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Antimicrobial (BN/PE) Film Combined with Modified Atmosphere Packaging Extends the Shelf Life of Minimally Processed Fresh-Cut Iceberg Lettuce

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This study was conducted to investigate the effect of modified atmosphere packaging (MAP) in combination with BN/PE film on the shelf life and quality of fresh-cut iceberg lettuce during cold storage. The total mesophilic population in the sample packed in BN/PE film under MAP conditions was dramatically reduced in comparison with that of PE film, PE film under MAP conditions, and BN/PE film. The O_2 concentration in the BN/PE film under MAP conditions decreased slightly as the storage period progressed. The coloration of the iceberg lettuce progressed the slowest when it was packaged in BN/PE film under MAP conditions, followed by BN/PE film, PE film, and PE film under MAP conditions. The shelf life of fresh-cut iceberg lettuce packaged in the BN/PE film under MAP conditions was extended by more than 2 days at 10°C as compared with that of the BN/PE film in which the extension effect was more than 2 days longer than that of PE, PET, and OPP films.

Keywords: Shelf-life extension, iceberg lettuce, BN/PE film, MAP

The processing and distribution of fresh-cut vegetables, such as lettuce, has increased dramatically over the past decades, and there is no sign of this trend slowing owing to the convenience these products provide to end-users [11].

The packaging of fresh-cut vegetables under antimicrobial films is a very well-known technique that offers a prolonged shelf life for respiring products [17, 21]. New natural biodegradable and edible packaging films have been researched owing to environmental concerns, and the development of an edible coating that can inhibit the growth of pathogenic bacteria in food products is an active area of research in the food science field [18, 19, 24].

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There have been a number of recent developments in the technologies applied to fresh agricultural products, such as the novel modified atmosphere packaging (MAP) technique and the technique that use ozone [3, 13, 15, 20]. Using the MAP technique, we can overcome the safety problem associated with these packaging processes because the growth of aerobic spoilage organisms in fresh processed vegetables is inhibited by these atmospheres [10].

Many studies concerning the microbiological control of fresh processed vegetables stored under MAP conditions have already been published. Almost 15% of all fresh-cut California iceberg lettuce [7] for retail is distributed under MAP conditions.

In general, nitrogen (N_2), oxygen (O_2), carbon dioxide (CO_2), or various mixtures of these three gases are used in MAP. The composition of the gas mixture in MAP depends on the types of vegetables and their expected shelf life [12]. An elevated CO_2 and reduced O_2 environment extends the shelf life of fruits and vegetables by inhibiting chemical, enzymatic, and microbial spoilage. This environment can selectively inhibit the growth of Gram-negative bacteria such as *Pseudomonas* sp., which typically grow rapidly under aerobic conditions and produce the off-flavors and odors associated with the spoilage of many foods [9].

Therefore, quick cooling and low-temperature maintenance after harvesting are absolutely necessary for maintaining the freshness of the lettuce. Functional materials, such as antimicrobial substances, and materials for gas-uptake and the alleviation of acidification are added to the master batch during film manufacturing in order to overcome physiological problems, such as discoloration and decomposition, and to protect the fruits and vegetables from microbes.

Iceberg lettuce (*Lactuca sativa* L), containing more than 95% of moisture, 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 [16].

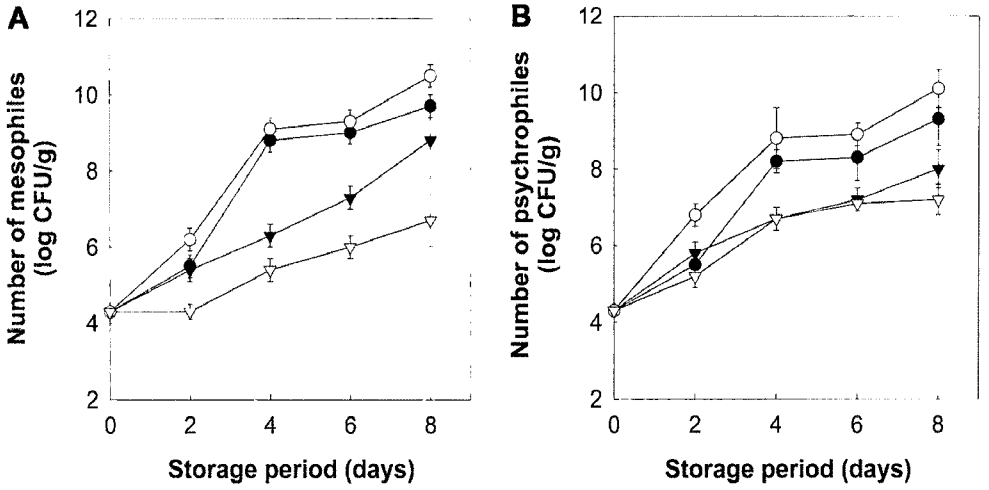

Fig. 1. Populations of bacteria (log CFU/g) in fresh-cut iceberg lettuce during storage when packaged under MAP in each type of film at 10°C for 8 days.
A. Mesophilic bacteria. B. Psychrotrophic bacteria. (-●-), PE film; (-○-), PE film+MAP; (-▼-), BN/PE film; (-▽-), BN/PE film+MAP.

In a previous study, we evaluated the possibility of improving the quality of iceberg lettuce using an antimicrobial film made by mixing antimicrobial material (Bactecide N: BN) with polyethylene (PE) [16]. The objective of this study was to investigate the effect of MAP in combination with BN/PE film on the shelf life and quality of fresh-cut iceberg lettuce during cold storage.

In this study, the MAP condition for storage of iceberg lettuce was composed of 95% N_2, 2.6% O_2, and 2.4% CO_2. The method of BN/PE film production, microbial populations determination, headspace analysis, sensory evaluation, and statistical analysis were as described in the previous paper [16].

When compared with those packaged in PE, PE+MAP, and BN/PE, the total mesophilic population was reduced by 3.0, 3.8, and 2.1 log units in the sample packed in BN/PE film under MAP conditions, respectively (Fig. 1). From the above points of view, it was supposed that BN/PE film under MAP is an appropriate packing condition to be used to extend the shelf life of fresh-cut iceberg lettuce by reducing the mesophilic microbial population. The synergy effect of microbial suppression activity by adding the MAP condition to the BN/PE film was weak, whereas the number of psychrophiles during 8 days of cold storage of fresh-cut iceberg lettuce at 10°C packaged in BN/PE film was strictly suppressed in comparison with the number of bacteria in PE films. 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, and these microorganisms could grow at low

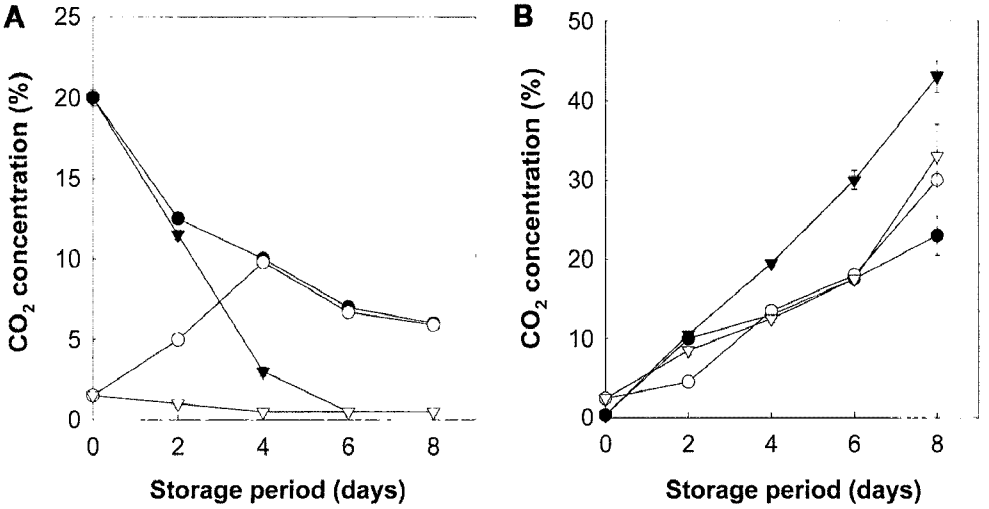

Fig. 2. Changes in O_2 and CO_2 concentrations during storage of fresh-cut iceberg lettuce when packaged under MAP in each type of film at 10°C for 8 days.
A, O_2; B, CO_2. (-●-), PE film; (-○-), PE film+MAP; (-▼-), BN/PE film; (-▽-), BN/PE film+MAP.

temperatures, even under MAP conditions [5]. Therefore, the storage of fresh-cut iceberg lettuce in BN/PE film under MAP conditions appeared to be very effective in controlling the number of psychrophiles. The microbial limit for the consumption of fresh processed vegetables by consumers is 8.0 log CFU/g for aerobic bacteria [8]. Therefore, when fresh processed iceberg lettuce was processed and stored under 10°C, the shelf life of the product was longer than 8 days in the BN/PE film package under MAP conditions, whereas the shelf life when using the other films tested, PE and PE+MAP, was no longer than 3 days.

The changes in the gas concentrations of fresh processed iceberg lettuce bags under MAP conditions at 10°C are shown in Fig. 2. Because CO_2 was produced by respiration and O_2 was consumed, their concentrations showed inverse courses within 8 days of cold storage. The O_2 concentration in the BN/PE film under MAP conditions, in which the initial concentrations adjusted to 2.6%, decreased slightly as the storage period progressed, whereas the O_2 concentration in the PE film under MAP conditions rapidly increased until 4 days of storage and then decreased. The O_2 concentrations in the BN/PE and PE films decreased with a steep slope. Low O_2 levels are considered to be favorable for the suppression of enzymatic browning and can be achieved passively by respiration of living tissues, or actively by injection of specific gas mixtures into film bags [4]. Since a decrease in quality is primarily based on visual appearance, such as brown coloration, a MAP condition with a low concentration of O_2 and a high concentration of CO_2 prolonged the shelf life of fresh-cut iceberg lettuce by reducing the rate of visual deterioration and browning [22].

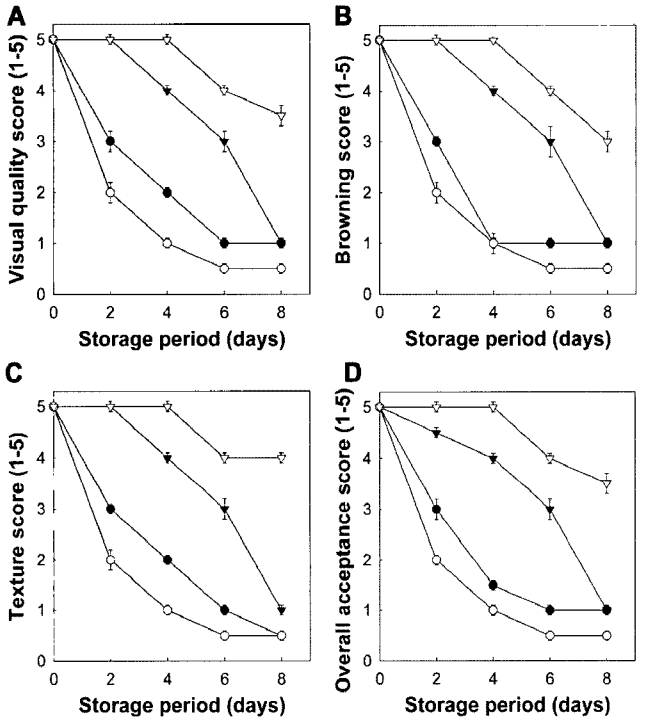

Fig. 3. Sensory evaluation of fresh-cut iceberg lettuce packaged under MAP in each type of film at 10°C for 8 days.
A. Visual quality score; **B**. Browning score; **C**. Texture score; **D**. Overall acceptance score. (-●-), PE film; (-○-), PE film+MAP; (-▼-), BN/PE film; (-▽-), BN/PE film+MAP; 1 point, worst; 2 point, bad; 3 point, moderate; 4 point, good; 5 point, very good.

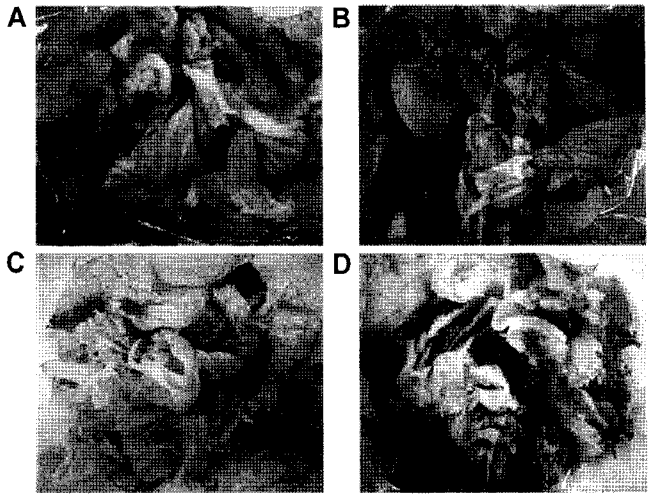

Fig. 4. Photographs of fresh-cut iceberg lettuce during storage packaged under MAP for each type of film at 10°C for 8 days.
A, PE film; **B**, PE film+MAP; **C**, BN/PE film; **D**, BN/PE film+MAP.

The changes in the organoleptic score of fresh-cut iceberg lettuce stored under different conditions are shown in Fig. 3, and the photographs are shown in Fig. 4. Physical appearance is one of the most important quality factors, because consumers first evaluate the quality of fruits and vegetables with their eyes [1]. Moreover, the fresh appearance and characteristic texture of fruits and vegetables are quickly lost when held in cold storage [6]. The sample packaged in BN/PE film under MAP conditions maintained an excellent visual appearance during the 4 days of storage. The visual appearance of the sample after 4 days of storage under these conditions was quite similar to the initial visual appearance of the sample (Fig. 3A). Moreover, the sample was maintained for more than 3 points, which was the limit of marketability, on the 8th day of preservation. The visual appearance of the sample packed in BN/PE film showed no significant differences during the 2 days of storage. However, the visual appearance of the sample deteriorated after 4 days of storage, and the sample fell below the marketability threshold at 6 days of storage. On the other hand, deterioration in the quality of the sample was observed on the first day of preservation in the PE film and the PE film under MAP conditions, and both samples fell below the marketability threshold at 2 days of storage. The browning on the surface of the vegetables was limited by the action of the polyphenol oxidase and peroxidase [23]. Because oxygen is an essential substrate for the

enzymatic reaction, a low-oxygen atmosphere reduces the enzymatic discoloration [14]. The browning of the lettuce appeared to be the most important factor in depreciating the consumer's interest in the lettuce, and it was rapidly advanced by cutting [16]. No browning was observed in the samples packed in BN/PE film under MAP conditions for up to 4 days, and the sample was maintained at more than 3 points (the limit of marketability) on the 8th day of preservation. The browning of the iceberg lettuce packed in BN/PE film was observed after the 4th day, and the sample decreased to below the marketability threshold on the 6th day. Browning of the lettuce packed in the PE and PE+MAP was found within the first day of preservation and became rapidly saturated as the preservation time progressed (Fig. 3B). The texture of fresh-cut iceberg lettuce packed in BN/PE film under MAP conditions remained unchanged until day 4, and was maintained above the 4-point level for up to 8 days (Fig. 3C). On the other hand, the samples stored in BN/PE, PE, and PE+MAP were inedible by days 6, 2, and 2, respectively. The overall acceptability of fresh-cut iceberg lettuce packed in BN/PE under MAP conditions remained unchanged for up to 4 days and then moderately decreased to 3.5 points an the 8th day of storage (Fig. 3D). The overall acceptability score on the 8th day of storage was below 1, which indicated that this condition was the worst.

Consequently, the shelf life of fresh-cut iceberg packaged in the BN/PE film under MAP conditions was extended by more than 2 days at 10°C compared with that of BN/PE film, which extended the shelf life of the lettuce by more than 2 days in comparison with that of PE, PET, and OPP films. Therefore, we infer that the extension of the shelf life of fresh-cut iceberg lettuce is greatest with the BN/PE film under MAP conditions in comparison with the other tested conditions, and that the extension effect of the BN/PE film is better than that of the PE film under MAP conditions.

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