

Effect of Chitosan Coating on Chilling Injury, Antioxidant Status and Postharvest Quality of Japanese Cucumber during Cold Storage

(Kesan Perlakuan Bahan Salut Kitosan ke atas Kecederaan Sejuk, Status Antioksidan dan Kualiti Lepas Tuai Timun Jepun semasa Penyimpanan Suhu Rendah)

NUR FATIN AFIFAH HASHIM, AZHANE AHMAD* & PAA KWESI BORDOH

ABSTRACT

Japanese cucumber (Cucumis sativus L.) could easily develop chilling injury when held at 7°C or below, thus limiting its storability and reduces consumer preference. Chitosan coating is known to be one of the methods used for preserving perishable fresh produce. This work was extended out to look into the efficacy of low molecular weight (LMW) chitosan coatings on chilling injury (CI), antioxidant levels and shelf life quality of Japanese cucumber. Fruit were coated with 0.5, 1.0 and 1.5% chitosan prior to cold storage at 7°C and 90-95% relative humidity (RH) for 12 days. The result showed that fruit coated with lowest concentration of chitosan (0.5%) was the most effective in alleviating chilling injury symptoms and reduced the increase of lipid peroxidation (MDA content) compared to higher concentrations (1.0 and 1.5%). Furthermore, when Japanese cucumbers were coated with 0.5% chitosan, it was able to maintain the postharvest quality and storability with higher firmness and delayed increase of weight loss. On the other hand, cucumber coated with 1.5% chitosan demonstrated high level of ascorbate peroxidase (APX) and catalase (CAT) activities than in 0.5 and 1.0% chitosan. This finding suggests a role for chitosan coating in alleviating oxidative stress that would lead to CI problems during cold storage.

Keywords: Antioxidant enzyme activity; chilling tolerance; chitosan; postharvest quality

ABSTRAK

Fenomena kecederaan sejuk (KS) seringkali berlaku ke atas timun Jepun (Cucumis sativus L.) yang disimpan pada suhu 7°C atau lebih rendah, sekaligus mengahadkan hayat simpanan di samping mempengaruhi kualiti buah untuk diterima oleh pengguna. Bahan salut kitosan dikenali sebagai salah satu kaedah lepas tuai yang digunakan untuk mengekalkan kualiti buah daripada mudah rosak. Kajian ini dijalankan untuk mengkaji keberkesanan bahan salut kitosan yang berberat molekul rendah ke atas kecederaan sejuk (KS), aktiviti antioksidan dan kualiti hayat simpanan timun Jepun. Buah diberikan perlakuan bahan salut kitosan dengan kepekatan 0.5, 1.0 dan 1.5% dan disimpan pada suhu rendah 7°C dengan kelembapan relatif 90-95% selama 12 hari. Keputusan kajian menunjukkan buah yang disalut dengan kepekatan kitosan paling rendah (0.5%) berupaya mengaleviasi simptom KS dan menindas aras peningkatan peroksidaan lipid (kandungan MDA) secara signifikan berbanding kitosan pada kepekatan lebih tinggi (1.0 dan 1.5%). Perlakuan 0.5% kitosan juga berupaya memelihara kualiti timun Jepun, memanjangkan hayat simpanan dengan memperlahankan kemerosotan ketegaran dan kehilangan berat. Sebaliknya, buah yang disalut kitosan pada kepekatan 1.5% menunjukkan aktiviti enzim katalase (CAT) dan askorbat peroksidase (APX) yang lebih tinggi berbanding buah yang disalut dengan 0.5 dan 1.0% kitosan. Keputusan kajian ini mencadangkan perlakuan bahan salut kitosan berupaya mengurangkan kepayahan oksidatif yang dipercayai menyebabkan masalah KS semasa penyimpanan suhu rendah.

Kata kunci: Aktiviti antioksidan enzimatik; kitosan; kualiti lepas tuai; toleransi kecederaan sejuk

INTRODUCTION

Japanese cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae family. Cucumber is a highly perishable and the crop quality can be greatly scaled down mainly due to moisture loss, skin shrinking and degreening that limit their postharvest lifespan (Al-Juhaimi et al. 2012). Storage at low temperature is used to arrest ripening related disorders and to prolong postharvest shelf life. However, Japanese cucumber easily manifested chilling injury (CI) symptoms when stored at cold storage that causes an early

deterioration of quality (Chen & Yang 2012). The most common CI symptoms observed are dark watery patches, surface skin pitting and pathogen vulnerability which leads to fruit deterioration (Qian et al. 2012; Yang et al. 2011).

Chilling damaging effect at low temperature may be regarded as an oxidative phenomenon related to an excess accumulation of reactive oxygen species (ROS) (Chen & Yang 2012). Apart from chilling oxidative damage, ROS owned a high affinity to interact with DNA molecules, proteins, lipids and membranes and resulting

in lipid peroxidation and subsequently plant cells death (Nukuntornprakit et al. 2015). Accordingly, significant level of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX) has been closely associated with defence mechanism toward chilling temperature and prevention of CI incidents (Zhang et al. 2009).

At present, consumers demand natural, safe and environmentally friendly alternative. Hence, it has become an increasing interest to look for the usage of natural essence such as polysaccharides chitosan as bioactive postharvest packaging materials to improve quality attributes and prolong shelf life of fresh commodities (Hong et al. 2012). Chitosan is an ubiquitous amino biopolymer derived from derivatives deacetylation of chitin, naturally most abundant mucopolysaccharides and supporting materials of crustaceans, insects and fungal cell walls (Badawy & Rabea 2009). This natural, biodegradable compound acts as an antimicrobial, antifungal and defense-related responses elicitor in plant tissue as well as promises an alternative approach without toxicological impacts on human health (Pushkala et al. 2013; Romanazzi et al. 2013).

Potentials of chitosan as an edible coating film has been proven to provide selective semi-permeable layer that can modify the gaseous exchange, reduce transpiration rate and delay ripening (Maqbool et al. 2011). Reduction in moisture loss and respiration rate can lead to the quality maintenance and a slower decrease in weight loss (El-Ghaouth et al. 1992). Apart from being coating agent, chitosan unique properties has been exploited in numerous industries such as agriculture (edible films, fungicide and elicitor) (Hoagland & Parris 1996; Lafontaine & Benhamou 1996; Makino & Hirata 1997; Ren et al. 2001), food preparation (coating, antimicrobial, preservative and antioxidant) (Benjakul et al. 2000; Fang et al. 1994; Pennisi 1992; Roller & Covill 1999; Sapers 1992; Shahidi et al. 2002), medicine and pharmacology (fibre, membranes, fabrics, drugs and artificial organs) (Kulpinsky et al. 1997; Liu et al. 2001; Nishimura 1997), biotechnology (emulsifier, chelator and flocculent) (Hirano & Nagao 1989; Uchida et al. 1989) and cosmetology (hair additives, lotions, facial and body care) (Lang & Clausen 1989).

This study aimed to examine the potentials and effect of chitosan as coating material on Japanese cucumber postharvest quality attributes and its ability to preserve the shelf life of fruit during cold storage. In addition, fruits were kept at 7°C in order to elucidate the association of chitosan coating with CI incidence. This paper also examined the effectiveness of chitosan coating in inducing the activity of ascorbate peroxidase (APX) and catalase (CAT) as well as to monitor the level of lipid peroxidation in the pulp tissues of Japanese cucumber.

MATERIALS AND METHODS

PLANT MATERIALS AND CHITOSAN COATING TREATMENT

Japanese cucumbers (*Cucumis sativus* L.) were obtained from a local supplier (Yuan Jin Plantation Enterprise, Cameron Highlands, Pahang, Malaysia) on the same day of

harvest. Uniformed shape and size (~300 g) and free from any diseases and mechanical damage were selected for this study. Next, fruits were rinsed with sodium hypochlorite (0.01%) solution, dipped with distilled water and air-dried at room temperature (22±1°C) before chitosan coating.

Low molecular weight chitosan (LMWC) (Sigma-Aldrich, USA) concentrations were selected based on preliminary screening. 0.5, 1.0 and 1.5% of chitosan solutions were adapted according to Maqbool et al. (2011). Approximately 0.5, 1.0 and 1.5 g of chitosan powder were in added into 100 mL distilled water comprising of 0.5 mL (v/v) glacial acetic acid, respectively. Solutions were dissolved by mild heating and stirred overnight and solutions were adjusted to pH values of 5.6 using 1 M NaOH. An emulsifier of Tween-80 (0.1 mL) was added into solutions.

After drying, Japanese cucumbers were randomly divided into four different groups and followed by dipping into 0.5, 1.0 and 1.5% chitosan (for approximately 2 min and were dried overnight at ambient temperature. Control cucumbers were dipped with acidic solution (pH5.6) of distilled water without chitosan. Following treatment, fruits were then placed in commercial corrugated boxes and kept with 90-95% RH at 7±0.1°C for 12 days for subsequent analysis. All the analysis were carried out at three day intervals and prior for tissue sampling, the whole fruits were cut into small cubes (approximately 1 cm³), freeze-dried with liquid nitrogen and kept at -80°C until further analysis.

WEIGHT LOSS, FIRMNESS AND EVALUATION OF CHILLING INJURY

Weight loss was determined by weighing fruits from each treatment using a digital balance (EK-600H, Japan). The same fruit were used for determining weight loss from day 0 at intervals of 3 days until final day of cold storage. The differences between relative initial and final fruit weight was considered as the total weight loss during the storage interval and expressed as percentage weight loss.

Fruit firmness was analyzed using the Instron texture analyzer (Model 5540, USA) equipped with 8 mm plunger tip. Firmness was evaluated by subjecting fruit to a puncture test at a constant speed of 20 mm min⁻¹ on three points along the surface of the cucumber. The average of these results was expressed in Newtons (N).

Chilling injury (CI) evaluation was carried out by visually observing the appearance of CI incidents on fruit during cold periods. The symptoms was recorded from day 0 at intervals of 3 days until final day of cold storage. The CI results was done according to Ali et al. (2004) in a three-stage scale. 0 = no signs of CI symptoms, 1 = CI symptoms covering <25% of the skin surface, 2 = CI symptoms covering 25-50% of surface and 3 = CI symptoms covering >50% of the surface. CI index was determined by the equation:

$$\text{CI Index} = \frac{\sum[(\text{CI scale}) \times (\text{number of fruit at the CI scale})]}{(\text{Total number of fruit})}$$

LIPID PEROXIDATION (MDA CONTENT)

Lipid peroxidation determination was carried out using the method described by Hodges et al. (1999) by homogenizing 1 g of sample tissues with ethanol: water in a ratio of 80:20 (v:v) in a test tube. The homogenized mixture was centrifuged for 10 min at 12,000 rpm (Eppendorf 5810R, Eppendorf AG, Hamburg, Germany). 1 mL of diluted supernatant was added with 1 mL of +TBA solution which containing 0.65% TBA, 0.01% BHT and 20.0% (w/v) TCA and 1 mL of -TBA solution that comprised of the above solutions without TBA. The reaction mixture was vortexed briefly and the reaction was initiated by placing the mixture in water bath (SWBD, Stuart, UK) at 95°C for 25 min. The tube containing reaction mixture was kept on ice for 10 min and the lipid peroxidation end products (MDA content) was estimated by measuring absorbances at 440, 532 and 600 nm using a UV-visible spectrophotometer (Multiskan Go, Thermo Fisher Scientific, USA).

EXTRACTION AND ASSAYS FOR ANTIOXIDANT ACTIVITIES

A crude enzyme was extracted using the method described by Tan et al. (2012). Plant sample (5 g of tissues) was grounded using mortar and pestle in the presence of liquid nitrogen followed by homogenization with 10 mL of potassium phosphate buffer (0.1 M, pH7.0) containing 1 mM EDTA and 1% polyvinylpolypyrrolidone (PVP).

APX activity in the crude enzyme extracts was assayed by the method of Nakano and Asada (1981). The assay mixture consisted of 1.91 mL of potassium phosphate (50 mM, pH7.0), 0.01 mL of H₂O₂ (0.1 mM), 0.05 mL of ascorbate (0.5 mM). The reaction was initiated by adding 0.03 mL of the crude enzyme extract and the decrease in absorbance was measured at 290 nm using a UV-visible spectrophotometer. Enzyme activity was expressed as mmol min⁻¹ fresh weight, where one unit is defined as the total of enzyme that catalyze the oxidation of 1 µmol of ascorbate per min under these assay conditions.

CAT activity was determined according to Beers and Sizer (1952) by measuring the diminution of H₂O₂. The

assay mixture comprised of 0.1 mL of crude enzyme extract mixed with 0.9 mL of potassium phosphate buffer (50 mM, pH7.0) and 5 mM H₂O₂. The absorbance was determined at 240 nm using a UV-visible spectrophotometer. CAT activity was expressed as units per gram of fresh weight, where one unit is defined as the total of enzyme required to decrease 1 µmol H₂O₂ in 1 min under these assay conditions.

STATISTICAL ANALYSIS

Experiments were arranged in a completely randomized design (CRD) with three replications consisted of 3 fruits each (*n*=9) except CI evaluation with four replications consisted of 10 fruits each (*n*=40). Data were subjected to analysis of variance (ANOVA) using Genstat 16 version (VSN International Ltd, UK), while Least Significance Difference (LSD) test was used to compare differences between treatment and storage periods at 95 % confidence level of each variable.

RESULTS AND DISCUSSION

EFFECTS OF CHITOSAN COATING ON WEIGHT LOSS AND TISSUE FIRMNESS

In all cases, Japanese cucumber weight loss increased throughout storage at 7°C (Figure 1). The lowest weight losses were observed in control (7.5%) and fruit coated with 0.5% chitosan (8.0%) on final day of storage. Meanwhile, higher chitosan concentrations 1.0 and 1.5% demonstrated higher weight loss of 8.8 and 9%, respectively (Figure 1). There was no significant (*p*>0.05) difference between all the chitosan treatments on weight loss and regardless the treatment, the weight loss increased significantly (*p*<0.05) until the final of storage periods.

Fruit weight loss is mainly contributed by high respiration rate and moisture evaporation (Garcia et al. 2014). Chitosan coating provides semi-transparent layer on the pericarp surface that act as a protective barrier to restrict respiration and delay transpiration rates over fruit

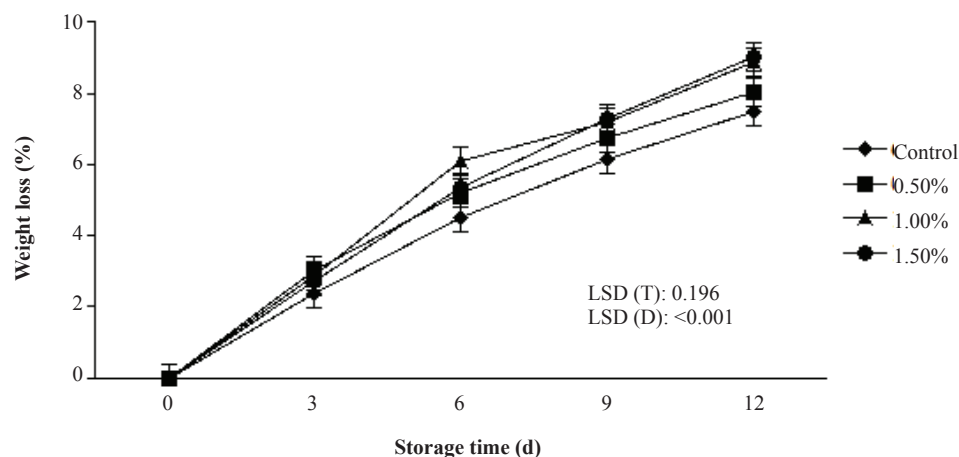


FIGURE 1. Effect of chitosan concentrations (0.5, 1.0 and 1.5%) on the weight loss of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars (*n* = 9). T=treatment, D=day

surfaces (Zhang et al. 2015). Cucumbers treated with chitosan regardless of the concentrations experienced a delayed weight loss indicating chitosan coating acted as an effective semi-permeable barrier against moisture loss which serve as a barrier to water vapour and suppressing the transpiration rate.

Our findings showed that the lowest chitosan concentration (0.5%) was more effective compared higher concentrations, explaining higher composite coating may promote higher weight loss due to the anaerobic respiration. As evidenced by studies with apricot (Ghasemnezhad et al. 2010) and banana (Maqbool et al. 2011) when coated with high chitosan concentration, experienced greater weight loss.

The firmness of Japanese cucumber declined from 76.7 to 68.8 N throughout cold storage (Figure 2). A constant declined of firmness in fruit has been demonstrated in all treatments, mostly during first three days of storage at the 7°C storage ($p>0.05$). Cucumber coated with higher concentrations of chitosan 1.0 and 1.5% experienced greatest losses in firmness with 7.3 and 10.4%, respectively, at the end of the experiment. In contrast, 2.9 and 4.7% of firmness losses were recorded in cucumbers coated with 0.5% chitosan and control, respectively. Regardless of chitosan treatment applied, fruit firmness was maintained on day 6 onwards (Figure 2). No significant difference ($p>0.05$) were found between the control and coated fruits throughout storage.

It has been postulated that loss of firmness was associated with solubilisation of cell wall composition accompanied with the burst of softening enzyme activities (Ali et al. 2004). However, firmness was maintained in cucumber coated with 0.5% chitosan than that in 1.0 and 1.5% chitosan. Apparently, firmness was declined with increasing chitosan concentrations, and cucumber coated with 1.0 and 1.5% chitosan lost their cell wall mechanical strength quicker than 0.5% chitosan and control fruits.

It is well documented that diminished postharvest quality probably due to accelerated respiration process and chitosan coating might have helped conservation of firmness

probably through internal atmosphere modification. Chitosan provides an excellent semipermeable film-forming that responsible for the low O_2 and/or high CO_2 of the vegetables and fruit, thus suppressing respiration rate and senescence related changes (Gol et al. 2013; Perdonés et al. 2012).

EFFECTS OF CHITOSAN COATING ON CHILLING INJURY AND LIPID PEROXIDATION

The chilling symptoms of Japanese cucumber stored at 7°C were reduced significantly ($p<0.05$) between chitosan coated and control fruits (Figure 3). With the advancement of the storage period, chilling injury (CI) manifested gradually up to 3 days of cold storage and CI symptoms evolved rapidly thereafter. The CI index of control cucumber was 0.3 and 0.38 during 9 and 12 days at 7°C, respectively. Chitosan treatment at 1.0 and 1.5% exhibited higher development and severity of CI meanwhile chitosan at 0.5% was most effective in alleviating chilling symptoms.

On day 9 of storage, both 1.0 and 1.5% demonstrated CI of 1.4 and the symptoms getting more pronounced and severe on day 12 with a CI index of 1.4 and 2.15, respectively. On the other hand, CI in 0.5% chitosan were 0.07 and 0.2 on the 9 and 12 days of storage, respectively (Figure 3). Skin shrivelling, surface pitting, dark-watery patches were amongst the CI symptoms observed in Japanese cucumber (data not shown). In fruit treated with 1.5% chitosan, fungal growth appeared on day 12 where the highest CI index was recorded.

Cucumber coated with higher chitosan concentrations exerted severe CI with damage generally being irreversible meanwhile, 0.5% chitosan showed the least injury. This result suggested that fruit treated with lowest chitosan concentration is much more tolerable against chilling than those in 1.0 and 1.5% chitosan. Coating with higher concentration of chitosan probably hasten the deterioration rate through anaerobic metabolism (Ghasemnezhad et al. 2010) thus gave rise to a faster senescence. The onset of

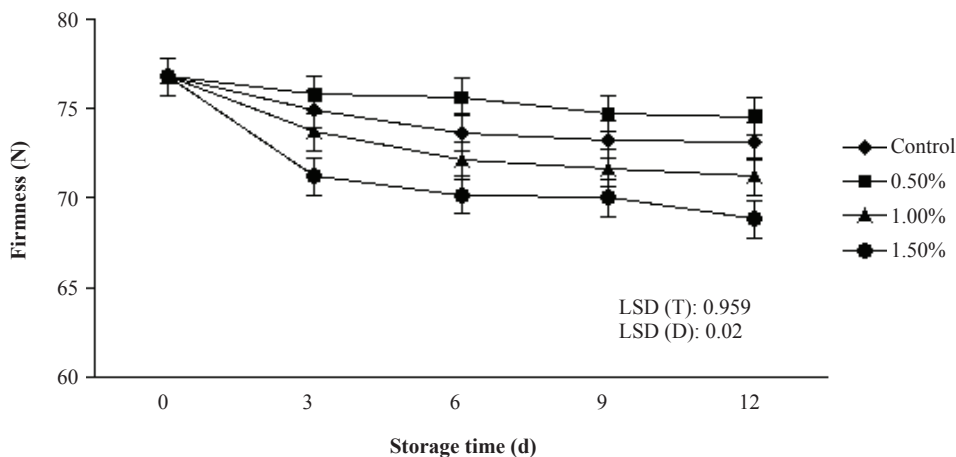


FIGURE 2. Effect of chitosan concentrations on the firmness of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars ($n=9$) T=treatment, D=day

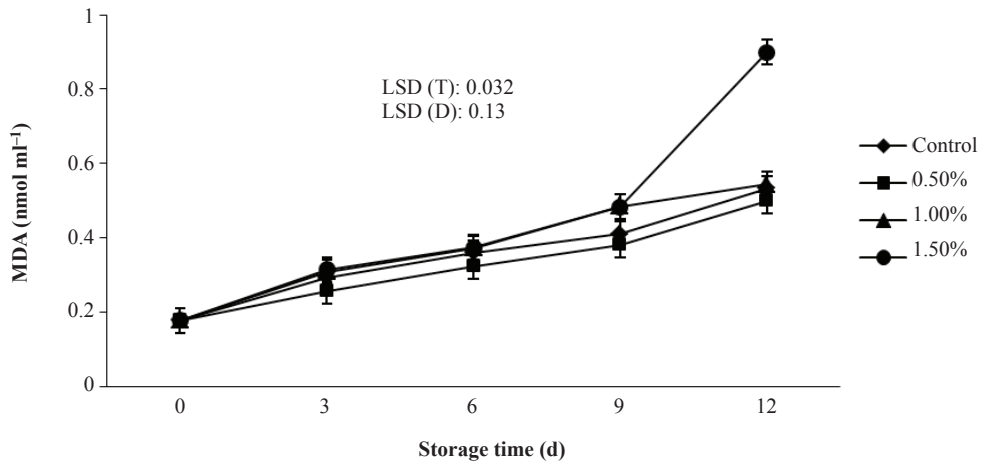


FIGURE 3. Effect of chitosan concentrations on the CI index of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars ($n=40$) T=treatment, D=day

CI has been associated with physiological disorders and abnormal cell metabolism (Qian et al. 2012). Apart from that, it also shifts the ROS balance and elevates the levels of ROS mainly hydrogen peroxides (H_2O_2), superoxide (O_2^-) (Wang et al. 2008).

Chitosan may induce defense responses which could boost the resistance to cold damage and reinforce the tolerance against chilling injury (Terry & Joyce 2004). Additionally, chitosan could act as exogenous elicitors in tomato tissue by triggering several defense mechanisms such as formation of phenolic compounds via *de novo* biosynthesis, accumulation of antifungal compounds such as phytoalexins and pathogenesis-related proteins (PR) such as chitinases and β -1,3-glucanases (Bautista-Banos et al. 2006, 2003; Lafontaine and Benhamou 1996; Xu et al. 2001).

As shown in Figure 4, MDA level in cucumber increased gradually throughout the cold period. Fruit treated with 0.5% chitosan showed lowest MDA content of 64.2% compared to other treatments over time. There was a sharp increment of 1.9-fold of lipid peroxidation (0.48 to 0.9 nmol mL⁻¹) in cucumber coated with 1.5% chitosan

on day 12. This pattern was coincident with severity of CI symptoms that appeared on the same day. Overall, the highest MDA content was recorded as 80.1% over time in cucumber treated with highest chitosan concentration (1.5%). Meanwhile, 1.0% chitosan and control fruit demonstrated an increase of 66.2% and 67% of the MDA content, respectively, over time (Figure 4). In all cases, there was a significance difference ($p<0.05$) between 1.5% chitosan coated cucumber and 0.5 and 1.0% concentrations.

Lipid peroxidation is a general response of plant tissues to abiotic stress including chilling (Imahori et al. 2008). The sharp increment of MDA in 1.5% chitosan at end of storage indicates that the membrane composition is compromised in the fruit tissue. With the advancement of chilling periods, a rapid rate of lipid peroxidation occurred as unsaturated fatty acids were oxidized due to attack by free radicals and oxygen leading to deteriorating changes associated with cell death (Hariyadi & Parkin 1991). Our finding suggesting that 0.5% chitosan was better at protecting and maintaining membrane structural integrity by showing a lower increase of MDA content throughout the storage.

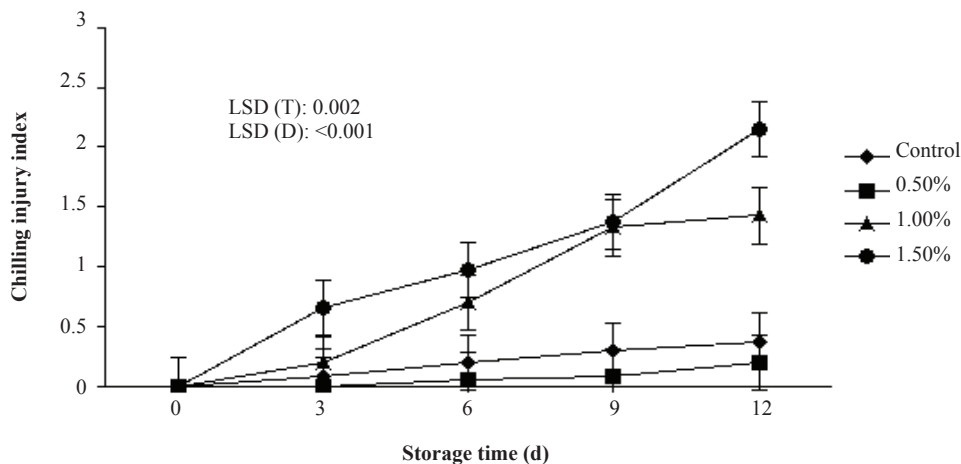


FIGURE 4. Effect of chitosan concentrations on the MDA content (lipid peroxidation) of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars ($n=9$). T=treatment, D=day

EFFECTS OF CHITOSAN COATING ON CAT AND APX ACTIVITIES

In all cases, catalase (CAT) activity exhibited a similar pattern, a sharp increase and reached a maximum value on day 3 before decreasing drastically until the end of the experiment (Figure 5). The antioxidant activity was 4.3 fold higher on day 3 (7.01 to 30.08 $\mu\text{mol}/\text{min}/\text{g}$) in 1.5% chitosan followed by 1.0% chitosan with 4 fold higher (7.01 to 28.9 $\mu\text{mol}/\text{min}/\text{g}$) indicating that treatment with higher chitosan improved the activity of CAT in Japanese cucumber throughout the cold storage. However, on final day storage, CAT activities in 1.5 and 1.0% chitosan declined tremendously to reach the same level as the control. No marked differences ($p>0.05$) were found between chitosan coated and control fruit.

In all cases, ascorbate peroxidase (APX) activity reached the highest peak on day 6 and declined rapidly thereafter (Figure 6), where control cucumber showed lower activity in contrast to fruit treated with chitosan. Among the chitosan concentrations examined, higher chitosan (1.5%) showed a greater increase in APX activity

than that in lower chitosan concentrations. After 6 days, APX decreased until end of storage regardless the treatments and showed no significance difference amongst treatments. Cucumber coated with 1.0% chitosan also displayed a high APX activity throughout cold storage in comparison to control and 0.5%, though it was relatively lower than 1.5% chitosan (Figure 6).

In control cucumber, both CAT and APX enzyme activities exhibited similar pattern of changes by increasing sharply during 3-6 days of storage, then activities decreasing drastically for the remaining storage time (Figures 5 & 6), which is in agreement with results reported previously (Cao et al. 2009; Xu et al. 2012). It has been agreed that the chilling tolerant plant possess high antioxidant potential would better withstand oxidative damage stress than a chilling sensitive plant with lower antioxidant capacities (Sala & Lafuente 2004).

CAT, which is localized in peroxisomes is the principal scavenging enzymes that catalyze the breakdown of H_2O_2 into harmless compounds; O_2 and H_2O (Wang & Gao 2013). Meanwhile, APX was mainly localized in

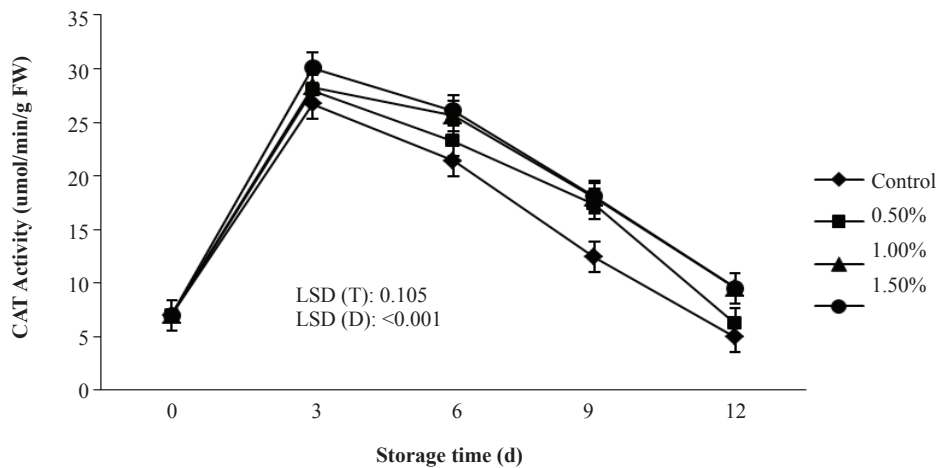


FIGURE 5. Effect of chitosan concentrations on the CAT activity of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars ($n=9$) T=treatment, D=day

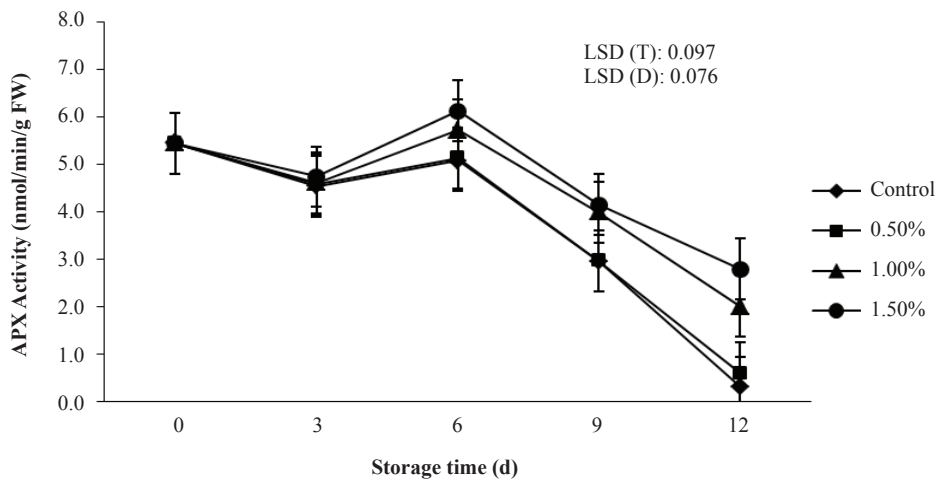


FIGURE 6. Effect of chitosan concentrations on the APX activity of Japanese cucumber stored for 12 days at 7°C. The \pm SE of the means was indicated by vertical bars ($n=9$) T=treatment, D=day

chloroplast, mitochondria, microbodies and cytosol might be responsible for regulation of the ROS signalling system (Imahori et al. 2008). H₂O₂ accumulation can be partly detoxified by AsA-GSH cycle, where APX has a crucial role by helping in the removal of H₂O₂ accumulation, which may account for the lower level of O₂^{•-} and H₂O₂ production (Qian et al. 2012).

The distinct increment of CAT and APX activity during early storage was observed in chitosan coated cucumber than that in control, suggesting chitosan could have enhanced both enzymes scavenging properties and then promoted protection from oxidative damage. The result also proposed that the enhanced activity of CAT and APX during the first 6 days at cold temperature were induced by chitosan coating might possibly be of benefit to ROS resistance in fruit. The result was in accordance with previous reports, indicating that chitosan coating in other crops such as tomato, sweet cherry, navel oranges, guava (Dang et al. 2010; Hong et al. 2012; Liu et al. 2007; Zeng et al. 2010) induced activities antioxidant defense enzymes, thereby trigger chilling resistance. However, CAT and APX activities declined sharply in later storage period coincident with the high CI index, such that the severity of tissue injury and accumulation of H₂O₂ was probably too far advanced to be contrasted.

CONCLUSION

Chitosan as coating material maintained higher firmness and suppressed weight loss. Furthermore, chitosan alleviated CI symptoms and reduced the increase of lipid peroxidation (MDA content) in cucumbers during cold exposure. The influence of chitosan on conferring chilling resistance in cucumber may be due to its ability to protect tissue membranes from oxidative damage by inducing higher CAT and APX activities. Hence, application of chitosan coating may become powerful postharvest tools to extend the quality and storability of cucumber fruits during cold storage.

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Nur Fatin Afifah Hashim, Azhane Ahmad* & Paa Kwesi Bordoh
School of Biosciences, Faculty of Science
University of Nottingham Malaysia Campus
43500 Semenyih, Selangor Darul Ehsan
Malaysia

Azhane Ahmad*
School of Food Science and Technology
Universiti Malaysia Terengganu
21030 Kuala Nerus, Terengganu Darul Iman
Malaysia

*Corresponding author: azhane_ahmad@yahoo.com.my

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