

## Shelf-Life Extension of Fresh-Cut Iceberg Lettuce (*Lactuca sativa* L) by Different Antimicrobial Films

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**Abstract** This study was conducted to investigate the antibacterial activity and shelf-life extension effect of iceberg lettuce packed in BN/PE film. The BN/PE film has a strong microbial suppression effect on pathogenic bacteria such as *Escherichia coli*, *Salmonella enteritidis*, and *S. typhimurium*. The number of psychrophiles and mesophiles during 5 days of cold storage of fresh-cut iceberg lettuce at 10°C packaged in BN/PE film was strictly suppressed in comparison with other tested films (OPP, PE, and PET film). When fresh processed iceberg lettuce was processed and stored under the current conditions, the shelf-life of the product was longer than 5 days in the BN/PE film package, whereas the shelf-life when using the other films tested, PE, OPP and PET, was no longer than 3–4 days. The decay rates of the iceberg lettuce packed in the BN/PE film was maintained at 29.8±2.1% on the 5th day of preservation. The samples packed in BN/PE film maintained an excellent visual quality during the 3 days of storage without significant differences in comparison with the initial visual quality. No browning was observed in the samples packed in BN/PE film for up to 3 days. The texture of shredded iceberg lettuce packaged in BN/PE film remained unchanged up to 3 days, and then a moderate decrease in texture was observed after 4 days of storage. In addition, the overall acceptability of fresh-cut iceberg lettuce packaged in BN/PE film did not change for up to 3 days, whereas the samples packaged in the other films were inedible by 3 days of storage. In conclusion, the shelf-life of fresh-cut iceberg lettuce packaged in the BN/PE film was extended to more than 5 days at 10°C, whereas that in the other films was 2 days at 10°C. Therefore, the shelf-life extension effect of the fresh-cut iceberg lettuce in BN/PE film packaging was very effective compared with the other films tested.

**Keywords:** Shelf-life extension, iceberg lettuce, BN/PE film, microbial suppression effect

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The demand for fresh-cut fruits and vegetables has been ceaselessly increasing over the past 10 years owing to the convenience of fresh-cut fruits and vegetables as ready-to-eat products coupled with the health benefits associated with their consumption [15, 33]. Fresh-cut lettuce, which represents more than 80% of the total production of fresh-cut produce, has been one of the more highly requested commodities by fast food services and salad bars over the past decade [7].

Iceberg lettuce (*Lactuca sativa* L) is composed of soft leaves that contain large amounts of moisture (more than 95%), and is used in fresh foods and salads because it not only improves the appearance of the food, but also increases the nutritional value of mixed salads.

Iceberg lettuce is currently consumed in a minimally processed fresh-cut and ready-to-use form. Minimally processed lettuce is defined as a maintained product, since it provides naturally maintained quality with the true effects of freshness, vitamins, minerals, fibers, etc. Stepwise operations, including trimming, core removal, cutting or slicing, washing, drying, and packaging, are generally applied in the processing of fresh-cut iceberg lettuce [28]. Several reports indicated that a bacterial population found in minimally processed lettuce was around 10<sup>5</sup> CFU/g, and it was detected in the range of 10<sup>5</sup> to 10<sup>7</sup> CFU/g in unprocessed lettuce [1, 6, 13, 18, 22, 24, 31]. The bacterial populations found during minimal processing were too high to control. Therefore, the food industry still needs to develop alternative processing technologies in order to meet the consumer expectations with safer and fresher vegetable products [23, 30].

New natural biodegradable and edible packaging films have been studied for environmental concern, and edible coating to inhibit the growth of pathogenic bacteria in food products is an active research interest in the food science field [26, 27, 32].

Fresh processed vegetables are usually developed in multi storage methods such as cold storage, reserve cooling

control, controlled atmosphere (CA), chemical control, ethylene occurrence control, plastic film packaging, and sealing-up packaging techniques [8, 17, 19, 34]. Some of the symptoms of iceberg lettuce associated with exposure to  $C_2H_4$  are premature senescence with a loss of chlorophyll and yellowing, tissue browning, and elevated levels of respiration. The effectiveness of antimicrobial films in preserving the quality of fresh processed vegetables, including lettuce, has been widely demonstrated [25, 29]. Packaging of fresh-cut vegetables under antimicrobial films is a very well-known technique offering a prolonged shelf-life of respiring products.

In this study, we evaluated the possibility of improving the quality of iceberg lettuce, using antimicrobial film made by mixing antimicrobial material (*Bactecide N*: BN) with polyethylene (PE).

## MATERIALS AND METHODS

### Materials

A 0.5% (w/w) concentration of pulverized Bactecide-N, ion-exchanged zeolite, was mixed in polyethylene (PE) resin, and then the mixture was liquified in a 125°C extruder (22 mM, Daechang Precision Machinery Co., Korea). Nylon turned into liquid in a 165°C extruder with the same method. Polyethylene resin blended with liquified Bactecide-N was positioned at the inner part of the BN/PE film, and nylon was placed at the outer part. The thickness of the BN/PE film was 60  $\mu\text{m}$  (antimicrobial polyethylene resin positioned at the inner section was 45  $\mu\text{m}$ , and nylon placed at the outer section was 15  $\mu\text{m}$ ). OPP, PE, and PET films were obtained from the department of packaging in Kyoungbuk College of Science.

### Physicochemical Properties of Films Tested in this Experiment

Film thickness was measured by using a digital micrometer (Model: ID-C112, Mitutoyo, Japan). The mechanical properties of the films under large deformations (Model: KTM-5, Universal Testing Machine, Korea) were measured in accordance with ASTM D882-00 [4]. All measurements and tests were performed at constant room temperature and 65% relative humidity. Films were kept under these conditions for 24 h. Initial grip separation and crosshead speed were set to 35 mm and 12 mm/min, respectively. The tested film strips were 70 mm long and 35 mm wide. Five measurements were taken from each film specimen.

Oxygen permeability (OP) of films was determined by using oxygen diffusion systems (OX-Tran 2-21 MH, U.S.A.) according to the ASTM F1770 Standard Method [5]. A value of 101,325 Pa (1 atm) was assumed for pressure for the OP (pure oxygen on one side of the film and pure nitrogen on the other side).

### Development of Master Batch and Manufacturing of Antimicrobial BN/PE Film

The source for the antimicrobial film was selected as *Bactecide N* (BN). The film was developed at the Lactic Acid Bacteria Laboratory of Genetic Engineering, Daegu University, and manufactured at the department of packaging in Kyoungbuk College of Science. A 0.5% antimicrobial substance was composed with inorganic matter,  $Ag^+/Zn^{2+}$  zeolite and mixed with granule PE, and compressed to nylon and finally extruded as thin film.

### Antibacterial Test for BN/PE Film

For the test of the antimicrobial activity of BN/PE and PE films, they were cut in the sizes of 0.5 (width) $\times$ 0.5 cm (length). Each film piece was dipped in 70% ethanol, and dried for sterilization. Bacteria tested were *Escherichia coli* KCTC 2195, *Salmonella typhimurium* ATCC 14028, and *Salmonella enteritidis* KCCM 12021. Each bacteria was inoculated in LB (Luria Bertani) medium for 16 h, and the films prepared were added to 50 ml of LB medium in a 200-ml flask with 100  $\mu\text{l}$  bacterial inoculum. Total bacterial populations were recorded by using the spreading surface method after 12 h cultivation.

### Determination of Microbial Populations

Samples of all the iceberg lettuce were homogenized for 30 seconds and serially diluted with peptone water (Difco, Detroit, MI, U.S.A.), as needed for the determination of microbial populations. The colony forming unit (CFU) of a particular microbial group was counted in the following media and at culture conditions: Plate Count Agar (Difco, Detroit, MI, U.S.A.) medium for mesophilic and psychrotrophic aerobic bacteria, and incubation at 30°C for 48 h and at 22°C for 48–72 h, respectively; de Man-Rogosa-Sharpe (MRS) agar (pH 6.2) (Scharlau, Spain) medium for lactic acid bacteria and incubation at 30°C for 3 days; Desoxycholate agar (Difco, Detroit, MI, U.S.A.) medium for coliform bacteria and incubation at 37°C for 24 h. Microbial populations were expressed as logCFU/g.

### Headspace Analysis

Prior to further sample preparation,  $O_2$  and  $CO_2$  concentrations in the packages were monitored during the shelf-life of the product. The gases were taken from the head space of each package and injected into the  $O_2$  and  $CO_2$  Gas Concentrations Check Machine.

### Quality Analyses

General appearance, color, weight retention, pH, and decay rate were evaluated by using the descriptive scales as described by Kader *et al.* [20, 21] and Arte's *et al.* [3]. The color changes ( $\Delta E$ ) were measured with a digital Chroma Meter (CR-200, Minolta Co., Japan). The pH was measured with a digital pH Meter (Millivolt Meter 611, Orion

Research, U.S.A.). The weight retentions for each lettuce package were determined at the interval of the storage time with a balance.

$$\text{Rate of weight retention (\%)} = \text{Wt/Wi} \times 100 \quad (1)$$

Wi: initial weight; Wt: weight at the interval of storage

### Sensory Evaluation

The organoleptic characteristics including color, texture, wilting, decay rate, and overall acceptance of fresh-processed iceberg lettuce in package were evaluated on day 0 and after 1 to 5 days of storage by a three membered expert panel. Lettuce visual quality included the appearance features of gloss, freshness, and color uniformity, and was intensity evaluated and scored on a 5-1 scale, where 5=excellent, 3=acceptable (limit of marketability), and 1=poor (inedible). Leaf-edge browning and leaf-surface browning were evaluated as browning on a 5-1 scale, where 5=no browning, 3=moderate browning, and 1=severe browning. Texture was scored on a scale of 5-1, where 5=very firm and turgid, 3=moderately firm, and 1=very soft. Overall acceptance was determined on a scale of 5-1, where 5=very good, 3=ordinary, and 1=bad.

### Statistical Analysis

There were three repetitions per treatment during the evaluation period. All data represent the mean of three replicates. Analysis of variance (ANOVA) followed by Duncan's multiple range test with a significance level of  $p \leq 0.05$  was performed using SPSS (Window 2000, Statistical Analysis).

## RESULTS AND DISCUSSION

### Physical Properties of the Films

The physical properties of the packaging films tested are summarized in Table 1. The thickness, tensile strength (TS), and oxygen transmission rate (OTR) were investigated to evaluate the differences in the physical properties of each type of film. It was shown that the OPP and PE films had high oxygen transmission properties in comparison

**Table 1.** Physical properties of the packaging films tested.

Packaging film	Thickness (mm)	Tensile strength (MPa)	OTR <sup>a</sup> (cc/m <sup>2</sup> ·day)
OPP <sup>b</sup>	15	12.2	7,000
PE <sup>c</sup>	30	27.6	1,260
BN/PE <sup>d</sup>	60	43.6	62
PET <sup>e</sup>	70	62.4	93

\*Superscripts describe the abbreviation of <sup>a</sup>oxygen transmission rate, <sup>b</sup>oriented polypropylene, <sup>c</sup>polyethylene, <sup>d</sup>bactecide N/polyethylene, and <sup>e</sup>polyethylene terephthalate, respectively.

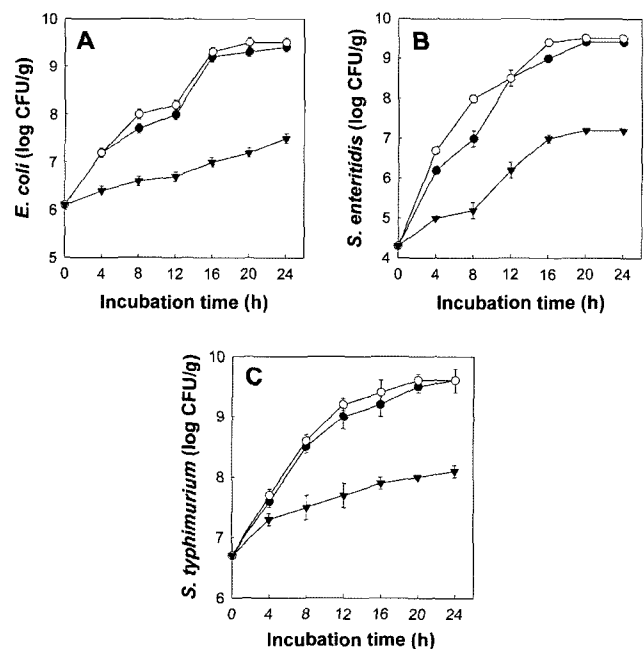
**Table 2.** Physicochemical properties of anti-microbial BN/PE film.

Chemical composition	SiO <sub>2</sub> , Al <sub>2</sub> O <sub>3</sub> , Ag, Zn, Na
90% of sterilization time	30 min
Average powder size	3–4 μm
Distribution of powder size (98% ON)	0.5–10 μm
Thermal stability	800°C
Density	0.39±0.05 g/cm <sup>3</sup>
Color	White
pH	7.0
Thickness	Nylon 15 μm+BN/PE 45 μm
Tensile strength	43.6 MPa
Oxygen transmission rate	61.8 cc/m <sup>2</sup> ·day

with the BN/PE and PET films. The oxygen transmission rate of approximately 61.8 cc/m<sup>2</sup>/day in the BN/PE film was the lowest of all the films tested. The detailed physicochemical properties of the BN/PE film were investigated and are summarized in Table 2. The BN/PE film, of which the density was 0.39±0.05 g/cm<sup>3</sup>, was composed of Ag<sup>+</sup>, Zn<sup>2+</sup>, and Na<sup>+</sup> in zeolite. The thermal activity of the BN/PE film was 800°C.

### Antibacterial Test for the BN/PE Film

Although the percentage of fresh-cut vegetables contaminated with foodborne human pathogens is very low, several



**Fig. 1.** Inhibitory effect of antimicrobial BN/PE film during liquid culture of pathogenic bacteria.

A. *E. coli* KCTC 2195. B. *S. enteritidis* KCCM 12021. C. *S. typhimurium* ATCC 14028. Each bacteria was cultivated at 37°C for 24 h. (●), Control (no addition of film); (○), PE film; (▼), BN/PE film.

outbreaks of foodborne illness have been found to be associated with the consumption of fresh vegetables [12, 35].

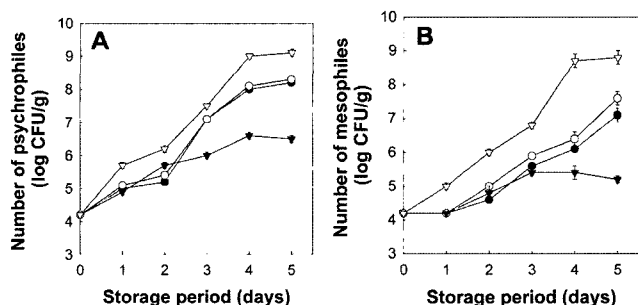
Fig. 1 shows the results of an antibacterial test for the BN/PE film using *Escherichia coli* KCTC 2195, *Salmonella enteritidis* KCCM 12021, and *Salmonella typhimurium* ATCC 14028. These results were used to determine the shelf-life of pathogenic bacteria. As shown in Fig. 1, the BN/PE film had a microbial suppression effect on pathogenic bacteria.

### Cold Storage of Fresh-cut Iceberg Lettuce in BN/PE Film Packaging

The number of psychrophiles was strictly suppressed during 5 days of cold storage of fresh-cut iceberg lettuce at 10°C packaged in BN/PE film in comparison with the number of bacteria in other tested films (OPP, PE, and PET films) in which the psychrophiles were not repressed at all. Generally, the number of aerobic bacteria on fresh processed iceberg lettuce and chopped lettuce increased to 8.0 log CFU/g after 5 days of storage at 10°C [3, 16]. These microorganisms can grow at low temperatures, even under modified atmosphere packaging [11]. Therefore, the storage of fresh-cut iceberg lettuce in BN/PE film appeared to be very effective in controlling the number of psychrophiles.

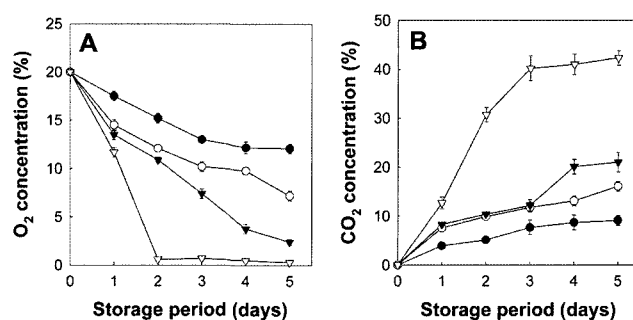
When compared with those packaged in OPP and PET films, the total mesophilic population was reduced by 1.9 and 3.6 log units in the sample packed in BN/PE film, respectively. From the above points of view, it was supposed that the BN/PE film is an appropriate packing material to be used to extend the shelf-life of fresh-cut iceberg lettuce by reducing the mesophilic microbial population.

The microbial limit for the consumption of fresh processed vegetables by consumers is 8.0 log CFU/g for aerobic bacteria [14]. Therefore, when fresh processed iceberg lettuce is processed and stored under the current conditions, the shelf-life of the product is longer than 5 days in the BN/PE film package, whereas the shelf-life when using the other films tested (PE, OPP, and PET), is no longer than 3–4 days.



**Fig. 2.** Populations of bacteria (logCFU/g) in fresh-cut iceberg lettuce during storage when packaged in each type of film at 10°C for 5 days.

A. Psychrotrophic bacteria. B. Mesophilic bacteria. (●), OPP film; (○), PE film; (▼), BN/PE film; (▽), PET film.



**Fig. 3.** Changes in O<sub>2</sub> and CO<sub>2</sub> concentrations during storage of fresh-cut iceberg lettuce when packaged in each type of film at 10°C for 5 days.

A, O<sub>2</sub>; B, CO<sub>2</sub>. (●), OPP film; (○), PE film; (▼), BN/PE film; (▽), PET film.

### Quantitation of O<sub>2</sub> and CO<sub>2</sub> Gas

The changes in the gas concentrations of fresh processed iceberg lettuce bags at 10°C are shown in Fig. 3. During storage, the CO<sub>2</sub> levels of all the tested samples steeply increased, whereas the O<sub>2</sub> concentration slowly decreased. These tendencies were found in all tested films; however, many differences in O<sub>2</sub> and CO<sub>2</sub> concentrations were also identified among the different films.

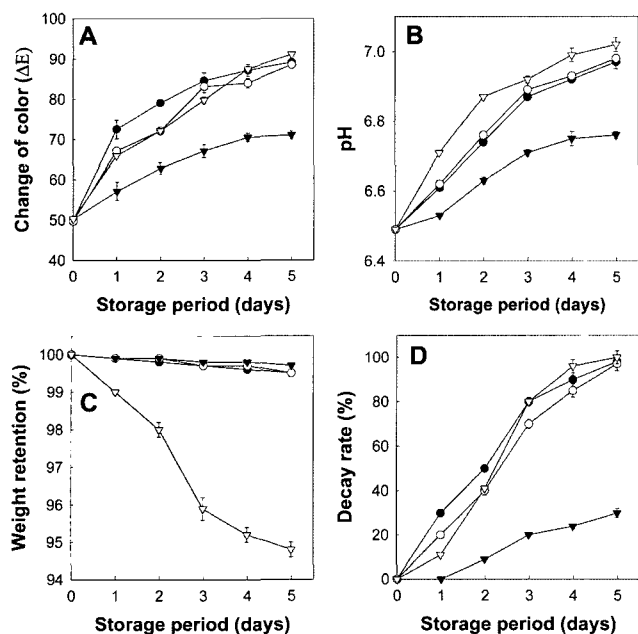
The packages using films with low gas transmission rates, the BN/PE and PET films, showed a tendency towards a low O<sub>2</sub> concentration and a high CO<sub>2</sub> concentration. This result was expected based on the gas transmission rates of the films, as shown in Table 1 [21]. Iceberg lettuce needs to be packaged in a manner that keeps the levels of respiration low; OPP and PE films showed high gas transmission rates and thus permit a high respiration rate.

### Quality Analyses

The changes in the coloration of fresh-cut iceberg lettuce packaged in various packing films are shown in Fig. 4A. The  $\Delta E$  value of the fresh-cut iceberg lettuce was 50.6 and increased with a gentle slope, indicating an increase in the degree of brown coloration in the fresh-cut iceberg lettuce leaves. The  $\Delta E$  value of the other three films precipitously increased with the passage of the storage period. The L (light) and b (blue-yellow) were increased and the a (green-red) was decreased (data not shown). These results showed the same tendencies as the result of the browning score tested by a three-expert panel as described in Fig. 4B.

The pH of the samples increased during the cutting step but decreased after washing. The washing of the iceberg lettuce with tap water carried out in this study showed some effectiveness [2, 9]. Furthermore, the increase in the pH was proportional to the increase in the decay rate.

Generally, the fresh-cut iceberg lettuce was highly susceptible to dehydration. However, the relative humidity is generally very high in film bags or containers wrapped with films [36]. Only slight changes in the weight retentions



**Fig. 4.** Changes in quality during storage of fresh-cut iceberg lettuce when packaged in each type of film at 10°C for 5 days. A, Color ( $\Delta E$ ); B, pH; C, weight retention; D, decay rate. (—●—), OPP film; (—○—), PE film; (—▼—), BN/PE film; (—▽—), PET film.

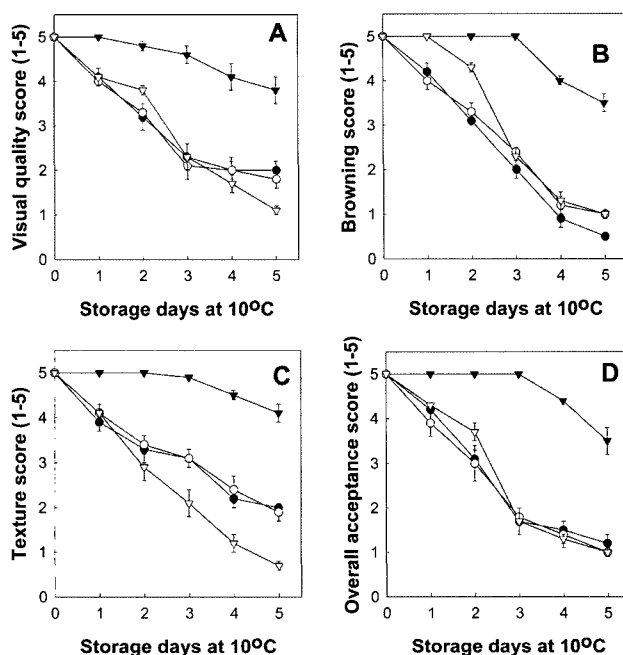
were found in this study, reaching to 99.7% in the OPP and PE films and 99.82% in the BN/PE film after 5 days of storage; however, the PET film weight retention was noted as 94.8% (Fig. 4C). Fruits and vegetables that are brown lose their fresh appearance and characteristic texture after being held in cold storage for just a short period of time [10].

The most important factor in maintaining the quality of fresh processed iceberg lettuce is the reduction of the microbial spoilage flora that cause both decay and safety problems. The decay rates of the iceberg lettuce packed in the OPP, PE, and PET films on the 2nd day of preservation were increased by 40%, and they continued to increase rapidly, reaching nearly 100% by the 5th day. On the other hand, the decay rate of the iceberg lettuce packed in BN/PE film was maintained at the 20% level until the 4th day of preservation, and it increased to  $29.8 \pm 2.1\%$  on the 5th day of preservation (Fig. 4D).

Therefore, it was verified that the preservation prolongation effect of the BN/PE film was predominantly from a decay rate point of view.

### Sensory Evaluation

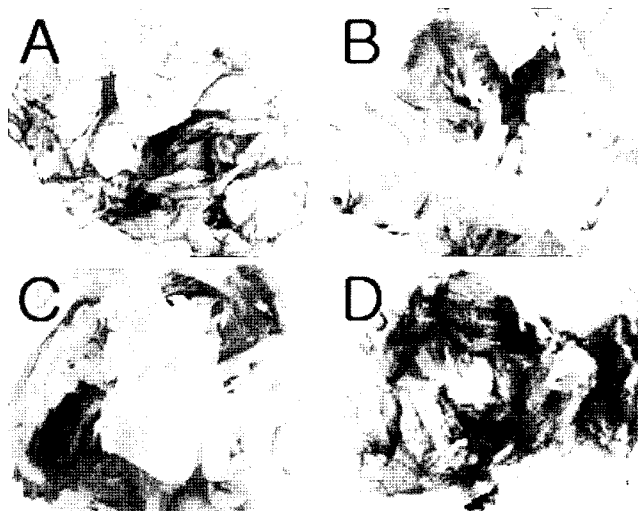
The changes in the organoleptic score of fresh-cut iceberg lettuce packaged in different films are shown in Fig. 5 and the photographs are shown in Fig. 6. The samples packed in BN/PE film maintained an excellent visual quality during the 3 days of storage, without significant differences in comparison with the initial visual quality. On the other



**Fig. 5.** Sensory evaluation of fresh-cut iceberg lettuce during storage packaged in each type of film at 10°C for 5 days. (—●—), OPP film; (—○—), PE film; (—▼—), BN/PE film; (—▽—), PET film.

hand, deterioration in the quality was observed on the first day of preservation in the OPP, PE, and PET films; moreover, it was decreased to below the 3 point, which was the limit of marketability, on the 3rd day of preservation (Fig. 5A).

Among the deteriorations in quality that were rapidly advanced owing to cutting, the browning of the lettuce appeared to be the most important factor depreciating the



**Fig. 6.** Photographs of fresh-cut iceberg lettuce during storage packaged in each type of film at 10°C for 5 days. A, OPP film; B, PE film; C, BN/PE film; D, PET film.

consumer's interest in the product. No browning was observed in the samples packed in BN/PE film for up to 3 days, and moderate browning was observed after 4 days of storage. On the other hand, browning was observed on the first day of preservation in the OPP, PE, and PET films; moreover, it was rapidly saturated by the passage of preservation time (Fig. 5B). The texture of shredded iceberg lettuce packaged in BN/PE film remained unchanged up to 3 days, and maintained above the 4 point for up to 5 days, and then a moderate decrease in texture was observed after 4 days of storage (Fig. 5C). In addition, the overall acceptability of fresh-cut iceberg lettuce packaged in BN/PE film did not change for up to 3 days, whereas the samples packaged in the other films were inedible by the 3rd day of storage (Fig. 5D).

Consequently, the shelf-life of fresh-cut iceberg lettuce packaged in the BN/PE film was extended to more than 5 days at 10°C, whereas the shelf-life of the lettuce packaged in the other films was extended to 2 days at 10°C. Therefore, we inferred that the fresh-cut iceberg lettuce shelf-life extension effect of BN/PE film was found to be best when compared with the other films tested.

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