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Effect of plastic films with different oxygen transmission rate on shelf-life of fresh-cut bok choy (*Brassica rapa* var. *chinensis*)

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Abstract

Freshly harvested bok choy (*Brassica rapa* var. *chinensis*) was minimally processed and packaged in three different kinds of plastic bags: (1) oriented polypropylene (OPP) perforated film (10 holes, 5 mm in diameter), (2) polyethylene (PE) film with a high oxygen transmission rate (OTR) of 3,200 ml.m^{-2}.day^{-1}, and (3) polyethylene (PE) film with a very high OTR of 6,000 ml.m^{-2}.day^{-1}. Results indicated that at 5^{o}C, the level of film OTR significantly affected the gas compositions in the package headspace, and consequently the quality of fresh-cut bok choy. The modified atmosphere created by the 3,200 OTR PE bag consisted of 3.9-4.9% CO_2 and 7.8-9.9% O_2 and the 6,000 OTR PE bag consisted of 2.3-3.1% CO_2 and 12.3-14.8% O_2. The atmospheres reached equilibrium after 3-4 days of storage, and they maintained this level throughout the storage period. The perforated OPP bag showed no difference in CO_2 or O_2 levels when compared to the outside air, suggesting that there was no modification occurred to the atmosphere. Changes in quality including weight loss, color (lightness, yellowness, and hue), chlorophyll content, sensory quality, and microbial growth were least in the 3,200 OTR PE film followed by the 6,000 OTR PE film and the perforated OPP film, respectively. The shelf-life of fresh-cut bok choy packaged in a 3,200 OTR PE bag was 25 days, compared with 20 days in the 6,000 OTR PE bag, and only 10 days for the perforated OPP bag.

Introduction

Fresh-cut vegetables are minimally processed by washing, sorting, peeling, and slicing. They are then packaged with plastic film or on a plastic tray wrapped with film (Kader, 2002). When fresh vegetables are cut in the preparation process, plant cells are ruptured, which induces physiological changes, such as increased respiration rate, ethylene production rate, and cut surface browning (Smyth *et al*., 1998). The susceptibility of fresh-cut vegetables to shriveling, yellowing and decay are the main factors leading the short shelf-life (Shen *et al*., 1999).

Low temperature and modified atmospheres in packaging can be beneficial for maintaining quality and extending of fresh cut vegetables shelf-life. A wide variety of vegetables have been kept under either low temperature storage or modified atmosphere packaging (MAP) systems, or in a combination of both (Kim *et al*., 2004; Able *et al*., 2005; Jia *et al*., 2009). It is well established that low temperature reduces the respiration rate and senescence, as well as the growth of spoilage microorganisms (Roura *et al*., 2000). However, optimum storage temperature depends on commodity types. Under MAP systems, the decreasing oxygen (O_2) and increasing carbon dioxide (CO_2) concentrations in the package atmosphere could be achieved by the interaction between the respiratory O_2 uptake and CO_2 production of the produce, and by the gas transfer from the packaging film (Zagory and Kader, 1988). Selecting a packaging film with a suitable oxygen transmission rate (OTR) plays a critical role in developing an effective MAP for fresh-cut vegetables.

Bok choy (*Brassica rapa* var. *chinensis*), also called pak choy or Chinese white cabbage, is becoming a highly valued fresh-cut vegetable. Its white stalks bear a strong resemblance to celery while the dark green crinkly leaves are similar to Romaine lettuce (Lu, 2007). It has a light, sweet flavor, crispy texture, and high nutritional values, rich in minerals and vitamins, and so it is highly popular with consumers. Conversely, the vegetable is highly perishable after harvest and vulnerable to the fresh-cut process (Able *et al*., 2003; Lu, 2007).

Some studies had been done on application of MAP system under low temperature storage on fresh-cut bok choy. O'Hare *et al*. (2001) reported that 0.5-2% O_2 and 2-10% CO_2 could extend shelf-life of bok choy, retarding the sugar depletion rate and

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consequently, yellowing of the leaves. Lu (2007) investigated the packaging effects on the quality of fresh-cut bok choy stored at 10°C. MAP flushed with 2% CO_2 and 5% O_2 in a polyethylene bag showed a reduction of respiration rate and minimized senescence over that in a perforated oriented polypropylene bag. However, it is rare to find a study investigating the effects of film OTR on package atmosphere in relation to the quality of fresh-cut bok choy. The objective of this study was to determine the effect of plastic film OTR on the quality of fresh-cut bok choy. Effects of OTR on sensory quality and microbial count, and hence on product shelf life are reported here.

Materials and Methods

Materials preparation

Bok choy (*Brassica rapa* var. *chinensis*) was freshly harvested at 6 weeks after planting from Siam Future Farm Co., Ltd. Chiang Rai Thailand. The vegetable was trimmed, washed in 200 ppm chlorine solution at 10°C, and centrifuged to remove excess water. Samples (250 g) were packaged in sealed 20 cm x 25 cm bags prepared with oriented polypropylene (OPP) film with perforations (10 holes, 5 mm in diameter), which served as the control. The other two types of film were polyethylene with high OTR (3,200 $ml.m^{-2}.day^{-1}$) and very high OTR (6,000 $ml.m^{-2}.day^{-1}$). The film OTR was determined by the film manufacturer (MTEC, NSTDA, Thailand) at 23°C, 0% RH using a MOCON apparatus according to an ASTM procedure (ASTM, 1986). At 5°C, the film OTRs were determined following the procedure from Moyls *et al.* (1992). The packaged samples were transferred to the laboratory within 1 hour and stored at 5°C and 90% RH for 25 days for the subsequent analysis. There were three sample bags for each type of film and the experiment was repeated three times.

Respiration rate

The measurement was carried out over 10 days at 5 and 25°C in a flow-through system. The bok choy samples (100 g each) were placed in glass containers. The containers were flushed with CO_2 and placed in humidified air (~90% RH) at a flow rate of 120 ml/min. The flow rate was calculated to permit the accumulation of CO_2 to~0.3%. The rate of CO_2 production was determined from 5 ml gaseous samples taken from the inlet and outlet port of the containers and analyzed by gas chromatograph (Agilent 7890N, Agilent technologies, USA). The gas chromatograph was equipped with 80/100 Heyesap Q and molecular sieve columns and a thermal chromatography detector (200°C), using nitrogen as the carrier gas.

Package atmosphere analysis

The CO_2 and O_2 concentration in the package headspace was measured. Silicone rubber was applied to the outer top surface of the bag and a needle sensor was attached to the Gas Analyzer (PBI Dansensor Check Mate 9900, Denmark). It penetrated the seal on the bag and the gas concentration was recorded.

Weight loss

Three sample packages from each package type were weighed and recorded on the day of packing and every five days of storage thereafter. The cumulative weight losses of the packages were expressed as percentage loss of original weight on the day of packaging.

Color

A colorimeter was used to measure the color in a CIELAB colorimetry system (Hunter Lab, UK). In order to have homogeneous color samples, the measurements used were only obtained from the green part of the bok choy leaf. The color coordinates ranged from L^* = 0 (black) to L^* = 100 (white), $-a^*$ (greenness) to $+a^*$ (redness), and $–b^*$ (blueness) to $+b^*$ (yellowness). A Minolta standard white plate (X = 83.6, Y = 81.2, Z = 93.8) and a black plate were used to standardize the instruments. The hue value was converted from a^* and b^* values (hue angle = $\tan^{-1} (b^*/a^*)$). The color measurements were taken in triplicate from each sample.

Chlorophyll content

Bok choy (about 2 g) were homogenized in 80% acetone/water ($v.v^{-1}$). The extract was filtered and the final volume was adjusted to 100 ml with the same 80% acetone/water solution. The filtered solution was used for the spectrophotometric determination of chlorophyll a (Chl a) and chlorophyll b (Chl b) at wavelength of 662 and 645 nm, respectively. These pigment amounts were calculated according to the formulas of Litchtentaler and Wellburn (1983) and expressed as milligrams per gram of fresh weight ($mg.g^{-1}$).

Microbiological analysis

The total microbial count was determined every 2 days of storage. A 25 g sample was added to 225 ml 0.1% of peptone water and then blended for 2 min using a stomacher. Serial decimal dilutions were made in dilution flasks with 90 ml 0.1% peptone solution. One ml sample solutions were poured in triplicate on PetrifilmTM aerobic count plates (3M, USA) and then incubated at 35°C for 48 h. Three samples of each group were analyzed. All counts were presented as average values over the samples.

Sensory evaluation

The fresh-cut bok choy's sensory quality was evaluated for discoloration intensity and off-odor on a 0–4 scale (0: none; 1: slight; 2: moderate; 3: strong and 4: severe). The acceptance in taste, texture and overall acceptability, were evaluated and scored on a 9-point hedonic scale (1: dislike extremely and 9: like extremely). The score of 6 was considered the limit of the bok choy salability. Samples were coded with three-digit numbers and presented in random order to 12 panelists. Training was given to the panel members on how to recognize and scale the quality attributes of the fresh-cut bok choy. Prior to each evaluation, the panel members were given reference samples to calibrate the scales.

Statistical analyses

The experiment was conducted by completely randomized design (CRD). The experiment was carried out two times. Three replications per treatment (packaging) per evaluation period were used. All determinations were conducted in triplicate. The mean values and standard deviations were calculated based on the data obtained. Statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (DMRT) (SAS, 2001). Differences were considered to be significant when the P-values were under 0.05.

Results and Discussion

Respiration rate

The respiration rate as determined by the amount of CO_2 produced is shown in Figure 1. The respiration rate estimated in term of CO_2 produced of the bok choy stored at 5°C ranged between 22-33 mg. kg-1. hr-1. This was low when compared to that of the samples stored at 25°C, over the first 5 days, which ranged from 68 to 130 mg. $kg^{-1}.hr^{-1}$. The respiration rate of bok choy can be classified as moderately high, which is similar to those of other green leafy vegetables (Hardenberg *et al*., 1986). Respiration rate is associated with product shelf-life potential as high rates of respiration are correlated with short shelf life (Watada *et al*., 1996). In the present study, a significant increase in respiration occurred at a high temperature of 25°C. The second peak of CO_2 was produced during days 6-10 of storage (147-224 mg. $kg^{-1}.hr^{-1}$) at 25°C storage. This was attributed to the increase in microbial activity resulting in the observed decay including brown discoloration and a development of off-odor and soft-rots. On the other hand, fresh-cut bok choy stored at a low temperature (5°C) showed a significantly lower respiration rate ($p<0.05$) and no microbial spoilage through 10 days.

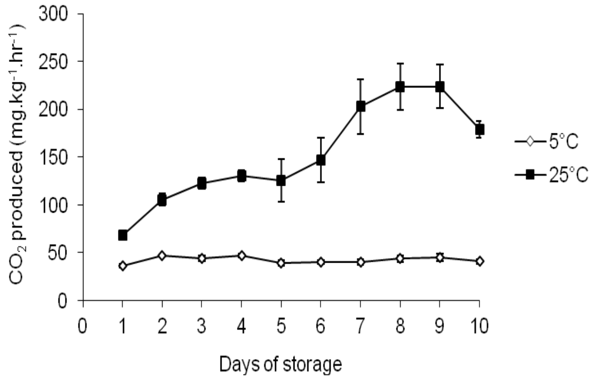

Figure 1. Respiration of fresh cut bok choy during storage at 5 and 25°C. Vertical bars represent ± S.D.

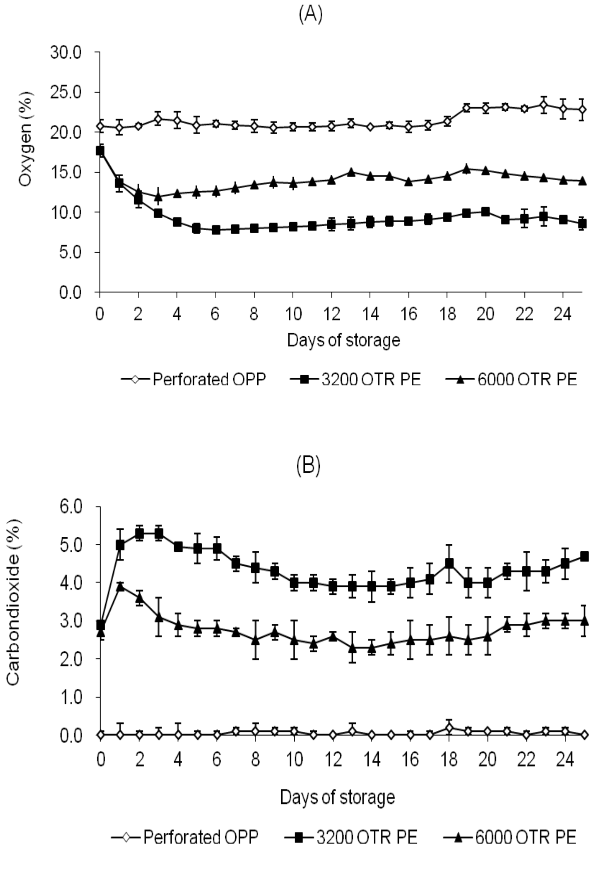

Figure 2. Oxygen (A) and Carbon dioxide (B) within package stored at 5°C. Vertical bars represent ±S.D.

Package atmosphere as affected by package film

Figure 2 shows the gas composition in the head space of different packages containing bok choy.

Table 1. Colors and chlorophyll content of fresh cut bok choy during storage at 5°C

Type of film	Days of storage						Days of storage					
	0	5	10	15	20	25	0	5	10	15	20	25
	Lightness						Chlorophyll a					
Perforated OPP	40.82a	42.94a	40.56b	40.29a	49.08a	49.37a	0.92	0.78	0.71	0.64	0.40	0.41
3200 OTR PE	40.82a	41.18a	39.00b	37.84a	41.31b	42.17b	0.92	0.98	0.77	0.77	0.65	0.71
6000 OTR PE	40.82a	41.65a	43.39a	40.58a	41.10b	47.48a	0.92	0.83	0.67	0.66	0.52	0.51
	Yellowness						Chlorophyll b					
Perforated OPP	5.57a	4.60a	4.48a	10.84a	16.81a	16.68a	0.29	0.31	0.27	0.25	0.18	0.21
3200 OTR PE	5.57a	3.38a	2.39b	6.84b	5.18b	6.55b	0.29	0.31	0.23	0.25	0.24	0.29
6000 OTR PE	5.57a	4.28a	6.19a	6.58b	5.49b	13.36a	0.29	0.30	0.23	0.24	0.21	0.23
	Hue angle						Total chlorophyll					
Perforated OPP	128.41a	132.46a	130.00a	115.43a	104.88a	100.48a	1.21	1.08	0.97	0.89	0.58	0.62
3200 OTR PE	128.41a	136.49a	144.12b	120.15b	128.81b	123.79b	1.21	1.30	1.01	1.02	0.89	1.00
6000 OTR PE	128.41a	132.94a	127.60a	123.83b	128.37b	111.29a	1.21	1.13	0.90	0.90	0.74	0.74

Data are means of replications Mean separation for each quality measured and day of storage by Duncan's multiple range test P≤0.05

The perforated OPP bag showed 20.6-21.4% O_2 and 0-0.03% CO_2, which was not different from the outside air, indicating that no modification occurred to atmosphere. A decrease in O_2 and an increase in CO_2 levels were observed in both bags. After 3-4 days of storage at 5°C, the O_2 concentration declined to 7.8-10.1% in the 3,200 OTR PE bag and to 12.0-15.4% in the 6,000 OTR PE bag, while the CO_2 concentration increased to 3.9-4.9% in the 3,200 OTR PE bag and to 2.4-3.1% in the 6,000 OTR PE bag. The relatively low O_2 and high CO_2 compared to the outside atmosphere indicated that both 3,200 and 6,000 OTR PE films modified the atmosphere within bags, and this was maintained throughout the 25 day storage period.

The modified atmosphere resulted in a reduced respiration rate of fresh produce. This could be explained by the inhibiting effects of CO_2 to enzymes actively participating in glycolysis and the Kreb's cycle (Peppelenbos and van't Leven, 1996). The concentrations of CO_2 (5-10%) and O_2 (1-3%) have been recommended for the storage of numerous green vegetables (Saltveit, 1997). These conditions efficiently retarded quality loss. In this study, the gas concentration in each MAP was not exactly within the recommended range. However, this was found superior for maintaining the quality of bok choy when comparing with control sample. In Lu (2007), package atmosphere conditions of 2% CO_2 and 5% O_2 were reported to minimize the senescence of fresh-cut bok choy to a significant extent.

Weight loss

The percentage weight loss of fresh-cut bok choy increased continuously during storage. The values observed in the perforated OPP bag increased substantially and reached the highest value of 4.8% on day 25, while in the 3,200 and 6,000 OTR PE bags changes were substantially lower throughout the storage period. At day 25, the percentages of weight loss were 0.39 and 0.55% in the 3,200 and 6,000 OTR PE bags, respectively. This difference was not significant (P>0.05). The high weight loss of the control sample was attributed to the dehydration and the loss of moisture through the bag perforations. Jia *et al.* (2009) reported a similar observation in that polyethylene bags without holes showed less weight loss than the bag with holes. In the present study, the 3,200 and 6,000 OTR PE bag without holes were better in retaining the weight loss than the OPP bag with holes.

Color

During storage, L^* and b^* increased while, the hue angle decreased (Table 1). From dark green to yellow, the color changes were most pronounced in the perforated OPP page, followed by the 6,000 OTR PE bag, and the 3,200 OTR PE bag, respectively. The L^* value, reflecting lightness of the bok choy, increased to a maximum value from 40.82 to 49.37 in the perforated OPP bag. It increased to 47.48 in the 6,000 OTR PE bag and to 42.17 in the 3,200 OTR PE bag, on day 25. The b^* value, reflecting yellowness, increased from 5.57 to 16.68 in the perforated OPP bag, to 13.36 in the 6,000 OTR PE bag, and to 6.55 in the 3,200 OTR PE bag. The hue angle slightly increased during the beginning of storage and then declined. On day 25 of storage, the hue angle values decreased from 128.41 to 100.48 in the perforated OPP bag, 111.29 in the 6,000 OTR PE bag, and 123.79 in the 3,200 OTR PE bag. The yellowing process of fresh-cut green vegetable was associated with both the decrease in hue angle and chlorophyll content (Toivonen and Brummell, 2008).

Chlorophyll content

Freshly harvested bok choy contained Chl a of 0.92 $mg.g^{-1}$ fresh weight, a Chl b of 0.33 $mg.g^{-1}$ fresh weight, and a total chlorophyll concentration of 1.21 $mg.g^{-1}$ fresh weight. During storage, the concentration of Chl a decreased by a greater extent than Chl b. Consequently, the total chlorophyll content gradually decreased to the lowest level on day 25 of storage (Table 1). Chlorophyll degradation of bok choy was highest when packed in perforated OPP bag, followed by the 6,000 OTR PE bag and the 3,200 OTR PE bag, respectively. Bok choy packaged in the perforated OPP bag showed a rapid decrease in Chl a to 0.41 $mg.g^{-1}$ fresh weight, in Chl b to 0.21 $mg.g^{-1}$ fresh weight, and a total chlorophyll content of 0.62 $mg.g^{-1}$ fresh weight. Results indicated that the ratio of Chl a to b decreased from 3:1 in the green leaves to 2:1 in the yellowed leaves. The reduction in Chl a was associated with the activity of Chlorophyllase which degrade the chlorophyll (Yamauchi and Watada, 1991).

Results of this study showed that the fresh-cut bok choy packaged in the 3,200 OTR PE bag had the least changes in the measured color values and chlorophyll content. This was in agreement with the lowest score for degree of discoloration as evaluated by the taste panelists. It was established that yellowing could be reduced by either storage under MAP conditions or low temperature storage (Shengmin, 2007). Ethylene accelerated the chlorophyll destruction through the enhancement of chlorophyllase activity (Shimokawa *et al.*, 1978). MAP system provided an atmospheric condition to limit ethylene production, therefore, limit yellowing or degreening of green fruits and vegetables. Able *et al.* (2005) reported that yellowing of bok choy leaves was associated with a depletion of sugar which is the main energy substrate. Low temperature storage significantly ($p<0.05$) slowed rate of glucose decline and, consequently retarded the yellowing of bok choy leaves with a minimal role of ethylene.

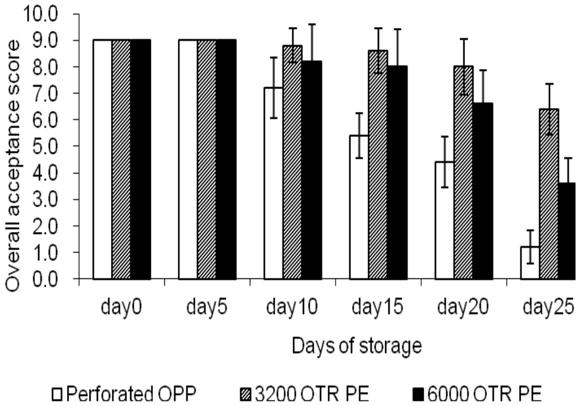

Figure 3. Overall acceptance score of fresh cut Bok choy during storage at 5°C. Vertical bars represent ± S.D.

Sensory quality

Evaluations on the extent of quality changes were performed at each storage time. Sample showed wilted yellow leaves by day 10 and onwards, and this was more marked at the end of storage. On day 25 of storage, the bok choy packaged in the perforated OPP bag and those packaged in the 6,000 OTR PE bag showed a strong discoloration with a score of 3.9 and 3.2, respectively (data not showed). On the contrary, the bok choy packaged in the 3,200 OTR PE bag showed a score of 1.8, indicating a slight discoloration.

An off-odor was first detected in the perforated bag on day 10. Bok choy packaged in the 3,200 and the 6,000 OTR PE bag did not develop an off-odor until day 15. The intensity of odor increased over time. On day 25, the intensity of off-flavor was 3.8, 1.7, and 2.9 for the samples in the perforated bag and the 3,200 and 6,000 OTR PE bag, respectively. Kim *et al.* (2004) reported that fresh-cut salad savoy packaged in low OTR film showed high intensity off-odor comparing with those of packaged in high OTR film. Under their conditions, the low OTR film exhibited a rapid depletion of O_2 to about 0%, contributing to the anaerobic respiration. This resulted in some volatile compounds, perceived as off-odor being produced. In the present study, a significant amount of O_2 (7.8-10.1%) was still observed in the 3,200 OTR PE bag; and therefore; no anaerobic respiration occurred. The detected off-odor, on the other hand, was high in the 6,000 OTR PE bag and in the perforated OPP bag. The detected off-odor might possibly be attributed to the decay of the vegetable under aerobic conditions.

For the acceptance test, samples were evaluated in terms of taste, texture and overall quality. During storage, a significant decrease in scores in overall sensory examination was observed in both the control and MAP samples (Figure 3). The score range for this test was 6, so the product above this score was still

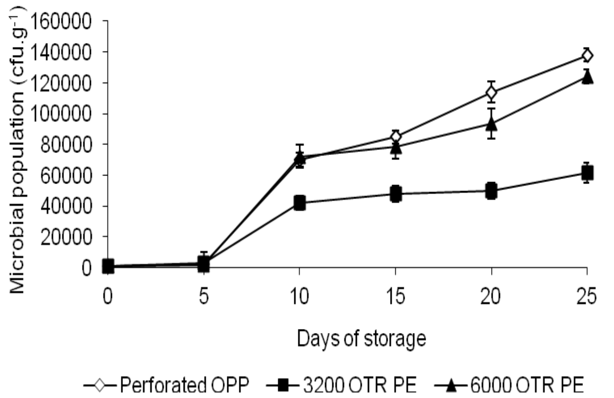

Figure 4. Total microbial count (cfu.g^{-1}) of fresh cut bok choy packaged in films with different OTR, and stored at 5°C. Vertical bars represent ± S.D.

acceptable. The loss of taste and texture contributed to the loss of the overall acceptance score. For overall acceptance, the fresh-cut bok choy packaged in the perforated OPP bag was still acceptable on day 10 (score 7.2), but at day 20, it was no longer acceptable (score 5.4). Those packaged in the 3,200 OTR PE bag were accepted until day 25 with a score of 6.4. Leaves in the 6,000 OTR PE bag were still acceptable on day 20 (score 6.2) but were rejected on day 25 (score 4.4). Overall, the 3,200 OTR PE bag was the most effective in retaining the inherent quality of the fresh-cut bok choy.

Total microbial count

Microbial populations increased over the storage period at 5°C (Figure 4). For the sample packaged in the perforated OPP bag, the colony counts substantially increased from 1.05x10^3 cfu.g^{-1} to 1.38x10^5 cfu.g^{-1} by day 25. However, leaves packaged in the 3,200 and 6,000 OTR PE film showed a lower increase in total bacteria count on day 25 of 6.15x10^4 and 1.24x10^5 cfu.g^{-1}, respectively.

The value of 10^6 cfu.g^{-1} is suggested by various authors as a microbiological value to determine the end of a product's shelf life (Gimeno and Cosano, 1997). In most countries, a criterion has been established whereby ready-to-eat products should contain less than 10^6 cfu.g^{-1} at the consumption stage, and that there should also be no pathogenic microorganism or their toxin, which are a serious risk to human health (Notermans *et al*., 1993). For the fresh-cut bok choy stored at 5°C, the acceptable period of storage time and microbial count was found to be as follows: the 3200 OTR PE bag (25 days) was 6.15x10^4 cfu.g^{-1}; the 6000 OTR PE bag (15 days) was 7.85x10^4 cfu.g^{-1}; and the perforated OPP bag (10 days) was 7.03x10^4 cfu.g^{-1}, respectively.

Conclusions

The combination of MAP and low temperature storage (5°C) for the fresh-cut bok choy effectively limited quality loss, including dehydration, yellowing, chlorophyll degradation, microbial growth, and sensory quality. This showed beneficial effects for retaining the quality upon storage and consequently, extending product shelf life to 10-15 days compared to control samples. Film oxygen transmission rates significantly affected the package atmosphere and the resulting quality under the tested conditions.

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