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PERFORMANCE OF IRRADIATED CHITOSAN AND FORMALIN TO PRESERVE BANANA AND BITTER GOURD

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Abstract

The demand for safe and hazard free agricultural products prompted investigating the potentiality of natural preservative for the preservation of fruits and vegetables. Chitosan is one of the most studied biopolymer as natural preservative. Research was conducted to assess the feasibility of extension of shelf life of banana (*Musa sapientum*) and two varieties (local and hybrid) of bitter gourds *(Momordica charantia. L)* were used for chitosan treatment. The fruits (bananas) were stored at ambient environment (30±1^{o}C and 75% relative humidity) and vegetables (bitter gourds) were stored both at ambient temperature as well as refrigeration condition (2-4^{o}C). The effects of chitosan coating on banana and bitter gourd were examined by means of shelf life, microbiological examination and sensory evaluation. The overall results showed that effectiveness of 40kGy irradiated Chitosan-1000ppm solution showed the best result in extending the shelf life of banana (60% increased) and deshi/local bitter gourd (about 65% increased) and chitosan-1500ppm acted most fruitfully only for hybrid bitter gourd (about 56% increased). On the other hand, 2.0% concentrations of chitosan have an ability to inhibit the load of microorganisms significantly with no cytotoxic effect. Finally, there was a significant effect of chitosan was observed with compare to formalin.

Keywords: Chitosan, Formalin, Banana, Bitter gourds

Introduction

Fruits and vegetables are highly valued in human diet mainly for vitamins and minerals. However, the present consumption of fruits and vegetables in Bangladesh is 126g/day/capita which are far below the minimum average requirement of 400g/day/capita (FAO/WHO, 2003). Bangladesh produces 4-5 million tons of horticultural produce. There is estimation that only 0.5% of the horticultural production is processed (Mazed, 1999). The post harvest losses for crops are huge in Bangladesh. For example, in 2004-2005, fruits produced 1.50 million M. tons, where post harvest losses was about 0.45 million M. tons which exhibits the loss in total production is 30% (GOB, 2000). A study primarily revealed that post harvest spoilage of some selected fruits and vegetables causes a loss of Taka 3,392 core every year in Bangladesh (Binh *et al.,* 1995).

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Many techniques have been studied in order to overcome these problems and extend the shelf life of fresh produce. Most fruit and vegetable sellers use formalin or formaldehyde, as an organic compound. Traders use this chemical as a preservative and to make fruits and vegetables look fresh for longer periods. In humans, the ingestion of formaldehyde has been shown to cause vomiting, abdominal pain, dizziness, cancer and in extreme cases can cause death. So, the food industries in many countries have been looking for natural and safe food preservatives (Sonti, 2003). Chitosan is non-toxic and easily biodegradable with gel forming ability at low pH (Muzzarelli, 1999). They can act as moisture and gas barriers, control microbial growth, preserve the color, texture and moisture of the product, and can effectively extend the shelf life of the product (Diep *et al.,* 2000). 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 post harvest losses and maintaining hygienic quality of fresh produce (Boylston *et al.,* 2002; Cheour and Mahjoub, 2003 and Gonzalez *et al.,* 2004). Bananas (*Musa sapientum*) are a leading tropical fruit in the world market and it is one of the most popular fruit eaten in Bangladesh. Moreover, it is a popular dessert cultivar, widely grown in the north and western areas of Bangladesh accounts for 40.7% of the total fruit production in the country (Islam and Hoque, 2004). Bitter gourd *(Momordica charantia.L)* is also one of the important cucurbitaceous vegetables due to its high nutritional and medicinal values. It is referred to 'King of gourds' because of its higher nutrient content (Saha *et al.,* 2004). To date, use of this irradiated coating material has not yet been reported on fresh banana and bitter gourd. 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 the quality of banana and bitter gourd.

Material and Methods

Study area: The experiment was conducted in the laboratory of FTNS, MBSTU, Tangail, Bangladesh and Institute of Radiation and Polymer Technology (IRPT), Bangladesh Atomic Energy Establishment, Dhaka. Fresh, healthy bitter gourd (local and hybrid) and naturally ripe banana were collected from Salimpur Upazila and Tangail Sadar.

Preparation of natural preservative (Chitosan): Prawn shell waste was used for the preparation of chitosan. After grinding and sieving the shell was deprotenized by 3% NaOH (w/v) for 2 hrs at 60^{o}C (solid: solvent/1:16). The extract was then washed and demineralized the chitin yield by 1N HCl for 2 hrs at 60^{o}C (solid: solvent/1:16). Followed by washing the extract was then decolorized by acetone and bleach bleached with 0.315% NaOCl for 5 min at room temp (solid: solvent/1:10). Deacetylation of chitin was done by 50% NaOH for 3 hours at 100^{o}C (solid: solvent/1:20). Finally the chitin was obtained through washing and drying.

Radiated chitosan: The prepared natural preservative (chitosan) was irradiated at gamma radiation. For banana and bitter gourd preservation 40kGy radiation doses was used.

Microbial analysis: Regarding microbial ecology, the levels of aerobic mesophilic microorganisms and fungal infection were determined as described in Lopez-Galvez *et al.* (2013).

Determination of cytotoxicity: In vitro cytotoxicity test was performed using Brine Shrimp Lethality Bioassay method.

Sensory analysis: For sensory analysis, quality was evaluated by a panel of three trained members. The samples were judged separately by a team of experienced judges for organoleptic parameters like colour, texture, taste, flavour and aroma using the Hedonic scale suggested by Krum (1955)**.**

Statistical analysis: Two replications of each experiment, separately in time, were carried out. Samples were evaluated in triplicate per treatment and evaluation period. ANOVA and Brown–Forsythe tests were used in order to compare different treatments depending on the homogeneity of the variances using SPSS 19.0 for Windows at a significant level of $P \leq 0.05$ for all the evaluations.

Results and Discussion

Extension of shelf life at Ambient and Refrigeration Environment: Banana

At ambient temperature: Single treated banana

The control and single treated bananas (sprayed once) were preserved at room temperature (30- 32ºC) and at normal humidity (77-85%) condition. After 3 days of preservation, result showed that sensational yellow color was developed in 90% of bananas at controlled atmosphere. On the other hand, 98% yellow and 2% greenish yellow for chitosan-1000ppm; 95% yellow and 5% greenish yellow for chitosan-1500ppm were developed and no fungal growth was found visually in any of the samples (Fig. 2). After 5 days of storage, bananas preserved at control atmosphere were rejected considering the visual quality. Chitosan-1000ppm showed 10% blackish area and Chitosan-1500ppm showed 30% blackish area. Formalin (1 %) showed 20% blackish area with ripening (Fig. 1). There was no significant change during storage among the treatments.

Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

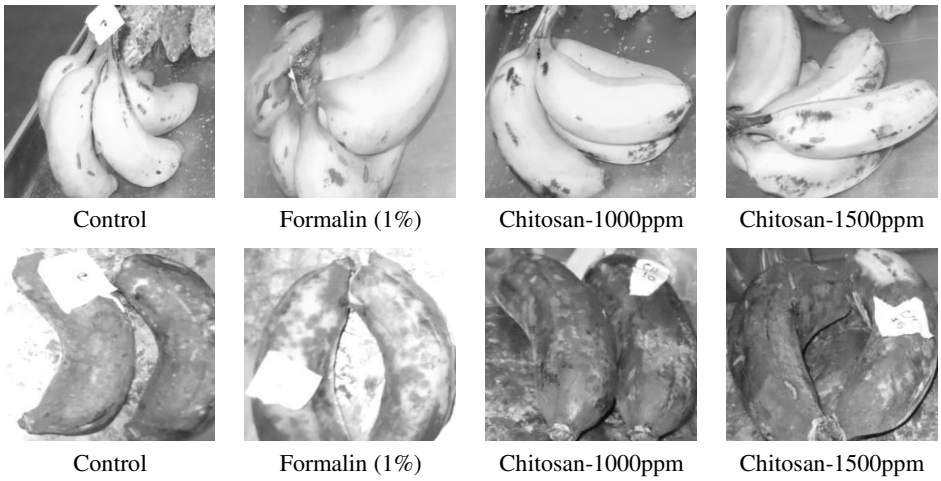


Fig. 1. Photographs showing the effects of different treatments on banana at ambient temperature (A- 0 days after treatment; B- 3 days after treatment and C- 5 days after treatment)

At ambient temperature: Multiple treated bananas

The control and multi treated bananas (sprayed multiple time) were preserved at room temperature and at normal humidity conditions. There were no significant changes during storages.

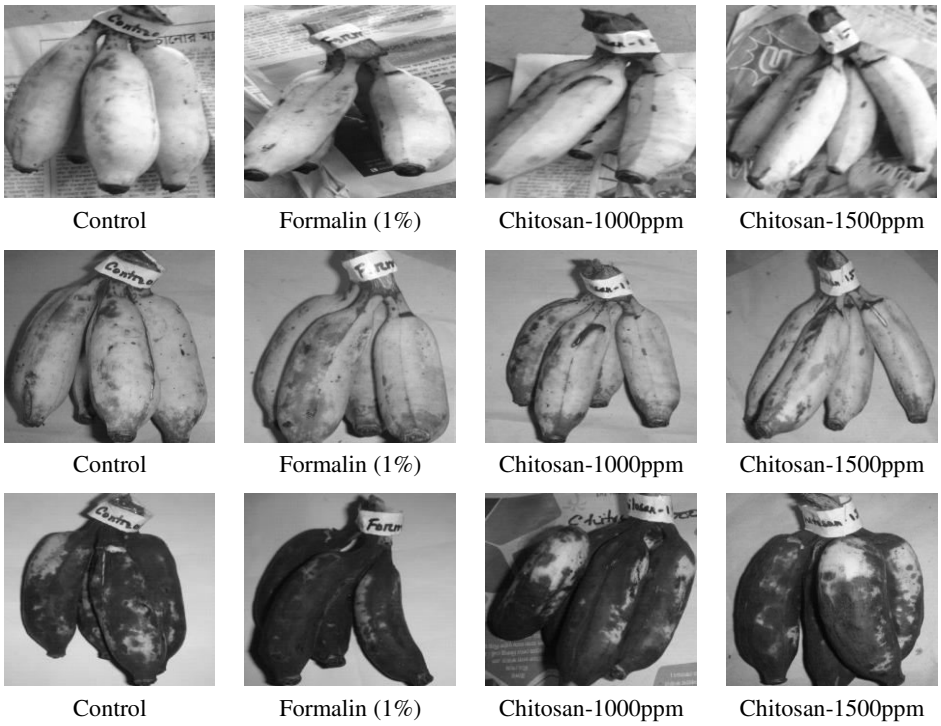


Fig. 2. Photographs showing the effects of different treatments on banana at ambient temperature (A- 0 days after treatment; B- 3 days after treatment and C- 5 days after treatment)

Biter Gourd

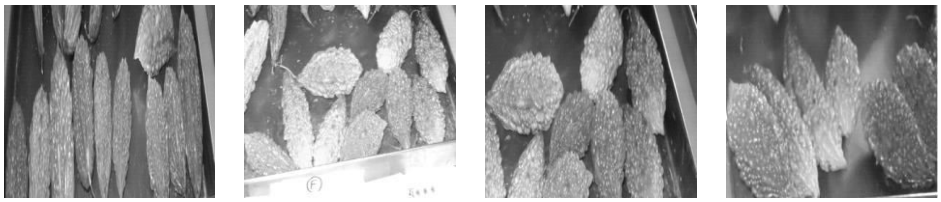
The control and treated bitter gourds were preserved at ambient and refrigeration environment, the shelf life was observed for 5 samples in each group.

At ambient temperature: Single treated bitter gourd (local & hybrid)

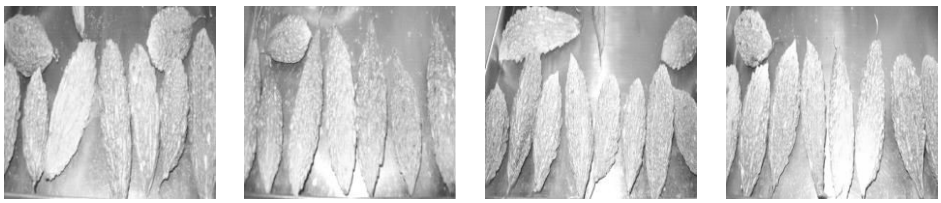
There were no significant changes occurred during the storage in both varieties of samples.

At ambient temperature: Multiple treated bitter gourd (local)

The shelf life was observed for 10 samples in each group for 3 days at normal atmospheres. After 3 days of storage visual fungal infection was observed in the controlled samples but there were no significant changes among the rest of the samples during the storage period (Fig.3).



Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

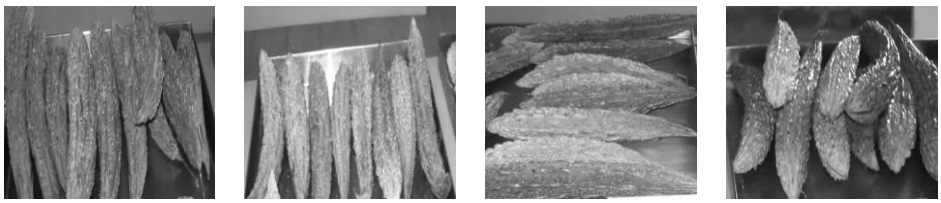


Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

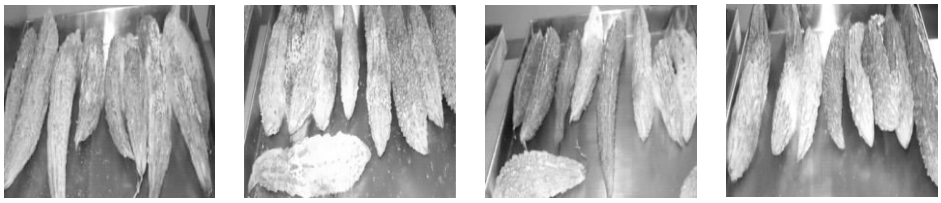
Fig.3. Photographs showing the effects of different treatments on bitter gourds at ambient temperature (A- 0 days after treatment; B- 3 days after treatment and C- 5 days after treatment)

At ambient temperature: multiple treated bitter gourd (hybrid)

There were no significant changes in case of visual and fungal infection during the storage periods (Fig.4).



Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

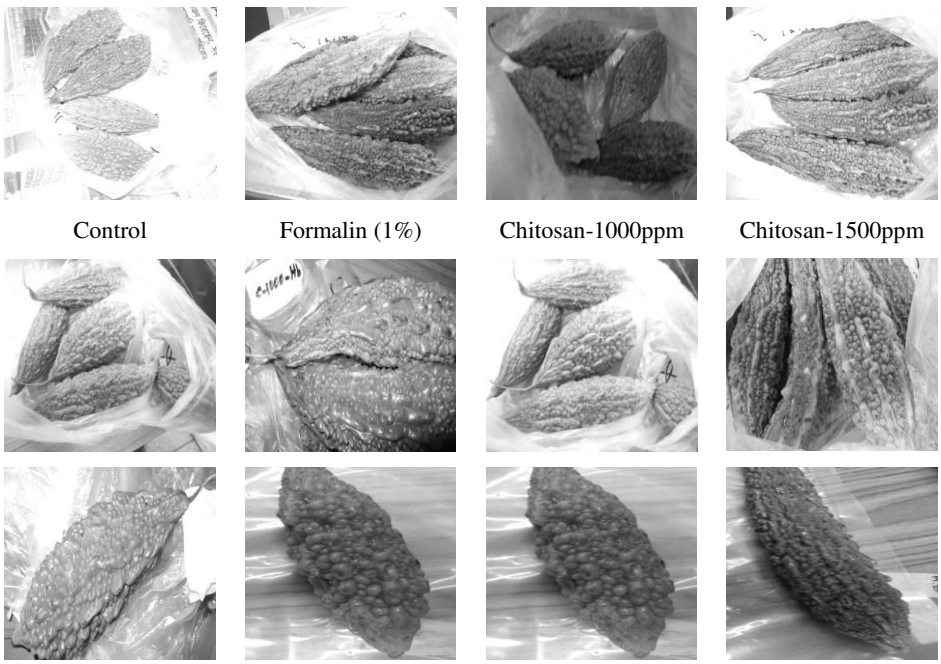


Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

Fig. 4. Photographs showing the effects of different treatments on bitter gourds at ambient temperature (A- 0 days after treatment; B- 3 days after treatment and C- 5 days after treatment)

At refrigeration temperature: single treatment of bitter gourd (local))

The shelf life was observed for 5 samples in each group. All groups of samples were wrapped with poly bag individually. The freezing temperature was 2-4^{o}C. At 0 day all samples were 100% green for control, Chitosan-1000ppm, Chitosan-1500ppm, formalin (1%) solution and no fungal growth was found visually. After 7 days of storage there were no significant changes in term of visual inspection but in every case only moisture loss was observed. After 14 days, control samples were rejected due to spoilage by fungal infection but still the other samples were accepted in term of sensory evaluation. After 23 days of storage, chitosan-1000ppm and chitosan-1500ppm treated samples were still showed well condition without fungal infection where as controlled and formalin treated samples were rejected visually.



Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

Control Formalin (1%) Chitosan-1000ppm Chitosan-1500ppm

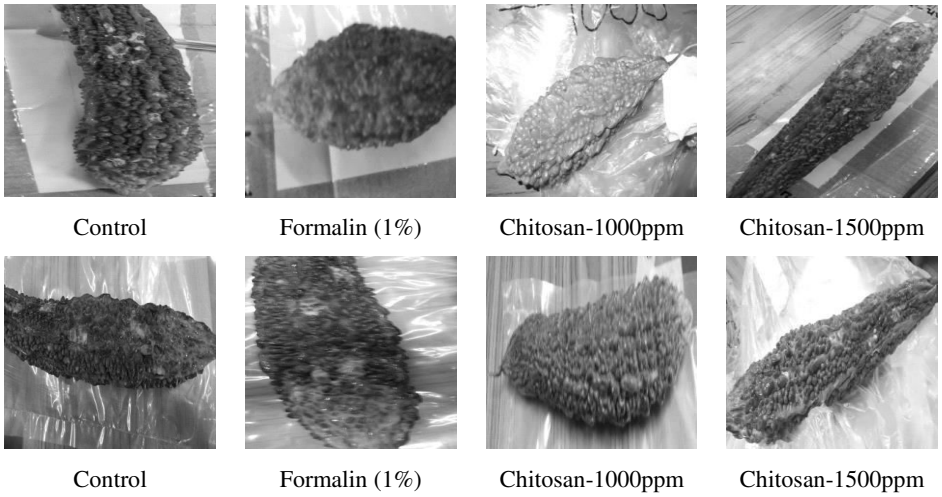
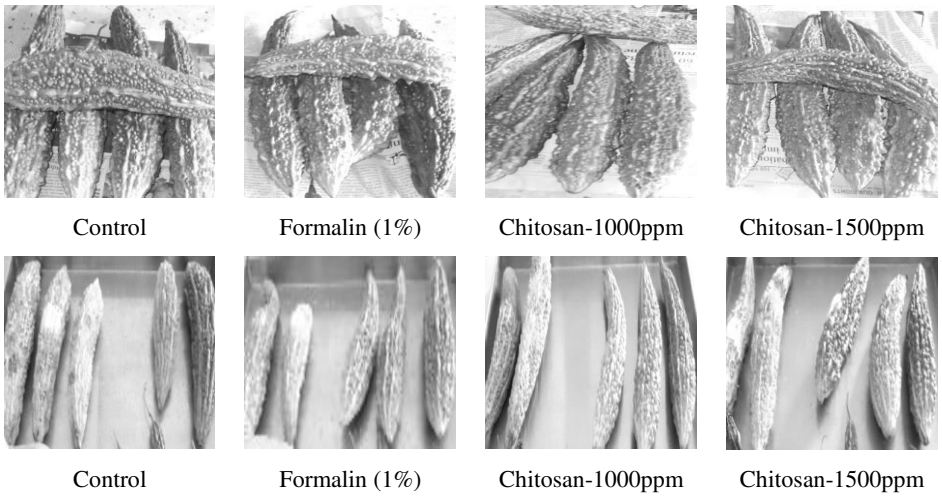


Fig. 5. Photographs showing the effects of different treatments on bitter gourds at refrigeration temperature (A- 0 days after treatment; B- 3 days after treatment, C-10 days after treatment, D-14 days after treatment, and E- 23days after treatment)

At refrigeration temperature: single treated bitter gourd freezing (hybrid)

The shelf life was observed for 5 samples in each group. All groups of samples were wrapped with poly bag individually. The refrigeration was 2-4^{o}C. At 0 day all samples were 100% green for control, Chitosan-1000ppm, Chitosan-1500ppm, formalin (1%) solution and no fungal growth was found visually. The samples were stored and evaluated up to 5 days and there were no significant changes among the treatments.



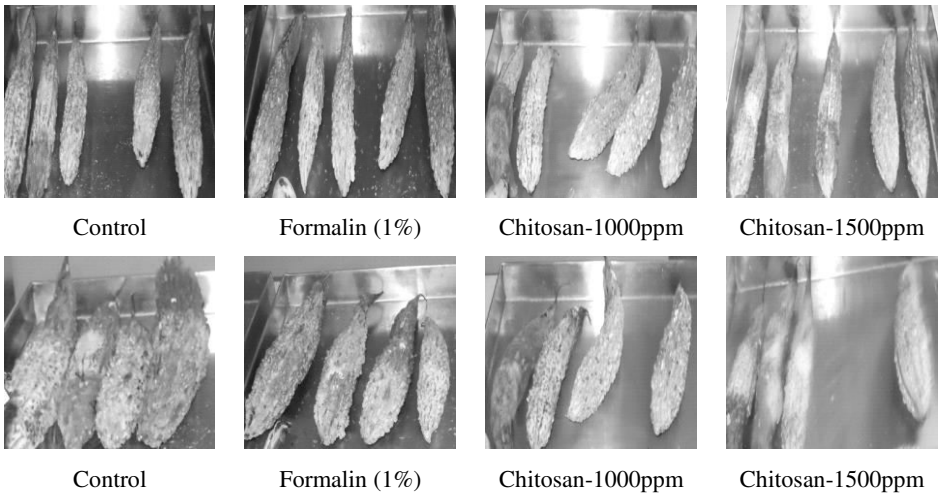


Fig. 6. Photographs showing the effects of different treatments on bitter gourds at refrigeration temperature (A- 0 days after treatment; B- 3 days after treatment, C-4 days after treatment and D-5 days after treatment)

Microbiological Analysis

Bitter Gourd: Bacterial Count

Table 1. Bacterial count of preserved bitter gourd (local & hybrid) at ambient environment

Sample Name	Outer Count (cfu/g)		Inner count (cfu/g)	
	Local sample	Hybrid sample	Local sample	Hybrid sample
Control	1.9×10^6	3.13×10^6	2.5×10^3	5×10^3
Formalin	9.5×10^6	1.1×10^7	3×10^5	3.5×10^5
Chitosan-1500ppm	5.75×10^6	6×10^6	6×10^6	9×10^4

Bitter Gourd: Fungal count

Table 2.Fungal count of preserved bitter gourd (local & hybrid) at ambient environment

Sample Name	Outer Count (cfu/g)		Inner count (cfu/g)	
	Local sample	Hybrid sample	Local sample	Hybrid sample
Control	5×10^3	3.15×10^3	2×10^2	$< 10^2$
Formalin	1.8×10^3	3.75×10^3	6.5×10^2	$< 10^2$
Chitosan-1500ppm	1.25×10^3	2.5×10^3	$< 10^2$	$< 10^2$

Cytotoxicity Test

Brine Shrimp Lethality Bioassay method was used for cytotoxic effect of the bitter gourds as a vegetable sample. Bitter gourd (local & hybrid) juice was dissolved in artificial sea water in which nauplii were inoculated. The number of death of nauplii was

found to increase at higher concentration of the juice (Table 3). It may be happened owing to three reasons: cytotoxic effect of the bitter gourds, decrease of dissolved oxygen concentration of the saline water, and formation of the viscous layer on the gills of nauplii.

Table 3. Mortality of brine shrimp (*A. salina*) nauplii at different concentrations of bitter gourd juice

Sample Name	Parameter	Dose (mg/ml)	Initial Nauplii Taken	No. of Nauplii Present after Incubation	Mortality (%)
Control (Negative)	Artificial saline water	_	10	9	10
Control (Positive)	vincristine sulfate	10ul/ml	10	0	100
Sample-1	Fresh bitter gourd control juice(Hybrid)	10ul/ml	10	4	60
Sample-2	Fresh bitter gourd control juice(Hybrid)	20ul/ml	10	0	100
Sample-3	Fresh bitter gourd control juice(Deshi)	10ul/ml	10	1	90
Sample-4	Fresh bitter gourd control juice(Deshi)	20ul/ml	10	0	100
Sample-5	4^{th} day control sample juice(Hybrid)	10ul/ml	10	0	100
Sample-6	4^{th} day control sample juice(Hybrid)	20ul/ml	10	1	90
Sample-7	4^{th} day control sample juice(Deshi)	10ul/ml	10	2	80
Sample-8	4^{th} day control sample juice(Hybrid)	20ul/ml	10	0	100
Sample-9	4^{th} day Chitosan-1500 sample juice(Hybrid)	10ul/ml	10	4	60
Sample-10	4^{th} day Chitosan-1500 sample juice(Hybrid)	20ul/ml	10	0	100
Sample-11	4^{th} day Chitosan-1000 sample juice(Deshi)	10ul/ml	10	6	40
Sample-12	4^{th} day Chitosan-1000 sample juice(Deshi)	20ul/ml	10	0	100

Sensory evaluation of preserved banana and bitter gourd

Banana: After analysis the scoring value, an increasing trend was observed first time and then decreasing respectively.

Color: The result showed that the single treatment color formalin (1%) showed the highest mean than other coated fruits. The lowest mean was observed in control (Table 4). Similar result was also found in multiple treated samples (result not shown).

Table 4. Color of banana at single treatment of natural preservative (Chitosan) and formalin (1%) at ambient environment

Storage Period (Days)	Color			
	Control	Formalin (1%)	Chitosan-A	Chitosan-B
0 day	10	10	9.5	9
3rd	6	8	9	7
5th	2	6	4	3
8th	2	3	1	2
Mean	5	7.25	5.9	5.25

A= 1000ppm chitosan solution and B=1500ppm chitosan solution

Texture: For single treatment 40kGy irradiated Chitosan-1000ppm solution showing the highest mean in texture. The lowest mean score was observed in control (Table 5). As Chitosan coating created modified atmosphere in fruits so it retained strong texture than uncoated fruits. Similar result was also found in multiple treated samples (result not shown).

Table 5. Texture of banana at single treatment of irradiated natural preservative (chitosan) and formalin (1%) at ambient environment

Storage Period (Days)	Texture			
	Control	Formalin(1%)	Chitosan-A	Chitosan-B
0 day	10	10	10	10
3	8	10	10	8
5	4	7	6.5	5
8	2	4	5	4
Mean	6	7.75	7.87	6.75

A= 1000ppm chitosan solution and B=1500ppm chitosan solution

Putrefied area (%): For single treatment between Chitosan-1000ppm and Chitosan-1500ppm, the first one has the highest score. On the other hand, among all samples control showed the lowest score (table 6). In multiple treated samples, controls had highest tasting score where 40kGy irradiated Chitosan-1500ppm solution had lowest score.

Table 6. Putrefied area of banana at different treatment of irradiated natural preservative (chitosan) and formalin (1%) at ambient environment

Storage Period (Days)	Putrefied area (%)			
	Control	Formalin (1%)	Chitosan-A	Chitosan-B
0 day	10	10	10	10
5	8	10	9	7
8	3	7	6	4.5
9	Rejected	Rejected	Rejected	Rejected
Mean	7.0	9	8.33	7.16

A= 1000ppm chitosan solution and B=1500ppm chitosan solution

Bitter Gourd

Color: In single treatment bitter gourd (local) chitosan-1500ppm solution dose showed the highest acceptability in color. Chitosan-1500ppm and 1000ppm solution dose have greater acceptability than 1% formalin solution. For single treatment (hybrid) chitosan-1000ppm solution dose showed the highest acceptability in color. Chitosan-1500ppm and 1000ppm solution dose have greater acceptability than 1% formalin solution.

Texture: Both chitosan-1000ppm and chitosn-1500ppm solution had hard texture than uncoated bitter gourd (local) for single treatment. Chitosan-1000ppm and Chitosan-1500ppm solution showed the highest acceptability than others. 1% formalin solution showed the lower acceptability. Also chitosan-1000ppm and chitosn-1500ppm solution had hard texture than uncoated bitter gourd (hybrid) for single treatment. 1% formalin solution showed the highest acceptability than others. Chitosan -1000ppm and Chitosan-1500ppm solution showed the lower acceptability (Table 7). Overall, there were no significant different between the samples and also in treatments.

Table 7. Texture of bitter gourd (local & hybrid) at different single treatment

	Texture							
		Local				Hybrid		
Storage Days	Control	Formalin (1%)	Chitosan-A	Chitosan-B	Control	Formalin (1%)	Chitosan-A	Chitosan-B
0 day	10	10	10	10	10	9	10	10
3	7	8	10	10	4	8	7	6
4	6.5	7	9	7	1	4	2	2
5	4	4	6	5	Reject	Reject	Reject	Reject
Mean	6.87	7.25	8.75	8.0	5.0	7.0	6.33	6.0

A= 1000ppm chitosan solution and B=1500ppm chitosan solution

Putrefied area (%): From this study, it was observed that chitosan-1500ppm solution had greater effectiveness than Chitosan-1000ppm solution, formalin (1%) and uncoated bitter gourd (local) samples. Chitosan-1500ppm and Chitosan-1000ppm solution showed the highest acceptability than others. 1% formalin showed the lower acceptability (Table 8). It was observed that Chitosan-1500ppm solution had greater effectiveness than Chitosan-1000ppm solution, formalin (1%) and uncoated bitter gourd (hybrid) samples. Chitosan-1500ppm and chitosan-1000ppm solution showed the highest acceptability than others. Control samples showed the lower acceptability.

Banana is a perishable fruit (Sardar *et al.,* 2008) but after application of irradiated chitosan solution as preservative the shelf life has extended. 40kGy radiation with 1000ppm showed the best result as it had no visual fungal growth for single treatment. On the other hand 1000ppm solution showed the best result as it had no visual fungal growth for multiple treatments. This radiation solution dose degraded the Chitosan in such molecular weight which act as perfect preservative film. At normal temperature in

single treatment bitter gourd (local) chitosan-1000ppm irradiate solution showed the best result than others and for bitter gourd (hybrid) chitosan-1500ppm solution showed the best result than other. Again multiple treatment bitter gourd (local) chitosan-1000ppm irradiate solution showed the best result than others and for bitter gourd (hybrid) chitosan-1500ppm solution showed the best result than other. In freezing treatment the bitter gourd (local) control was spoiled at 14th day but the treated bitter gourd (local) sample spoiled at 23th day for single treatment where chitosan-1000ppm showed the best result .In addition to, bitter gourd (hybrid) control was spoiled after 9 days but the treated bitter gourd (Hybrid) sample spoiled at 14th day for single treatment where Chitosan-1500ppm showed the best result. These results are consistent with other scientists in case of mango, carrot and tomato (Sardar *et al.,* 2008; Barzegar *et al.,* 2008 and Casariego *et al.,* 2008).

Table 8. Putrefied area (%)) of bitter gourd (local & hybrid) at different single treatment

Storage Days	Sensory Evaluation Putrefied area (%)							
		Local			Hybrid			
	Control	Formalin (1%)	Chitosan-A	Chitosan-B	Control	Formalin (1%)	Chitosan-A	Chitosan-B
0 day	10	10	10	10	10	9	10	10
3	9	9	10	10	3	8	6	8
4	9	8	10	9	2	1	3	2
5	4	3	5	7	Reject	Reject	Reject	Reject
Mean	8.0	7.5	8.75	9.0	5.0	6.0	6.33	6.66

A= 1000ppm chitosan solution and B=1500ppm chitosan solution

Study showed that chitosan extend the shelf life of litchi (Sun *et al.,* 2010). Chitosan coating was seen to delay fruit senescence of strawberry fruits stored at 10ºC and 70±5% relative humidity (Hernandez *et al.,* 2008). In the present study, the possibility of death of nauplii owing to toxicity is very low as the number of death was nil for lower concentrations, suggesting no cytotoxic effect. Moreover, chitosan was used as parent materials of the bitter gourds. Hence, the most possible reason for the death of nauplii is the formation of the viscous layer on the gills of the nauplii as the highly concentrated juice of the bitter gourds led to high viscosity and thus tends to the formation of gel like structure on the gills which eventually inhibit oxygen permeability of the gills and caused death.

The mean values showed that in general, the color score was vary from day to day of storage in banana and bitter gourd. In our studies, the application of chitosan coating delayed the change in eating quality and partially inhibited the oxidative enzymes activity, phenylalanine ammonia lyase (PAL), peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO) of banana and in bitter gourd which is associated with discolouration.

This implies that the chitosan coating may form a protective barrier on the surface of the samples and reduce the supply of oxygen. Therefore, color variation highly occurred in control sample than chitosan coating sample. Superior variation of color scores might be due to chitosan coating, which maintained color and retained the quality of fruit until 1 week and vegetable until 4 weeks of storage the decay started. Jiang & Li (2001) reported that chitosan treated long an fruit had good eating quality even after 30 days of storage at 2°C. Chitosan retained fruit and vegetable quality and no off color was developed than control. These results tally with Munoz (2006) who reported the influence of the chitosan on strawberries stored at 20°C for 4 days showing better maintenance of eating quality. Also, chitosan delayed texture changes by inhibiting the increase of the enzyme activity of cellulose in Chitosan treated samples compared with the untreated sample. Doreyappa & Huddar (2001) reported that texture of mangoes after ripening showed significantly decreasing trend as the storage period proceeded when stored at 32-35°C. It might be due to fluctuations in acids, pH and sugar/acid ratio (Jitareerat *et al.,* 2007). Fruit and vegetable treated without chitosan coating did not develop texture while Chitosan coated fruits and vegetable showed best results. Untreated control in banana fruits and bitter gourd vegetables had lowest textural scores. It might be due to the change in moisture, carbohydrates, proteins, amino acids, lipids and phenolic compounds that can influence the texture of fresh fruits and vegetable (Malundo *et al.,* 1997).

The beneficial effect of the elevated Chitosan concentration on firmness has also been reported for tomato (Ghaouth *et al.,* 1992a), peach, Japanese pear, kiwifruit (Du *et al.,* 1997), 'Murcott' tangor (Chien *et al.,* 2007a) and also in strawberry (Jiang & Li, 2001). The application of irradiated chitosan coating (with optimum concentration 1000ppm) could be beneficial and considered for commercial application in extending the shelf-life and assuring quality to some extent, controlling decay of banana and bitter gourd. In using chitosan for decay control, we consider that it may be suitable in the treatment of banana and bitter gourd stored for shorter periods (e.g. 8 and 5 days) or for short-distance transport and distribution. However, for longer storage (e.g. 23 and 14 days) and marketing, we adopted cold storage temperature (2-4^{o}C) with plastic packaging for bitter gourd (local and hybrid) sample to control discoloration and decay. Study also showed that 2.0% concentrations of chitosan have an ability to inhibit the load of microorganisms significantly. This treatment can ideally be adopted for enhancing storage life of highly perishable fruits and vegetables like banana and bitter gourd to restrict the use of harmful preservatives by the growers and traders.

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