

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Effect of Chitosan Coating on Physical and Microbial Characteristics of Fresh-Cut Green Peppers (*Capsicum annuum* L.)

L.V. Raymond, M. Zhang and S.M. Roknul Azam
State Key Laboratory of Food Science and Technology,
School of Food Science and Technology, Jiangnan University, 214036 Wuxi, China

Abstract: Edible coatings, thin layer formed on the surface of a product, have been used to preserve fresh-cut fruits and vegetables since they can improve quality by affecting respiration and moisture loss. Green pepper slices were subjected to chitosan coating treatment achieved by dipping, afterwards stored at 5°C for a period of 15 days. The effect of various chitosan concentrations (0%, 0.5%, 1.0% and 2% (w/v) chitosan) on some physico-chemical and microbial characteristics of the slices was subsequently analyzed during storage. The data indicated that the performance of chitosan treatments was better than that of control. The decrease of surface lightness was delayed by chitosan treatments; and the surface green colour was kept under marginal changes compared to control. On the other hand, a greater reduction of fungal incidence, carbon dioxide concentration and electrolyte leakage of the chitosan treated samples was observed with increased chitosan concentration treatment. Microbiological evaluation revealed that total viable cells count decreased with increasing chitosan concentration. This correlated well with the external changes which were affected to a lesser extent in the 2% chitosan compared to the control; in addition, delaying of changes was significantly chitosan concentration dependent.

Key words: Green peppers, chitosan coating, fresh-cut fruits and vegetables, physical and microbial properties

INTRODUCTION

Fresh fruits and vegetables, once harvested and/or processed, quickly deteriorate because of physiological ageing, biochemical changes and microbiological spoilage. Those deterioration are dependent of the changes in their components (proteins, lipids, carbohydrates, water) owing to environmental and/or processing factors (Cha and Chinnan, 2004). For peeled, cut and sliced fresh fruits and vegetables, such deterioration is accelerated because of the wounding and lesions of the tissues that occur during the operations (Tovar *et al.*, 2001). Microbial growth and enzyme activity causing overall quality loss are enhanced by the release of cellular substrates. Peppers (*Capsicum annuum* L.) are well known for their edible colourful juicy and crispy flesh of the pungent or sweet variety, as well as their nutritious content (Raffo *et al.*, 2007). Fresh peppers are often eaten raw and supplied pre-cut to manufacturers as ready-to-use ingredients. However, the main problems limiting their shelf life occur by shriveling, decay development on the cut surface, as well as degreening of the vegetable among different degraded quality characteristics (Sakaldas and Kaynas, 2010). Those problems are correlated to an undesirable loss of water during metabolism or diffusion through the skin and respiration.

To minimize post cutting loss, reduction of the storage temperature combined or not with other techniques is often achieved. Appropriate pre treatment and storage conditions can prolong the shelf life. One of the major advance reached to increase the shelf life of minimally processed products include edible coatings (Rojas-Grau *et al.*, 2009). Edible coatings, defined as a thin layer of material, can be applied on fresh and minimally processed fruits and vegetables and have been reported on their capacity to form a wrapping film acting as a protective barrier. This allows a control of biochemical changes in the plant metabolism by preventing water loss or creating a modified atmosphere surrounding the product. The purpose of application of edible coatings is to inhibit migration of moisture or gas as well as serving as a food additives carrier like antioxidants or antimicrobials and reduce the decay without affecting quality of the food. Chitosan is a modified natural carbohydrate polymer [β - (1,4) - glucosamine] derived from chitin and has numerous uses. Its film forming capacity (Shahidi *et al.*, 1999), gas barrier ability and antimicrobial activity against various microorganisms (Muzzarelli and Muzzarelli, 2003) justify its use for production of edible coating to prolong the shelf life and maintain the quality attributes. Chitosan may be used as a surface coating on fresh fruits and

vegetables to modify the internal atmosphere, to decrease the transpiration loss and to delay the ripening. Chitosan coating has been successfully applied to preserve cut product such as cut Chinese water chestnut (Pen and Jiang, 2003), cut "Fuji" apples (Qi *et al.*, 2011), or minimally processed broccoli (Ansorena *et al.*, 2011).

Studies have been reported on the use of chitosan coating on several fresh products. To our investigation, cut peppers edges dehydrated, turned brown and overall quality degraded within 5 days without any treatment at cool conditions (unpublished data). Therefore, in this study, dipping in chitosan solution was assayed to improve quality of cut green peppers storability during cold storage; investigation and assessment of some physical attributes as quality indicators included the determination of overall acceptability, fungal tolerance, colour changes, respiration rate, cell membrane integrity and microbial loading.

MATERIALS AND METHODS

Plant material and sample preparation: Green peppers (*Capsicum annuum* L.) obtained from a local farm (Wuxi, Jiangsu, China) were used in the present study. The fruits were sanitized with chlorinated water (20 mL/L) and rinsed with tap water. Similarity of edible flesh green colour was evaluated with a colorimeter Konica Minolta Chroma Meter CR-400 Series Version 1.11 (Konica Minolta, Tokyo, Japan). The edible portions of the fruits were thus manually sliced into longitudinal pieces along the longitudinal axis with a stainless steel knife. Subsequent washing followed-on and cellular fluids were drained with tissue paper. Peppers slices (94.1% Fresh Weight (FW) moisture content, 1.0 g citric acid/L titratable acidity, 4.5°Brix and 139.61 mg/100 g FW vitamin C) were divided in random into different group for chitosan treatments.

Treatments and storage condition: The cut green peppers were dipped for 5 min into a solution of 0% (control), 0.5%, 1% or 2% (w/v) chitosan (Chitosan, 80-95% deacetylation degree, medium molecular weight). The coating solution was prepared by dispersing 0, 5, 10 and 20 g of chitosan powder into 1L of distilled water containing 1% (v/v) glacial acetic acid (Kyu Kyu Win *et al.*, 2007) and final pH of the solution adjusted to pH 5.0 with 0.1 M NaOH. Coating solution of 0%, 0.5%, 1% and 2% chitosan were respectively prepared and coded as Control, CS 1, CS 2 and CS 3. After being air dried for 2 hrs at Room Temperature (RT), samples (150 g) were placed in glass container, tightly closed and stored at 5°C, 85-90% relative humidity to be later assessed for further analyses intended for 15 days.

Analytical procedures: Pepper slices quality was visually assessed by the extent of external changes

during the storage period at 5°C. On day 0, 3, 5, 7, 10 and 15, changes were evaluated on a 1-5 scale according to the percentage of surface area decayed and appearance change, where 1=extreme (>50%), 2=moderately severe (20-50%), 3=moderate (5-20%), 4=slight (up to 5%) and 5=none. Results were expressed as an overall quality index over time.

Before and during storage, samples were subjected to inspection of visible sign of fungal infection. These observations were made each day for 15 days. Samples were considered infected when the first visible lesion or infection was detected.

The respiration rate was estimated by the CO₂ production analyzed with a Gas Analyzer Cyes-II (Jiading Federation Instrument, Shanghai, China). The changes of atmosphere in the container containing ambient air as the initial atmosphere was measured by means of the CO₂ released by the samples. On day 0, 3, 6, 10 and 15, gas samples, withdrew with a 20 ml syringe, were injected into the instrument and CO₂ concentration was read. The changes of CO₂ concentration was calculated considering the initial concentration.

Colour changes of the skin surface were measured with a hand colorimeter Konica Minolta Chroma Meter CR-400 Series Version 1.11 (Konica Minolta, Tokyo, Japan). The instrument was first calibrated with a white ceramic plate. Measurement was performed on day 0, 5 and 10 on samples placed in a single layer on a tray, skin side up. The colour coordinates L* (-100 = dark to +100 = light), a* (-a* = green to +a* = red), b* (-b* = blue to +b* = yellow) were recorded.

The membrane permeability was evaluated by the leak of electrolyte. Five grams of diced peppers (0.8 x 0.8 x 0.4 cm) were put at 30°C and in boiling water (Zhang *et al.*, 2008). Electrical conductivity was estimated with a conductivity meter (DDS-11A, Shanghai Huaguang Co., Shanghai, China). The membrane permeability was characterized by the electrical conductivity ratio of the pre-heated to post-heated samples; measurements were done in triplicate on day 0, 3, 6 and 10.

Initially after washing (day 0) and during the storage period, total viable cell was enumerated. Ten grams of sample in 1:10 of sterile 0.1% (w/v) peptone water were subsequently 10-fold diluted. The total count was assessed using the pour plate method and prepared plate count agar as medium after incubation at 35°C for 48 hrs. Samples were analyzed on day 0, 3, 5, 10, 15 and microbial counts were expressed as log CFU.g⁻¹.

Analyses were preformed in triplicate. Changes in quality indicators as influenced by the chitosan concentration were analyzed by Analysis of Variance (ANOVA) using the SPSS 16 statistical software (SPSS, Chicago, Illinois, USA). Mean separations were conducted using the Duncan's multiple range test, with a level of significance of p≤0.05.

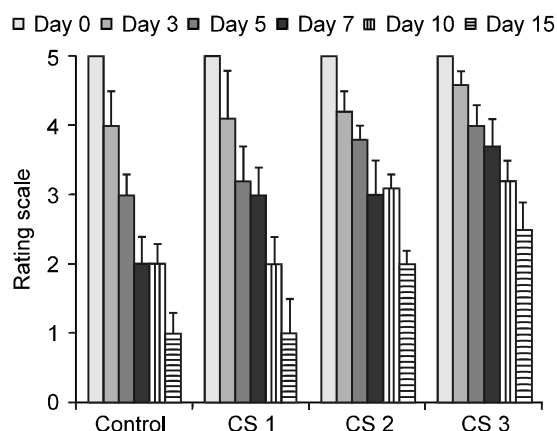


Fig. 1: Changes of overall visual quality of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

RESULTS AND DISCUSSION

The overall external appearance significantly ($p \leq 0.05$) changed with increasing storage period but the chitosan coating delayed the quality drop. The quality changes were more gradual in the samples coated with 2% chitosan ($p \leq 0.05$) compared to other samples; besides, a clear decline with decreasing chitosan concentrations was observed which was more accentuated in uncoated samples (Fig. 1). For the first 3 days, no significant changes occurred in the chitosan-coated samples at different concentration (0.5%, 1% and 2%, respectively CS 1, CS 2 and CS 3). Significant difference ($p \leq 0.05$) came up between the coating treatments after the 5th day when at 0.5% chitosan, samples presented cut edges browning. Up to 50% of damaged control fruits was reached within a week, whereas this percentage occurred on day 15 in the 2% coated fruits. In this investigation, browning of the cut surfaces of control and 0.5% chitosan coated samples limited the shelf life in term of visual quality. These browning can results from dehydration or microbial infection or action of PPO enzymes that are freed by the cutting process and oxidize phenols into quinines in the presence of molecular oxygen. Enzyme spreading and reaction was

less important when coating form a barrier to oxygen, limiting and reducing enzymatic reaction. Similarly, chitosan on minimally processed water caltrop fruit diminish browning and extended their shelf life (Zhan *et al.*, 2011). Better quality and extended shelf life of sliced mango or peeled litchi fruit were also reported to be owed to the chitosan coating (Dong *et al.*, 2004; Chien *et al.*, 2007) forming film protecting the fruit surface. Likewise, in this work, the increasing and sufficient amount of 2% chitosan on cut peppers improved its quality and prevented decay development.

Green pepper is sensitive to fungal infection and microbial attack, especially for the post cut fruit. The time elapsed between the application of the coating and the apparition of a visible decay varies in function of chitosan concentration (Table 1). In evaluating decay incidence of all samples, it was found that coated samples at 1% and 2% chitosan showed a significant ($p \leq 0.05$) delay of fungal infection. The samples treated at 0.5% had a similar fungal incidence to that of control (5-6 days). On the other hand, the gray mold and white microbial spots were hardly detected in the 2% chitosan coated samples for an average of 13 days. Fungal decay contributes to decline the visual quality score that limits the shelf life. It is known that microorganisms have negatively charged cell, so by means of the positively charged amino group, chitosan can interact with the negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Dutta *et al.*, 2009). The present results indicate that the protection of the surfaces to contamination can be achieved by the coating to delay contamination.

Freshness of fruits and vegetables can be preserved by reducing respiration rate in order to avoid rapid quality deterioration. The effect of chitosan coating on the respiration behaviour of cut peppers stored at 5°C for 15 day was shown in Fig. 2. In general, CO₂ concentration increased ($p \leq 0.05$) in all samples, nevertheless respiration of coated samples was reduced compared to control. In the beginning, the chitosan coating treatment had a stimulatory effect on the respiration rate, but thereafter maintained the respiration rate under lower concentration than that of control which increased

Table 1: Apparition of fungal decay (days) and microbial count (log CFU/g) of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

	Control	CS 1	CS 2	CS 3
Fungal incidence				
First day of visible infection	5.660±0.570	6.330±0.570	9.330±0.570	13.330±0.570
Microbial count (log CFU/g)				
Day 0	0.312±0.059 ^a	0.312±0.059 ^a	0.312±0.059 ^a	0.312±0.059 ^a
Day 3	0.380±0.012 ^{ab}	0.372±0.033 ^{ab}	0.316±0.004 ^a	0.318±0.003 ^a
Day 5	0.452±0.024 ^b	0.410±0.010 ^b	0.413±0.004 ^b	0.402±0.000 ^b
Day 10	0.665±0.022 ^c	0.579±0.004 ^c	0.541±0.048 ^c	0.505±0.007 ^c
Day 15	1.115±0.077 ^d	0.842±0.040 ^d	0.681±0.015 ^d	0.609±0.009 ^d

Values = means ± standard deviations (n = 3), Values with different superscript letters in the same column are significantly different ($p \leq 0.05$)

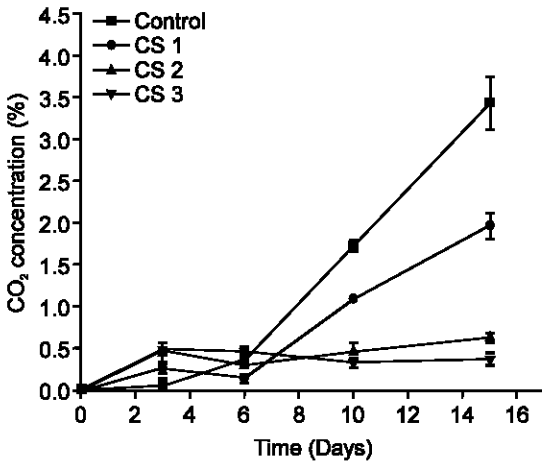


Fig. 2: Changes of carbon dioxide (CO₂) concentration of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

towards the end of storage time. Accumulation of CO₂ in control can be related to the fungal decay and flesh damage that increase without the additional layer of coating for protection. Among the coated samples, those treated with 2% chitosan showed the lowest respiration rate after the 6th day and CO₂ concentration was 5 times reduced at the end of storage (0.38% of CO₂) compared to those treated with 0.5% chitosan (1.96% of CO₂). It was observed that 1% and 2% chitosan treatment clearly reduced the respiration rate after the 6th day of storage ($p \leq 0.05$). A semipermeable film on the fruit surface can be formed by the chitosan, consequently modifying the internal atmosphere of the fruit. With limited gas exchange due to the coating barrier, enzymatic activity and metabolism involving respiration can be thus affected, thereby resulting in lower changes of the coated slices.

Colour differences measured by lightness L* and green colour a* between control and chitosan coated cut peppers were determined during storage and are presented in Fig. 3. Chitosan coated cut peppers at 1 and 2% presented higher L* value during storage, probably due to the added glossiness of the coating. On the other hand, the surface lightness of the control and 0.5% chitosan coated samples clearly reduced after being stored for 5 days. The surface lightness of the fruits treated with 2% chitosan was significantly different ($p \leq 0.05$) from that of control and other coated samples after 10 days; additionally the highest L* value of the 2% chitosan coated samples indicate the lowest colour changes. Coatings are used as semi permeable barrier to delay colour loss and browning by enzymatic reaction or by dehydration that are part of the response to the cutting process (Siomos *et al.*, 2010).

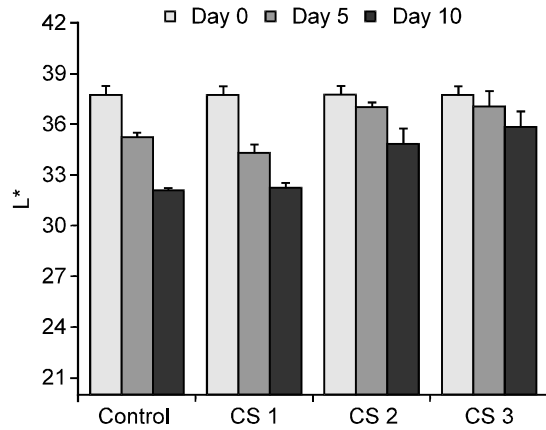


Fig. 3: Changes of surface lightness L* of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

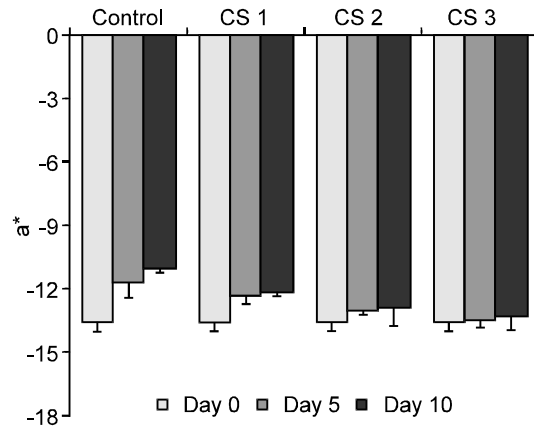


Fig. 4: Changes of surface green colour of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

During the storage period also, the skin surface green colour (a*) of the cut peppers decreased. When in the control batch the difference was spotted at the 5th day, there was no significant changes of the a* value of the coated cut peppers for 10 days (Fig. 4). The decrease in a* value associated with chitosan coating treatment was lower than that of the control, but did not significantly change after the different concentration (0.5, 1.0 and 2% chitosan). Less colour change can be attributed to the modification of the internal atmosphere in the fruit, when higher level of CO₂ and lower level of O₂ can delay metabolism process. In previous study, it was found that fresh cut papaya treated with chitosan were able to maintain the highest colour values during the storage time (Gonzalez-Aguilar *et al.*, 2009).

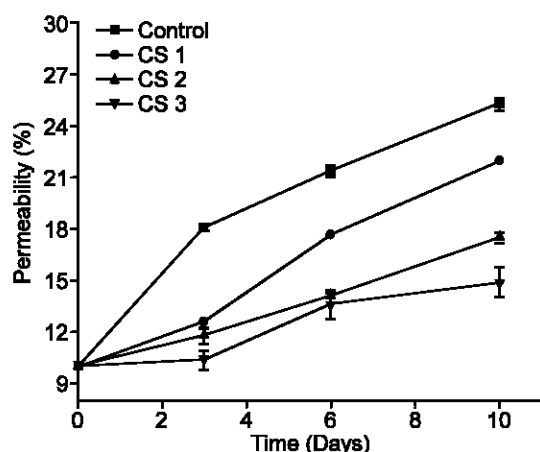


Fig. 5: Changes of cell membrane permeability of fresh cut green peppers during storage treated with different concentration of chitosan coating (Control: 0%, CS 1: 0.5%, CS 2: 1%, CS 3: 2%)

As shown in Fig. 5, the relative conductivity of all the samples increased during storage of cut peppers. The membrane properties accordingly changed during the storage period and turned out to be less protected to leakage with increasing time. The increased electrolyte leakage was statistically significant ($p \leq 0.05$) in all the samples throughout storage, however, the coating treatment diminishes the leakage rate and revealed different degree of reduction. By the 3rd day only, the relative conductivity of the control was significantly higher ($p \leq 0.05$) than those of the coated samples: an increase of 8.1% was noted compared to 0.4% in the 2% chitosan coated samples. The deformation of the fruit tissue was less affected when samples were exposed to the coating treatment, thereby delaying electrolyte leakage. Additionally, among all samples, the 2% chitosan prevented the most the electrolyte leakage during storage. After 10 days of storage at 5°C, the relative conductivities of the coated samples were 14.9%, 17.5% and 22%, respectively for the 2, 1 and 0.5% coating compared to 25.3% of the control. From this, it is clear that the coating influenced membrane integrity and delayed electrolyte leakage than that of the control. Enhanced antimicrobial activity in fruits and vegetables by means of treatments or storage conditions would reduce the microbial incidence to improve the overall storage life. Table 1 shows the evolution of total microbial load during storage of coated and uncoated cut peppers. In the present study, an increase of the microbial load was noted; however coated samples exhibited the lowest total viable cells count along the storage ($p \leq 0.05$). Moreover, increasing reduction effect was observed in more concentrated coating treatment. In comparing the data, it was revealed that after 15 days of storage, the highest load was 1.115 log CFU/g in Control and the lowest was 0.609 log CFU/g in 2% chitosan coated samples. Limited oxygen for

microorganism growth due to the coating barrier probably inhibited the microorganism development or penetration from surrounding environment. Higher count in control can be due to the access of microorganisms to the freed cellular content after cutting, easing their proliferation. Results reported in previous studies showed that chitosan delayed the accumulation of viable cells in minimally processed garlic (Geraldine *et al.*, 2008), as well as the effectiveness of antimicrobial activity of chitosan coating was demonstrated on coated broccoli thereby presented improved quality (Moreira *et al.*, 2011).

Conclusion: Chitosan applied as coating on cut green peppers provided favourable effect during storage at 5°C. The chitosan treatments reduced respiration rate by reduced CO₂ accumulation culminating in improved external appearance. By means of the chitosan treatments also, fungal incidence, surface green colour, electrolyte leakage and microbial count were lower than that of control. On the other hand, it was realized that chitosan concentration greatly influenced the outcome of the sample quality retention. Thereby, higher chitosan coating concentration (2%) exhibited a beneficial impact on the quality of fresh cut green peppers kept at 5°C, where that concentration (2%) proved to be effective in improving visual quality.

REFERENCES

- Ansorena, M.R., N.E. Marcovich and S.I. Roura, 2011. Impact of edible coatings and mild heat shocks on quality of minimally processed broccoli (*Brassica oleracea* L.) during refrigerated storage. *Postharvest Biol. Technol.*, 59: 53-63.
- Cha, D.S. and M.S. Chinnan, 2004. Biopolymer-based antimicrobial packaging: A review. *Food Sci. Nutr.*, 44: 223-237.
- Chien, P.J., F. Sheu and F.-H. Yang, 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *J. Food Eng.*, 78: 225-229.
- Dong, H., L. Cheng, J. Tan, K. Zheng and Y. Jiang, 2004. Effect of chitosan coating on quality and shelf life of peeled litchi fruit. *J. Food Eng.*, 64: 355-358.
- Dutta, P.K., S. Tripathi, G.K. Mehrotra and J. Dutta, 2009. Perspectives for chitosan based antimicrobial films in food applications. *Food Chem.*, 114: 1173-1182.
- Geraldine, R.M., N.F. Ferreira Soares, D.A. Botrel and L.A. Goncalves, 2008. Characterization and effect of edible coatings on minimally processed garlic quality. *Carbohydrate Polymers*, 72: 403-409.
- Gonzalez-Aguilar, G.A., E. Valenzuela-Soto, J. Lizardi-Mendoza, F. Goycoolea, M.A. Martinez-Téllez, M.A. Villegas-Ochoa, I.N. Monroy-García and J.F. Ayala-Zavala, 2009. Effect of chitosan coating in preventing deterioration and preserving the quality of fresh-cut papaya maradol. *J. Sci. Food Agric.*, 89: 15-23.

- Kyu Kyu Win, N., P. Jitareerat, S. Kanlayanarata and S. Sangchote, 2007. Effects of cinnamon extract, chitosan coating, hot water treatment and their combinations on crown rot disease and quality of banana fruit. *Postharvest Biol. Technol.*, 45: 333-340.
- Moreira, M.d.R., S.I. Roura and A. Ponce, 2011. Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. *LWT - Food Sci. Technol.*, 44: 2335-2341.
- Muzzarelli, C. and R.A.A. Muzzarelli, 2003. Chitin related food science today and two centuries ago. *Agro-Food Industry Hi-Tech*, 14: 39-42.
- Pen, L.T. and Y.M. Jiang, 2003. Effects of chitosan on shelf life and quality of fresh-cut chinese water chestnut. *Lebensmitte Wissenschaft und Technologie*, 36: 359-364.
- Qi, H., W. Hu, A. Jiang, M. Tian and Y. Li, 2011. Extending shelf-life of fresh-cut fuji apples with chitosan-coatings. *Innovative Food Sci. Emerging Technol.*, 12: 62-66.
- Raffo, A., I. Baiamonte, N. Nardo and F. Paoletti, 2007. Internal quality and antioxidants content of cold-stored red sweet peppers as affected by polyethylene bag packaging and hot water treatment. *Eur. Food Res. Technol.*, 225: 395-405.
- Rojas-Grau, M.A., G. Oms-Oliu, R. Soliva-Fortuny and O. Martín-Belloso, 2009. The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: A review. *Int. J. Food Sci. Technol.*, 44: 875-889.
- Sakaldas, M. and K. Kaynas, 2010. Biochemical and quality parameters changes of green sweet bell peppers as affected by different postharvest treatments. *Afr. J. Biotechnol.*, 9: 8174-8181.
- Shahidi, F., J.K.V. Arachchi and Y. Jeon, 1999. Food applications of chitin and chitosan. *Trends Food Sci. Technol.*, 10: 37-51.
- Siomos, A.S., D. Gerasopoulos, P. Tsouvaltzis and A. Koukounaras, 2010. Effects of heat treatment on atmospheric composition and color of peeled white asparagus in modified atmosphere packaging. *Innovative Food Sci. Emerging Technol.*, 11: 118-122.
- Tovar, B., H.S. Garcia and M. Mata, 2001. Physiology of pre-cut mango. II. Evolution of organic acids. *Food Res. Int.*, 34: 705-714.
- Zhan, L., J. Hu and Z. Zhu, 2011. Shelf life extension of minimally processed water caltrop (*trapa acornis nakano*) fruits coated with chitosan. *Int. J. Food Sci. Technol.*, 46: 2634-2640.
- Zhang, M., Z.G. Zhan, S.J. Wang and J.M. Tang, 2008. Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT - Food Sci. Technol.*, 41: 686-691.