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POSTHARVEST QUALITY OF MANGO (*MANGIFERA INDICA* L.) FRUIT AS AFFECTED BY CHITOSAN COATING

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Abstract

The effect of coating with irradiated Crab and Shrimp chitosan (CHIirr, Mv = 5.14 × 104) and un-irradiated Crab chitosan (CHIun, Mv = 2.61 × 105) on postharvest preservation of mango (*Mangifera indica* L.) fruit was studied. Irradiation at 100 kGy and 200 kGy of both Crab chitosan and Shrimp chitosan were used and the fruits were stored at 15°C ± 1°C and 85% relative humidity for 6 weeks. The effect of various chitosan coatings on fruit ripening behaviour, biochemical and organoleptic characteristics were evaluated during storage. The incidence of disease attack was also observed. The overall results showed the superiority of irradiated Crab chitosan (200 kGy) in extending the shelf-life of mango fruit as compared to control. The irradiated Crab chitosan (200 kGy) treated fruits also maintained their eating quality up to 4 weeks of storage. Only 6% disease incidence was observed in fruits coated with irradiated Crab chitosan (200 kGy) as compared to control (25%) after 4 weeks of storage. The results of this study showed that irradiated chitosan coatings have an excellent potential to be used on fresh produce to maintain quality and extending shelf-life.

Introduction

Mango being a highly perishable fruit possesses a very short shelf life and reach to respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature (Narayana *et al*., 1996). The shelf life of mango varies among its varieties depending on storage conditions. It ranges from 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13ºC (Carrillo *et al*., 2000). This short period seriously limits the long distance commercial transport of this fruit (Gomer-Lim, 1997). Usually after harvesting, the ripening process in mature green mango takes 9-12 days (Herianus *et al*., 2003). The ripening process of mango fruit involves a series of biochemical reactions, resulting into increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll, developing pigments by carotenoids biosynthesis, change in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolics and volatile compounds, thus leading to ripening of fruit with softening of texture to acceptable quality (Herianus *et al*., 2003).

Fruit sensitivity to decay, low temperature and general fruit perishability due to the rapid ripening and softening limits the storage, handling and transport potential (Hoa *et al*., 2002). On the other hand, application of modified atmosphere (MA) or controlled atmosphere (CA) is not always compatible with this fruit. Although CA storage has been shown to extend the shelf-life of mango (Bender *et al*., 2000; Noomhorm & Tiasuwan, 1995), it is cost prohibitive. MA storage was also reported to slow mango ripening, but was often accompanied by high CO_2 and off flavor (Gonzalez-Aguilar *et al*., 1997).

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Films and edible coatings are defined as "a thin application of material that forms a protective barrier around an edible commodity and can be consumed along with the coated product" (Guilbert, 1986). Films and coatings have been used traditionally to improve appearance and to conserve food products. The most common examples are the wax coatings for fruits, which were reportedly used in China as far back as 12^{th} century (Dalal *et al*., 1971).

Edible coatings are used to create a modified atmosphere and to reduce weight loss during transport and storage (Baldwin, 1994). In fact, the barrier characteristics to gas exchange for films and coatings are the subjects of much recent interest (Tripathi & Dubey, 2004). Development of films with selective permeability characteristics, especially to O_2, CO_2 and ethylene allow some control of fruit respiration and can reduce growth of microorganisms (Cuq *et al*., 1995).

Coatings have long been used on citrus, apples (shellac and carnauba wax), tomatoes (mineral oil) and cucumbers (various waxes). However, these coatings are less studied for use on apricots, pineapples, bananas, cherries, dates, guavas, mangoes, melons and nectarines or peaches (Baldwin, 1994). Nevertheless, the postharvest use of polysaccharide and protein coating materials on several types of fruit has been developed in the past few years including cellulose-sucrose fatty acid esters on apricot (Sumnu & Bayindirli, 1995), cellulose on mango (Baldwin *et al*., 1999), guava (McGuire and Hallman, 1995), chitosan on strawberry (El-Ghaouth *et al*., 1991), tomato (El-Ghaouth *et al*., 1992) and the corn protein (Zien) on tomato (Park *et al*., 1994).

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae (Tolamite *et al*., 2000) and is used in medical or industrial products as a bioactive material (Cho *et al*., 2008; Matsuhashi & Kume, 1997). Its structure resembles with the cellulose except that the hydroxyl groups in position 2 have been replaced by acetyl amino groups (Peniston & Johnson, 1980). Chitosan is very reactive polysaccharide having three different functional groups (primary −OH, secondary −OH and $-NH_2$) and the water soluble with organic acids (Sanford, 1989). It inhibits the growth of a wide variety of bacteria (Sudarshan *et al*., 1992; Yalpani *et al*., 1992) and fungi (Allan & Hadwiger, 1979, Stossel & Leuba, 1984; Kendra & Hadwiger, 1984; Fang *et al*., 1994). Eriksson & Hardin (1987) and Uchida (1988) have ascribed the function of high-molecular-weight chitosan as an antimicrobial material or flocculent to either amino groups in the molecule or hydrogen bonding between chitosan and extra cellular polymers in addition to an electrostatic interaction with the cell surface. Hughes *et al*., (1994) showed the combined effect of chitosan and cellulosic material on the flocculation. Uchida (1988) reported the antimicrobial activity of chitosan degraded by chitosanase and showed that the activity of 5% degraded chitosan was higher than that of un-degraded one.

Radiation causes the changes in the physico-chemical properties of chitosan (Kume & Takehisa, 1982). It is considered that irradiation is a useful method for producing lower molecular weight products from carbohydrates by degradation.

Chitosan is well known coating material used in several fruits for prolonging their shelf life (Graham, 1990). Similarly, irradiation is an economically viable technology for reducing postharvest losses and maintaining hygienic quality of fresh produce (Boylston *et al*., 2002; Cheour & Mahjoub, 2003; Gonzalez-Aguilar *et al*., 2004). To date, use of this irradiated coating material has not yet been reported on fresh mango fruits. Therefore, in this study it was attempted to evaluate different types of locally developed irradiated chitosan coatings most suitable for enhancing the shelf life and improving quality of mango fruits.

Materials and Methods

Plant material: Uniform mango fruits of cv "Summer Bahisht Chaunsa" were harvested at physiologically mature stage from Mango Research Station Shujabad, district Multan (30° 10'N, 71° 36'E), Punjab province, Pakistan. Fruits were washed using distilled water, air-dried, packed into corrugated boxes and then brought to the Post Harvest Lab, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi. The various concentrations of irradiated chitosans [T_1 = Control, T_2 = 1.5% Crab chitosan irradiated (100 kGy), T_3 = 1.5% Crab chitosan irradiated (200 kGy), T_4 = 1.5% Crab chitosan Un-irradiated, T_5 = 1.5% Shrimp chitosan irradiated (100 kGy)] were applied to the fruits. The chitosan of different types used in this study was prepared by Pakistan Radiation Services (PARAS) in the Pakistan Institute of Applied Sciences (PIAS), Rawalpindi. After application of treatments, fruits were air dried, packed in corrugated boxes and placed in storage at 15°C ± 1 and 85% RH for six weeks. Mangoes were analyzed for different parameters after regular intervals of 7 days for 6 weeks.

Physico-chemical characteristics and organoleptic evaluation

Physical characteristics: Fruit samples (5 fruits /replication) were weighed at the start of experiment and at the end of each storage interval. The difference between initial and final fruit weight was considered as total weight loss during that storage interval. The calculations were made in percentages on fresh weight basis. Fruit weight was recorded on weekly interval by using digital balance. Firmness of the pulp was recorded (N) with a penetrometer (Model FT-327) using an 11 mm plunger tip. The data regarding disease incidence was also recorded during whole storage period.

Chemical characteristics: The total soluble solids (TSS) levels of the fruit were determined according to AOAC method (Anon., 1990) by using hand refractometer. Titratable acidity was determined by the standard methods of AOAC (Anon., 1990). The pH of mango fruit juice was recorded according to AOAC (Anon., 1984) method No. 981. 12b by using digital pH meter (Model: Knick 646). Ascorbic Acid (Vitamin C) contents were determined by the Indophenol's titration method used by Ruck (1963). To estimate the sugars in juice of each treated sample, the method described by Hortwitz (1960) was used.

Organoleptic evaluation: Organoleptic evaluation of the fruit for taste, flavor and aroma for all the samples was done using the Hedonic scale suggested by Krum (1955). A panel of seven judges with age ranging from 25-40 years was made on their consistency and reliability of judgment. Panelists were asked to score the difference between samples by allotting the numbers from 0-9, where 0-2 represent disliked extremely, 3-5 for fair, 6-8 for good and 9 for excellent aroma, taste and flavor.

Statistical analysis: Experiment was conducted in Completely Randomized Design (CRD) with three replications. The data were subjected to analysis of variance using the computer software MSTAT-C (Freed & Scott, 1986), while Least Significant Difference (LSD) test was used to compare differences between treatments at 95% confidence level of each variable (Chase & Bown, 1997).

Results and Discussion

Physical characteristics: Percent weight loss during storage showed significant results (Fig. 1a). The minimum weight loss occurred in fruits treated with Crab Chitosan 200 kGy (T_3) followed by the fruit treated with Crab Chitosan 100 kGy (T_2) as compared with untreated fruit (T_1). It was found that as the storage time proceeded the weight loss percentage was also increased and the maximum weight loss was recorded after 6 weeks of storage.

The results associated with fruit firmness as influenced by chitosan coatings showed that maximum fruit firmness was retained in fruits treated with Crab Chitosan 200 kGy (T_3) followed by the fruits treated with unirradiated Crab Chitosan and minimum fruit firmness was noticed in untreated control fruits (T_1). The data presented revealed that there was a similar decreasing trend in fruit firmness in all treatments towards the end of storage. Minimum firmness was calculated after 6 weeks of storage while maximum, recorded at the time of harvest (Fig. 1b).

Minimum weight loss in fruits treated with Crab Chitosan 200 kGy (T_3) could be due to coating with irradiated chitosan, which acted as barrier between inner and outer environment of the fruit. Maximum weight loss in untreated control fruits (T_1) may be due to high rate of respiration and transpiration. The results get support with conclusion of Chien *et al*., (2005), the group of scientists worked on effect of irradiated (low molecular weight chitosan: LMWC) and unirradiated (high molecular weight chitosan: HMWC) chitosan on cut fruits and stated that irradiated chitosan retained much moisture contents of fruit, so maintained fruit quality for longer time. There are some reports correlating the application of irradiated and unirradiated chitosan on mango. Irradiated chitosan prolonged the storage life of mango from 7 to 15 days with fairly acceptable quality and unirradiated chitosan could not ripen fruit, whereas control was spoiled (Lan *et al*., 2000). The irradiation increased degree of deacetylation and lowered down the molecular weight of chitosan which in turn delayed internal changes of fruit that was not much delayed by unirradiated chitosan because it had less degree of deacetylation and high molecular weight (Lan *et al*., 2000; Kume & Takehisa, 1982). This is why maximum moisture in the fruits was retained in fruits treated with 200 kGy irradiated chitosan because it has ability to act on outside and inner side of the fruit while the fruits coated with 200 unirradiated chitosan only affected outer side of the fruit.

The ripening of mango fruits is characterized by a loss of firmness due to cell wall digestion by pectinesterase, polygalacturonase and other enzymes (Narain *et al*., 1998). Maximum fruit firmness in Crab Chitosan 200 kGy coated fruits (T_3) could be attributed to the permeability property of the coating and its effects on the fruits (Buescher, 1979) and provided better way to reduce the evaporation and avoided shrinkage (Medlicott *et al*., 1987). It might be outcome of irradiated chitosan which has ability to effect inside of the fruit (Lan *et al*., 2000; Kume & Takehisa, 1982). Due to wax coatings there was reduction in cell wall loosening which in turn maintained cell integrity (Salunkhe & Desai, 1984). While minimum values in untreated control fruits (T_1) could be due loosening of cell wall, reduction of pectic enzymes which reduced the firmness of mango fruits (Jitareerat *et al*., 2007). Overall better retention of firmness in coated fruits as compared to untreated can be explained by retarded degradation of insoluble proto-pectins to the more soluble pectic acid and pectin. During fruit ripening, de-polymerization or shortening of chain length of pectin substances occurs with an increase in pectin-esterase and polygalacturonase activities (Kashappa & Hyun, 2006). Less

availability of oxygen to the coated fruit may be responsible for reduction in the activities of these enzymes and hence retention of the firmness of fruits during storage (Salunkhe *et al*., 1991). According to the studies by Intalook *et al*., (2006) chitosan coating materials affected postharvest quality changes of mango fruit cv. Chok Anan. One other factor involved in maintaining the structure of fruits is chitosan coating contained calcium which demonstrated the best results, probably because calcium may interact with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining structure of the fruit (Rolle & Chism, 1987).

Chemical characteristics: For all chitosan coated mangoes, there was an increase in TSS during storage compared with the unirradiated chitosan treatment and control (Fig. 1c). The mango fruits treated with Crab chitosan 100 kGy (T_2), Crab chitosan 200 kGy (T_3) and Shrimp chitosan 100 kGy showed statistically higher values of TSS followed by unirradiated Crab chitosan (T_4) and control (T_1). The higher levels of total soluble solids in the fruit coated with chitosan may be due to protective O_2 barrier reduction of oxygen supply on the fruit surface which inhibited respiration (Yonemoto *et al*., 2002). Du *et al*., (1997) reported that application of chitosan coating inhibited respiration rates of fruit. The decrease of total soluble solids is caused by a decline in the amount of carbohydrates and pectins, partial hydrolysis of protein and decomposition of glycosides into sub-units during respiration (Ball, 1997). Further, it was observed that fruit treated with any chitosan had a high total soluble solid content, titratable acidity and ascorbic acid content, but no significant difference in total soluble contents between irradiated and unirradiated mango fruits was observed during storage (Jiang & Li, 2000).

The pH increased and the titratable acidity decreased significantly ($p<0.05$) along with increased storage time in both coated and uncoated fruits (Fig. 2a). These results agreed with those reported by El-Ghaouth *et al*., (1991) and Garcia *et al*., (1998a) that the decrease of acidity during storage demonstrated fruit senescence. It was determined as a small change in pH represents a large change in hydrogen ion concentration (Ball, 1997). The change in pH is associated with number of reasons; it might be due to the effect of treatment on the biochemical condition of the fruit and slower rate of respiration and metabolic activity (Jitareerat *et al*., 2007). Coatings slowed the changes on pH and titratable acidity, effectively delaying fruit senescence. This was probably because the semi-permeable chitosan film formed on the surface of the fruit might have modified the internal atmosphere i.e., the endogenous CO_2 and O_2 concentration of the fruit, thus retarding ripening (Lowings & Cutts, 1982; Bai *et al*., 1988). The increase in pH may be due to the breakup of acids with respiration during storage (Pesis *et al*., 1999) and the higher levels of titratable acidity in the fruit coated with unirradiated chitosan (T_4) may be due to protective O_2 barrier or reduction of O_2 supply to the fruit surface which inhibited respiration rate (Jiang & Li, 2000). Increased activity of citric acid during ripening or reduction in acidity may be due to their conversion into sugars and their further utilization in the metabolic processes of the fruit. Doreyappa & Huddar (2001) reported the similar pattern in different varieties of mango fruits stored at 18-34°C. They observed a series of physico chemical changes during ripening and the major changes were decrease in acidity. The acidity of the fruit is an important character to determine its quality and acceptability. Very high or very low values of the acidity are not recommended for good fruits. Jiang *et al*., (2004) also reported the effect of chitosan coatings on longan fruit and found that titratable acidity decreased during storage.

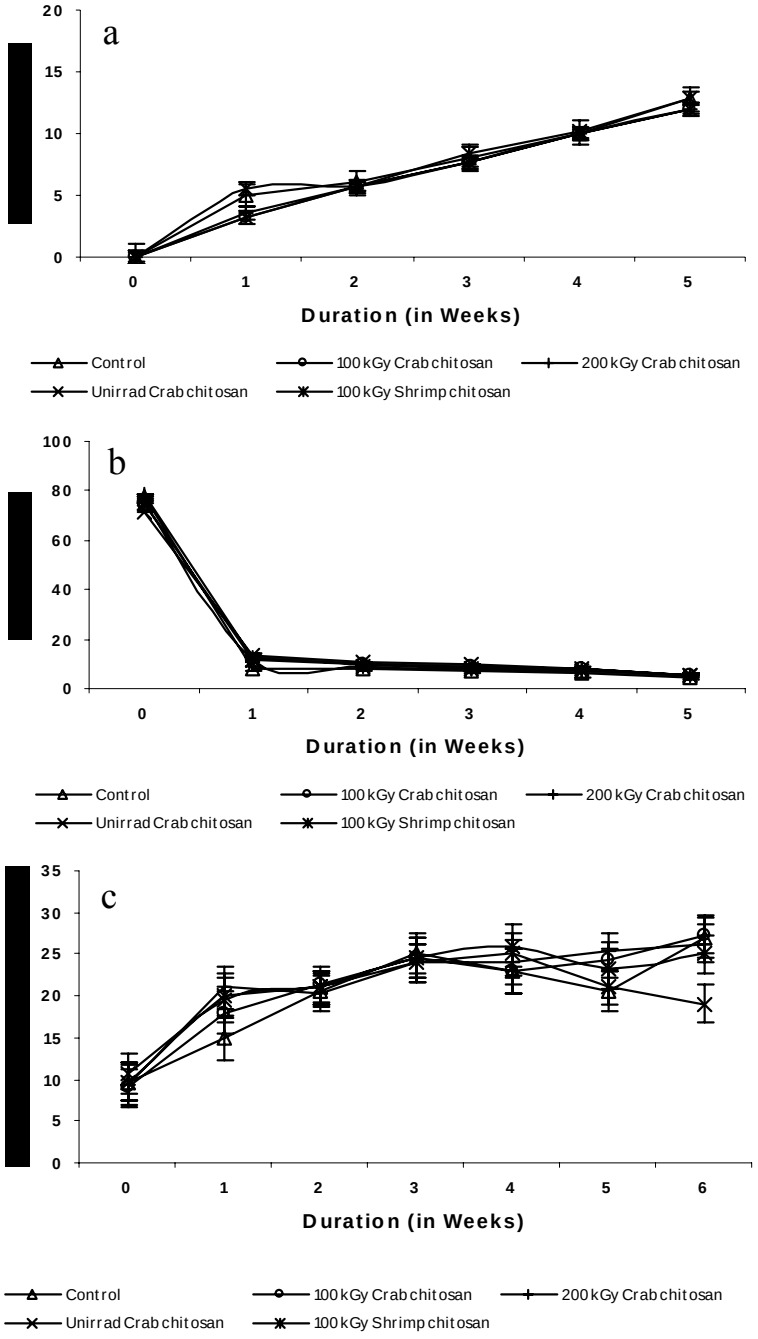

Fig. 1. Effect of chitosan coatings on a) weight loss, b) firmness and c) total soluble solids of mango fruit during storage (Vertical bars represents ± SE of means for three replicates).

The results illustrated in (Fig. 2b) revealed that there was a significant decrease in ascorbic acid values of chitosan coated fruits along with the storage period. However, the rate of decrease in vitamin C was significantly higher in untreated control fruits as compared with coated fruits. Present studies showed that vitamin C was mostly high in mature but unripe mango fruits and it decreased as the ripening progressed. The reason for high vitamin C content in coated fruit can be attributed to slow ripening rate of chitosan treated fruit. Oxidation of ascorbic acid may be caused by several factors including exposure to oxygen, metals, light, heat and alkaline pH (Sritananan *et al*., 2005). Coatings served as a protective layer and control the permeability of O_2 and CO_2 (Srinivasa *et al*., 2002). The ascorbic acid contents in irradiated Crab chitosan 200 kGy coated fruits were higher than unirradiated chitosan coated fruits at the end of time of 6 weeks storage. The effect of radiations on chitosan was reported with the break of glycosides link to produce different lower molecular weight fragments, which help in protecting the outer and inner surface of fruits (Park *et al*., 1993). The results congregates with the findings of Jiang *et al*., (2004) who narrated that ascorbic acid content decreased when longan fruit was coated with chitosan at low temperature 2°C.

Total sugars of the fruit are considered one of the basic criteria to evaluate the fruit ripening. It is clear from the results that at the time of harvest the sugars were very low but with the passage of time ripening enhances and ultimately total sugars increased (Fig. 3c). However during storage of mango fruits total sugars significantly increased in all treatments except control, as storage prolonged the rate of respiration, transpiration and other metabolic changes (Gul *et al*., 1990). Gradual increase in reducing sugars in coated mango fruits as compared to control treatment (Fig. 3a) might be due to its slow ripening process (Youssef *et al*., 2002). Maximum amount of reducing sugars in untreated control fruits might be due to rapid conversion of starch to sugars as a result of moisture loss and decrease in acidity by physiological changes during storage (Wills & Rigney, 1979). This view is supported by Khalid (1974) who studied the effect of wax coating and irradiation on the shelf life of mangoes at room temperature and observed that the pH, TSS and sugars (reducing, non-reducing and total sugars) increased while acidity and moisture decreased.

Sensory evaluation: The statistical analysis showed that in general, the taste score was increased from 3.64 to 8.42 after four weeks of storage and gradually decreased to 3.65 after 6 weeks of storage. Therefore, the results showing an increasing trend first and then decreasing significantly (Fig. 4a). It might be due to fluctuations in acids, pH and sugar/acid ratio (Malundo *et al*., 1997). Although there are many different tastes mostly appear to primarily represent four dominant chemical sensations, sweet, sour, bitter and salty in which sweet and sour predominate, thus sweet due to sugar and sour from organic acids (Kays, 1997). This fine variation of taste scores might be due to chitosan coating, which maintained taste and retained the quality of fruit until 4 weeks of storage the decay started. Jiang & Li (2001) reported that chitosan treated longan fruit had good eating quality even after 30 days of storage at 2°C. Chitosan retained fruit quality and no off flavor was developed than control. These results tally with Munoz *et al*., (2006) who reported the influence of the chitosan on strawberries stored at 20°C for 4 days showing better maintenance of eating quality.

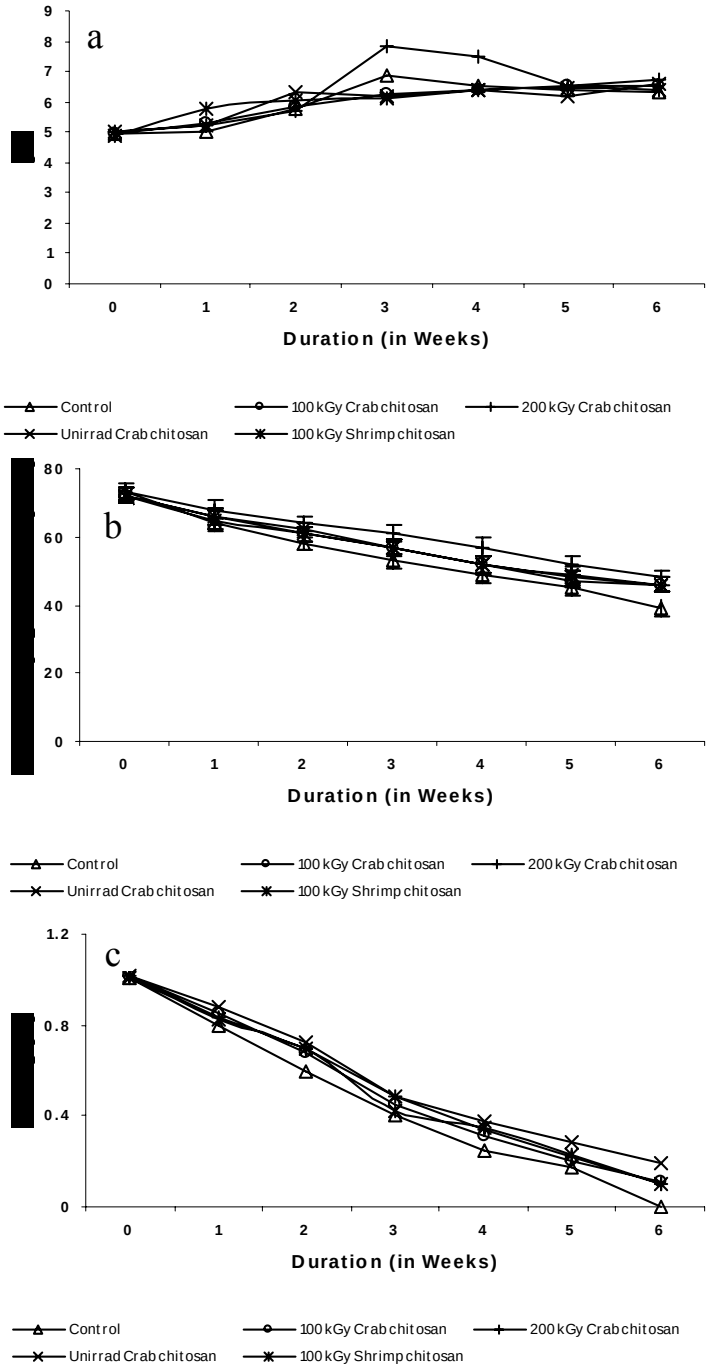

Fig. 2. Effect of chitosan coatings on a) pH, b) vitamin C and c) titratable acidity of mango fruit during storage (Vertical bars represents ± SE of means for three replicates).

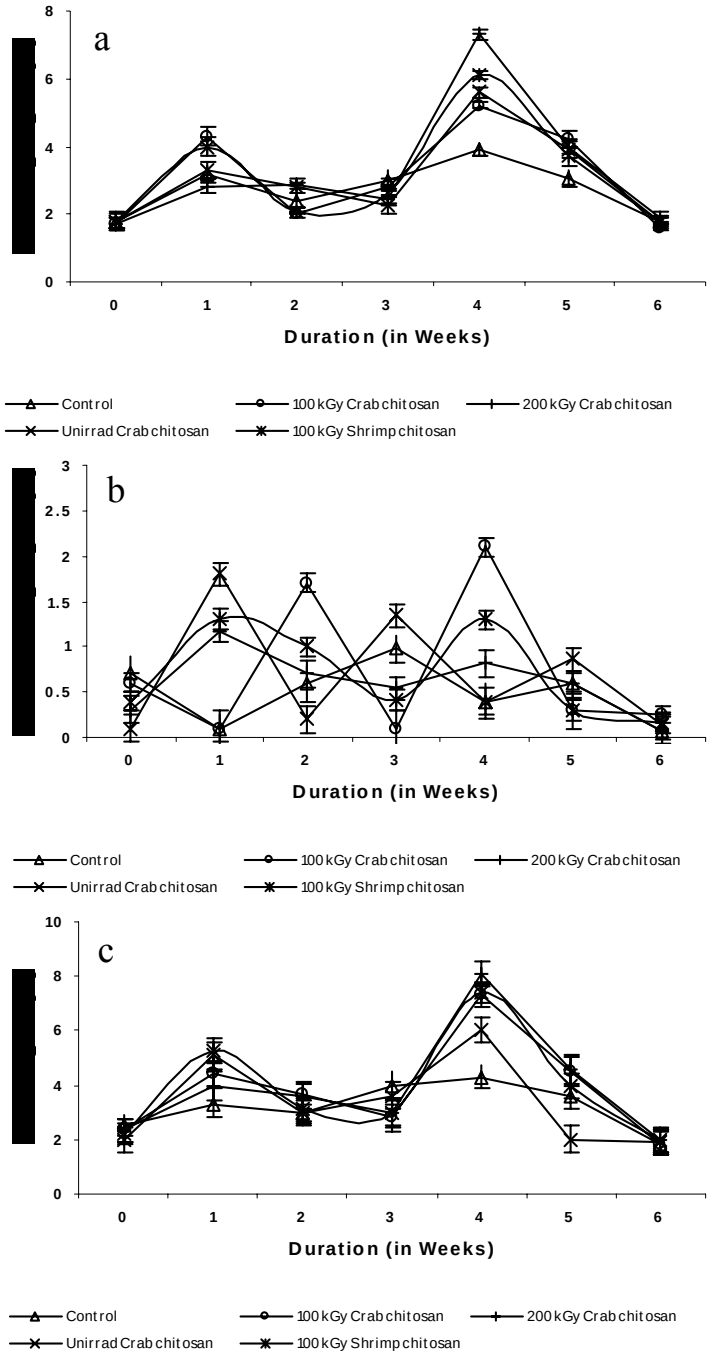

Fig. 3. Effect of chitosan coatings on a) reducing sugars, b) non-reducing sugars and c) total sugars of mango fruit during storage (Vertical bars represents ± SE of means for three replicates).

It was observed from the result that the flavor score was increased from 1.92 to 7.31 after third weeks of storage and gradually decreased to 4.58 after 6 weeks of storage (Fig. 4b). Similarly, the maximum aroma score was observed after third week of storage and the fruits treated with irradiated Crab Chitosan 200 kGy (T_3) performed very well during storage. It was observed from the result that the aroma score was increased from 2.27 to 6.37 after 3 weeks of storage and gradually decreased to 2.43 after 6 weeks of storage (Fig. 4c). It seems that the biochemical changes were slower and conversion of organic compounds into esters, aldehydes, acids, alcohols and ketones did not take place that contributed significantly to flavor and aroma of the fruits (No *et al*., 2007). Whereas in control, decline in flavor and aroma score was started after 2nd and 3rd weeks of storage respectively, and after that the fruits started spoiling. It might be due to the volatile compounds in free atmosphere. Doreyappa & Huddar (2001) reported that flavor of mangoes after ripening showed significantly decreasing trend as the storage period proceeded when stored at 32 to 35°C. It might be due to fluctuations in acids, pH and sugar/acid ratio (Jitareerat *et al*., 2007). Fruit treated without chitosan coating did not develop flavor while chitosan coated fruits showed best results. Desirable flavors may be produced by loss of organic acids during senescence (Baldwin *et al*., 1999). Untreated control fruits had lowest flavor scores. It might be due to the change in carbohydrates, proteins, amino acids, lipids and phenolic compounds that can influence the flavor of fresh fruits (Malundo *et al*., 1997).

Incidence of disease attack: In this study it was found that that the decay controls of irradiated chitosan on mango fruits was better as compared with uncoated fruits. Chitosan treated fruit inhibited the growth of a wide variety of bacteria and fungi as compared to the control treatments. The fruit-spoiling fungi (*Colletotrichum gleosporioides)* were observed in untreated control fruits after 2 weeks and in irradiated chitosan coated fruits after 5 weeks of storage. The control fruits were affected 13.3%, after 14 days of storage while irradiated chitosan coated fruits were affected only 6.9%. At the end of storage control fruits were fully spoiled. However, irradiated chitosan coated fruits were still having 75% fruits not having disease attack. El-Ghaouth *et al*., (1991) suggested that chitosan induces chitinase, a defense enzyme (Mauch *et al*., 1984), which catalyzes the hydrolysis of chitin, a common component of fungal cell walls (Hou *et al*., 1998), thus preventing the growth of fungi on the fruit. The results suggest that irradiated chitosan coating is effective on preservation of fresh fruits. It can extend the shelf life (Eissa, 2007), limit the growth of fungi, and decrease the spoilage without affecting on ripening characteristics of fruit (Lam & Diep, 2003). Microbial inhibition caused by radiation-formed chitosan fragments is stronger than that by original chitosan molecule due to the contribution of both mechanisms that occurred simultaneously in case of irradiated chitosan (Jitareerat *et al*., 2007). Action mechanism of original chitosan abides mainly by only one mechanism in which chitosan molecule stack to cell wall (Lan *et al*., 2000). It has been proposed that when chitosan is liberated from the cell wall of fungal pathogens by plant host hydrolytic enzymes chitosan penetrates the nuclei of fungus and increases with RNA and protein synthesis (Duan *et al*., 2007; Devlieghere *et al*., 2004). According to El-Ghaouth *et al*., (1992a) Chitosan coated tomatoes were prevented by attack of *Penicillium* spp., *Aspergillus* spp., *Rhizopus stolonifer* and *Botrytis cinerea*. Moreover, Chitosan has itself ability to control some fungal diseases, which deteriorate fruit quality during storage (Muzzarelli & Rocchetti 1985).

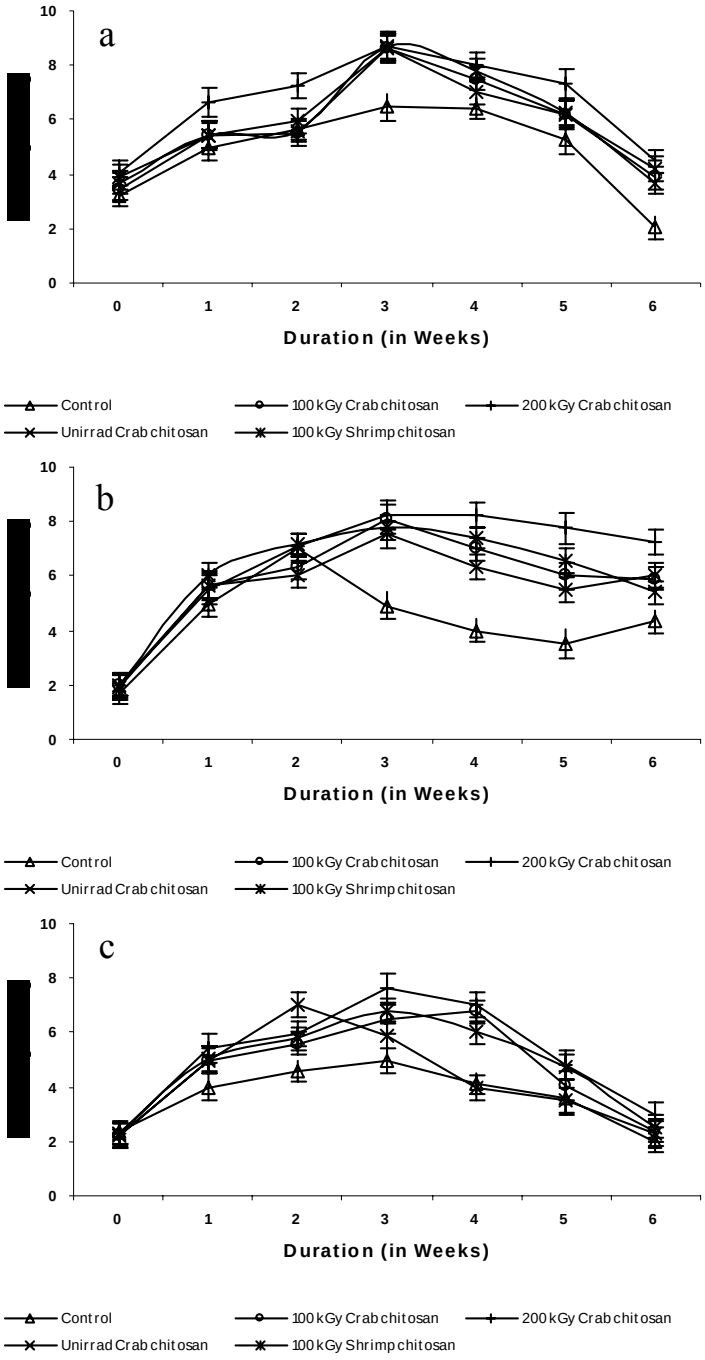

Fig. 4. Effect of chitosan coatings on a) taste, b) flavor and c) aroma of mango fruit during storage (Vertical bars represents ± SE of means for three replicates).

Conclusion

The Crab chitosan irradiated (200 kGy) had extended the shelf life of mango fruits, showed best behavior through out storage period with minimum loss of weight, shrivel, increased ascorbic acid content and able to conserve better sensory characteristics. Irradiated chitosan coating also protected the mango fruits from disease attack. This study recommends chitosan as the best edible coating material that is very effective in improving the overall quality of mango fruits.

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