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Full Length Research Paper

Extension of the shelf-life of fresh oyster mushrooms (*Pleurotus ostreatus*) by modified atmosphere packaging with chemical treatments

Gongnian Xiao^{1,2}, Min Zhang^{1*}, Liang Shan¹, Yuru You² and Vilas M. Salokhe³

¹State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, People's Republic of China.

²Key Laboratory of Agricultural Products, Chemical and Biological Processing Technology, Zhejiang University of Science and Technology, Hangzhou 310023, People's Republic of China.

³Agriculture, Aquatic Systems and Engineering Program, Asian Institute of Technology, Pathumthani 12120, Thailand.

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The objective of this study was to improve the preservation of fresh oyster mushrooms by modified atmosphere packaging (MAP) and chemical treatments. The MAP effects and treatments on organoleptic quality, weight loss, cell permeability, texture changes and polyphenol oxidase (PPO) activity were studied. In addition, three packaging materials were used for MAP mushroom storage. Low-density polyethylene (LDPE) proved to be a more suitable material than polyvinyl chloride (PVC) and LDPE-PVC as MAP materials. On the other hand, for mushrooms storage, MAP in combination with chemical treatments (sorbitol 0.05 g/100 g, CaCl₂ 1.0 g/100 g and citric acid 3.0 g/100 g) showed inhibitory effects on weight loss and mushrooms cell permeability. Furthermore, the results of polyphenol oxidase (PPO) activity, texture and organoleptic analysis also showed that active MAP, composed of 1.5% O₂ and 20% CO₂ and combined with chemical treatments, was found to be beneficial in maintaining oyster mushrooms quality and shelf-life.

Key words: Modified atmosphere packaging (MAP), oyster mushroom, shelf-life, preservation, chemical treatments.

INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*), also called *Pinggu* mushroom in China, is widely cultivated in China, Japan and Thailand. It is a highly perishable mushroom with a normal shelf-life of 1 to 3 days at ambient temperature during marketing (Xiao and Zhang, 2003). Modified atmosphere packaging (MAP) was demonstrated in some reports to be an effective method of extending the shelf-life of this mushroom and other mushrooms' varieties (such as *Agaricus bisporus*) (Gabriela et al., 2008; Borton et al., 1987); however, the gas compositions used were quite different. In other reports, an optimum O₂ level of 6% was used for white mushrooms MAP storage, although the white mushrooms had an increased shelf-life, modifying the moisture within a conventional package

(without modifying the atmosphere) with positive effects on their quality and shelf-life (Roy et al., 1995a, 1995b, 1996). It was also shown that low O₂ (1.0 ~ 1.6%) and high CO₂ (17 ~ 18%) levels could effectively extend the shelf-life of white mushrooms (Deepak et al., 2000). According to other authors, the use of MAP technology cannot preserve the organoleptic quality at room temperature beyond one week (Jones et al., 1963; Zhang et al., 2006).

Mushrooms packed and unwrapped in a conventional hardboard box loose marketability early during the storage period due to weight loss, shrinkage, browning and spore formation (Tianjia et al., 2010). Packaging with modified atmosphere in combination with sorbitol pretreatment resulted in mushrooms with better colour and lower moisture loss (<18%) during 9 days storage at 12°C, when compared with the control group without sorbitol pretreatment under other conditions (Roy et al., 1995). The utilization of MAP in post-harvest mushrooms

*Corresponding author. E-mail: min@sytu.edu.cn. Fax: 0086-(0)510-85807976.

preservation is one important technique used to reduce losses and maintain quality, considerably extending the effect of low temperature storage. As MAP is a dynamic system a gradient is set up between the outside and inside of the package (Zhang and Sundar, 2005). So, an inappropriate packaging material permeability, surfacing from the product, can produce O₂ and CO₂ levels that will affect the extended respiration rate, thereby inducing physiological injuries, which in the case of mushrooms, results in severe browning. Thus, the suitable MAP material for specific foods must be selected through a scientific research.

Many works have focused on the use of chemical treatments to extend and improve the shelf-life of fruits and vegetables (Guijarro et al., 2007; Wilawanet al., 2003). In this study, since MAP can control the respiration, we used water holding agents and antioxidants. The water holding capacity of sorbitols is well known, and these compounds are used in several countries mainly to reduce drip of fruits and vegetables. Citric acid is widely used in the food industries, especially for its preservative action due to pH lowering and its synergetic effect for antioxidants due to its metal-chelating action (Martine et al., 2000).

Mushrooms senescence is characterized by morphological maturation. It may be accessed directly by sensory method, where organoleptic quality, off-odors and off-colour can be tested, as being an index of the mushrooms storage effects (Zhang and Sundar, 2005; Li et al., 2006). Weight loss of mushroom can affect the major postharvest loss mainly due to the high metabolic rate, which resulted into short shelf-life. However, the mushroom cell membrane integrity is destroyed, resulting in increased permeability of the cells. Thus, conductivity reflects the concentration of ions in the solution, in that the greater the conductivity, the greater the ion concentration. Nevertheless, using electrical conductivity represents the membrane permeability.

Mushrooms, by the way, can be physically evaluated by the texture, where the maximum stress, deformation degree and power can be considered as the texture indices measures (Xiao and Zhang, 2003; Zhang and Duan, 2005). Since the interior tissues have a hierarchical and loose spongy structure, they are not homogeneous and compacted as other solid materials, such as apple and cucumber.

During storage, PPO enzymatic action seriously affects the sensory quality regularly. PPO exists in many advanced plants and it catalyzes two different reactions (Li et al., 2006): (i) Monophenol hydroxylation, which generates the corresponding ortho-dihydroxide radical compounds; and (ii) ortho-diphenol oxidation, which generates ortho-diquinone. The ortho-diquinone continues to change into a brownish pigment, although discoloration and browning have been reported in carrots, apples, apricots, peaches, lettuces, etc., and are caused mainly by oxidation of natural phenolic compounds in quinines in the presence of PPO (Zhang et al., 2005).

The objective of this work was to find the best combined effects of the modified atmosphere packaging, using different film types, and chemical treatment on the quality and shelf-life of fresh oyster mushrooms.

MATERIALS AND METHODS

Oyster mushrooms of XIAFENG962 strain, grown on traditional manure-based compost, were picked from a local farm in Suzhou, Jiangsu province, China.

Packaging equipment

An ADFM-V300 packaging system for MAP, made by Zhangjiagang HengZhong Machine Company, Suzhou, China, is shown in Figure 1. The capacity of the precision distribution and mixing cylinders were 1 and 10 L, respectively. The nominal accuracy of the system was $\pm 0.5\%$. This system was successfully used for pork MAP research (Zhang and Sundar, 2005; Li et al., 2006). A DDS-11A type electrical conductivity measuring apparatus was used for determining the permeability of the mushrooms cells (Xiao and Zhang, 2003; Zhang et al., 2006; Li et al., 2006).

Procedure for packaging and storage

Mushrooms were sorted by size and appearance. Diseased, damaged, open-veiled and very large or small mushrooms (cap diameter <25 mm or >40mm) were discarded, and the mushroom stems were trimmed by hand to a stipe length of 7 \pm 1 mm. Acceptable mushrooms were randomly selected and dipped in the following solutions for 3 min: (i) Treatment 1: phytic acid (0.1/100 g), sorbic acid (0.05/100 g), sorbitol (0.05/100 g) and potassium sorbate (0.05/100 g); (ii) Treatment 2: sorbitol (0.05/100 g), CaCl₂ (1.0/100 g) and citric acid (3.0/100 g); and (iii) Treatment 3: sub-nanoparticle silver (0.1 μ g/g), whose level was referred to from the previous research in the study's lab by Zhang and Duan Wilawan et al. (2003). At the 4th, 6th, 8th and 10th day of MAP storage, in a low temperature incubator, six mushrooms were chosen and evaluated at random from each group of MAP. Three package materials were used for the tests and they included: (i) LDPE, (ii) PVC and (iii) LDPE-PVC composite. The size and packages of wall thickness were 25 x36 mm and 0.022 mm, respectively. For LDPE-PVC composite, each composition occupied half of the thickness, while each package contained 100 \pm 5 g mushrooms. The materials for LDPE, PVC and LDPE-PVC composite had an oxygen permeability of 50 to 80, 3 to 5 and 0.2 to 0.8 ml/m².d.atm at 25°C and 100%RH, and a water vapour transmission rate of 87 to 120, 5 to 13, 2 to 7 g/m².d. at 40°C and 90%RH, respectively. A gas mixture of 1.5% O₂ and 20% CO₂ was introduced and balanced in the packages, the mixture was sealed and then stored at 6 \pm 1°C.

Organoleptic evaluation

Organoleptic evaluation was performed based on four aspects, that is, color, hardness, degree of rot and flavor. Ten students, who have the knowledge of food science and sensory evaluation of food, and who were studying in the university as graduate students were requested to score the samples. Each organoleptic aspect was scored with reference to fresh mushrooms according to the following scale: 15 (no change); 10 (slight change); and 5 (obvious change). Two controls were established: (i) C.K (check) was packaged only in the air and was stored at 6°C; (ii) C.K' was stored without packaging at 6°C.

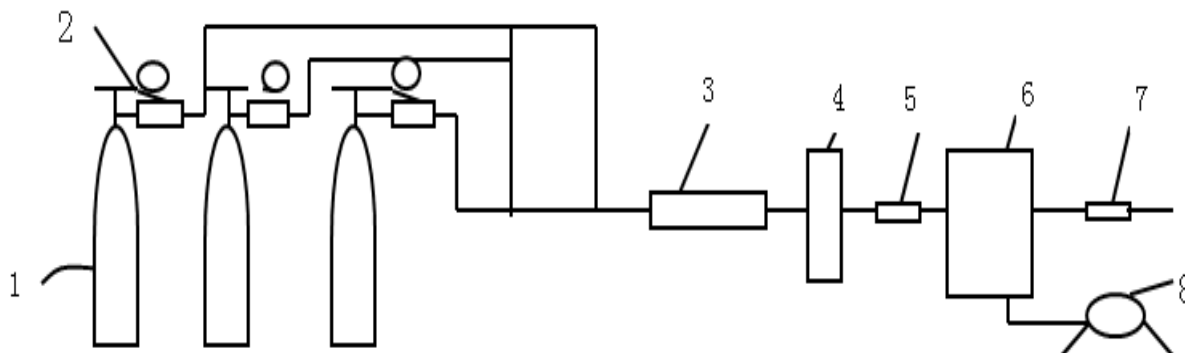


Figure 1. Schematic diagram of the gas mixing system for MAP. 1, Gas bottle; 2, pressure control valve; 3, three way valve; 4, precision distribution cylinder; 5, filling valve; 6, mixing cylinder; 7, filling valve; 8, vacuum pump.

Quality attributes

Weight loss

Weight loss was calculated according to the weights of each package before and after storage, and expressed as a percentage of the initial weight of mushrooms (Xiao and Zhang, 2003; Zhang et al., 2001, 2003).

Permeability of the cell membranes

Cell permeability was expressed as the electrical conductivity ratio of pre-heated and post-heated samples (Xiao and Zhang, 2003; Carole et al., 2010). 10 g samples were cut into dices of 0.8 x 0.8 x 0.4 cm, and then washed in deionized water. After absorbing water (about 10 min later), the mushroom dices were soaked in 50 ml distilled water at a constant temperature of 30°C. The electrical conductivity was determined on a DDS-11A measuring apparatus (Shanghai Huaguang Co, Shanghai, China). The dices were then dipped in boiling water for 15 min and the electrical conductivity was again determined.

Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) activity was measured on a 721-type spectrophotometer (Lengguang Technological Company, Shanghai, China) as described by Deepak and Shashi (2007). The increase in optical density upon addition of an aliquot of 0.1 ml of enzyme solution to 2.9 ml of substrate mixture solution was measured at 430 nm, setting the temperature at 25°C for every 15 s over 2 min. The initial reaction rate ($\Delta OD \text{ min}^{-1}$) was used to express enzyme activity and was calculated by the linear regression of the measured value of absorbance, which increased over 2 min. The substrate mixture solution was composed of 0.1 ml hydrogen peroxide with 0.3% (m/m), 0.2 ml o-benzene diamine with 1% (m/m) in alcohol solution, and 2.6 ml phosphate buffer with the concentration of 0.1 mol at pH 7.0.

Mushrooms texture

The mushrooms texture was analyzed on a TA.XT2i (Stable Micro Systems Ltd, Surrey, UK) texture analyzer with suitable parameters (probe: P/2N; puncture rate: 2.0 mm/s). After 10 days of storage, mushroom pieces of 0.2 x 0.2 cm size were cut from the kernel of

sporophores. The maximum stress (F_{\max}) of break-away was used to represent the hardness of the mushrooms. Each determination was repeated 10 times, and the results were averaged.

Data analysis

The experiment was designed randomly with three replicates (except for the mushroom texture, which had 10 replicates). Data analysis was carried out by the analysis of variance (ANOVA), with mean separation by Duncan's multiple range tests, and significant differences were established at $P < 0.05$ level.

RESULTS AND DISCUSSION

Effects of different packaging materials on mushrooms organoleptic quality

The results (Figure 2) showed that MAP improved the preservation effect on mushrooms using any of the three package materials. However, the effects of the LDPE films were better when compared with the other two packaging materials after 8 days storage, which became evident in the organoleptic score of 13 versus 10 and 9, respectively. This may be due to higher LDPE gas permeability. The selection of these films was based on the irrelative low O_2/CO_2 permeability and the special gas selectivity. The mushrooms had a low respiratory activity, while the permeability properties were considered appropriate for the tested storage conditions.

Effect of different treatments on organoleptic quality and weight loss

As demonstrated by these trials (Table 1), the mushrooms of C.K' were rotten at the 6th day of storage. Furthermore, the organoleptic quality of C.K samples was also lower than that of treatments 1, 2 and 3. So, we can conclude that treatments 1 and 2 provided satisfactory preservation effects.

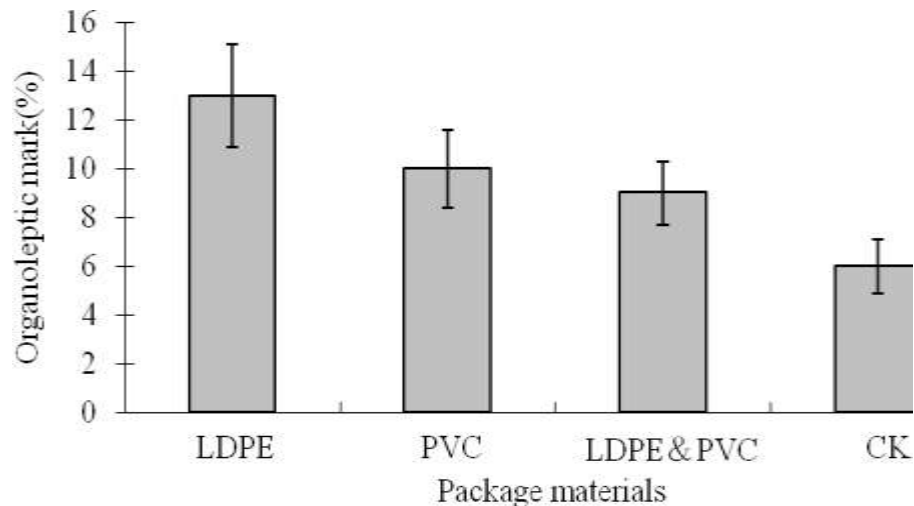


Figure 2. Effects of different packaging materials on the preservation of oyster mushrooms [treatment 2 with MAP (1.5% O₂ and 20% CO₂)]. The mean and standard deviation values were obtained by three replicates.

Table 1. Organoleptic evaluation of oyster mushrooms in different storage times of MAP (1.5% O₂ and 20% CO₂) combined with different treatments.

Treatment	Organoleptic evaluation score			
	4th day	6th day	8th days	13th day
1	14.0±0.2	13.0±0.8	10.0±0.1	9.0±0.2
2	13.0±0.3	13.0±0.3	13.0±0.1	13.0±0.2
3	14.0±0.4	13.0±0.4	9.0±0.2	9.0±0.3
CK	14.0±0.7	12.0±0.4	10.0±0.2	7.0±0.3
CK'	12.0±0.1	7.0±0.6	6.0±0.2	5.0±0.3

1, Stored at 6 ± 1°C; 2, MAP conditions were LDPE film (thickness: 0.022 mm); 3, the sensory scoring system as in 2 and 3. The mean and standard deviation values were obtained by three replicates.

In addition, the MAP and treatments controlled better the mushrooms weight loss (Table 2).

Effects of MAP and treatments on mushrooms cell permeability change

Zhang et al. (2006) used this method for evaluating the effect of strawberries preservation. In this study, with the extension of storage time after harvest, gradients were gradually established in the mushroom cells so that the cell permeability was increased. MAP and the chemical treatments showed obvious prevention effects on cell permeability, in comparison with the C.K and C.K' groups from day 8 onward (Figure 3). With the post-harvest storage time, the mushroom cell membrane integrity was destroyed, resulting in increased permeability of the cells. MAP and the chemical treatments also effectively inhibited the rate of change of cell permeability; hence, the shelf-life of mushrooms was extended.

Effects of MAP and treatments on mushrooms texture changes

As demonstrated in Figure 4, C.K' had a number of break-away peaks with an F_{max} around 954 g, while treatment 2 exhibited an F_{max} around 2764 g, which represented a big difference in hardness between the treatment groups and C.K'. Phytic acid can inhibit microbiological infection, as citric acid can also inhibit microbiological infection and adjust its pH (Carole et al., 2010). Sorbitol can inhibit water loss (Zhang and Duan, 2005; Carole et al., 2010), and all these chemicals can inhibit enzyme activity and carbohydrate degradation (Xiao and Zhang, 2003; Zhang et al., 2006). The preservation effects of the chemical treatments could be attributed to their combined functions that were mentioned. Furthermore, since the F_{max} of C.K was 1541 g, the MAP treatment on its own maintained mushroom quality, although the effects were less than the treatments with MAP.

Table 2. Effects of different treatments on weight loss (%) of oyster mushrooms in MAP (1.5% O₂ and 20% CO₂).

Treatment	Weight loss (%)			
	4th day	6th day	8th days	13th day
1	1.07±0.02	0.90±0.01	0.69±0.01	2.30±0.05
2	1.37±0.03	1.02±0.01	0.84±0.01	1.38±0.03
3	1.20±0.03	0.33±0.00	0.40±0.00	1.90±0.04
CK	1.20±0.02	1.04±0.01	0.91±0.00	2.26±0.01
CK'	3.34±0.98	5.51±0.43	5.29±0.43	9.67±1.11

The mean and standard deviation values were obtained by three replicates.

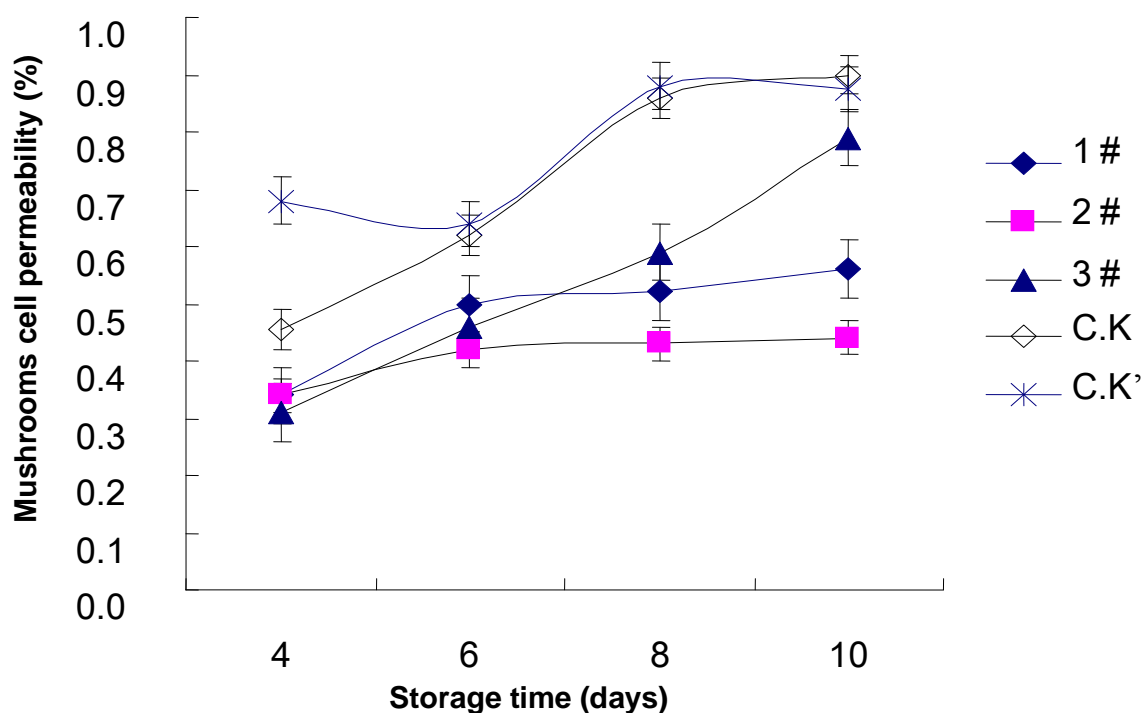


Figure 3. Effects of different treatments and MAP (1.5% O₂ and 20% CO₂) on the cell permeability ratio of oyster mushrooms. The mean and standard deviation values were obtained by three replicates.

Effects of MAP and treatments on mushrooms polyphenol oxidase (PPO) activity

In these trials (Figure 5), treatments 1 and 2, were obviously better than C.K and C.K' in controlling PPO activity. It may be that the addition of sorbitol and citric acid affected the pH values, which were related to the PPO activity. In addition, the values of PPO activity fluctuated as the results in cool storage (Zhang et al., 2001; Zhang, 2004). The effects of sub-nano-particle silver treatment were not very desirable, and were different from the results of the previous study by the authors in tomato juices. As such, it may be that the respiration of mushrooms can be combined with sub-nano-particle silver (Zhang and Duan, 2005).

Conclusions

According to the results, it was clearly indicated that:

1. LDPE was preferred to PVC and LDPE-PVC as the MAP film material for oyster mushrooms storage.
2. MAP in combination with the chemical treatments can extend oyster mushroom shelf-life from 4 to 6 days. Treatment 2 [sorbitol (0.05/100 g), CaCl₂ (1.0/100 g), citric acid (3.0/100 g)] had the best effect, in that it inhibited weight loss, and increased cell permeability and PPO activity.
3. Chemical treatments improved the economical benefits of preserving agricultural products due to their desirable effects and low cost. At the same time, all the chemical

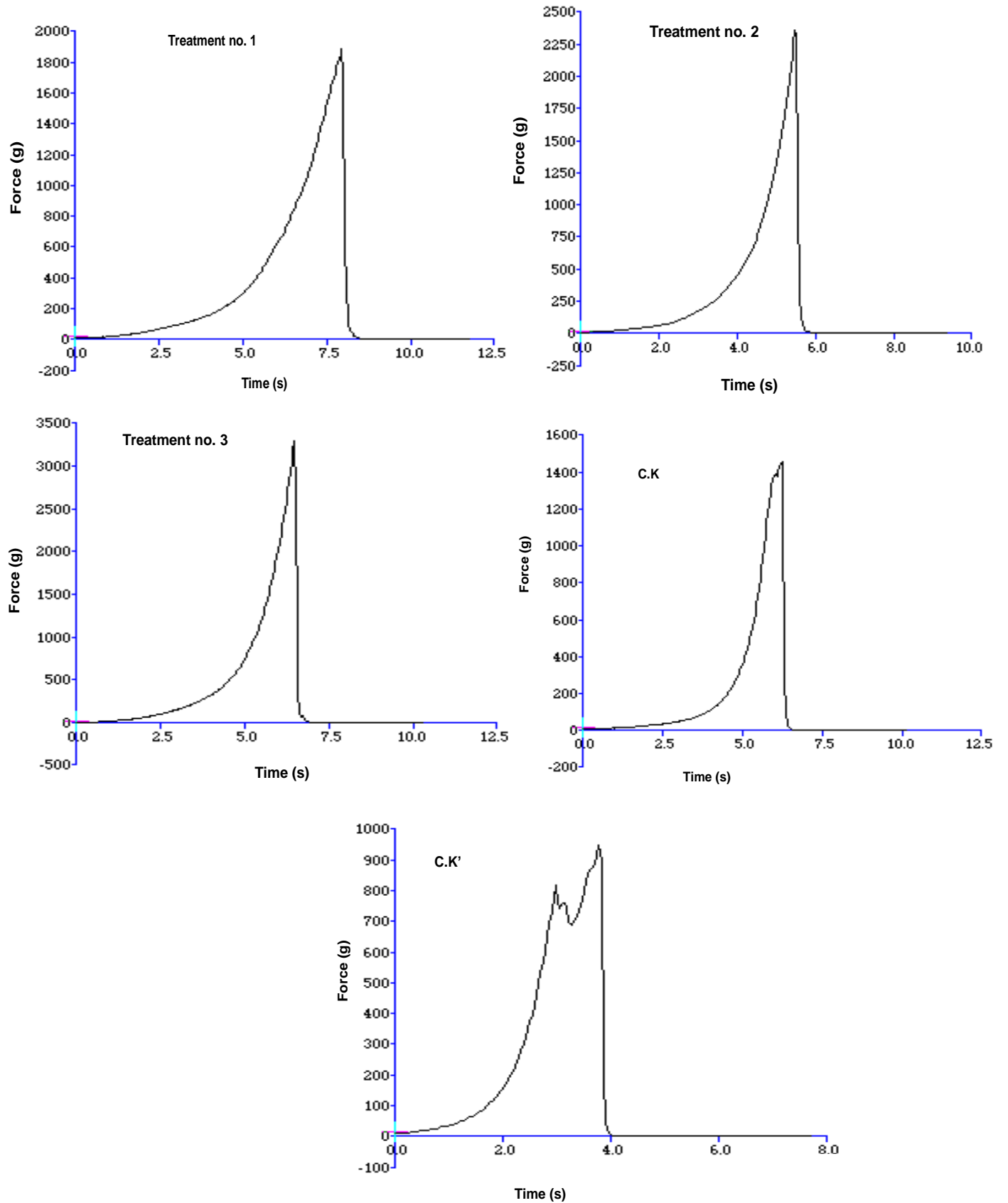


Figure 4. Force-time diagrams for oyster mushrooms in MAP (1.5% O₂ and 20% CO₂) with different treatments on day 10 at 6°C. The mean and standard deviation values were obtained by ten replicates.

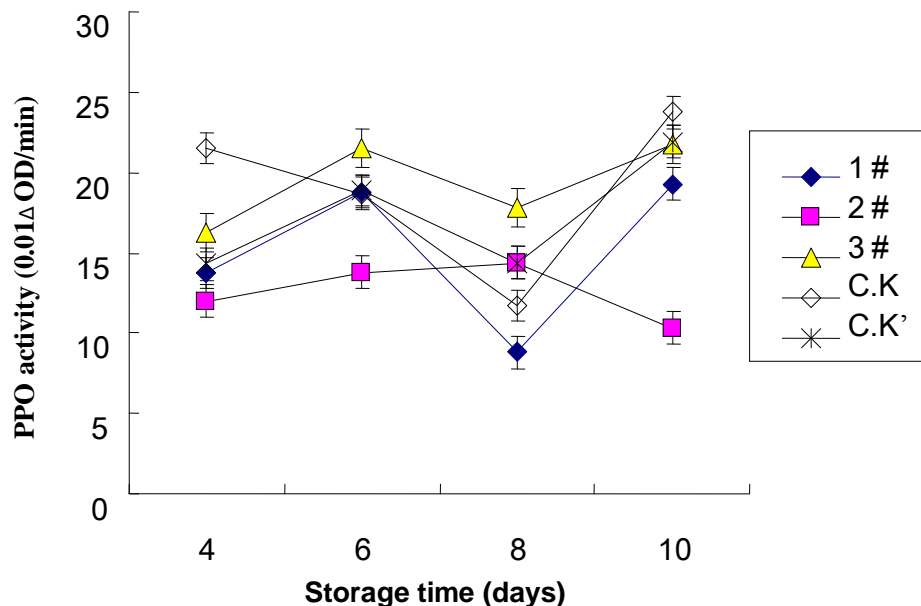


Figure 5. Effects of different treatments on the polyphenol oxidase (PPO) activity of oyster mushrooms in storage [packaging film: LDPE, MAP (1.5% O₂ and 20% CO₂)]. The mean and standard deviation values were obtained by three replicates.

treatment substances selected in this study were allowed by the Food Additives Law and were suitable for inclusion in a HACCP plan.

4. MAP technology, combined with chemical treatments, is promising for practical application to oyster mushrooms.

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