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# Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP

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**Abstract**

Silver nanoparticles have recently gained increasing interests due to their antimicrobial activities in food processing applications. The aim of this study was to evaluate the effect of silver nanoparticles-PVP coating on weight loss, ascorbic acid, total chlorophyll, crude fiber, color, firmness and microbial qualities of asparagus spears stored at 2 and 10 °C. Asparagus samples were first sanitized with 100 mg $l^{-1}$ sodium hypochloride solution for 15 min. They were then immersed in coating solution containing silver nanoparticles for 3 min at room temperature. During 25-day storage at 2 or 10 °C, the coated asparagus demonstrated lower weight loss, greener color and tender texture compared with the control samples. The growth of microorganism was significantly hindered by the coating. Based on comprehensive comparison and evaluation, asparagus spears coated by silver nanoparticles could be kept in good quality for 25 days at 2 °C and for 20 days at 10 °C.


*Keywords:* *Asparagus officinalis* L.; Silver nanoparticles; Polyvinylpyrrolidone; Coating; Storage; Quality

## 1. Introduction

As one of the important fresh vegetables, green asparagus (*Asparagus officinalis* L.) is becoming increasingly popular due to its special flavor, taste and the high economic value in terms of export in recent years in China. However, it is also an extremely perishable vegetable. Freshly harvested asparagus deteriorates rapidly leading to a short shelf life of 3–5 days under normal postharvest handling at ambient temperature (Baxter & Waters, 1991; Lipton, 1990) and 14–15 days at the normal cold storage temperature 1–3 °C (An, Zhang, Lu, & Zhan, 2006; Esteve, Farre, & Frigola, 1995; Li, Zhang, & Yu, 2006; Villanueva, Tenorio, & Sagardoy, 2005). In a previous research, the effects of modified atmosphere package (MAP) and hypobaric pressure on shelf-life of green asparagus were studied (An, Zhang, & Lu, 2007; Li & Zhang, 2006). It was concluded that more combination methods need to be studied to improve the storage quality of green asparagus.

Edible coatings have long been known to protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavor compounds and reducing microbial growth (Debeaufort, Quezada-Gallo, & Voilley, 1998). Specially formulated edible coatings may provide additional protection against contamination of microorganism while serving the similar effect as modified atmosphere storage in modifying internal gas composition (Park, 1999). Among noble-metal nanomaterials, silver nanoparticles have received considerable attentions due to their attractive physicochemical properties. It is well known that silver in various chemical forms has strong toxicity to a wide range of microorganisms (Liau, Read, Pugh, Furr, & Russell, 1997). In particular, silver nanoparticles have been shown to be a promising antimicrobial material (Sondi & Salopek-Sondi, 2004). The larger surface area of silver nanoparticles can improve

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their antibacterial effectiveness against 150 types of microbes.

Some researchers investigated the safety of solutions containing small concentrations of silver ion (USEPA, 2001; Zhang, Duan, & Shan, 2005). The US Environmental Protection Agency (USEPA) proposed that the secondary maximum contaminant level of silver ion in drinking water must be less than 0.10 mg $l^{-1}$ (USEPA, 2001). In our previous study (Zhang et al., 2005), we found that the optimal preservation for vegetable juices was to use 0.04 mg $kg^{-1}$ doses of 'quasi-nanoscale' silver particles (101–109 nm).

Although the coating has been extensively studied to increase the shelf life of many fresh fruits and vegetables, no information is available regarding the application of silver nanoparticles-polyvinylpyrrolidone (PVP) coating for green asparagus. The objectives of this study were to evaluate the effect of a silver nanoparticles-PVP coating on the weight loss, ascorbic acid, total chlorophyll, crude fiber, color, firmness and microbial quality of green asparagus stored at 2 and 10 °C.

## 2. Materials and methods

### 2.1. Preparation of silver nanoparticles and determination of their characterization

In our previous study (Zhang et al., 2005), we compared the effect of 'quasi-nanoscale' silver particles and normal silver solution with same concentration on reduction of total microorganism accounts in the preliminary tests and found the 'quasi-nanoscale' silver particles to be much more effective. In this study, therefore, we chose to focus only on the effect of nano-silver particles on some physical, chemical and microbiological changes in stored green asparagus spears.

All chemical materials were reagent grades (GR). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Shanghai Chemical Reagent Company, Shanghai, China. Polyvinylpyrrolidone (PVP, K30, polymerization degree 360) was purchased from the national group of chemical reagent in Shanghai, China. Double distilled and deionized water ($>15$ MΩcm$^{-1}$) was used throughout the experiment.

$AgNO_3$, $NaBH_4$ and PVP were dissolved in deionized water to form aqueous solution of $AgNO_3$ (0.1 M), $NaBH_4$ (0.01 M) and PVP (0.01 M), respectively. The aqueous solutions of PVP (0.01 M) and $NaBH_4$ (0.01 M) were mixed at a volume ratio of 1:1. About 500 ml of this solution was transferred to a beaker, and agitated with a magnetic stirrer (85-2, Shanghai Scientific Instruments Co., Ltd., Shanghai, China) before adding the $AgNO_3$ (0.1 M) solution. Upon addition of silver nitrate drop by drop, the colorless solution of $NaBH_4$-PVP slowly changed from yellow to pale brown indicating the formation of silver nanoparticles. Glycerol (Shanghai Chemical Reagent Company, Shanghai, China) was added to the solution of silver nanoparticles as a plasticizer (1.5/100 ml) and the solution was stirred over a magnetic stirrer for 15 min under magnetic stirring at ambient temperature. The final practical silver concentration 0.06 mg $l^{-1}$ for coating was used, which is safe to consumer according to USEPA (2001).

Absorption spectra of the solution of silver nanoparticles were taken with a UV–vis spectrophotometer (UV-240, Shimadzu Corporation, Kyoto, Japan) at a rate of 50 nm $min^{-1}$. Quartz cells had a path length of 1 cm.

Micrographs of silver nanoparticles were obtained with a transmission electron microscope (H7000, Hitachi, Tokyo, Japan). A drop of the silver dispersion was placed on a carbon-coated copper grid, which was allowed to dry before observation under the microscope.

### 2.2. Plant material and handling

Freshly harvested green asparagus (*A. officinalis* L. cv. 'UC800') spears were obtained from a commercial farm in Suzhou, Jiangsu Province, China. The spears were cut at ground level between 8:00 and 9:30 AM, placed in crushed ice and transported to the laboratory within 3 h on the day of harvest. Straight, undamaged spears, 8–20 mm in diameter and 22 cm in length with closed bracts were used.

The green asparagus was submerged in a 100 mg $l^{-1}$ sodium hypochloride solution for 15 min at room temperature and briefly dried by ambient air at ambient temperature to remove surface liquid. The asparagus was then immersed in the coating solution for 3 min at room temperature and drained. The treated asparagus was dried in a cold-air draft for 10 min (10 m $s^{-1}$ and 15 °C) for 10 min. Non-coated asparagus served as the control for this study. All the asparagus samples were stored for up to 25 days at 2 and 10 °C with 90–95% relative humidity (RH). Both the control and coated samples were analyzed at a 5-day interval from the day of coating. Twelve spears were used in each experiment, and the tests were conducted in triplicates.

### 2.3. Measurement of weight loss and ascorbic acid

Weight loss was determined by weighing the samples on a digital balance (FA/JA, Shanghai precision & Scientific Instrument Co., Ltd., Shanghai, China) and was reported as percentage loss based on the original mass.

Ascorbic acid in asparagus was determined according to AOAC official method (AOAC, 1995). In brief, a 5 g sample of asparagus was blended with 50 ml of metaphosphoric-acetic acid solution to extract ascorbic acid. The mixture was centrifuged (at $4000 \times g$ for 5 min), and then the supernatant was taken and transferred to a volumetric flask. It was rapidly titrated with indophenols solution until light distinct rose pink color persisted for more than 5 s.

### 2.4. Total chlorophyll and crude fiber contents

Chlorophyll was extracted from 5 g macerated spears by homogenizing it in 20 ml of 80% acetone with a tissue

homogenizer (DS-1, Shanghai Sample Model Factory, Shanghai, China) at 35000 rpm for 30 s (Zhang, De Baerdemaeker, & Schrevens, 2003). The homogenate was filtered through four layers of cheesecloth, centrifuged at $15\,000 \times g$ for 15 min, and absorbance (Abs.) was read at 647 and 664.5 nm with an UV–vis recording spectrophotometer (UV-754, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). The total chlorophyll content was calculated by chlorophyll ($\mu g\,g^{-1}$) = 17.95 × Abs. at 647 nm + 7.9 × Abs. at 664.5 nm (Inskeep & Bloom, 1985; Zhang, Huan, Tao, & Wang, 2001). The crude fiber content of the spears was determined each four days of storage according to Sosa-Coronel, Vest, & Herner (1976).

### 2.5. Firmness

The firmness was determined as the maximum shear force measured by a texture analyzer (TA.XT2i, Stable Micro Systems Ltd., Godalming, UK) with a HDP/VB shear cell. The shear force was measured at three different locations of a spear, namely, 20 cm (apical), 10 cm (middle) and 3 cm (lower) from the base. We expected that the increasing lignin content from the tip to the base of the stalk should cause the texture to change along asparagus spears. The descending speed of the probe and the strain to penetrate the asparagus were $1.0\,mm\,s^{-1}$ and 90%, respectively. The measured peak force in $g$ served as a direct indication of firmness.

### 2.6. Color measurements

A Shenguang colorimeter (WSC-S, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) was used to determine the exterior color changes for the surface of asparagus spears. The color changes were quantified by the tristimulus color values ($L^*, a^*, b^*$). $L^*$ refers to the lightness of the spear, and ranges from black = 0 to white = 100. A negative value of $a^*$ indicates green, while a positive one indicates red–purple color. Positive $b^*$ indicates yellow and negative blue color (McGuire, 1992). The hue angle [$h^\circ = \tan^{-1}(b^*/a^*)$] was calculated from $a^*$ and $b^*$ values (Lancaster, Lister, Reay, & Triggs, 1997). The deviation from the raw material color was represented as $\Delta E$, and calculated as $\sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$, where $L_0$, $a_0$ and $b_0$ were the color parameters of fresh asparagus spears. The meter was calibrated using the manufacturer's standard white plate ($L^* = 95.73$; $a^* = -0.68$; $b^* = 3.25$). On each spear, three readings were taken at 1, 2, and 3 cm from the tip (Siomos, Dogras, & Sfakiotakis, 2001).

### 2.7. Microbial analysis

Thirty grams of asparagus were removed aseptically from each treatment. The sample was then homogenized in peptone saline solution ($8.5\,g\,l^{-1}$ NaCl + $1\,g\,l^{-1}$ peptone (Oxoid, L34)) for 1 min in a stomacher (S400, Shanghai Scientific Instrument Co., Ltd., Shanghai, China). After dilution, series were made in peptone water, the samples were plated on the media as follows: (1) plate count agar (PCA, OxoidCM325) for total aerobic psychrotrophic count and incubated at 30 °C for 72 h; (2) Sabouraud media (Oxoid CM41) for yeast and moulds. The Petri dishes were incubated at 25 °C for 120 h.

### 2.8. Statistical analysis

Experimental data were analyzed using the SAS analysis of variance test (ANOVA) procedure (SAS Institute, Cary, NC, USA). Significant differences among mean values were identified using least significant difference at $p < 0.05$.

## 3. Results and discussion

### 3.1. Characterization of silver nanoparticles

Fig. 1 shows a typical UV–vis absorption spectrum of the coating solution containing silver nanoparticles. A broad emission peak was centered at about 420 nm. The presence of this peak was the result of the surface plasmon resonance of silver nanoparticles (Zheng, Wang, Xu, Wu, & Xu, 2003). According to Fritsche, Porwol, & Wiegand (1998), the emission peak at 400 nm corresponded to the silver particles of less than 5 nm diameters. Increasing silver particles would shift peaks to longer wavelengths. Based on the position of the emission peak and the relation between the width of shift and the size of particles, we estimated that the diameter of the silver nanoparticles prepared in this research was about 20 nm. This estimation was confirmed by transmission electron micrograph (TEM) imaging of the silver nanoparticles (Fig. 2). It was clear from Fig. 2 that the morphology of silver nanoparticles was

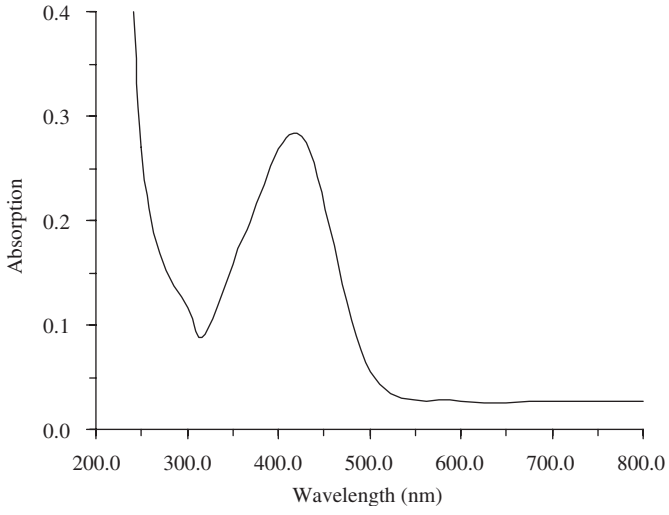


Fig. 1. Typical UV–vis absorption spectrum of the solution of silver nanoparticles dispersion.

almost spherical with a mean diameter around 15–25 nm. This result was in agreement with the other research about the characteristics of silver nanoparticles prepared with the chemical reduction method (Li, Ji, & Xie, 2004). The results shown in Figs. 1 and 2 revealed the evidence for the formation of silver nanoparticles in the coating solutions prepared under the experimental condition. The solutions with PVP formed a thin coating on the surface of asparagus when water evaporated, leaving the nanoparticles evenly distributed in the coating matrix.

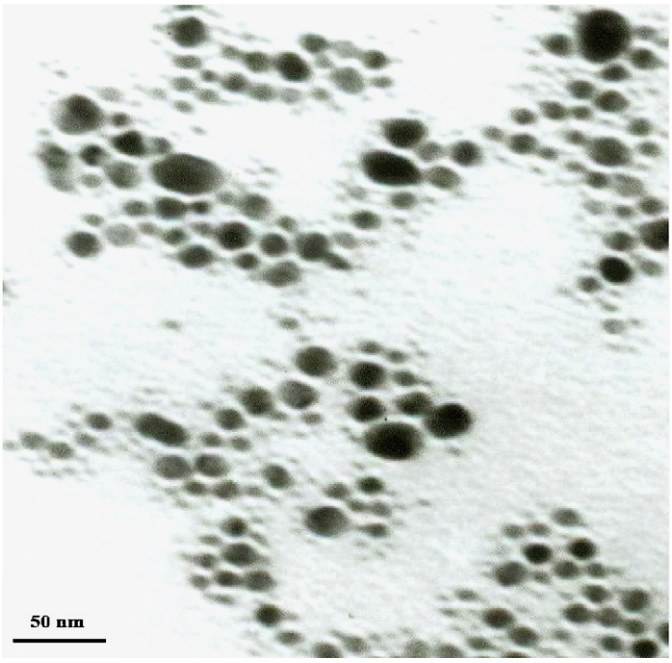


Fig. 2. Transmission electron microscopy (TEM) of silver nanoparticles ( × 100,000).

### 3.2. Weight loss, ascorbic acid, total chlorophyll and crude fiber

The changes of weight loss, ascorbic acid, total chlorophyll and crude fiber contents in the green asparagus samples stored at 2 and 10 °C are shown in Fig. 3. Over the 25 days of storage, the weight losses were reduced from 9.2% and 13.8% in controlled samples to 1.9% and 4.6% in the coated asparagus at 2 and 10 °C, respectively (Fig. 3A). The coating significantly reduced the weight loss over the storage period at both temperatures. The largest weight loss reduction was obtained from coated application of silver nanoparticles-PVP at the end the storage time of asparagus (Fig. 3A).

Significant increases ($p < 0.05$) in ascorbic acid loss after treatments took place at 2 °C during the storage time from 5 to 25 days but at 10 °C only for the storage time of 20 and 25 days (Fig. 3B). The reduction (76.3%) in controlled samples was larger than that (52.0%) in the coated samples

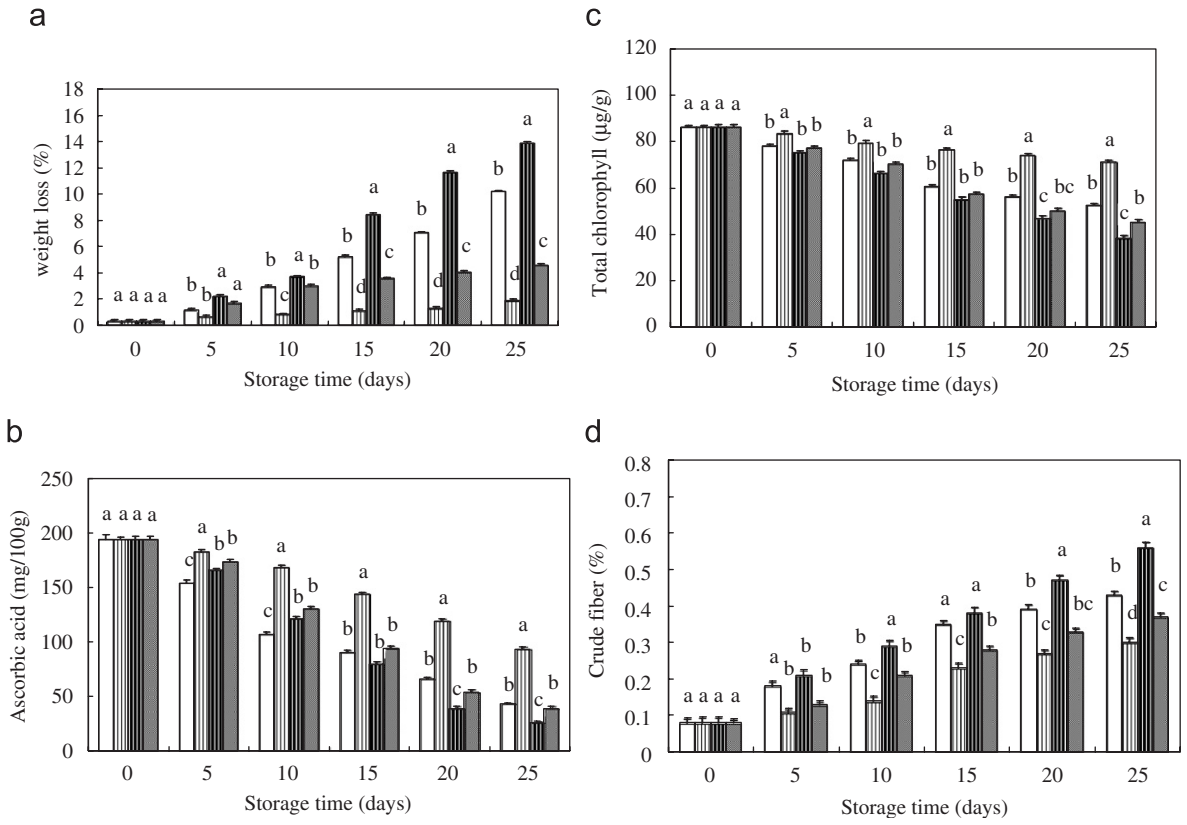


Fig. 3. Changes of weight loss (A), ascorbic acid (B), total chlorophyll (C) and crude fiber (D) in green asparagus stored at 2 and 10 °C. Control, stored at 2 °C □. Coated, stored at 2 °C (▥).Control, stored at 10 °C (▮). Coated, stored at 10 °C (▩). Different letters within the same storage day indicate that means are different at the 0.05 level of significance.

over the 25-day storage at 2 °C. When increasing the storage temperature to 10 °C, the reduction of the ascorbic acid loss increased to 86.9% and 78.1% in controlled and the coated samples, respectively.

Significant differences ($p<0.05$) between the coated asparagus and the control samples in total chlorophyll content of the green spears were observed after stored at 2 °C from 5 to 25 days but at 10 °C only for 25 days (Fig. 3C). The presence of silver nanoparticles-PVP coating had a positive effect on chlorophyll content only at 2 °C, this is probably because chlorophyllase or dioxygenase is more active under higher temperatures and chlorophyll is easy to be degraded in this condition (Heaton & Marangoni, 1996).

Asparagus with silver nanoparticles-PVP coating had lower crude fiber content compared to the control samples over the whole 25-day storage at 2 and 10 °C (Fig. 3D). This reducing effect of silver nanoparticles-PVP coating on crude fiber is the same as the former study by modified atmosphere packaging in white asparagus (Siomos et al., 2001).

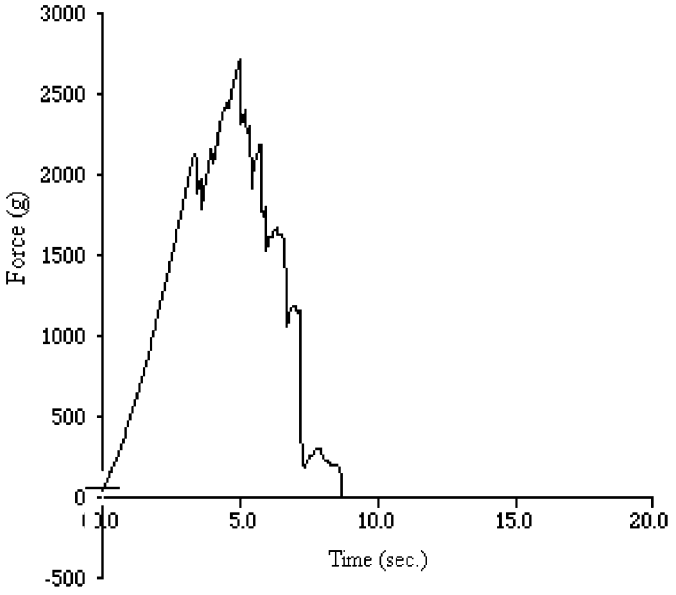


Fig. 4. Typical force/time curves for the middle section of green asparagus.

### 3.3. Firmness

The firmness is an important factor used by consumer to assess asparagus quality (Siomos, Sfakiotakis, & Dogras, 2000). Fig. 4 shows a typical force/time curve for the middle section of a green asparagus spear. The maximum shear force from this curve indicates the firmness (or tenderness) of the tested sample. Fig. 5 summarizes changes of the firmness of the middle section of green asparagus spears stored at 2 or 10 °C. The firmness of the middle section with the apical and lower sections (data not shown) all increased with storage time. The increases in the firmness of asparagus spears were positively correlated with observed increases in crude fibers (Fig. 3D). This increase was due mainly to lignin development and the activity of the phenylalanine-amonialyase (PAL), which decreases in concentration from the lower to the apical section (Lau, Tang, & Swanson, 2000; Lipton, 1990). Regardless of the storage temperature (i.e., 2 or 10 °C), after being stored for 15 days the middle section of the coated asparagus was less tough or tender (lower shear force) ($p<0.05$) than the control samples (data not shown). The same trend was observed in the apical and lower sections.

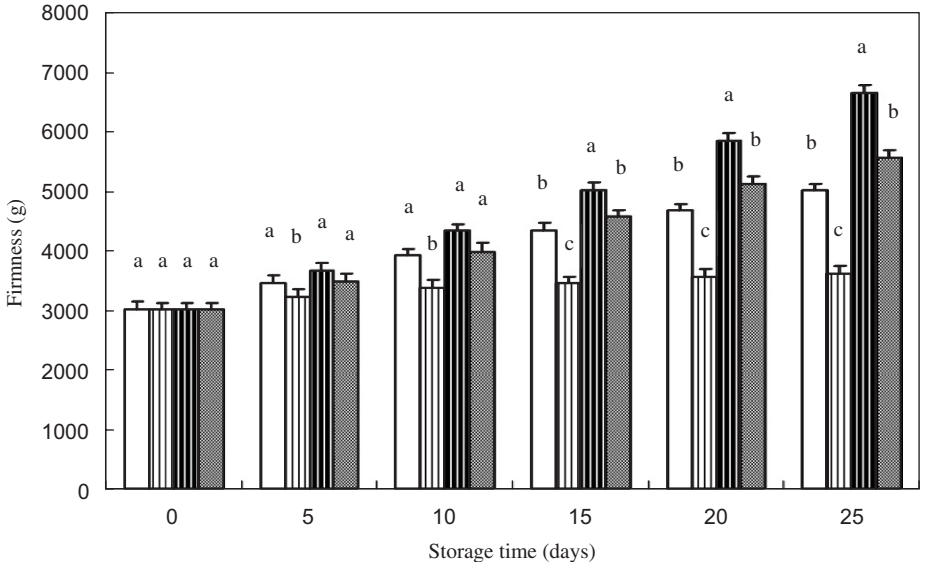


Fig. 5. Changes of firmness on green asparagus (middle section) stored at 2 and 10 °C during storage. Control, stored at 2 °C □. Coated, stored at 2 °C (▥).Control, stored at 10 °C (▮). Coated, stored at 10 °C (▩). Different letters within the same storage day indicate that means are different at the 0.05 level of significance.

### 3.4. Color

An increase in the total color difference $\Delta E$ and a decrease in hue angle were observed with storage time. The storage time-related changes in $\Delta E$ were significant with both coated and control samples ($p<0.05$). The statistical results showed significant differences ($p<0.05$) in $\Delta E$ after being stored for 15 days but not in hue angle values between the coated and control samples (Fig. 6). The total color difference $\Delta E$ for the coated asparagus changed at a much lower rate than in the control samples. Overall, hue angles changed from about 130° (a 180° hue angle indicating blue green) to about 100° (a 90 hue angle indicating pale yellow), with coated samples showing slightly, but significantly, smaller reductions. The reduction of hue angles of the samples correlated well with the reduction of total chlorophyll concentration over the storage (Fig. 3C). From both results, coating demonstrated certain degree of beneficial effect on the reduction of color changes in asparagus.

### 3.5. Microbial accounts

The predominant microflora which influence the shelf life of vegetables are psychrotrophic bacteria (Garcia-Gimeno & Zurera-Cosano, 1997; Hotchkiss & Banco,

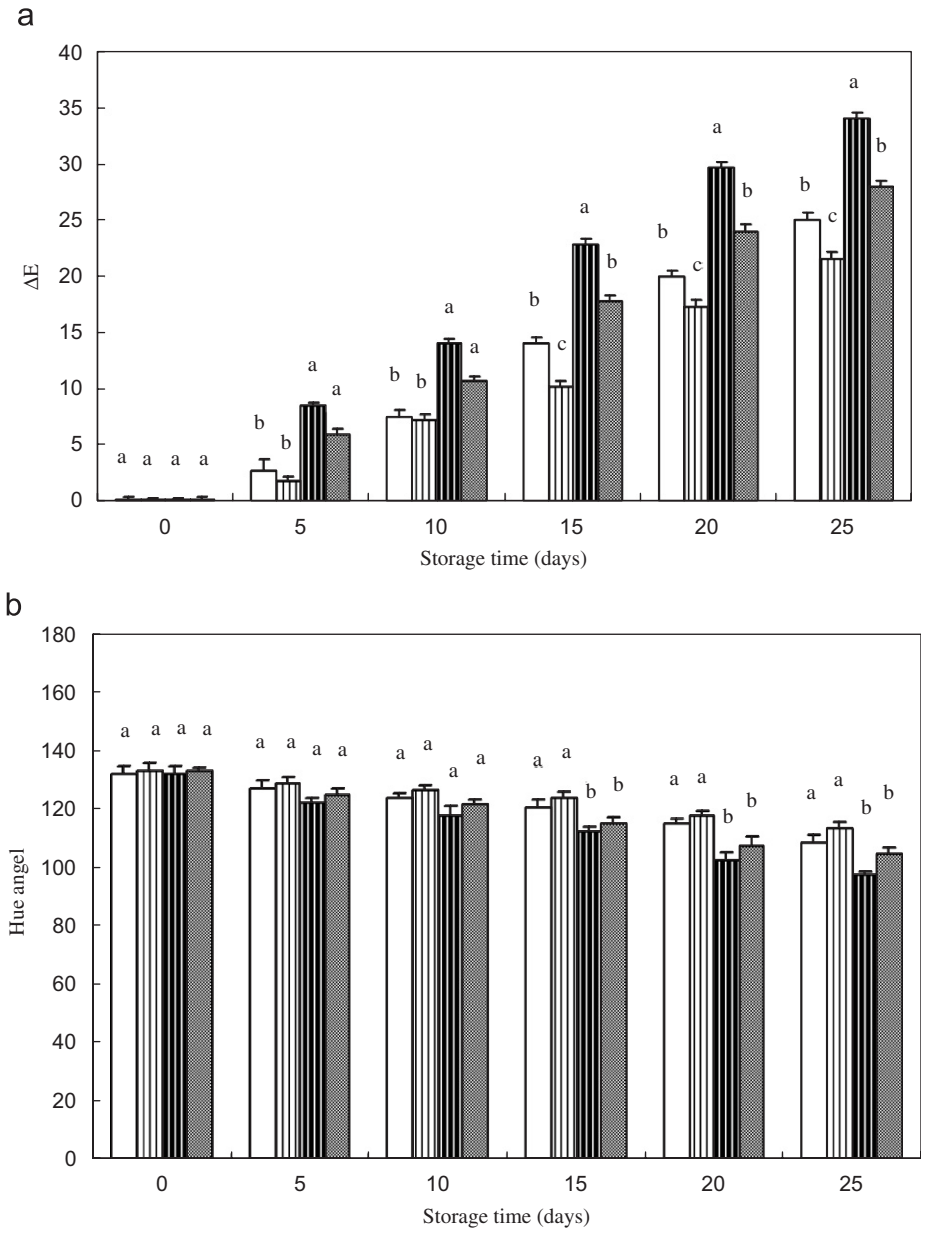


Fig. 6. Changes of $\Delta E$ (A), hue angel (B) on green asparagus stored at 2 and 10 °C during storage. Control, stored at 2 °C (□). Coated, stored at 2 °C (▥).Control, stored at 10 °C (▮). Coated, stored at 10 °C (▩). Different letters within the same storage day indicate that means are different at the 0.05 level of significance.

1992). Changes in the total aerobic Psychrotrophic count, total number of yeasts and moulds in asparagus stored at 2 and 10 °C are shown in Fig. 7. At both temperatures, the silver particles-PVP coating significantly hindered the increase in total aerobic psychrotrophic count compared with the control samples (Fig. 7A). Similar effect of coating was observed in reducing the growth of yeasts and moulds during the storage (Fig. 7B). At the end of 25-day storage, visually apparent differences were observed between coated asparagus and the control samples ($p<0.05$) at both temperatures.

According to our previous studies (An et al., 2007; Li & Zhang, 2006), green asparagus (cv. 'UC800') could retain good quality in storage up to 14–15 days at the normal cold storage temperature 1–3 °C. The corresponding values for the quality parameters, such as weight loss, ascorbic acid, total chlorophyll, crude fiber, firmness, color, and micro-organism counts, of asparagus after 15-day storage at 2 °C were used in this study as the references for evaluating the extended storage time for the coated samples. It is clear from Fig. 3 that weight losses of the coated samples stored both at 2 and 10 °C on the 25th day were less than that of the control asparagus stored at 2 °C for 15 days (Fig. 3A). Ascorbic acid content in the coated samples stored at 2 °C on the 25th day was comparable to the control asparagus stored at 2 °C for 15 days (Fig. 3B). The total chlorophyll of the coated samples stored at 2 °C was stable over the 25-day storage period, while the crude fiber of the same group samples was much lower than that of the control asparagus stored at the same temperature for 15 days (Fig. 3C and D). Similar observation was made with firmness results.

The color of the coated samples, however, changed more rapidly than the above mentioned quality parameters. Measured $\Delta E$ values of the coated samples stored at 2 and 10 °C at the 20th day were higher than the that of the control stored at 2 °C for 15 days (Fig. 6A), while the hue angle of the coated samples stored at 2 °C on the 20th day was similar to that the control stored at 2 °C for 15 days.

At 2 °C, the coating was able to maintain aerobic psychrotrophic count, as well as yeasts and moulds counts accounts in samples stored over 25 days to a level much less than that in the control stored for 15 days (Fig. 7A and B).

Based on the above analyses, it is apparent that the silver nanoparticles-PVP coating was able to extend the shelf-life of green asparagus spears by about 10 days at 2 °C. It should, nevertheless, be noted that this research was exploratory in nature. More research is needed to assess the effect of nano-silver particles to human health, and the acting principle of nano-silver particles is also to be studied in further.

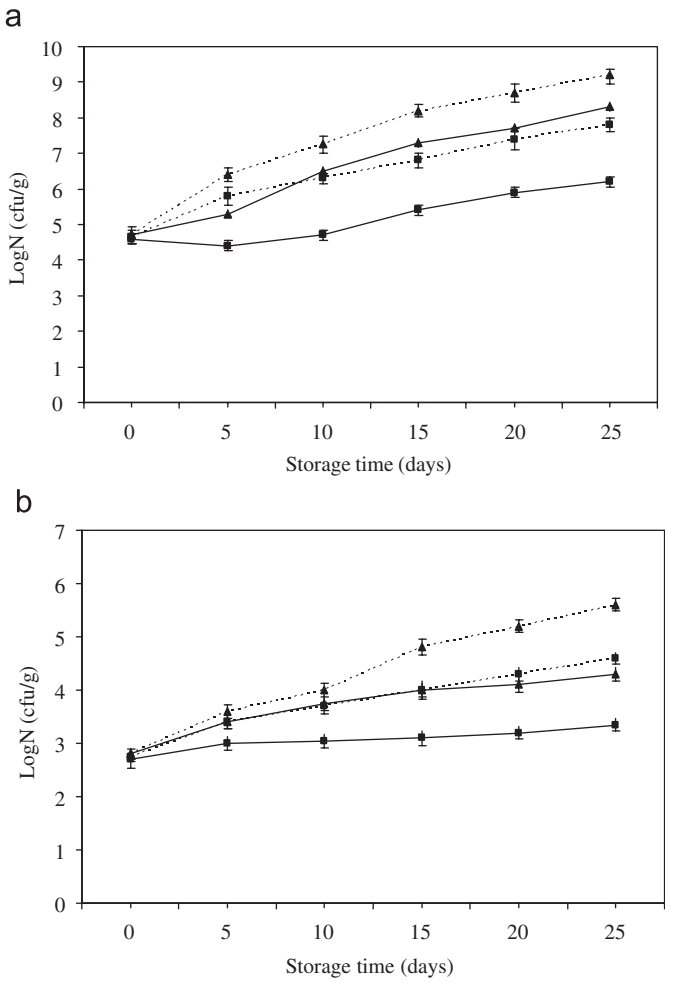


Fig. 7. Total aerobic psychrotrophic count (A), yeasts and moulds (B) on asparagus stored at 2 and 10 °C. Control, stored at 2 °C (—▲—). Coated, stored at 2 °C (—■—). Control, stored at 10 °C (- - -▲- - -). Coated, stored at 10 °C (- - -■- - -).

## 4. Conclusion

Applications of silver nanoparticles-PVP coating to green asparagus were shown to be beneficial in keeping the quality of the storage. Coating of silver nanoparticles-PVP slowed down the weight loss, ascorbic acid and total chlorophyll, reduced the color changes in the skin of asparagus, inhibited the increasing of the tissue firmness, the growth of microorganism and increased the shelf-life of asparagus by about 10 days at 2 °C.

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