

Effects of Packaging Materials and Disinfectants on Quality Changes of Ceylon Spinach (*Basella alba* L.) during Storage

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Abstract

The objectives of the study were to determine the effective washing disinfectant (chlorine dioxide and peracetic acid) in reducing total aerobic counts, *E. coli* and coliform, and then to test different packaging materials, namely polypropylene (PP), high density polyethylene (HDPE), and low density polyethylene (LDPE), on the quality and shelf-life of Ceylon spinach. The results showed that washing Ceylon spinach with 100 ppm peracetic acid solution for five minutes significantly reduced *E. coli* and coliform counts (reduction of 1.1 and $>2 \log \text{CFU g}^{-1}$, respectively). Ceylon spinach packed in HDPE material might optimally prolong the shelf-life up to 7 days whereas spinach packed in LDPE was recommended if used within 6 days at $10 \pm 1^\circ\text{C}$.

Keywords

Ceylon spinach, microbial counts, packaging materials, peracetic acid, quality, storage

Introduction

Basella alba L. is an important green leafy vegetable commonly found in the tropical regions of the world (Deshmukh & Gaikwad, 2014; Varalakshmi, 2016). In Vietnam, there are more than 100 industrial sectors of vegetable production with a total capacity of 300,000 tons each year (MARD, 2018). Fresh vegetables are sold in local markets, supermarkets, and retailers, etc. in Vietnam. Most of the vegetables marketed are produced as primary production and are rinsed and stored under ambient temperatures in local markets or refrigerated conditions in supermarkets. For convenience and safety reasons, it is necessary to produce ready-to-use vegetable products as minimally processed vegetables, especially *Basella alba* (Ragaert *et al.*, 2007; Kakade *et al.*, 2015).

Minimally processed vegetables, which includes vegetables being selected, washed and dried, and packaged with sealed. These steps may not be efficient to eliminate contamination

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on minimally processed vegetables (Graça *et al.*, 2017). Hence, washing vegetables with disinfectants could potentially reduce or eliminate the microbial loads on vegetable products as much as possible (Tong *et al.*, 2015; Tong *et al.*, 2018). Chlorine (the most common disinfectant) and peracetic acid (an alternative disinfectant) have been studied intensively on vegetables (Arturo-Schaan *et al.*, 1996; Li *et al.*, 2001; Allende *et al.*, 2004; Jin & Lee, 2007). Though the use of chlorine is reasonable regarding cost and efficiency, the chlorination process has some negative effects, such as the formation of carcinogenic by-products, skin irritation, and negative respiratory tract effects (Tong, 2015). Unlike chlorine, peracetic acid is harmless and produces environmentally friendly residues; it is a promising and widespread substitute for chlorine.

In addition to using sanitizing treatments as chemical interventions in the reduction of microbial loads on vegetables, packing vegetables in plastic containers as a physical intervention could be inacted to extend the shelf-life of minimally processed vegetables. The storage of the minimally processed vegetables can be combined with refrigeration in order to reduce the respiration rate and inhibit microbial growth (Barbosa *et al.*, 2011). However, the types of packaging materials and packaging methods also partly affect the quality changes and the shelf-life of products during storage (Suryawanshi, 2008; Costa *et al.*, 2011; Muizniece-Brasava *et al.*, 2013), particularly for vegetables (Piagentini & Güemes, 2002; Jacobsson *et al.*, 2004), meat (Kotzekidou & Bloukas, 1996), and fish products (Ly & Tong, 2017).

The objectives of the present study were to determine the most effective disinfectant in reducing the total aerobic counts of *Escherichia coli* (*E. coli*) and coliform on Ceylon spinach vegetables (*Basella alba*) in preliminary treatments and identify the proper packaging materials that can minimize the changes in quality and organoleptic properties of Ceylon spinach vegetables during storage.

Materials and Methods

Experimental design

Decontamination efficacy

Ceylon spinach (*Basella alba*) was grown on a farm-scale in Chau Thanh A district, Hau Giang province, Vietnam and was harvested after 30-35 days of cultivation. The Ceylon spinach plants (35-50cm in length) were harvested from January to April 2018 and transported to the laboratory at the Food Technology Department, Can Tho University. Preparation and disinfection were carried out in a cleanroom at 28-30°C. Three separate experiments were repeated independently. The white rust, downy mildew, and yellow leaves were removed prior to washing the plants in tap water (for 60 seconds) to remove the soil, sand, and foreign matters. The excess water on the surface of the Ceylon spinach was drained manually using a salad spinner (Japan) for one minute. Thereafter, the Ceylon spinach was dipped in chlorine dioxide (5%, Thailand; pH adjusted to 6.5 via acetic acid) or peracetic acid (12%, Korea) at a concentration of 100ppm for five minutes with the ratio of 1:10 of fresh-cut spinach and the solution. Then, the Ceylon spinach was rinsed with potable water (commercial drinking water produced by Can Tho University) with the same ratio used with the disinfectant for one minute to remove any disinfectant residues and centrifuged for another minute.

In each experiment, the decontaminated Ceylon spinach was compared to the unwashed and water washed samples. Effective decontamination was evaluated based on the reduction of microbial counts, i.e. total mesophilic counts, *E. coli* and coliform counts, and organoleptic quality.

Storage of the Ceylon spinach

Similar to the procedure described above, the Ceylon spinach plants were washed in the selected disinfectants and were packed in different packaging materials: PP (polypropylene, thickness of 25µm), HDPE (high density polyethylene 67%, thickness of 35µm), or LDPE (low density polyethylene, thickness of 40µm) with package sizes of

18x21cm. Each independent experiment, twenty-four sealed bags of the Ceylon spinach plants packed on three above materials weighing 50g each were placed in a refrigerator (Sanaky, Vietnam) at $10 \pm 1^\circ\text{C}$. The control samples were placed in plastic disks but were not sealed, and then stored in the same refrigerator. The three different storage experiments were repeated independently.

Analytical methodology

Physical and chemical qualities of the Ceylon spinach

The initial weight and storage weight of each sample were determined on the sampling day prior to analysis using a balance (OHAUS, China). The extent of spoilage was calculated by weighing the amount of spoiled leaves from each stored sample.

The pH of the Ceylon spinach was determined by an electronic pH meter (Vernier, USA).

The vitamin C content (mg/100g dry weight) of the Asian spinach was analyzed by titration methods with 2,6-dichlorophenol-indophenol reagents.

The color of the whole leaves was measured using a spectrophotometer-colorimeter (model NR60CP, China). The overall color difference (ΔE) in color spaces L^* , a^* , and b^* was calculated according to the following equation (1):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (1)$$

where L^* , a^* , b^* and L , a , b are the measured values of the samples on day 0 and day of sampling, respectively.

Microbiological analysis

A 15g composite sample collected from different parts of each spinach sample was transferred aseptically to a Stomacher bag by means of sterile scalpels and tweezers. One hundred thirty-five (135) mL of sterile Maximum Recovery Diluent (MRD; Merck, Darmstadt, Germany) was added and the mixture was homogenized for 1min. A tenfold serial dilution was made from the sample in the MRD. The total aerobic mesophilic counts

(TMC), total yeasts and molds, and total coliform and *E. coli* counts were determined by pour plating the decimal dilutions onto Plate Count Agar (PCA, India, Himedia), Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, Darmstadt, Germany), and Chromocult® Coliform Agar ES (Merck, Darmstadt, Germany), respectively. The pour plates were incubated for 2-3 days at 37°C to determine the TMC and total yeasts and molds, and for 24h to determine the total coliform and *E. coli*. Microbial counts were reported as log CFU g^{-1} of tissue.

Sensory evaluation

After the spinach samples were washed with one of the two disinfectants mentioned above at a concentration of 100ppm for 5min (the preliminary treatment), the sensory quality of the spinach samples, general appearance, color, odor, and texture, were evaluated by 18-19 panelists who participated in triangle tests. After being washed with a disinfectant, the samples were rinsed in tap water for one minute before the sensory tests. In all cases, a set of three samples of the vegetable (each sample *ca.* 50g), randomly coded with 3-digit numbers, was presented to each panelist. The panelists were told that two of the samples were similar and were asked to identify the odd sample. The number of correct answers was compared to the number expected by using a statistical table to achieve a significant answer (5% level) according to the BS ISO 4120:2004.

The sensory evaluation of the Ceylon spinach during storage was implemented by scoring methods. The spinach was scored on a scale from 0 to 5, with 0 being the lowest and 5 being the highest. A descriptive table with the lowest and highest quality attributes was provided. Each assessment was carried out by ten panelists and they were also asked to state whether or not the spinach was acceptable. This was used to determine the shelf-life of the products.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using the Statgraphics Centurion 15.1 program (U.S.A.). LSD (Least

Significant Difference) test was applied for pairwise comparisons of means at $P \leq 0.05$. All results were reported as the mean values \pm standard deviations.

Results and Discussion

Effects of the chlorine dioxide and peracetic acid disinfectants on the microbial counts of the spinach

Chlorine dioxide and peracetic acid are considered alternative sanitizers because of their strong oxidation capacities and lower risk of forming harmful by-products (Monarca *et al.*, 2002; Silveira *et al.*, 2008). Both disinfectants are safe and create environmentally friendly residues (Demirci & Ngadi, 2012).

The antimicrobial effects of chlorine dioxide and peracetic acid (PAA) were evaluated on the Ceylon spinach (**Figure 1**). The initial total mesophilic counts (TMC), coliform counts, and *E. coli* counts on unwashed Ceylon spinach were 7.1 ± 0.12 , 4.9 ± 1.23 , and 2.9 ± 1.02 log CFU g^{-1} , respectively. The TMC on the Asian spinach washed in tap water, chlorine dioxide, and PAA were 6.9 ± 0.41 , 6.4 ± 0.81 , and 6.5 ± 0.06 log CFU g^{-1} , respectively. The coliform counts on the Ceylon spinach washed in tap water, chlorine dioxide, and PAA were 4.7 ± 0.7 , 4.1 ± 1.14 , and 3.8 ± 0.3 log CFU g^{-1} , respectively. The *E. coli* counts on the Ceylon spinach washed in tap water, chlorine dioxide, and PAA were 1.6 ± 1.13 , 1.1 ± 0.15 , and <1 log CFU g^{-1} , respectively. These data indicate that the microbial counts on Ceylon spinach washed in tap water (control samples) were not reduced significantly. The chlorine dioxide and peracetic acid solutions were not effective in reducing TMC but significantly reduced coliform and *E. coli* counts on the Ceylon spinach. Surprisingly, a significant reduction of *E. coli* was observed (detection limits, <1 log CFU g^{-1}) when the Ceylon spinach was washed with peracetic acid. This result was probably because peracetic acid has a stronger oxidation capacity and is less affected by organic matter than chlorine dioxide (Kitis, 2004). The obtained results are in agreement with the previous study of Sapers (2001) who

reported that cucumbers washed in chlorine dioxide water resulted in less than 1 log unit of various microbes (i.e. total aerobic counts, lactic acid bacteria, yeast and molds) on product surfaces. Additionally, the microbial reductions for aerobic bacteria coliform, yeast and molds on fresh-cut celery, cabbage, and potatoes washed in 80ppm of peracetic acid were less than 1.5 log units (Joshi *et al.*, 2013).

The sensory qualities of the Ceylon spinach samples after being washed with chlorine dioxide or peracetic acid were evaluated using triangle tests. The results in **Figure 2** show that the Ceylon spinach samples washed with the two solutions were not significantly different compared to those washed with tap water. The number of correct responses was lower than the required number of correct responses to achieve a significant difference. Based on these results, peracetic acid is recommended as a suitable sanitizer to wash Ceylon spinach for further storage.

Physical and chemical changes of the spinach during storage

Weight loss and rate of spoilage during storage

The weight loss and rate of spoilage of the spinach were measured by the change in weight of the samples over the storage period at $10 \pm 1^\circ\text{C}$. The percent of weight loss and rate of spoilage of the Ceylon spinach are shown in **Figure 3** and **Figure 4**, respectively. The highest weight loss (17.28%) and almost complete spoilage (99.24%) were observed in the control samples (not packed) after 3 days. The direct contact of the Ceylon spinach with cool air resulted in a rapid loss of moisture during storage. After 7 days of storage, the weight loss (6.89%) and rate of spoilage (44.21%) of the Ceylon spinach packed in LDPE were higher than the samples packed in PP (2.56% and 22.53%, respectively) and HDPE (4.27% and 12.53%, respectively). It is well known that the rate of water vapor transmission of LDPE is higher than the other packaging materials, thus, the samples packed in LDPE had a higher loss of moisture and higher spoilage rate (Piagentini & Güemes,

2002). In addition, the loss of moisture can cause undesirable changes in color, soft structure, and loss of nutritional quality (Nagar *et al.*, 2012).

Color and pH changes of the spinach during storage

The color changes of the spinach during storage shown in **Table 1** reveal that the overall

color difference ($\Delta E = 2.94$) of the control samples was not different with the samples contained in the PP and HDPE packages ($\Delta E = 1.95$ and 1.94 , respectively) at day 3. The statistical results also showed that all three packaging materials used did not have an impact on the overall color difference of the Ceylon spinach during storage at $10 \pm 1^\circ\text{C}$.

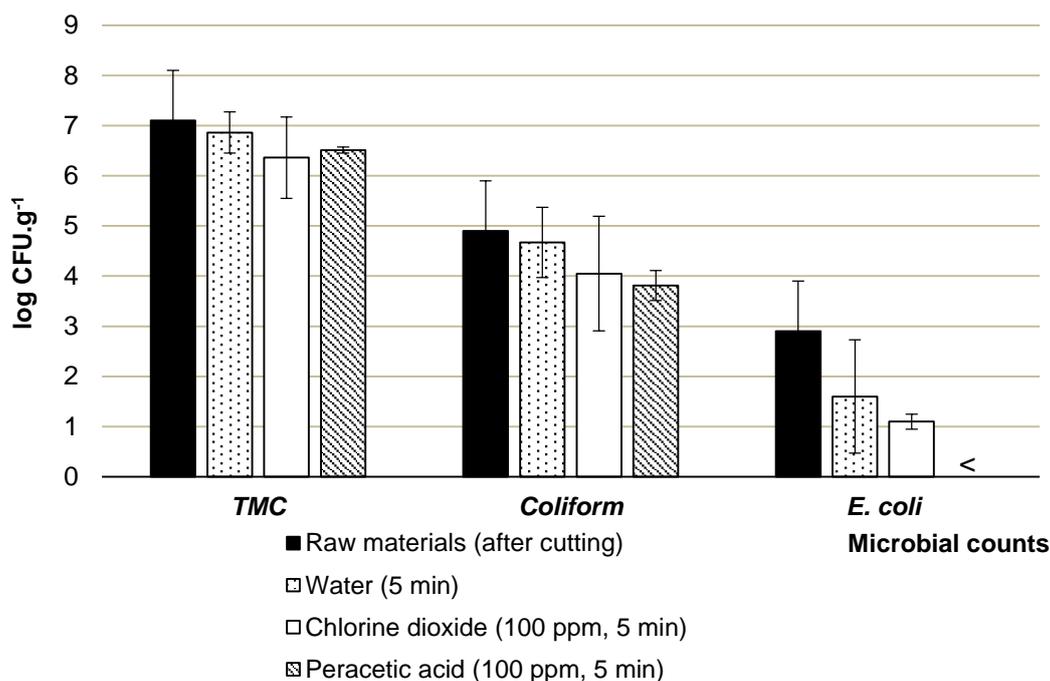


Figure 1. Microbial counts on the Ceylon spinach after being washed with water, chlorine dioxide, or peracetic acid (PAA)

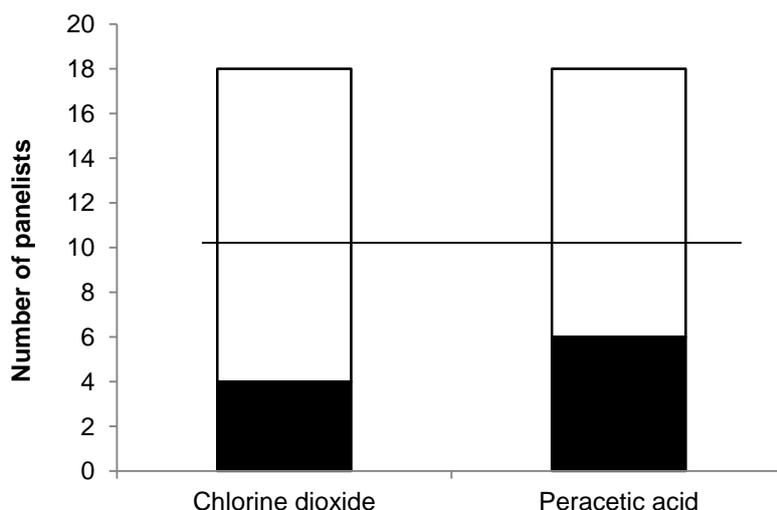


Figure 2. Sensory test of Ceylon spinach treated with chlorine dioxide or peracetic acid water at 100ppm for 5 minutes. □ number of panelists choosing the wrong sample as the odd sample, ■ number of panelists choosing the correct sample as the odd sample. The line (–) shows the minimum number of correct answers needed to have a significant difference in sensory qualities between series ($\alpha = 0.05$).

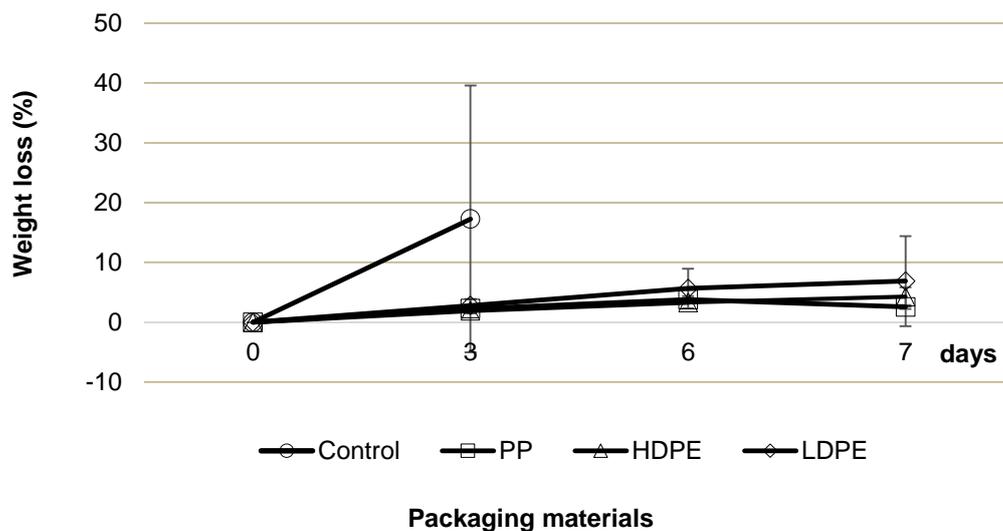


Figure 3. Weight loss of the Ceylon spinach during storage

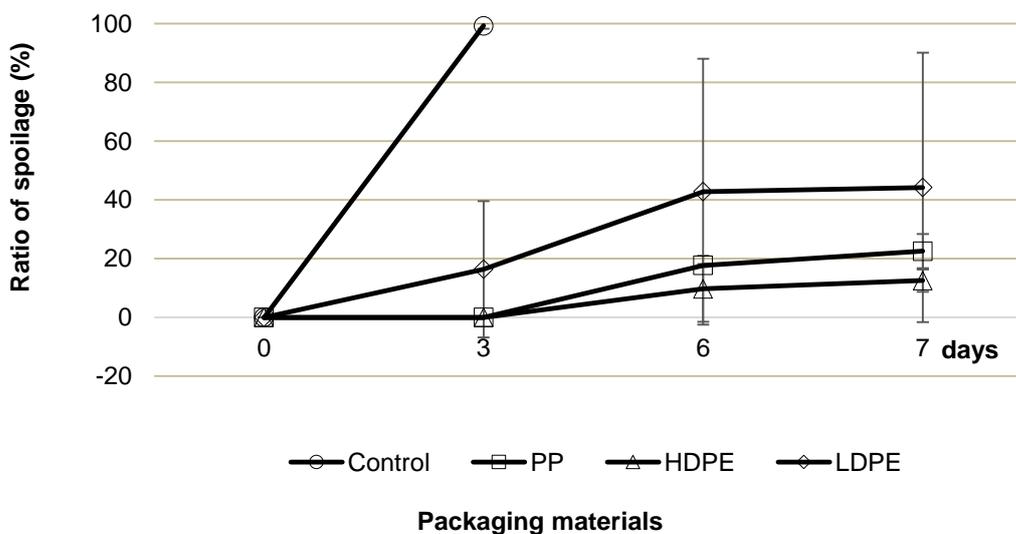


Figure 4. Rate of spoilage of the Ceylon spinach during storage

The effects of the packaging materials on the pH value of the spinach during storage at $10 \pm 1^\circ\text{C}$ are shown in **Figure 5**. The initial pH value of the spinach was 6.4 ± 0.14 . The pH values ranged from 6.36 to 6.5 during storage. The type of packaging material did not influence the pH value of the Ceylon spinach during storage.

Vitamin C changes of the spinach during storage

Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. More than 90% of vitamin C in the human diet is supplied by fruits and vegetables, especially green leafy

vegetables (Lee & Kader, 2000; Citak & Sonmez, 2010). In this experiment, changes in vitamin C contents of the Ceylon spinach were also determined during storage (**Table 2**).

Generally, a decrease in the vitamin C content of the Ceylon spinach was observed in all the packed samples after 7 days at $10 \pm 1^\circ\text{C}$ (**Table 2**). The vitamin C content of the unpackaged samples reduced rapidly after 3 days (loss of 82%). Although the packaging materials delayed the loss of vitamin C of the Ceylon spinach during storage, the vitamin C content was not significantly different among the three packaging materials. At day 7, the loss of vitamin C of the Ceylon spinach was 78-86% compared to day 0. Gil *et al.* (1999) found that the decrease of vitamin C of fresh-cut spinach both in air and MAP packaging was up to 50% after 3 days of storage at 10°C .

Microbial quality of the spinach during storage

Total aerobic mesophilic counts

The results of the total aerobic mesophilic counts (TMC) of the spinach during storage at $10 \pm 1^\circ\text{C}$ (with a relative humidity of 44-60%) in different packaging materials are shown in **Figure 6**. The initial total mesophilic counts of the Ceylon spinach were from 7.0 to 7.3 log CFU g^{-1} . This is in agreement with previous studies that showed initial TMC ranged from 2 to 8 log CFU g^{-1} (Lee *et al.*, 2013; Ottesen *et al.*, 2013; Gu *et al.*, 2018). Samples washed with peracetic acid (100ppm, 5min) had initial TMC values (at day 0) of 5.3 ± 0.4 log CFU g^{-1} . As recommended by Debevere *et al.* (2006), the microbiological criteria of TMC for fresh vegetables was targeted at 5 log CFU g^{-1} at the end of shelf-life, and an acceptable limit of 8 log CFU g^{-1} . Therefore, the initial microbiological

Table 1. Effects of the packaging materials on the overall color difference (ΔE) of the Ceylon spinach during storage at $10 \pm 1^\circ\text{C}$

Day	Overall color difference (ΔE)			
	Control	PP	HDPE	LDPE
0	0 ^a	0 ^a	0 ^a	0 ^a
3	$2.94^b \pm 0.23$	$1.95^{ab} \pm 0.49$	$1.94^{ab} \pm 1.14$	$1.72^a \pm 0.13$
6	NA	$2.25^a \pm 1.59$	$1.22^a \pm 0.53$	$1.52^a \pm 0.60$
7	NA	$1.72^a \pm 0.13$	$0.99^a \pm 0.56$	$1.01^a \pm 0.21$

Note: Unpackaged samples served as the control; Values with the same letter within the same row are not significantly different at $P < 0.05$; NA: Not analyzed.

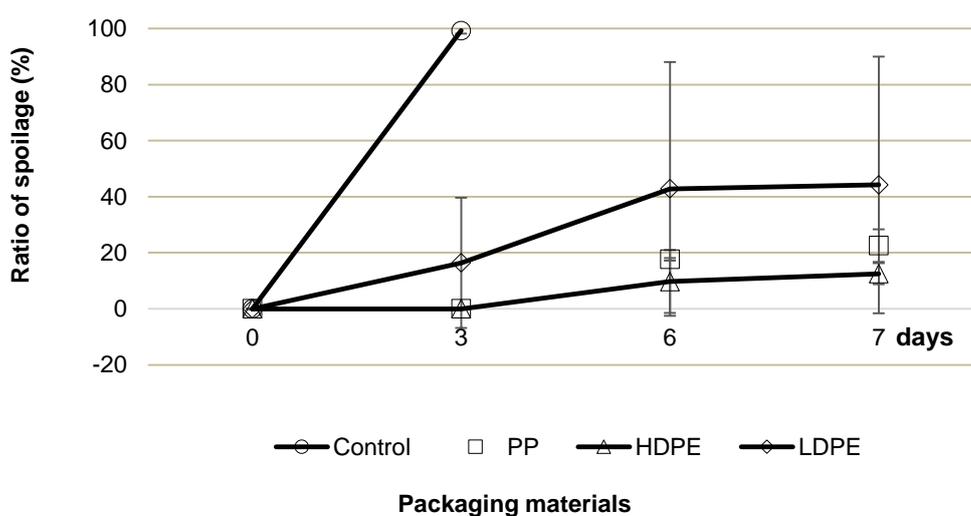


Figure 5. Changes in the pH of the Ceylon spinach during storage

Table 2. Changes in vitamin C contents of the Ceylon spinach in different packaging materials during storage at $10 \pm 1^\circ\text{C}$

Day	Changes in vitamin C (mg/100g dry weight)			
	Control	PP	HDPE	LDPE
0	30.79 ^a ± 2.77	30.79 ^a ± 12.77	30.79 ^a ± 12.77	30.79 ^a ± 12.77
3	5.53 ^a ± 3.36	22.55 ^a ± 14.70	16.05 ^a ± 10.30	15.36 ^a ± 12.46
6	NA	8.07 ^a ± 1.06	5.93 ^a ± 2.76	6.21 ^a ± 2.17
7	NA	6.42 ^a ± 2.27	6.54 ^a ± 1.28	4.06 ^a ± 2.38

Note: Unpackaged samples served as the control; Values with the same letter within the same row are not significantly different at $P < 0.05$; NA: Not analyzed.

quality of the Asian spinach was acceptable. No stored samples exceeded $8 \log \text{CFU g}^{-1}$ after 6 days of storage. The shelf-life of the samples in the PP and HDPE packages can be extended to day 7 with TMC counts of 7.8 and $7.7 \log \text{CFU g}^{-1}$, respectively.

Total yeasts and molds

Generally, yeast and mold counts of the samples in all the packaging materials increased gradually during storage at $10 \pm 1^\circ\text{C}$ (Figure 7). The initial counts of yeasts and molds were $3.6 \pm 0.7 \log \text{CFU g}^{-1}$. This was in line with the study of Debevere *et al.* (2006) who reported a tolerance limit of $4 \log \text{CFU g}^{-1}$. During storage, all the samples showed rapid growth of yeasts and molds after day 3. Particularly, the total yeasts and molds of the samples in the PP package increased rapidly during storage. At day 7, the yeast and mold counts in the PP were $5.2 \pm 0.1 \log \text{CFU g}^{-1}$, whereas the counts in the HDPE and LDPE packaging were 4.5 ± 0.4 and $4.4 \pm 0.6 \log \text{CFU g}^{-1}$, respectively. The recommended acceptable limit of yeasts and molds is $5 \log \text{CFU g}^{-1}$ (Debevere *et al.*, 2006). The Ceylon spinach stored in LDPE as well as HDPE can be maintained up to 7 days at $10 \pm 1^\circ\text{C}$ before becoming unacceptable for human consumption.

Sensory evaluation

Sensory evaluation was implemented on the characteristics of the Asian spinach to evaluate the color, odor, texture, and overall changes during storage (Figure 8). At day 0, the color, odor, and texture of the Asian spinach showed very high acceptability with scores of 4.6–4.8. Rapid changes in terms of the sensory aspects were observed in the control samples during storage (scores of 2.1–3.4 at day 3).

The spoilage of vegetables may result from the degradation of physiological and sensory characteristics (color, texture, and odor) as well as from microbiological degradation (Kakade *et al.*, 2015). In this study, the sensory quality had a high correlation with the chemical and microbiological quality. The samples stored in the PP and HDPE packages (both had an overall score of 4) were better than those packed in LDPE (overall score of 3). According to the results of this study, packaging the Ceylon spinach in LDPE at $10 \pm 1^\circ\text{C}$ is recommended if the spinach is used within 6 days, while packaging the Ceylon spinach in the PP or HDPE packages extends the shelf-life up to 7 days. More importantly, after 7 days of storage, the Ceylon spinach in the HDPE package had better quality compared to those packed in PP based on the microbiological quality and spoilage rate. Therefore, the HDPE package is suggested to prolong the quality of the Ceylon spinach during storage.

Conclusions

Ceylon spinach should first be decontaminated with peracetic acid (100ppm for 5 minutes) before being packed then stored at $10 \pm 1^\circ\text{C}$. The results indicated that the use of PP or HDPE packages might optimally prolong the shelf-life of the Ceylon spinach up to 7 days. The Ceylon spinach in the LDPE packaging is recommended if the produce is used within 6 days, while the Ceylon spinach in HDPE had better quality compared to those packed in PP based on the microbiological quality and spoilage rate. Therefore, the HDPE packaging material is recommended for storage of Ceylon spinach at $10 \pm 1^\circ\text{C}$.

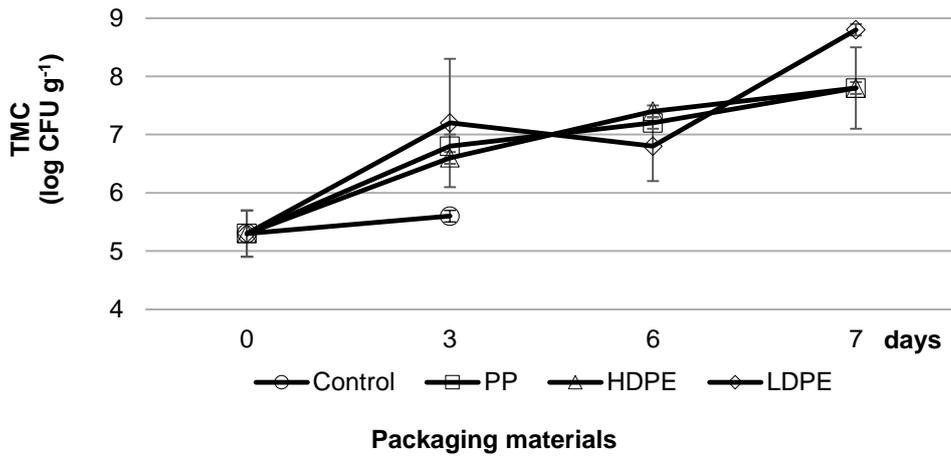


Figure 6. Total aerobic mesophilic counts (TMC) on the spinach during storage

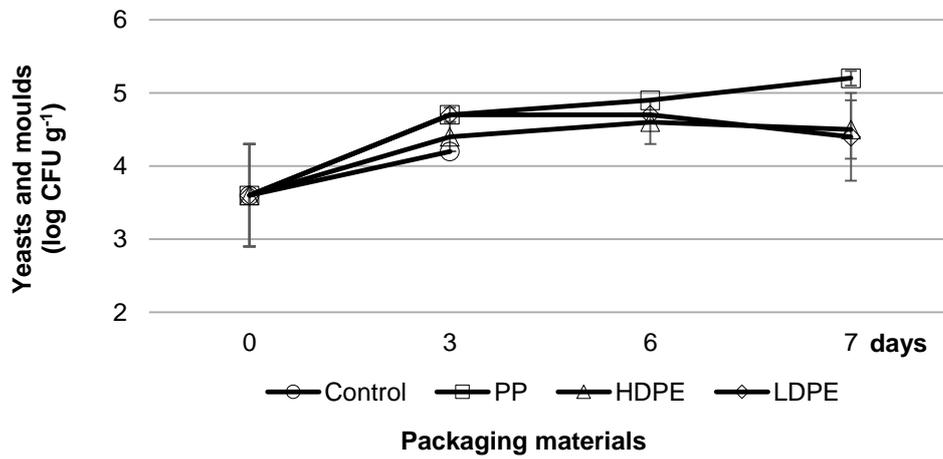
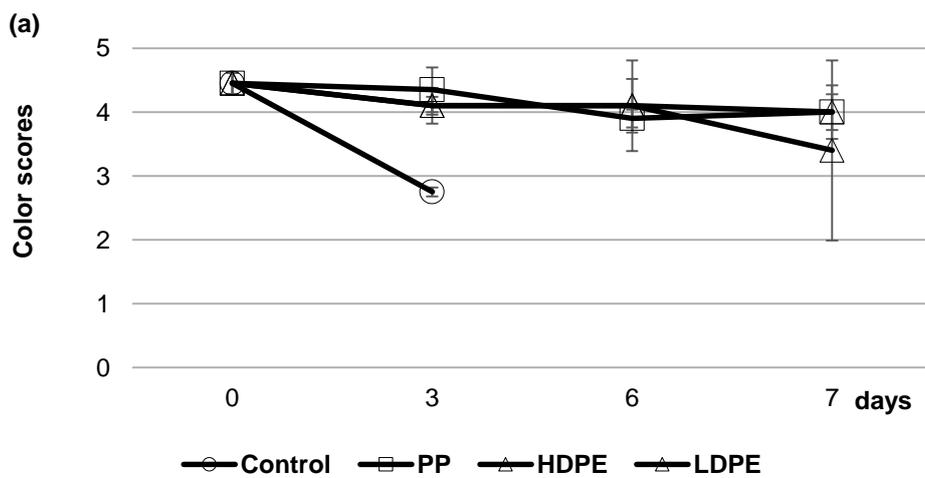


Figure 7. Total yeast and mold counts of the Ceylon spinach during storage



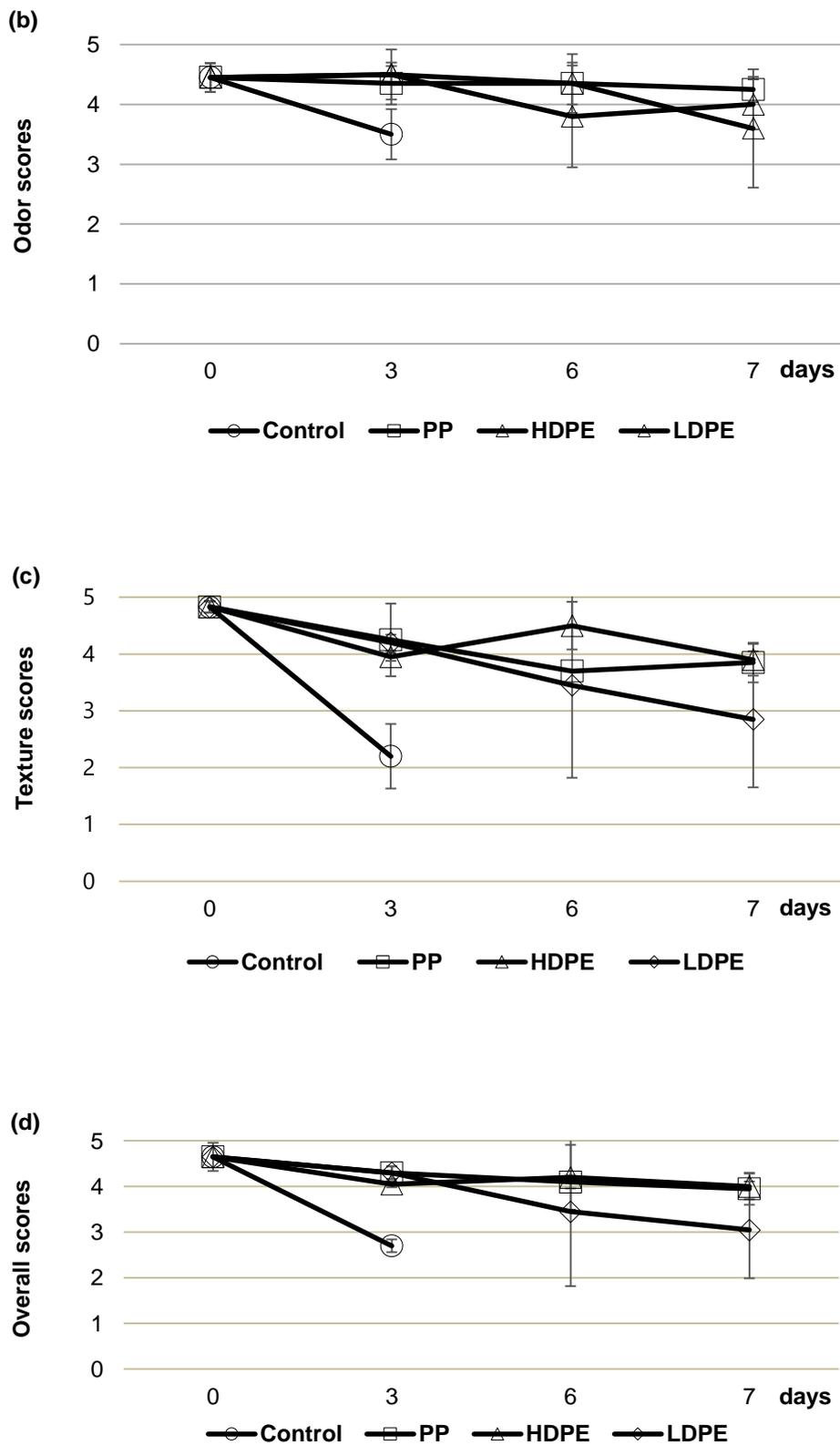


Figure 8. Sensory changes of (a) color, (b) odor, (c) texture, and (d) overall attributes of the Ceylon spinach during storage

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