International Letters of Natural Sciences
ISSN: 2300-9675, Vol. 60, pp 30-37
doi:10.18052/www.scipress.com/ILNS.60.30

Submitted: 2016-07-31
Revised: 2016-10-11
Accepted: 2016-10-17
Online: 2016-11-14

Gamma Radiation Treated Chitosan Solution for Strawberry Preservation: Physico-Chemical Properties and Sensory Evaluation

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Keywords: Strawberry fruit, Shelf life, Chitosan, Natural preservative.

Abstract. The research was designed to extend shelf life of stored strawberry by bioactive chitosan coating. Strawberry fruits were dipped for 2 min in different concentration of irradiated chitosan (300, 500, 1000 and 1500 ppm) solution which were subjected to different gamma radiation doses (30, 40, 50 and 60 kGy) while uncoated fruits were served as control. Samples were packed in zip bags and stored in 4°C and kept for observation. Fruits treated with 50 kGy irradiated chitosan at 1500 ppm concentration showed significant delays in the change of weight loss, decay percentage, pH, and ascorbic acid content as compared with the uncoated control fruits. Samples stored in 4°C and packed in zip bags showed an increased shelf life up to 21 days whereas control samples with same condition could maintain edible quality only up to 3 days. Compared to the controls, all coating formulations had positive effect on the inhibition of cell wall degrading enzymes. These findings suggest that the use of irradiated chitosan coatings can be very useful for extending the shelf-life and maintaining quality of strawberry fruit.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a fruit of unique taste with a source of high levels of vitamins (C and E), beta carotene, antioxidents and phenolic compounds such as anthocyanins which can exert a beneficial effect on health [1]. Being the most important phenolic compound, anthocyanin causes the red color of strawberry [2].

Strawberry is getting popular in Bangladesh. But due to the high degree of perishability and infection caused by bacteria and fungus, strawberry fruit has a very short shelf-life. Its sensitive peel has also made it very vulnerable and the fruit quality degrades rapidly. It is also very susceptible to mechanical injury that can reduce its quality rapidly thus making marketing a challenge [3]. At 0°C strawberry can be preserved up to 2 weeks and after bringing out these fruits can be consumed up to 3-4 days maintaining at ambient temperature (20°C) [4]. Some techniques like Chemical treatment, hot water treatment, ultraviolet (UV) light and controlled atmosphere are being used which adversely affect the flavor, color, aroma and texture of this fruit [5]. Treating strawberries with fungicides has been reported to leave harmful residual effect [6]. Moreover, consumer's preference for more natural way to preserve with a view to conserving the nutritional value has made natural preservation an increasing interest [7]. These can be done by using of bio-based edible coating that has the antimicrobial property to prolong shelf-life by creating a semipermeable barrier to gas transfer between the fruit and the surrounding atmosphere and thus can maintain taste, aroma, texture and appearance [8-10]. Irradiated chitosan can reduce mycelial growth and spore germination of gray mold and *Rhizopus*, and induce morphological change in causal organisms [11]. Moreover, chitosan elicits plant's resistance against pathogens [12]. That is why chitosan has achieved much attention to preserve fruit and vegetables for its biodegradable and non-toxic nature along with its preservation capability against the microorganisms [13]. Islam *et al.*

[14] and Ebrahim *et al.* [15] showed the effect of low molecular weight chitosan coating to extend the shelf life of mango and pineapple respectively. There are few papers reported the quality improvement of strawberry by various chitosan formulation [16-17] but so far, there is no data was reported on the effectiveness in the control of postharvest decay of strawberry of low molecular weight chitosan formulations, either alone or with other additives. In this paper, low molecular chitosan was prepared by irradiating chitosan solution by gamma radiation and the functionality of irradiated chitosan coating on strawberry preservation has been discussed.

2. Materials and Methods

2.1. Fruit source

Fresh strawberries (*Fragaria* × *ananassa* Duch*)* were collected from a garden at Savar, Dhaka, Bangladesh. These fruits were transported to the laboratory of Institute of Radiation and Polymer Technology (IRPT), Atomic Energy Research Establishment, Savar and were graded according to their size, shape and color, ripening and defects.

2.2. Chemicals

High molecular weight Chitosan solution (deacetylation degree 82.7%, viscosity less then 200maps, 2% acetic acid in 55°C) was extracted from prawn shell in Institute of Radiation and Polymer Technology (IPRT) laboratory. All other chemicals and solvents used were of laboratory grade.

2.3. Treatments

Gamma radiation of chitosan solution (2% w/v in 2% acetic acid) was done by a 120 kCurie radiation source at 3.2 kGy per hour dose rate. The experiment had two stages. At first different doses (30, 40, 50 and 60 kGy) of gamma irradiated chitosan solutions were applied on strawberries samples. The test samples were kept at 4°C in zip lock bags for five successive days and physical changes like weight loss, smell, color or texture change were observed. Best results were derived from 50kGy chitosan treated samples. At the second stage, various concentration of 50kGy solution was tested over a wide range of samples. Strawberry samples were dipped into the solution of 50kGy chitosan with 300ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm concentrations and then packed by zip lock bags. Samples were then stored in refrigerator at 4°C up to 21 days. Physical changes like weight loss, smell, color or texture changes were observed day to day.

2.4. Weight loss percentage

Strawberry samples (fifteen fruit per replication) were weighed at the beginning of the experiment (i.e. 0 day) and at the end of each storage interval. The difference between the initial and final weight of the fruit was considered as a total weight loss and the results were expressed as the percentage loss of the initial weight as per the standard method of AOAC [18].

2.5. Decay percentage

The number of decayed fruit due to fungal or any microorganism infection was recorded at 5 days intervals and calculated as a percentage. For each coating, a group of 4fruits (in three replicates) was observed. The decay percentage of coated and uncoated fruit was calculated as the number of decayed fruit divided by the initial number of all fruit multiplied by 100.

2.6. Measurement of pH

The pH of the fruit samples was assessed using a digital pH meter of type H1 98106, manufactured by HANNA, UK at ambient temperature according to the standard method described in AOAC [18].

2.7. Microbial count reduction

The bacterial and fungal count was estimated by Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) media respectively. 1g fruit sample was dissociated in 10 mL of saline water and then 10μL sample was spread in PCA and PDA plates. The plates were incubated for 24 hours in 37°C and 25°C respectively and colony forming units (cfu) were counted.

2.8. Sensory evaluation

The sensory analysis of the coated and uncoated (control) strawberry fruit was carried out according to the Bai *et al.* [19] with some modifications. The sensory quality was evaluated by color, taste, texture, flavor and overall acceptability for all the samples after 0, 5, 10, 15 and 21 days storage at 4°C. Samples of coated and uncoated strawberries were randomly presented to seven non-trained panelists for sensory evaluations and were asked to score the differences between the samples where 0–2 represented extreme dislike; 3–5 fair; 6–8 good; and 9 excellent for color, taste, texture, flavor and overall acceptability.

3. Results and Discussion

3.1. Effect of Gamma radiation on chitosan solution

The average molecular weight (Dalton) and degree of deacetylation of irradiated chitosan chitosan was determined by viscometric method through Mark Houwink equation and from FTIR spectroscopy (transmittance mode) respectively. The intrinsic viscosity and molecular weight of non- irradiated chitosan was found 153.1 mL/g and 188,014 Da respectively. But after radiation, both the intrinsic viscosity and molecular weight of the chitosan decreased significantly. In case of 50 kGy gamma irradiated chitosan the molecular weight was declined up to around 80000 Da. The decrease in molecular weight occurred due to the chain scission of chitosan molecules at glycosidic linkage with high ionizing gamma radiation.

Figure 1 shows significant differences in FTIR spectra of non irradiated and irradiated chitosan at 3450- 3500 cm^{-1} and 1650-1670 cm^{-1}. From the spectra, it was noticed that the ratio of absorbance at wave number 1665 cm^{-1}. A_{1655} (due to stretching of C=O of amide groups) to absorbance at wave number 3450 cm^{-1}, A_{3450} (due to O-H stretching overlapping the N-H stretching) decreased with increasing radiation dose which indicated the increase in degree of deacetylation (DD) of chitosan according to the method described by Baxter *et al.* [20]. Percentage of DD was found 72.73% for untreated chitosan and 80.54% for the chitosan treated by 50kGy gamma radiation. The increase of DD due to irradiation can be explained due to the fact that hydrolysis of the acetamide group to amine group occurs in presence of bound moisture by ionizing radiation thus DD is increased.

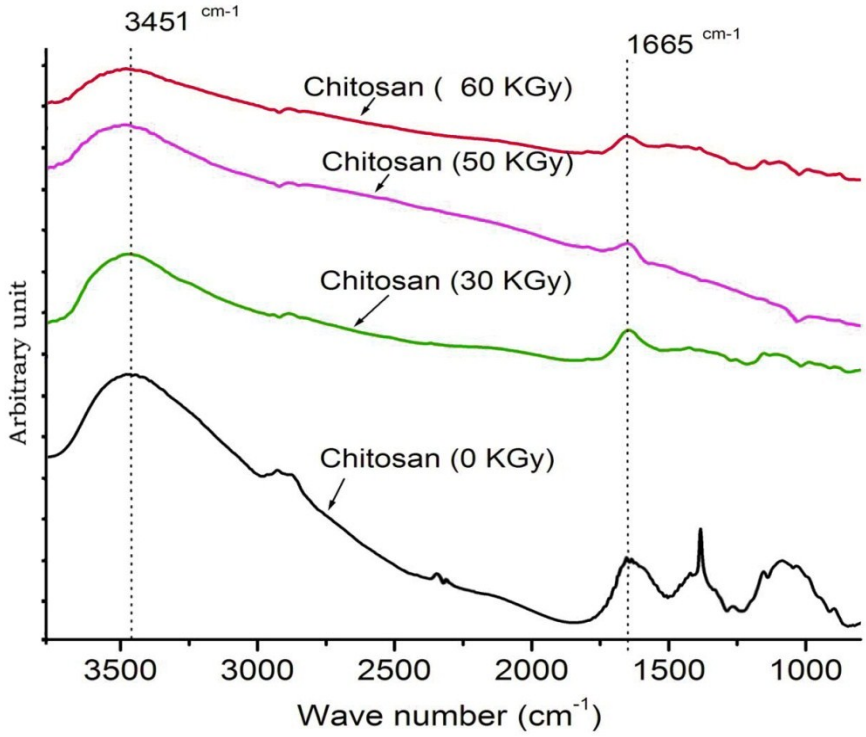

Figure 1: FTIR spectra (transmittance mode) of non irradiated chitosan and irradiated chitosan

3.2. Weight loss percentage

Figure 2 shows the percent of weight loss after three and five days storage period respectively for the strawberries samples treated with 1000ppm irradiated chitosan solution and stored in 4°C and packed in zip bags. Result suggested that 50 kGy irradiated chitosan solution is the most potential to increase shelf life of strawberry. It was also found that the shelf life can be increased up to 15 days whereas control samples with same condition could maintain edible quality only up to 3 days. So, considering these results further studies are done to optimize the concentration of 50 kGy irradiated chitosan.

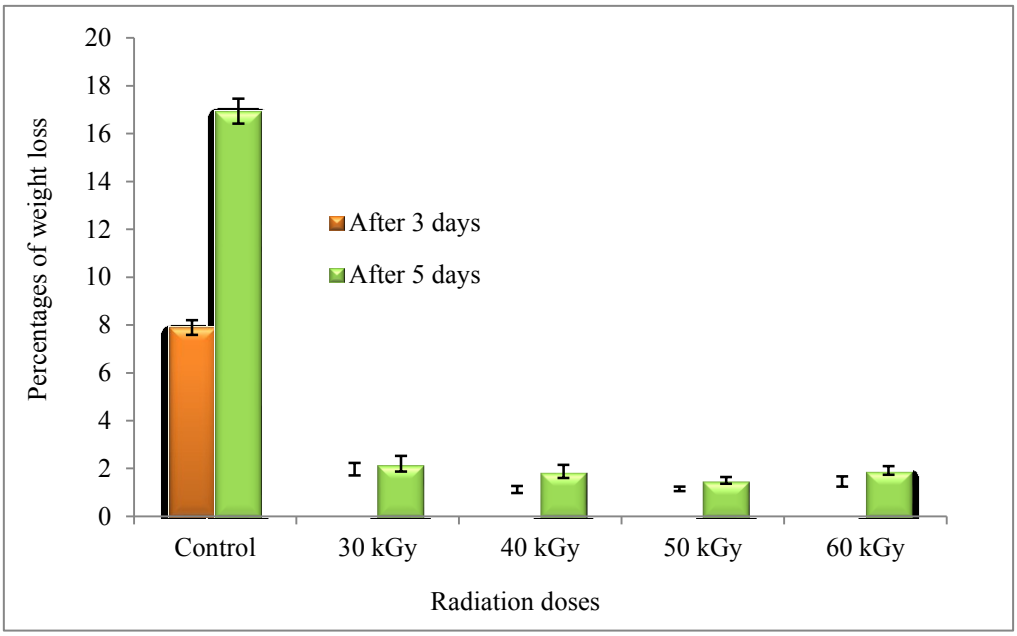

Figure 2: Percentage of weight loss for different radiation doses of chitosan treated strawberries

Table 1 shows the percentage of weight loss of strawberry samples treated by different concentration of 50 kGy gamma radiated chitosan solution. It was found that the percentage of weight loss showed a decreasing trend up to 1500 ppm for the treated strawberries. On the other hand, the weight losses were found about similar for the treatment of 1500ppm and 2000ppm. Whereas, control samples showed a sharp increase of weight loss with time. And after 10 days the fluid leakage from the fruits was so intense that it was not possible to calculate the weight loss. That's why, weight loss after 10 days was not done (ND). So, these results suggested that 1500ppm of 50 kGy gamma radiated chitosan is the most potential for the preservation of strawberries.

Table 1: Percentage of weight loss for different concentrations of chitosan treated strawberries

Properties	Concentration	Weight loss in start day	Weight loss in 5 days	Weight loss in10 days	Weight loss in 15 days	Weight loss in 21 days
Inside zip bags at 4°C	Control	0%	18.29%	22.39%	ND	ND
	300ppm	0%	1.96%	3.08%	7.63%	10.56%
	500ppm	0%	1.78%	2.68%	5.97%	8.37%
	1000ppm	0%	1.46%	2.59%	5.03%	7.40%
	1500ppm	0%	1.03%	2.34%	4.54%	5.88%
	2000ppm	0%	1.0%	2.41%	4.49%	5.81%

3.3. Decay percentage

From the results it can be observed that the decay percentage increased with storage time for all treatments (**Table 2**). The initial decay percentage in uncoated strawberries was 11.50% after 5 days, and after 21 days of storage that was found as 95.59%. Almost all portions of the uncoated strawberries were damaged after that period of storage.

In contrast, the incidence of decay for coated fruit was in the range of 14.45–28.57% at 21 days of storage. This longer storage was possible due to the antibacterial and antifungal property of chitosan [21]. Its film forming property creates a mechanical barrier to preserve fruit from pathogen attack, contributing to longer storage. This film also inhibited the gas exchange required for fruit decay. Thus chitosan is a potential agent that can be used to reduce postharvest losses of strawberry which was previously observed by Hernández-Munõz *et al.* [22] using untreated chitosan solution.

Table 2: Decay percentage for different concentrations of chitosan treated strawberries

	Concentration	5 Days	10 Days	15 Days	21 Days
% of Decay	Control	11.50%	56.29%	77.68%	95.59%
	300ppm	5.89%	12.90%	20.17%	28.57%
	500ppm	6.39%	9.35%	12.78%	19.42%
	1000ppm	5.07%	7.54%	10.80%	17.58%
	1500ppm	4.15%	6.78%	9.15%	14.45%
	2000ppm	4.56%	7.12%	9.06%	15.22%

3.4. Measurement of pH

The pH value increased within the range from 4.6 – 5.2 of different strawberry sample both treated and untreated ones (**Table 3**). Uncoated fruits showed higher increasing rate than coated fruits but no significant difference was encountered. For 1500ppm 50 kGy chitosan treated fruits showed better result (pH 5.1) than other treatments as higher pH may be an indication of better sweetness of the fruit because the lesser the pH of the fruit sample the higher presence of acidic compounds which are responsible for sour taste of the fruits.

Table 3: pH values of different concentrations of chitosan treated strawberries

	Concentration	1st Day	5 Days	10 Days	15 Days	21 Days
Value of P^H	Control	4.6	4.7	5	4.7	4.5
	300ppm	4.6	5.1	4.5	4.7	4.2
	500ppm	4.6	5	4.8	4.6	4.5
	1000ppm	4.7	4.8	4.6	4.8	5.0
	1500ppm	4.8	5.2	5.1	5.0	4.7
	2000ppm	4.7	5.2	5.2	5.1	4.9

The change in pH may be related to the biochemical condition of the fruit. The altered metabolic and respiration activity could be another cause behind the pH change [23].

3.5. Microbial count reduction [24]

The fruit sample was subjected to bacterial and fungal counts in agar media after 10 days of preservation by 50 kGy 1500 ppm chitosan solution and compared with the untreated strawberry after same days and conditions of preservation. The bacterial count of untreated strawberry was 2×10^5cfu/g whereas the bacterial count was 1.04×10^4cfu/g in chitosan treated strawberry (**Table: 4**).

Table 4: Bacterial count in untreated strawberry and chitosan treated strawberry

	Colony count on Plate Count Agar media at different dilutions				Results of Total Bacterial Count
Dilution factor	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Untreated strawberry	TNTC*	200	46	3	2×10^5cfu/g
Chitosan treated strawberry	104	10	1	1	1.04×10^4cfu/g

On the other hand, the fungal count of untreated strawberry was 8×10^2cfu/g while it was 7×10^2cfu/g in chitosan treated strawberry (**Table: 5**). The log value shows that chitosan

significantly reduces the bacterial count in the preserved strawberry. However, the reduction of fungal count was not significant. Although chitosan has good antibacterial and antifungal property [21] the developed sample did not work as a good antifungal agent on the strawberry fruits. This might be happening due to over growth chitosan resistant fungus on the strawberry.

Table 5: Fungal count in untreated strawberry and chitosan treated strawberry

	Colony count on Potato Dextrose Agar media at different dilutions				Results of Total Fungal count
Dilution factor	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
Untreated strawberry	8	5	ND**	ND	8×10^{2}cfu/g
Chitosan treated strawberry	7	1	ND	ND	7×10^{2}cfu/g

* TNTC= too numerous to count, ** ND = not done, cfu= colony forming unit

3.6. Sensory evaluation

The overall visual appearance of strawberries after different days storage at 4°C are presented by hedonic scale. Here 0–2 represented extreme dislike; 3–5 fair; 6–8 good; and 9 excellent for color, taste, texture, flavor and overall acceptability. The average points for the samples preserved by 1500ppm 50kGy chitosan solution up to 15 days was 5 which was marked as fair enough to maintain the edible quality. Whereas, the control samples got the score of 4.25 after 5 days of storage. These results indicated the overall quality of the stored strawberry samples which suggested that chitosan treated strawberry can be stored up to 15 days with conserving its fair edibility whereas untreated strawberry losses its edibility within five days of storage period.

Table 6: Comparison of overall visual appearance of untreated strawberries and chitosan treated strawberries

Properties	**Conc^{n}/type**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day10**	**Day 15**
Smell	Control	10	8	7	5	4	3	1
	1500ppm	10	9	8	7	7	6	5
Colour	Control	10	8	7	5	4	3	1
	1500ppm	10	10	9	8	7	6	5
Texture	Control	10	8	7	5	4	3	1
	1500ppm	10	10	8	7	7	5	4
Spoilage	Control	10	8	7	5	5	3	1
	1500ppm	10	10	9	8	8	7	6

4. Conclusion

The treatment of irradiated (50 kGy) chitosan in 1500ppm concentration was found fruitful to extend shelf life of strawberries according to our results. Treated strawberries showed best behavior throughout storage period with minimum loss of moisture, shrivel, and were able to conserve better sensory characteristics. Irradiated chitosan coating also protected strawberry fruits from visual microbial growth. So, this study recommends chitosan as a very promising edible coating material that can be very effective and safer way in maintaining the overall quality of strawberry.

Acknowledgement

The authors would like to acknowledge Governance Innovation Unit (GIU) of Prime Minister Office, Bangladesh for providing support for the research.

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