

RESEARCH ARTICLE

Postharvest Application of *Aloe Vera* gel improved shelf life and quality of strawberry (*Fragaria x ananassa* Duch.)

Muhammad Adil Rehman^{1,2*}, Amjad Hameed¹, Zulfiqar Ahmad², Shabbir Ahmad³, Muhammad Imran Tipu^{1,4}, Faiz-ul-Hassan Shah², Tahir Mehmood², Leslie D. Bourquin⁵, Shabbir Hussain^{1,6}

¹Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences, Faisalabad, Pakistan, ²Department of Food Science & Technology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan, ³Department of Food Science and Technology, Muhammad Nawaz Shareef University of Agriculture, Multan, ⁴Department of Botany, University College of Life Sciences, The Islamia University of Bahawalpur-Pakistan, ⁵Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI, 48824-1224, USA, ⁶Central Analytical Facility (CAF) Division, PINSTECH Nilore, Islamabad

ABSTRACT

Strawberry is highly appreciated for its aroma and texture, but rapid detrimental changes lead to decay and loss of quality in strawberries, which are the main reasons for huge economic losses. To tackle this major concern, this research focused on assessing the efficacy of edible coatings based on aloe vera (AV) gel to maintain quality parameters and postharvest storage of strawberries. Fruits were coated with gel containing one of five different concentrations of AV gel (0%, 20%, 40%, 60% and 80%) and stored at 1 °C and 95% relative humidity (RH) for up to 20 days. The weight loss, total soluble solids and malondialdehyde content was higher in untreated fruits, whilst these parameters were reduced in treated fruits. The AV gel treated fruits also maintained firmness, titratable acidity, ascorbic acid and total antioxidant activity, whereas, a significant decrease in these parameters was observed in untreated fruits. Furthermore, AV gel coated fruits had better superoxide dismutase, catalase and ascorbate peroxidase activities than uncoated fruits. Additionally, AV gel coatings reduced the rate of loss of total phenolic and flavonoid contents during storage. Consequently, it is concluded that AV gel edible coating is recommended for improving postharvest life and for maintaining maximum quality characteristics of strawberry stored for 20 days stored at 1 °C and 95% RH. Among all treatments, 80% AV gel revealed the best results in maintaining the overall fruit quality and delaying ripening throughout the storage period.

Keywords: Strawberry; Edible coating; *Aloe vera* gel; Postharvest management; Antioxidant enzymes; HPLC

INTRODUCTION

Strawberries, (*Fragaria x ananassa* Duch.) among the most admired summer fruits globally, are appreciated for their distinctive and exceptionally appealing aroma and flavor, and are also nutritionally rich in many health benefits compounds such as polyphenols, anthocyanins, vitamins and amino acids (Campaniello, Bevilacqua et al. 2008). In Pakistan, the total cultivation area for strawberry is 179 hectares with the production of 609 tons annually (Shahzad, Ahmad et al. 2020). However, the strawberry fruits have very short postharvest life because of their high perishability due to loss in water, fungal decay, higher respiration rate and physiological deterioration, and are also vulnerable to mechanical injury (Vargas,

Albors et al. 2006). Methods used such as controlled or modified atmospheres have been used to increase shelf life of strawberries (Martínez-Romero, Guillén et al. 2003), however, more effective techniques with efficacy for maintaining postharvest quality are in need to investigate.

Previous research on edible coatings has demonstrated promising outcomes in enhancing postharvest life and maintaining the quality of many fruits such as strawberries (Vargas, Albors et al. 2006) and papaya (Tapia, Rojas-Graü et al. 2008). These coatings act as moisture and gas barriers, slowing the rate of respiration and preserving color and texture (Bourtoom 2008). *Aloe vera* plants contain glutinous, colorless gel from the parenchyma origin cells (He, Changhong et al. 2005).

*Corresponding author:

Muhammad Adil Rehman, Nuclear Institute for Agriculture and Biology College, Pakistan Institute of Engineering and Applied Sciences, Faisalabad, Pakistan, Department of Food Science & Technology, Faculty of Agriculture and Environmental, The Islamia University of Bahawalpur-Pakistan. **E-mail:** adil.rehman@iub.edu.pk

Received: 26 October 2021; **Accepted:** 14 June 2022

In fruits like nectarines, papaya, table grapes and sweet cherries, *Aloe vera* (AV) gel coating favors modified atmosphere of internal gases, which decreases moisture loss, respiration rate, oxidative browning, tissue softening, and microorganism proliferation (Ahmed, Singh et al. 2009); (Valverde, Valero et al. 2005); (Martínez-Romero, Alburquerque et al. 2006). In addition, AV gel coatings in conjunction with additives help to keep the color and firmness of fresh-cut apple slices (Chauhan, Raju et al. 2011).

Calcium salts have been used widely in the fruit and vegetable manufacturing as a firming and preservative agent. Calcium is important for preserving cell wall structure by keeping the pectic acid interactive inside the cell wall, resulting in calcium pectate (Javed, Randhawa et al. 2016). Loquat fruits showed higher firmness and postharvest life when treated with CaCl_2 in comparison to untreated fruits (Akhtar, Abbasi et al. 2010). (Manganaris, Vasilakakis et al. 2007) found that immersion of peaches in 62.5 mM CaCl_2 treatment increased the firmness of tissues compared to untreated peaches. Additionally, the efficacy of ascorbic acid (AA) either used alone or its derivatives (0.5-4 % (w/v) to maintain fruit postharvest quality has been studied extensively. It is used as an ingredient in the formulation of edible coatings because of its anti-browning and antimicrobial properties (Tapia et al., 2008). Considerable research has examined the effects of AA on antimicrobial activity for fruits, both whole and fresh-cut sliced, such as apple (Perez-Gago, Serra et al. 2006), jackfruit (Acedo, Varron et al. 2012) and papaya (Tapia et al., 2008).

The current study was designed to evaluate the effect of *Aloe vera* (AV) gel application as an edible coating with the addition of AA and CaCl_2 as additives on postharvest life and quality of fresh strawberry fruit.

MATERIALS AND METHODS

Plant material

Fine-quality strawberry fruits of Chandler cultivar (*Fragaria ananases*) were harvested from a Farm near Samundari, District Faisalabad, Punjab, Pakistan. Fully mature, uniform size and marketable fruits were harvested without any signs of disease and injury. After harvesting strawberry fruits were transported to laboratory. The fruits were sorted and evaluated according to size, shape, and color uniformity. Strawberry fruits were sampled promptly to determine its quality traits at the time of harvest (0 day).

Aloe vera (AV) gel preparation

Aloe vera plants were cultivated in clay pots (5 kg soil) in a net house at the Nuclear Institute for Agriculture and Biology College (NIAB-C) Faisalabad, Pakistan. At

maturity (180 days), leaves were cut with a sharp knife and taken to the laboratory. The leaves (averaging 415 g) were washed with distilled water, dried in the shade, and the gel was separated. The gel matrix was blended in high-speed blender for 15 min at 25 °C to obtain a homogeneous mixture and filtered through filter cloth of normal mesh size (1-2 mm) to remove any coarse rind particles and to yield a fiber free gel. The gel was batch pasteurized at (70 °C, 40 min) and then stabilized by cooling to room temperature (25 °C). Ascorbic acid (4% w/v) and CaCl_2 (3% w/v) were added, respectively, to the *aloe vera* gel (Sogvar, Saba et al. 2016; Rehman, Asi et al. 2020). Glycerol (1%) was added to improve coating efficiency and viscosity. Tween 20 was applied to the gel at a concentration of 0.03 % (v/v) to aid in adhesion. Distilled water was used to prepare all the coating solutions containing different levels of AV gel (0, 20, 40, 60 and 80%). All ingredients were homogenized to obtain smooth mixture of coating gel solutions.

Coating treatments and storage conditions

Five AV gel treatments: 0% (control), 20%, 40%, 60%, and 80% AV gel were used. Control samples were treated with autoclaved distilled water. Fruits were treated with AV gel solutions by following the method of (Hassanpour 2015). All fruits were dipped for 1 min in AV gel solutions, dried with air at ambient temperature and packed in a polystyrene box (each contained 10 fruits) and stored up to 20 days at 1 °C and 95% relative humidity. The control fruits were dipped in distilled water for 1 min and stored under the same conditions. After every 4th day, the data on quality parameters and shelf life were recorded up to 20 days of storage.

Percent loss in weight

By weighing the fruits on a digital balance, the percent weight loss was determined. After dipping each dried fruit in the respective treatments before storage (W_1), the first reading was taken, and the second reading was taken at the end of each sampling day (W_2). The percent weight loss was calculated as followed:

$$\text{Loss in weight \%} = \frac{W_1 - W_2}{W_1} \times 100$$

Firmness

For measuring hardness of fruits, a GY-4 Fruit Penetrometer (Zhejiang Top Cloud- Agri Technology Co., Ltd., Zhejiang, China) was used. Two different probes of 3.43 mm and 7.87 mm diameter were used. Mean force values (N) were recorded (Martínez-Romero et al., 2006).

Titrateable acidity (TA) and total soluble solids (TSS)

The TA of strawberry fruit pulp was determined by neutralizing the acid present in known quantity of pulp against 0.1 N NaOH (Yen, Yang et al. 2008). TSS was

calculated by using digital handheld refractometer and expressed as °Brix. The pH meter (Loviband pH-200) was used to determine the pH value of fruits.

Ascorbic acid (AA)

Ascorbic acid (AA) content was measured by using two reagents 2,6-dichlorophenol indophenol and metaphosphoric acid were used with sample; then after diluted with distilled water, mixed thoroughly and absorbance determined at 520 nm (Pisoschi, Danet et al. 2008)

Lipid peroxidation (Malondialdehyde, MDA)

The amount of lipid peroxidation in strawberry fruits treated with AV gel formulations and control were determined by malondialdehyde (MDA) content measured by the action of thiobarbituric acid following the method (Sun, You et al. 2011) with slight modifications. A 25 µL sample was homogenized in 0.1% TCA and centrifuged at 10,000 × g for 5 min. TBA was added to 1 mL aliquot of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifuging at 10,000 × g for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the nonspecific absorption at 600 nm was subtracted.

Total phenolic (TP) concentrations

Total phenolic concentration (TPC) in strawberry fruits was measured by the Folin-Ciocalteu (F-C) colorimetric method by following the procedure of (Nair, Saxena et al. 2018).

Total antioxidant activity (TAA)

The DPPH (2,2-diphenyl-1-picryl-hydrazil) radical scavenging method with spectrophotometric (UV-2100) measurement of light absorbance at 517 nm was used to determine total antioxidant activity (TAA), according to the method of (Sánchez-Moreno, Larrauri et al. 1998). TAA presented as inhibition (%) of DPPH radical formation and is calculated using the following formula.

$$\text{TAA (\%)} = \frac{\text{Absorbance (sample)} - \text{Absorbance (control)}}{\text{Absorbance (sample)}} \times 100$$

Assay of enzyme activities

Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities were examined by homogenizing the sample of fruit in 50 mM potassium phosphate buffer (pH 7.0). The mixtures were then centrifuged at 10,000 rpm for 10 min and supernatant was collected to determine the enzyme activity assays. In addition, nitroblue tetrazolium (NBT) technique was

used for the assay of SOD activity as described by earlier researchers (Ali, Khan et al. 2016). SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT. Whereas, an absorbance change of 0.01 unit's min⁻¹ was defined as one unit CAT activity. Additionally, the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 3 min for APX enzyme assay.

Determination of flavonoids compounds (rutin, myricetin, quercetin and kaempferol)

Sample preparation

Flavonoids were extracted from strawberry juice samples for the analysis through high performance liquid chromatography (HPLC) by placing the sample (10 mL) in 50 mL eppendorf tubes and adding 100% methanol (25 mL) to each tube (Tewari, Gupta et al. 2017). The tubes were sonicated by using an Ultrasonic bath (Sigma-Aldrich, USA) for 20 min and then centrifugation (10,000 rpm for 10 min) was achieved at 25 °C for 10 min. The resultant supernatant was collected and methanol (5 mL) was added to the residual and centrifuged again at 10,000 rpm. Combined supernatants for each sample were filtered using Whatman No. 42 filter paper. 05 g anhydrous sodium sulfate was added to each sample before filtration to eliminate surplus water. These filtered samples were analyzed to determine the concentrations of rutin, quercetin, myricetin and kaempferol in each strawberry sample by HPLC.

HPLC system and conditions

The HPLC (LC-10A, Shimadzu, Japan) was used for the quantitative and qualitative determination of rutin, quercetin, myricetin and kaempferol. The mobile phase comprises of two solvents (trifluoroacetic acid 3% and combination of acetonitrile and methanol at ratio of 80:20 v/v) were mixed (50:50 v/v) and used in isocratic mode at 0.8 mL/min flow rate and 360 nm. The UV-Vis (SPD 10-A) detector was used and column temperature was set at 30 °C. The C18 (250 × 4.6 mm, 5 µm particle size, Supelco, USA) analytical column was used for separation of flavonoids. The retention time and peak area of reference standards were compared to measure individual flavonoids (Rehman, Asi et al. 2020).

Standard solution

Flavonoid standards (quercetin, rutin, myricetin and kaempferol) were imported from Sigma-Aldrich, USA, and were prepared in 100% methanol at various concentrations (Fig. 1). To evaluate the detector's limit of detection (LOD) and linearity (response) for each flavonoid standard, a series of solutions (0, 10, 20, 50, 100, 200, and 300 g/ml) were prepared.

Statistical analysis

The data were analyzed by Analysis of Variance as a completely randomized design with a factorial arrangement

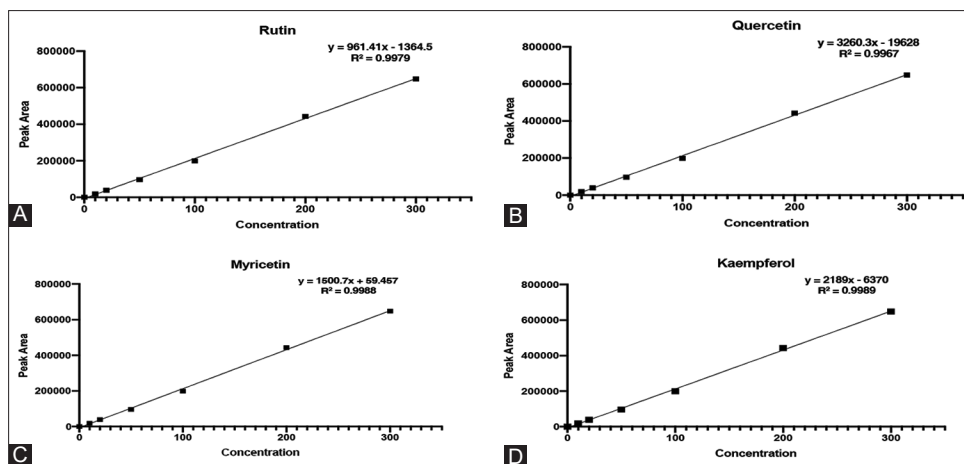


Fig 1. (A-D) Linear curves of standards (rutin, quercetin, myricetin and kaempferol)

of treatments (coating concentrations and storage days) using SAS 9.4 software. All the parameters were assessed at 5% significance level. Treatment effects were determined using Tukey's (HSD) multiple comparison test. Results are presented as means \pm SD. GraphPad Prism 8 software (<http://www.graphpad.com>) was used to generate Figures.

RESULTS AND DISCUSSION

Weight loss

Weight loss was observed regardless of the treatments during storage, but its percentage was higher in untreated fruits than AV gel treated fruits (Fig. 2A). AV gel 60% treatment inhibited weight loss than any other treatment. Although, AV gel 40% also significantly reduce fruit weight loss compared to controls. At the completion of the storage study, higher weight loss was recorded in control samples (1.19%) compared with 0.81% in AV gel 60% and 0.87% in AV gel 40%.

Loss in water due to transpiration and respiration rates encourages loss of carbon reserves, which are the main reasons for weight loss in fruits (Vogler and Ernst 1999). AV gel showed significant effect on strawberry fruits in developing a physical barrier which reduces moisture loss and, therefore, hinders the dehydration and shriveling of fruits (Fig. 2A). AV gel also reduced weight loss of grapes in as demonstrated by Valverde et al. 2005. It has also been stated that chitosan (1%) with 2% AA and 0.5% CaCl₂ reduced weight loss in coated apple fruits compared to untreated apples (Qi, Hu et al. 2011). These presented results clearly recommend that AV gel has significant effect in decreasing weight loss of the strawberry fruits and, hence, increasing the shelf life of strawberries.

Firmness

Aloe vera (AV) gel treated fruits soften slowly in contrast to non-treated fruits, although firmness of fruits decreased

in all treatments during storage time (Fig. 2B). All AV gel treatments maintained strawberry fruit firmness compared to untreated controls. The AV gel 80% treatment reduced fruit softening to the greatest extent compared to other treatments.

The most important factor that appeals the consumer's acceptability is firmness. Strawberries has a tendency to soften (Perkins-Veazie 1995) which makes strawberry fruit highly susceptible to fungal contamination during postharvest storage. Coating with AV gel slows down softening because of its barrier properties reducing O₂ uptake, hence it reduces the metabolic rate and ultimately the process of ripening in fruits. Similar findings were reported by (Reddy, Belkacemi et al. 2000), who sprayed chitosan coating on strawberry fruits. These results indicate that AV gel can be used to maintain firmness of strawberry fruits for enhancement of shelf life.

Total soluble solids (TSS) and titratable acidity (TA)

During storage, TSS increased in uncoated fruit but in 60% and 80% AV gel treated fruits showed stable TSS concentration through 4th days storage and started to increase slightly thereafter (Fig. 2C). TSS in uncoated fruits were significantly higher compared to coated fruit after 4th days of storage. At the termination of experiment, all AV gel treatments significantly reduced TSS relative to controls, with 60% and 80% AV gel treatments being more effective than 20% and 40% AV gel. A decline in TA was observed in both uncoated and coated fruits but all AV gel treatments had greater TA contents than uncoated fruits at the end of the experiment (Fig. 2D).

The primary reason for decline in TA during storage is due to metabolic changes impacting organic acids during the respiratory process (Sogvar, Saba et al. 2016). Moreover, solubilization of the cell wall polyuronides and hemicelluloses in fully ripe strawberry also facilitates

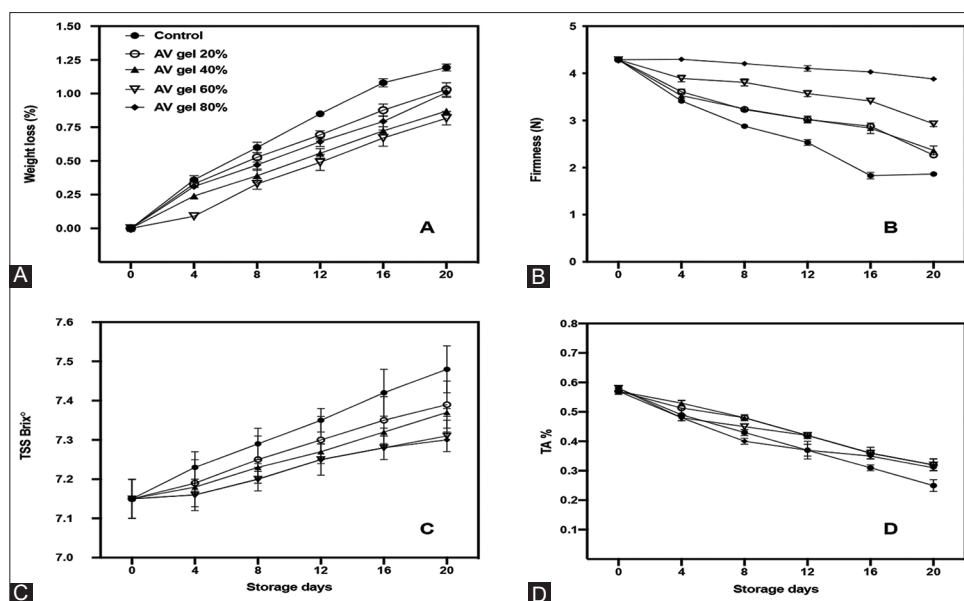


Fig 2. Effect of AV gel treatments at different concentrations on weight loss (%) (A), firmness (N) (B), total soluble solids (Brix°) (C) and titratable acidity (%) (D) of strawberry fruits. Vertical bars represent standard errors.

an increase in TSS (Tanada-Palmu and Grosso 2005). AVG coating provides a modified internal atmosphere, (Martínez-Romero et al. 2003), and also its gas barrier property reduces oxygen uptake which, consequently, reduces fruit respiration (Arowora, Williams et al. 2013).

Ascorbic acid (AA)

The AA content in non-coated fruits decreased significantly during the storage period, but AA loss was significant reduced in AV gel coated fruits, with greatest reduction observed in fruits treated with 80% AV gel (Fig. 3A). Although all other AV gel treatments tried to mitigate the decrease in AA content during storage (Fig. 3A). The AA content in control fruits decreased from 700 $\mu\text{M}/\text{g}$ to 340 $\mu\text{M}/\text{g}$ during the storage period, whereas in 80% AV gel treated fruits, AA concentration was 599 $\mu\text{M}/\text{g}$ at the termination of the experiment.

The possible reason for loss of AA is auto-oxidation, which occurs when AA mixes with oxygen present in the atmosphere (Owusu-Yaw, Marshall et al. 1988). AV gel coating performs as a protecting sheet and also controls the permeability of O_2 and CO_2 , hence, lowering the auto-oxidation of AA (Mditshwa, Magwaza et al. 2017). These outcomes in this study are consistent with those of Sogvar, Saba et al. (2016) who demonstrated that AV gel along with addition of ascorbic acid were effective in maintaining the ascorbic acid levels in strawberries.

Malondialdehyde (MDA) and total phenolic (TP) concentrations

Application of AV gel coatings suppressed MDA formation in fruit treated with AV gel (20%, 40%, 60% or 80%) as

compared to non-treated samples (Fig. 3B). It was evident that AV gel 80% treatments were much efficacious in reducing the levels of MDA and inhibiting the shriveling of the fruit. Overall, MDA content was increased gradually in treated fruits whereas rapid increase was exhibited in control samples.

Lipid in the epidermis of fruits can be oxidized into MDA, which is indicative of damage to the fruit membrane. Hence, increased MDA content is associated with membrane injury in fruit. This membrane injury can ultimately manifest as ruptures or shriveling of the skin of the fruit (Sun, You et al. 2011). It was also reported by (Hong, Xie et al. 2012) that the effect of chitosan coating on guava fruit was efficacious to reduce MDA formation during storage.

TP concentrations at harvest was 37.25 mM/g (gallic acid equivalents) and decreased swiftly throughout storage in uncoated fruit (Fig. 3C) and was 22.85 mM/g after 20 days of storage. Coating fruits with AV gel reduced losses of TP concentrations, with 80% AV gel treated fruits having TP concentration of 35.43 mM/g after 20 days of storage. Fruits treated with 40% and 60% AV gel also responded well in contrast to control or 20% AV gel treated fruits.

Phenolic compounds are secondary metabolites that possess antioxidant activity which can inhibit free radical formation produced during oxidative stress (Wasim Aslam, Inam-ur-Raheem et al. 2018). TPC content retards oxidative degradation of lipids, and nutritional value of food is maintained (Shah, Khan et al. 2017). Our results are in line with the work of (Anjum, Akram et al. 2020) as in their

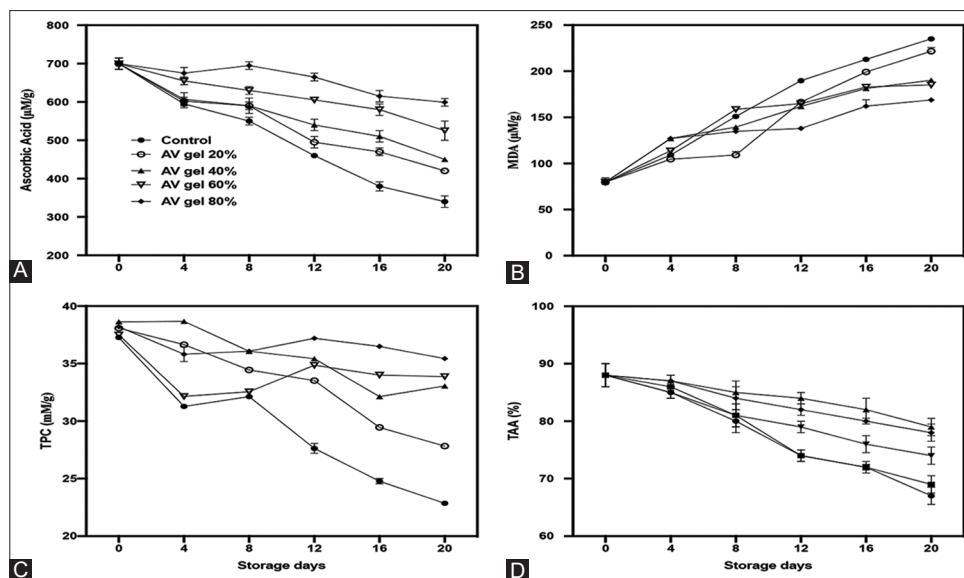


Fig 3. Effect of AV gel treatments at different concentrations on ascorbic acid µM/g (A), MDA µM/g (B), total TP concentration mM/g and TAA % (D) of strawberry fruits. Vertical bars represent standard errors.

findings TP concentration was also higher in response to AV gel application. Comparable results were reported in gabriroba fruit grown in Brazil and stored at different storage temperatures (Silva, Cardoso et al. 2013).

Total antioxidant activity (TAA)

TAA decreased in all fruits regardless of treatment (Fig. 3D). Antioxidant activity at start of storage was 88% and it decreased to 67% in untreated fruits. Fruits treated with 80% AV gel showed relatively stable antioxidant activity until the 8th day of storage before it began to decline. The TAA decreased in 80% AV gel treated fruits from 88% to 79% during the storage period. Fruits treated with 60% AV gel also had minimal declines in TAA from initiation to termination of the experiment (88% to 78%).

AV gel coating increases the resistance of fruit to deterioration through improving antioxidant capacity and free radical scavenging capability (Hu, Hu et al. 2005). TAA is directly proportion to the presence of effective oxygen radical scavengers (Tulipani, Mezzetti et al. 2008). Our findings are well-supported with research by other groups who reported that fruits treated with AV gel coatings had maintained better antioxidant capacity in contrast to untreated grapes (Serrano, Valverde et al. 2006) and raspberry (Hassanpour 2015).

Enzyme activities

SOD activity decreased in both untreated and AV gel treated fruits. SOD activity at the start of storage was 150 units/g and it decreased to 60 units/g in non-coated fruits. AV gel 60% treated fruits showed relatively stable antioxidant activity till 8th day of storage before its sudden decline. The

SOD decreases in AV gel 60% ranged from 160 units/g to 103 units/g during storage period (Table 1). AV gel 40% and 60% treated fruits also presents appreciable attempt in maintaining the SOD ranging from 152 units/g to 88 units/g and 151 to 94 units/g, respectively, during storage period. CAT activity decreased in both untreated and AV gel treated fruits. CAT activity at start of storage was 530 units/g and it decreased to 160 units/g in non-coated fruits. The SOD decrease in AV gel 60% and 80% treated fruits was observed same ranged from 530 units/g to 260 units/g during storage period (Table 2). APX activity decreased in both untreated and AVG treated fruits. APX activity at start of storage was 1420 units/g and it decreased to 380 units/g in control fruits. The APX decrease in AV gel 80% ranged from 1420 units/g to 540 units/g during storage period (Table 3). AV gel 60% treated fruits presents better results in maintaining the APX ranging from 1420 units/g to 600 units/g during the storage period.

Antioxidative enzymes such as CAT, POD and AP stimulate the plant defense system against different biotic and abiotic stress. In anaerobic species, SOD is a powerful defence enzyme that inhibits superoxide damage. Same as APX and CAT facilitates the free radicals eradications which can cause stress damage to cells. AV gel coating restricts senescence and preserves higher antioxidant enzymes activities (Hassanpour 2015); (Mirshekari, Madani et al. 2019). Our results are in accordance to the author (Ali, Khan et al. 2019) who determined that anti-oxidative enzymes such as SOD, CAT and APX purify many varieties of free radicals and lower browning of litchi fruit by alleviating oxidative damage.

Table 1: Effect of AV gel treatments at different concentrations on superoxide dismutase (SOD) of strawberry. Letters shows significant differences ($p \leq 0.05$)

	0 Days	4 Days	8 Days	12 Days	16 Days	20 Days
Control	150 ± 0.30 b	90 ± 0.10 k	89 ± 0.50 k	88 ± 0.50 k	72 ± 0.30 m	60 ± 0.40 n
AV Gel 20%	126 ± 0.50 d	112 ± 1.00 g	112 ± 3.00 g	96 ± 0.70 j	81 ± 2.20 l	63 ± 0.60 n
AV Gel 40%	152 ± 1.50 a	122 ± 0.50 e	117 ± 0.50 f	106 ± 0.40 h	101 ± 1.50 i	88 ± 1.10 k
AV Gel 60%	162 ± 1.00 a	141 ± 0.20 b	139 ± 0.90 b	127 ± 0.90 d	114 ± 0.50 g	103 ± 2.50 hi
AV Gel 80%	151 ± 1.75 a	133 ± 0.10 c	114 ± 2.00 g	106 ± 1.00 h	102 ± 1.20 i	94 ± 1.30 j

Table 2: Effect of AV gel treatment at different concentrations on catalase (CAT) of strawberry. Letters shows significant differences ($p \leq 0.05$)

	0 Days	4 Days	8 Days	12 Days	16 Days	20 Days
Control	530 ± 10 ab	380 ± 50 d-h	340 ± 0 f-j	300 ± 20 g-l	250 ± 40 i-m	190 ± 80 lm
AV Gel 20%	570 ± 70 a	460 ± 0 a-e	380 ± 50 d-h	270 ± 30 h-m	230 ± 20 j-m	170 ± 50 m
AV Gel 40%	520 ± 40 ab	530 ± 160 ab	400 ± 100 c-g	310 ± 90 g-k	260 ± 80 i-m	210 ± 50 km
AV Gel 60%	500 ± 60 a-c	480 ± 40 a-d	480 ± 30 a-d	390 ± 80 c-g	320 ± 50 g-k	260 ± 10 i-m
AV Gel 80%	540 ± 80 ab	520 ± 40 ab	450 ± 20 b-f	360 ± 50 e-i	290 ± 20 g-l	260 ± 70 i-m

Table 3: Effect of AV gel treatment at different concentrations on ascorbate peroxidase (APX) of strawberry. Letters shows significant differences ($p \leq 0.05$)

	0 Days	4 Days	8 Days	12 Days	16 Days	20 Days
Control	1420 ± 20 a	1240 ± 40 ab	920 ± 60 c-e	700 ± 80 e-i	640 ± 80 g-j	400 ± 20 k
AV Gel 20%	1320 ± 60 ab	1140 ± 40 bc	900 ± 60 de	900 ± 100 de	700 ± 80 e-i	440 ± 80 jk
AV Gel 40%	1360 ± 20 ab	900 ± 60 de	540 ± 100 i-k	540 ± 80 i-k	540 ± 100 i-k	380 ± 60 k
AV Gel 60%	1440 ± 80 a	1240 ± 80 ab	1140 ± 20 bc	860 ± 20 d-g	720 ± 40 e-i	600 ± 20 h-k
AV Gel 80%	1360 ± 20 ab	980 ± 40 cd	880 ± 40 d-f	800 ± 40 d-h	660 ± 80 f-j	540 ± 40 i-k

Total flavonoids content (TFC)

The contents of four types of flavonoids compounds in strawberry fruits were qualitatively and quantitative analyzed by HPLC. On the bases of their retention times with the standard solutions, peaks were recognized as rutin, myricetin, quercetin, and kaempferol, respectively (Fig. 4).

According to the Fig. 5, rutin, myricetin, quercetin and kaempferol were detected in all fruits regardless of treatments. The content of all four flavonoids compounds decreased in both control and AV gel treated fruits. Rutin content at the start of storage was 23.39 $\mu\text{g/g}$ and it decreased to 8.23 $\mu\text{g/g}$ in non-treated fruits at the completion of storage. However, fruits coated with AV gel 80% ranged from 23.39 $\mu\text{g/g}$ to 13.98 $\mu\text{g/g}$ from initiation to termination of experiment (Fig. 5A). In non-coated fruits myricetin content during storage decreased from 8.65 $\mu\text{g/g}$ to 2.09 $\mu\text{g/g}$, whereas, fruits coated with AV gel 80% maintained myricetin content from 8.65 $\mu\text{g/g}$ to 5.62 $\mu\text{g/g}$ (Fig. 5B). Quercetin and kaempferol contents were also decreased in non-coated fruits during storage ranged from 0.038 $\mu\text{g/g}$ to 0.011 $\mu\text{g/g}$ and 0.17 $\mu\text{g/g}$ to 0.047 $\mu\text{g/g}$, respectively, whereas this decrease in fruits coated with AV gel 80% ranged from 0.038 $\mu\text{g/g}$ to 0.25 $\mu\text{g/g}$ and 0.17 $\mu\text{g/g}$ to 0.11 $\mu\text{g/g}$ during storage period (Fig. 5C, D).

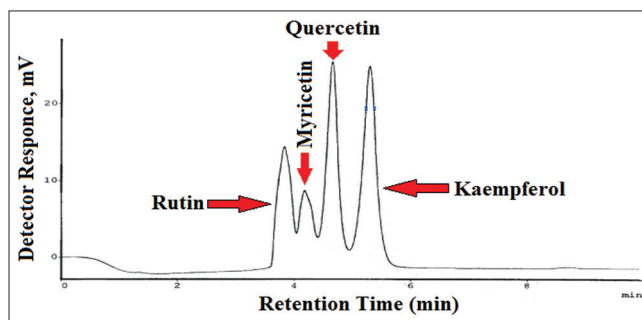


Fig 4. HPLC chromatogram of four compounds by their retention times (min): rutin (3.82), myricetin (4.17), quercetin (4.65) and kaempferol (5.29).

Phenolic and flavonoid compounds act as primary antioxidants (Viera, Piovesan et al. 2017), and they are known to react with hydroxyl radicals, superoxide anion radicals (Márquez, Contreras et al. 2019). AV gel facilitates flavonoids to induces phenylalanine ammonia lyase biosynthesis to the fruit which might be allow the fruit to maintain flavonoid content for longer period of time (Khalil, Khan et al. 2018). These outcomes are in close agreement with the authors who applied different coatings on fruits and found effective for flavonoids contents in tomato (Firdous, Khan et al. 2020) and guava fruits (Rehman, Asi et al. 2020).

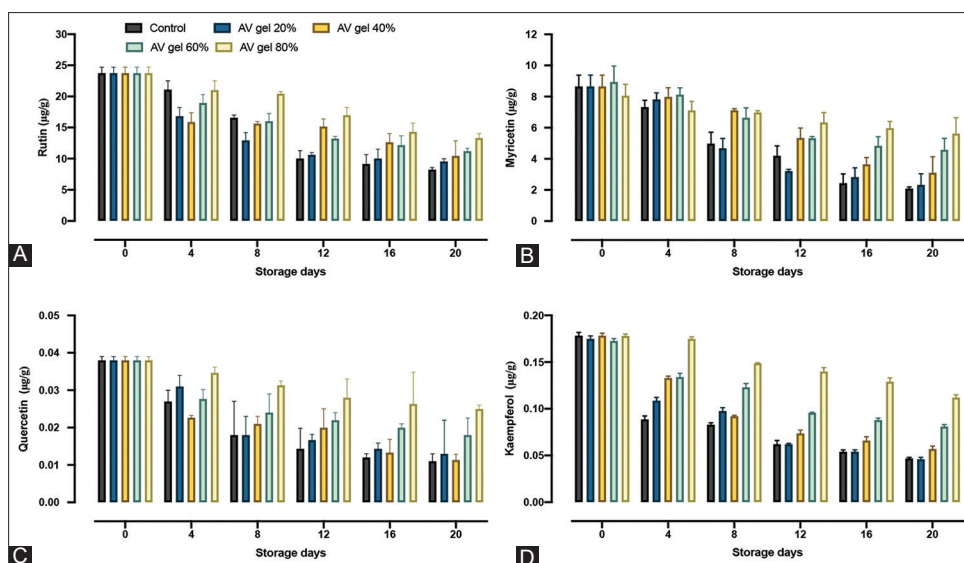


Fig 5. Effect of AV gel treatments at different concentrations on rutin µg/g (A), myricetin µg/g (B), quercetin µg/g (C) and kaempferol (D) of strawberry fruits. Vertical bars represent standard errors.

CONCLUSION

In conclusion, the present study suggests that AV gel concentrations could extend shelf life of strawberry fruits by preserving the quality attributes including fruit firmness, TSS, TA, ascorbic acid (AA) and total antioxidant activity (TAA). It reduces the decay by maintaining the activities of SOD, CAT and APX. It also has respectable effects on TPC and flavonoids content. These results propose that *aloe vera* gel treatments, particularly AV gel 60% and 80% could be considered an effective and environment friendly application for delaying ripening processes and retaining quality of strawberry fruit through decreasing oxidative stress during postharvest storage conditions.

ACKNOWLEDGMENTS

Authors are highly thankful to NIAB and PIEAS management to provide the analytical facilities to carry out research work in Food Toxicology Laboratories.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Author Contributions

A.H. conceptualized the work and conceived the experimental design; M.A.R. and S.H. collected samples and carried out formal analyses; A.H. performed statistical analyses and helped in the interpretation of the results and in the revision of the manuscript; A.H., Z.A. and L.D.B. contributed to the writing and interpretation of the

results; M.A.R., F.U.H., T.M., and M.I.T. read the paper and approved the published version of the document; S.A. reviewing, editing, and writing the final draft. All authors have read and agreed to the published version of the manuscript.

REFERENCES

- Acedo, J., D. Varron, I. Emnace, R. Lauzon and A. Jr. Acedo. 2012. Antimicrobial effects of ascorbic acid and calcium lactate in freshcut jackfruit (*Artocarpus heterophyllus* Lam.). vol. 989. In: Southeast Asia Symposium on Quality Management in Postharvest Systems and Asia Pacific Symposium on Postharvest Quality, pp. 199-208.
- Ahmed, M. J., Z. Singh and A. S. Khan. 2009. Postharvest *Aloe vera* gel-coating modulates fruit ripening and quality of 'Arctic Snow' nectarine kept in ambient and cold storage. *Int. J. Food. Sci. Technol.* 44: 1024-1033.
- Akhtar, A., N. A. Abbasi and A. Hussain. 2010. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Pak. J. Bot.* 42: 181-188.
- Ali, S., A. S. Khan, A. U. Malik and M. Shahid. 2016. Effect of controlled atmosphere storage on pericarp browning, bioactive compounds and antioxidant enzymes of litchi fruits. *Food. Chem.* 206: 18-29.
- Ali, S., A. S. Khan, A. Nawaz, M. A. Anjum, S. Naz, S. Ejaz and S. Hussain. 2019. *Aloe vera* gel coating delays postharvest browning and maintains quality of harvested litchi fruit. *Postharvest Biol. Technol.* 157: 110960.
- Anjum, M. A., H. Akram, M. Zaidi and S. J. S. H. Ali. 2020. Effect of gum arabic and *Aloe vera* gel based edible coatings in combination with plant extracts on postharvest quality and storability of 'Gola' guava fruits. *Sci. Hortic.* 271: 109506.
- Arowora, K., J. Williams, C. Adetunji, O. Fawole, S. Afolayan, O. Olaleye, J. Adetunji and B. Ogundele. 2013. Effects of *Aloe vera* coatings on quality characteristics of oranges stored under cold storage. *Greener J. Agric. Sci.* 3: 39-47.
- Bourtoom, T. 2008. Edible films and coatings: Characteristics and

- properties. *Int. Food Res. J.* 15: 237-248.
- Campaniello, D., A. Bevilacqua, M. Sinigaglia and M. R. Corbo. 2008. Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries. *Food. Microbiol.* 25: 992-1000.
- Chauhan, O., P. Raju, A. Singh and A. Bawa. 2011. Shellac and aloe-gel-based surface coatings for maintaining keeping quality of apple slices. *Food. Chem.* 126: 961-966.
- Firdous, N., M. R. Khan, M. S. Butt and M. Shahid. 2020. Application of aloe vera gel based edible coating to maintain postharvest quality of tomatoes. *Pak. J. Agric. Sci.* 57: 245-249.
- Hassanpour, H. 2015. Effect of *Aloe vera* gel coating on antioxidant capacity, antioxidant enzyme activities and decay in raspberry fruit. *LWT-Food Sci. Technol.* 60: 495-501.
- He, Q., L. Changhong, E. Kojo and Z. Tian. 2005. Quality and safety assurance in the processing of *Aloe vera* gel juice. *Food Control.* 16: 95-104.
- Hong, K., J. Xie, L. Zhang, D. Sun and D. Gong. 2012. Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. *Sci. Hortic.* 144: 172-178.
- Hu, Q., Y. Hu and J. Xu. 2005. Free radical-scavenging activity of *Aloe vera* (*Aloe barbadensis* Miller) extracts by supercritical carbon dioxide extraction. *Food Chem.* 91: 85-90.
- Javed, M. S., M. A. Randhawa, M. S. Butt and H. Nawaz. 2016. Effect of calcium lactate and modified atmosphere storage on biochemical characteristics of guava fruit. *J. Food Process. Preserv.* 40: 657-666.
- Khalil, A. A., M. R. Khan and M. A. Shabbir. 2018. *In vitro* antioxidant activity and punicalagin content quantification of pomegranate peel obtained as agro-waste after juice extraction. *Pak. J. Agric. Sci.* 55: 197-201.
- Manganaris, G., M. Vasilakakis, G. Diamantidis and I. Mignani. 2007. The effect of postharvest calcium application on tissue calcium concentration, quality attributes, incidence of flesh browning and cell wall physicochemical aspects of peach fruits. *Food. Chem.* 100: 1385-1392.
- Márquez, K., D. Contreras, P. Salgado and C. Mardones. 2019. Production of hydroxyl radicals and their relationship with phenolic compounds in white wines. *Food. Chem.* 271: 80-86.
- Martínez-Romero, D., N. Alburquerque, J. Valverde, F. Guillén, S. Castillo, D. Valero and M. Serrano. 2006. Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. *Postharvest Biol. Technol.* 39: 93-100.
- Martínez-Romero, D., F. Guillén, S. Castillo, D. Valero and M. Serrano. 2003. Modified atmosphere packaging maintains quality of table grapes. *J. Food. Sci.* 68: 1838-1843.
- Mditshwa, A., L. S. Magwaza, S. Z. Tesfay and U. L. Opara. 2017. Postharvest factors affecting Vitamin C content of citrus fruits: A review. *Sci. Hortic.* 218: 95-104.
- Mirshekari, A., B. Madani and J. B. Golding. 2019. *Aloe vera* gel treatment delays postharvest browning of white button mushroom (*Agaricus bisporus*). *J. Food. Meas. Charact.* 13: 1250-1256.
- Nair, M. S., A. Saxena and C. Kaur. 2018. Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). *Food. Chem.* 240: 245-252.
- Owusu-Yaw, J., M. Marshall, J. Koburger and C. Wei. 1988. Low pH inactivation of pectinesterase in single strength orange juice. *J. Food. Sci.* 53: 504-507.
- Perez-Gago, M. B., M. Serra and M. Del Rio. 2006. Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest. Biol. Technol.* 39: 84-92.
- Perkins-Veazie, P. 1995. Growth and ripening of strawberry fruit. *Hortic. Rev.* 17: 267-297.
- Pisoschi, A. M., A. F. Danet and S. J. J. o. A. M. Kalinowski. 2008. Ascorbic acid determination in commercial fruit juice samples by cyclic voltammetry. *J. Autom. Method. Manage.* 937651: 8.
- Qi, H., W. Hu, A. Jiang, M. Tian and Y. Li. 2011. Extending shelf-life of fresh-cut 'Fuji' apples with chitosan-coatings. *Innov. Food. Sci. Emerg. Technol.* 12: 62-66.
- Reddy, M. B., K. Belkacemi, R. Corcuff, F. Castaigne and J. Arul. 2000. Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit. *Postharvest Biol. Technol.* 20: 39-51.
- Rehman, M. A., M. R. Asi, A. Hameed and L. D. Bourquin. 2020. Effect of postharvest application of *Aloe vera* gel on shelf life, activities of anti-oxidative enzymes, and quality of 'Gola'Guava fruit. *Foods.* 9: 1361.
- Sánchez-Moreno, C., J. A. Larrauri and F. Saura-Calixto. 1998. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food. Agric.* 76: 270-276.
- Serrano, M., J. M. Valverde, F. Guillén, S. Castillo, D. Martínez-Romero and D. Valero. 2006. Use of *Aloe vera* gel coating preserves the functional properties of table grapes. *J. Agric. Food Chem.* 54: 3882-3886.
- Shah, H. M. S., A. S. Khan and S. Ali. 2017. Pre-storage kojic acid application delays pericarp browning and maintains antioxidant activities of litchi fruit. *Postharvest Biol. Technol.* 132: 154-161.
- Shahzad, S., S. Ahmad, R. Anwar and R. Ahmad. 2020. Pre-storage application of calcium chloride and salicylic acid maintain the quality and extend the shelf life of strawberry. *Pak. J. Agric. Sci.* 57: 339-350.
- Silva, E. P. D., A. F. L. Cardoso, C. Fante, C. M. Rosell and E. V. D. B. Vilas Boas. 2013. Effect of postharvest temperature on the shelf life of gabirola fruit (*Campomanesia pubescens*). *Food. Sci. Technol.* 33: 632-637.
- Sogvar, O. B., M. K. Saba and A. Emamifar. 2016. *Aloe vera* and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit. *Postharvest Biol. Technol.* 114: 29-35.
- Sun, J., X. You, L. Li, H. Peng, W. Su, C. Li, Q. He and F. Liao. 2011. Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of litchi fruit. *Postharvest Biol. Technol.* 62: 288-294.
- Tanada-Palmu, P. S. and C. R. Grosso. 2005. Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (*Fragaria ananassa*) quality. *Postharvest Biol. Technol.* 36: 199-208.
- Tapia, M., M. Rojas-Graü, A. Carmona, F. Rodríguez, R. Soliva-Fortuny and O. Martín-Belloso. 2008. Use of alginate-and gellan-based coatings for improving barrier, texture and nutritional properties of fresh-cut papaya. *Food Hydrocoll.* 22: 1493-1503.
- Tewari, R., M. Gupta, F. Ahmad, P. K. Rout, L. Misra, A. Patwardhan and R. Vasudeva. 2017. Extraction, quantification and antioxidant activities of flavonoids, polyphenols and pinitol from wild and cultivated *Saraca asoca* bark using RP-HPLC-PDA-RI method. *Ind. Crops Prod.* 103: 73-80.
- Tulipani, S., B. Mezzetti, F. Capocasa, S. Bompadre, J. Beekwilder, C. R. de Vos, E. Capanoglu, A. Bovy and M. Battino. 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J. Agric. Food. Chem.* 56: 696-704.
- Valverde, J. M., D. Valero, D. Martínez-Romero, F. Guillén, S. Castillo

- and M. Serrano. 2005. Novel edible coating based on *Aloe vera* gel to maintain table grape quality and safety. *J. Agric. Food. Chem.* 53: 7807-7813.
- Vargas, M., A. Albors, A. Chiralt and C. González-Martínez. 2006. Quality of cold-stored strawberries as affected by chitosan-oleic acid edible coatings. *Postharvest. Biol. Technol.* 41: 164-171.
- Viera, V., N. Piovesan, J. Rodrigues, R. O. Mello, R. Prestes, R. dos Santos, R. de A Vaucher, T. Hautrive and E. Kubota. 2017. Extraction of phenolic compounds and evaluation of the antioxidant and antimicrobial capacity of red onion skin (*Allium cepa* L.). *Int. Food. Res. J.* 24: 990-999.
- Vogler, B. and E. Ernst. 1999. *Aloe vera*: A systematic review of its clinical effectiveness. *Br. J. Gen. Pract.* 49: 823-828.
- Wasim Aslam, H. K., M. Inam-ur-Raheem, T. Zahoor and M. Shahid. 2018. Integrated effect of ascorbic acid, citric acid and calcium lactate on quality and shelf-life of fresh-cut papaya cubes. *Pak. J. Agric. Sci.* 55: 183-189.
- Yen, M. T., J. H. Yang and J. L. Mau. 2008. Antioxidant properties of chitosan from crab shells. *Carbohydr. Polym.* 74: 840-844.