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Irradiated chitosan as a potential natural preservative for extending shelf life of green mango

Md. Abdul Kader[1], Nehar Parvin[2], Md.Elias Molla[3], Mubarak A Khan[4]

Abstract: Irradiated chitosan solutions of different concentrations (500, 750 and 1000 ppm) were used to preserve green mango stored in open and in zip bag at room and freezing temperature respectively. The percentage of weight loss, spoilage rate and microbiological properties (such as, total bacteria count and total mold count) of treated and untreated mango were investigated. It was observed that the shelf life of green mango greatly prolonged due to the antimicrobial activity of irradiated chitosan. In addition, the results also revealed that 750 ppm irradiated chitosan solution performed superior extending shelf life of mango at freezing temperature. Thus, 750 ppm irradiated chitosan solution can be used as most promising and safest natural preservative for extending the shelf life of green mango fruits at low temperature.

Key words: Antimicrobial activity of Chitosan, Natural preservative, Irradiated chitosan, Green mango.

**Corresponding author: Md. Abdul Kader, e-mail: makbiopoly@gmail.com*

1. Introduction

Amropali (local name) green mango (*Mangifera indica* L.) is prominent as tropical fruits for its vitamin C content and others nutritional values throughout the world. It is generally called 'King of fruits'native to southern Asia.Mango is available in all parts of Bangladesh. According to the FAO report in 2009, the world wide production of mango was nearly 38.6 million tons whose world export was approximately 11 million tons. Unluckily, because of mismanagement, inadequate storage or lack of technical knowledge producers and traders have to face about 20-30% losses [1] and loss of this perishable commodity is estimated up to 320.7 thousand tons annually [2]. Damage of mango due to stem end and anthracnose, limits its storage potential and the shelf life is depended on the basis of spoilage (10%) during storage [3]. Mango being a climacteric fruit possesses a very short shelf life.The shelf life of mango differs among its varieties depending on storing conditions. It ranges from 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13 °C [4]. The use of edible coatings is a promising alternative to develop the quality and extend shelf life of fresh and nominally processed produce [5]. Irradiated chitosan is a right promising edible coating material that revealed effective and safer way in preserving the overall quality of pineapple [6]. Chitosan possesses biochemical properties, inherent antifungal properties, enzyme activity (chitinase), and due to excellent film forming ability it is proved to be effective atextending the shelf life of fruits and vegetables [7-11]. These results were consistent with other scientists in case of mango, carrot and tomato [12-14]. It has been observed and reported that chitosan extended the shelf life of litchi [15]. Recently, another report showed that irradiated chitosan was very effective and safer way

1 Ahsanullah University of Science and Technology, Department of Arts and Sciences, Faculty of Engineering, Dhaka-1208, Bangladesh
2 Jahangirnagar University, Department of Chemistry, Savar, Dhaka-1342
3 Jahangirnagar University, Department of Chemistry, Savar, Dhaka-1342
4 Institute of Radiation and Polymer Technology(IRPT), Bangladesh Atomic Energy Commission, Savar, Dhaka

in maintaining the overall quality of strawberry [16].So far, the application of irradiated chitosan has not yet been reported on the preservation of green mango fruits. Thus, the aim of this study was to investigate the effect of irradiated chitosan solutions on the extension of shelf life of green mango.

2. Materials and methods

2.1. Source of green mango fruit

Fresh green amropali (local name) mangoes (*Mangifera indica* L.) were directly collected from garden, Manikganj district, Bangladesh and brought to the laboratory. The mangoes were selected without any infection and damage.

2.2. Extraction and Irradiation of chitosan

Extraction of chitosan from prawn shell was done in Institute of Radiation and Polymer Technology (IRPT) laboratory. High molecular weight chitosan was dissolved in 2% acetic acid solution to make a homogeneous chitosan solution. The homogeneous chitosan solution (2% w/v in 2% acetic acid) was gamma irradiated at 40 kGy by a 120 k Curie radiation sources at 3.2 kGy per hour dose rate. Other chemicals and solvent used were analytical grade.

2.3. Preparation of irradiated chitosan solution

Chitosan solutionsof different concentrations (500, 750 and 1000 ppm) were prepared by dissolving it in distilled water and applied for coating over a wide range of green mango samples.

2.4. Preservation of green mango samples by irradiated chitosan solutions

The green mango samples were treated by immersing them into 500, 750 and 1000 ppm irradiated chitosan solution for two minutes respectively. The control sample comprised of mangoes without washing or immersing into chitosan solution. After air dried at room temperature, the mango samples were stored at room and freezing temperature in open and in zip polybag condition respectively. The control samples were also stored at the same conditions. The room temperature was in the range of 28-30 °C and the relative humidity (RH) of the air was ranged from 75 to 85% during the whole storage period.

2.5. Physical analysis

2.5.1. Percentage of weight loss

Theinitial weight of all irradiated chitosan treated and untreated (control) green mango samples were recorded initially. In a definite time, the weight losses of all mango samples were checked and recorded up to fresh condition. The percentage of weight losses in a given time intervals were calculated as the total weight loss divided by initial weight multiplied by 100.

2.5.2. Spoilage rate

The spoilage of treated and untreated mangoes due to fungal or any other microorganism infection was observed in a given time interval and recorded. Each batch consisted of 6 pcs green mangoes (average weight 600g) for observation. The percentage of spoilage rate was calculated as the number of spoiled or partly infected mangoes divided by the initial number of all mangoes multiplied by 100.

2.6. Microbiological analysis

Bacterial and fungal counts were performed by Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) media respectively. 1 g mango sample was mixed in 10 mL of saline water and the 10 μL sample was spread in PCA and PDA plates. The plates were incubated at 37 °C and 25 °C for 24 hours respectively. Then the colony forming units (cfu/g) were counted. All cfu counts were performed in triplicate.

3. Results and discussions

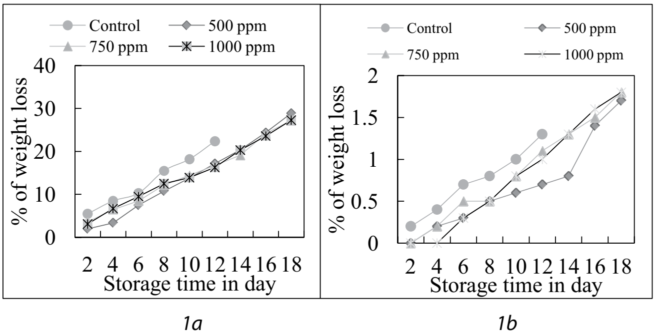
3.1. Characterization of irradiated chitosan solution

Viscometric method through Mark Houwink equation and FTIR spectroscopy were used to determine the average molecular weight and the degree of deacetylation (DD) of irradiated chitosan respectively. The effect of various gamma radiation doses on the average molecular weight and degree of deacetylation of chitosan solution were reported previously by our research group [17]. The degree of deacetylation of irradiated chitosan at 40 kGy radiation was approximately 79%.

3.2. Observation of shelf life extension

3.2.1. Percentage of weight loss

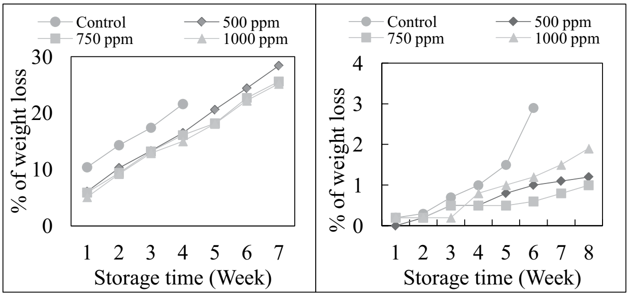
The percent of weight loss of several chitosan solutions treated and untreated (control) green mango samples in open and in zip bag at room (Fig.1a and 1b) and freezing (Fig.2a and 2b) temperature were observed during the whole storage periods respectively. It was observed that chitosan solution treated mango samples in open maintained weight significantly. The rate of change of weight losses for all treated samples were more or less same during six-day storage period; although significantly less than that of untreated (control) sample. After six days, the percent of weight loss of control sample sharply increases with increasing storage period, whereas it was slowly increased for treated samples in open conditions. 12 days later, all the control samples were become shrinkage and completely spoilt. But 18 days later, both 750 and 1000 ppm chitosan treated samples exhibited a weight loss of 27.3%, while 500 ppm treated sample showed weight loss of 28.9%. This results may be due to the slow evaporation and transpiration rate of mango. In addition to reducing respiration rate, chitosan coating can form a film on fruit surfaces that reduces respiratory rate by controlling the permeability of carbon dioxide and oxygen [18]. Chitosan also act as a hydrophobic barrier and thus prevent evaporation of water [19] from inner cell. On the other hand, insignificant changes of weight loss were observed for all treated and control mango samples in zip bag at room temperature during 18-days and 12-days storage periods (Fig 1b) respectively. But 12 days later, all the control samples were completely spoilt. This result may be attributed due to no evaporation and transpiration in zip bag. The weight changes of different chitosan treated and untreated (control) green mango samples in open and in zip bag at freezing temperature were observed on a weekly basis during 7-weeks and 8-weeks storage periods (Fig 2a and 2b) respectively. The observation showed that 750 and 1000 ppm chitosan solution treated mango samples in open at freezing temperature significantly maintained weight compared to control and other samples. It was observed that the rate of change of weight loss of treated mangoes in open are more or less same during first three weeks; although less than untreated (control) sample.



1a *1b*

***Fig 1.** Evaluation of weight loss (%) of chitosan treated and untreated green mango stored in open (Fig.1a) and in zip bag (Fig.1b) at room temperature*

After 4 weeks, all the control samples were become shrinkage, dried and completely spoilt. However, the treated samples were recorded at 25.2% weight loss for both 750 and 1000 ppm while 28.4% for 500 ppm chitosan after 7-weeks storage periods respectively.In case of all treated and untreated green mango samples in zip bag at freezing temperature, no significant changes of weight loss were found during 8-weeks storage periods (Fig. 2b). This result was attributed due to irradiated chitosan coating act as a hydrophobic barrier and thus prevent evaporation of water from inner cell of mangoes [19] and also insignificant evaporation and transpiration in zip bag. Chitosan coating improves the other quality such as slower softening, texture changes and color retention.



2a *2b*

Fig 1. *Evaluation of weight loss (%) of chitosan treated and untreated green mango stored in open (Fig.2a) and in zip bag (Fig.2b) at freezing temperature*

After taking both temperatures into account, it was observed that 750 ppm chitosan solution confirmed the least weight loss on average and hence extended the shelf life significantly.

3.2.2 Spoilage rate

The untreated (control) green mango samples both in open and in zip bag at room temperature begun to shrinkage, spoilage and dryness after 6-days. Whereas all the treated mangoes were completely fresh up to 12-days and then begun to partially shrinkage, spoilage and dryness. It was observed that after 12-days, all the control samples were completely spoiled; whereas treated samples showed extending shelf life up to 7days during 18-days storage periods (Table 1). In addition, mango samples in zip bag at room temperature had the highest spoilage rate. Thus, 750 ppm chitosan treated mango samples at room temperature had the highest shelf life during the whole storage periods. These results may be attributed to form a protective film on chitosan coated fruit surfaces and consequently the NH^{3+} group of chitosan may also be attributed to confine the growth of harmful germs, thus effectively controlling the fruit spoilage [18].

Table 1. Spoilage rate (%) of Green mango samples in open and in zip bag at room temperature during 18-days storage periods

Chitosan concentration	Spoilage rate (%)											
	3rd day		6th day		9th day		12th day		15th day		18th day	
	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag
Control	0	0	10.5	16	50	60	100	100	-	-	-	-
500 ppm	0	0	0	0	16	32	48	60	64	80	80	96
750 ppm	0	0	0	0	0	16	32	45	45	60	64	80
1000 ppm	0	0	0	0	0	16	32	50	48	70	70	90

Table 2. Spoilage rate (%) of Green mango samples in open and in zip polybag at freezing temperature during 8-weeks storage periods

Chitosan concentration	Spoilage rate (%)											
	3rd weeks		4th weeks		5th weeks		6th weeks		7th weeks		8th weeks	
	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag	Open	Zip bag
Control	50	16	70	45	100	66	-	80	-	-	-	-
500 ppm	0	0	16	0	32	16	75	32	100	64	-	80
750 ppm	0	0	16	0	32	16	64	32	96	48	-	60
1000 ppm	0	0	16	0	32	16	70	32	100	60	-	75

The spoilage rate of irradiated chitosan treated green mango samples both in open and in zip bag at freezing temperature was greatly reduced than that of control samples during the 8-weeks storage periods (Table 2). The untreated samples in open and in zip bag were spoilt after 4 and 6 weeks respectively (Table 2).Whereas all the treated mango samples at freezing temperature were almost fresh up to 5 weeks in zip bag and then begun to partially spoil. It was observed that chitosan treated samples in zip bag at freezing temperature showed relatively lower spoilage rate and thus exhibited the extending shelf life up to 3 weeks during 8-weeks storage periods (Table 2). Moreover, 750 ppm chitosan treated samples in zip bag at freezing temperature showed the best result. All these results might be ascribed due to the antimicrobial activity of chitosan and thus effectively controlling the fruit spoilage [18]. Therefore, it has been observed that 750 ppm chitosan solution was not the favorable condition for growth of microorganism to spoil mango samples rapidly and hence, extending the shelf life in both conditions during the whole storage periods.

3.3. Microbiological analysis

3.3.1 Total Bacteria Count (TBC) and Total Mold Count (TMC)

Fig. 3a and 3b represent the TBC and TMC of 750 ppm chitosan solution treated and untreated green mango samples at room and freezing temperature respectively. At room temperature, the TBC was found 10,000 and 730 cfu/g for untreated and 750 ppm chitosan treated mango samples respectively; whereas at freezing temperature it was found 3160 and 200 cfu/g for untreated and 750 ppm chitosan treated mango samples respectively. The lowest TBC was found to be 200 cfu/g for 750 ppm chitosan treated mango sample at freezing temperature.

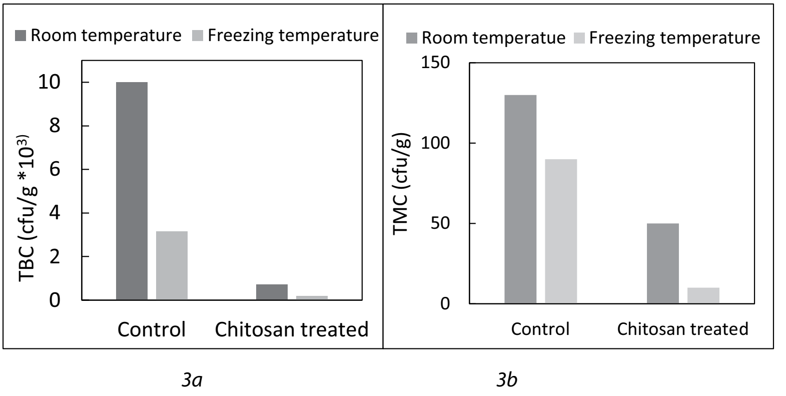


Fig 3. *TBC (Fig.3a) and TMC (Fig.3b) of 750 ppm chitosan treated and untreated (Control) green mango after 3-weeks storage periods at room and freezing temperature respectively*

This observation suggested that the variation of TBC depends on the storage temperature and thus microbial growth significantly decreased at lower temperature. The TMC was found 130 and 50 cfu/g for untreated and 750 ppm chitosan treated mango samples at room temperature respectively; while at freezing temperature it was found 90 and 10 cfu/g respectively. 750 ppm chitosan treated green mango sample showed the lowest TMC of 10 cfu/g at freezing temperature. This observation further suggested that the variation of TMC also depends on the storage temperature and hence microbial growth significantly decreased at lower temperature. Based on the report, irradiated chitosan can be characterized as antimicrobial agent. These results could be ascribed to highly effective antimicrobial activity of irradiated chitosan. Many scientists reported the similar results about the antimicrobial activity of chitosan against different groups of microorganisms, such as bacteria, yeast and fungi, and have received great attention [20-22]. These findings further supported that irradiated chitosan showed the extending shelf life of green mango duringthe preservation periods. Moreover, 750 ppm chitosan treated mango samples in zip bag at freezing temperature exhibited the best result.Therefore, it has been observed that 750 ppm chitosan solution was not the favorable condition for growth of microorganism.

4. Conclusion

In this investigation, the preservation of green mango by irradiated chitosan of 750 ppm solution was found to prolong the shelf life by the highest degree than that of others treated and untreated one. In addition, 750 ppm chitosan solution treated mango at freezing temperature showed the lowermost percentage of weight loss, spoilage rate, total bacteria count (TBC) and total mold count (TMC) during the storage periods; thus significantly extend shelf life of green mango. This observation suggested that the lower TBC and TMC depends on the storage temperature and hence microbial growth significantly decreased at lower temperature. Moreover, the antimicrobial activity of irradiated chitosan greatly increased the shelf life of green mango.Therefore, finally this research recommended that 750 ppm irradiated chitosan solution can be used as most effective and safest natural preservative for extending the shelf life of green mango fruits at low temperature.

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