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RESEARCH PAPER

Preservation of table grapes through innovative techniques

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Key Message: This study concludes that the quality of table grapes can be preserved with the help of chitosan and salt solution ($CaCl_2$ and $NaHCO_3$). These techniques are proved to be useful in reducing the post-harvest losses if their capacity is increased at large scale production.

Abstract: Worldwide population is increasing day by day due to which the demand for food production will be increased by 60% in 2050. However, a huge portion of food is being lost through pre-harvest and postharvest factors. Grape production is increasing in Pakistan during the recent years. Due to the minimum shelf life, it has significant postharvest losses. Therefore, the present study was designed to find out new techniques for improving the quality and overcoming the post-harvest losses of grapes. Chitosan at 0.5% and salts ($CaCl_2$ and $NaHCO_3$) at 2% were sprayed at the pre-harvest stage of King's Ruby grapes. On complete maturity, grapes were harvested and stored at ambient temperature (30-35°C) for 15 days. The results of the study revealed that 0.5% chitosan solution particularly improved the quality of table grapes. After 15 days of storage, the decline in antioxidant and decay incidence was recorded at 13.44 and 13.43%, respectively in treated samples as compared to 20.61 and 20.1% in control. A two-time greater reduction in acidity loss was achieved by Chitosan. Similarly, the antioxidant and phenolic contents of grapes were also preserved to a greater extent. Salts solutions were moderately effective in improving the quality of the shelf life of grapes. Hence, the pre-harvest application of the Chitosan solution proved to be a successful strategy in preserving the quality and shelf life of grapes.

Keywords: Chitosan, Grapes, GRAS chemicals, Pre-harvest, Storage life

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Introduction

Due to increase in world population, the living standard, health consciousness, fruit production and consumption have also increased. The fruit production, consisting of citrus fruits, tropical fruits, stone fruits, bananas, avocados, tomatoes, and melons, was 2,587,570 metric tons in the year 2007 which increased to 34,622,004 metric tons in 2017 (Food and Agriculture Organization [FAO], 2019). Such overall increase in fruit production is important in accordance with the global population growth. It has been predicted that there will a 60% increase in food production by 2050. But the depletion due to post-harvest losses and a decline in the fruit quality poses a crucial challenge to the farmer. Losses to fruits are caused by poor management and storage, improper packaging, as well as fungal and microbial contaminations. Several approaches and techniques to manage post-harvest losses have been established over the last few decades (Alexandratos & Bruinsma, 2012).

Grapes (*Vitis vinifera*) are important fruit commodity belonging to the Vitaceae family. They are one of the main prevalent agricultural crops being cultivated since prehistoric times. There is an evidence of its origin near the Caspian and the Black Sea. Now a day's grapes are produced everywhere subject to a suitable environment (Szalay, 2016). Grapes contain significant quantities of functional substances and phenolic compounds including quercetin, catechin and gallic acid (Wang et al., 2002). The grapes are very perishable due to their high moisture and sugar contents which reduce its shelf life. Therefore they are stored under suitable refrigerated conditions. They are highly susceptible to contamination with pathogenic and spoilage microorganisms. That is why grapes should be consumed or converted to other derived products within few weeks after harvest. The development of management techniques, advances in processing and flow in marketing of grapes are needed. It is much required to adopt cost-effective preservation technologies (Khiari et al., 2019). They fall in the category of non-climacteric fruits having a reduced rate of physiological activity and results in greater post-harvest losses of the produce. The global harvest losses of vegetables and fruits account for 30 to 40% of the produce. In the case of grapes, the post-harvest loss is 16-23% of the product and the most of it is borne by the consumer (Aujla et al., 2011). *Botrytis cinerea* is a major causal organism for gray mold in grapes. Pre-harvest and post-harvest pathological incidences along with the physical and physiological factors are major agents of post-harvest deterioration in grape (Zoffoli et al., 2009). Considering the importance of grapes as fruit and their infection-prone nature, many pre-harvest treatments have

been applied to grapes by scientists and researchers. These include the use of SO_2 pads, CA storage, improved packaging schemes, spraying of certain chemicals and fungicides etc. Over the past decades, there has been a great emphasis on natural substances and materials to increase the storage life of fruits and vegetables (Sanchez-Gonzalez et al., 2011). Recently, safer technologies that have been utilized like microbe mediated biological control, use of GRAS (Generally recognized as safe) botanical pesticides, and innovative physical approaches like cold plasma, and pulsed light techniques. These newly developed technologies are being utilized in the multiple hurdle models (Dukare et al., 2020).

One of the new modified approaches for maintaining the consistency of the fruits is the application of an edible coating that has shown promising results (Guimaraes et al., 2018). To avoid gaseous diffusion, the process uses biological materials as a covering layer on the drug surface and hence prevents the cycle of mixing. Several academic studies have demonstrated this method. The nature of the coating is known as a thin layer that is placed on the fruit surface to establish the barrier that can be consumed as part of the whole product between the fruits and the atmosphere (Baldwin & Hagenmaier, 2011). Physical chemistry of the comestible coatings of the whole fruits requires the increased strength, titrated acidity and vitamin C improvement. In this way, water losses are delayed, the soluble solids in the fresh cuts are enhanced and the color of the products is preserved. Some effects can occur both in entirety and in fresh fruits. This increased researchers ' curiosity, which resulted in extensive research. For example, the use of an edible coating has been reported to reduce the production of polyphenol oxidase (PPO) and peroxidase (POD) and eventually to reduce the physiological effects such as discoloration and anti-browning of strawberry (Vishwasrao & Ananthanarayan, 2017). Kumar et al. (2018) confirmed a visible decrease in PPO and POD development in coated fresh-cut apples consistent with the maintenance of browning symptoms.

Among GRAS substances used to increase the shelf life of foods, edible coating holds a prominent place. Edible coatings not only inhibit the growth of micro-organisms but at the same time prevent the moisture loss through transpiration and keep a firm texture of the fruits and vegetables. Among polysaccharides, chitosan is cationic and is derived from crustaceans or fungi (Fajardo et al., 2010). Chitosan forms an attractive edible layer due to its antibacterial and antimicrobial properties. Based on their antibacterial properties and coating or film-forming ability, chitosan-based coatings and film have been tested on several varieties of cheeses with the goal of minimizing microbiological growth and thus increasing the shelf life of the cheese. Chitosan is known to be effective as a broad-spectrum antimicrobial agent (Plascencia-Jatomea, 2003; Barka et al., 2004). It has many applications in food preservation as a coating material. Grape bunches inoculated with pathogen *Botrytis cinerea* got effective preservation from fungal infection especially when chitosan was applied after the inoculation of the pathogen in the berries. Chitosan in the concentration of 1.5 and 2% showed the best result when the conditions were maintained at 90% RH and 25 ºC (Camili et al., 2007). Like chitosan and many other food preservatives, salts also possess antimicrobial activity. Organic and inorganic salts are given GRAS status by the US regulatory authorities (Youssef & Ruberto, 2014). Salts are safely applied to foods; they are inexpensive, pose no environmental hazards and are non-toxic. Pre-harvest treatments with different salt solutions have been tested by several researchers. Among various salts used as pre-harvest treatments for grapes, potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$) and calcium chloride ($CaCl_2$) have shown the promising results. These salts are found to be the most effective in controlling post-harvest decay of grapes caused by grey mould (Nigro et al., 2006).

Keeping in view all these facts the present study was designed to find out the potential of pre-harvest chitosan and salt solution application in improving the quality of grapes at ambient temperature. It was also desired to search for suitability of novel techniques that could increase the shelf life of grapes and decrease the post-harvest losses.

Material and Methods

Grapes for the experimental purpose were obtained from Barani Agricultural Research Institute (BARI) Chakwal Punjab (Pakistan). Pre-harvest trials were carried out at BARI where grapes were planted. The plants of the variety King's Ruby were used as material. For post-harvest treatments, the grapes were harvested early in the morning and shifted to the Post-harvest laboratory of PMAS Arid Agriculture University Rawalpindi (Pakistan) on the same day and stored at 5 ºC.

Preparation of chitosan solution

Chitosan solution was prepared following the method of Meng et al. (2008). Acetic acid solution at 0.5% was used as a solvent to prepare the chitosan solution. Powdered chitosan was added in dilute acetic acid and the concentration of chitosan solution was also maintained at 0.5%. 1M NaOH was used to adjust the pH of solution at 5.6. Tween-80 at the concentration of 0.05% was added in the solution to act as a surfactant.

Pre-harvest treatments

Grapes were sprayed with different treatments comprising of chitosan solution, salt solutions and combination of both. Fully ripe bunches of grapes were selected on the plant. Bunches of different plants were used as replication. The treatments were applied 15 days prior to harvest.
The following treatments were followed.
Treatment 1: Control (Sprayed with distilled water)
Treatment 2: 2% $CaCl_2$ solution
Treatment 3: 2% $NaHCO_3$ solution
Treatment 4: 0.5% Chitosan solution

Storage of samples

On complete maturity, grapes were harvested and stored at ambient temperature (30-35 ºC) for 15 days by placing them separately in trays and packaged with polythene.

Extraction of grape juice

Few grape bunches from every replication were crushed to extract the juice. It was used for carrying out various quality and physicochemical tests. The skin was separated from grapes and pulp of the berries was put in a juice extractor. Juice from all treatments was separately stored in small-sized glass bottles.

Physiochemical analysis

Different physiochemical tests were carried out to analyze the change in the quality of grapes. These include the following parameters.

Moisture loss %

Moisture loss or weight loss from samples was determined by accurately weighing them before and after the storage period. The difference in weight indicated the loss of moisture from the grapes. Moisture loss was expressed in percentage. The following formula was used in weight loss determination.

$$\text{Moisture loss (\%)} = \frac{InitialWeight - FinalWeight}{InitialWeight} \times 100$$

The research was carried out in the month of July and the ambient temperature of the post-harvest laboratory was 30-35 ºC. So, both types of treatments were stored for 10 days at ambient temperature and moisture loss was determined at the end of 10 days of storage.

Percent decay

The decay percentage was calculated by examining the grape bunches carefully after an interval of 3 days. Infected and decayed berries were counted from each bunch and its ratio to a total number of berries served as the decay percentage.

$$\text{Percent decay (\%)} = \frac{Decayed\ Berries}{Total\ number\ of\ Berries} \times 100$$

Titratable acidity

Titratable acidity was measured by potentiometric titration following (Association of Official Analytical Chemists [AOAC], 2000) official method No. 942.15. 1 ml juice was diluted with 25 ml distilled water and then titrated against 0.1 N NaOH solution to a pH of 8.1. Titratable acidity of grape juice was expressed in terms of tartaric acid using the following formula:

$$\text{\% Acid} = \frac{(\text{ml Base titrant}) \times (\text{Normality of base}) \times (\text{Equivalent weight of acid})}{Sample\ volume\ (ml) \times 10}$$

Total phenolics

The total phenolic content of grapes was measured according to Folin–Ciocalteu method as used by Ivanova et al. (2010). One ml of grape juice was taken in 10 ml volumetric flask and 5 ml distilled water was added in it. 0.5 ml Folin–Ciocalteu reagent was added in the juice mixture and shaken properly. After a few minutes, 1.5 ml of 0.5 % sodium carbonate was added in the flask. Different solutions of gallic acid in a concentration ranging from 0 to 100 % were prepared. Absorbance values of these solutions were taken from spectrophotometer (UV-9200 Biotech Engineering Management Co. Ltd. UK) at 765 nm and a standard curve was plotted. The absorbance of sample values was taken afterward and then compared with readings of the standard curve to determine the concentration of total phenolics.

Antioxidant activity

The changes in antioxidant activity were measured by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. DPPH solution was prepared by mixing 0.03g DPPH solution (Sigma–Aldrich) per liter of liquid methanol (Sigma–Aldrich). 0.1 ml of grape juice was taken in a glass test tube and 3.9 ml of freshly prepared DPPH solution was added in it. The mixture was kept in dark for 30 minutes. The mixture was then transferred to cuvet and antioxidant activity was measured on a spectrophotometer (UV-9200 Biotech Engineering Management Co. Ltd. UK) at the wavelength of 517 nm (Sanchez-Moreno et al., 2003). The following formula was used for calculating the percent antioxidants in the samples.

$$\text{DPPH (Scavenging ability) \%} = \left(\frac{1 - Sample\ Absorbance}{Blank\ Absorbance}\right) \times 100$$

Statistical analysis

Experimental data were analyzed using a two-way analysis of variance (ANOVA) at a 5% level of significance (Steel & Torrie, 1997). The differences in means were further analyzed using the Least Significant Difference test.

Results and Discussion

Moisture loss

Fig. 1 shows a summary of percent moisture loss from grapes during a storage period of 15 days at ambient temperature. It is evident from the results that loss of moisture increased as time progressed. The lowest moisture loss was observed in grapes sprayed with Chitosan. After a period of about 2 weeks, 13.44% of water was lost in these treatments as compared to 20.61%

in control. Salt treatments were also successful in reducing water loss but their efficiency was not comparable to Chitosan. Chitosan spray formed a protective barrier to the entrance of heat and sunlight which ultimately resulted in lower water loss. These findings are in accordance with the studies of Meng et al. (2010); Gao et al. (2013).

Decay assessment

The decay percent for all pre-harvest treatments stored at ambient temperature after 5, 10 and 15 days are shown in Fig. 2. With the increase in storage time, the decay percentage increased with pace. Maximum decay (20.15%) was seen in control after 15 days of storage at ambient temperature. Chitosan owing to its anti-fungal properties was successful in maintaining decay incidence to 13.43% was about 33% less than control. Salts also possess the ability to reduce rots and control fungal growth (Nigro et al., 2006). $CaCl_2$ and $NaHCO_3$ lowered the decay incidence to about 25% and 20% respectively. Meng et al. (2008) applied chitosan solution to grapes both in pre-harvest and post-harvest conditions. A greater reduction in decay rates was recorded at a temperature of 20 ºC. Moreover, other important quality parameters also showed improved results. Chitosan in different concentrations of 1%, 0.5%, and 0.1% had a direct effect on increasing PAL (Phenyl Ammonia Lyase) activity in table grapes. Infection caused to grape bunches and individual berries by grey mould, was significantly reduced in chitosan treated grapes in comparison with the control sample. The greatest inhibition of pathogen was observed when the concentration of the chitosan solution was maintained at 1% (Romanazzi et al., 2002).

Titratable acidity

Titratable acidity of grape samples showed a continuous and significant decline during storage (Table 1). The decrease in titratable acidity of grape juice during storage is considered as deterioration in quality. In pre-harvest treatments, the lowest decline in TA was witnessed in grapes treated with chitosan (8.47%) followed by $CaCl_2$ (12.51%) and $NaHCO_3$ (14.94%). Control samples presented the highest decline in TA (20.01%). Chitosan coating results in a lesser decline of TA of grapes (Jiang et al., 2005; Hong et al., 2012; Gao et al., 2013). The organic acids responsible for the titratable acidity of fruits were slowly consumed during the storage resulting in a decreased value of TA. Chitosan coating slowed down the respiration and metabolic processes of berries resulting in lesser loss of titratable acidity (Hagenmaier, 2005). Edible coating helps to preserve the TA by decreasing the respiration rate. This assertion was confirmed by Melo et al. (2018) which showed that the edible coating extracted from fungal chitosan nanoparticles resulted in low TA values for grapes that have been stored at 12°C and 25°C. This result was compatible with the findings of Gao et al. (2018), in which they claimed that coating of cinnamaldehyde-chitosan on navel orange (*Citrus sinensis* L., Osbeck) might induce low degradation levels of titratable acidity during 120 d of storage at RH of 80-90% and temperature of 10 ± 1 °C.

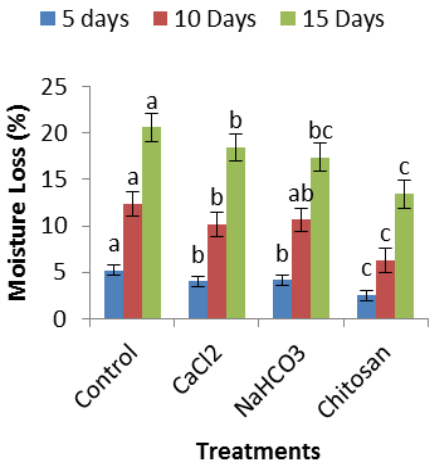

Fig. 1 Moisture loss (%) in grapes during storage

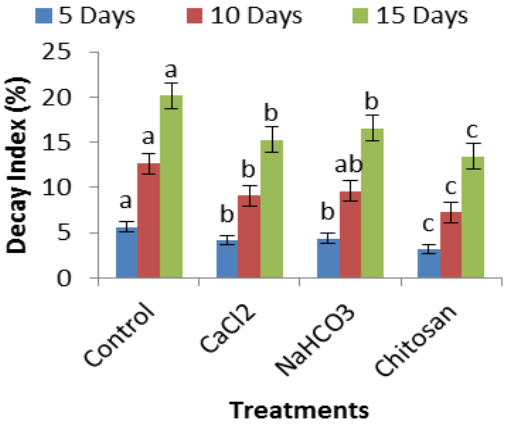

Fig. 2 Decay index (%) in grapes during storage

Total phenolic contents

Total phenolic contents (TPC) in grape samples at the start of storage ranged from 2101 to 2536 mg GAE / liter of grape juice (Table 2). As the storage time progressed, TPC started decreasing sharply and then increased afterward reaching a constant state. The greatest loss in TPC was recorded in controlled samples reaching a value of 31.2%. Salt treatments were effective in preserving phenolics to a little extent. The best reduction in loss of TPC was measured in 0.5% chitosan. The decline in TPC in this treatment was recorded at 16.7%. Meng et al. (2008) confirmed these results. Valero et al. (2006) found the same capability of chitosan to reduce the rate of loss in TPC. This might be due to the fact that chitosan coating provided a barrier to air and light. The oxidation of phenolic compounds and their loss was not as higher in chitosan-coated berries as was in other treatments. The phytonutrients of fresh-cut fruit can be preserved with the application of edible coating. The primary and secondary metabolites of 1.5 per cent coated strawberry with carboxy methyl cellulose solution and 1.0 per cent chitosan solution produced a small decrease in glucose, carotenoid, amino acids, fatty acids, terpenoid, flavonoid and phenyl propanoid after being preserved (Yan et al., 2019). The edible cover is added by dipping or spraying the covering solvent to the surface of the fruit. An optimal edible layer will create a partial movement obstruction to water which can limit fruit moisture loss and alter the fruit's environment by serving as a gas exchange barrier (Jongsri et al., 2016). It slows down in breathing and senescence without causing anaerobiosis, as well as slowing down growth of fungus and increasing the exterior of fruit (Dhall, 2013; Mahajan et al., 2014).

Antioxidant activity

The antioxidant content of grape samples varied from 61.65 to 67.81% at the start of the storage interval (Table 3). During storage, these values fluctuated showing a random increase and decrease but recorded an overall decline at the end of 15 days storage. Control samples displayed the highest levels of antioxidant loss (9.4%) in pre-harvest coated samples. On the other hand, grapes that had the only chitosan applied to them showed the lowest antioxidant loss at 3.43%. Despite this greater protection, the results are in contradiction with the findings of Sanchez-Gonzalez et al. (2011); Ponce et al. (2008). The previous studies have shown a rapid increase in antioxidant content of fruit coated with chitosan during initial days of storage. This contradiction might due to be the fact that the concentration of chitosan solution in the present study was maintained which is about four times lower than the concentration in which these researchers have employed chitosan. A reduced strength coating could not increase or otherwise fully prevent the loss of antioxidants. Mannozzi et al. (2018) stated that procyanidin-rich chitosan-based coatings preserved the antioxidant properties of fresh blueberries that are stored at 4 ° C for 14 days. Recently some researchers investigated the use of ascorbic acid added chitosan enhanced the antioxidant efficacy of mango (*Mangifera indica* L.) compared to the control samples at 15 ± 2 °C during the 24-day storage time with 85–90 percent RH (Zahedi et al., 2019).

Table 1 Titratable acidity of grape samples at different storage stages

Treatments	TA before storage	TA after storage	TA reduction (%)
Control	0.85 ± 0.04^{a}	0.68 ± 0.04^{ab}	20.01 ± 2.5^{a}
$CaCl_2$	0.72 ± 0.05^{ab}	0.63 ± 0.03^{b}	12.5 ± 1.3^{bc}
$NaHCO_3$	0.87 ± 0.05^{a}	0.74 ± 0.04^{a}	14.94 ± 1.6^{b}
Chitosan	0.59 ± 0.04^{b}	0.54 ± 0.05^{c}	8.47 ± 1.1^{c}

TA denotes titratable acidity; The values after ± are standard deviations; Values in a column not sharing the same letters are significantly different at $p<0.05$

Table 2 Total phenolics of grape samples at different storage stages

Treatments	Total phenolics before storage	Total phenolics after storage	Total phenolics decline (%)
Control	2376 ± 87^{b}	1634 ± 68^{c}	31.23 ± 3.24^{a}
$CaCl_2$	2101 ± 76^{c}	1652 ± 65^{c}	21.41 ± 2.71^{b}
$NaHCO_3$	2447 ± 82^{ab}	1902 ± 64^{b}	22.27 ± 2.62^{b}
Chitosan	2536 ± 93^{a}	2110 ± 61^{a}	16.79 ± 1.97^{c}

The values after ± are standard deviations; Values in a column not sharing the same letters are significantly different at $p<0.05$

Table 3 Antioxidant acidity of grape samples at different storage stages

Treatments	Antioxidant activity before storage	Antioxidant activity after storage	Decline (%)
Control	65.32 ± 2.62^{ab}	59.17 ± 2.57^{c}	9.4 ± 0.91^{a}
$CaCl_2$	67.81 ± 2.94^{a}	63.72 ± 2.28^{a}	6.03 ± 0.53^{b}
$NaHCO_3$	61.65 ± 2.97^{c}	56.77 ± 2.24^{d}	7.91 ± 0.75^{c}
Chitosan	64.31 ± 1.14^{b}	62.1 ± 1.99^{ab}	3.43 ± 0.62^{d}

The values after ± are standard deviations; Values in a column not sharing the same letters are significantly different at $p<0.05$

Conclusion

The improved cultural practices and more cultivation of fruits like grapes necessitate the use of certain techniques that can improve the quality and shelf life of the cultivated fruits. Chitosan solution and salts have shown promising results in reducing the decay and rots of fruits. Chitosan solution, in particular, proved to be an effective pre-harvest treatment that positively affects the quality parameters of grapes. These techniques can be useful in reducing post-harvest losses if scaled up to production at large scale.

Authors Contribution Statement: Anwar Ahmad and Naeem Khalid conceived the idea and designed the study. Umer Sarwar and Shahmeen Khalid performed experiments in the lab. Sahar Shibli supervised the research in lab. Hafiz Muhammad Rizwan Abid wrote the manuscript. Arshad Mahmood Malik added his inputs in reviewing the manuscript.

Conflict of Interest: The authors have no conflict of interest for the study entitled "Preservation of table grapes through innovative techniques".

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