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Chitosan is a new target of chemical replacement to formalin in food preservative

Md. Arfat Sharif, Ajoy Kumer, Md. Bosir Ahmed and Sunanda Paul

Md. Arfat Sharif
Lecturer, Department of Textile Engineering, European University of Bangladesh, Dhaka, Bangladesh

Ajoy Kumer
Lecturer, Department of Chemistry, European University of Bangladesh, Dhaka, Bangladesh

Md. Bosir Ahmed
Lecturer, Department of Chemistry, European University of Bangladesh, Dhaka, Bangladesh

Sunanda Paul
Post graduate Researcher, Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong, Bangladesh

Correspondence
Ajoy Kumer
Lecturer, Department of Chemistry, European University of Bangladesh, Dhaka, Bangladesh

Abstract
The conventional way of food preservation by using synthetic chemicals like formalin has been used for many years. But the fact that they have been found as health hazardous/poisonous, also having some demerits like cancer, brain cancer, skin diseases, trouble in respiration, vomiting. Chitosan is a natural biopolymer produced from the exoskeletons of shrimp and crab, which shows antimicrobial activities against microbial pathogens. This study aimed to evaluate the propensity of chitosan to replace of formalin compared to untreated control. Six doses of chitosan solution viz. (0, 125, 250,500, 750 and 1000 ppm) were emerged in alternate day on banana (Champa), tomato, apple, brinjal, palwal, cauliflower and carrot. Then observe one to ten days and record the decay of results respectively. It revealed that chitosan extended shelf life of all fruits/ vegetable by delaying the decay; however, level of protection varied in different kinds of fruits and vegetable as well as in doses of the chitosan used. So we can say that chitosan acts as an alternative preservative to formalin without hampering health and low toxicity.

Keywords: chitosan, fruits, vegetable, decay

Introduction

Chitosan a most available plentiful natural polymer after the cellulose is a linear polysaccharide composed of arbitrarily dispersed β-(1-4)-linked D-glucosamine (deactivated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide [1]. Other names of chitosan are poliglusam, deacetylchitin, and Poly-(D) glucosamine. As a bi product of the fishery industry, it is measured as regenerating raw material. Cellulose, chitin and chitosan are similar inner high molecular natural polymer [2]. It is a white or light red solid powder, not soluble in water, however soluble in acid medium. It consists of alkaline polysaccharide with lower to high viscosity in aqueous convoy which forms a clear film [3]. It is a contradictory to cellulose, which represent as a homo polymer, it concern with chitosan a hetero-polymer chain connection, which is a high degree of crystalline, long and bendable chains forms. In low P^H solution it is positive charge acted as cationic charge. The -OH and $-NH_2$ reactive groups are dependable for the changes of characteristic of new products by different reaction [4]. Chitosan and its derivatives, such as trimethyl chitosan (where the amino group has been trimethylated), have been used in non viral gene delivery. trimethyl chitosan, or quaternised chitosan, has been shown to transfect breast cancer cells, with increased degree of trimethylation increasing the cytotoxicity at approximately 50% trimethylation [3, 5]. oligomeric delivatives (3-4 kDa) are relatively nontoxic and have good gene delivery properties.

More than 700 types of food in traditional and modern markets proved using formalin (BPOM 2006). Formalin is a chemical compound formaldehyde-based are commonly used for the bodies preservatives. Formalin is suspected to cause cancer (carcinogens agent) [6] lung cancer, asthma [7]. Upon intraperitoneal, oral, or inhaler exposure, formalin promptly diffuses into many tissues, including the brain, testis, and liver [8]. Formaldehyde is swiftly absorbed from the gastrointestinal tract following intake and from the respiratory tract following inhalation which makes it a dodgy chemical to be used as preservative. In addition to its abundant uses (such as pressed wood products, paper, textile fibers, adhesives and plastics, carpeting, foam insulation, cosmetics, nail hardeners, disinfectant, some finger paints, and some cleaning products) in our convenient life, it is dishonestly used to preserve various kinds of food stuff [9].

The toxicological effects including histopathological adaptation in the stomach (i.e., gastrointestinal lesions (such as papillomata's hyperplasia and hyperkeratosis,), caused gastrointestinal cancer, allergy, asthma, abdominal pain and vomiting [10]. Formaldehyde is a highly toxic systemic poison that is engrossed in good health by inhalation. The vapor is a severe respiratory territory and skin irritant and may cause dizziness or suffocation [11]. Contact with formaldehyde solution may cause severe burns to the eyes and skin. For that, need a natural preservative as an alternative to substitute synthetic preservatives having low toxic and detrimental consequence and chitosan is the one of the most important natural biopolymer which acts as a antimicrobial agent and also important because of its eco-friendly behavior and biodegradability [12].

Chitosan is not soluble without additives in water because of the protonizing of the free amino groups of chitosan. It needs an acid medium. Therefore chitosan is soluble with organic acid like acetic, formic, lactic, citric acid etc and inorganic acid like HCl, HBr, HNO_3 and $HClO_4$. Chitosan with sulfuric and phosphoric acid is not soluble. Solution stability is low if the p^H value is more than 7, then Chitosan sediment. The characteristics of chitosan solutions depend on the concentration, the p^H value, the charge density of chitosan, type of acid used as well as solution temperature [3]. Chitosan have been of interest in the past few decades due to their potential broad range of industrial applications [4]. Chitosan can also be used in water processing engineering as a part of a filtration process. Chitosan causes the fine sediment particles to bind together, and is subsequently removed with the sediment during sand filtration [5]. It also removes phosphorus, heavy minerals, and oils from the water. Food is a basic need for human being. The food needs will continue to increase in line with population growth. Food will undergo changes among other undesirable spoilage and rancidity. The process of decay and rancidity is caused by the chemical reaction that comes from within and from outside of the food [13].

Food preservatives included in the group of food additives that are pharmacologically inert (effective in small quantities and not toxic). The use of preservatives is very wide, almost the entire industry to use it, including the pharmaceutical, cosmetic, and food [12]. However, the use of preservatives on the market there are still many materials that are prohibited by BPOM (Agency for Food and Drugs supervisor), such as formaldehyde, which is commonly used for meatballs and tofu, with low cost reasons and the products look better and last longer.

The use of synthetic chemicals as preservatives in food is very worrying, because it causes many the health and environment problems. At this time there are still many the use of prohibited preservatives such as formaldehyde and borax.

2. Materials and Methods

2.1. Materials and Chemicals Used in the Experiment

Chitosan [2-Amino-2-deoxy-(1→4)-beta-D-glucopyranan] from shrimp shells was purchased from Sigma. Fresh fruits/vevetable used viz. (banana (Champa), tomato, apple, brinjal, palwal, cauliflower and carrot were collected from the local market of Mirpur-1 district of Dhaka, Bangladesh. Other materials and chemicals used in the investigation were PET bottles (1.0 L and 500 mL), plastic container (1000 ml), distilled water, hydrochloric acid (HCl), sodium hydroxide (NaOH), and pipette tips were available in the laboratory of Department of Chemistry of European University Of Bangladesh. The glassware and minor equipment used were test tubes, round bottom flasks, pH meter, micro-pipette, spray head and a digital balance.

2.2. Preparation of Chitosan Solution

Ten grams of chitosan from shrimp shells (Sigma) was taken in a round bottom flask dissolved in 100 ml of 0.5N hydrochloric acid. The pH of the solution was adjusted to 7.0 by adding 0.5N sodium hydroxide and then diluted with sterilized distilled water (H_2O) to make the stock solution of 1 L (10,000 ppm). Five working solutions, 125, 250,500, 750 and 1,000 ppm prepared by appropriate dilution of the stock solution with distilled water. Only distilled water was used as control (0 ppm chitosan).

2.3. Application of Chitosan Solution

Each fruits/ vegetable were washed with tap water. The excess water from the surface of the fruits was absorbed by paper towel tissues before placing them into a 500 ml plastic container. Six chitosan solutions (0,125, 250,500, 750 and 1,000 ppm) were evenly sprayed onto the fruits until wet and then loosely covered the container by a piece of newspaper. Spray application of chitosan on fruits was done in every alternate day and the morphological features of the fruits were observed every day. The observation of fruits decay over time was recorded in a notebook and also photographed every day by a digital camera. Statistical Analysis Data obtained were analyzed by using Microsoft Excel software and bar graphs were drawn using the mean values of five replicated experiments.

3. Results and Discussion

Chitosan generally delayed the decay of fruits compared to the untreated control. However, the protection of fruits by varying doses of chitosan varied among the tested fruits except tomatoes. Table-1, shows the time course protection (%) of different fruits by varying doses of chitosan.

Table 1: Data of percentage of decay in different concentration

		Percentage of decay of sample in different days, %									
	Amount of chitosan, ppm	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	**8th day**	**9th day**	**10th day**
Banana (Champa)	0 ppm	0	0	0	10	18	26	30	45	56	70
	125ppm	0	0	0	6	15	20	25	35	42	48
	250ppm	0	0	0	5	10	18	24	30	38	45
	500ppm	0	0	0	4	8	15	20	25	35	40
	750ppm	0	0	0	2	5	12	13	17	19	27
	1000ppm	0	0	0	0	3	8	10	13	16	22
Tomato	0 ppm	0	0	5	15	40	55	70	100	--	--
	125ppm	0	0	0	5	15	25	40	80	90	95
	250ppm	0	0	0	0	10	15	30	50	70	80

	500ppm	0	0	0	0	5	10	20	30	40	60
	750ppm	0	0	0	0	0	5	12	20	30	40
	1000ppm	0	0	0	0	0	0	10	12	20	15
Apple	0 ppm	0	0	0	0	0	5	15	30	50	65
	125ppm	0	0	0	0	0	0	10	20	30	50
	250ppm	0	0	0	0	0	0	5	15	25	40
	500ppm	0	0	0	0	0	0	0	5	20	35
	750ppm	0	0	0	0	0	0	0	0	10	25
	1000ppm	0	0	0	0	0	0	0	0	0	10
Brinjal	0 ppm	0	0	10	20	40	60	90	100	--	--
	125ppm	0	0	5	10	20	40	70	85	100	--
	250ppm	0	0	0	10	20	30	45	60	85	95
	500ppm	0	0	0	0	10	20	30	35	60	80
	750ppm	0	0	0	0	0	10	20	30	50	60
	1000ppm	0	0	0	0	0	0	5	15	35	40
Palwal	0 ppm	0	0	0	20	30	50	80	100	--	--
	125ppm	0	0	0	5	20	30	60	80	100	--
	250ppm	0	0	0	0	10	20	40	60	80	95
	500ppm	0	0	0	0	0	5	20	50	55	70
	750ppm	0	0	0	0	0	5	10	30	50	55
	1000ppm	0	0	0	0	0	0	0	10	20	35
Cauliflower	0 ppm	0	0	0	5	10	30	50	80	100	--
	125ppm	0	0	0	0	5	20	40	60	75	90
	250ppm	0	0	0	0	0	10	30	45	60	80
	500ppm	0	0	0	0	0	0	20	40	50	60
	750ppm	0	0	0	0	0	0	0	10	40	50
	1000ppm	0	0	0	0	0	0	0	0	20	30
Carrot	0 ppm	0	0	0	5	10	20	40	60	100	
	125ppm	0	0	0	0	5	10	30	40	60	90
	250ppm	0	0	0	0	0	5	20	30	50	80
	500ppm	0	0	0	0	0	0	10	25	40	70
	750ppm	0	0	0	0	0	0	0	20	30	50
	1000ppm	0	0	0	0	0	0	0	0	10	30

Table 2: Bar graph of decay with chitosan for apple, brinjal, palwal and tomato.

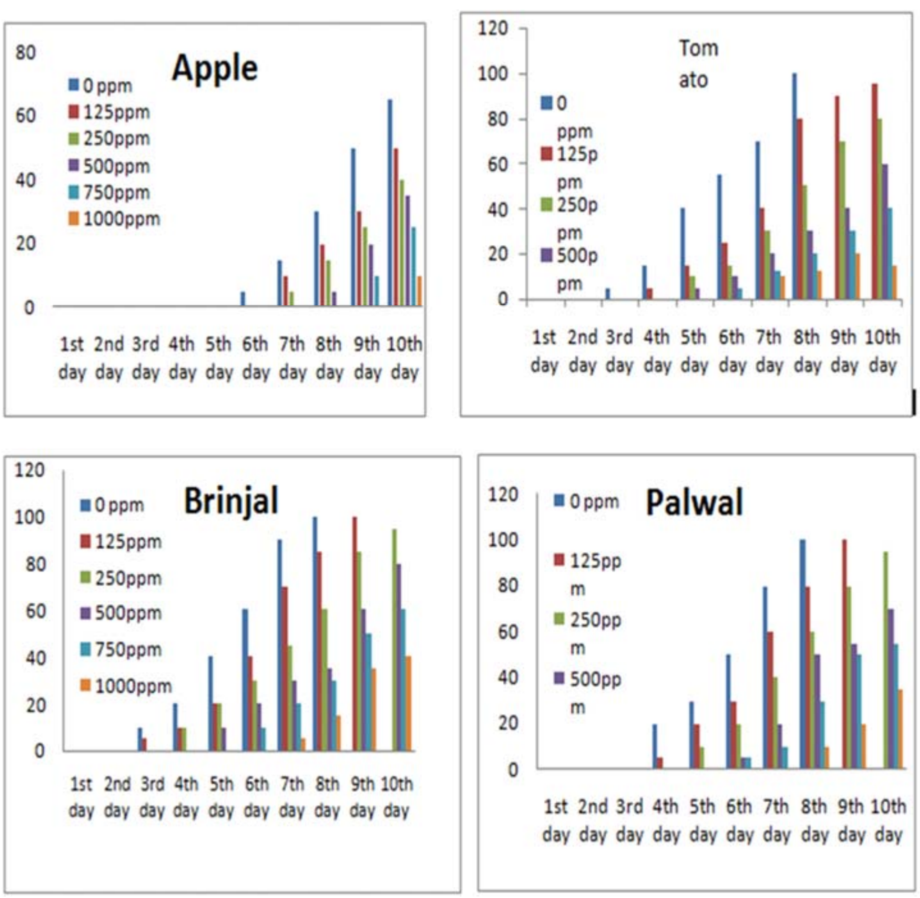
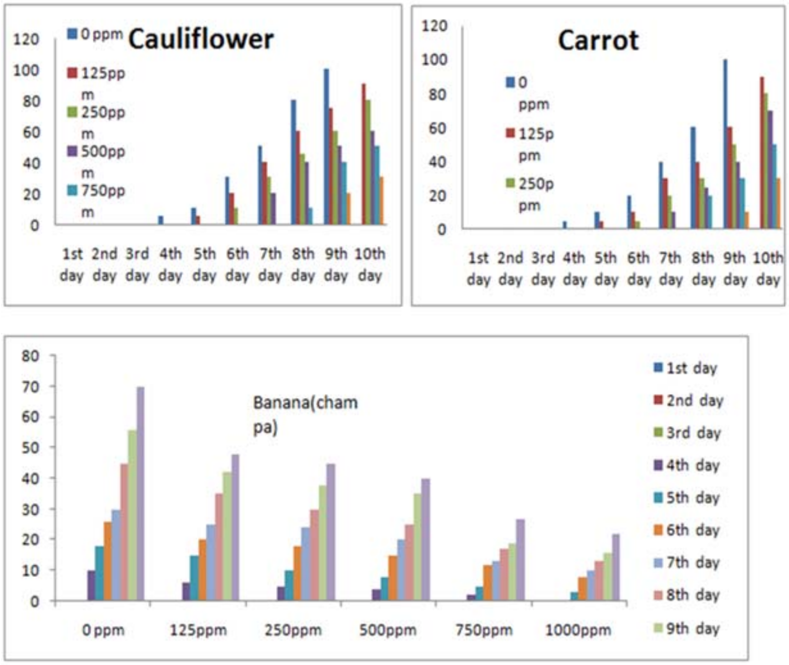


Table 3: Bar graph of decay with chitosan cauliflower, carrot and Banana



The appraisal of the protection of all samples by the application of chitosan was show in graph. This study demonstrated that chitosan protected vegetable from decay at room temperature. Bangladesh is rich in production of shrimp in the Southern region. Exoskeleton of the exported shrimp is a waste. It can be utilized to produce chitosan and utilize this waste into a safe fruit preservative. As chitosan is an antimicrobial agent against many plant pathogenic fungi and bacteria, protection of fruits and vegetable decay observed in this study is likely to be linked with its antimicrobial activity [12]. Moreover, chitosan induces systemic resistance in plants. Formalin is a health perilous chemical, which has been suspected to use in fruit perpetuation in Bangladesh. The preliminary findings of delay in fruit decay by chitosan verified. However, a further investigation is needed with varying doses of chitosan on higher amount of varying fruits under different storage conditions for precise recommendation of its use in fruit preservative.

4. Conclusion

Chitosan squirt extremely enhanced shelf life of strawberry, tomato, orange and bananas under room temperature. However, the type of fruit seemed to be an important factor, as strawberries showed more signs of decay then bananas. Interestingly, the fruits infused with 1000 ppm chitosan possibly had some side effects as some rotted even faster. The fruits which were soaked with 500 ppm produced the best results, as they still looked fresh with no signs of rotting. The fruits treated with only water decayed faster and normally. Too much of chitosan may actually be very bad for some fruits such as strawberries or tomatoes. The findings of the current study might be useful for replacing the use of health hazardous formalin by natural alternative and eco-friendly chitosan.

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