

RESEARCH ARTICLE

Effect of irradiated chitosan treatment on storage life of fruits of two commercially grown papaya (*Carica papaya* L.) varieties**

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Abstract: Potential use of gamma irradiated chitosan for control of the anthracnose disease causing organism, *Colletotrichum gloeosporioides*, and extension of storage life of papaya varieties Rathna and Red Lady were investigated. Chitin extracted from shrimp waste was converted into chitosan and used as an antifungal treatment against fungal strains isolated from both papaya varieties. Chitosan in powder form and as 1% solution were irradiated with different doses -5, 10, 25, 50, 75, 100 and 150 kGy of ⁶⁰Co gamma rays-in order to enhance antifungal activity. The effective irradiation dose for the inhibition of radial mycelial growth of respective fungal strains was selected via a series of *in vitro* experiments using potato dextrose agar (PDA). A complete inhibition of growth in both fungal strains was observed on PDA plates incorporated with 1% chitosan solution at all irradiated doses compared to the control (distilled water). The plates incorporated with non-irradiated chitosan (0 KGy) and inoculated with the fungus isolated from Red Lady papaya showed slight mycelial growth while Rathna isolate showed complete inhibition of growth with this treatment. However, irradiated chitosan powder was found to be less effective in inhibiting *in vitro* growth of both fungal strains, 1% chitosan solution (in 10% acetic acid) irradiated at the lowest dose (5 kGy) was selected for *in vivo* experiments on storage life extension of papaya fruits. Eighty percent marketable quality fruits were observed in Rathna papaya while 70% marketable quality fruits were observed in Red Lady subjected to irradiated chitosan treatment and stored at 13.5°C , 95% RH for 14 days followed by 2 days at ambient temperature (28°C ± 2).

Keywords: Antifungal activity, chitosan, *Colletotrichum gloeosporioides*, Papaya, irradiation, Rathna, Red Lady Papaya.

INTRODUCTION

Chitosan is used as a natural coating for fruit and is derived from crab or prawn shell after deacetylation of chitin. Next to cellulose it is the second most abundant, naturally available, easily degradable biopolymer. Chitosan is known to form a semi permeable film, which modifies the internal atmosphere of the fruit and decreases transpiration losses. It is known to lower the rate of respiration and delay the ripening process. Chitosan shows antifungal activity by inducing the defense barrier in host tissue¹. Chitosan thus helps to retain the quality of fruits during storage and is non toxic and biologically safe². Previous studies ^{3, 4} revealed that chitosan – extracted from locally available head and shell waste of farm shrimps - at a concentration of 1 % could be used to extend the storage life (with 70 % marketability) of papaya var. Rathna up to 14 days at 13.5° C and 95 % relative humidity. However, 1 % chitosan dip treatment was not effective in controlling the anthracnose disease in papaya var. Red Lady that was introduced recently to Sri Lanka (*authors' unpublished data*).

Biological activity of chitosan depends significantly on its physico-chemical properties such as molecular weight and the molecular fraction of glucosamine units in the chitosan polymer chain. Hence, the application of this polysaccharide is limited in some fields due to its high molecular weight resulting in low solubility in aqueous diluted acids and high viscosity of the solution even at low concentrations⁵. Ionization radiation is known as an effective process in the modification of polymers⁶. The

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two main reactions, which determine the final properties of a polymer, include (a) main chain scission leading to the reduction of molecular weight (b) cross linking the opposite process, resulting in formation of macroscopic insoluble material.

Chitosan belongs to the class of natural polymers that undergoes depolymerization when exposed to ionization radiation^{7, 8}. Previous studies⁹ have investigated effects of γ -irradiation on chitosan fibres and films, and observed that the viscosity, average molecular weight of the polymer decreased with increasing irradiation dose and radiation induced scission of the chitosan chain. Low molecular weight chitosan has wide application as a fungicide. In Thailand irradiated chitosan fruit coating is used to eliminate some fungi on the skin as well as to preserve the texture by reducing respiration and water evaporation in Salak, *Salacca wallichina*¹⁰. Mango coated with irradiated chitosan (in India) showed extended shelf life from 7-15 days (*Personal communication: A.G. Chmielewski International Atomic Energy Authority, Vienna, 2005*). However, studies on postharvest application of irradiated chitosan are limited. The objectives of the present investigation are to determine the antifungal activity of irradiated chitosan on the anthracnose causing fungal strains isolated from papaya and to test the efficacy of irradiated chitosan as a fruit coating to extend the storage life of papaya.

METHODS AND MATERIALS

Extraction of chitosan: Chitin was extracted from prawn shell and head waste at the Fish Technology Laboratory, Industrial Technology Institute (ITI). Extraction process (ITI patent pending for the method of extraction) included deproteinization with 5 % NaOH and demineralization with 2 % HCl. Extracted chitin was thereafter converted to chitosan by deacetylation with 40 % NaOH for 2 d. The resulting chitosan was ground into a fine powder (particle size < 1 mm) by extensive grinding in an ultra centrifuge mill (Retsch, Germany).

Antifungal activity of irradiated chitosan powder: Chitosan powder was irradiated by gamma rays from a ⁶⁰Co source (Atomic Energy Authority) at a dose rate of 5 kGy/h. A triplicate of each pack (3 g) was subjected to different irradiation doses such as 25, 50, 75, 100 and 150 kGy. Chitosan powder (3 g) without irradiation was used as the control. The colour of the irradiated chitosan powder was determined using a Minolta Chromameter (CR 200, Japan). Lightness (L) values were measured and the degree of change for each colour index, such as delta 'L' was expressed as the difference between value of control and value after irradiation at a specific dose¹¹.

Each sample (1 g) was dissolved in 10% (v/v) acetic acid (100 mL) and the pH of the solution was adjusted to 5.6 with 50% sodium hydroxide. Chitosan solution of (1%w/v) was obtained by dissolving 1 g of chitosan in 100 mL 10% (v/v) glacial acetic acid and homogenizing at 6500 rpm for 5 min using IKA-Ultra-Turrax-T25 homogenizer. The pH of the solution was adjusted to 5.6 with 50% NaOH. The relative viscosity of each sample was measured using a rotational type viscometer (Fungi Lab Spain) at 25°C with the number 2 spindle at 60 rpm. The viscosity measurement was expressed in Centipoises (CP). The viscosity decrease rate (VDR) was calculated as $VDR = (V / V_0) \times 100$, where V_0 was the viscosity of initial chitosan (non irradiated control) in 10 % acetic acid solution and V was the viscosity of the chitosan solution prepared from irradiated chitosan.

Two different strains of *Colletotrichum gloeosporioides* were isolated from diseased fruit of papaya var. Rathna and Red Lady respectively. Pathogenicity was proved in each case by establishing Koch's postulates. Pure cultures of respective strains were maintained separately on PDA at 25°C. A mycelial disc (0.9 cm, diameter) was cut from the peripheral region of 5d old cultures of both fungal strains grown on PDA and transferred to the centre of 9 cm diameter, PDA plates. Aqueous solutions of chitosan prepared from irradiated powder (subjected to different doses) were incorporated separately into the medium at 50°C before plating. Plates incorporated with non-irradiated chitosan (0 kGy) in 10 % acetic acid and sterile distilled water (without chitosan) served as controls. Growth was assessed after 10 d by measuring the colony diameter in centimetres.

Antifungal activity of irradiated chitosan solution: Chitosan powder (1 g) was dissolved in 10 % acetic acid solution (100 mL), homogenized at 6000 rpm for 5 min and filtered through muslin cloth. The resulting solution (Triplicate per irradiation dose, 100 mL per replicate) was irradiated directly by ⁶⁰Co gamma rays (Atomic Energy Authority) with doses of 5, 10, 25, 50, 75, 100 and 150 kGy at a dose rate of 5 kGy / h. The pH of each solution was adjusted to 5.6 with 50 % sodium hydroxide after the irradiation treatment. The colour and relative viscosity of the irradiated chitosan solutions were measured with a Chromameter (Minolta ,CR 200, Japan) and rotational type viscometer (FungiLab, Spain) at 25° C respectively. Antifungal activity of the irradiated chitosan solution on both fungal isolates was carried out as mentioned above. Plates incorporated with non-irradiated chitosan (0 kGy) in 10% acetic acid and sterile distilled water (without chitosan) served as controls.

Effect of irradiated chitosan on infection of fresh papaya fruits: Fresh papaya fruits from both Rathna and Red Lady varieties were harvested at colour index 2 (10% yellow stage) from a commercial cultivation in Neelabamma, and transported to the laboratory on the same day. Twenty fruits of each variety were dipped in 4 L of 1% chitosan (non irradiated) for 5 min (T1) and another set was separately dipped in 1% chitosan solution irradiated at 5 kGy (at a dose rate of 5 kGy per h.) for 5 min (T2). After dipping, the fruits were allowed to air-dry¹² for 5 min at 28°C. Twenty fruits of each variety dipped in sterile distilled water for 5 min served as the control (T3). The treated and untreated fruits were then lined with styrofoam netting and placed vertically (stem end facing down) in corrugated fiberboard cartons. Each carton contained 10 fruits. The packaged fruits were stored at 13.5°C and 95% RH for 2 and 3 wks. The fruits were removed from cold storage at the completion of the specified storage period, and ripened at 28°C using Ethrel (ripening agent) in a ripening chamber. Ethrel (1.6 mL) was used in 93 L volume glass cubicles allowing 20% headspace. After ripening, fruits were held at 25°C for 24 h. Overall visual quality was assessed according to the score based on disease severity (measuring the lesion diameter) and colour where 1 - 2 = fruit not marketable, 3 = poor quality but limited marketability, 4 - 5 = fair quality and marketable, 6 - 7 = good quality and marketable, 8 - 9 = excellent quality¹³. The percentage marketability was calculated as a ratio of the number of fruit with overall quality scores of 6 - 7 or 8 - 9 against the total number of fruit used per treatment¹³.

Statistical method: The *in vitro* studies were conducted as a CRD (Completely Randomised Design) with ten replicates and repeated twice to confirm the results. Data were analyzed by analysis of variance and mean separation was done by Duncan's Multiple Range Test (DMRT) at $p < 0.05$. *In vivo* treatments were arranged in a completely randomized design (CRD) in the low temperature storage with 20 replicates per treatment. This experiment was repeated twice. Percentage marketability data were analyzed by a non-parametric Friedman two-way analysis (Minitab Inc., 1996).

RESULTS

Antifungal activity of irradiated chitosan powder

The change in colour of chitosan powder was calculated and presented as a 'Delta L' value. 'Delta L' (ΔL) value is widely adopted as a tool for measurement of browning of food¹¹. The colour of the powder changed to a more intense brown with increasing irradiation dose (Figure 1). It was

observed that the browning discolouration at irradiation dose over 100 kGy was not desirable. The relationship between the viscosity of the chitosan solution prepared from irradiated powder and irradiation dose is illustrated in Figure 2. Viscosity measurements are widely used to calculate the average molecular weight of chitosan^{9, 11}. Viscosity decrease rate was high between 0 to 75 kGy but stabilized thereafter.

Significant growth ($p < 0.05$) of the fungus (isolated from Red Lady) was observed in plates incorporated with chitosan irradiated at all doses including controls (Figure 3). However, the fungal strain isolated from Rathna behaved in a different manner and showed no mycelial growth in plates incorporated with 0 kGy and 25 kGy chitosan solutions (prepared from irradiated powder) while control plates (without chitosan) were covered with mycelial growth at the end of 5 day incubation (Figure 3). A significant ($p < 0.05$) increase in growth of the fungus (isolated from Rathna) was observed in plates incorporated with chitosan irradiated at 50, 75, 100 and 150 kGy doses (Figure 3).

Antifungal activity of irradiated chitosan solution

No significant ($p < 0.05$) browning was observed in chitosan solutions irradiated at dose rates 5 and 10 kGy compared to the original sample (control) as shown in Figure 4. However, browning increased thereafter with the increase of irradiation dose. The 100 kGy treated sample showed the highest browning (high ΔL value). Viscosity decrease rate (VDR %) was higher from 0 to 5 kGy and stabilized at 5, 10 and 25 kGy doses (Figure 5). A slight decrease in VDR was observed again from 25 to 50 kGy and stabilized thereafter.

No fungal growth was observed with either of the isolates in plates incorporated with chitosan solutions irradiated at 5, 10, 25, 50, 75 and 100 kGy and incubated at 28°C for 10 days (Figure 6). Maximum growth of the pathogen was observed in control plates. However, the plates incorporated with non-irradiated chitosan (0 kGy) and inoculated with the fungus isolated from Red Lady papaya showed a slight mycelial growth compared to the Rathna isolate. As the 1% chitosan irradiated in aqueous solution was found to be highly effective in controlling both isolates of *C. gloeosporioides in vitro*, it was decided to select this concentration for further *in vivo* experiments with fresh papaya. No significant ($p < 0.05$) growth of the fungus was observed when the irradiation dose was increased from 5 to 100 kGy. Therefore, 5 kGy was selected as the effective dose for *in vivo* application for fresh papaya.

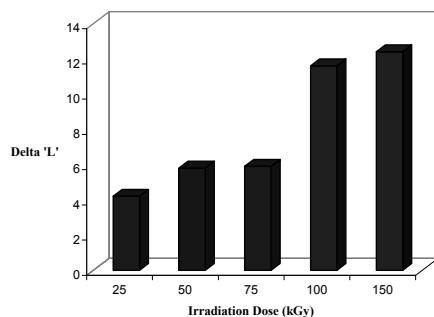


Figure 1: Change in Delta 'L' values (colour in relation to non irradiated controls-'L' value = 70) of the irradiated chitosan powder

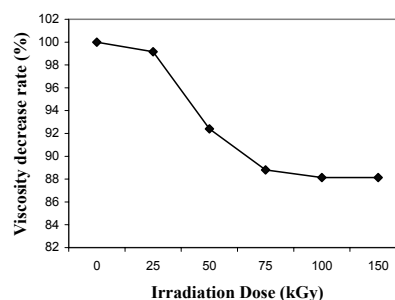


Figure 2: Effect of irradiation on viscosity decrease rate of chitosan powder in 10% acetic acid solution - dose range from 0 - 150 kGy

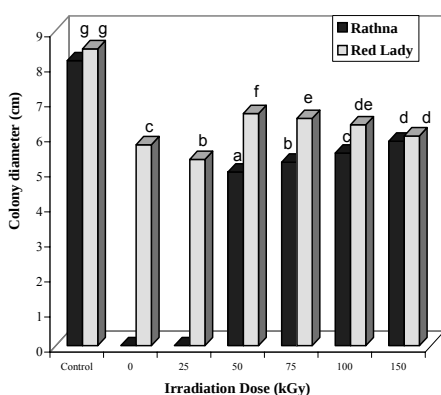


Figure 3: Antifungal activity of irradiated chitosan powder on the growth of anthracnose causing fungi isolated from Rathna and Red Lady papaya incubated for 5 days at 28 °C (control = in distilled water, 0 = in non irradiated chitosan)

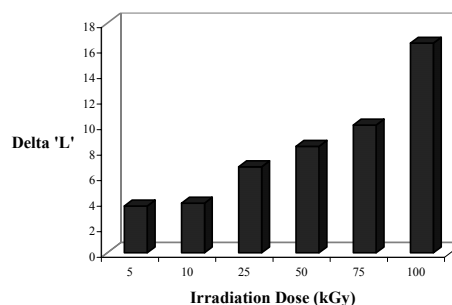


Figure 4: Change in Delta 'L' values (colour in relation to non irradiated controls-'L' value = 50) of the irradiated chitosan solution

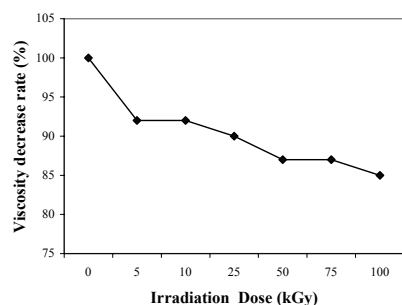


Figure 5: Effect of irradiation on viscosity decrease rate of chitosan solution directly irradiated at doses ranging from 0-100 kGy

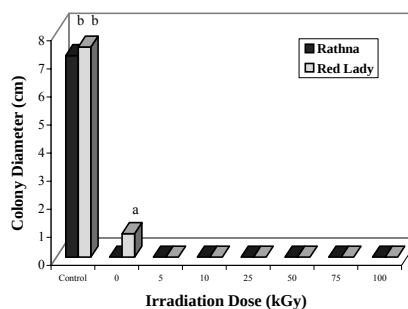


Figure 6: Antifungal activity of irradiated chitosan solution on the growth of anthracnose causing fungi isolated from Rathna and Red Lady papaya and incubated for 5 days at 28 °C (control = in distilled water, 0 = in non irradiated chitosan)

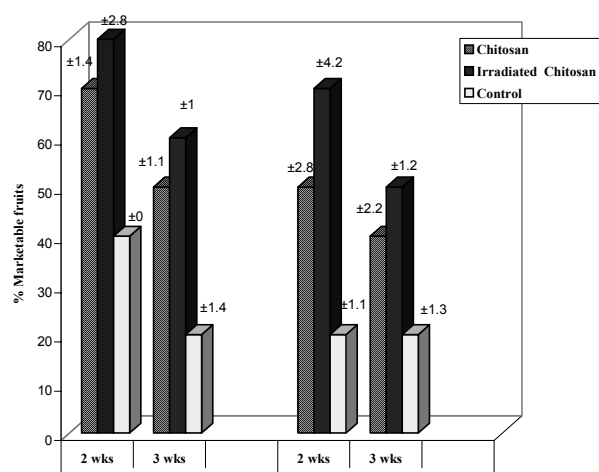


Figure 7: Percentage of the marketable fruit of papaya var. Rathna and Red Lady in relation to storage period and treatments applied (with $\pm$ Standard deviation)

Effect of irradiated chitosan on infection of fresh papaya fruit

Eighty percent marketable quality fruit was observed in Rathna papaya subjected to irradiated chitosan dip treatment while non-irradiated chitosan treated fruit showed 70% marketability after 2 weeks storage at 13.5 °C (Figure 7). When storage period was extended by a further week the quantity of marketable fruit in both treatments was reduced by 20%. Overall fruit quality was better with less disease severity (indicated as higher marketability) when fruits were treated with irradiated chitosan than non-irradiated chitosan. In var. Red Lady the percentage of marketable fruits was lower than in var. Rathna with respect to both irradiated (70%) and non-irradiated (50%) chitosan treatments. However, control fruit dipped in distilled water of both varieties showed least number of marketable quality fruit (20 %).

DISCUSSION

Low molecular weight chitosan has been demonstrated to be an effective plant protector against infectious diseases and environmental stress¹¹. It is also reported that the molecular weight and molecular fraction of chitosan, influence the solubility and interaction with the cell walls of target micro-organisms⁵. Ionization radiation is known as a useful tool for degradation of chitosan polymer¹⁴ and reducing molecular weight¹⁵. Present results indicated that the % VDR of chitosan solutions decreased rapidly when subjected to lower irradiation doses and it decreased or stabilized with the increase in irradiation dose. Similar results were also obtained from irradiation treatment of chitosan and chitosan oligomer in 2002¹¹. The rapid

decrease in VDR with a low dose of irradiation (5 - 25 kGy) observed in the present study could be due to the irradiation-induced scissions of 1 - 4 glycosidic bonds of chitosan polysaccharide that caused a reduction of the molecular weight of the polymer¹¹. An irradiation dose of over 75 kGy was not suitable due to the development of an intense brown colour in chitosan solution that can affect the cosmetic appearance of coated fruits.

Chitosan solution (1 %) prepared from irradiated chitosan powder was found to be less effective in controlling the growth of *C. gloeosporioides* irrespective of the irradiation dose applied. Significant ($p < 0.05$) increase in the growth of the fungus was observed when irradiation dose was increased from 50 kGy to 150 kGy in irradiated chitosan solutions prepared from irradiated powder. Similar observations were made with increasing irradiation dose over 500 kGy with respect to antimicrobial activity of irradiated chitosan powder by other scientists¹⁶. However, chitosan solution (1 %) directly subjected to irradiation doses even at 5 kGy was found to be highly effective in *in vitro* control of both anthracnose causing fungal strains isolated from Rathna and Red Lady papaya. Degradation of chitosan by irradiation in aqueous solution is reported as an indirect effect due to the generation of hydroxyl radicals which in turn attack chitosan molecules by H-abstraction, thus forming radicals at carbon atoms¹⁵. It is also reported¹⁵ that irradiation in solution (at 10 kGy) allows for easy and fast reduction of the molecular weight down to oligomer range. Higher antifungal activity seen in the present study with respect to the directly irradiated chitosan solution could be due to the above reasons. However, irradiation treatment with very high doses could break most of the bonds leading to complete degradation with monomer and short-chain oligomers. These fragments may not create inhibition effects on the growth of micro-organisms and they might even stimulate the growth as observed in some plate-culture conditions. These findings are consistent with the work reported in previous studies¹⁶. The differential response shown by the two fungal strains (isolated from Rathna and Red Lady) to irradiated chitosan could be due to the variation in cell wall characteristic (thickness) of these two fungal strains.

Previous studies conducted on the biological activity of chitosan suggested two different mechanisms of chitosan and target organism interactions. The first mechanism describes adsorption of chitosan to cell walls leading to cell walls covering, membrane disruption and cell leakage. The second mechanism proposed is the penetration of chitosan into living cells leading to inhibition of various enzymes and interference with

synthesis of mRNA and proteins¹⁷⁻¹⁹. The action of original (non-irradiated) chitosan is likely to be the first mechanism that is the adhesion of the macromolecules to the cell wall of the target organism¹⁶. However, ionization radiation breaks the glycosidic links of chitosan to produce lower molecular weight fragments of varying size. The microbial inhibition caused by radiation-formed chitosan fragments could be stronger than that by original (non irradiated) molecules due to the contribution of both mechanisms (adsorption to cell wall and penetration to the cell) that occurred simultaneously in irradiated chitosan¹⁶. Irradiation of chitosan in the solid state leads to chain scission, and also causes side reactions such as oxidation and deamination¹⁵.

In the *in vivo* study, 80% fruit with marketable quality was observed when Rathna papaya was subjected to irradiated chitosan treatment while non-irradiated chitosan treated fruit showed 70 % marketability after 2 weeks storage at 13.5°C. Sixty percent fruit with marketable quality was observed even when storage life was extended for 3 weeks with irradiated chitosan treated Rathna papaya. It was observed that irradiated chitosan treatment (70% marketable fruit) was suitable for Red Lady variety where non-irradiated chitosan treatment (50% marketable fruit) was less effective in controlling the anthracnose disease of this variety. *In vivo* studies with fresh Rathna and Red Lady papayas showed that shelf life could be extended up to 3 weeks (with 60% marketable fruit) when chitosan solution is subjected to irradiation treatment at 5 kGy.

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