, 4.68 and 4.62 at application of NLE, AVG, and AVG+NLE, respectively. AVG+NLEshows best results of pH in the present study because the variation in their results is very less than other treatments at storage duration. The results of AVG+NLE for day 0 were (4.42), day 3 (4.57), day 6 (4.57), and day 9 (4.62), respectively (Fig. 1A).

Titratable Acidity

In the present study, TA showed a decreasing trend with storage period. EC0 showed highest variation in their values from day 0 (0.65%), day 3 (0.47%), day 6 (0.43%) and day 9 (0.40%) while AVG+NLE shows best results of TA in the present study because the variation in their results is very less than all the other treatments at storage duration. The results of AVG+NLE were for day 0 (0.65%), day 3 (0.58%), day 6 (0.53%) and day 9 (0.52%) (Fig. 1B).

Total soluble solids

Total soluble solids showed an increasing trend from zero day to day nine of the storage. TSS contents were found maximum (10.96) when peach fruits were tested on 9 days of storage and found minimum (9.55) at 0 days of storage (Fig. 1C). Interaction showed maximum TSS (11.43) in the control treatments (EC_0) followed by NLE (11.20), AVG (10.70), and AVG+NLE, respectively (Fig. 1C).

Ash content

The data derived from the experiment revealed that the ash content of the Indian blood Peach variety was not significantly different in most of the treatments. Highest value was found in AVG+NLE (0.70 %) followed by AVG (0.69 %) while lowest values were found in EC0 (0.66) (Fig. 1D).

Total sugar

An increasing trend was observed in the total sugar contents of the peach fruits with storage period in the present study. We observed maximum TS of (11.14%) at 9 days of storage, while minimum TS contents (9.20%) of peach fruits were observed at 0 days of storage (Fig. 1E). Interaction analysis showed maximum TS of 11.14% at EC0 followed by 10.93%, 10.40% and 10.03% at NLE, AVG, and AVG+NLE, respectively (Fig. 1E). AVG+NLE was found best among all other treatments in the present study because the variation in their results wasvery less than all the other treatments at storage duration. The results of AVG+NLE for day 0 is (9.30%), day 3 (9.53%), day 6 (9.73%) and day 9 (10.03%), respectively.

Reducing sugars and non-reducing sugars

Like TS, both reducing sugars and non-reducing sugars of peach fruits were also increased with respect to their storage days. Maximum reducing sugar content of 4.55

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was observed at 9 days of storage period while minimum reducing sugar 3.60 of peach fruits were found at 0 days storage period (Fig. 1F). AVG+NLE showed better results of reducing sugars in the present study because the variation in their results was found less as compared with other treatments at storage duration. The results of AVG+NLE for day 0 was (3.53%), day 3 (3.73%), day 6 (3.86%) and day 9 (4.20%), respectively (Fig. 1F). Similarly non-reducing sugar contents was also increased with storage duration and found a maximum of 6.11 at 9 days of storage while a minimum of 5.58 at 0 days of storage (Fig. 1G). Again, AVG+NLE showed better results than other treatments as variation was found less at different storage durations.

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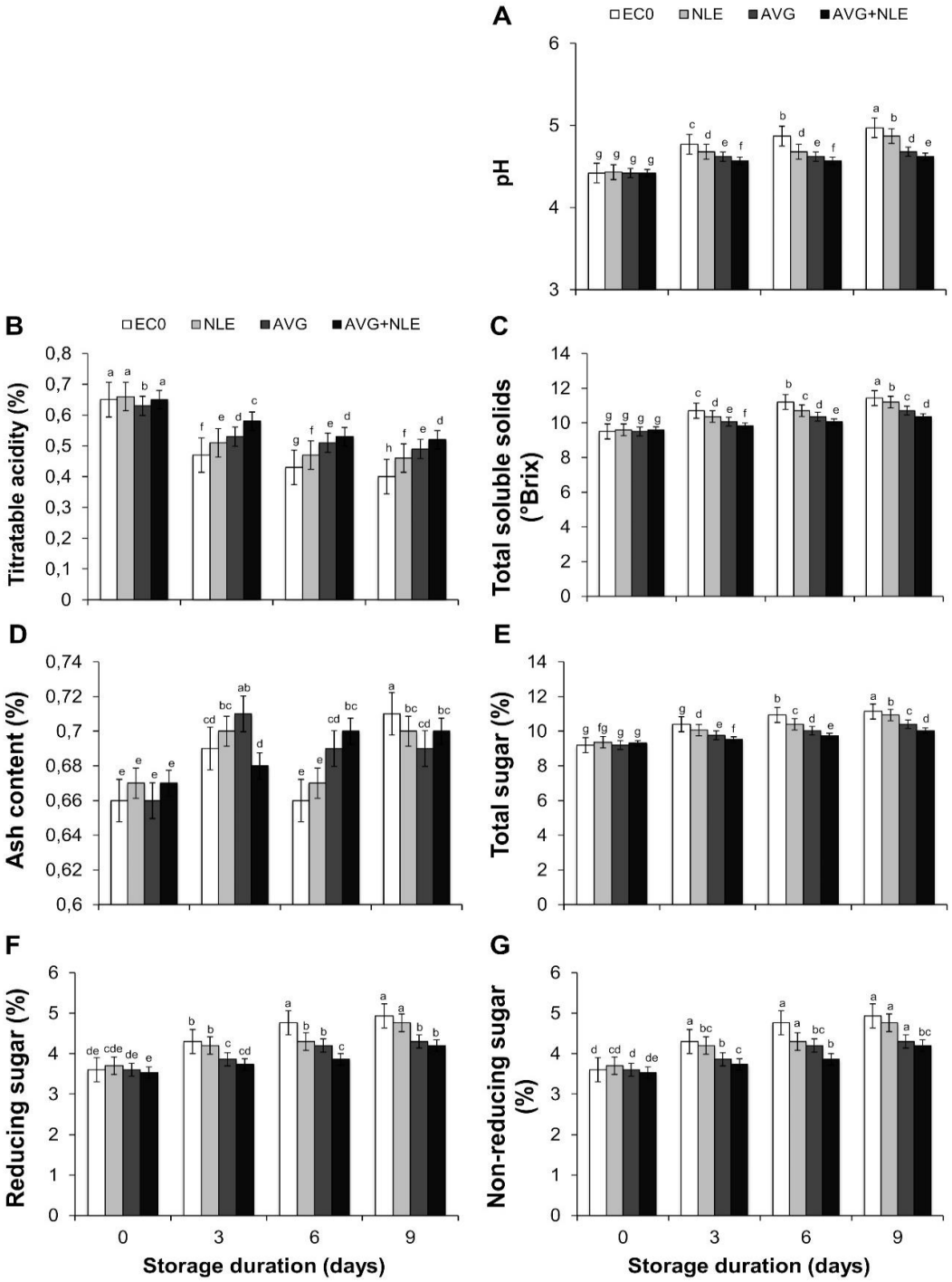

Fig 1. Effect of Aloe vera gel coating and Neem leaves extract on pH (A), Titratable acidity (B), Total soluble solids (C), Ash content (D), Total sugar content (E), Reducing sugar

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content (F), and Non-reducing sugar content (G) of peach fruit during storage at 25 °C for 9 days. Abbreviations: EC_0: Control (untreated); NLE: Neem pure leaves extract (100 ml L^{-1}), AVG: Aloe vera gel extracts (100 ml L^{-1}), AVG+NLE: Neem pure leaves extract (100 ml L^{-1}) and Aloe vera gel extracts (100 ml L^{-1}).

Total carotenoids, phenolic, and flavonoids contents

Decreasing trends in carotenoids and TPC of peach fruits were observed with different storage durations. Maximum value of 83.1 for carotenoids was observed at EC_0 at 0 days of storage, while the lowest value of 70.5 was recorded at AVG+NLE at 9 days of storage (Fig. 2A). EC0 showed the highest variation in their values from day 0 (70.96), day 3 (74.82), day 9 (77.42) and day 6 (77.49), while AVG+NLE showed the best results of carotenoids in the present study because the variation in their results is very less than all the other treatments at storage duration (Fig. 2A). TPC showed a decreasing trend from zero day to day nine of the storage. EC0 shows highest variation in their values from day 0 (300.4 mg g^{-1}), day 3 (308.25 mg g^{-1}), day 6 (312.7 mg g^{-1}) and day 9 (323.0 mg g^{-1}), while AVG+NLE showed the best results of TPC in the present study because the variation in their results is very less than all the other treatments at storage duration (Fig. 2B). The results of AVG+NLE for day 0 (340.0 mg g^{-1}), day 3 (329.0 mg g^{-1}), day 6 (314.0 mg g^{-1}), and day 9 (308.0 mg g^{-1}).

Results pertaining to the total flavonoids content showed a decreasing trend from zero day to day nine of the storage. EC_0 shows highest variation in their values from day 0 (50.94 mg g^{-1}), day 3 (54.74 mg g^{-1}), day 6 (57.35 mg g^{-1}) and day 9 (57.74 mg g^{-1}) while AVG+NLE showed the best results of TFC in the present study because the variation in their results is very less than all the other treatments at storage duration (Fig. 2C). The results of AVG+NLE for day 0 (63.41 mg g^{-1}), day 3 (58.71 mg g^{-1}), day 6 (51.07 mg g^{-1}), and day 9 (mg g^{-1}).

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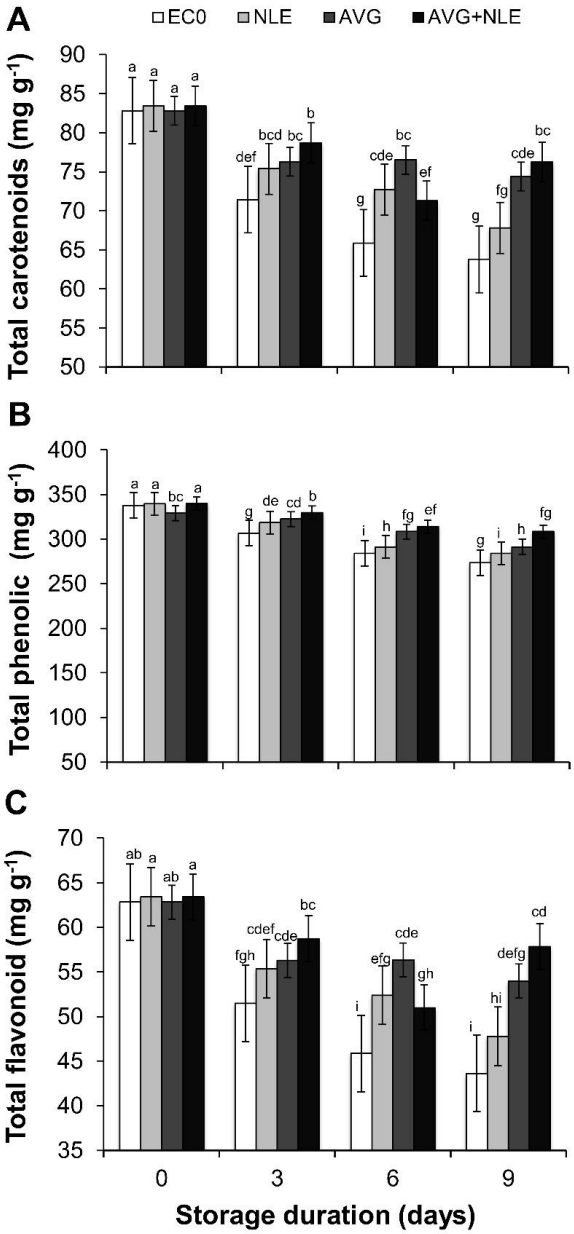

Fig 2. Effect of Aloe vera gel coating and Neem leaves extract on Total carotenoids content (A), Total phenolic contents (B), and Total flavonoid contents (C) of peach fruit during storage at 25 °C for 9 days. Abbreviations: EC_0: Control (untreated); NLE: Neem pure leaves extract (100 ml L^{-1}), AVG: Aloe vera gel extracts (100 ml L^{-1}), AVG+NLE: Neem pure leaves extract (100 ml L^{-1}) and Aloe vera gel extracts (100 ml L^{-1}).

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Radical Scavenging Assay

Analysis of mean values for all the treatments with respect to their storage days revealed that mean values were in the range of 40.95% to 47.87% for DPPH. DPPH showed a decreasing trend from zero day to day nine of the storage. EC0 showed the highest variation in their values from day 0 (40.95 %), day 3 (44.83%), day 6 (47.44%) and day 9 (47.87%), while AVG+NLE showed the best results of DPPH in the present study because the variation in their results is much less than all the other treatments at storage duration (Fig. 3). The results of AVG+NLE for day 0 (53.41%), day 3 (48.71%), day 6 (41.28%), and day 9 (48.07%).

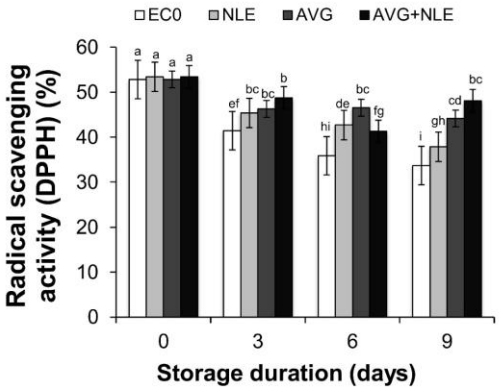

Fig 3. Effect of Aloe vera gel coating and Neem leaves extract on Radical scavenging assay (DPPH) of peach fruit during storage at 25 °C for 9 days. Abbreviations: EC_0: Control (untreated); NLE: Neem pure leaves extract (100 ml L^{-1}), AVG: Aloe vera gel extracts (100 ml L^{-1}), AVG+NLE: Neem pure leaves extract (100 ml L^{-1}) and Aloe vera gel extracts (100 ml L^{-1}).

Catalase

Catalase showed a decreasing trend from zero day to day nine of the storage. EC_0 showed the highest variation in their values from day 0 (72.13), day 3 (58.14), day 6 (52.71), and day 9 (48.14) while AVG+NLE showed the best results of CAT in the present study because the variation in their results was much less than all the other treatments at storage duration (Fig. 4A). The results of AVG+NLE for day 0 (71.97), day 3 (67.72), day 6 (63.81) and day 9 (59.76).

Superoxide dismutase

Analysis of mean values for all the treatments with respect to their storage days revealed that mean values were in the range of 45.84 to 24.53 for SOD. SOD showed a decreasing trend from zero day to day nine of the storage. EC0 showed the highest variation in their values from day 0 (45.84), day 3 (35.94), day 6 (29.73), and day 9 (24.53), while AVG+NLE showed thebest results of SOD in the present study because the variation in

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their results was much less than all the other treatments at storage duration (Fig. 4B). The results of AVG+NLE for day 0 (44.29), day 3 (41.24), day 6 (38.13), and day 9 (34.43).

Peroxidase

Analysis of mean values for all the treatments with respect to their storage days revealed that mean values were in the range of 55.72 to 39.53 for POD. POD showed a decreasing trend from zero day to day nine of the storage. EC_0 shows highest variation in their values from day 0 (55.24), day 3 (45.14), day 6 (39.53) and day 9 (34.13) while AVG+NLE showed the best results of POD in the present study because the variation in their results was much less than all the other treatments at storage duration (Fig. 4C). The results of AVG+NLE for day 0 (54.99), day 3 (51.84), day 6 (48.23), and day 9 (44.23).

Fig 4. Effect of Aloe vera gel coating and Neem leaves extract on Catalase enzyme activity (A), Superoxide dismutase activity (B), and Peroxidase activity (C) of peach fruit during storage at 25 °C for 9 days. Abbreviations: EC_0: Control (untreated); NLE: Neem pure leaves extract (100 ml L^{-1}), AVG: Aloe vera gel extracts (100 ml L^{-1}), AVG+NLE: Neem pure leaves extract (100 ml L^{-1}) and Aloe vera gel extracts (100 ml L^{-1}).

Qualitative phytochemical analysis

The present study on the Indian Blood Peach variety revealed the presence of medicinally active constituents. The phytochemical active compounds of Indian Blood Peach variety were qualitatively analyzed, and the results are presented in Table 1. In these screening processes, tannins, flavonoids, terpenoids, saponins, steroids, phlorotannins, carbohydrates, glycosides, proteins showed different results based on availability and non-availability in peach cv. Indian blood (Table 1) revealed that terpenoids, steroids, phlorotannins, are not present in peach cv. Indian Blood, while tannins, flavonoids, saponins, carbohydrates, glycosides, proteins, alkaloids, are present in peach cv. Indian Blood. Duration of storage period and the application of treatments NLE, AVG and AVG+NLE do not affect the availability of these phytochemicals in the peach cv. Indian blood.

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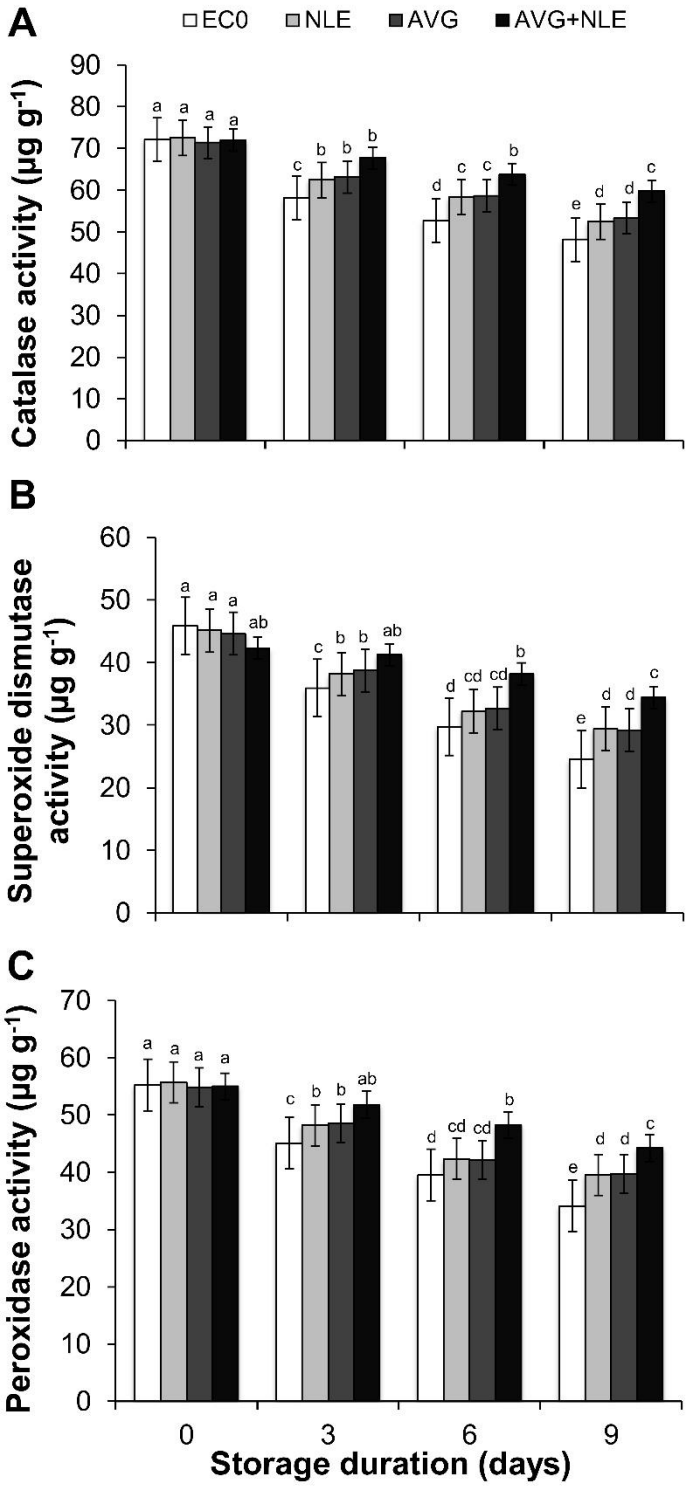

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Table 1. Qualitative phytochemical screening of Peach fruit during storage at 25 °C for 9 days

Treatment	Storage durations	Tannins	Flavonoids	Terpenoids	Saponins	Steroids	Alkaloids	Carbohydrates	Proteins
EC_0	0	+	+	-	+	-	+	+	+
NLE	0	+	+	-	+	-	+	+	+
AVG	0	+	+	-	+	-	+	+	+
AVG+NLE	0	+	+	-	+	-	+	+	+
EC_0	3	+	+	-	+	-	+	+	+
NLE	3	+	+	-	+	-	+	+	+
AVG	3	+	+	-	+	-	+	+	+
AVG+NLE	3	+	+	-	+	-	+	+	+
EC_0	6	+	+	-	+	-	+	+	+
NLE	6	+	+	-	+	-	+	+	+
AVG	6	+	+	-	+	-	+	+	+
AVG+NLE	6	+	+	-	+	-	+	+	+
EC_0	9	+	+	-	+	-	+	+	+
NLE	9	+	+	-	+	-	+	+	+
AVG	9	+	+	-	+	-	+	+	+
AVG+NLE	9	+	+	-	+	-	+	+	+

(+) Showed highly presence (-) showed lowest presence. EC_0: Control (untreated); NLE: Neem pure leaves extract (100 ml L^{-1}), AVG: Aloe vera gel extracts (100 ml L^{-1}), AVG+NLE: Neem pure leaves extract (100 ml L^{-1}) and Aloe vera gel extracts (100 ml L^{-1}).

Discussion

Peach is considered the second most important and famous fruit of Pakistan after plum having a tremendous taste. Unfortunately, Pakistan is facing a drastic reduction in peach quality due to improper post-harvest management. In this context, the present study was carried out to replace the unsafe synthetic costly fungicide with environment-friendly and cheap botanical extract like Aloe Vera and neem to enhance the storage life of Peach cv. Indian blood.

The measurement of hydrogen ion activity (pH) is a useful method of expressing the acidity of peach fruit. It is an important factor of fruit where farmers and consumers pay more attention to the fruit quality than to its size and yield (Paudyal and Haq 2008). The pH of peach juice increased throughout storage durations, as was expected with the decline in fruit acidity following application of various coatings. The AVG+NLE coating formulation had the lowest pH. The different coatings tested in this study had varying impacts on pH. These findings are similar to those of Baldwin et al. (1999), who observed that pH value depends on the kind of coating. Changes in pH might be caused by the changes in biochemical condition of the fruit. The coating solution may have an effect on metabolic activity, particularly the rate of respiration (Jitareerat et al. 2007).

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Titratable acidity (TA) is another important fruit quality parameter which reflects the storage life. In our study, fruit TA was significantly affected by treatment and storage duration. TA progressively decreased in all fruit samples during the whole storage duration. However, Aloe vera gel and neem plant coatings (AVG+NLE) delayed the change in TA compared to other treatments. TA shows opposite trend with respect to pH and TSS of the fruit, this indicates the sweeter taste if the value of pH and TSS is increased and more tartness in cv. Indian Blood confirms the high value of TA (Seymour et al. 2012). It has been reported earlier that TA acidity decreased with increase in storage period (Dey, Ghosh, Bhowmick, & Ghosh, 2014; Bahmani et al. 2015). Plant extracts as coatings may retain high TA during storage (Yanng et al. 2014), while reduction in respiration rate with plant extract coatings might be a reason for high TA in treated fruits in this study.

Fruit TSS content is an important quality index, which can reflect the maturity stage of any fruit (Khaliq et al. 2019). The quality parameter like TSS attracts the consumer due to the sweeter taste that is also an important economic factor in an industry that buys its fruit based on the sugar content and processes over 95% (Rouse 2000). TSS can be increased during the storage period as the complex sugar is converted into simple sugar (Hajilou and Fakhimrezaei 2011). In the present study, application of Aloe Vera coating in concoction with neem extract minimized TSS. Reduction in TSS might be due to the reduction in fruit senescence and metabolic activity (Khaliq et al. 2019). These findings are in agreement with results of researchers who reported that Aloe Vera coating minimizes TSS in fruits (Sophia et al.2015; Chauhan et al.2014; Brishti et al.2013), while the ability of Aloe vera gel coatings improved by incorporation of other ingredients like gelatin and other essential oils (Bill et al. 2014; Chauhan et al. 2015).

The total ash content in the sample is the sum of all the minerals present in the fruit. The primary goal of ash determination is to identify the mineral content of food materials. We found out that the average ash content of the studied Peach fruit was 0.68 % ± 0.01. This conclusion is acceptable because it is well known that high-quality fruits should have less than 1% ash. Ash in fresh fruits ranges from 0.2 to 0.8% and is generally inversely related to moisture content. Some dried fruits (e.g., apricots) may contain as much as 3.5% ash (Pomeranz and Meloan 1994).

In the present study, we observed an increasing trend for TS and reducing sugars for all the treatments, though AVG+NLE showed a significantly low increase at 9 days of storage. Our results are in accordance with previous findings in tomato coated with Aloe vera (Chrysargyris et al. 2016). In the Peach fruit, the sugars prominent are glucose, fructose and sucrose (Aliman et al. 2019). Sugar composition influences the perceived level of fruit sweetness and is also an instant source of energy for metabolic activities. It also acts as maturity index for peach fruit. The present result indicated a high level of total reducing and non-reducing sugars in Indian blood variety. High level of sugars persists sweet in taste and low level of sugars persists bitter in taste, this bitterness is also due to the opposite relation between low sugar level and high acidic content in peach fruit.

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Reducing and non-reducing sugars showed the same trends as shown by TSS, this indicates that all the soluble solids present in Indian blood variety were mainly comprised of these sugars. An overall view of the obtained variation in sugar levels might be due to the influence of treatments application on the storage of Indian blood variety (Abbasi et al. 2011).

In our study, we found out that carotenoids and total phenolic contents of Peach fruits were within the adequate range. Vizzotto et al. (2007) showed that carotenoids and TPC should be ranged between 0.1-1.9 mg $g^{-1}$and 158-495 mg g^{-1}, respectively in different Peach varieties. Carotenoids and total phenolic contents of Peach fruits decreased regarding storage duration. The decrease in TPC of Peach fruit might be due to oxidation (Ullah et al. 2018). However, Aloe Vera gel and Neem extracts in concoction strongly inhibited the loss of TPC of Peach fruit during storage as compared with control. Our results were in accordance with the findings of Chauhan et al. (2014) and Yang et al. (2014), who reported that plant extracts application as edible coatings retain the TPC in fruits. TPC reduces the oxidative degradation of lipids and preserves nutritional food value (Shah et al. 2017). Coatings may have an effect by delaying senescence or the ripening process, reducing TPC losses, or enhancing the defense system and maintaining fruit quality during storage after harvest (Khaliq et al. 2016). These findings are congruent with those of other researchers who have performed extensive research on the phenolic content of fruits during storage and ripening (Chidtragool et al. 2011; Chauhan et al. 2014; Yang et al. 2014).

TFC of peach fruit was also decreased with the progress of storage duration. In present study, TFC in control treatment showed more decline rate as compared with other treatments, while Aloe Vera and Neem plant extracts (AVG+NLE) as coatings showed better results for TFC. Flavonoids increase fruit shelf life as it has antioxidant activity (Khaliq et al. 2019). The more accumulation of TFC in Peach fruits in AVG+NLE might be associated with the stimulation of Phenylalanine ammonia lyase (PAL) enzyme activity as Hassan pour (2015) reported that during storage Aloe Vera gel coating stimulate PAL activity in raspberry fruit.

The antioxidant activity (DDPH) of peach fruit was affected by different treatments in the present study, and found to decrease with varying periods of storage. DDPH activity was found significantly higher in fruit of AVG+NLE as compared with control. Plant extracts as edible coating maintain high DPPH activity with storage in fruits (Khaliq et al. 2019; Nair et al. 2018; Hassan pour 2015). High DDPH value in AVG+NLE might be due to the compound Aloe-emodin found in Aloe Vera gel that greatly contributes to DDPH activity (Zou et al. 2016).

Plant tissues contain several enzymes scavenging like CAT, POD and SOD (Ali et al. 2020) which not only help to control the level of reactive oxygen but also protect plant cells during stress conditions (Hong et al. 2012). In our study, this enzymatic activity of Peach fruit decreased with different storage periods. However, all the fruits treated with

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Aloe vera gel enriched with Neem extracts (AVG+NLE) significantly delayed the activity of these enzymes as compared with control which is not strange as previous studies confirmed that plant extracts coatings could induce hosts to boost up anti-oxidative activity. For example, Hassan pour (2015) observed that Aloe vera gel coatings enhanced the activity of SOD and POD in raspberry fruit. In addition, Ali et al. 2020 reported higher SOD and CAT enzyme activities in lotus root slices with the application of Aloe vera gel coatings in combination with ascorbic acid. Our results were also corroborated with those of Hong et al. (2012); they reported that the activities of SOD, POD and CAT in guava fruit increased with chitosan application as coating. In our study we found that fruit samples coated with Aloe vera gel and Neem extracts had higher SOD, POD and CAT activities as compared with control one, this clearly indicating that these edible plants extract coatings delayed the senescence of Peach fruit.

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals discovered in the Indian Blood Peach variety have a wide range of activities that may help in the prevention of chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties and are also responsible for central nervous system activities. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility (Chunget al. 1998). The presence of flavonoids in Indian blood variety shows that this variety involved in addition to having effects on mammalian metabolism. Flavonoids have received considerable attention because of their beneficial effects as antioxidants preventing human diseases such as cancer and cardiovascular diseases, and some pathological disorders of gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections (Yao et al. 2004).

Conclusion

Application of chemical-free edible coatings like Aloe vera gel and Neem leaf extract has the ability to prolong Peach fruit marketability. The effect of Aloe vera gel and Neem plant extract was found more promising in reducing the loss of total phenolic and total flavonoid content and increased the antioxidant enzymes of Peach fruit. Therefore, Aloe vera gel and Neem plant extracts could be an eco-friendly way, non-chemical treatment for overcoming the physicochemical changes and keeping the nutritional values during postharvest storage of Peach.

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