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Effect of Natamycin in Combination with Pure Oxygen Treatment on Postharvest Quality and Selected Enzyme Activities of Button Mushroom (*Agaricus bisporus*)

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ABSTRACT: The combined effects of natamycin (NA) and pure oxygen (PO) treatment on microbial and physicochemical characteristics of button mushroom (*Agaricus bisporus*) stored at 4 ± 1 °C for 16 days was investigated. Mushroom respiration rate, weight loss, firmness, color, percent open caps, total soluble solids, microbial and activities of polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and peroxidase (POD) were measured. The results indicate that treatment with natamycin + pure oxygen (NAPO) maintained tissue firmness, inhibited increase of respiration rate, delayed browning and cap opening, and reduced microorganism counts of yeasts and molds compared to control treatment. The efficiency was better than that of NA or PO treatment. Furthermore, NAPO inhibited the activities of PPO, PAL, and POD throughout the storage period. Our study suggests that NAPO treatment has the potential to improve the quality of button mushroom and extend the shelf life.

KEYWORDS: *button mushroom, natamycin, high oxygen, browning, microbiological quality, storage life*

INTRODUCTION

Consumption and production of button mushroom (*Agaricus bisporus*) have grown continuously in the past years. It is the most extensively cultivated edible mushroom, comprising 32% of the worldwide production.[1] Mushrooms are a good source of of vitamin B_2, niacin, folates, and many mineral elements.[2] However, button mushrooms only have a short shelf life of 3−4 days; accelerated physiological, morphological, and microbial changes lead to browning and sliminess as well as early breaking of the veil, expansion and darkening of the cap and gills, and elongation of the stem.[3]

Atmospheres with high O_2 levels (≥70 kPa) have recently been suggested as an innovation of modified atmosphere packaging (MAP) for postharvest fruits and vegetables, to inhibit physiological metabolism, maintain quality and safety, and prolong shelf time.[4,5] Day[6] suggested that high O_2 treatment can inhibit microbiological growth, enzymatic discoloration, and the anaerobic fermentation reactions of fruits and vegetables. However, only a small portion of the work has been applicable to fresh-cut fruits and vegetables. Gorny et al.[7] found that 80 kPa O_2 alone effectively reduced the respiration rates of pear slices over 4 days at 10 °C, while it did not inhibit the browning and softening. Exposure to 70 kPa O_2 seemed to clearly reduce the growth of microorganisms on fresh-cut pears, but the shelf life may be limited due to the browning and off-odors that develop beyond 14 days of storage.[8] Nevertheless, Lu et al.[9] reported the use of 100 kPa O_2 to pretreat apples before slicing and noted that this technique may inhibit browning, off-flavors, and the respiration rate of the fresh-cut apple slices. These different conclusions depend not only on the gas compositions but also on the storage temperature, variety, tissue structure, and fruit aging.

Natamycin (NA) is a natural antifungal agent produced by *Streptomyces natelensis*. It inhibits the fungi by binding to cell membrane sterols, especially ergesterol; therefore, membrane permeability is enhanced, resulting in lyses. NA was approved as a generally recognized as safe (GRAS 21 CFR 172.155, FDA-ASP/1577, 007681-93-8) agent by Food and Drug Administration (FDA) in the United States, and was assigned as a natural preservative in European Union (EEC no. 235). Its effectiveness in preventing mold growth on cheese and sausage has been reported.[10]

To our knowledge, there are no published data on the use of natamycin for enhancing the storage life of button mushrooms. Therefore, the goal of this research was to develop an integrated approach that could be used to extend the shelf life of button mushrooms and to investigate the combination effects of natamycin and pure oxygen treatments on microbiological and physicochemical properties of button mushroom.

MATERIALS AND METHODS

Mushroom Treatment and Storage. Button mushroom used in this study were harvested from a local farm in Hangzhou, China. The mushrooms were transported to the laboratory in 1 h after picking and then stored in darkness at 4 ± 1 °C and 90% relative humidity (RH). The day after, mushrooms were screened for uniform size and maturity and the absence of mechanical damage. Button mushrooms treated with 0.5 mM natamycin solutions exhibited the most benefit in maintaining mushroom quality in our preliminary investigation. Mushrooms were divided into four samples of 60 each. Four different treatments were used: (1) control, (2) pure oxygen (PO), (3) natamycin (NA), and (4) natamycin + pure oxygen (NAPO). The mushrooms were dipped in 0.5 mM natamycin solutions for 10 min at 20 °C, with dip in distilled water used as a control. Four jars were used for treatment. The jars were placed at 4 ± 1 °C and 90% relative humidity (RH), two jars were linked by separate lines to continuous

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flow (100 mL/min) of 100% O_2. The gases were checked regularly with SCY-2A O_2 and CO_2 analyzer (Xinrui Instrument Co., Shanghai, China) and maintained at ±2% for the duration of the experiment. Samples were then stored for 16 days, and subsequently, every 4 days three replicates from each treatment group were analyzed.

Respiration Rate. A closed system was chosen to measure respiration rate of the product.[11] At each storage time, approximately 50 g of mushrooms from the four treatments were placed under normal air for 1 h. Then, mushrooms were held in a closed container that contained 15 mL of 0.05 mol L^{-1} $Ba(OH)_2$ and were stored at 20 °C for 1 h. Then, 2 drops of phenolphthalein were added, and the solution was titrated with 1/44 mol L^{-1} oxalate. Measurements were replicated three times. Respiration rates of samples were (expressed as CO_2 production rate) calculated with the following formula:

$$\mathrm{RI} = \frac{(V_1 - V_2)c \times 44}{Wt}$$

In the formula, V_1 is the volume of oxalate of control (mL), V_2 is the volume of oxalate of samples (mL), c is the concentration of oxalate (mol/L), 44 is the molecular weight of CO_2, W is the weight of samples (g), and t is the test time (h).

Texture, Weight Loss, and Total Soluble Solids. A penetration test was performed on the button mushroom cap using a TA.XT Express-v3.1 texture analyzer (Stable Micro Systems), using a 5 mm diameter cylindrical probe. Samples were penetrated 5 mm in depth. The speed of the probe was 2.0 mm s^{-1} during the pretest as well as during penetration. Force and time data were recorded with Texture Expert (Version 1.0) from Stable Micro Systems. From the force vs time curves, firmness was defined as the maximum force. Weight loss was determined by weighing the whole mushroom before and after the storage period. Weight loss was expressed as the percentage of loss of weight with respect to the initial weight. Mushrooms were ground in mortar and squeezed with a hand press, and the juice was analyzed for total soluble solids (TSS). TSS was measured at 25 °C with a refractometer (Taiguang 405225, Taihua Optical Co., Ltd. Chengdu, China).

Color. The surface color of mushroom caps was measured with a WSC-S Colorimeter (Shanghai Precision Instrument Co. Ltd., Shanghai, China). To analyze the L^* (light/dark), a^* (red/green), and b^* (yellow/blue) values, each mushroom was measured at three equidistant points of the cap and compared to the ideal mushroom color values of $L^* = 97$, $a^* = -2$, and $b^* = 0$. The browning index (BI), which represents the purity of brown color,[12] was calculated according to the following equations:

$$\mathrm{BI} = [100(x - 0.31)]/0.172,$$

$$\text{where } x = (a + 1.75L)/(5.645L + a - 3.012b)$$

Percent Open Caps and Overall Acceptability. Criteria for judging the percentage of open caps were based on the development of umbrella-like shape of the cap followed by detachment of the veil. The percent open caps was determined from a known number of mushrooms as

$$\%\ \text{open caps} = N_{oc}/N_t \times 100$$

where N_t = total number of mushrooms and N_{oc} = number of open-caped mushrooms.

The overall acceptability based on color, texture, and percent open caps was done by a panel of four judges on a round table basis using a four-point scale, where 1 = poor, 2 = fair, 3 = good, and 4 = excellent.

Enzyme Assays. For analysis of enzymatic activities, mushroom tissues (4.0 g) were homogenized with 12 mL of 50 mM K-phosphate buffer (pH 7.3) containing 1 mM EDTA, 2 mM DTT. After centrifugation for 15 min at 10000*g* and 4 °C, the supernatant was collected and used as the crude enzyme extract for the polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and peroxidase (POD) assays. Protein content was determined according to the method of Bradford,[13] with bovine serum albumin used as the standard.

PPO (EC 1.10.3.2) activity was measured by incubating 0.5 mL of enzyme extract to 2.5 mL of buffered substrate (100 mM sodium phosphate, pH 6.4 and 50 mM Catechol), and then monitoring the change of absorbance at 398 nm.[14] One unit of activity of PPO was defined as the amount of enzyme causing 0.01 absorbance increase per minute under the conditions of assay. The specific PPO activity was expressed as U/mg protein.

PAL (EC 4.3.1.5) activity was assayed by measuring the absorbance of *trans*-cinnamic acid at 290 nm.[15] The reaction mixture (3 mL), which contained 0.8 mL supernatant and 50 mM L-phenylalanine in sodium borate buffer (200 mM, pH 8.8), was incubated at 37 °C for 90 min, and the reaction was terminated by ice water. One unit of PAL activity was defined as the amount of enzyme that caused the increase in absorbance at 290 nm of 0.01 in 1 h under the specified conditions. The specific PAL activity was expressed as U/mg protein.

POD (EC 1.11.1.7) activity was measured spectrophotometrically using substrate guaiacol in this work.[16] The reaction mixture for the determination of POD activity consisted of 50 mM sodium phosphate buffer (pH 6.0), 5 mM guaiacol, 5 mM H_2O_2, and 50 μL of tissue extract. One unit of POD activity was defined as the amount of enzyme that caused the change in absorbance at 470 nm of 0.01/min under the specified conditions. The specific POD activity was expressed as U/mg protein.

Microbiological Analysis. All samples were analyzed for mesophilic, psychrophilic, pseudomonad bacteria and yeasts and molds. Twenty-five grams of mushrooms were removed aseptically from each pack and diluted with 225 mL of 0.1% peptone water. The samples were homogenized by a stomacher at high speed for 2 min. Serial dilutions (10^{-1}–10^{-9}) were made in serial dilution tubes by taking 1.0 mL with 9.0 mL of 0.1% peptone water. Aerobic counts were determined on plate count agar (PCA; Merck) following incubation at 35 °C for 2 days for mesophilic bacteria and at 4 °C for 7 days for psychrophilic bacteria. *Pseudomonas* was counted on cephaloridin fucidin cetrimide agar (CFC; Difco), with selective supplement SR 103 (Oxoid). The incubation temperature was 25 °C and plates were examined after 48 h. Yeasts and molds were estimated on potato dextrose agar (PDA; Merck) and incubation conditions were 28 ± 1 °C for 5–7 days.

Statistical Analysis. Experiments were performed using a completely randomized design. Data were subjected to one-way analysis of variance (ANOVA). Mean separations were performed by Tukey's multiple range test (DPS version 6.55). Differences at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Natamycin in Combination with Pure Oxygen Treatment on Respiration Rate, Texture, Weight Loss, and Total Soluble Solids. The respiratory rate of button mushroom measured as CO_2 evolution at harvest was around 43 mg CO_2 kg^{-1} h^{-1} (Figure 1A). During the 4 °C storage period, respiration rate gradually increase in the first 8 days. No significant differences were observed between control and NA samples; however, lower respiration rate was found in PO than control samples, and NAPO treatment was the most efficient in reducing respiration during the storage period. At the end of storage, respiration rates of button mushrooms stored in control, NA, PO, and NAPO, were 33.2, 31.0, 20.1, and 17.6 mg CO_2 kg^{-1} h^{-1}, respectively. Similar results were obtained in other previous research, in which 80% or 100% O_2 reduced ethylene production rates, delayed ripening of mature green and breaker tomatoes at 20 °C, and also 40, 60, or 80 kPa O_2 inhibited the respiration rates of Bartlett pear slices and ethylene production during 4 days at 10 °C.[17] These data suggest that PO treatment could inhibit the respiration of button mushroom, and the lower respiration rate in the NAPO mushrooms might be correlated with a delayed senescence and a reduced susceptibility to decay.

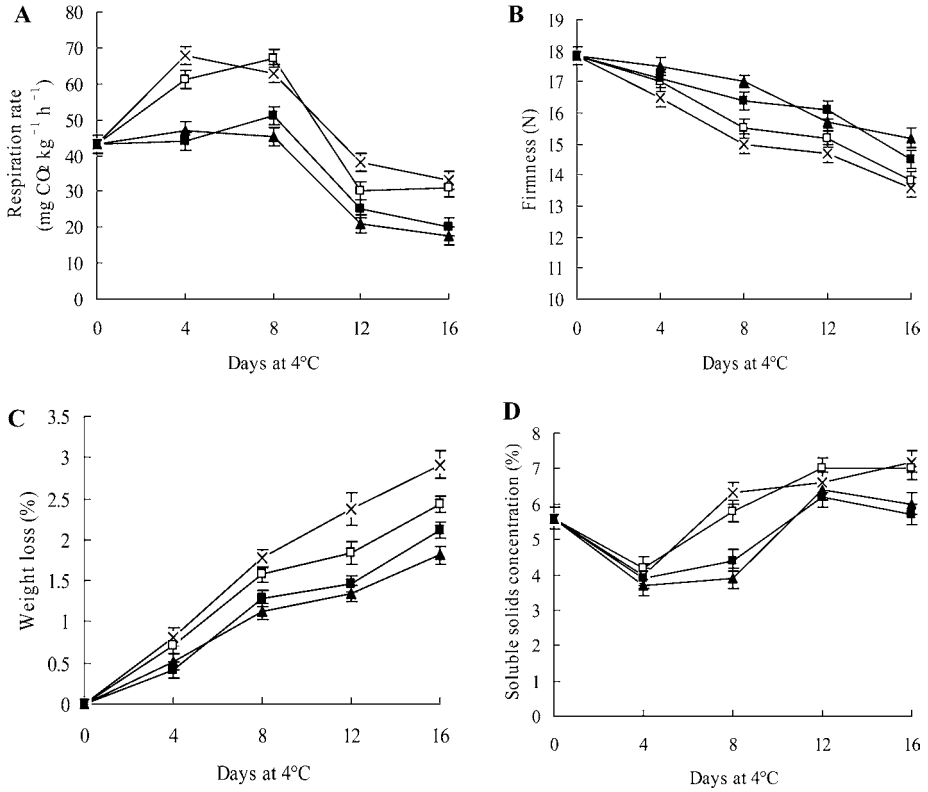

Figure 1. Changes in respiration rate (A), firmness (B), weight loss (C), and total soluble solids (D) of button mushrooms packaged in control (×), PO (■), NA (□), and NAPO (▲) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

The texture of button mushroom is often the first of many quality attributes judged by the consumer and is, therefore, extremely important in overall product acceptance. Button mushroom suffers a rapid loss of firmness during ripening, which contributes greatly to its short postharvest life and susceptibility to fungal contamination. Figure 1B shows that the control mushrooms had the fastest softening rate, losing about 23.7% of their firmness in about 16 days. The firmness of mushrooms treated with PO, NA, or NAPO also decreased but to a lesser extent. NAPO treatment led to a significantly higher firmness than that in the control sample, due to a synergistic effect of NA and PO. There were no significant variations between NA and control samples. Oms-Oliu et al.[18] reported that high oxygen preserved the initial firmness of fresh-cut Piel de Sapo melon better than low O_2 concentrations or nonmodified atmosphere. For mushrooms, softening can occur because of the degradation of cell walls by bacterial enzymes and increased activity of endogenous autolysins.[19] Microorganisms such as *Pseudomonas* degrade mushrooms by breaking down the intracellular matrix and reducing the central vacuole, resulting in partially collapsed cells and a loss of turgor. This kind of bacterial-induced softening was observed in control samples but was inhibited by NAPO treatment. Additionally, softening is often related to water loss, which is responsible for the loss of turgor of fresh mushrooms. Here, the control demonstrated the largest firmness depletion and weight loss, and the NAPO and PO samples presented the smallest loss of firmness and weight loss.

As shown in Figure 1C, weight loss increased as the storage period progressed in all the treatments. The highest weight loss was observed in the control samples; it reached 2.91% at the end of storage, suggesting that dehydration is an important process in mushroom quality loss during postharvest storage. On day 8, the NAPO and PO mushrooms showed the lowest weight loss (1.13% and 1.28%, respectively), followed by NA samples (1.58%). In this study, the NAPO significantly reduced the weight loss of mushrooms as compared with control treatment and therefore delayed mushroom shriveling and quality deterioration. Nevertheless, there was no significant difference between NAPO and PO samples ($P > 0.05$).

Changes in the soluble solids content (SSC) of button mushrooms over storage time are shown in Figure 1D. The SSC of control mushrooms increased after 4 days of storage while PO and NAPO mushrooms experienced a slight increase during the same period. The lowest levels of SSC were recorded in NAPO and PO mushroom at end of the storage. Tao et al.[20] have reported an increase in SSC in button mushrooms stored at 4 ± 1 °C and 75% RH. The effect of PO in reducing SSC of shiitake mushroom was probably due to the slowing down of respiration and metabolic activity, hence retarding the ripening process. Indeed, the greater changes in SSC occurred in those mushrooms, which suffered the greatest water loss. The solubilization of the cell wall polysaccharide and hemicelluloses in senescent mushroom might also contribute to the increase in SSC. A suppressed respiration rate also slows down the synthesis and the use of metabolites, resulting in lower SSC due to the slower hydrolysis of carbohydrates to sugars.[21] Our results are similar with Wszelaki et al.,[22] who found significantly lower SSC values in high oxygen treated strawberries than fruit held in air. However, other studies have reported significantly higher SSC values in high oxygen treated blueberry.[23]

Effect of Natamycin in Combination with Pure Oxygen Treatment on Color. Different values obtained after

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Table 1. Changes in Color of Button Mushrooms Packaged in Natamycin + 100% O_2 Stored at 4 °C for 16 Days[a]

treatments	L	a	b	ΔE	BI
			0 Days		
control	91.12 ± 0.14 a	0.34 ± 0.01 c	15.26 ± 0.25 c	16.52 ± 0.53 b	17.97 ± 0.38 b
PO	90.34 ± 0.08 b	1.03 ± 0.04 a	15.43 ± 0.43 b	17.07 ± 0.24 a	18.93 ± 0.14 a
NA	90.45 ± 0.15 b	0.56 ± 0.04 b	15.65 ± 0.16 b	17.16 ± 0.18 a	18.80 ± 0.42 a
NAPO	91.35 ± 0.24 a	0.43 ± 0.10 bc	14.89 ± 0.07 a	16.11 ± 0.31 c	17.52 ± 0.52 c
			4 Days		
control	84.47 ± 0.38 c	2.66 ± 0.27 a	18.36 ± 0.31 a	22.71 ± 0.24 a	26.02 ± 0.61 a
PO	85.13 ± 0.43 b	1.54 ± 0.13 c	15.76 ± 0.20 c	20.05 ± 0.50 c	21.11 ± 0.47 d
NA	86.21 ± 0.32 a	2.13 ± 0.04 b	18.34 ± 0.14 a	21.68 ± 0.26 a	24.94 ± 0.36 b
NAPO	86.54 ± 0.21 a	1.65 ± 0.06 c	17.54 ± 0.52 b	20.75 ± 0.32 b	23.29 ± 0.32 c
			8 Days		
control	80.35 ± 0.11 d	2.88 ± 0.07 a	19.32 ± 0.32 a	25.97 ± 0.13 a	29.24 ± 0.29 a
PO	82.43 ± 0.24 b	2.14 ± 0.14 c	17.48 ± 0.05 c	23.13 ± 0.26 c	24.95 ± 0.52 c
NA	81.60 ± 0.52 c	2.32 ± 0.22 b	18.26 ± 0.32 b	24.27 ± 0.47 b	26.59 ± 0.32 b
NAPO	83.54 ± 0.23 a	1.46 ± 0.18 d	17.22 ± 0.08 c	22.13 ± 0.12 d	23.59 ± 0.45 d
			12 Days		
control	77.56 ± 0.05 d	4.85 ± 0.24 a	23.78 ± 0.26 a	31.47 ± 0.36 a	40.04 ± 0.62 a
PO	80.31 ± 0.17 b	3.55 ± 0.13 c	21.43 ± 0.13 c	27.72 ± 0.40 c	33.29 ± 0.39 c
NA	79.30 ± 0.15 c	4.13 ± 0.07 b	22.46 ± 0.16 b	29.25 ± 0.14 b	36.06 ± 0.25 b
NAPO	81.34 ± 0.26 a	4.21 ± 0.05 b	19.69 ± 0.06 d	25.91 ± 0.24 d	30.64 ± 0.70 d
			16 Days		
control	74.35 ± 0.48 c	5.43 ± 0.13 a	24.67 ± 0.32 a	24.31 ± 0.26 d	44.39 ± 1.05 a
PO	76.45 ± 0.26 b	4.78 ± 0.27 b	23.55 ± 0.26 b	31.98 ± 0.53 b	40.24 ± 0.77 b
NA	76.47 ± 0.06 b	5.32 ± 0.05 a	25.53 ± 0.15 c	33.57 ± 0.26 a	44.42 ± 1.03 a
NAPO	77.80 ± 0.13 a	4.67 ± 0.13 c	22.60 ± 0.25 c	30.40 ± 0.27 c	37.64 ± 0.80 c

[a]PO, pure oxygen; NA, natamycin; NAPO, natamycin + pure oxygen; ΔE, the degree of overall color change in comparison to color values of an ideal mushroom; BI, browning index. Values are the mean of three replications ± standard deviation. Means in same column with different letters are significantly different ($P < 0.05$).

Table 2. Changes in Percent Open Caps and Overall Acceptability of Button Mushrooms Packaged in Natamycin + 100% O_2 Stored at 4 °C for 16 Days[a]

treatments	0 days	4 days	8 days	12 days	16 days
			Percent Open Caps		
control	0	15.32 ± 0.11 a	38.33 ± 0.12 a	65.12 ± 0.45 a	85.47 ± 0.58 a
PO	0	14.43 ± 0.08 b	33.78 ± 0.16 b	57.36 ± 0.24 b	79.60 ± 0.46 b
NA	0	14.35 ± 0.29 b	34.43 ± 0.27 b	55.35 ± 0.42 c	77.50 ± 0.60 c
NAPO	0	14.27 ± 0.16 b	31.27 ± 0.33 c	52.86 ± 0.37 d	73.21 ± 0.72 d
			Overall Acceptability		
control	5	3.75 ± 0.03 c	2.34 ± 0.17 d	1.15 ± 0.06 d	1.00 ± 0.00 c
PO	5	3.82 ± 0.06 b	2.58 ± 0.07 c	1.46 ± 0.04 c	1.13 ± 0.05 c
NA	5	4.04 ± 0.11 a	2.89 ± 0.10 b	1.84 ± 0.04 b	1.37 ± 0.03 b
NAPO	5	4.06 ± 0.05 a	3.11 ± 0.12 a	2.12 ± 0.08 a	1.76 ± 0.03 a

[a]PO, pure oxygen; NA, natamycin; NAPO, natamycin + pure oxygen. Values are the mean of three replications ± standard deviation. Means in same column with different letters are significantly different ($P < 0.05$).

application of NAPO, compared to the control treatment, are shown in Table 1. From this table, higher luminosity (L) values and lower total color variation (ΔE) were observed in NAPO samples compared to control after 4 days. The L value of control samples decreased sharply after the first 4 days, and it was 80.35 at day 8 and 77.56 at day 12; the last value may not be considered as commercially acceptable if a L value of 80 for wholesalers was taken into account.[24] The browning index (BI) of mushrooms was higher in control than NA, PO, and NAPO during the whole storage. On day 12, mushrooms treated with NAPO browned slightly, but they also had commercial value and edibility. Compared with control, NAPO inhibition of browning could be due to the synergistic effect of NA combined with PO treatment. The browning of mushrooms is attributed to the action of polyphenol oxidase (PPO) enzyme and the action of bacteria and mold on the mushroom tissue. As NA treatment causes reduction of spoilage organisms, it prevents the formation of brown patches, hence improving the color and appearance. On the other hand, Lu et al.[9] found lower browning of Spartan apples slices under 100 kPa O_2 for 12 days at 1 °C before slicing. Lower discoloration was observed when pepper dices were exposed to high O_2 combined with 20 kPa CO_2.[25]

Effect of Natamycin in Combination with Pure Oxygen Treatment on Percent Open Caps and Overall Acceptability. The percentage of open-cap mushrooms increased as the storage period advanced in all the treatments and was higher in control samples. The percentage of open-cap mushrooms in control was 85.47% after 16 days of storage (Table 2). On the other hand, the percentage of open-cap mushrooms for NA, PO, and NAPO treatment were in the

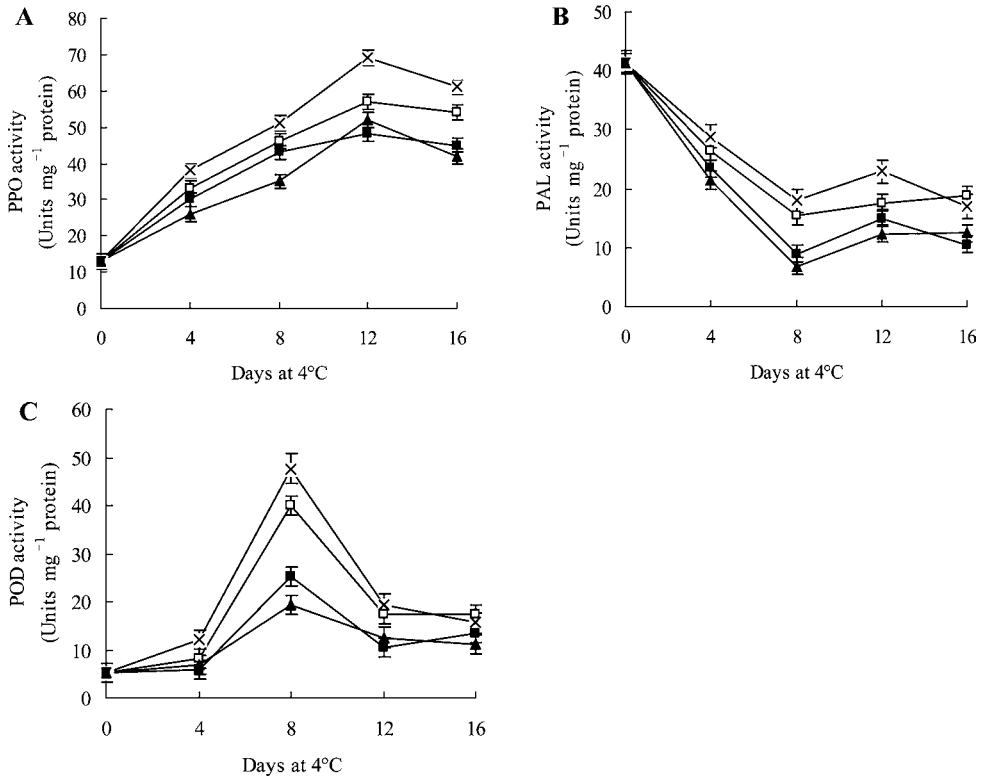

Figure 2. Changes in PPO (A), PAL (B), and POD (C) of button mushrooms packaged in control (×), PO (■), NA (□), and NAPO (▲) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

range of 73.21−79.60% after 16 days. Among the treatments, the minimum percentage (73.21%) was recorded for NAPO samples. The cap opening of mushrooms is related to the dryness of mushrooms as a result of water loss during storage. The increased water loss during storage causes a decrease in cohesive forces of water and other hydrophilic molecules, such as proteins responsible for the intact position of the caps and veil in mushrooms. As NA, PO, and NAPO reduces water loss, the cap opening of mushrooms was less, particularly in NAPO. The overall acceptability based on color, texture, and percent open caps of mushrooms decreased as the storage period advanced in all the treatments. On the basis of judgements made by sensory panel members, the control samples were unacceptable after 12 days of storage. However, mushrooms in NAPO and NA did not exhibit these characteristics even on day 16; the NAPO samples were acceptable and in marketable condition and recorded an overall acceptability of 2.12 after 12 days of storage. These results suggest that the NAPO was effective in retarding mushroom sensory deterioration.

Effect of Natamycin in Combination with Pure Oxygen Treatment on PPO, PAL, and POD Activities. Browning is the consequence of a chain of reactions in which the first step is catalyzed by the enzyme PAL, which degrades phenylalanine to ammonia and *trans*-cinnamic acid. Subsequently, *trans*-cinnamic acid is hydroxylated, its aromatic group is methylated, and the carboxyl group is reduced to give phenolic compounds. These compounds are then oxidized by PPO. POD could also be involved, although to a lesser extent owing to the low availability of H_2O_2 within the cell.[26] PPO activity increased in the control samples during the first 12 days of storage and then decreased (Figure 2A). Treatment with NAPO and PO significantly suppressed the increase in PPO and POD activity, promoted the decrease in PAL activity, with NAPO being more effective than PO (Figure 2B,C). There was no significant difference between the treatments with NA and the control. The rapid increase in activities of PPO and POD possibly accelerated the oxidation of polyphenols and thus led to rapid browning of button mushroom. Increase in PAL activity was also related to tissue browning of fresh fruits and vegetables during storage.[27] Treatment with NAPO inhibited activities of PPO, POD, and PAL, which may account for the inhibition of the mushroom browning. Day[6] suggested that high O_2 may cause substrate inhibition of the enzyme PPO or, alternatively, that high levels of colorless quinones subsequently formed may cause feedback inhibition of PPO. Gómez et al.[28] also found that superatmospheric O_2 concentrations could be effective in preventing enzymatic browning by PPO. In this study, the reduced activities of PPO, POD, and PAL in NAPO mushrooms could be due to the synergistic effect of PO combined with NA treatment.

Effect of Natamycin in Combination with Pure Oxygen Treatment on Microbiological Quality. The effects of different treatments compared to control on the counts of mesophilic, psychrophilic, pseudomonad bacteria and yeasts and molds are shown in Table 3. It is evident from this study that treatment with NA and NAPO was more effective in reducing yeasts and molds counts than PO and control. NA and NAPO treatment reduced yeasts and molds counts to below detection limits (10 cfu/g) for at least 8 days, with recovery being observed at later time points. These organisms are more susceptible to NA than other types of spoilage bacteria. Therefore, microbial degradation resulting in changes such as browning and softening was clearly delayed in NA and NAPO samples. According to Eastwood et al.,[3] the organisms usually responsible for spoilage of mushrooms are Gram-negative, psychrotrophic bacteria, particularly belonging to the *Pseudomonasae* family, because of

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Table 3. Changes in Microbial Counts (log_{10} cfu g^{-1}) of Button Mushrooms Packaged in Natamycin +100% O_2 Stored at 4 °C for 16 Days[a]

days at 4 °C	control	PO	NA	NAPO
		Mesophilic Bacteria		
0	4.11 ± 0.14 a	4.16 ± 0.21 a	4.10 ± 0.08 a	4.13 ± 0.12 a
4	5.32 ± 0.11 a	5.26 ± 0.25 a	5.25 ± 0.24 a	5.20 ± 0.31 a
8	6.70 ± 0.25 a	6.72 ± 0.14 a	6.59 ± 0.18 ab	6.42 ± 0.28 b
12	7.47 ± 0.36 a	7.36 ± 0.27 ab	7.20 ± 0.25 b	6.89 ± 0.14 c
16	7.86 ± 0.30 a	7.81 ± 0.29 a	7.75 ± 0.35 a	7.47 ± 0.26 b
		Psychrophilic Bacteria		
0	3.56 ± 0.05 a	3.54 ± 0.16 a	3.57 ± 0.19 a	3.50 ± 0.26 a
4	5.74 ± 0.20 a	5.77 ± 0.18 a	5.58 ± 0.07 b	5.50 ± 0.14 b
8	6.31 ± 0.17 b	6.45 ± 0.23 a	6.13 ± 0.12 c	5.83 ± 0.10 d
12	6.89 ± 0.26 a	6.74 ± 0.15 b	6.47 ± 0.25 c	6.25 ± 0.23 d
16	7.32 ± 0.28 a	7.30 ± 0.20 a	6.87 ± 0.37 b	6.50 ± 0.18 c
		Pseudomonad Bacteria		
0	5.13 ± 0.26 a	5.17 ± 0.25 a	5.10 ± 0.21 a	5.15 ± 0.08 a
4	5.80 ± 0.13 a	5.67 ± 0.16 b	5.55 ± 0.16 c	5.45 ± 0.20 d
8	6.43 ± 0.37 a	6.40 ± 0.17 a	6.25 ± 0.27 b	6.02 ± 0.17 b
12	7.21 ± 0.42 a	7.17 ± 0.23 a	6.87 ± 0.14 b	6.40 ± 0.24 c
16	7.87 ± 0.26 a	7.65 ± 0.29 b	7.54 ± 0.32 c	7.13 ± 0.27 d
		Yeasts and Molds		
0	3.16 ± 0.21 a	3.11 ± 0.16 a	ND	ND
4	5.20 ± 0.25 a	4.92 ± 0.30 b	ND	ND
8	6.31 ± 0.18 a	5.83 ± 0.04 b	ND	ND
12	6.86 ± 0.15 a	6.47 ± 0.08 b	2.36 ± 0.07 c	ND
16	7.35 ± 0.23 a	6.88 ± 0.16 b	3.79 ± 0.04 c	2.27 ± 0.06 d

[a]PO, pure oxygen; NA, natamycin; NAPO, natamycin + pure oxygen. Values are the mean of three replications ± standard deviation. Means in same row with different letters are significantly different ($P < 0.05$). ND = Not detected; detection limit for mesophilic, psychrophilic, pseudomonad bacteria and yeast and mold counts was 1 log_{10} cfu g^{-1}.

contamination of the product from compost. Mushrooms from control treatment exhibited tiny brown spots on day 4 that developed into dark zones, characteristic of *Pseudomonas* spoilage by day 8. Mushrooms were highly decayed at this point and the end of shelf life was due to microbial spoilage. The NAPO samples did not exhibit these characteristics of microbial degradation, even on day 10.

In summary, the results presented in this study indicated that a combination of NA and PO was more effective in maintaining tissue firmness, inhibiting respiration rate and browning, and reducing microbial counts like yeasts and molds on button mushroom than applying NA or PO alone. Moreover, NAPO treatment also reduced weight loss and delayed cap opening during the storage period. Therefore, NAPO treatment may be a promising method of maintaining button mushroom quality and extending their postharvest life. Further studies are required in order to maintain high oxygen atmosphere when a plastic film is used.

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REFERENCES

(1) Chang, S. T. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China. *Int. J. Med. Mushrooms.* **1999**, *1*, 291−300.

(2) Mattila, P.; Konko, K.; Eurola, M.; Pihlava, J. M.; Astola, J.; Vahteristo, L.; Hietaniemi, V.; Kumpulainen, J.; Valtonen, M.; Piironen, V. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 2343−2348.

(3) Eastwood, D.; Burton, K. Mushrooms—A matter of choice and spoiling oneself. *Microsc. Today* **2002**, *29*, 18−19.

(4) Jacxsens, L.; Devlieghere, F.; Van der Steen, C.; Debevere, J. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *Int. J. Food Microbiol.* **2001**, *71*, 197−210.

(5) Oms-Oliu, G.; Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Antioxidant content of fresh-cut pears stored in high-O_2 active packages compared with conventional low-O_2 active and passive modified atmosphere packaging. *J. Agric. Food Chem.* **2008**, *56*, 932−940.

(6) Day, B. P. F. High oxygen modified atmosphere packaging for fresh prepared produce. *Postharvest News Inf.* **1996**, *7*, 31−34.

(7) Gorny, J. R.; Hess-Pierce, B.; Cifuente, R. A.; Kader, A. A. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biol. Technol.* **2002**, *24*, 271−278.

(8) Oms-Oliu, G.; Raybaudi-Massilia Martínez, R. M.; Soliva-Fortuny, R.; Martín-Belloso, O. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control.* **2008**, *19*, 191−199.

(9) Lu, C.; Toivonen, P. M. A. Effect of 1 and 100 kPa O_2 atmospheric pretreatments of whole 'Spartan' apples on subsequent quality and shelf life of slices stored in modified atmosphere packages. *Postharvest Biol. Technol.* **2000**, *18*, 99−107.

(10) Stark, J. Natamycin: An effective fungicide for food and beverages. In *Natural Antimicrobials for the Minimal Processing of Foods*; Roller, S., Ed.; Woodhead Publishing: Cambridge, GBR, 2003; pp 82−97.
(11) Li, W. X.; Zhang, M.; Yu, H. Q. Study on hypobaric storage of green asparagus. *J. Food Eng.* **2006**, *73*, 225−230.
(12) Palou, E.; Lopez-Malo, A.; Barbosa-Canovas, G. V.; Welti-Chanes, J.; Swanson, G. B. Polyphenoloxidase activity and colour of blanched and high hydrostatic pressure treated banana puree. *J. Food Sci.* **1999**, *64*, 42−45.
(13) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(14) Wang, Y. S.; Tian, S. P.; Xu, Y.; Qin, G. Z.; Yao, H. J. Changes in the activities of pro- and anti-oxidant enzymes in peach fruit inoculated with Cryptococcus laurentii or Penicillium expansum at 0 or 20°C. *Postharvest Biol. Technol.* **2004**, *34*, 21−28.
(15) Koukol, J.; Conn, E. E. The metabolism of aromatic compounds in higher plants. IV. Purification and properties of the phenylalanine deaminase of *Hordeum vulgare*. *J. Biol. Chem.* **1961**, *236*, 2692−2698.
(16) Moerschbacher, B. M.; Noll, U. M.; Flott, B. E.; Reisener, H. J. Lignin biosynthetic enzyme in stem rust infected resistant and susceptible near-isogenic wheat lines. *Physiol. Mol. Plant Pathol.* **1988**, *33*, 33−46.
(17) Kader, A. A.; Ben-Yehoshua, S. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biol. Technol.* **2000**, *20*, 1−13.
(18) Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Physiological and microbiological changes in fresh-cut pears stored in high oxygen active packages compared with low oxygen active and passive modified atmosphere packaging. *Postharvest Biol. Technol.* **2008**, *48*, 295−301.
(19) Zivanovic, S.; Buescher, R. W.; Kim, K. S. Textural changes in mushroom (*Agaricus bisporus*) associated tissue ultrastructure and composition. *J. Food Sci.* **2000**, *65*, 1404−1408.
(20) Tao, F.; Zhang, M.; Yu, H.; Sun, J. Effects of different storage conditions on chemical and physical properties of white mushrooms after vacuum cooling. *J. Food Eng.* **2006**, *77*, 545−549.
(21) Rohani, M. Y.; Zaipun, M. Z.; Norhayati, M. Effect of modified atmosphere on the storage life and quality of *Eksotika papaya*. *J. Trop. Agric. Food Sci.* **1997**, *25*, 103−113.
(22) Wszelaki, A. L.; Mitcham, E. J. Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biol. Technol.* **2000**, *20*, 125−133.
(23) Zheng, Y. H.; Yang, Z. F.; Chen, X. H. Effect of high oxygen atmospheres on fruit decay and quality in Chinese bayberries, strawberries and blueberries. *Food Control.* **2000**, *19*, 470−474.
(24) López-Briones, G. L.; Varoguaux, P.; Chambroy, Y.; Bouquant, J.; Bureau, G.; Pascat, B. Strorage of common mushroom under controlled atmospheres. *Int. J. Food Sci. Technol.* **1992**, *27*, 493−505.
(25) Conesa, A.; Verlinden, B. E.; Artés-Hernández, F.; Nicolaï, B.; Artés, F. Respiration rate of fresh-cut bell peppers under superatmospheric and low oxygen with or without high carbon dioxide. *Postharvest Biol. Technol.* **2007**, *45*, 81−88.
(26) Artés, F.; Castañer, M.; Gil, M. I. Enzymatic browning in minimally processed fruit and vegetables. *Food Sci. Technol Int.* **1998**, *4*, 377−389.
(27) Fujita, N.; Tanaka, E.; Murata, M. Cinnamaldehyde inhibits phenylalanine ammonia-lyase and enzymatic browning of cut lettuce. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 672−676.
(28) Gómez, P. A.; Geysen, S.; Verlinden, B. E.; Artés, F.; Nicolaï, B. M. Modelling the effect of superatmospheric oxygen concentrations on in vitro mushroom PPO activity. *J. Sci. Food Agric.* **2006**, *86*, 2387−2394.