Antifungal Properties of Gamma-Irradiated Chitosan from Sea Crab Shells

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Short Communication

Abstract: Chitosan extracted from sea crab shells was used to determine antifungal properties against *Aspergillus niger*. Chitosan powder irradiated at 100 kGy and dissolved in 1 % acetic acid (v/v) with pH adjusted to approximately 6.0 was used in preparing chitosan concentrations of 2 %, 1.5 %, 1 % and 0.5 %. The agar dilution method was used to test the antifungal activity of the various chitosan solutions at concentrations of 0.20 %, 0.15 %, 0.10 % and 0.05 %. Both media containing irradiated and unirradiated chitosan inhibited the mycelial growth of *Aspergillus niger* and the degree of inhibition was dependent on the concentration of the chitosan in the fungal growth medium. Results show that the media containing irradiated chitosan inhibited the mycelia growth of *Aspergillus niger* to a greater extent than the media containing unirradiated chitosan.

***Key words**:* Antifungal property, chitosan, inhibition, *Aspergillus niger*, irradiation.

Introduction:

The preservation of food by physical and chemical methods is an important area to the food industry. Food preservatives have to meet several regulatory standards, for example, they must be (i) efficient over a broad range of spoilage organism; (ii) tasteless and odorless, and (iii) non toxic, safe and inexpensive. When physical means of preservation are not available or desirable, chemical preservative(s) must be used, and the choice of one or more preservative is primarily based on the chemical composition of the food, its pH, and other characteristics. No preservative is universal, and the list of GRAS (generally recognized as safe) preservatives is short [1]. There is also a growing demand from the public for natural preservatives, and for these reasons, alternative sources of safe, effective, and acceptable preservatives need to be developed. Chitosan is the deacetylated form of the natural polymer chitin, a polysaccharide composed of N-acetyl D-glucosamine and D-glucosamine units, and extracted mainly from crustacean waste by demineralization and deproteinization. Over 60% deacetylation of chitin yields chitosan [2]. Recently, research on chitosan and its properties have shown its potential for use as a food preservative. Chang *et al*. [3] achieved a 10 day shelf life extension of “mulkimchi” (pickle type kimchi, i.e., chinese cabbage) at 5°C by incorporating 0.2% chitosan compared to control samples.

Low molecular weight polysaccharides and/or oligosaccharides can be produced by degradation of corresponding polysaccharides including marine polysaccharides such as alginate, chitin/chitosan, and carrageenan. Chemical, enzymatic and radiation processing technologies can be applied for degradation process. Recently much attention has been paid to the application of radiation processing technology for degradation of natural polysaccharides. Research has shown that low molecular weight polysaccharides have antimicrobial properties.

A number of pathogenic moulds such as *Fusarium* spp., *Aspergillus* spp, *Penicillium spp and Rhizopus spp.* have been reported as causal agents of foodborne disease and food spoilage [4]. There has been considerable interest in radiation-processed low molecular weight natural polysaccharides with antimicrobial properties. Matsuhashi and Kume [5] reported that irradiated chitosan having molecular weight of 10^5 to 3×10^5 exhibited highly antimicrobial activities. Similarly, Ha *et al*. [6] noted the enhancement of antifungal activity of irradiated chitosan for different fungi strains. This study therefore is aimed at investigating the antifungal properties of radiation-processed chitosan from crab shells on *Aspergillus niger*.

Materials and Methods

Sample collection and preparation

Crab shells were purchased from fishermen in Accra, Ghana. Crab shells were washed and then dried in the oven at 60^0C overnight. The dried shells were ground

in a moulinex blender, sieved to a particle size of 90 µm and then packaged in polyethylene bag for storage at ambient temperature until used.

Chitosan preparation and irradiation:

Chitosan was prepared using the procedure described by Ocloo *et al.* [7] having degree of deacetylation of 80 %. The chitosan powder was then irradiated at 100 kGy using the Co-60 gamma irradiator of the Radiation Technology Centre of Ghana Atomic Energy Commission at room temperature with dose rate of 1.43 kGy/h.

Antifungal activity of irradiated chitosan:

Chitosan powder was dissolved in 1 % acetic acid (v/v) and the pH adjusted to approximately 6.0 by addition of 2N NaOH. Chitosan concentrations of 2 %, 1.5 %, 1 % and 0.5 % were prepared. Chitosan solutions were filtered and autoclaved at 121 °C for 15 min. Using the agar dilution method [8] with some modifications, the different formulations of the sterile chitosan were mixed with Potato Dextrose Agar and poured into petri dishes to obtain final concentrations of 0.20 %, 0.15 %, 0.10 % and 0.05 %. Discs (4 mm) of fungal mycelia from the growing edges of a 5-day old culture of *Aspergillus niger* (isolated from dehydrated cassava powder) were placed on agar plates and incubated for 5 and 6 days at 28 °C. The growth diameters (mm) were measured.

Results and Discussion:

The growth (in terms of colony diameter) of *Aspergillus niger* on media containing different concentrations of irradiated chitosan are shown in Table 1 and Plate A can be seen that both media containing irradiated and unirradiated chitosan inhibited the mycelial growth of *Aspergillus niger*. The results indicate that the degree of inhibition was dependent on the concentration of the irradiated and un-irradiated chitosan in the fungal growth medium. The media containing 0.2 % irradiated and unirradiated chitosan recorded the highest inhibitions of mycelial growth with maximum colony diameters of 6.50 cm and 7.65 cm respectively after 6 days. The results clearly revealed that the media containing irradiated chitosan inhibited the mycelia growth of *Aspergillus niger* to a greater extent than the media containing unirradiated chitosan. It is noteworthy that the mycelia growth of *Aspergillus niger* on media containing acetic acid and media without acetic acid or chitosan did not differ greatly since the recorded colony diameters were 8.33 and 8.50 cm respectively. This clearly indicates that the inhibitory action of the media is due to the presence of chitosan. Balicka-Ramisz *et al.* [9] established a minimal inhibitory concentration (MIC) of 0.6 mg/cm^3 against *Candida albicans*. Also, Allan and Hadwiger [10] reported that 1 % solution of chitosan in 1 % acetic acid had completely inhibited growth of *Candida tropicalis*.

Conclusion:

The presence of chitosan in the media inhibited the mycelia growth of Aspergillus *niger*. The media containing irradiated chitosan inhibited the mycelia growth of *Aspergillus niger* to a greater extent than the media containing unirradiated chitosan.

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Table 1: Growth (colony diameter, cm) of *Aspergillus niger* on Potato Dextrose Agar containing different concentrations of irradiated chitosan.

Concentrations	0	0	0.05%	0.1%	0.15%	0.2 %	0	0	0.05%	0.1 %	0.15 %	0.2 %
Sample	DAY 5						DAY 6					
Control [1]	6.90	-	-	-	-	-	8.50	-	-	-	-	-
Control [2]	-	6.75	-	-	-	-	-	8.33	-	-	-	-
Irradiated chitosan	-	-	6.33	6.68	5.5	4.75	-	-	8.00	7.50	7.50	6.50
Unirradiated Chitosan	-	-	6.9	6.5	6.35	5.83	-	-	8.30	8.20	8.17	7.65

[1] only PDA ; [2] contains 1% acetic acid of pH 6

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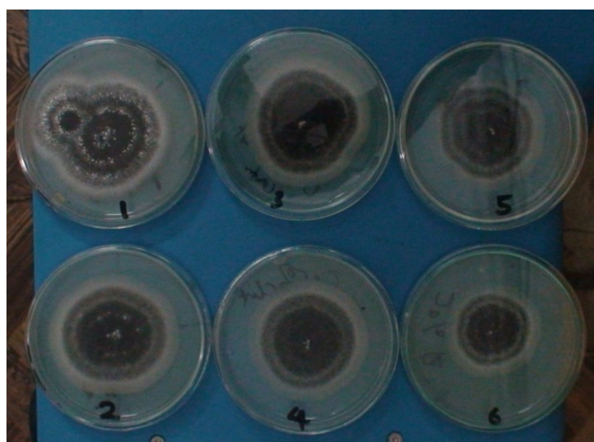

Plate A

1. *Aspergillus* on PDA media; 2 *Aspergillus* on media +1 % Acetic acid; 3. *Aspergillus* on 0.05 % Unirradiated chitosan; 4. *Aspergillus* on 0.05 % Irradiated chitosan; 5. *Aspergillus* on 0.2 % Unirradiated chitosan; 6. *Aspergillus* on 0.2 % Irradiated chitosan

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