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Influence of Irradiated Chitosan Coating on Postharvest Quality of Kinnow (*Citrus reticulata* Blanco.)

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The effect of coating with irradiated chitosan (CHIirr, Mv = 5.14×10^4) and unirradiated chitosan (CHIun, Mv = 2.61×10^5) on postharvest preservation of Kinnow (*Citrus reticulata* B) has been studied. 1 and 2 % coatings of both CHIirr and CHIun were used and the fruits were stored at 4 °C in 80 % relative humidity for 12 weeks. Changes in weight loss, ascorbic acid, titratable acidity, total soluble solids, reducing, non-reducing and total sugars were periodically measured during storage. Physical characteristics such as general appearance in colour and shape of the fruits and organoleptic evaluation were also carried out. All chitosan coating reduced the weight loss and respiration rate during storage. It also delayed and minimized the changes in: ascorbic acid contents, titratable acidity, *etc.* and maintained eating quality of fruits till 3 months as compared to untreated fruits.

Key Words: Chitosan, Postharvest preservation, Radiation, Kinnow, Coatings.

INTRODUCTION

In 1811, Henri Braconnot observed that a certain substance called chitin found in mushrooms did not dissolve in sulfuric acid. Since then, the exploration of chitin has taken on many different forms. It is a very simple natural biodegradable substance that has been around for ages. Researchers continue to build on the original finding of Braconnot, discovering new uses for chitin. The deacetylation of chitin converts it into another miraculous compound called "chitosan". It is cationic polysaccharide with high molecular weight soluble in dilute organic acids[1]. Meyers *et al.*[2] observed that the antibacterial activity of chitosan was affected by its molecular weight. The molecular weight of chitosan can be easily degraded by radiation in liquid state and in solid state[3]. Irradiation of chitosan changes the physico-chemical properties

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and also affects its antibacterial activity[4]. Irradiated chitosan showed antibacterial activity and it has been tested against *Escherichia coli* B/r[5]. Irradiation of chitosan at 100 kGy in the dry state was effective in increasing the activity and inhibited the growth of *E. coli* completely at a concentration of 3 mg/mL[4]. Chitosan also exhibit antifungal activity and it has been proven to control numerous pre- and postharvest diseases and prolong the storage life in various horticultural commodities such as strawberries[6], tomatoes[7], pears and peaches[8], apples[9], longan[10] and mangoes[4]. Chitosan is non-toxic, safe and has the ability to form a semi permeable coating, which extends the shelf life of treated fruits and vegetables by minimizing the rate of respiration and reducing water loss as compared to the untreated one. Coating citrus fruits (*Murcott tangor*) with low molecular weight chitosan increased postharvest quality and shelf life and also decreased fungal decay[11].

In this study, a native Southeast Asian variety of citrus fruit called Kinnow was selected. Kinnow has the following features, which differentiates it from other citrus varieties. The rind, containing numerous oil glands, comes off easily with bare hands since it is loosely bound compared to other type of citrus fruit. It consists of several easily separated sections containing high juice content. It is one of the most important fruit crop successfully grown in Southeast Asian countries. Citrus fruits are very susceptible to be attacked by pathogenic fungi due to its low pH, high moisture content and many nutrients. This causes decay, produces mycotoxins and thus makes them unfit for consumption[12].

For increasing its shelf life as well as maintaining its quality, irradiated and unirradiated chitosan coatings on Kinnow mandarin have been tried and their effect up to 12 weeks storage period has been investigated.

EXPERIMENTAL

Commercially available Kinnow (*Citrus reticulata* Blanco.) fruit approximately of the same size, shape, colour and maturity level were harvested from Kinnow orchard, in Sargodha, Pakistan. All fruits were sorted carefully as damaged and bruised ones were rejected. All fruit lots were washed carefully, so as dirt spots were cleaned while washing and allowed to dry at room temperature under sterile conditions. The clean dried fruits were separated into five main lots each containing 144 fruits. Fifteen fruits per treatment were analyzed for weight loss determination in separate cartons.

Chemical and radiation process: All chemicals were of analytical grade and were used as such. Tween 80 was purchased from Sigma Chemical Co. St Louis, USA. Chitosan flakes, degree of deacetylation 80 % was provided by Advanced Polymer Lab, PIEAS. This chitosan was prepared from crab shell. The viscosity average molecular weight of chitosan flakes was 2.61 × 10^5. These chitosan flakes were irradiated in air at room temperature by a 200 kCi gamma Irradiator (Pakistan Radiation Services, Lahore) with dose rate of 3.32 kGy/h to a total absorbed dose of 200 kGy (Table-1). The molecular weight of CHIirr is 5.14 × 10^4.

TABLE-1
COATING MATERIALS USED

Code	Type of materials	Concentration of coated solution (w/v)
CHIcon	Control Uncoated	–
CHIun1	Unirradiated Chitosan	1 %
CHIun2	Unirradiated Chitosan	2 %
CHIirr1	Irradiated Chitosan	1 %
CHIirr2	Irradiated Chitosan	2 %

Preparation and application of coating solution: The solutions of chitosan were prepared by dissolving chitosan (1 %, 2 % w/v) in 0.25 N HCl and pH was adjusted to 5.6 with 1 N NaOH. Purpose of this level of pH is to gain maximum antifungal property. Tween 80 was used with concentration of 0.1 % to improve the wettability. Fruits were dipped individually into the aqueous solutions of chitosan of different concentration. All fruits were dried again at room temperature under sterile conditions. Fruits were carefully placed in cartons and were stored at 4 °C with a relative humidity of 80 %.

Weight loss measurements: 15 Fruits per treatment were analyzed for weight loss determination in separate cartons. Fruits were marked according to treatment number and replications. The fruits were weighed on weekly basis to determine the weight loss. On each sampling date, weight of all marked fruits were taken from each treatment and compared with weight of the first day/initial day. Weight loss was calculated by using following formula:

$$\text{Weight loss (\%)} = \frac{Wo - Wt}{Wo} \times 100$$

where Wo = weight (g) of fruit on first day and Wt (g) = weight after't' interval.

Measuring amount of soluble solids, pH, titratable acidity and ascorbic acid: After every seven days of storage, a sample of 12 fruits was removed from each group and the amount of soluble solids, titratable acidity and ascorbic acid contents of the fruits were analyzed. Pulp from 12 fruits was extracted with Phillips Citrus Presser and filtered. This filtered juice was used for different tests. The amount of soluble solids was measured by using a hand refractometer (ATAGO, Model N1). Titratable acidity of fruits were noticed by taking juice and titrated against 0.1 N NaOH. The same comparison was done to observe the citric acid contents between treatments. The ascorbic acid contents were calculated by methods[13]. Fruit juice was titrated with 2,6-dichloroindophenol.

Determination of reducing, non-reducing and total sugars: Reducing, non-reducing and total sugars were measured by the method described[14].

Statistical analysis: Statistical analysis was carried out by using analysis of variance techniques (ANOVA) and means were compared using least significant difference test (LSD) at 5 % level of significance as recommended[15].

RESULTS AND DISCUSSION

Applications of coating materials on different fruits have been applied for the last 50 years to reduce moisture loss, respiration rate and to restore gloss and luster. Some physiological effects of delaying deterioration of citrus fruits by individual seal packaging in high density polyethylene film are also in practice[16]. Various types of skin coatings and treatment have been used to restrict moisture loss from the surface of citrus fruits such as Kinnow, oranges and Valencia[17-19]. In this study, the influence of irradiated and unirradiated chitosan coating with different parameters on postharvest quality of Kinnow has been carried out.

Effect of chitosan coatings on weight of fruits: Weight loss of fruit and vegetable is the main aspect which is of prime concern and related to the shelf life of the produce. In citrus fruit, the major requirement for extending the postharvest life is to slow down transpiration rate[16]. Wax coating is presently being used for this purpose which decreases the rate of respiration by degrading ethylene produced by the fruit into carbon dioxide and water[20]. On each sampling date, weight of all marked fruits were taken from each treatment and compared it with the first day weight of the fruit and the results are shown in Fig. 1. It can be seen from the figure that weight loss increases with increase in storage period. Furthermore, minimum weight loss was observed in chitosan coated fruits as compared to uncoated one. Moisture loss through respiration is the main process leading to weight loss of pulp and peel of Kinnow fruit. Weight loss in fruit coated with CHIcon fruits was 3 % on 1st week and 41 % on 12th week. This is a maximum weight loss as compared to chitosan treated fruits because chitosan coating provides barrier between inner and outer environment of the fruit and decreases rate of transpiration. By comparing chitosan coated fruits, it can be concluded that minimum weight loss of 25 % was observed in CHIirr2 on 12th week. The minimum weight loss in CHIirr2 might be due to its high rate of respiration[21] which in turn increases the metabolic activities inside the fruit as a result of the ripening of the fruit[22]. Kume and

Takehisa[3] found that the irradiation increased the degree of deacetylation and lower down the molecular weight of chitosan which in turn delayed internal changes of fruit that is not much delayed by unirradiated chitosan because it has less degree of deacetylation and high molecular weight.

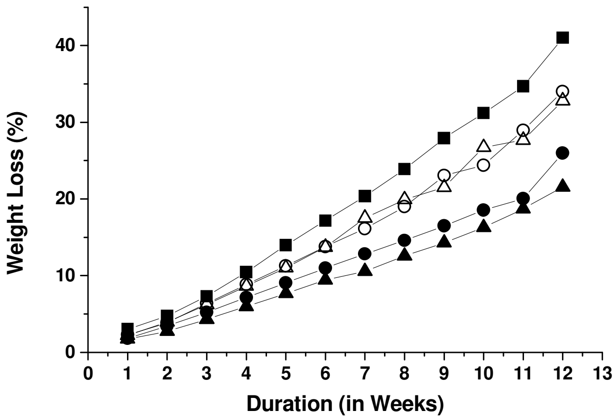

Fig. 1. Changes in percentage weight loss of citrus fruits coated with unirradiated, irradiated chitosan and the control during storage at 4 °C (-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

Effect of chitosan coatings on ascorbic acid contents: In citrus fruit, changes like ascorbic acid content, acidity and other internal changes are affected as fruits are kept in storage so these undesirable changes reduce and eventually damaged the quality of the fruit. Chitosan has the ability to minimize these changes to successive extent. Fig. 2 explains the effect of different chitosan coatings on ascorbic acid during storage. This figure shows a decrease in ascorbic acid contents as storage period increases. A similar result showed that ascorbic acid decreased with increasing period of storage in fruits of Kinnow[23]. Chitosan coated fruits demonstrated a small decrease in ascorbic acid as compared to controlled ones. This decrease in ascorbic acid reduces the eating quality of citrus fruits. Ascorbic acid contents of 54.77 mg/100 mL of juice were obtained at the start of the experiment and maximum loss of 16.01 mg/100 mL was observed in CHIcon on 12th week. Minimum loss in ascorbic acid was observed in 2 % coated fruits. In CHIirr2, ascorbic acid contents at the end of experiment were 26.00 mg/100 mL of juice whereas in CHIun2 it was only 22.12 mg/100 mL of juice. Will *et al.*[24] observed that degradation of ethylene into carbon dioxide and water resulted in decrease in oxygen level, which might lead to lesser loss of ascorbic acid from fruit. Irradiated chitosan might favour this reaction and accelerate the degradation of ethylene which in turn retained best possible ascorbic acid contents as compared to unirradiated chitosan.

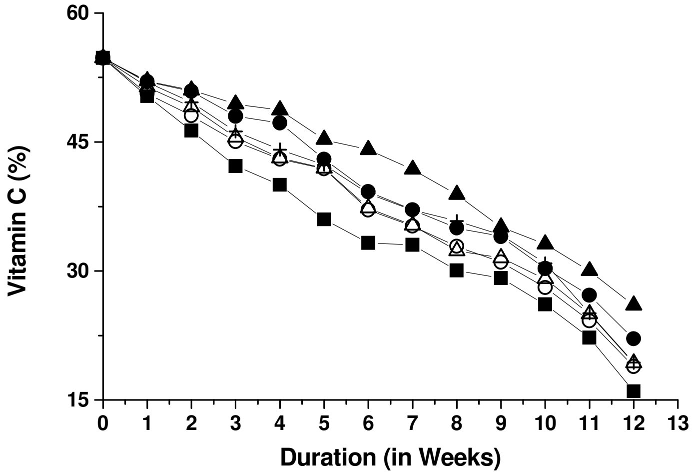

Fig. 2. Effect of unirradiated, irradiated chitosan coatings on ascorbic acid contents of Kinnow and the control during storage at 4 °C (-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

Effect of chitosan coating on titratable acidity: Titratable acidity of fruits were noticed by taking 10 mL juice and titrated against 0.1 N NaOH by standard method[25]. Titratable acidity is directly related to the concentration of organic acids present in the fruits. Acidity of citrus juice is due to content of citric acid, malic acid, small amount of benzoic acid, oxalic acid, tartaric acid, succinic acid as well as formic acid. Organic acids exist as free acids (citric acid), anions (citrate) or are combined as salt[26]. The same comparison was done to observe the citric acid contents between treatments. Among all treatments titratable acidity was calculated after one week interval as it has been described. At each week interval, it was observed that citric acid contents were decreasing with increasing period of storage due to changes in biochemical constituents resulted during respiration. Again it is evident from Fig. 3 that maximum loss showed untreated fruits CHIcon with value 0.83 g/100 mL in the start of experiment, which is same for all treatments. In CHIcon maximum loss was noticed from 0.83 to 0.16 g/100 mL and the maximum citric acid contents were retained by CHIirr2 from 0.83 to 0.32 g/100 mL of juice. It might be the effect of coating type that retained reasonable amount of acidity in Kinnow fruits because irradiated chitosan has the ability to delay these changes than unirradiated chitosan[21]. Acidity is used as an indication of maturity that is acid decreases on ripening of fruit. Sheikh *et al.*[27] observed the reduction in total acidity while studying the preservation of mangoes with fungicidal emulsion.

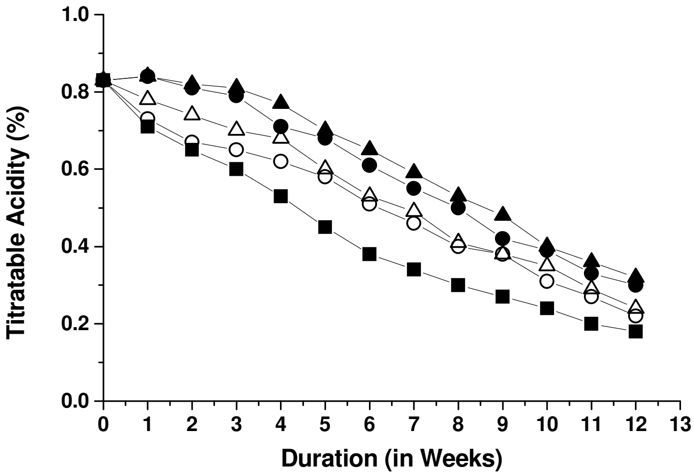

Fig. 3. Effect of unirradiated, irradiated chitosan coatings on titratable acidity of Kinnow and the control during storage at 4 °C
(-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

Effect of chitosan coatings on total soluble solid of fruits: The changes in total soluble solid are directly related with hydrolytic changes occurred in the starch concentration during the postharvest period[28]. These changes result into the conversion of starch to sugars, which is an important index of ripening process[26]. Fig. 4 shows that there is a variable pattern with the onset of storage life but generally it shows an increasing trend in total soluble solids of the fruit during storage in both coated and uncoated fruits. After 7 weeks of storage, this increase was rapid in all treatments and on 12th week, CHIirr2 coated fruits showed minimum value of 12.82°Brix which was at par with CHIun2 with value of 12.86°Brix. Maximum values of13.40°Brix was recorded in CHIcon. This increase in total soluble solid is caused by hydrolysis of starch into sugars and their accumulation in vacuoles of cells[29]. Minimum increase in case of CHIirr2 might be due to the fact that these retard the hydrolysis of starch into sugars and also the conversions of polysaccharides into disaccharides and monosaccharide by changing the biochemical activities[26]. Similar results were reported that chitosan coated red pitayas exhibited less increase in total soluble contents than uncoated ones[30]. Jiang *et al.*[31] also reported similar results in case of chitosan coated litchi which exhibited good quality including less increase in total soluble contents and less decrease in acidity. Thimula[32] worked on carboxymethyl chitosan and stated that there was a sharp rise in TSS in uncoated tomatoes at room temperature but gradual increase was noticed in fruits at low temperature.

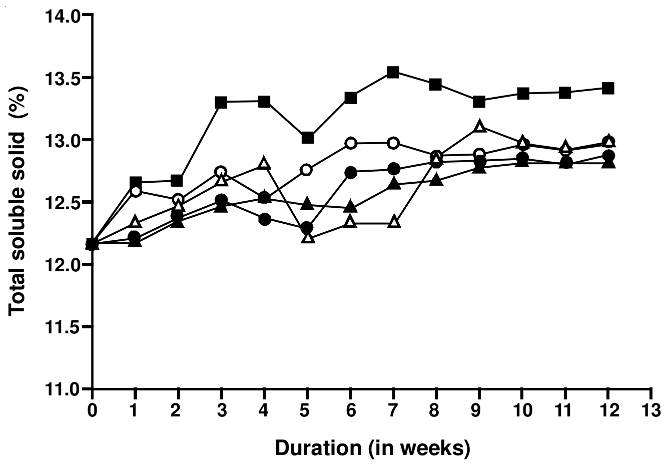

Fig. 4. Effect of unirradiated, irradiated chitosan coatings on total soluble contents of Kinnow and the control during storage at 4 °C (-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

Determination of reducing, non-reducing and total sugars: Three parameters of determining the sugars were measured by reported method[14]. Figs. 5-7 show the effect of chitosan coating on contents of reducing, non-reducing and total sugar, respectively. The values of reducing, non-reducing and total sugars on first day of experiment were 3.52, 4.37 and 7.89, respectively but as storage period enhanced these values were increased. After 12th week maximum increase in sugars contents were observed in CHIcon as compared to coated fruits and the values of reducing, non-reducing and total sugars in CHIcon fruits were as 5.42, 5.84 and 11.57, respectively. In CHIcon, higher rate of sugars is affected by the enzymes present in the fruit, especially due to the decreasing activity of pectinase enzyme which might also affect the activity of other enzymes. In CHIirr2, minimum loss is observed in all kinds of sugars and it was the best coating to control these changes, which are high in rest of the treatments. The slow rate of increase in sugar in CHIirr2 might be due to chitosan coating that affects the activity of enzymes which are responsible for the starch accumulation and may affect the conversion of starch into sugar[33]. Similarly, the rate of non-reducing sugars was comparatively slow in coated fruits than uncoated one. The same pattern is for total sugars that also increase as storage prolongs[34]. This gradual increase in total sugar may be due to the hydrolysis of polysaccharides and/or due to the less acidity and ascorbic acid contents[35]. Similar results were reported on sweet orange[36].

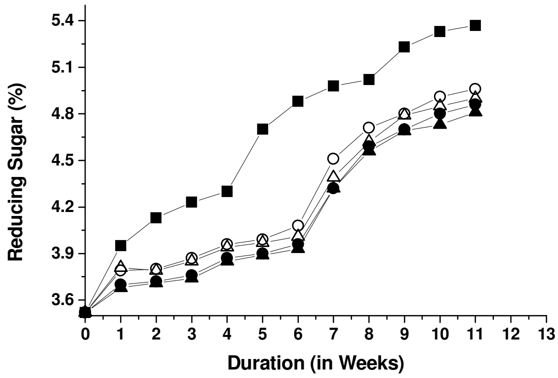

Fig. 5. Effect of unirradiated, irradiated chitosan coatings on reducing sugar (%) of Kinnow and the control during storage at 4 °C
(-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

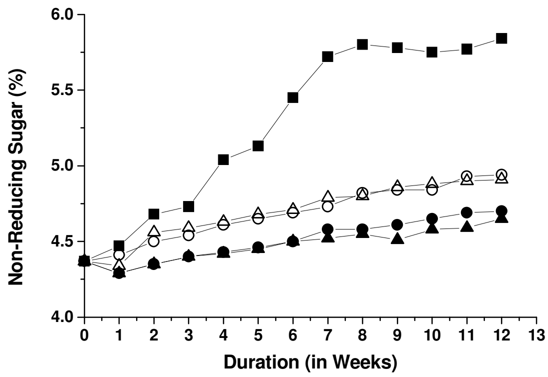

Fig. 6. Effect of unirradiated, irradiated chitosan coatings on non-reducing sugar (%) of Kinnow and the control during storage at 4 °C
(-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

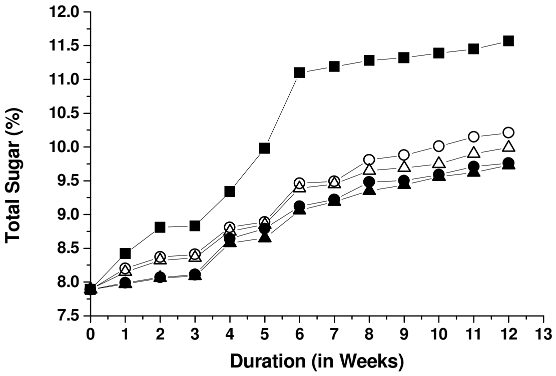

Fig. 7. Effect of unirradiated, irradiated chitosan coatings on total sugar (%) of Kinnow and the control during storage at 4 °C
(-■- CHIcon, -○- CHIun1, -●- CHIun2, -△- CHIirr1, -▲- CHIirr2)

Sensory evaluation: The taste and colour in fruits are very important, as these are the main factors that determine the consumer's acceptability. The taste in fruit is due to sugar acid ratio and optimum proportion of this ratio is essential for maintaining the sweetness in taste[37]. In this study, sensory evaluation of fruit showed better results even after 12 weeks of storage. The major advantage of this coating was that the fruits were consumable at every judgment day. CHIirr2 showed maximum scores of taste at 12th week than CHIcon fruits. It might be due to the sugar acid ratio that was maintained due to effect of thick layer of irradiated chitosan whereas minimum scores in untreated control fruits might be due to fluctuations in acids, pH and sugar/acid ratio because control fruits could not balance between the sugar acid ratios[38]. The scores were 6.03 and 6.03, respectively as compared to 1st day that were 9.80 scores for taste. Other treatments lie between these minimum and maximum values. Similarly, in case of colour, maximum value (scores) 6.23 were found in CHIirr2 on 12th week whereas, minimum value (scores) 3.10 were recorded in CHIcon fruits, which was at peak *i.e.* 9.00 on 1st day. It is uncertain but it might be the reason that the CHIirr2 reduced the evaporation rate which maintained the moisture in fruit; it might delay the enzymatic oxidation and photo degradation. The principal changes responsible for degradation system might be the oxidation system[39].

Conclusion

In conclusion, coating of Kinnow with chitosan provides an active package on fruits. Coating of 2 % irradiated chitosan was effective enough to decrease the respiration rates and delays ripening by suppressing the evolution of ethylene and carbon dioxide. It maintained the active ingredients of the fruit with minimum loss and also kept its organoleptic properties. In addition, against the growing awareness of consumers around the world about the chemical preservatives, chitosan can be a good alternate due to its non-toxic and biocompatible nature.

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