

Extension of Shelf-Life of Tomato Using Irradiated Chitosan and its Physical and Biochemical Characteristics

Nehar Parvin^{1,2}, M. A. Kader³, Roksana Huque⁴, M. E. Molla²,
Mubarak A. Khan^{1*}

¹Institute of Radiation and Polymer Technology(IRPT), Bangladesh Atomic Energy Commission, Savar, Dhaka.

²Jahangirnagar University, Department of Chemistry, Savar, Dhaka-1342.

³Ahsanullah University of Science and Technology, Department of Arts and Sciences, Dhaka.

⁴Institute of Food and Radiation Biology(IFRB), Bangladesh Atomic Energy Commission, Savar, Dhaka

*makhan.inst@gmail.com

Keywords: Chitosan, Natural preservative, Gamma radiation, Antimicrobial activity

Abstract. The effect of irradiated chitosan coating on post-harvest preservation of tomato was observed in this study. Irradiated chitosan (40 kGy) solution of various concentrations (500, 750, 1000, 1500 and 2000 ppm) were applied on post-harvest preservation of tomato. Both chitosan treated and untreated (control) tomato were stored at room temperature in open and zip bag conditions. The effect of coating of various chitosan solutions on tomato was observed during storage period. The percentage of weight loss and spoilage rate of the preserved and control tomato samples were investigated. Several parameters (such as total bacteria count, total mold count, moisture, ash, acidity, vitamin C, sugar, protein and fat) were analysed for irradiated chitosan coated tomato in open condition after 3-weeks storage period. In addition, the same parameters were also analysed for control tomato. Considering all parameters, the results revealed that 1500 ppm chitosan solution performed better in extending the shelf-life of tomato as compared to the control and other treated samples. Thus, this observation recommend that irradiated chitosan coating have the potential to be used as natural preservative to maintain quality and extending shelf-life of tomato.

1. Introduction

Tomato (*Lycopersicon esculentum*) is the most common vegetable crop in all areas of tropical countries in the world and is available throughout the year [1]. It is produced mainly for the consumption of fresh vegetable, juice, jams, sauces or salad. As a tropical commodity, the storage suffers from severe problems, which results in their rapid deterioration and high economic losses. It is found that the main culprit is microbiological diseases. A careful management is necessary to control diseases and safely preservation of post-harvest tomato for consumer. However, the shelf-life of tomatoes vary depending on storage conditions. It ranges from 4 to 8 days at room temperature [2]. This short period seriously limits the long distance commercial transport of tomato. Researchers are working on several methods in order to minimize the postharvest losses of fruits and vegetables and consequently extend its shelf-life, such as, application of fungicides, heat treatment, irradiation, use of bio-control agents, use of natural coating (essential oil, chitosan) and others [3-7]. In addition, the use of irradiated chitosan coatings on strawberry fruits were reported that greatly extend the shelf life due to antimicrobial properties of irradiated chitosan [8]. These coatings act as barriers to water loss and gas exchange by creating a micro-modified atmosphere around the product [7, 9, 10]. The use of chitosan coatings has been successfully investigated to extend the shelf life of several food products, such as, mangoes [11], water caltrop [12], Chinese water chestnut [13], fruit based salad [14]. Recently, another review reported that chitosan films and coatings prevent losses of fresh fruit nutritional quality [15].

Chitosan is a linear amino polysaccharide of glucosamine and *N*-acetyl glucosamine units and is obtained by alkaline deacetylation of chitin extracted from the exoskeleton of crustaceans such as shrimps and crabs, as well from the cell walls of some fungi [16]. Chitosan, as the most abundant naturally occurring amino-polysaccharide, possesses many of these attributes and has attracted attention because of its unique physiochemical characteristics and biological activities [17, 18, 16]. From a biological standpoint, chitosan and its derivatives are very attractive for agriculture applications, which are closely related to human safety and fitness. Owing to its high biodegradability, nontoxicity, and antimicrobial properties, chitosan is widely-used as an antimicrobial agent either alone or blended with other natural polymers. The antimicrobial activity depends on several factors such as molecular weight, degree of deacetylation, solubility, positive charge density, chemical modification, pH, concentration, hydrophilic/hydrophobic characteristic, chelating capacity, and type of microorganism. Early research reported the antimicrobial potential of chitin, chitosan, and their derivatives dated from the 1980-1990s [16, 19-23]. The antimicrobial activity of chitin, chitosan and their derivatives against diverse groups of microorganisms, such as bacteria, yeast and fungi have been receiving great attention over recent years [24-26]. In addition, the antimicrobial activities of chitosan are greatly depending on molecular weight and degree of deacetylation (DD). Another report suggested that chitosan having a higher degree of deacetylation (DD) tends to have higher antimicrobial activity [27]. There are some related works reported the preservation of tomato [28-32] but no specific observation has been reported yet for extending shelf life of post-harvest tomato treated with irradiated chitosan (40 kGy) solution in this manner. This research was aimed to investigate the effect of several concentrations of irradiated chitosan coating on post-harvest tomato for extending shelf-life as well as evaluating the quality changed during its storage period at ambient temperature in open condition.

2. Materials and Methods

2.1. Extraction and Irradiation of chitosan

Chitosan was extracted from prawn shell in Institute of Radiation and Polymer Technology (IRPT) laboratory. High molecular weight chitosan was dissolved in 2% acetic acid solution to make a homogeneous chitosan solution. Chitosan solution (2% w/v in 2% acetic acid) was gamma irradiated at 40 kGy by a 120 kCurie radiation sources at 3.2 kGy per hour dose rate. Other chemicals used were laboratory grade.

2.2. Preparation of irradiated chitosan solution

Irradiated chitosan solution varying the concentrations (500, 750, 1000, 1500 and 2000 ppm) were prepared in distilled water to apply for coating over a wide range of tomato samples.

2.3. Source of tomatoes and preservation by irradiated chitosan solution

Matured green tomatoes were directly collected from local cultivator and brought to the laboratory. The tomatoes were selected without any infection and damage. The selected tomatoes were coated by immersing them in 500, 750, 1000, 1500 and 2000 ppm irradiated chitosan solution for two minutes respectively. The control consisted of tomatoes without washing or immersing in chitosan solution. After air drying at room temperature, tomatoes were stored at ambient temperature in open and in zip bag. The control samples were also stored at the same conditions. The room temperature variation was 25-28 °C and the relative humidity of the air ranged from 70 to 85 % during the whole storage period.

2.4. Physical analysis

2.4.1. Percentage of weight loss

At the first day of sampling, the initial weight of all chitosan coated and control tomatoes were recorded. In a definite time, the weight losses of tomatoes were checked and recorded up to fresh

condition. The percentage of weight losses in a given time intervals were calculated as the total weight loss divided by initial weight multiplied by 100.

2.4.2. Spoilage rate

The spoilage of tomatoes due to fungal or any other microorganism infection was counted every 7 days interval and recorded as percentage. 500 g (average 6 pieces) tomatoes were observed for each batch. The percentage of spoilage rate was calculated as the number of spoiled samples divided by the initial number of all tomatoes multiplied by 100.

2.5. Microbiological (Total bacteria count and Total mold count) analysis

Bacterial and fungal counts were performed by Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) media respectively. 1 g tomato sample was mixed in 10 mL of saline water and the 10 μ L sample was spread in PCA and PDA plates. The plates were incubated at 37 $^{\circ}$ C and 25 $^{\circ}$ C for 24 hours respectively. Then the colony forming units (cfu) were counted. All cfu counts were performed in triplicate.

2.6. Chemical analysis

Various irradiated chitosan solution coated tomato samples in open condition were analyzed for different parameters (such as moisture content, ash content, acidity, vitamin C, sugar, protein, fat) after three-weeks storage periods by standard methods. On the other hand, the control tomatoes were also analyzed for the same parameters within a week after preservation. All experiments were performed in triplicate.

3. Results and Discussions

3.1. Characterization of irradiated chitosan solution

The average molecular weight and the degree of deacetylation (DD) of irradiated chitosan was determined by viscometric method through Mark Houwink equation and FTIR spectroscopy respectively. The effect of various gamma radiation doses on the average molecular weight and degree of deacetylation of chitosan solution were reported previously by Khan et.al [8, 33] research group in the institute of radiation and polymer technology (IRPT) laboratory, Bangladesh Atomic Energy Commission. The degree of deacetylation of chitosan at 40 kGy radiation dose was approximately 79 %.

3.2. Evaluation of shelf life extension

3.2.1. Percentage of weight loss

The weight changes of different chitosan solution coated and uncoated (control) postharvest tomatoes during 6-weeks storage (Fig. 1a) in open and 4-weeks storage (Fig. 1b) in zip bag were observed weekly at ambient temperature respectively. The result showed that 2000 ppm chitosan solution coated tomatoes in open significantly maintained weight compared to control and other treated samples. It was found that the treated and untreated tomatoes exhibited the same rate of weight loss during one-week storage period. One week later, the percent of weight loss of control samples sharply increased with increasing storage period however, it was increased slowly for treated tomatoes. In addition, it was recorded that 29.87 % weight loss for control (shrinkage and dried condition), whereas 10.71 to 18.66 % weight loss for treated tomatoes (almost fresh) depending on chitosan concentration during 3-weeks storage periods. Owing to the high spoilage, the weight loss of control tomatoes was not recorded after 3-weeks storage periods. Although the weight loss of 1500 and 2000 ppm chitosan treated tomatoes were found only 35.68 and 30.48 % at 6-weeks storage periods respectively. In case in zip bag condition, no significant weight loss was found during 4-weeks storage periods but all samples were spoilt (Fig. 1b) due to no evaporation and transpiration. In addition to reducing respiration rate, chitosan coating acts as a hydrophobic barrier and thus prevent evaporation of water from inner cell of tomatoes. Chitosan coating

improves the other quality such as slower softening, texture changes and color retention. This was similar with the recent study reported that the loss of firmness during storage of tomatoes that could be the result of endogenous enzymes; was linked to the cell wall degradation [28]. Another report showed that chitosan films and coatings prevent losses of fresh fruit [15]. According to the method used in this study, control tomatoes both in open and in zip polybag at ambient temperature lost its shelf life as firmness after 3-weeks and 2-weeks storage periods respectively.

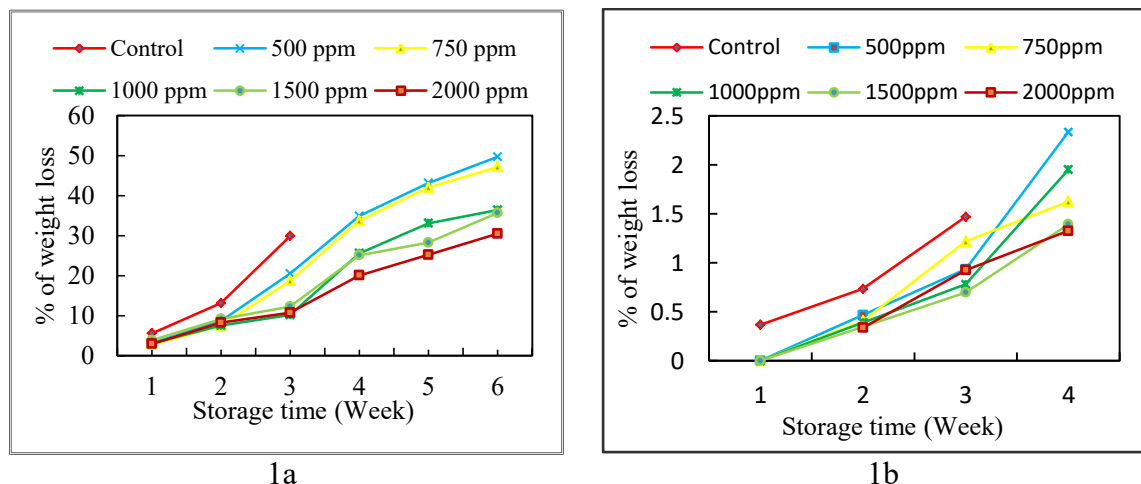


Figure 1. Evaluation of percentage of weight loss of treated and control tomatoes during 6-weeks storage (1a) in open and 4-weeks storage (1b) in zip bag at room temperature respectively

Moreover, the tomatoes treated with 1500 and 2000 ppm chitosan were found relatively lower weight loss and sustained up to 6-weeks as edible condition in open. Therefore, it can be recommended that irradiated chitosan coating greatly prolong the shelf life of tomatoes.

3.2.2. Spoilage rate

One week later, control tomatoes in open condition started suffering from shrinkage, spoilage and dryness but all treated tomatoes were almost fresh up to 4-weeks storage periods. Chitosan treated tomatoes begun to shrinkage, spoilage and dryness after 4-weeks storage period but few control tomatoes were found in compressible and dried condition at 3-weeks storage period. Moreover, chitosan treated tomato samples showed excellent extending shelf life up to 7-weeks (Table 1).

Table 1. Spoilage rate (%) of tomato samples in open condition during storage periods

Sample applied	Percentage of spoilage rate							
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
Control	0	50	83.33	-	-	-	-	-
500 ppm	0	0	0	16.66	50	66.66	66.66	100
750 ppm	0	0	0	33.33	50	66.66	83.33	83.33
1000 ppm	0	0	0	0	16.66	33.33	50	83.33
1500 ppm	0	0	0	0	16.66	16.66	33.33	83.33
2000 ppm	0	0	0	16.66	16.66	66.66	66.66	66.66

Table 2. Spoilage rate (%) of tomato samples in zip bag condition during storage periods

Sample applied	Percentage of spoilage rate					
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week
Control	0	42.85	100	-	-	-
500 ppm	0	16.66	33.33	83.33	100	-
750 ppm	0	14.28	57.14	57.14	100	-
1000 ppm	0	20.00	60.00	100	-	-
1500 ppm	0	16.66	50.00	50.00	83.33	100
2000 ppm	0	16.66	33.33	50.00	50.00	100

On the other hand, the control tomatoes in zip bag at ambient temperature started spoilage after a week and were completely spoiled after two weeks (Table 2). Whereas the chitosan treated tomatoes in zip bag were found to be edible condition up to 4-weeks storage periods. This finding were attributed due to the antimicrobial activity of irradiated chitosan resulting in increasing the shelf life of tomatoes. This result supported by the previous study of several research groups that reported the antimicrobial activity of chitosan and received great attention [24-27].

3.2.3. Microbiological analysis (TBC and TMC)

The total bacteria count (TBC) and total mold count (TMC) of control and various chitosan solutions coated tomato in open and in zip bag shown in Fig. 2a and 2b respectively after 3-weeks storage periods.

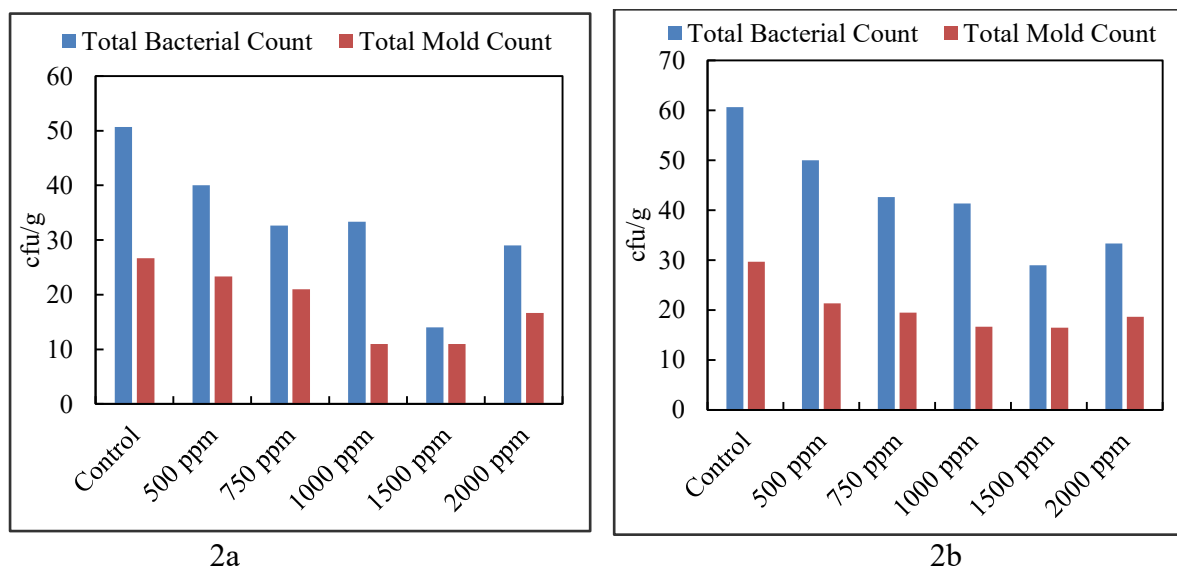


Figure 2. Total bacteria count (TBC) and total mold count (TMC) of control and various chitosan solution treated tomatoes in open (2a) and in zip bag (2b) after 3-weeks storage periods

It was observed that the TBC and TMC sharply decreases with the increasing of chitosan concentration for both in open and in zip bag condition. In addition, the TBC was 50.66 and 60.66 cfu/g for control tomatoes in open and in zip bag respectively, whereas it was 14.0 and 29.0 cfu/g for 1500 ppm chitosan treated tomato in the same condition respectively. The TMC was found at 26.66 and 29.66 cfu/g for control in open and in zip polybag respectively. On the other hand, the TMC was 11.0 and 16.5 cfu/g for 1500 ppm chitosan solution coated tomato in the same conditions. Based on the results, it was suggested that irradiated chitosan significantly performed as antimicrobial agent. Hence, this result supported by early reported work that suggested the mechanism of antimicrobial activity of irradiated chitosan is that it prevents the bacteria and mold growth and thus killing them by reducing nutrients and oxygen in the culture medium [29].

3.2.4. Chemical analysis (Nutritional facts)

Moisture content: The moisture content of control and irradiated chitosan (500, 750, 1000, 1500 and 2000ppm) coated postharvest tomato within a week and after 3-weeks storage period shown in Table 3 respectively. The lowest value of moisture content (92.7 %) was observed for control within a week while highest value was 94.2 % for 1500 ppm chitosan coated tomato after 3-weeks storage period. The highest moisture content for treated tomato may be due the fact that irradiated chitosan coating acts as a better protective shield between inner and outer environment of the tomato during preservation period. The similar result was reported that the loss of moisture may be due to high respiration and transpiration rate [2, 30] and also reported that chitosan coatings decreased the water vapor permeability [15].

Table 3. Nutritional analysis of control and several chitosan solutions treated tomato in open condition within a week and after 3-weeks storage period respectively

Sample applied	Nutritional facts (%)						
	Moisture	Vitamin C	Acidity	Sugar	Ash	Protein	Fat
Control	92.7	17.6	0.12	1.4	0.4	8.8	0.45
500ppm	93.2	7.6	0.21	2.7	0.6	8.8	0.40
750ppm	93.3	8.8	0.25	2.5	0.8	9.5	0.40
1000ppm	93.4	9.5	0.33	2.3	0.8	7.4	0.38
1500ppm	94.2	13.9	0.33	2.2	0.8	8.7	0.37
2000ppm	94.0	14.9	0.38	1.7	0.6	9.2	0.38

Vitamin C: It was found that vitamin C content remained satisfactory level with the increases of chitosan concentration for a long (after 3-weeks) storage period. The value of ascorbic acid (vitamin C) was 17.6 % for control tomato within a week, whereas it was 14.9 % for 2000 ppm chitosan treated tomato after 3 weeks (Table 3). The cause for maintaining vitamin C content by chitosan coating may be due to slow ripening rate. In addition, ascorbic levels may be decreased with time due to oxidation of vitamin C. This finding was also supported by a research work on mango that reported the vitamin C was mostly high in mature but unripe mango fruit and it decreased as the ripening time progressed [2]. This finding was also supported by the report that revealed chitosan based edible coatings prevent vitamin C losses in fruits during storage [15].

Acidity: The values of acidity indicate the quality and acceptability of fruits and vegetables, very low and very high values of acidity are not acceptable for good food. This effort revealed that the value of titrable acidity increases with increasing chitosan concentration (Table 3). The highest value was observed for 2000 ppm chitosan coated tomato due to acidic nature of chitosan solution, and the lowest value was observed for control. In addition, the increasing trend of acidity may have occurred due to an elevation of CO₂ concentration and a reduction of O₂ during its storage period in a modified atmospheric condition caused by the chitosan coating.

Sugar: It was observed that the sugar content (Table-3) of chitosan coated tomato slightly decreased with increasing chitosan concentration after 3-weeks storage time. The decreasing trend of sugar in high chitosan concentration coated tomato might be due to slow ripening process. This result agreed with the report that suggested by Moneruzzaman et al. [32].

Ash content: This observation revealed that control sample contained the lowest ash, whereas chitosan coated samples showed ash content in increasing trend with respect to control sample (Table-3).

Protein: No significant changes of protein were observed for all tomatoes after 3-weeks storage periods (Table-3). This result might be due to slow metabolism conversion during preservation process.

Fat content: Changing of fat were observed chitosan coated tomato after 3-weeks storage periods (Table-3). The lowering of fat content for chitosan treated tomato with respect to control may be due to the facts that chitosan acted as a fat reducer.

4. Conclusion

Based on this study, the application of irradiated chitosan appeared to bear a significant potential as a natural preservative for extending the shelf life of postharvest preservation of tomato. It was revealed from the observation that irradiated chitosan coating maintained the quality of preserved tomato during storage period. Moreover, 1500 ppm chitosan solution showed the best result compared to control and others treatment in terms of percentage of weight loss, spoilage rate, total bacterial counts (TBC), total mold counts (TMC) and nutritional facts. Thus, these results recommended that the application of irradiated chitosan solution on postharvest tomato can be a promising natural preservative for extending shelf life as well as maintaining the desired quality during storage in open at room temperature.

Acknowledgement

The authors are grateful to Governance Innovation Unit (GIU) of Prime Minister Office, Government of the people's republic of Bangladesh for encouraging this research. They would like to thank the Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Commission, Savar, Dhaka for laboratory facilities. Also they would like to thanks Mr. Jubair Bin Habib for his technical assistant during the thesis writing.

References

- [1] FAO STAT, (2009). Available: <http://faostat.fao.org/site/339/default.aspx>. Accessed 2 Dec 2010.
- [2] N.A. Abbasi et al., Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating, Pak. J. Bot. 41(1) (2009) 343-357.
- [3] A. Ali et al., Potential of chitosan coating in delaying the postharvest anthracnose (*Colletotrichum gloeosporioides* Penz.) of Eksotika II papaya, International journal of food science & technology. 45(10) (2010) 2134-2140.
- [4] S. Bautista-Baños et al., A review of the management alternatives for controlling fungi on papaya fruit during the postharvest supply chain, Crop Protection. 49 (2013) 8-20.
- [5] P. Cia et al., Effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose, Postharvest Biology and Technology. 43(3) (2007) 366-373.
- [6] M. Maqbool et al., Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage, Postharvest Biology and Technology. 62(1) (2011) 71-76.
- [7] M. Rojas-Graü et al., The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: a review, International Journal of Food Science & Technology. 44(5) (2009) 875-889.
- [8] S. Jesmin et al., Gamma radiation treated chitosan solution for strawberry preservation: physico-chemical properties and sensory evaluation, International Letters of Natural Science. 60 (2016) 30-37.
- [9] C. Han et al., Effects of chitosan coating on postharvest quality and shelf life of sponge gourd (*Luffa cylindrica*) during storage, Scientia Horticulturae. 166 (2014) 1-8.
- [10] S. Zhang et al., Effect of ultraviolet irradiation combined with chitosan coating on preservation of jujube under ambient temperature, LWT-Food Science and Technology. 57(2) (2014) 749-754.
- [11] T. Djioa et al., Combined effects of postharvest heat treatment and chitosan coating on quality of fresh-cut mangoes (*Mangifera indica* L.), International Journal of Food Science & Technology. 45(4) (2010) 849-855.
- [12] L. Zhan, J. Hu, Z. Zhu, Shelf life extension of minimally processed water caltrop (*Trapa acornis* Nakano) fruits coated with chitosan, International Journal of Food Science and Technology. 46(12) (2011) 2634-2640.
- [13] Y. Peng, Y. Li, L. Yin, Effects of edible coatings on some quality parameters of Chinese water chestnut (*Eleocharis tuberosa*) during storage, International Journal of Food Science and Technology. 48(7) (2013) 1404-1409.
- [14] D. D'Amato, M. Sinigaglia, M.R. Corbo, Use of chitosan, honey and pineapple juice as filling liquids for increasing the microbiological shelf life of a fruit-based salad, International Journal of Food Science & Technology. 45(5) (2010) 1033-1041.
- [15] G. Kerch, Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review, Trends in Food Science & Technology. 46(2) (2015) 159-166.

-
- [16] H.K. No, S.P. Meyers, Preparation of chitin and chitosan, in: Chitin Handbook, Muzzarelli, RAA; M.G. Peter (Ed.), European Chitin Society, 1997.
- [17] E.I. Rabea et al., Chitosan as antimicrobial agent: applications and mode of action, *Biomacromolecules*. 4(6) (2003) 1457-1465.
- [18] R.C. Goy, D.D. Britto, O.B. Assis, A review of the antimicrobial activity of chitosan, *Polímeros*. 19(3) (2009) 241-247.
- [19] P. Beaney, J. Lizardi-Mendoza, M. Healy, Comparison of chitins produced by chemical and bioprocessing methods, *Journal of Chemical Technology and Biotechnology*. 80(2) (2005) 145-150.
- [20] M.N.R. Kumar, A review of chitin and chitosan applications, *Reactive and Functional Polymers*. 46(1) (2000) 1-27.
- [21] K. Kurita, Chitin and chitosan: functional biopolymers from marine crustaceans, *Marine Biotechnology*. 8(3) (2006) 203.
- [22] G. McKay, H.S. Blair, J. R. Gardner, The adsorption of dyes onto chitin in fixed bed columns and batch adsorbers, *Journal of Applied Polymer Science*. 29(5) (1984) 1499-1514.
- [23] K.M. Vårum et al., Determination of the degree of N-acetylation and the distribution of N-acetyl groups in partially N-deacetylated chitins (chitosans) by high-field n.m.r. spectroscopy, *Carbohydrate Research*. 211(1) (1991) 17-23.
- [24] A. Khanafari, R. Marandi, Sh. Sanatei, Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods, *Iranian Journal of Environmental Health Science and Engineering*. 5(1) (2008) 19-24.
- [25] Q. Li et al., Applications and properties of chitosan, *Journal of Bioactive and Compatible Polymers*. 7(4) (1992) 370-397.
- [26] Z. Liman, S. Selmi, A. El-abed, Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physico-chemical properties, *African Journal of Biotechnology*. 10(4) (2011) 640-647.
- [27] B. Acharya et al., Characterization of chito-oligosaccharides prepared by chitosanolytic with the aid of papain and pronase, and their bactericidal action against *Bacillus cereus* and *E. coli*, *Biochemical Journal*. 391(2) (2005) 167-175.
- [28] A.B. Wold et al., Colour of post-harvest ripened and vine ripened tomatoes (*Lycopersicon esculentum* Mill.) as related to total antioxidant capacity and chemical composition, *International Journal of Food Science & Technology*. 39(3) (2004) 295-302.
- [29] M.S. Benhabiles et al., Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste, *Food Hydrocolloids*. 29(1) (2012) 48-56.
- [30] M.S. Benhabiles et al., Assessment of coating tomato fruit with shrimp shell chitosan and N, O-carboxymethyl chitosan on postharvest preservation, *Journal of Food Measurement and Characterization*. 7(2) (2013) 66-74.
- [31] S. Bautista-Baños, R. González-Soto, M.D.L.R. Garcia, Physical properties of chitosan films with lemon essential oil added and their impact on the shelf life of tomatoes (*Lycopersicon esculentum* L.), *Revista Mexicana de Ingeniería Química*. 17(1) (2017) 1-11.
- [32] K.M. Moneruzzaman et al., Effect of harvesting and storage conditions on the post harvest quality of tomato (*Lycopersicon esculentum* Mill) cv. Roma VF, *Australian Journal of Crop Science*. 3(2) (2009) 113.
- [33] T.U. Rashid et al., A new approach for the preparation of chitosan from γ -irradiation of prawn shell: effects of radiation on the characteristics of chitosan, *Polymer International*. 61(8) (2012) 1302-1308.