

Comparison and evaluation of effect of Chitosan as edible coating to maintain the postharvest quality and enhance shelf-life of *Psidium guajava* and *Fragaria ananassa* fruits.

Comparación y evaluación del efecto del quitosano como recubrimiento comestible para mantener la calidad poscosecha y mejorar la vida útil de los frutos de *Psidium guajava* y *Fragaria ananassa*

Rama Krishna Konda & Dr. Kamala Golla*

Bhavan's Vivekananda college of science, humanities and commerce, Sainikpuri, Secunderabad, India.

* Author for correspondence: kamala_arudra81@yahoo.com

ABSTRACT

Preservation of fruits post-harvest has always been a major problem for farmers and industries because of their short-shelf life and high perishability. Several methods are followed for preservation such as cold storage, use of wax coatings, each of these has their own disadvantages. So, in search of a perfect alternative for these preservative methods, use of edible coatings has shown a significant effect and benefit on fruit preservation. Chitosan, a natural polymer was chosen as an edible coating because of its properties such as biodegradability, non-toxicity. Guava and strawberry are the fruit with short shelf life due to intense metabolic activity. In attempt to minimize these post-harvest problems, chitosan (1%) was used as an edible coating for a period of 96hrs and at a temperature of 25 ± 5 °C on guava and strawberry and evaluated its physiochemical characteristics and anti-oxidant system. It was observed that there was a less decrease in total sugar content (simple sugars), increase in peroxidase and catalase activity and also decrease in the fresh weight loss. These results suggested that chitosan effectively prolonged the quality attributes in guava and strawberry post-harvest, by delaying processes like ripening and increase of anti-oxidant activity, upon storing at a temperature of 25 ± 5 °C.

Keywords: postharvest, shelf-life, edible coating, Biodegradability, Peroxidase and catalase activity, Total sugar content, fresh weight loss.

RESUMEN

La conservación de las frutas después de la cosecha siempre ha sido un problema importante para los agricultores y las industrias debido a su corta vida útil y su alta caducidad. Se siguen varios métodos para la conservación, como el almacenamiento en frío, el uso de recubrimientos de cera, cada uno de estos tiene sus propias desventajas. Por lo tanto, en la búsqueda de una alternativa perfecta para estos métodos de conservación, el uso de recubrimientos comestibles ha demostrado un efecto y un beneficio significativos en la conservación de la fruta. El quitosano, un polímero natural, se eligió como recubrimiento comestible debido a sus propiedades, como la biodegradabilidad y la no toxicidad. La guayaba y la fresa son las frutas con vida útil corta debido a la intensa actividad metabólica. En un intento por minimizar estos problemas poscosecha, se utilizó quitosano (1%) como recubrimiento comestible por un período de 96 horas y a una temperatura de 25 ± 5 °C en guayaba y fresa y se evaluaron sus características fisicoquímicas y sistema antioxidante. Se observó que hubo una menor disminución en el contenido de azúcares totales (azúcares simples), aumento en la actividad de peroxidasa y catalasa y también disminución en la pérdida de peso fresco. Estos resultados sugirieron que el quitosano efectivamente prolongó los atributos de calidad en poscosecha de guayaba y fresa, al retrasar procesos como la maduración y el aumento de la actividad antioxidante, al almacenarse a una temperatura de 25 ± 5 °C.

Palabras clave: poscosecha, vida útil, recubrimiento comestible, biodegradabilidad, actividad peroxidasa y catalasa, contenido de azúcar total, pérdida de peso fresco.

INTRODUCTION

Guava (*Psidium guajava* L.) is a well-known tropic fruit, belonging to phylum magnoliophyta, class magnoliopsida, and myrtaceae family. This fruit is considered to be very rich in nutritional elements such as, levels of vitamin-C are found to be 3 to 6 times higher than in orange as well as the lycopene content is twice that of tomato fruits. Aider, M. (2010) mentioned that it also contains saponin, oleanolic acid, lyxopranoids, alabopyranoids, guaijavarin, quercetin and flavonoids (Aider, M. 2010). Karagozlu M.Z(2014) mentioned that ascorbic acid and citric acid are the major ingredients of guava that play an important role in anti-mutagenic activity (Karagozlu M.Z., Kim S.-K 2014). Guava fruit also contains terpenes, caryophyllene oxide and p-selinene in large quantity which produce relaxation effect. It is employed to treat a lot of sickness like diarrhoea, reducing fever, dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief and wounds. It also contains secondary metabolites like antioxidants, polyphenols, antiviral compounds, anti-inflammatory compounds. The presence of terpenes, caryophyllene oxide

and p-selinene produces relaxation effects and its high antioxidant content has radio-protective ability.

Strawberry (*Fragaria ananassa*), belongs to family Rosacea. Heinonen (1998) mentioned that it is a good source of natural antioxidants (Heinonen, I. M., Meyer, A. S., & Frankel, E. N 1998). In addition to the usual nutrients such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, phenolic acids. The high antioxidant activity of strawberry is mainly attributed by vitamin c and polyphenol components are anthocyanins, ellagic acid derivatives and flavanols. It can also contribute in regulating blood sugar levels by slowing digestion because of its dietary fibre and fructose contents. Its fibre content also helps in control caloric intake by its satiating effect. These are also rich in folate. Folate along with vitamin c plays a crucial role in supplementing this essential micro-nutrient. It is also rich in manganese, by consumption of eight medium berries (144g) more than 20% of the daily adequate intake for this mineral can be full-filled. Clifford(2000) mentioned that anthocyanins are the best known and quantitatively the most imp poly phenolic compounds in strawberry with its value ranging between 150-600mg/kg of fresh weight (Clifford 2000, Lopes-da-Silva et al . 2002). Pelagonidin-3-glucoside is the major anthocyanin in strawberries, irrespective of its genetic and environmental factors. Santos-Buelga(2000) mentioned that it also contains cyanidin-3-glucoside in constant amounts, but only in smaller proportions (Santos-Buelga C, Scalbert A 2000). Flavanols in strawberry found in monomeric (catechins) and polymeric forms (condensed tannins or procyanidins). Santos-Buelga (2000) mentioned that Procyanidins was reported to possess antioxidant, antimicrobial, anti-allergic and antihypertensive properties and inhibit activities of some phenolic enzymes and receptors (Santos-Buelga C, Scalbert A 2000).

These are the fruits with a short shelf-life post-harvest which limits their transportation and storage period. Hong (2012) mentioned that the major reason behind this short shelf life and early spoilage post-harvest is fast ripening, high respiration rate (Hong et al. 2012, xisto et al. 2004) and also reactive oxygen species (ROS). To counter these ROS, cells develop anti-oxidant activity and these antioxidants play a major role in scavenging these free radicals and hence are also known as "free-radical scavengers". Antioxidants are compounds that undergo oxidation terminating the chain reaction by reacting with free radicals and chelating catalytic metals and results in neutralization of free radicals and simultaneous inhibition of oxidation of other vital molecules. These free radicals are created as a consequence of ATP synthesis in mitochondria using oxygen. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. The levels of these species decide their effect on the cell, under low or moderate levels it shows a beneficiary effect on cellular responses and immune functions, whereas higher concentrations lead to

damage of cellular structures creating oxidative stress. Oxidative stress is the major cause for damage of fruits. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. In foods, antioxidants have been defined as 'substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats, therefore, in food science antioxidants are usually equated with chain-breaking inhibitors of lipid peroxidation, but not exclusively so. Antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods. There are both synthetic and natural antioxidants available. Synthetic antioxidants are chemically synthesized compounds since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. Butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) were originally developed to protect petroleum from oxidative gumming. Other examples of synthetic antioxidants are propyl gallate (PG), dodecyl gallate (DG), octyl gallate (OG) and ethylene diaminetetraacetic acid (EDTA).

Giovannoni (2017) mentioned that ripening process causes changes in physiological and biochemical parameters such as chlorophyll degradation, enzymatic cell wall degradation, changes in sugar content, respiratory activity, ethylene production and in levels of aromatic compounds (Giovannoni et al. 2017), all together contribute to the early spoilage of fruits. Cheng (2010) mentioned that ROS, mainly produced due to the electron transport in mitochondrial respiration, when released in excess, mainly under stress or during fruit ripening can directly damage cells and reduce quality of fruits and vegetables (Cheng et al. 2010). Chitosan, a high molecular weight cationic linear polysaccharide composed of randomly distributed β -linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan's are recognized as versatile biomaterials because of their non-toxicity, low allergenicity, biocompatibility and biodegradability. Chitosan is reported to have other biological properties, Karagozlu (2014) mentioned the antitumor property (Karagozlu M.Z., Kim S.-K 2014), Martins (2014) mentioned the antimicrobial property (Martins et al. 2014), Ngo D.H.(2014) mentioned the antioxidant activity (Ngo D.H., Kim S.K 2014). Several hypotheses were also proposed regarding the reason behind the antibacterial activity of chitosan. Sudarshan N.R (1992) mentioned that Low molecular weight chitosan can penetrate bacterial cell walls, bind with DNA and inhibit DNA transcription and mRNA synthesis (Sudarshan N.R et al 1992), Zheng L.Y.(2003) while high molecular weight chitosan can bind to the negatively components on the cell wall, thus forming an impermeable layer around the cell, changes cell permeability and blocks

transport into the cell (Zheng L.Y., Zhu J.F 2003). Park S.C(2015) mentioned that data from an *in vivo* experiment employing a mouse model of bacterial infection provided evidence that low molecular weight water soluble beta-chitosan may find anti-infective and wound healing applications (park S.C et al. 2015). Tayel A.A(2015) mentioned that chitosan was also found to exhibit antifungal activity against several phytopathogenic fungi such as *Penicillium* sp. in citrus fruit(tayel A.A et al. 2015), Ben-shalom N (2003) mentioned the antifungal activity of chitosan on *Botrytis cinerea* in cucumber plants(Ben-shalom N et al.2003), Atia M.M.M(2005) mentioned the antifungal activity of chitosan on *Phytophthora infestans* (Atia M.M.M et al 2005), Saharan V (2015) mentioned the antifungal activity of chitosan on *Alternaria solani* and *Fusarium oxysporum* (Saharan V et al. 2015) in tomatoes. Bai R.K(1998) suggested mechanism involved a permeable chitosan film formed on the crop surface which interfered with the fungal growth and activated several defence processes like chitinase accumulation, proteinase inhibitor synthesis, callus synthesis and lignification (Bai R.K et al 1998). Chito oligosaccharides are nontoxic and water-soluble compounds derived from chitosans by enzymatic degradation. Sulphated chito oligosaccharide III with a molecular weight of 3–5 kDa potently suppressed HIV-1 replication, HIV-1-induced syncytium formation, lytic action, and p24 antigen production. Artan M (2010) mentioned that Sulphated chito oligosaccharide III obstructed viral entry and virus-cell fusion probably by interfering with the binding of HIV-1 gp120 to CD4 cell surface receptor. Unsulfated chito oligosaccharides did not have similar actions (Artan M et al. 2010). Tokoro A (1988) mentioned that recent investigations revealed that chitosan and its derivatives exhibited antitumor activity in both *in vitro* and *in vivo* models (Tokoro A et al.1988) and also observed that the antitumor effect of chitosan derivatives was due to the increase in secretion of interleukin-1 and 2 which caused maturation and infiltration of cytolytic T-lymphocytes. Li B(2015) mentioned that *N*-methacryloyl chitosan, produced as a result of a single-step chemo selective *N*-acylation reaction, acquires the desirable features of hydro solubility, UV crosslink ability and injectability which facilitates quick and cost-effective construction of patterned cell-loaded polysaccharide microgels with distinctive amino groups as building materials for tissue engineering and quick transdermal curing hydrogel *in vivo* for localized and sustained protein delivery (Li B et al.2015).chitosan is used in pharmaceutical industry as drug delivery system. Chameettachal S (2015) mentioned the use of chitosan in different forms like vaccines, micelles, microspheres, tablets and they also facilitate transmucosal absorption which is important in nasal and oral delivery of some polar drugs like peptides along with protein vaccines for their administration (Chameettachal S et al.2015). Ueno H (2001) mentioned that chitosan also exhibits the property of wound healing because of its various properties such as biodegradability, biocompatibility, low immunogenicity which favours the process of wound healing and it was also observed to promote the activity of polymorphonuclear

leukocytes, macrophages and fibroblasts that enhance granulation as well as the organization of the repaired tissues (Ueno H et al.2001). Chitosan also shows water treatment activity and is one of the most efficient material for water treatment. Prabhu S.M(2015) mentioned that protonated polyamidoamine grafted chitosan beads loaded with Zr (IV) ions, produced by amination of chitosan beads by ethylenediamine through Michael addition and followed by protonation, eliminated fluoride ions from aqueous solutions with higher selectivity than other metal ions. The adsorption was spontaneous and endothermic (Prabhu S.M., Meenakshi S. A 2015).Pereira P(2015) mentioned that glycol chitosan nanogels may be useful as drug delivery vectors for targeting different intracellular compartments (Pereira P et al.2015).Sayed S(2015) mentioned that quaternary tetraalkylammonium chitosan derivatives can be utilized in the form of an inexpensive perchlorate-specific solid-phase extraction anion exchange cartridge in conjunction with colorimetric analysis for perchlorate removal or analysis (Sayed S et al.2015). Chitosan is also given as a food supplement or nutraceutical as it helps in lowering cholesterol and reducing obesity. Heber D (2003) mentioned that chitosan swells up giving the feeling of satiety by physically filling the stomach (Heber D 2003). They are also found to show antioxidant activity and many studies have been performed to understand its antioxidant activity. Low-molecular-weight chitosan is more active in scavenging free radicals, such as hydroxyl, superoxide, alkyl and 2,2-diphenyl-1-picrylhydrazyl radicals. Younes I (2015) mentioned that the mechanism is due to the reaction of unstable free radicals with amino and hydroxyl groups on the pyranose ring, which form the stable radicals (Younes I., Rinaudo M 2015). The electron spin resonance data demonstrated that medium-molecular-weight hetero-chitooligosaccharides prepared from 90% deacetylated chitosan manifested the highest radical scavenging potency. The radical-scavenging activity of hetero-chitooligosaccharides was related to the degree of deacetylation values and the molecular weight. In addition to these applications, they are also used in the treatment of cardiovascular diseases, age-related diseases, dry mouth syndrome, food industry and as mucosal immunity enhancer. Cosme silva G M (2017) mentioned that despite of its many applications in engineering, biotechnology and medicine (Cosme silva G M et al.2017), it was also used as coating and as an effective preservative in case of papaya, mango. In this work, effect of chitosan was evaluated on guava and strawberry at a temperature of 25 ± 5 °C for a period of 96hrs.

MATERIALS AND METHODS

Chitosan, strawberry and Guava: Strawberry, camarosa variety were obtained from orchards of Karnataka and guava, VNR bihi variety were obtained from VNR nursery group, araku. Both of them were brought by land transportation to department of biochemistry, bhavans Vivekananda college, secunderabad. Commercial chitosan was purchased from banglore fine chemicals.

Chitosan solution: 1g of chitosan was dissolved in 100ml of distilled water and 2ml of glacial acetic acid was added to dissolve the chitosan and the solution was buffered to pH nearly 6.9 with 0.1M NaOH.

Chitosan treatment: Fruits were dipped in chitosan solution for 1 min and air dried for 30 minutes and now they were stored at a temperature of 25 ± 5 °C for 96hrs.

Preparation of fruit extraction: 10g of strawberry and guava was homogenised separately in presence of 50ml of 0.1M phosphate buffer (pH 7.0) under cool conditions and the extract was centrifuged at 10000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant was represented as "crude enzyme".

Peroxidase activity: To the test tubes taken add 0.5ml of solution A (Solution A- 2.2ml of hydrogen peroxide 35% in 10 ml of distilled water, added to 50 ml of phosphate buffer) and 0.5ml of solution B (Solution B - 83.3 mg of Amino anti pyrene in 10ml of distilled water, added to 163 mg of 25 phenol in 70ml of distilled water). The reaction starts with the addition of 1ml of enzyme extract to the above test tubes. The tubes were kept in water bath for 5 minutes at 30°C. To this 2ml of Absolute ethyl alcohol was added (to stop the reaction). Absorbance was read using spectrophotometer at 505nm and enzyme activity was expressed in units/mg.

Catalase activity: In the control cuvette, enzyme solution and hydrogen peroxide free phosphate buffer were added. In the experimental cuvette, 3ml of hydrogen peroxide phosphate free and 0.01- 0.04ml of sample was used. Time interval was noted for the decrease in the absorbance and the time should be 60 seconds. Absorbance was read at 280nm and enzyme activity was calculated and expressed in units/mg/min.

Glucose estimation: Into a series of test tubes labelled 1-5 working standard maltose solution was pipetted out with concentration ranging from (200- 1000µg) in volume 0.2 to 1.0ml. To all the test tubes distilled water was added to make up the volume to 1.0ml. 1.0ml of distilled water separately serves as blank. Both the enzyme extracts were taken in two aliquots that is 0.5ml and 0.7ml. 1.0ml of DNS reagent was added to all the test tubes including blank and unknown. Mix the contents. Place all the tubes in boiling water bath for 5 minutes. Tubes were removed and 0.5ml of 40% sodium

potassium tartrate was added when the tubes were still hot. Cool the tubes at room temperature and add 3.5ml of distilled water and vortex the contents. Measure the absorbance against reagent blank at 540nm.

RESULTS AND DISCUSSION

Physical parameters-Fresh weight loss: Fig 1 suggests that there was a decrease in the fresh weight loss in strawberry test compared to that of strawberry control, a total decrease of 8.65 gm was observed in strawberry control, total decrease of 4.05 gm was observed in strawberry test before and after the interval of 96hr treatment. Similar results were observed in fig 2 in which a decrease in the fresh weight loss in guava test compared to guava control, a total decrease of 10.33 gm was observed in guava control, total decrease of 9.63 gm was observed in guava test before and after the interval of 96hr treatment.

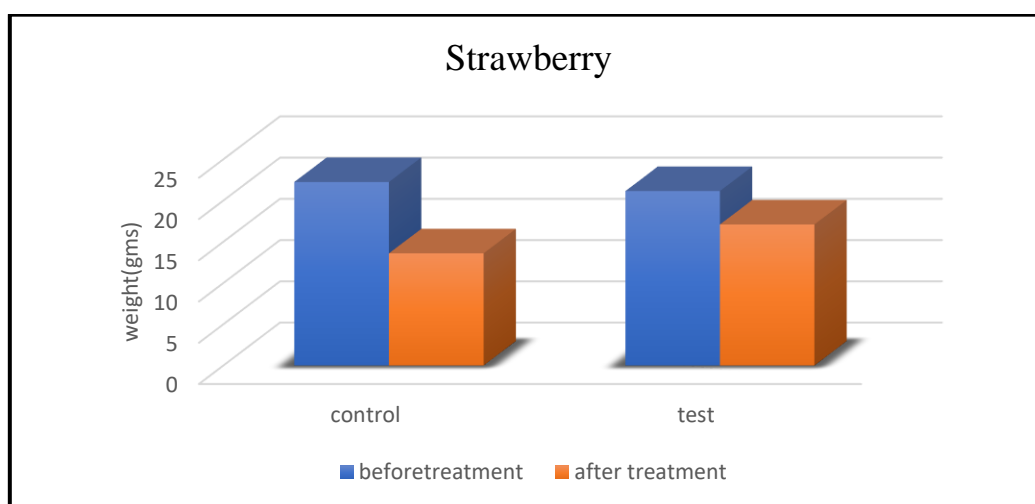


Fig 1: fresh weight loss of Strawberry

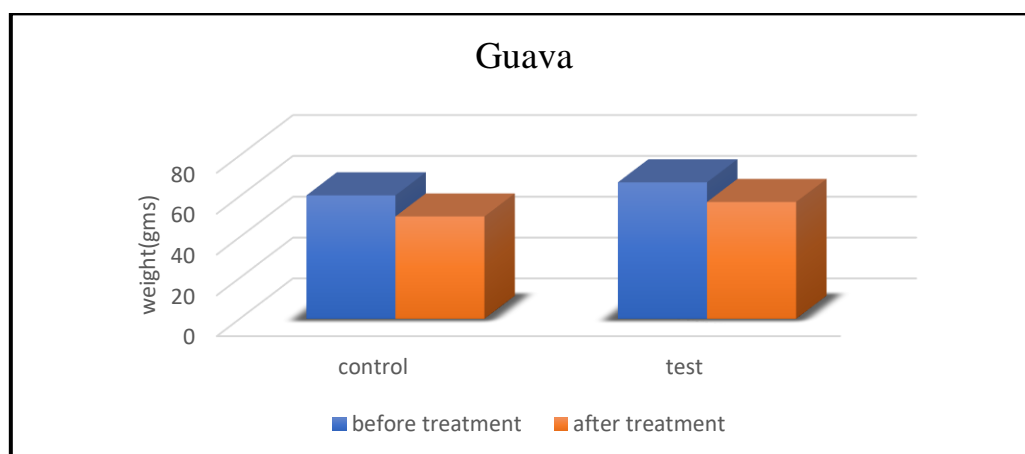


Fig 2: Graph for fresh weight loss of Guava

There are many factors influencing the fresh weight loss of the fruit such as rapid water loss, higher respiration rate which may lead to the loss of fresh weight and shrinkage of the fruit reducing its quality and making it unfit for consumption. The movement of air surrounding the fruit plays a major role in water loss, faster the surrounding air movement, quick the water is lost from fruit and as there is no continuous water supply to the harvested fruit to replace the lost water, it utilises the water content remaining at the time of harvest which was found to approximately 65-95% of fresh weight. This loss of water is very rapid and soon leads to shrinkage and death of the fruit. The other factor which affects the fresh weight loss is higher respiration rate. During the process of respiration in postharvest fruits, there is a continuous utilisation of starch reserves which stops once they are exhausted, eventually ageing follows and fruit dies and decays. Air supply to the fruit shows a significant influence on the respiration rate, as it contributes to nearly 20% of the oxygen essential for normal plant respiration. As chitosan forms a film and acts as a packaging material successfully limiting the exposure of the fruit to the surrounding air movement and air supply thus reducing both water loss and decreasing respiration rate respectively maintaining its quality and increasing its shelf life during storage at a temperature of $25\pm 5^{\circ}\text{C}$.

Upon comparing the effect of chitosan on fresh weight loss between guava and strawberry, it was observed that chitosan showed a very significant effect on strawberry as the difference in the fresh weight loss between control and test was observed to be 4.15 gm in case of strawberry, which was limited to only 0.70 gm in case of guava, because the rate at which water is lost from strawberry was very high due to its thin waxy skin with more number of pores compared to guava with thick corky skin with few pores.

Anti-oxidant activity: The major part of the plants antioxidant system involves antioxidant enzymes. These antioxidant enzymes are catalysts, involved in the scavenging of the free-radicals and also interrupt oxidising chain reactions to minimize damage caused by free-radicals [7]. Catalase and peroxidase are the most common antioxidant enzymes found in almost all living organisms. Since, both peroxidase catalase utilizes the same substrate i.e., hydrogen peroxide, activity of one enzyme may influence the activity of the other enzymes. When there is an increased activity of peroxidase, a decrease in the activity of catalase was observed.

Fig 3 suggests a higher peroxidase activity in guava test compared to guava control, in both test and control, highest enzyme activity was observed with enzyme concentration of 1.5 ml. Fig 5 suggests that catalase activity of guava control was high compared to guava test, highest enzyme activity was observed at 60 mins and activity reduced with respect to time.

Fig 4 suggests a higher peroxidase activity in strawberry control compared to strawberry test, in both control and test highest enzyme activity was observed at enzyme concentration of 0.5 ml. Fig 6 suggests that catalase activity in strawberry test was high compared to strawberry control, highest enzyme activity was observed at 60 mins and activity reduced with respect to time.

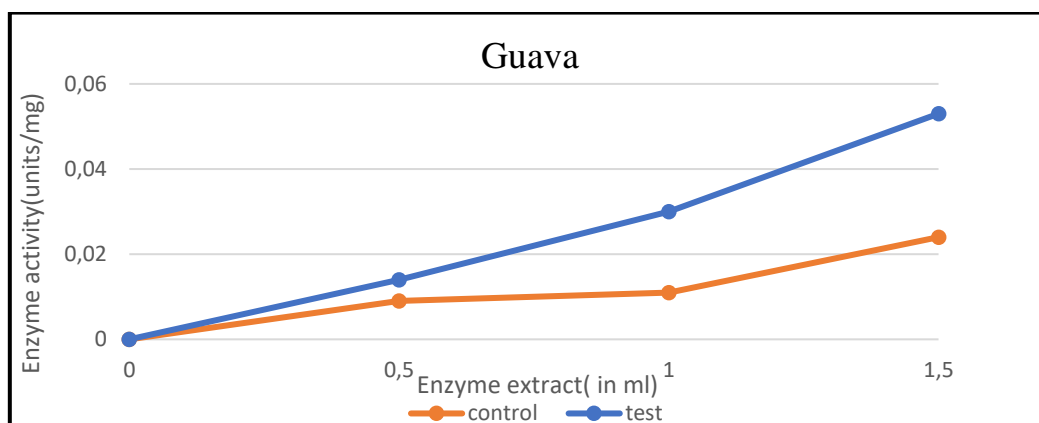


Fig 3: peroxidase activity of guava after 96 hr treatment.

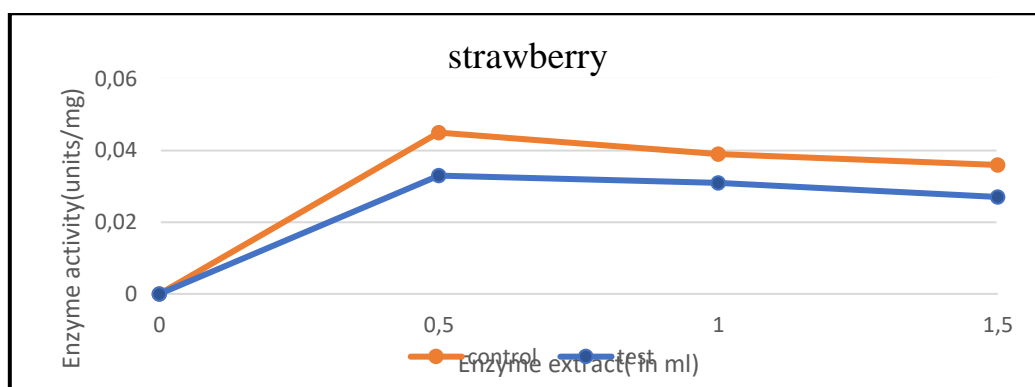


Fig 4: peroxidase activity of strawberry after 96 hr treatment

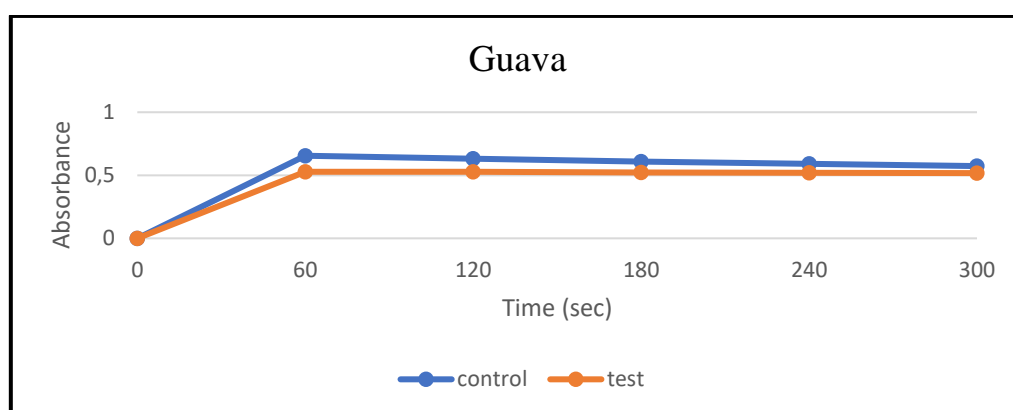


Fig5: Catalase activity of Guava after 96 hr treatment

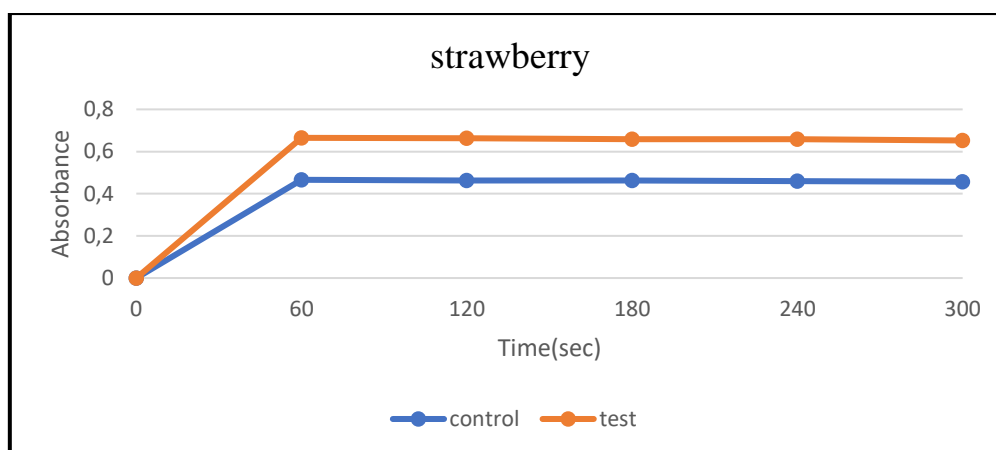


Fig 6: Catalase activity of Strawberry after 96 hr treatment

In both the fruits, as results suggested either peroxidase and catalase activity increased in case of test fruits, which suggested there was an increase in antioxidant enzyme activity in coated fruits. Among the various properties possessed by chitosan the ability to act as bio stimulant and exogenous elicitor property was a very important which helped the fruits to trigger its defence system protecting the fruit from pathogen attack and helped in maintaining quality and shelf life of the fruits. Its elicitor activity increases the physiological response and stimulates antioxidant enzymes via nitric oxide and hydrogen peroxide signalling pathways which helped in scavenging ROS system and ultimately improves the performance under stress conditions.

The other factor which contributed to increase in antioxidant enzyme activity was dehydration stress which was observed due to the rapid water loss in postharvest fruits. Antioxidant enzyme activity played a very important role in countering the dehydration stress and ROS scavenging. It was clearly evident from the in both the fruits, either peroxidase or catalase activity of the coated fruit (test) was increased compared to that of uncoated fruit, this is because chitosan successfully acted as an exogenous elicitor and improving the endogenous antioxidant activity. This antioxidant activity helped in scavenging the free-radicals which play a role in the spoilage of fruits by damaging the membrane integrity and lipid peroxidation.

Glucose estimation: By estimation of glucose concentration using DNS method, it was observed that coated fruit (test) showed less glucose levels compared to that of uncoated fruit (control) in case of both guava and strawberry. During the process of respiration, stored starch reserves are converted to simple sugars such as glucose, precursor in respiration and this may lead to increase in the glucose concentration because of its higher respiration rate. Similar mechanism is observed during the process of respiration in which complex carbohydrates (sucrose) get hydrolysed to simple carbohydrates (glucose) and this contributes to the sweetness of a ripened fruit making it

more susceptible to the microbial attack. As chitosan formed a film around the fruit it helped in reducing the respiration rate and delaying the process of ripening which was responsible for the less glucose concentrations observed in both guava and strawberry test fruits compared to control fruits.

Upon comparing the glucose concentrations of both guava and strawberry after 94 hr treatment, in fig 7 it was observed that difference in the values of guava control and test was observed to be 0.01mg/ml. In fig 8, it was observed that difference in the values of strawberry control and test was observed to be 0.035 mg/ml. These results were obtained because strawberry showed a higher respiration rate because of its thin skin, whereas in case of guava the major effect of chitosan was in delaying the process of ripening. Hence, in both the fruits chitosan successfully delayed the process of conversion of complex sugars to simple sugars which was responsible for the lower glucose concentrations observed.

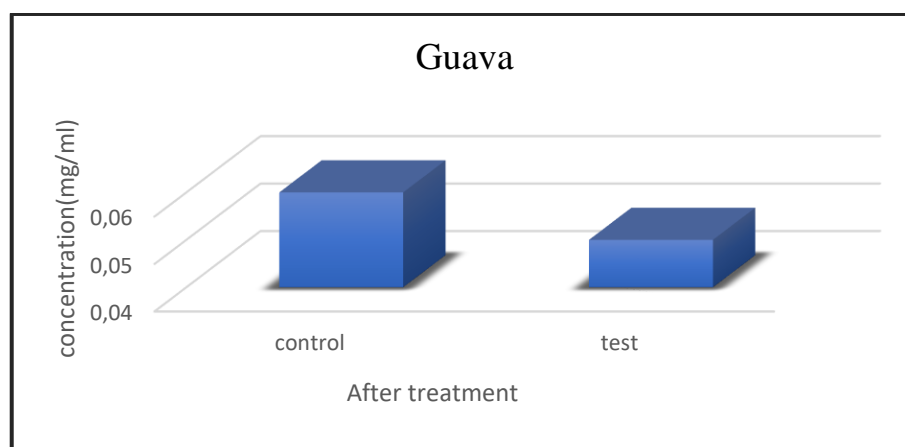


Fig 7: Glucose estimation of Guava after 96 hr treatment

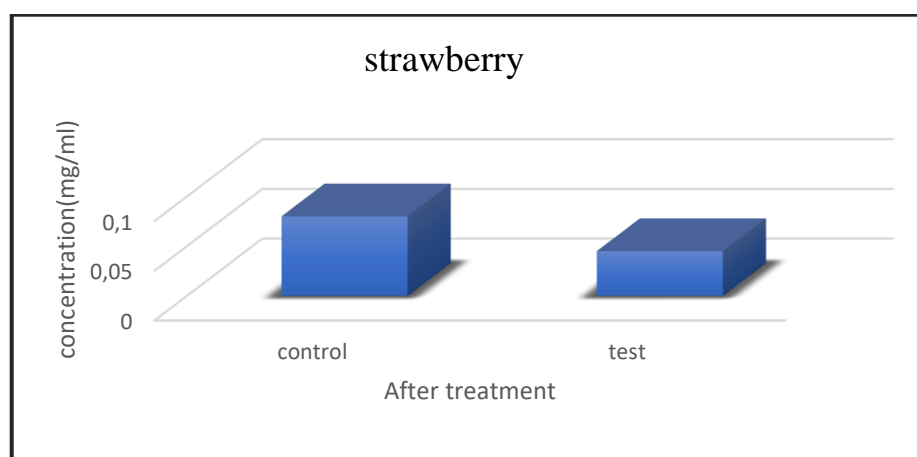


Fig 8: Glucose estimation of strawberry after 96 hr treatment

SEM analysis: As observed in fig 9, results of the SEM analysis suggested the particle size of chitosan in chitosan solution was found to be in the range of 1-5 μm , which proved the colloidal, polymer and film forming nature of the chitosan. This film helped in forming a barrier between the fruit and external environment reducing the fruits exposure to the surrounding air movement and reducing the respiration rate, water loss, thus helping the fruits in maintaining the postharvest quality of the fruit.

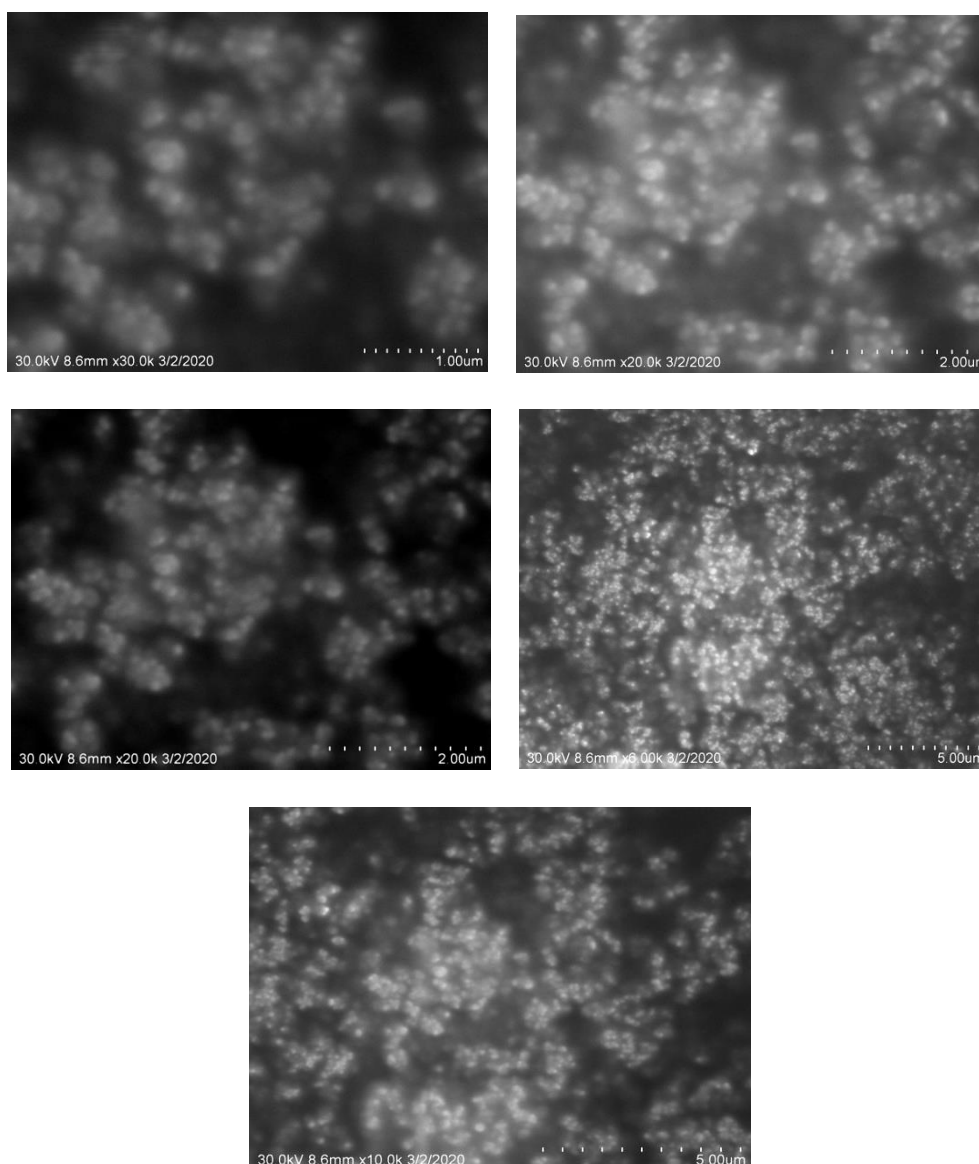


Fig 9: SEM analysis of chitosan solution

As conclusion, in search of several alternatives for wax coatings, use of chitosan as an edible coating was found to be effective as it formed a film on the surface of fruit, limiting the exposure of the fruit to atmosphere and modifying the exchange of gases like

carbon dioxide and oxygen, affecting the respiration rate and also restricted the oxidation process and also reducing the water loss. It was also observed that chitosan also acted as an exogenous elicitor and increased the antioxidant activity and inducing the resistance in the host by increasing the activities of several defence-related enzymes, such as chitinase & β -1,3-glucanase in orange, strawberry and raspberries, in the similar manner, there was an increase in the antioxidant activity i.e. activity of POD and catalase which helped to scavenge the excess ROS released and protected the cells from damage such as lipid peroxidation and loss of membrane integrity. Bai R.K(1988) mentioned that chitosan can effectively delay ripening by changing the carbohydrate metabolism and controlling the sugar levels (Bai R.K et al.1988). Similar results were observed in our study as there was a less decrease in the total sugar content because of delayed ripening process. Based on these observations, it was concluded that chitosan can be considered as a commercial application to improve the shelf-life and maintain the quality attributes in case of guava and strawberry during the storage at a temperature of $25\pm 5^{\circ}\text{C}$ which is generally considered as the room temperature by delaying the process of ripening and increasing anti-oxidant activity, main factors which contribute to maintain the quality of fruits.

ACKNOWLEDGEMENT

We would like to express my sincere thanks to our Head of the Department, Dr. A. Sai padma, who gave me an opportunity to undertake and complete this project in the Biochemistry Lab. We are grateful for her encouragement, understanding and support in the development process. We respect and thank, Prof. Y. Ashok, Principal for providing me an opportunity to do the project work in Biochemistry lab and giving us all support and guidance, which made me completely the project duly. We are extremely thankful to him for providing such a nice support and guidance. we also thank Mr. Kishan babu, and Ms. Vijaya lakshmi, Lab assistant, Department of Biochemistry for their immense help in providing me with the necessary requirements during my project work

REFERENCES

- Aider, M. (2010) Chitosan application for active bio-based films production and potential in the food industry: review. *LTW-Food Science and Technology* 43(6):837-842.
- Ali, A., Muhammad, M. T. M., Sijam, K., & Siddiqui, Y (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. *Food chemistry* 124(2):620-626.

- Artan M., Karadeniz F., Karagozlu M.Z., Kim M.M., Kim S.K.(2010). *Anti-HIV-1 activity of low molecular weight sulfated chitooligosaccharides*. Carbohydrate Research 345(5):656-662.
- Atia M.M.M., Buchenauer H., Aly A.Z., Abou-Zaid M.I.(2005). *Antifungal activity of chitosan against *Phytophthora infestans* and activation of defence mechanisms in tomato to late blight*. Biological Agriculture and horticulture 23(2):175-197.
- Bai R.K., Huang M.Y., Jiang Y.Y.(1988). *Selective permeabilities of chitosan-acetic acid complex membrane and chitosan-polymer complex membranes for oxygen and carbon dioxide*. Polymer Bulletin 20:83-88.
- Ben-Shalom N., Ardi R., Pinto R., Aki C., Fallik E (2003). *Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan*. Crop protection 22(2):285-290.
- Bridle P, Garcia-Viguera C (1997). *Analysis of anthocyanins in strawberries and elderberries. A comparison of capillary zone electrophoresis and HPCL*. Food Chemistry 59(2):299-304.
- Campaniello, D., Bevilacqua, A., Sinigaglia, M., & Corbo, M (2008). *Chitosan: Antimicrobial activity and potential applications for preserving minimally processed strawberries*. Food Microbiology 25(8):992-1000.
- Chameettachal S., Murab S., Vaid R., Midha S., Ghosh S (2015). *Effect of visco-elastic silk-chitosan microcomposite scaffolds on matrix deposition and biomechanical functionality for cartilage tissue engineering*. Journal of Tissue engineering and Regenerative Medicine 11(4):1212-1229.
- Cheng, Y., Zhu, P., Yang, J., Liu, X., Dong, S., Wang, X., Chun, B., Zhuang, J., & Zhang, C (2010). *Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4*. Cardiovascular research 87(3):431-439.
- Clifford MN (2000). *Anthocyanins, nature, occurrence and dietary burden*. Journal of the Science of Food and Agriculture 80(7):1063-1072.
- Cosme Silva, G. M., Silva, W. B., Medeiros, D. B., Salvador, A. R., Cordeiro, M. H. M., da Silva, N. M., Santana, D. B., & Mizobutsi, G. P. (2017). *The chitosan affects severely the carbon metabolism in mango (*Mangifera indica* L. cv. Palmer) fruit during storage*. Food chemistry 237:372-378.

- Giovannoni, J. J., Nguyen, C., Ampofo, B., Zhong, S., & Fei, Z (2017). Epigenome and Transcriptional dynamics of fruit ripening. *Annual Review of Plant Biology* 68(1):61-84.
- Heber D 2003. Herbal preparations for obesity: Are they useful? *Primary Care: clinics in office practice* 30(2):441-63.
- Heinonen, I. M., Meyer, A. S., & Frankel, E. N (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry* 46(10):4107-4112.
- Hong, K., Xie, J., Zhang, L., Sun, D., & Gong, D (2012). Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. *Scientia horticulturae* 144:172-178.
- Jongsri, P., Wangsomboondee, T., Rojsitthisak, P., & Seraypheap, K (2016). Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit. *LWT-Food Science and Technology* 73:28-26.
- Karagozlu M.Z., Kim S.-K (2014). Chapter Twelve—Anticancer effects of chitin and chitosan derivatives. In: Kim S.K., editor. *Advances in Food and Nutrition Research* 72:215-225.
- Li B., Wang L., Xu F., Gang X., Demirci U., Wei D., Li Y., Feng Y., Jia D., Zhou Y (2015). Hydrosoluble, UV-crosslinkable and injectable chitosan for patterned cell-laden microgel and rapid transdermal curing hydrogel *in vivo*. *Acta Biomaterialia* 22:59-69.
- Lopes-da-Silva F, de Pascual-Teresa S, Rivas-Gonzalo JC, Santuos-Buelga C (2002). Identification of anthocyanin pigments in strawberry (cv. Camarosa) by LC using DAD and ESI-MS detection. *European food research and technology* 214:248-253.
- Martins A.F., Facchi S.P., Follmann H.D., Pereira A.G., Rubira A.F., Muniz E.C. (2014). Antimicrobial activity of chitosan derivatives containing *N*-quaternized moieties in its backbone: a review. *International Journal of Molecular sciences* 15(11):20800-20832.
- Ngo D.H., Kim S.K.(2014). Chapter Two—Antioxidant effects of chitin, chitosan, and their derivatives. In: Kim S.K., editor. *Advances in Food and Nutrition Research* 73:15-31.
- Park S.C., Nam J.P., Kim J.H., Kim Y.M., Nah J.W., Jang M.K.(2015). Antimicrobial action of water-soluble beta-chitosan against clinical multi-drug resistant bacteria. *International Journal of Molecular sciences* 16(4):7995-8007.

- Pereira P., Pedrosa S.S., Wymant J.M., Sayers E., Correia A., Vilanova M., Jones A.T., Gama F.M. (2015). siRNA inhibition of endocytic pathways to characterize the cellular uptake mechanisms of folate-functionalized glycol chitosan nanogels. *Molecular Pharmaceutics* 12(6):1970-1979.
- Prabhu S.M., Meenakshi S (2015). A dendrimer-like hyper branched chitosan beads toward fluoride adsorption from water. *International journal of biological macromolecules* 78:280-286.
- Saharan V., Sharma G., Yadav M., Choudhary M.K., Sharma S.S., Pal A., Raliya R., Biswas P (2015). Synthesis and *in vitro* antifungal efficacy of Cu-chitosan nanoparticles against pathogenic fungi of tomato. *International journal of biological macromolecules* 75:346-353.
- Santos-Buelga C, Scalbert A (2000). Proanthocyanidins and tannin like compounds: nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture* 80(7):1094-1117.
- Sayed S., Jardine A (2015). Chitosan derivatives as important biorefinery intermediates. Quaternary tetraalkylammonium chitosan derivatives utilized in anion exchange chromatography for perchlorate removal. *International Journal of Molecular sciences* 16(5):9064-9077.
- Shull, S., Heintz, N. H., Periasamy, M., Manohar, M., Janssen, Y. M., Marsh, J. P (1991). Differential regulation of antioxidant enzymes in response to oxidants. *The Journal of Biological Chemistry* 266(36):24398-24403.
- Sudarshan N.R., Hoover D.G., Knorr D (1992). Antibacterial action of chitosan. *Food Biotechnology* 6(3):257-272.
- Tayel A.A., Moussa S.H., Salem M.F., Mazrou K.E., El-Tras W.F.(2015). Control of citrus molds using bioactive coatings incorporated with fungal chitosan/plant extracts composite. *Journal of the Science of Food and Agriculture* 96(4):1306-1312.
- Tokoro A., Tatewaki N., Suzuki K., Mikami T., Suzuki S., Suzuki M (1988). Growth-inhibitory effect of hexa-*N*-acetylchitohexaose and chitohexaose against Meth-A solid tumor. *Chemical and Pharmaceutical Bulletin* 36(2):784-790.
- Ueno H., Mori T., Fujinaga T (2001). Topical formulations and wound healing applications of chitosan. *Advanced Drug Delivery Reviews* 52(2):105-115.
- Xisto, A., Abreu, C. M. P. d., Corrêa, A. D., & Santos, C. D. d (2004). Texture of Pedro Sato guavas submitted to the application of calcium chloride. *Ciencia e Agrotechnologia* 21(8):113-118.

Younes I., Rinaudo M (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs* 13(3):1133-1174.

Zhang, D., & Quantick, P. C (1998). Antifungal effects of chitosan coating on fresh strawberries and raspberries during storage. *The Journal of Horticultural Science and Biotechnology* 73(6):763-767.

Zheng L.Y., Zhu J.F(2003). Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate Polymers* 54(4):527-530.

Received: 22th May 2021; Accepted: 05th November 2021; First distribution: 03th April 2022.