International Journal of Agricultural Technology 2018 Vol. 14(7): 1987-1998
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Effect of preharvest chitosan application on bioactive compounds of and sunflower sprouts during storage

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Supapvanich, S., Chimsoontorn, V., Anan, W., Boonyaritthongchai, P., Tepsorn, R. and Techavuthiporn, C. (2018). Effect of preharvest chitosan application on bioactive compounds of and sunflower sprouts during storage. International Journal of Agricultural Technology 14(7): 1987-1998.

Abstract The effects of preharvest chitosan treatments on antioxidant activities and bioactive compounds of sunflower sprouts during cold storage were investigated. The sprouts were watered with 0, 0.1, 0.5 and 1.0 % (w/v) chitosan solutions prior harvest 24 h. After harvest, the sprouts were stored at 4 ± 1 ℃ for 9 d. The investigated parameters were visual appearance, antioxidant activities such as ferric reducing antioxidant potential (FRAP), DPPH radical scavenging (DFRS) activity and bioactive compounds including total phenols, flavonoids and ascorbic acid concentrations. The visual appearance of the sprouts of all treatments was maintained during storage at 4 ± 1 ℃ for 9 d. Preharvest chitosan treatment enhanced antioxidant and DFRS activities, total phenols, flavonoids and ascorbic acid contents of the sprouts, especially at 0.1 % chitosan. During the storage, 1.0 % chitosan treatment induced FRAP, DFRS activity and all bioactive compounds rather than others treatments. These suggest that preharvest chitosan treatment is an effective alternative improving nutritional quality of the sunflower sprouts during cold storage.

Keywords: Sunflower sprouts, chitosan, elicitor

Introduction

In view of increased interests in health-promoting foods, people have been recently concerning to consume healthy food from plant origin. High intake of plant products has been approved to reduce the risk of a number of chronic diseases and obesity (Gosslau and Chen, 2004). These benefit effects are attributed to biologically active compounds possessing antioxidant potential such as phenolic compounds, ascorbic acid, carotenoids and dietary fibres

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(Podsędek, 2007; Pandey and Rizvi, 2009). Several epidemiological studies suggest that long-term consumption of plant products rich in biologically active compounds, especially plant polyphenols, offer protection against cancer, cardiovascular diseases and neurodegenerative diseases (Scalbert *et al*., 2005; Pandey and Rizvi, 2009). Daikon and sunflower sprouts are accepted as good sources of biologically active compound exhibiting antioxidants such as phenolic compounds, flavonoids and vitamins (Hanlon and Barnes, 2011; Cho *et al.,* 2008). According to increased health concern, utilization of the radishes and sunflower sprouts in diets appears realistic and has become popular.

Chitosan , a natural carbohydrate polymer composed of randomly distributed β-(1-4) D-glcosamine and N-acetyl-D- glucosamine, is commonly obtain from shellfish byproducts (Bautista *et al.,* 2003). It has received much interest for wide application in food and agriculture. Chitosan stimulates various enzymes relating to defense mechanisms such as phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase, superoxide dismutase (SOD), catalase (CAT), and peroxidases (PODs) activities (Cho *et al.,* 2008; Katiyar *et al.,* 2015). Moreover, previous work revealed that chitosan application increased yield, growth rate and vitamin C as well as decreased the rot of soybean sprouts (Lee *et al.,* 2005; Choi *et al.,* 2000; No *et al.,* 2003). Cho *et al.* (2008) suggested that soaking sunflower seed in 0.5% chitosan with 28 kDa increased free radical scavenging activity and certain biologically active compounds such as total phenolic compounds, total isoflavone and melatonin contents in sunflower sprouts. Pérez-Balibrea *et al*. (2011) had reported that 0.01% chitosan treatment induced vitamin C and glucosinolate contents in broccoli sprouts during 7 days old seedlings. Hence, chitosan may be used as an alternative to maintain postharvest quality and to enhance healthy properties of sprouts.

The objective of this work was to investigate the effect of preharvest chitosan treatment on antioxidant activities and bioactive compounds contents such as total phenols, flavonoids and ascorbic acid of sunflower sprouts during refrigerated storage at 4 ± 1 ℃.

Materials and methods

Sprouts material and Chitosan applications

Sunflower seeds were derived from a commercial seed company, Lopburi province, Thailand. Sixty grams of sunflower seeds were washed and then soaked with sterilized water for 12 h. They were then wrapped with a wet-cloth sheet for 24 h and cultivated in dark at room temperature (28 ±2 ℃) for 4 d. After 4 d of cultivation in the dark place, the sprouts were exposed to sun light

for 2 d. A 500 mL of tapped water was sprayed every 12 h for 5 d. On day 6 of cultivation, the sprouts were watered with 500 mL of food grade chitosan solutions at 0.1, 0.5 and 1.0 % (w/v) and the controls were watered with 500 mL of tapped water. The sprouts were harvested by using a sharp blade on day 7 of cultivation. The sprouts were cleaned by dipping in 200 ppm NaOCl for 1 min and then rinsing with pasteurized water. The surface water was removed and 150 g of the sprouts were packed in a zip-locked LLDPE bag (a commercial plastic bag for fresh fruit and vegetables and then storage at 4 ± 1 ℃ and 87 ± 2 % RH for 9 d. Four replicates of the sunflower sprouts were sampled in every 3 d of storage, respectively. Visual appearance, Ferric reducing antioxidant potential (FRAP), DPPH free radical scavenging (DFRS) activity and bioactive compounds including total phenols, flavonoids and ascorbic acid concentrations were determined.

FRAP and DPPH free radical scavenging activity assays

A 3 g of sprout was homogenized with 60 % (v/v) ethanol and stirred for 30 min at 4 ℃. The homogenate was then filtered through a Whatman no. 1 filter paper. The filtrate was collected to determine FRAP, DFRS activity, total phenols and flavonoids concentrations. FRAP was determined using the method described by Benzie and Strain (1996). A 0.1 mL of filtrate was reacted with 2.9 mL of FRAP reagent which consisted of acetate buffer pH 3, 10 mM 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) and 20 mM ferric chloride hexahydrate in the ratio of 10:1:1 (v/v/v). The reaction was stand at room temperature at least 30 min. The absorbance at wavelength of 630 nm was measured. The FRAP value was calculated and reported as mmole trolox equivalents per kilogram fresh weight (mmol kg-1). DFRS activity was measured according to the method of Brand-Williams *et al.* (1995) with slight modification. The reaction was begun when 1.0 mL of filtrate was added into 2.0 mL of 1 mM DPPH in methanol. The reaction was held at dark place for 5 min and the absorbance at the wavelength of 517 nm was measured. The DFRS activity was calculated using the equation as shown below.

DFRS activity (%) = [(A0min – A5min)/A0min] x 100

A0min = the absorbance of the sample at 0 min; A5min = the absorbance of the sample at 5 min.

Total phenols and flavonoids concentrations assays

Total phenols concentration was determined using the method of Slinkard and Singleton (1977). One mL of the filtrate was reacted with 1.0 mL of 50% (v/v) Folin–Ciocalteu reagent solution and 2.0 mL of saturated Na2CO3

solution. The absorbance of the sample was measured at the wavelength of 750 nm. Total phenols concentration was calculated and expressed as mg gallic acid per kilogram fresh weight (mg kg-1). Flavonoids concentration was assayed using the method described by Jia *et al*. (1999). A 0.25 mL of the filtrate was mixed with 1.25 mL of distilled water and 75 µL of 0.5% NaNO2. The solution was mixed and stand at room temperature for 6 min before 150 µL of 10% AlCl3-6H2O was added. The mixed solution was again stand for 5 min and then 0.5 mL of 1.0 M NaOH was mixed. The absorbance of the mixture was determined at the wavelength of 510 nm. Flavonoids concentration was calculated and expressed in term of mg catechin equivalent per kilogram fresh weight (mg kg-1).

Total ascorbic acid concentration assay

A 5 g of sprouts was extracted with 30 mL of cold 5% metaphosphoric acid and then filtered through Whatman no 1. filter paper. The filtrate was collected to determine total ascorbic acid concentration according to the method of Hashimoto and Yamafuji (2001). The reaction was started when 1.6 mL of the filtrate was mixed with 0.8 mL of 2.0% di-indophenol. Afterwards, the mixture was mixed with 1.6 mL of 2 % thiourea and 0.4 mL of 1% dinitrophenol hydrazine, respectively. The reaction was incubated for 3 h and then 2 mL of 85% sulphuric acid was added. After that, the mixture was again incubated at room temperature for 30 min before the absorbance at 540 nm wavelength was recorded. Ascorbic acid concentration was calculated and expressed in the term of gram of ascorbic acid per kilogram fresh weight (g kg-1).

Statistical analysis

The data was presented as the average of four replications ± standard deviation (SD) bar. Statistical analysis was carried out using Analysis of Varian (ANOVA) and the difference between the means was performed with DMRT at $P < 0.05$ by using SPSS version 14.0 software.

Results

Visual appearance

The effect of preharvest chitosan treatments on visual appearance of the sunflower sprouts after storage for 9 d were shown in figure 1. No cotyledon shrinkage was found over the storage for 9 d (Fig. 1). All preharvest chitosan treatments had no effect on colour of the sprouts over the storage. These

suggest that cold storage at 4 ± 1 ℃ could maintain visual appearance of sunflower sprouts during for 9 d and all preharvest chitosan treatment had no influence on visual appearance of the sprouts throughout the storage**.**

Antioxidant activities

The FRAP and DFRS activity of sunflower sprouts during the storage were shown in figure 2. On first day of the storage, FRAP and DFRS activity of the sunflower sprouts treated with 0.1 % chitosan were slightly higher than other treatments while those of other chitosan treatments were similar to the control. During the storage, The FRAP value of all treatment remained constant during storage for 3 d and then increased reaching to the peak on day 6. The highest FRAP value was found in 1.0 % chitosan-treated sprouts which was significantly higher than other treatments until the end of storage ($P < 0.05$). In the similar vein, DFRS activity of the sunflower sprouts also remained constant during storage for 3 d and then peaked on day 6 which the highest value was found in 1.0 % chitosan-treated samples followed by 0.5 % and 0.1% chitosan-treated and untreated sprouts, respectively. On day 9 of the storage, DFRS activity of all treatments declined and was similar. These suggest that preharvest chitosan treatment prior harvest 24 h induced antioxidant activities of sunflower sprouts, especially at 1.0 % chitosan.

Total phenols and flavonoids contents

The changes in total phenols and flavonoids contents of sunflower sprouts were shown in figure 3. On the initial day of storage (24 h after treatment), total phenols content of 0.1 % chitosan treated sprouts was significantly higher than that of the control ($P < 0.05$). The increases in total phenols and flavonoids concentrations were found during storage for 6 d and then decreased. Preharvest chitosan treatment at the concentration of 1.0 % significantly enhanced the both total phenols and flavonoids concentrations compared to 0.5 % chitosan and 0.1 % chitosan treatments, respectively. The lowest concentration of total phenols concentration was found in the control. No significant difference in flavonoids concentration of the control, 0.5 % and 0.1 % chitosan-treated sunflower sprouts was found during the storage.

Ascorbic acid concentration

It is widely acknowledged that ascorbic acid is an essential compound in plant and functions as antioxidant and a co-substrate of plant peroxidases (Pandey and Rizvi, 2009). Ascorbic acid concentration of sunflower sprouts

treated with chitosan during the storage was presented in figure 4. The results revealed that 0.1 % chitosan could induce ascorbic acid concentration after treated 24 h and maintained a high level of ascorbic acid concentration for 3 d of the storage. After day 6 of the storage, no significant difference in ascorbic acid concentration of all treatments was found.

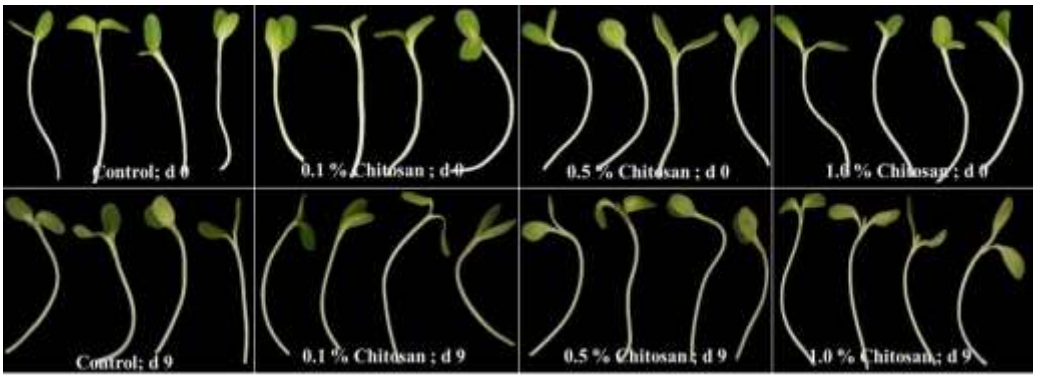

Figure 1. Visual appearance of preharvest 0 (control), 0.1%, 0.5% and 1.0% chitosan treated sunflower sprouts during storage at 4 ± 1 ℃ for 9 d

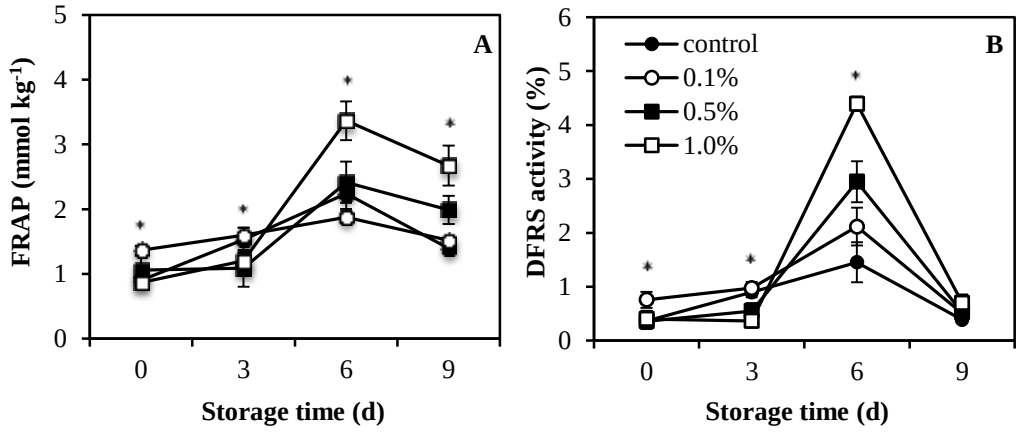

Figure 2. Ferric reducing antioxidant potential (FRAP) (A) and DPPH free radical scavenging (DFRS) activity (B) of sunflower sprouts pre-harvest treated with chitosan at various concentrations during storage at 4 ± 1 ℃ for 9 d. Data represent the mean of four replications ± SD. Significant differences between treatments are indicated with asterisk [*($P < 0.05$)]

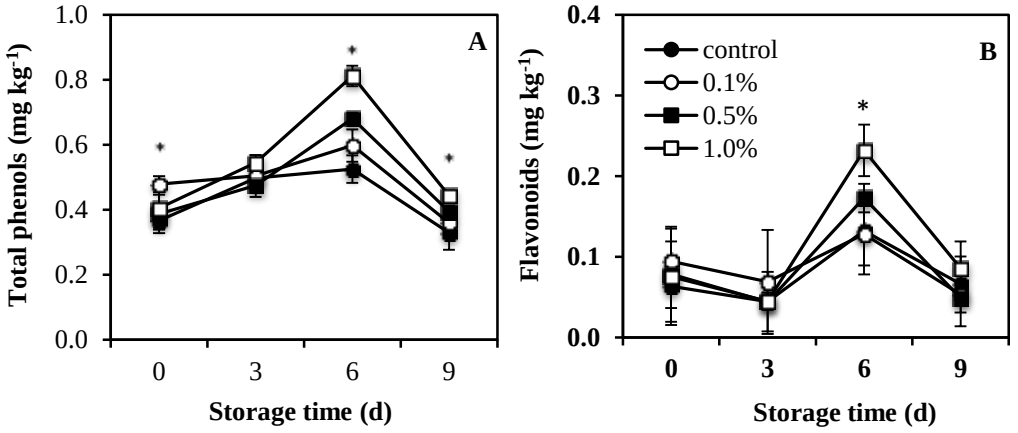

Figure 3. Total phenols (A) and flavonoid (B) contents of sunflower sprouts pre-harvest treated with chitosan at various concentrations during storage at 4 ± 1 ℃ for 9 d. Data represent the mean of four replications ± SD. Significant differences between treatments are indicated with asterisk [*($P < 0.05$)]

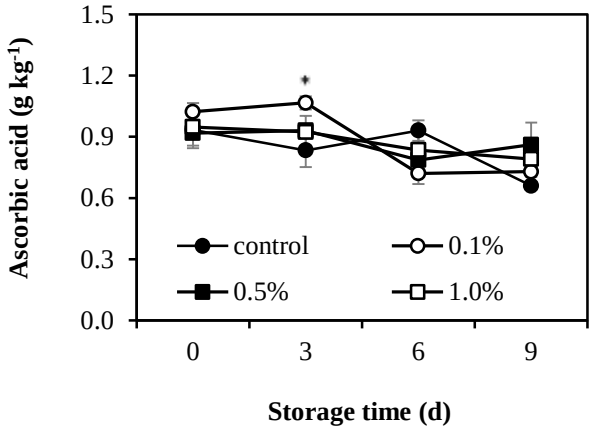

Figure 4. Total ascorbic acid content of sunflower sprouts pre-harvest treated with chitosan at various concentrations during storage at 4 ± 1 ℃ for 9 d. Data represent the mean of four replications ± SD. Significant differences between treatments are indicated with asterisk [*($P < 0.05$)]

Discussion

As the results shown above, the use of chitosan as an elicitor did not affect visual appearance of the sprouts throughout storage. The visual appearance of the sprouts on 9 d of the storage did not obviously different from

the sprout on initial day. This reveals that the storage conditions especially low temperature and package could maintain freshness appearance the sprout during storage. It is commonly acknowledged that cold storage (at proper cold temperature) can reduce total metabolisms and delay physiological changes of fresh commodities resulting to prolong shelf-life and postharvest quality (Stanley, 1991). However, low storage temperature alone is not enough to control postharvest quality. The loss of moisture content during storage is also the main factor limiting shelf-life and visual appearance of leafy vegetables including sprouts (Wills *et al.,* 2007). Generally, the shrinkage of leafy vegetables is revealed when the moisture loss from the vegetables is higher than 4% (Supapvanich *et al.,* 2012). The use of LLDPE plastic bag, a commercial plastic bag for fresh fruit and vegetables, could prevent the loss of moisture from the sprouts which the weight loss of sprouts was approximately 3% (data not shown). Mangaraj *et al.* (2009) suggested the use of LLDPE film is suitable for fresh fruit and vegetables as it is soft, flexible and has good moisture barrier property in low temperature.

Chitosan is commonly used as coating agent for postharvest produces. Recently, many previous researches have been confirmed that it is also an elicitor (Bohland *et al.,* 1997; Ruiz-Garcá and Gómez-Plaza, 2013). The recent work shows that chitosan stimulated antioxidant activity and free radical scavenging activity and certain bioactive compounds such as total phenolic compounds and flavonoids of the sprouts. The increased antioxidant activity and free radical scavenging activity are commonly accepted that they are associated with the enhancement in bioactive compounds such as total phenols, flavonoids and ascorbic acid concentrations. These phytochemicals are commonly acknowledged as the major sources of antioxidants in foods from plant origin. This result was confirmed by Cho *et al.* (2008) which found that the increase in DFRS activity of sunflower sprouts dipped in chitosan solution was associated with the increase in phytochemicals concentrations such as total phenols, melatonin and total isoflavone contents during storage. Moreover, Katiyar *et al.* (2015) and Guo *et al.* (2003) also addressed that chitosan treatment could enhance free radical scavenging activity in plant by inducing antioxidant enzyme activities such as superoxide dismutase, catalase and peroxidase. The increased total phenols and flavonoids content of the sprouts might be associated with the signalling phenylpropanoid biosynthesis which stimulated by chitosan (Khan *et al.,* 2003; Ruiz-Garcá and Gómez-Plaza, 2013; Katiyar *et al.,* 2015). Previous works had confirmed that chitosan stimulated the activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (Carvacho *et al.,* 2014; Khan et al., 2003) which these enzymes participate in the biosynthesis of phenolic compounds and flavonoids (Vogt, 2010).

Moreover, Carvacho *et al.* (2014) found that chitosan obviously increased total phenolic compounds of broccoli sprouts and maintained its high level during storage. Viacava and Roura (2015) also reported that exogenous daily spray of chitosan induced phytochemicals biosynthesis including phenolic compounds and flavonoids of lettuce sprouts. Certain previous works had reported the maintenance or increment of ascorbic acid content in postharvest produces by chitosan application (No *et al.*, 2007; Hong *et al.*, 2012). Kerch *et al.* (2011) suggested that chitosan could promote vitamin C synthesis and has scavenging activity resulting to prevent negative effect of reactive oxygen species activity on vitamin C in fresh commodities during refrigerated storage. Moreover, previous work reported that chitosan treatment induced the vitamin C content of soybean sprouts (No *et al.*, 2003) and broccoli sprouts (Pérez-Balibrea *et al.*, 2011). However, the recent work showed that chitosan at the concentration of 0.1 % could retard the reduction of ascorbic acid content at the first 3 d of storage period. This suggest that preharvest chitosan application could delay the loss of vitamin C of sunflower sprouts during storage.

Conclusion

Preharvest chitosan treatments before harvest 24 h had no effect on visual appearance of sunflower sprouts throughout the storage for 9 d compared to the control which its visual appearance was maintained by the cold storage. The 0.1% chitosan treatment could induce antioxidant activities and bioactive compounds concentrations after treated for 24 h. During storage, antioxidant activities such as FRAP and DFRS activity and bioactive compounds concentrations such as total phenols and flavonoids, were obviously enhanced by 1.0 % chitosan during the storage. Preharvest 0.1 % chitosan treatment could maintain higher concentration of ascorbic acid for 3 d of the storage compared to other treatments. In conclusion, preharvest chitosan treatment, especially at 1.0 % chitosan, is a potential alternative improving nutritional value involving antioxidant activities and bioactive compounds of sunflower spouts during cold storage.

Acknowledgement

The project was funded by Faculty of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang.

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(Received: 10 September 2018, accepted: 31 October 2018)