Research on Modified Atmosphere Packaging (MAP) with High Oxygen Concentration of Fresh Shiitake Mushrooms (*Lentinus edodes*)

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Yanjie LI

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ABSTRACT

Modified atmosphere packaging (MAP) is a beneficial tool to prolong the shelf life of fresh vegetables and fruit. MAP technique has been researched on some produce. Temperature as a critical factor to affect the respiration rate of fresh produce needs to be taken into consideration when designing the MAP. Shiitake mushroom as a produce with affluent nutrients and typical taste is very perishable and easy to get brown. MAP on fresh shiitake mushrooms isn't widely researched and applied, which requires further and deeper research to obtain the optimum packaging conditions. Therefore, the effect of temperature on the respiration rate was researched and modeled. And the effect of gas composition on respiring product, the change of physiology and nutrient components of fresh shiitake mushrooms was also studied.

In the first chapter, the general information of modified atmosphere packaging and the physiology, nutrients and storage characteristics after postharvest of shiitake mushrooms were introduced. In addition, researches about the modified atmosphere packaging application on some fruits and vegetables, as well as the storage and packaging methods of fresh shiitake mushrooms were reviewed.

In the second chapter, seven selected vegetables with various package types were investigated, and the effect of temperature on O_2 consumption rate (RO_2), CO_2 production rates (RCO_2) and respiratory quotient (RQ) of seven selected vegetables were also studied. The results showed that packages of Shiitake, Maitake and Enokitake mushroom were not suitable because the oxygen concentration has decreased to lower than 1 % and the anaerobic fermentation would be soon induced. Temperature greatly influenced the O_2 consumption rate (RO_2) of Shiitake mushroom, Enokitake mushroom and Green hot pepper, and significantly influenced CO_2 production rates (RCO_2) of all these seven kinds of vegetables. It was also found that RQ was dependent on the temperature, and it became

high as the temperature increasing from 10 to 20 °C. And the gas transmission rate change of all the films with reciprocal of Kelvin temperature well fitted the exponential regularity. Among the packages of 7 vegetables, in-package oxygen concentrations were extremely low of Shiitake, Maitake and Enokitake mushrooms, for the reason of high oxygen consumption by respiration and low oxygen transmission from film. The suggestive solution is to improve the film gas transmission rate or increase the initial oxygen concentration. Decreasing the mass of produce is not suggested because of the waste of package materials. For Long bean, Green hot pepper and Baby leaf, the MA atmosphere was not created for the too high oxygen concentration inside the packages, and the respiration won't be inhibited, and the suggestions for these three vegetables are to select the package film with lower gas permeability or increase the mass of being packaged produce. For Edamame, perforation-mediated method was suggested, but the number of perforations should be decreased.

In the third chapter, active modified atmosphere packaging with different initial O_2 concentrations (HOP, MOP, LOP and AIR) were applied on the storage of postharvest shiitake mushrooms. LOP with the initial 3 % O_2 /5 % CO_2 and AIR treatment induced the anaerobic fermentation immediately after packaging and the high accumulation of ethanol concentration during the storage. Ethanol release was well retarded in MOA with 50 % O_2 initially and HOP with 100 % O_2 initially. Shiitake mushrooms' tissue was affected significantly by LOP for a high value of electrolyte leakage after the storage and a consequent high total phenolic content. No packaging could prevent the polysaccharide content decreasing in this research. And the various packaging methods did not show the big difference of decrease of polysaccharide content. All the active modified atmosphere packaging had the significant effect on the increase of free amino acid content during the storage. Therefore, all the active modified atmosphere packaging showed the advantageous

effect on the free amino acid increase, but the LOP had the detrimental effect on shiitake mushrooms.

In the fourth chapter, high oxygen atmosphere with 100% O_2 and 80% O_2 were applied on preserving shiitake mushrooms packaged in polyethylene packages. The result showed that both 100% O_2 and 80% O_2 had beneficial effect on the sensorial quality of fresh shiitake mushrooms by retarding the anaerobic metabolism occurrence and contributing the better aroma scores compared with air packaging. HOP can not reduce the respiration rate or prevent the fermentation metabolism of shiitake mushrooms completely, but HOP with initial 100% O_2 could retard the ethanol and acetaldehyde accumulation. Mushroom's hardness, color parameters of L^* and a^* got significant higher values in HOP than in air packaging after the storage, but TSS content did not show any significant difference between treatments. Aroma played an important role when judging the sensorial quality of fresh shiitake mushrooms, and aroma scores in HOP samples were significantly higher than AIR samples. During the storage, samples stored in HOP especially 100 % O_2 atmosphere, showed a lower deterioration rate and higher acceptable quality than those stored under air condition.

In the fifth chapter, the change in qualities using high oxygen packaging (HOP), with an initial 100% O_2 concentration, was investigated at 10 °C and 90% RH and compared to perforation-mediated modified atmosphere packaging (PM-MAP) with 4 (P4), 8 (P8) and 20 (P20) perforations (Diameter = 234 ± 26μm). Gas composition, color, hardness, TSS concentration, mass loss and sensory evaluation were determined during the 8 days' storage. The result showed that P4 and P8 created the low oxygen and high carbon dioxide atmospheres and effectively maintained shiitake mushrooms quality within 4 days at 10 °C. However, with extended storage, severe fermentation atmosphere with a high ethanol concentration happened in P4 and obvious browning happened in P8. So P4 and P8 did not

show the effect on keeping freshness of shiitake mushrooms at the end of the storage. However, HOP with 100% initial O_2 concentration maintained a significantly higher L^*, hardness and sensory quality than PM-MAP. Therefore, it could be concluded that HOP showed the potential to maintain the quality of shiitake mushrooms when prolonging the storage period more than 8 days.

In further research, the effect of gas composition and storage time on the respiration rate of various vegetables or fruits should be studied and modeled, and the optimum conditions for designing the MAP should be obtained. The enzyme activity changes of shiitake mushrooms during the storage under different packaging conditions also will be researched. In addition, the effect of packaging film with different permeabilities on the quality change of shiitake mushrooms should also be taken into consideration.

CHAPTER 1

General Introduction and Literature Review

1.1 General introduction

1.1.1 Importance of vegetables and fruits to humans

Vegetables and fruits are very important to humans for the indispensable nutrients. These nutrients guard the human's body from the disease and maintain the health to humans. The nutritive constituents include various vitamins, such as vitamin C (ascorbic acid), A, thiamine (B1), niacin (B3), pyridoxine (B6); also include some minerals such as calcium, potassium, magnesium. The source and the effects of these nutritive constituents are depicted in Table 1.1.

In addition, non-nutritive constituents in vegetables and fruits provide advantageous help to humans and prevention to disease. For instance, anthocyanidins including cyanidin, malvidin, delphinidin contained in some colorful fruits and vegetables (blueberry, grape, strawberry, pomegranate) had the inhibitory effect on heart disease, cancer initiation, diabetes. Besides, flavanones contained in citrus and flavones contained in celery, pepper also were proved to effectively inhibit cancer or allergies. Table 1.2 detailedly depicts non-nutritive plant constituents and their effect on human's wellness. Both the nutritive and non-nutritive constituents are very important for their supplementary and indispensable effect to the human's health. In these constituents, many of them are very easy to get decreased or destroyed after the storage. For instance, Vitamin C (ascorbic acid) of the plant will be soon depleted if stored inappropriately or damaged mechanically. Polyphenolic compounds is also easy to be depleted enzymatically catalyzed by polyphenol oxidase.

Table 1.1 Nutritive constituents of fruits and vegetables that have a positive impact on human health and their sources (Kader 2001)

Constituent	Sources	Established or proposed effects on human-wellness
Vitamin C (ascorbic acid)	broccoli, cabbage, cantaloupe, citrus fruits, guava, kiwifruit, leafy greens, pepper, pineapple, potato, strawberry, tomato, watermelon	prevents scurvy, aids wound healing, healthy immune-system, cardiovascular-disease
Vitamin A (carotenoids)	dark-green vegetables (such as collards, spinach, and turnip greens), orange vegetables (such as carrots, pumpkin, and sweet potato), orange-flesh fruits (such as apricot, cantaloupe, mango, nectarine, orange, papaya, peach, persimmon, and pineapple), tomato	night blindness prevention, chronic fatigue, psoriasis, heart disease, stroke, cataracts
Vitamin K	nuts, lentils, green onions, crucifers (cabbage, broccoli, brussel sprouts), leafy greens	synthesis of pro-coagulant factors, osteoporosis
Vitamin E (tocopherols)	nuts (such as almonds, cashew nuts, filberts, macadamias, pecans, pistachios, peanuts, and walnuts), corn, dry beans, lentils and chickpeas, dark-green leafy vegetables	heart-disease, LDL-oxidation, immune-system, diabetes, cancer
Fiber	most fresh fruits and vegetables, nuts, cooked dry beans and peas	diabetes, heart disease
Folate (folicin or folic acid)	dark-green leafy vegetables (such as spinach, mustard greens, butterhead lettuce, broccoli, brussels sprouts, and okra), legumes (cooked dry beans, lentils, chickpeas and green peas), asparagus	birth defects, cancer, heart disease, nervous system
Calcium	cooked vegetables (such as beans, greens, okra and tomatoes) peas, papaya, raisins, orange, almonds, snap beans, pumpkin, cauliflower, rutabaga	osteoporosis, muscular/skeletal, teeth, blood pressure
Potassium	baked potato or sweet potato, banana & plantain, cooked dry beans, cooked greens, dried fruits (such as apricots and prunes), winter (orange) squash, and cantaloupe	hypertension, stroke arteriosclerosis
Magnesium	spinach, lentils, okra, potato, banana, nuts, corn, cashews	osteoporosis, nervous system, teeth, immune system

Table 1.2 Non-nutritive plant constituents that may be beneficial to human health (Kader 2001)

Constituent	Compound	Sources	Established or proposed effects on human-wellness
Proanthocyanins	tannins	apple, grape, cranberry, pomegranate	cancer
Anthocyanidins	cyanidin, malvidin, delphinidin, pelargonidin, peonidin, petunidin	red, blue, and purple fruits (such as apple, blackberry, blueberry, cranberry, grape, nectarine, peach, plum & prune, pomegranate, raspberry, and strawberry)	heart disease, cancer initiation, diabetes, cataracts, blood pressure, allergies
Flavan-3-ols epicatechin,	epigallocatechin catechin, gallocatechin	apples, apricots, blackberries, plums, raspberries, strawberries platelet	aggregation, cancer
Flavanones	hesperetin, naringenin, eriodictyol	citrus (oranges, grapefruit, lemons, limes, tangerine)	cancer
Flavones	Luteolin, apigenin	celeriac, celery, peppers, rutabaga, spinach, parsley, artichoke, guava, pepper	cancer, allergies, heart disease
Flavonols	quercetin, kaempferol, myricetin, rutin	onions, snap beans, broccoli, cranberry, kale, peppers, lettuce	heart disease, cancer initiation, capillary protectant
Phenolic acids	caffeic acid, chlorogenic acid, coumaric acid, ellagic acid	blackberry, raspberry, strawberry, apple, peach, plum, cherry	cancer, cholesterol
Carotenoids	Lycopene	tomato, watermelon, papaya, Brazilian guava, Autumn olive, red grapefruit	cancer, heart disease, male infertility

1.1.2 Nutrients and physiology of fresh shiitake mushrooms (*Lentinus edodes*)

1.1.2.1 Nutrients contained in shiitake mushrooms

Shiitake mushrooms belong to Fungi Kingdom, Basidiomycota Division, and Lentinula Genus. They have been cultivated for over 1000 years. Shiitake mushrooms are traditionally well-known edible mushrooms of high nutritious value. For example, their fruit bodies had high content protein (20–23% in dry weight), lipids, carbohydrate (58–60% in dry weight), vitamins (B1 (thiamine), B2 (riboflavin), B12 (niacin), and pantothenic acid) and minerals (Fe, Mn, K, Ca, Mg, Cu, P, and Zn). The water-soluble polysaccharide which has been proved to have inhibitive effect on cancer, oxidation has also been found in shiitake mushrooms. For instance, lentinan, D-glucans, xyloglucans etc, have been identified. Shiitake mushrooms also contain some indigestible polysaccharides which provide dietary fiber, such as heteroglycan, polyuronide, and chitin (Solomon P. Wasser, 2005, Mizuno 1995, Hobbs 2000).

1.1.2.2 Cultivation, consumption fresh shiitake mushrooms

Shiitake mushrooms are native to Japan, China and Korea and have been grown in all three countries since prehistoric times. And today, their cultivation and consumption widely spread to all over the world. In Japan, the consumption of shiitake mushrooms increases by tons of amount yearly, and the price also goes up rapidly. From Fig. 1.1, it could be known that the consumption amount of shiitake mushrooms changed from 20.76 kton in 1965 to 87.71 kton in 2005 with the four times increase in 40 years. With their increase of consumption demand, the price also rose up to 1056 yen per kg in 2005 from 370 yen per kg in 1965. The significant increase of consumption and price indicates that the nutritional value of shiitake mushrooms is being realized gradually.

1.1.2.3 Physiological characteristics and storage property of fresh shiitake mushrooms

Although Shiitake mushrooms (*Lentinus edodes*) have affluent nutritive compounds,

they are fast-respiring, highly perishable and tend to lose quality immediately after harvest. The shelf life is short because of the high respiration rate, tendency to turn brown and having no physical protection to avoid water loss or microbial attack. If the fresh shiitake mushrooms were stored or packaged inappropriately, the shelf life will be shortened because of the perishability or browning (Jiang, Feng and Li 2012, Simón, González-Fandos and Tobar 2005). Therefore, the storage or packaging is very critical for the preservation of fresh shiitake mushroom. According to our investigation, shiitake mushrooms sold in present market in Japan (average temperature of 8-10°C) with the trey and film packages, the shelf life was less than 3 days for the severe browning and deterioration. Therefore, the appropriate storage method and optimum packages are very necessary to shiitake mushrooms.

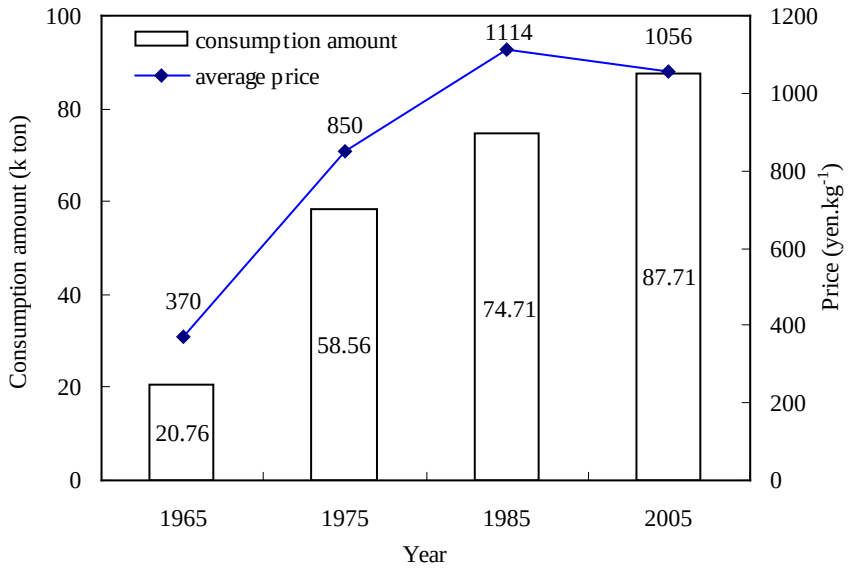

Fig. 1.1 Fresh shiitake mushrooms consumption and average price in Japan
(Source: Data base of Forestry agency)

1.1.3 Modified atmosphere packaging (MAP)

Modified atmosphere Packaging (MAP) is the practice of modifying the composition of the internal atmosphere of a package in order to prolong the shelf life of fresh product. MAP as a technique used for prolonging the shelf-life period of fresh or minimally processed foods can be used to maintain the freshness of fresh produce. The atmosphere surrounding the food inside the package is modified determined by fresh product respiration and the gas transmission from packages. If the permeability (for O_2 and CO_2) of the packaging film is adapted to the product respiration, an equilibrium modified atmosphere will establish in the package and the shelf life of the food will be increased. But the optimum modified atmosphere varies according to the packaged product. MAP is used with various types of products, where the mixture of gases in the package depends on the respiration of product, and packaging film permeability. The suitable gas composition is very dependent upon the product being packaged. Used singly or in combination, these gases are commonly used to balance safe shelf-life extension. Until today, MAP technology has been applied on some meat, fresh vegetables and fruits, but it still needs to be developed to further widely used for food as many as possible.

1.2 Literature review

1.2.1 Modified atmosphere packaging (MAP) and its application

Modified atmosphere packaging (MAP) technology has been researched for many years, and applied on a variety of fruits and vegetables (Table 1.3). In-package gas composition and concentration could reach the equilibrium by adjusting the package permeability and product packaged. The package permeability could be modified throughout applying the permeable package film or perforating on the packages (Rennie and Tavoularis 2009). MAP was researched that it reduced the mass loss, inhibited the quality deterioration and microbiology, and prolonged the shelf life of fresh food. Gómez and Artés (2005) reported that green sticks of 15 cm length of “Trinova” cv. were placed in hermetically sealed plastic bags: low-density polyethylene (LDPE), oriented polypropylene (OPP) and polyethylene-perforated bags as control (air). The O_2 and CO_2 concentrations, soluble solid content, pH, titratable acidity, color, sensorial quality and sugar and organic acids contents were monitored. One of the major benefits of MAP is the prevention or retardation of fruit ripening and associated biochemical and physiological changes. Compared to the control, both MAP treatments improved the sensory quality, avoided the loss of green color, decreased the development of pithiness and retarded the growth of microorganisms. In any treatment neither off-odors nor off-flavors were detected. But after 15 days at 4°C within the OPP bags a steady-state atmosphere of 6 kPa O_2+7 kPa CO_2 was reached and celery sticks stored under these bags showed the best quality. In this research, the authors also pointed out that lower than 2 % O_2 level may result in anaerobic respiration and the development of off-flavors and off-odors. Fruits exposed to such low O_2 levels may also lose their ability to attain uniform ripeness upon removal from MAP. MAP was also researched on coleslaw (Cliffe-Byrnes, Mc Laughlin and O'Beirne 2003).

The effects of six packaging treatments on the quality of dry coleslaw packaged in modified atmospheres and stored at 4 and 8 °C for 9 days were determined. The coleslaw was packaged within either OPP or one of four micro-perforated OPP films, PA-120, PA-160, PA-190, and PA-210 (gas permeability was OPP < PA-120 < PA-160 < PA190 < PA-210). It was also stored within punctured OPP (i.e. in air). Packaging within OPP resulted in an atmosphere with very low O_2 (<1%) and extremely high CO_2 (25–35%) levels. These storage conditions had detrimental effects on the quality of coleslaw: loss of firmness, falling pH, high cell permeability and exudate, high surface moisture and poor acceptability of aroma. By contrast, the micro-perforated films generated less atmosphere modification; in some cases this was insufficiently modified to be technically useful. The relatively high O_2 levels in these micro-perforated packs resulted in lower appearance and color scores, increased surface dryness and higher firmness values. Increasing storage temperature from 4 to 8 °C resulted in a reduction in shelf-life for all film types. In addition, when the MAP was applied on green bell pepper, the quality characteristics were investigated (Manolopoulou et al. 2010). Three packaging films (LDPE of 60 μm, MDPE of 30 μm and PVC) and two storage temperatures 5 and 10 °C were tested. The in-package O_2 concentration did not go below the 2 % level which is considered as the lowest recommended. The in-package CO_2 concentration ranged between 2 and 5 % in the polyethylene (PE) packaging. Packaging resulted in limited mass loss (< 2% of the initial mass) and firmness reduction was small at both storage temperatures. Limited wilting and shriveling at the end of shelf-life was noted in the unpackaged peppers initially stored at 10 °C. Peppers packaged with the PE films did not exhibit significant changes in ascorbic acid content during the storage period including shelf-life. Peppers packaged with the two PE films at 5 °C, showed significant less chilling injuries compared to the unpackaged

peppers.

The effects of passive and active modified atmosphere packaging were also proved to effectively prolong the shelf life of some fresh fruit and vegetables. In the research of MAP application on table grapes (Costa et al. 2011), table grapes were packaged with three films made up of OPP and characterized by a different thickness (20, 40 and 80 μm, respectively) were used to package the grape in air (passive MAP) and under three different initial headspace gas compositions (active MAP) with O_2:CO_2:N_2 of 5:3:92, 10:3:87 and 15:3:82. As controls, grape samples were also stored without packaging. During a prolonged storage period at 5 °C, the headspace gas concentrations, the mass loss, the microbiological stability and the sensory acceptability were monitored. Results obtained highlight that all selected packaging films significantly prevent product decay, thus promoting a substantial shelf life prolongation, if compared to the unpackaged product. In particular, the best results were recorded with the thickest polymeric matrix sealed in air, which assured a shelf life more than 70 days. The active MAPs were not found significant for a shelf life prolongation, due to the fast equilibrium of gas reached in the bags and due to more pronounced product dehydration. Normally the lower oxygen concentration inside the packages is taken as the potential to inhibit the respiration rate of the fresh product. However, the high oxygen packaging (HOP) was recently researched to be effective to inhibit the anaerobic respiration happening, browning and microbiology growth. Jacxsens et al. (2001) researched three types of ready-to-eat vegetables (mushroom slices, grated celeriac and shredded chicory endive), which are sensitive to enzymatic browning and microbial spoilage, and the effect of EMA (3% O_2/5% CO_2, balance N_2) and high O_2 atmospheres (HOA: 95% O_2 /5% N_2) on their quality and shelf life was compared. High O_2 atmospheres were found to be particularly effective in inhibiting enzymatic browning of the tested vegetables. Also, the microbial quality was better as a reduction in yeast growth

was observed. The HOA can be applied as an alternative for low O_2 modified atmospheres for some specific types of ready-to-eat vegetables, sensitive to enzymatic browning and spoilage by yeasts. Oms-Oliu, Soliva-Fortuny and Martín-Belloso (2008) also researched the effect of HOA active packaging (70 kPa O_2, balance N_2), conventional low O_2 atmosphere (LOA) active modified packaging (2.5 kPa O_2 + 7 kPa CO_2, balance N_2), and traditional passive atmosphere (PA) packaging on fresh-cut "Flor de Invierno" pears. Results showed that HOA did not prevent the production of acetaldehyde and ethanol during storage of fresh-cut pears but their accumulation was promoted under anoxic conditions. Although LOA reduced CO_2 production and inhibited ethylene production, moderate CO_2 concentrations in combination with excessively low O_2 levels inside packages accelerated the accumulation of fermentative metabolites. Both LOA and HOA significantly reduced the growth of microorganisms during storage. In addition, HOA had an inhibitory effect on some spoilage microorganisms isolated from fresh-cut "Flor de Invierno" pears. Microbiological stability of fresh-cut pears stored under HOA was assured throughout storage, but commercial shelf-life may be limited by the browning appearance of the cut surfaces and off-odors beyond 14 days storage. However, the high oxygen packaging may induce the biological disorder of the some fresh food. Kader and Ben-Yehoshua (2000) reviewed that an atmosphere of 100 kPa O_2 potentiated the effect of 0.5 μl l^{-1} C_2H_4 on isocoumarin formation in carrots, resulting in a 5-fold increase over that found in carrots treated with C_2H_4 in air. Superatmospheric O_2 levels increased ethylene production and the incidence and severity of pink rib and C_2H_4-induced russet spotting on lettuce. Mature-green tomatoes exposed to 80 or 100 kPa O_2 for more than 5 days exhibited dark-brown spots on their skin. The severity of this high-O_2 injury depended on duration of exposure at 20 °C and type of wax used on the tomatoes. Therefore, the application of high oxygen packaging should be considered according to the biological

characteristics and sensitivity to oxygen of product.

Table 1.3 Recommended MA conditions for selected fruits and vegetables

Product	O_2 (%)	CO_2 (%)	N_2 (%)
Fruits			
Apple	1-2	1-3	95-98
Banana	2-5	2-5	90-96
Grape	2-5	1-3	92-97
Grapefruit	3-10	5-10	80-92
Kiwifruit	1-2	3-5	93-96
Lemon	5-10	0-10	80-95
Orange	5-10	0-5	85-95
Peach	1-2	3-5	93-96
Pineapple	2-5	5-10	85-93
Pear	2-3	0-1	96-98
Strawberry	5-10	15-20	70-80
Raspberry	10	15-20	70-75
Mango	5	5	90
Vegetables			
Broccoli	1-2	5-10	88-94
Cabbage	2-3	3-6	91-95
Carrot	5	3-4	91-92
Cauliflower	2-5	2-5	90-96
Corn (sweet)	2-4	10-20	76-88
Cucumber	3-5	0	95-97
Chili peppers	3	5	92
Tomatoes	3-5	0	95-97
Onion	1-2	0	98-99
Lettuce	1-3	0	97-99
Celery	2-4	0	96-98
Spinach	Air	10-20	59-69
Brussels sprouts	1-2	5-7	91-94
Asparagus	Air	5-10	69-74

Source: (Lee et al. 1996, Sandhya 2010, Exama et al. 1993)

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1.2.2 Packaging materials for MAP

The MA packaging technique consists of the enclosure of respiring produce in polymeric films in which the gaseous environment is actively or passively altered to slow respiration, reduce moisture loss and extend the shelf life of the products. The permeability of the film plays a very important role when creating the MAP. O_2, CO_2 and N_2 permeability and water vapor transmission rate of some common plastic films are shown in Table 1.4. Among the films shown this table, low density polyethylene (LDPE) film has a relative higher O_2 and CO_2 permeability than others, and polyester has a higher water vapor transmission rate.

Table 1.4 Permeability of selected films applied on MAP (Sandhya 2010)

Film	Permeability ($ml \cdot m^{-2} \cdot d^{-2} \cdot atm^{-1}$ for 25 mm film at 25 °C)			Water vapor transmission, $g \cdot m^{-2} \cdot d^{-1} \cdot atm^{-1}$ (38°C and 90% RH)
	O_2	CO_2	N_2	
Low density polyethylene (LDPE)	7800	42,000	2800	18
High density polyethylene (HDPE)	2600	7600	650	7-10
Oriented polypropylene (OPP)	2000	8000	400	6-7
Cast polypropylene (CPP)	3700	10,000	680	10-12
Rigid PVC	150-350	450-1000	60-150	30-40
Plasticized PVC	500-30,000	1500-46,000	300-10,000	15-40
Ethylene vinyl acetate (EVA)	12,500	50,000	4900	40-60
Polystyrene, oriented (PS)	5000	18,000	800	100-125
Ethylene vinyl alcohol (EVOH)	3-5	-	-	16-18
Polyvinylidene chloride (PVDC)	9-15	20-30	-	-
Polyurethane (Polyester)	800-1500	7000-25,000	600-1200	400-600
Polyamide (Nylon-6)	40	150-190	14	84-3100

1.2.3 MAP and other storage methods applied on fresh shiitake mushrooms

Shiitake mushrooms are very perishable which calls for the effective preservation and packaging methods to prolong the shelf life. Antmann et al. (2008) reported when fresh shiitake mushrooms were packaged in active modified atmospheres (initial 15 % O_2 or 25 % O_2) and passive modified atmosphere in two macro-perforated packages A (9.0×10^3 perforations·m^{-2}, 0.1 mm^2 surface) and B (17 perforations·m^{-2}, 0.1 mm^2 surface) with polyethylene films, and stored at 5 °C. The result suggests that during the first 6 days of storage all the evaluated packaging conditions were useful for reducing mushroom deterioration rate. However, mushrooms in active modified atmosphere packages developed off-odors after 12 days, and mushrooms packaged in macro-perforated packaging A showed the smallest deterioration rate. However, weight loss after 6 days of storage reached 15%, which would be unacceptable. Sensory quality of fresh shiitake mushrooms packaged in passive modified atmosphere packaging was also researched (Ares et al. 2006) and the result indicated that mushrooms stored under modified atmosphere had a higher deterioration rate than those stored in PP macro-perforated films. In another research of Parentelli et al. (2007), sensory analysis of shiitake mushrooms was carried out and the result showed that mushrooms stored under modified atmosphere (active with 5 % O_2 and 2.5 % CO_2 initially and passive) had a higher deterioration rate than those stored in PP macro-perforated films, and lower sensory quality values during the entire storage time. These results suggest that mushroom deterioration was probably due to shiitake mushrooms' sensitivity to high CO_2 concentrations.

Shiitake mushrooms stored under controlled atmosphere (CA) with high oxygen atmosphere (80 % O_2 and 100 % O_2) and air was also performed by Liu et al. (2010). The results showed that high oxygen, especially 100% O_2 treatment was effective at reducing browning degree and electrolyte leakage of mushroom.

1.3 Objectives of this study

Quality deterioration of product is induced easily due to inappropriate packaging and storage methods. The fresh fruits and vegetables have many limitations for storage, such as the mass loss, discoloration, softening, microbiology growth, and nutrient loss. Fresh shiitake mushroom, is a fast respiring product and easy to deteriorate. The inappropriate packaging methods may induce the loss of nutrient value and big waste of shiitake mushrooms. Until now, modified atmosphere packaging technology has not been widely applied on the fresh fruits and vegetables, because there are still many unclear aspects to be researched, such as the optimum gas compositions and package films. Therefore, the MAP technology still needs to be further developed, and the preservation technology of shiitake mushrooms also need to be researched. The objectives of this study were as follows:

1) To investigate the commercial package types of some vegetables, clarify their applicability and analyze their characteristics.
2) To further clarify the physiology and nutritional compounds change of shiitake mushrooms packaged in various atmospheres.
3) To determinate effect of high oxygen modified atmosphere packaging on respiratory physiology and sensorial qualities of fresh shiitake mushrooms
4) To compare the effect of high-oxygen packaging and perforation-mediated modified atmosphere packaging on the qualities of fresh shiitake mushrooms.

CHAPTER 2

Analysis of Present Commercial Packages Applied on Fresh Vegetables

2.1 Introduction

Modified atmosphere packaging (MAP) which modifies the O_2 and CO_2 concentrations in the packages could prolong the shelf life of fresh produce (McLaughlin and O'Beirne 1999). Desired MAP is achieved through the interaction between two processes: the respiration of produce and the transfer of gases through the packaging material (Mahajan et al. 2007, Caleb et al. 2012, Fonseca, Oliveira and Brecht 2002a). The suitable in-package atmosphere could prolong the shelf life of the fresh product. In present market, the plastic films are common used to package the fresh product. But whether they are suitable to the product or whether the atmospheres inside the packages could be modified by the interaction of gas permeability of the film and respiration rate of product is still unknown. Temperature plays a very important role in designing MAP for its dramatic influence on respiration rate and gas permeability. Respiration rate of fresh produce is influenced by temperature dramatically during the whole distribution and retailing procedures. Therefore, the analysis of the gas permeability and respiration rate which are influenced by temperature is very important for the establishment of the in-package atmosphere and should be researched.

The objective of this study is to investigate the effect of temperature on respiration rate of some selected vegetables, and analyze the adaptability of present plastic packages.

2.2 Investigation of MAP of selected vegetables

2.2.1 Produce and sample preparation

All the samples were bought from the market in Japan and soon transported to the lab.

The samples in this study contained seven categories of vegetables: Shiitake mushroom, Maitake mushrooms, Enokitake mushrooms, Long bean, Green hot pepper, Edamame and Baby leaf (mixed young leaves). The harvest places of vegetables were depicted in Table 2.1. The samples were stored in the 10, 15 and 20 ℃ instant temperature and humidity storage rooms for 2 h till the core temperature reached to the storage room temperature. Then the gas concentration and respiration rate were detected.

Table 2.1 Harvest places of selected vegetables

Produce	Harvest place
Shiitake mushroom	Hokkaido
Maitake mushroom	Niigata Prefecture
Enokitake mushroom	Yamagata Prefecture
Long bean	Fukushima Prefecture
Green hot pepper	Chiba prefecture
Edamade	Akita prefecture
Baby leaf	Fukushima Prefecture

2.2.2 Film identification, thickness detection and gas transmission rate detection

The package films unknown were identified by Fourier Transform Infrared Spectrometer (Magna-IR Spectrometer 560, Nicolet, USA) with the analysis software of OMNIC. And spectrums were compared with the reference spectrum and the film could be identified.

The film thickness was detected by digital thickness tester (0-25 mm, 0.001 mm, Mitutoyo, Japan).

O_2 transmission rate (OTR) and CO_2 transmission rate (CTR) at 10, 15 and 20 °C with Gas Barrier Testing System (GTR-30X, GTR Tec Corporation, Japan) along with a Gas Chromatograph (GC 2700, Yanaco, Japan).

2.2.3 Determination of gas concentration and respiration rate

The O_2 and CO_2 concentrations were determined by a gas chromatograph (GC-8A, Shimadzu, Japan) with a thermal conductivity detector (TCD), and the packed column ZY-2 consisting of Molecular Sieve 5A column, Porapak Q column and Shimalite Q column. The carrier gas was helium with 0.67 $ml \cdot s^{-1}$ flow rate. Column temperature was 75 °C; injector and detector temperature was 80°C.

The oxygen consumption rate (R_{O2}) and carbon dioxide production rate (R_{CO2}) of whole vegetables were measured using the closed system method. Air-tight organic glass jars of 3 L with a lid and rubber septum in the middle were used to and the samples were stored at different temperatures (10, 15 and 20 °C). Gas samples of 1 ml were withdrawn from the head space through rubber septum and injected to the GC hourly for 2 hours. R_{O2} and R_{CO2} were calculated with the following equations (1) and (2).

$$R_{O2} = (K_{O2} \times V_f) / m \quad (1)$$

$$R_{CO2} = (K_{CO2} \times V_f) / m \quad (2)$$

where R_{O_2} is oxygen consumption rate and R_{CO_2} is carbon dioxide production rate which can be expressed as $mg \cdot kg^{-1} \cdot h^{-1}$; K_{O_2} K_{CO_2} are the slopes of oxygen and carbon dioxide concentration percent versus time curve respectively which can be expressed as $\% \cdot h^{-1}$; V_f is the free volume of container that subtracted the sample's volume from the container volume and expressed as liter; m is the mass of the produce and expressed as kg.

Respiration rate measurements may also be used to calculate the respiratory quotient (RQ), which is the ratio of CO_2 produced to O_2 consumed (R_{CO_2}/ R_{O_2}). Changes in RQ can indicate the nature of the substrates utilized by the tissue, and also whether anaerobic respiration is occurring (Bower et al. 1998, Joles et al. 1994).

2.2.4 Statistical analysis

All the results were expressed as mean values± standard deviation (SD). All data were analyzed by analysis of variance (ANOVA).

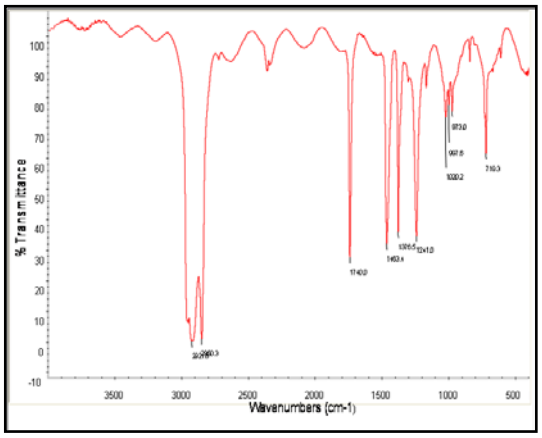
2.3 Result and discussion

2.3.1 Comparison of packages of the vegetables and in-package gas concentration

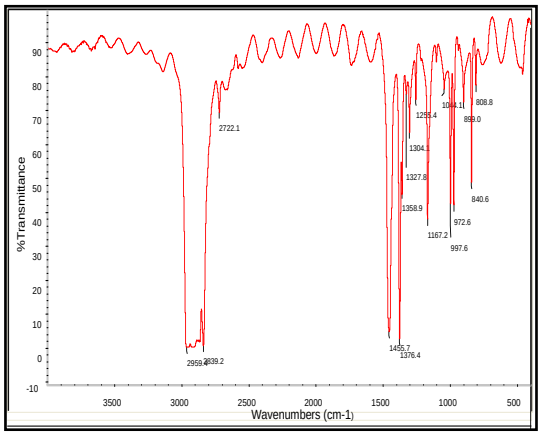
From the spectra, the film's type could be identified by comparing with the standard spectra (Fig. 2.1). From the authentic spectrum of EVA and PP, it could be known that the wavenumbers of main peaks of Ethylene Vinyl Acetate (EVA) film were about 2921, 2850, 1740, 1463, 1376, 1241, 1020, 997, 973 and 719 cm^{-1}, and the wavenumbers of main peaks of Polypropylene (PP) film were 2959, 2839, 1455, 1376, 1167, 997, 972, and 840 cm^{-1}. Compared with the peaks of authentic films, the film applied on Shiitake mushroom, Maitake mushroom, Long bean and Green hot pepper could be judged as EVA film, and on Enokitake mushroom, Edamame and Baby leaf could be judged as PP film. The packages types of the vegetables were shown in Fig. 2.2 and details of the packages were depicted in Table 2.2. The packages of Shiitake mushroom, Maitake mushroom, Long bean and Green

hot pepper were plastic treys with the transparent films.

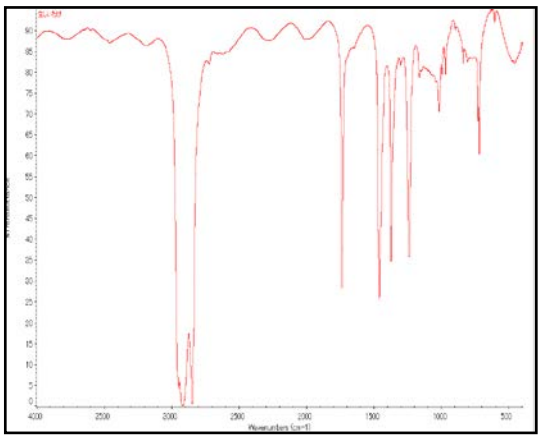
O_2 concentrations inside the packages were described in Table 2.4. It is obvious that the packages of Shiitake mushroom, Maitake mushroom and Enokitake mushroom were not suitable because the O_2 concentration has been decreased to lower than 1 %, so the anaerobic fermentation will be soon induced. The O_2 concentration in the packages of Long bean, Green hot pepper, Edamame and Baby leaf are > 10%, and this atmosphere did not contribute any modified effect on inhibiting respiration of these vegetables although the anaerobic fermentation did not be created. The reasons of the oxygen concentrations were different in packages of these products will be stated in detail in the later content.



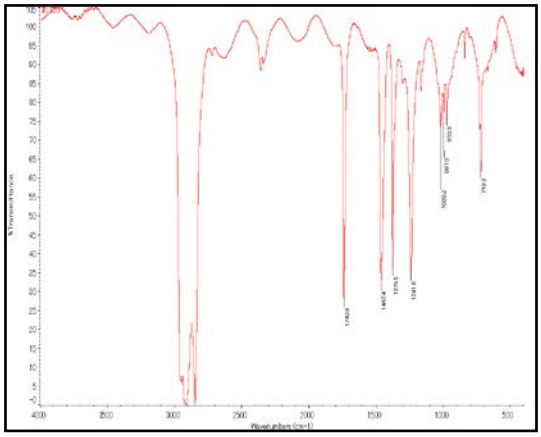
(a) EVA authentic spectrum



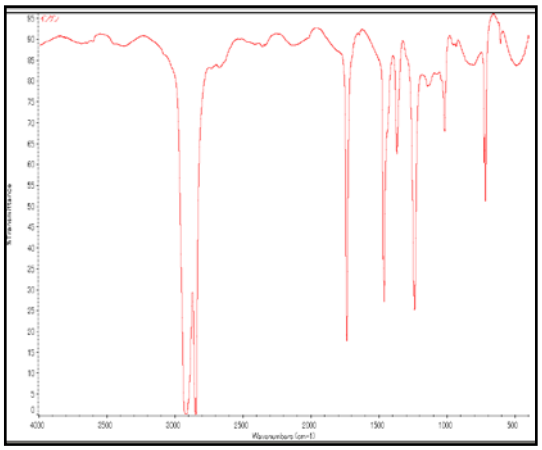
(b) PP authentic spectrum



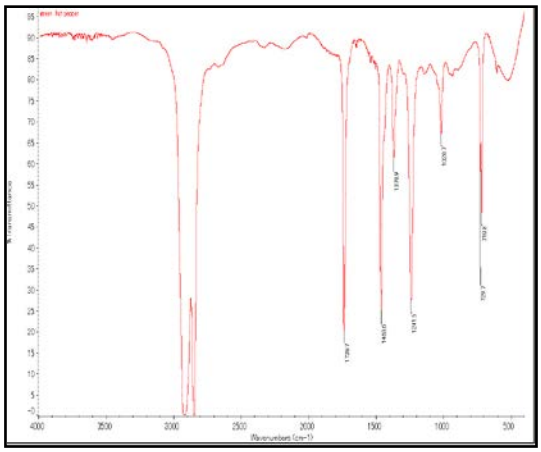
(c) Spectrum of film for Shiitake mushroom



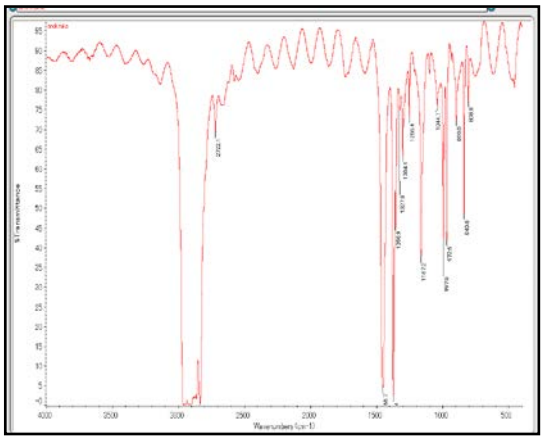
(d) Spectrum of film for Maitake mushroom



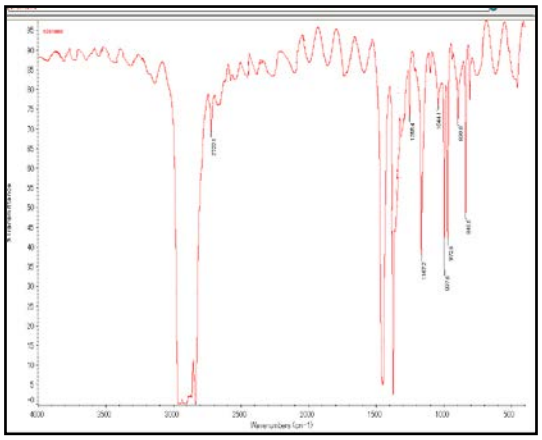
(e) Spectrum of film for Long bean



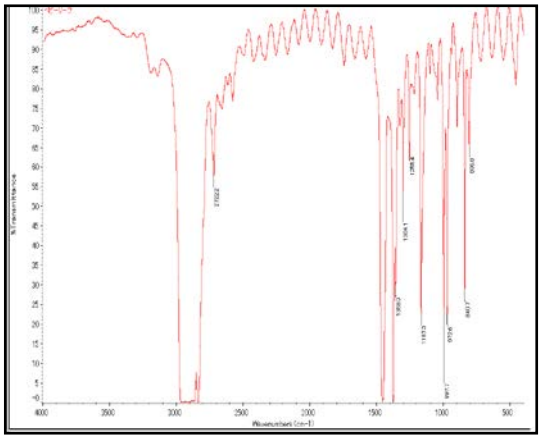
(f) Spectrum of film for Green hot pepper



(g) Spectrum of film for Enokitake mushroom



(h) Spectrum of film for Edamame



(i) Spectrum of film for Baby leaf

Fig. 2.1 Spectra of package films of the vegetables

Shiiitake mushroom

Maitake mushroom

Enokitake mushroom

Long bean

Green hot pepper

Edamame

Baby leaf

Fig. 2.2 Package types of the vegetables

Table 2.2 Description of package film and gas concentration of the vegetables used in the experiment

Product	Package type	Film					Trey	
		Material	Area (cm^2)	Thickness (μm)	OTR at 10 °C ($ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$)	CTR at 10 °C ($ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$)	Material	Volume (cm^3)
Shiitake mushroom	Film + trey	EVA	135	15	8005.11	68539.4	PS	312
Maitake mushroom	Film + trey	EVA	176	20	5924.83	25117.01	PS	563
Enokitake mushroom	Film	PP	613.6	25	1052.78	3849.2		
Long bean	Film + trey	EVA	197.8	20	6108.52	25853.17	PS	356
Green hot pepper	Film + trey	EVA	148	20	5439.87	24393.68	Foamed PS	636
Edamame	Film	PP	641	40	885.45	3263.12		
Baby leaf	Film	PP	698	40	682.69	2532.96		

OTR: Oxygen transmission rate; CTR: Carbon dioxide transmission rate
EVA: Ethylene Vinyl Acetate, PP: Polypropylene, PS: Polystyrene

2.3.2 Gas transmission rate change of different films affected by temperature

The O_2 transmission rate and CO_2 transmission rate were detected at 10, 15 and 20 °C. The gas transmission rate of plastic films changed with temperature and followed the exponential regularity with reciprocal of Kelvin temperature (Beaudry et al. 1992). From the Fig. 2.3, it is shown that OTR and CTR well fit the exponential relations with the reciprocal of Kelvin temperature. OTR and CTR increased with the temperature increasing, and EVA 15 and EVA 20 got the higher OTR and CTR than PP 25 and PP 40.

2.3.3 Effect of temperature on respiration rate and respiratory quotient

Fig 2.4 showed that the R_{O_2} and R_{CO_2} increased with the temperature increasing for all the 7 kinds of vegetables. And Table 2.3 also described the significance of differences of respiration rate and respiratory quotient (RQ) at different temperatures. But among them, Shiitake mushroom, Enokitake mushroom and Green hot pepper showed significant increases of O_2 consumption rate with the temperature increasing. Especially shiitake mushroom had a R_{O_2} increase from 114.16 $mg \cdot kg^{-1} \cdot h^{-1}$ to 312.40 $mg \cdot kg^{-1} \cdot h^{-1}$ and a R_{CO_2} increase from 98.22 $mg \cdot kg^{-1} \cdot h^{-1}$ to 359.74 $mg \cdot kg^{-1} \cdot h^{-1}$ when temperature increased from 10 °C to 20 °C, and a significant difference between 10, 15 and 20 °C as well as an extremely significant difference between 10 and 15 °C. The huge increment indicated that respiration rate of shiitake mushroom is susceptible to temperature which called for a proper storage and distribution temperature to prolong the shelf life. But the CO_2 production rates of all the seven kinds of vegetables were significantly influenced by temperature. Normal respiratory quotient (RQ) values limit with 0.7 to 1.3 for aerobic respiration (Kader et al. 1989). Shiitake mushroom, Enokitake mushroom, long bean showed the extremely significant increases and green hot pepper showed the significant increase of RQ with the temperature increasing. Therefore, Shiitake mushroom, Enokitake mushroom, long bean are very easy to be induced to anaerobic respiration when the O_2 concentration depletes to low level.

Normal respiratory quotient (RQ) values limit with 0.7 to 1.3 for aerobic respiration (Kader et al. 1989). Shiitake mushroom, Enokitake mushroom, Long bean showed the extremely significant increases and Green hot pepper showed the significant increase of RQ with the temperature increasing. Therefore, Shiitake mushroom, Enokitake mushroom, Long bean have greater possibility to induce the anaerobic respiration when the temperature increasing.

2.3.4 Comparison of the total O_2 transmission rate and total O_2 consumption rate

The O_2 concentration and the comparison of total O_2 transmission rate through the package films and total O_2 consumption rate by respiration of product were shown in Table 2.4. In this study, the extremely low O_2 concentration was created in packages of Shiitake mushroom, Maitake mushroom and Enokitake mushroom. According to the respiration rates of them, it could be explained that these three kinds of mushrooms had very high respiration rates compared with other vegetables. The oxygen transmission of the package films of these three products were 22.69, 21.90 and 13.57 ml·package^{-1}·d^{-1}, but the oxygen consumption rate of them were 211.43, 205.48 and 517.62 ml·package^{-1}·d^{-1} respectively. Although the package films are permeable, the high respiration induced the oxygen inside the package was soon consumed. The oxygen concentration was high in the packages of Long bean, Green hot pepper, Edamame and Baby leaf. For Long bean and Baby leaf, high oxygen concentration was attributed to their low respiration rate and accordingly low oxygen consumption. Although Green hot pepper had a higher respiration rate, the mass in the package was only 10.05g, which did not induce the much oxygen consumption. For edamame, although the oxygen transmission rate of the PP film applied on it was 11.92 ml·package^{-1}·d^{-1} and the oxygen consumption rate was 421.90 ml·package^{-1}·d^{-1}, still higher oxygen concentration inside the package could be created. The reason is that the package of edamame was perforated. The size of 1 mm in diameter and numbers of 30 of the perforations on each package were observed, which induced a high gas transmission and no MA effect. So the perforations of the packages for edamame should be decreased and the optimal modified atmosphere condition would be created.

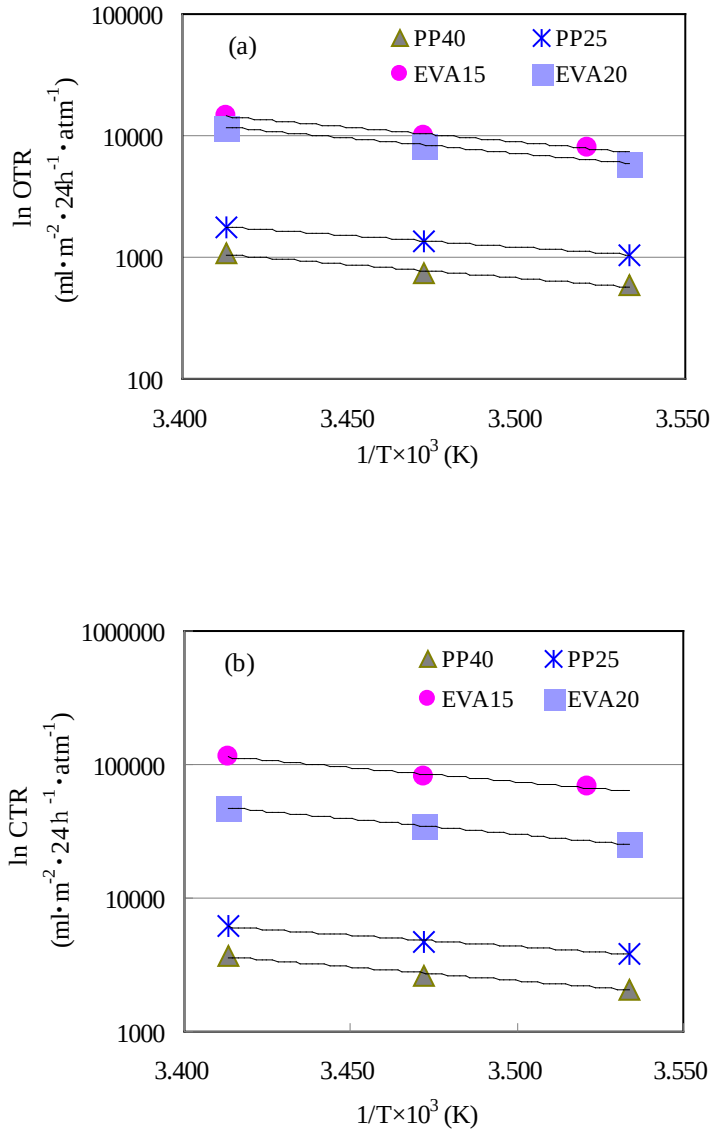


Fig. 2.3 O_2 transmission rate (OTR) and CO_2 transmission rate (CTR) change of different films at different temperatures

(a) OTR, (b) CTR

Temperature was expressed as kelvin temperatue (K).

PP 40 and PP 25 are Polypropylene film with 40 and 25 μm thickness respectively; EVA 15 and EVA 20 are Ethylene Vinyl Acetate film with 15 and 20 μm thickness respectively

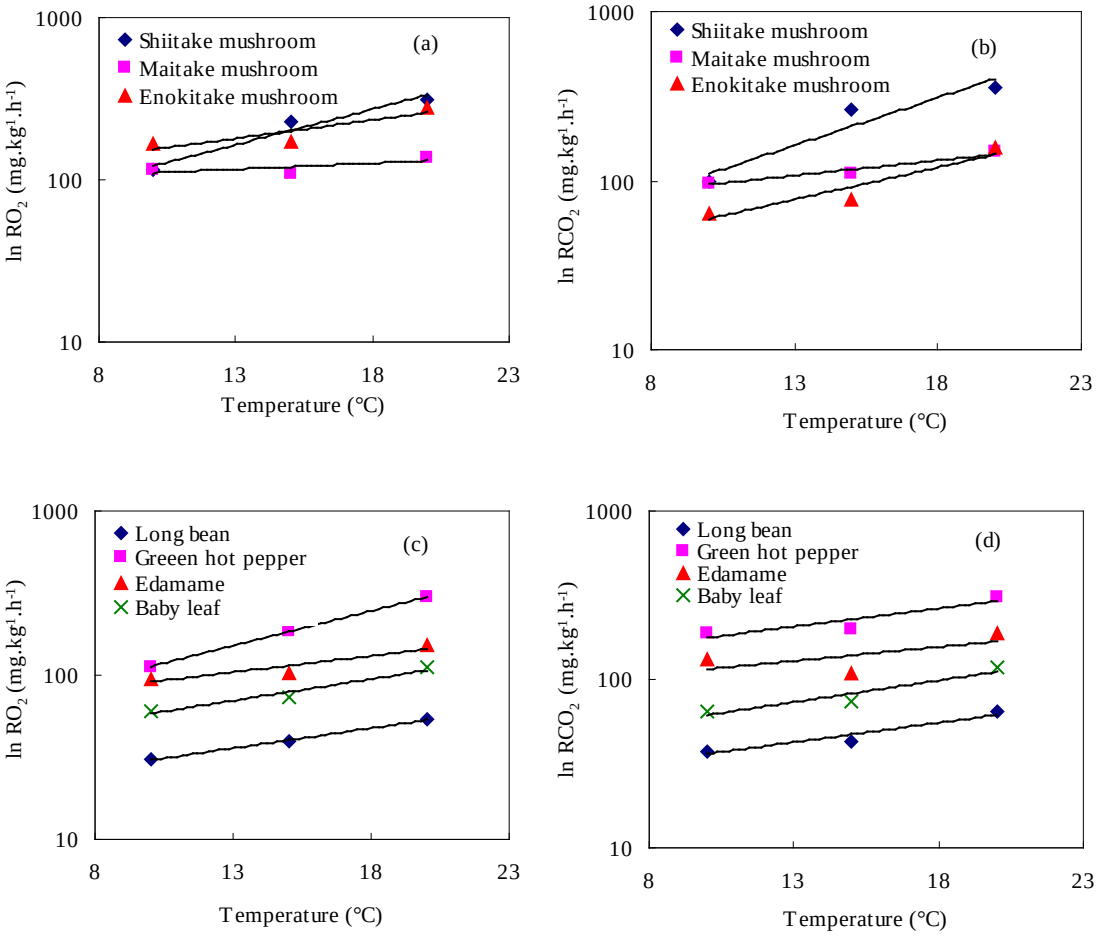


Fig. 2.4 O_2 consumption rate (RO_2) and CO_2 production rate (RCO_2) of seven vegetables at 10, 15 and 20 °C.
(a) and (b) were R_{O2} and R_{CO2} of shiitake, maitake and enokitake mushroom respectively; (c) and (d) were R_{O2} and R_{CO2} of long bean, green hot pepper, edamade and baby leaf respectively.

Table 2.3 Changes in O_2 consumption rate (RO_2), CO_2 production rate (RCO_2) and respiratory quotient (RQ) at 10, 15 and 20 °C

	T	Shiitake mushroom	Maitake mushroom	Enokitake mushroom	Long bean	Green hot pepper	Edamame	Baby leaf
RO_2 ($mg \cdot kg^{-1} \cdot h^{-1}$)	10	114.16±10.26 ^{cB}	115.57±23.74 ^{aA}	165.01±18.02 ^{bB}	30.75±0.65 ^{cC}	112.10±15.03 ^{cB}	95.52±7.43 ^{bB}	60.23±16.18 ^{bB}
	15	228.87±33.66 ^{bA}	109.71±6.14 ^{aA}	173.67±1.42 ^{bB}	39.08±0.49 ^{bB}	185.82±57.76 ^{bB}	103.12±2.52 ^{bB}	73.87±3.63 ^{bB}
	20	312.40±39.63 ^{aA}	137.28±9.08 ^{aA}	278.21±2.87 ^{aA}	54.06±3.10 ^{aA}	296.75±0.78 ^{aA}	150.89±1.94 ^{aA}	110.60±11.66 ^{aA}
RCO_2 ($mg \cdot kg^{-1} \cdot h^{-1}$)	10	98.22±4.54 ^{cB}	97.16±8.87 ^{bB}	64.53±13.33 ^{bB}	37.76±0.41 ^{bB}	188.27±21.75 ^{bB}	130.48±23.43 ^{bB}	64.57±10.74 ^{bB}
	15	260.91±40.51 ^{bA}	110.70±3.95 ^{bB}	78.20±12.73 ^{bB}	43.08±1.44 ^{bB}	200.45±15.20 ^{bB}	108.42±5.07 ^{bB}	74.15±6.27 ^{bB}
	20	359.74±47.85 ^{aA}	146.51±14.64 ^{aA}	154.78±8.29 ^{aA}	64.36±4.37 ^{aA}	310.73±26.27 ^{aA}	189.70±8.51 ^{aA}	117.06±14.04 ^{aA}
RQ	10	0.86±0.04 ^{bB}	0.87±0.19 ^{aA}	0.39±0.04 ^{bB}	1.23±0.02 ^{aB}	1.70±0.29 ^{aA}	1.36±0.14 ^{aA}	1.14±0.39 ^{aA}
	15	1.14±0.02 ^{aA}	1.01±0.02 ^{aA}	0.45±0.07 ^{bAB}	1.10±0.04 ^{bAB}	1.13±0.24 ^{bA}	1.05±0.04 ^{aA}	1.00±0.04 ^{aA}
	20	1.15±0.02 ^{aA}	1.07±0.04 ^{aA}	0.56±0.02 ^{aA}	1.19±0.04 ^{aA}	1.05±0.09 ^{bA}	1.26±0.04 ^{aA}	1.06±0.08 ^{aA}

Mean values± standard deviations with different lowercase letters are significant different ($p \leq 0.05$),
different capital letters are extremely significantly different ($p \leq 0.01$);
T: temperature (°C).

Table 2.4 Comparison of the total O_2 transmission rate and total O_2 consumption rate

	Mass of product (g)	O_2 (v/v %)	Total O_2 transmission rate from film (ml·package^{-1}·d^{-1})	Total O_2 consumption rate by respiration (ml·package^{-1}·d^{-1})
Shiitake mushroom	110.24±3.45	0.87±0.25	22.69±2.56	211.43±20.57
Maitake mushroom	105.83±3.56	0.24±0.15	21.90±2.87	205.48±35.63
Enokitake mushroom	186.72±6.78	0.28±0.13	13.57±3.12	517.62±30.25
Long bean	135.33±6.24	15.79±2.54	25.37±3.58	69.91±2.77
Green hot pepper	10.05±2.12	16.74±5.32	16.90±2.09	18.93±3.54
Edamame	262.91±10.65	13.99±3.66	11.92±3.45	421.90±15.35
Baby leaf	28.85±3.01	15.00±4.85	10.01±1.36	29.19±6.54

Storage temperature: 10 ℃

2.4 Conclusion

Temperature greatly influenced the O_2 consumption rate (RO_2) of Shiitake mushroom, Enokitake mushroom and Green hot pepper, and significantly influenced CO_2 production rates (RCO_2) of all these seven kinds of vegetables. It was also found that RQ was dependent on the temperature, and became high when the temperature increased from 10 to 20°C. Temperature also influenced the gas composition of MAP systems for many fruit and vegetables. The respiration rate of the product and the gas transmission rate of package film were also affected by temperature. Therefore, the O_2 and CO_2 levels depend on film permeability and product respiration, and the temperature dependence of these two processes is determined by film type, commodity physiology, respectively (Beaudry et al. 1992).

In this study, for the Long bean, Edamame, Green hot pepper and Baby leaf, the oxygen concentration was higher than 10 % which could not create the MA condition to prolong the shelf life. For the vegetables with high respiration rate (Shiitake, Maitake and Enokitake mushroom), the packages are not suitable either for the reason of the extremely low oxygen concentration in the packages and anaerobic respiration would be induced soon. It was suggested that the appropriate package types should be considered to modify the in-package atmosphere and to avoid the anaerobic fermentation and prolong the shelf life of them.

Therefore, for Shiitake, Maitake and Enokitake mushrooms, in-package oxygen concentrations were extremely low for the reason of high oxygen consumption by respiration and low oxygen transmission from film. So the suggestive solution is to improve the film gas transmission rate or increase the initial oxygen concentration. Decreasing the mass of produce is not suggested because of the waste of package materials and the high cost. For Long bean, Green hot pepper and Baby leaf, the MA atmosphere

was not created for the too high oxygen concentration inside the packages, and the respiration won’t be inhibited, and the suggestions for these three vegetables are to select the package film with lower gas permeability or increase the mass of being packaged produce. For Edamame, perforation-mediated method was suggested, but the number of perforations should be decreased.

In the further study, we will select the shiitake mushroom as the experimental samples and research for the optimal packaging condition for fresh shiitake mushrooms.

CHAPTER 3

Effect of Modified Atmosphere Packaging with Different Initial Gas Compositions on Physiology and Nutritional Compounds of Shiitake Mushrooms (*Lentinus edodes*)

3.1 Introduction

Modified atmosphere packaging (MAP) has been successful applied on some vegetables and fruits. In active MAP, the desired initial gas was flushed into the packages to modify the physiology to prolong the shelf life of fresh food (Charles, Guillaume and Gontard 2008, Horev et al. 2012). MAP with depleted oxygen inside the package could reduce the respiration rate of fresh product. However, the extremely low oxygen will induce the anaerobic fermentation with the accumulation of off-odors. In recent years, high oxygen modified atmosphere packaging as an alternative to the traditional MAP has also been used to maintain the shelf life of some product. High-oxygen MAP has been proved to effectively inhibit the microbiology and browning, retard the anaerobic fermentation. However, Low-oxygen packaging may reduce the respiration rate and keep the shelf life longer or quality better than normal air packaging. But low oxygen may also have potential to induce the anaerobic fermentation, disagreeable flavors, reduction in aroma biosynthesis or tissue injury.

Therefore, MAP with different oxygen concentrations may have different effects on the quality of fresh food. Until today, although there have been researches about the application of MAP on fresh vegetables and fruits, few researches have been conducted about physiology and nutritive constituents changes under MAP with low and high oxygen atmospheres.

Shiitake mushroom (*Lentinus edodes*) is one of the most common popular mushrooms

cultivated all over the world, especially in Asian countries (ÇaglarIrmak 2007). Its production and consumption are growing rapidly because of the special taste and the nutritional compounds such as polysaccharides, antioxidants, dietary fiber, ergosterol, minerals, vitamin B1, B2 and C (Antmann et al. 2008, ÇaglarIrmak 2007). However, fresh shiitake mushrooms are very perishable and easy to become discoloration after the post-harvest which requests the appropriate preservation or packaging methods to prolong their shelf life and maintain the quality and nutritive constituents. There have been some researches about the application of modified atmosphere packaging (MAP) on the shiitake mushrooms. But there are few studies about the change of nutritive constituents of shiitake mushrooms such as polysaccharide, phenolics or amino acid during the storage influenced by in-package gas with different oxygen concentrations.

The objective of this study is to investigate the effect of modified atmosphere packaging with different initial gas compositions on the physiology and nutritive constituents (polysaccharide, total phenolics and free amino acid content) of fresh shiitake mushrooms during the storage.

3.2 Materials and methods

3.2.1 Packaging and storage

Fresh shiitake mushrooms were harvested from Ibaraki Prefecture in Japan, and transported to the lab within 24 hours of harvest. The mushrooms were stored before packaging at 10 °C with 90% relative humidity (RH) for 2 hours until the core temperature was the same as the storage chamber.

Selected shiitake mushrooms for uniform size and color were packaged under each of the following conditions: (1) High oxygen packaging (HOP): 100% oxygen initially; (2) Medium oxygen packaging (MOP): 50% O_2 and balanced with N_2 initially; (3) Low

oxygen packaging (LOP): 3% O_2/ 5 % CO_2, balanced with N_2 initially. The packaging material employed was polyethylene film with 21.5 cm × 15 cm in area and 100 μm in thickness. The O_2 transmission rate under 10 °C is 750 $ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$ and CO_2 transmission rate under 10 °C is 3480 $ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$ for this film. Three replicates were evaluated per each packaging condition. All the packaged samples were stored at 10°C with 90% RH for 7 days.

3.2.2 Gas composition analysis

The gas composition (O_2, CO_2 and ethanol) under all conditions was measured everyday during the 7 days' storage. CO_2 and O_2 concentrations in the packages were determined by withdrawing a gas sample (1 ml) from the package headspace and injecting into gas chromatograph (GC-8A, Shimadzu, Japan) with a thermal conductivity detector (TCD) and the packed column ZY-2 consisting of Molecular Sieve 5A column, Porapak Q column and Shimalite Q column. The carrier gas was helium with 0.67 $ml \cdot s^{-1}$ flow rate. Column temperature was 75 °C; injector and detector temperature was 80 °C.

Ethanol concentrations were detected by injecting gas samples (1 ml) into a gas chromatograph (GC-14B, Shimadzu, Japan) with a flame ionization detector (FID) and PEG 20M column, and the carrier gases were helium with 0.89 $ml \cdot s^{-1}$ flow rate, hydrogen with 0.67 $ml \cdot s^{-1}$ flow rate and air with 8.17 $ml \cdot s^{-1}$ flow rate. Column temperature was 65 °C, injector temperature was 250 °C and detector temperature was 275 °C.

3.2.3 Electrolyte leakage determination

Sample pretreatment and detection for electrolyte leakage followed the method of Liu et al. (2010) with some modifications. Shiitake mushroom tissue discs (5 mm thick, 10 mm diameter) were prepared from the mushroom fruit bodies using a cork borer. 1 g cap tissue discs and 1 g stipe tissue (total 2 g) were weighted, then put into deionised water and

immersed at the condition of constant temperature of 25 °C for 1h. Conductivity of the surrounding solution was determined with a conductivity meter (ES-51, Horiba, Kyoto, Japan). The tissue was then boiled for 30 min and the total conductivity was also determined. Electrolyte leakage was expressed as a percentage of total electrolytes in the tissue.

3.2.4 Crude water-soluble polysaccharide, total phenolics and free amino acid determination

3.2.4.1 Sample preparation

Before and after the storage, shiitake mushroom samples were sliced and freeze-dried (FDU 830, Eyela, Tokyo. Japan), then were ground to a fine powder. The mushroom powder was collected in sterile glass sample bottles with the cap tightened and stored in desiccators at room temperature for analysis.

3.2.4.2 Polysaccharide extraction and determination

Crude water-soluble polysaccharide was extracted and determined according to the methods of Zhang et al. (2007), Han et al. (2011), XuJie and Wei (2008) with some modifications. 0.2 g freeze-dried powder was weighted and extracted by 10 ml deionized water in 100 °C water bath. The triplicate extractions were carried out. After the centrifugation at the speed of 10,000 rmp for 10 min with the centrifuge (GS-15R, Beckman, USA), the supernatant was collected and added with 4 volumes of 99.5 % EtOH at 4 °C for 12 h. Then after the centrifugation (10,000 rpm for 15 min), precipitate was diluted with deionized water to a final volume of 250 ml. 2 ml of crude polysaccharide extract solution was added to 1 ml 6% phenol solution and 5ml dense sulfuric acid. After 20 min, absorbance at 490 nm wavelength was measured by spectrophotometer (V-550, JASCO, Tokyo, Japan). Glucose was used as the standard to create the calibration curve by plotting absorbance versus glucose standard solution concentration. The result was

expressed as mg glucose per gram dry weight ($mg \cdot g^{-1}$).

3.2.4.3 Total phenolics (TP) contents determination

TP concentrations were measured by Folin-Cioaclteu Reagent (FCR) method. The extraction and analysis methods referenced the method described by Dubost, Ou and Beelman (2007) with some modifications. 0.2 g freeze-dried mushroom powder was added 10 ml of 80 % ethanol and heated in 60 °C water bath for 30 min, and the triplicate extractions were carried out. After the centrifugation (8,000 rpm, 5 min) and filtration, the supernatant was diluted with deionized water to a final volume of 200 ml. 2 ml of total phenolics extract solution was added to 0.1 ml 50% FCR. After 8 min, 2 ml 2.5 % Na_2CO_3 solution was added, and after 1 h, absorbance was measured by an UV-VIS spectrophotometer (V-550, JASCO, Tokyo, Japan) at 750 nm wavelength. Gallic acid was used as the standard to create the calibration curve by plotting absorbance versus gallic acid concentration. The total phenolics contents were expressed as gallic acid equivalents per gram dry weight (mg $GAE \cdot g^{-1}$).

3.2.4.4 Free amino acid determination

Free amino acid extraction and analysis followed the methods of Kim et al. (2009), and with some modifications. 0.2 g freeze-dried mushroom powder was added with 10 ml 75 % ethanol, then heated in 70 °C water bath for 30 min. After centrifugation (10,000 rpm, 10 min), the supernatant was collected. The triplicate extractions were carried out. The collected supernatant then evaporated to dryness. The residue was dissolved by 4 ml deionized water and diluted with 8 ml Lithium Citrate solution to reach a final pH value of between 2 to 3. After that, 3 ml 3% trichloroacetic acid solution was added and left at 4 °C for 1 h to remove the protein and then centrifuged for 15 min at 10,000 rpm. The collected supernatant liquid was filtrated with Millipore 0.2 μm syringe filters (Waterman, USA).

The filtrate was used for analysis. The standard amino acid solution was the mixture of type ANII, type B, 1.25 $mmol \cdot l^{-1}$ aspartic acid solution, 2.5 $mmol \cdot l^{-1}$ glutamic acid solution and 2.5 tryptophan solution (Wako, Japan). The standard solution and prepared filtrate were analyzed by automatic amino acid analyzer (JLC-500/V2, JEOL, Tokyo, Japan) equipped with a cation exchange resin column. The results were expressed as the milligram amino acid per gram dry weight ($mg \cdot g^{-1}$).

3.3 Statistical analysis

All the results were expressed as mean values± standard deviation (SD). All data were analyzed by analysis of variance (ANOVA). Differences between treatments were analyzed by LSD tests and differences at $p \leq 0.05$ were considered to be significantly different.

3.4 Result and discussion

3.4.1 Change in headspace gas composition

O_2 and CO_2 concentration change were shown in Fig. 3.1 and ethanol concentration change was shown in Fig. 3.2 under various packaging conditions. O_2 concentration decreased and CO_2 concentration increased continuously under all the packaging conditions as expected. However, O_2 concentration decreased to lower than 1 % on Day 1 under LOP condition and AIR condition, so from the Day 1 onward, the ethanol accumulated under LOP and AIR condition. O_2 concentration fell down to extremely low level from the 3rd day of the storage under MOP condition with 50 % O_2 concentration initially and the ethanol could also be detected from Day 3 consequently. HOP kept the aerobic atmosphere and no ethanol gas could be detected until the Day 6, but on the Day 7, ethanol concentration also reached 10 $\mu l \cdot l^{-1}$. CO_2 concentration under LOA and AIR increased to about 20 % on the Day 2, and from Day 2 on, it changed little until the end of the storage. At the end of the storage, CO_2 accumulated to 78.60 % in HOP and 59.59 % in

MOP, which was higher than LOP and AIR treatments. This result indicated that HOP and MOP stimulated the respiration rate of shiitake mushrooms to produce more CO_2.

Therefore, the respiration rate of shiitake mushrooms was not inhibited under all the packaging conditions for the increasingly accumulated CO_2. Although modified atmosphere packaging with low oxygen initially (LOP) had weaker effect to stimulate the respiration than HOP and MOP, severe anaerobic fermentation with a high concentration of ethanol induced the obvious quality deterioration. Meanwhile, although modified atmosphere packaging with elevated oxygen initially (HOP and MOP) did not inhibit the anaerobic fermentation completely, the accumulation of ethanol was well retarded. High oxygen packaging was also found to have the effect to inhibit of off-odor, browning, decaying and microbiology growth, maintain the sensory quality in the sliced mushroom, strawberry and blueberry (Liu et al. 2010, Mizuno 1995, Kader and Ben-Yehoshua 2000).

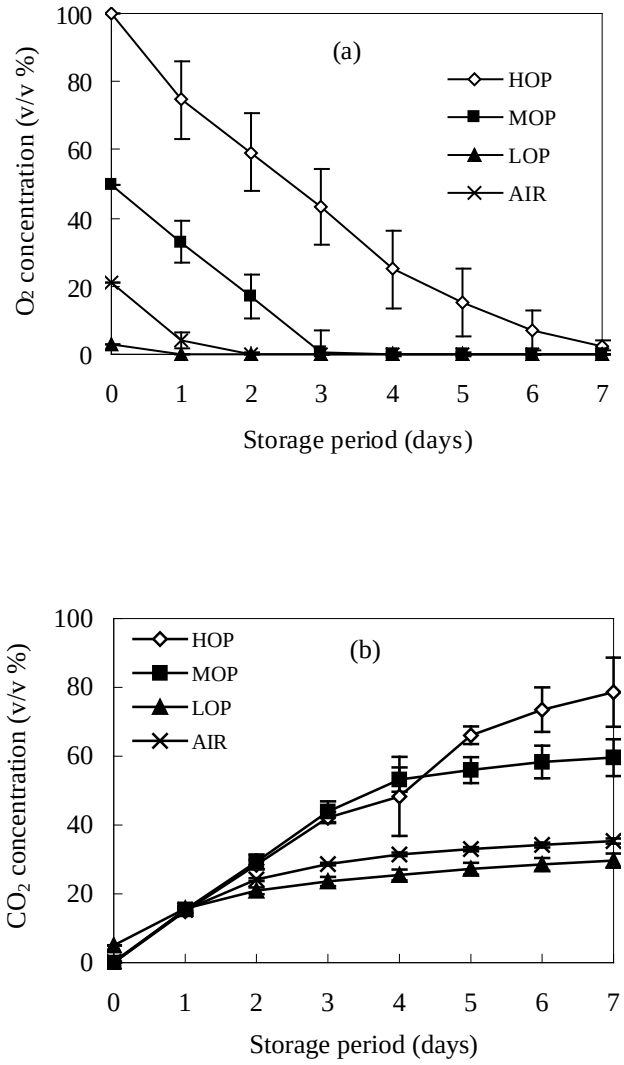


Fig. 3.1 O_2 (a) and CO_2 (b) concentrations in packaged shiitake mushrooms stored at 10 °C. Vertical bars represent standard deviation (n=3).

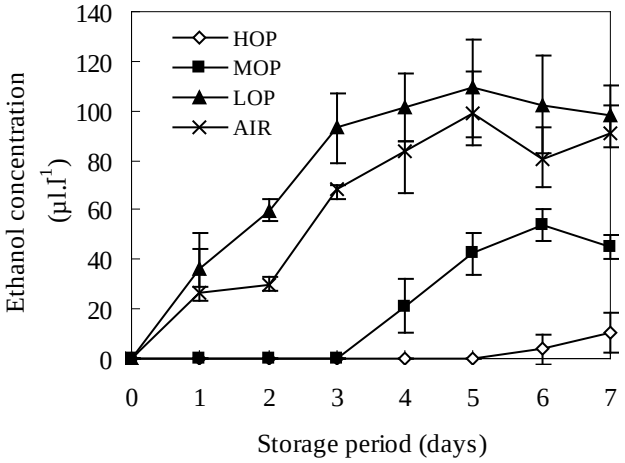


Fig. 3.2 Ethanol concentration in packaged shiitake mushrooms stored at 10 °C. Vertical bars represent standard deviation (n=3).

3.4.2 Changes in electrolyte leakage

Electrolyte leakage of plant tissues has been used as an indicator for tissue and membrane integrity in many researches (Marangoni, Palma and Stanley 1996, Jiang et al. 2001, Liu et al. 2010). Result showed in Fig. 3.3 indicated that after the storage, electrolyte leakage increased compared with the initial samples, but only little increase in HOP, MOP, AIR and initial samples, and there is not any significant difference between HOP, MAP and AIR. However electrolyte leakage in LOP significantly increased to 46.16 % after 7 days' storage. Therefore, it could be known that membrane stability and permeability of shiitake mushrooms was mildly influenced under HOP, MOP and AIR treatments but significantly destroyed by LOP. That is also related to the anaerobic atmosphere. The anaerobic respiration started from the first storage day in LOP and the extremely high ethanol concentration also accumulated then, which would do harm or have detrimental effect for the product.

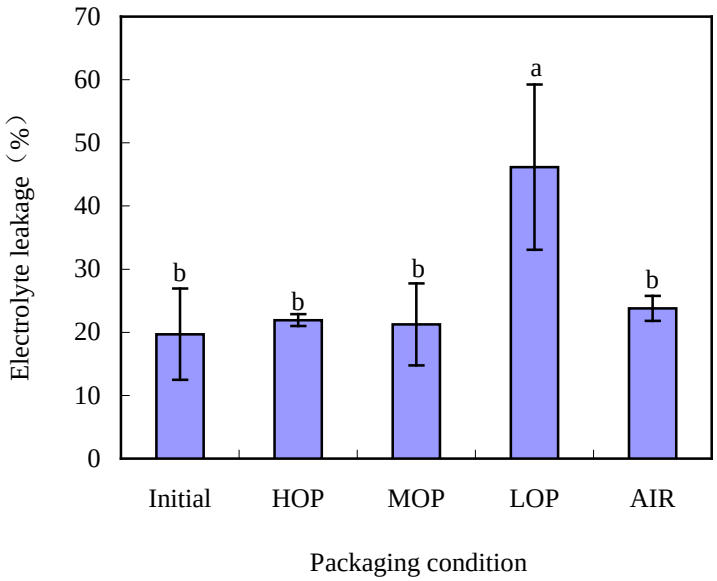
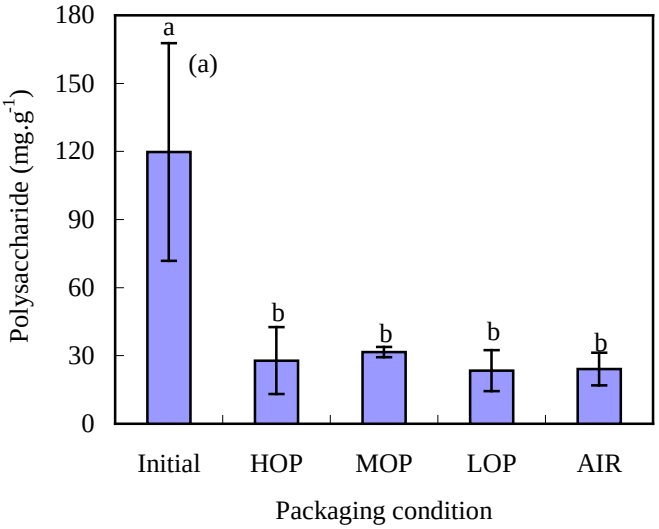


Fig. 3.3 Electrolyte leakage change between initial samples and samples after storage under different packaging conditions. Different letters are significantly different ($p \leq 0.05$).Vertical bars represent standard deviation (n=3).

3.4.3 Changes in water-soluble polysaccharide (WSP) and total phenolics (TP) content

Water-soluble crude polysaccharide content decreased significantly under all the packaging conditions from initial 119 $mg \cdot g^{-1}$ to lower than 35 $mg \cdot g^{-1}$ after storage (Fig. 3.4 (a)). And no significant change could be detected between the treatments. This result indicated that all the modified atmosphere packaging methods in this study could not prevent the sugar consumption caused by respiration of shiitake mushrooms during the storage, and the various initial O_2 concentrations inside the package did not show the different influences on the consumption of sugar content of shiitake mushrooms.

In this study, total phenolics content of shiitake mushrooms increased under all the packaging conditions after the storage (Fig. 3.4 (b)). Low oxygen packaging contributed to a significantly higher value than HOP, MOP and AIR. This increase could be related to the developmental changes and a physiological response to infections or injuries (Amanatidou et al. 2000, Alasalvar et al. 2005). This result is also corresponding to the electrolyte leakage result, which also increased after the storage. Therefore the highest content of total phenolics was determined in LOP could be explained as the result of the change of the membrane permeability treated by low oxygen and the accompanying severe anaerobic respiration.



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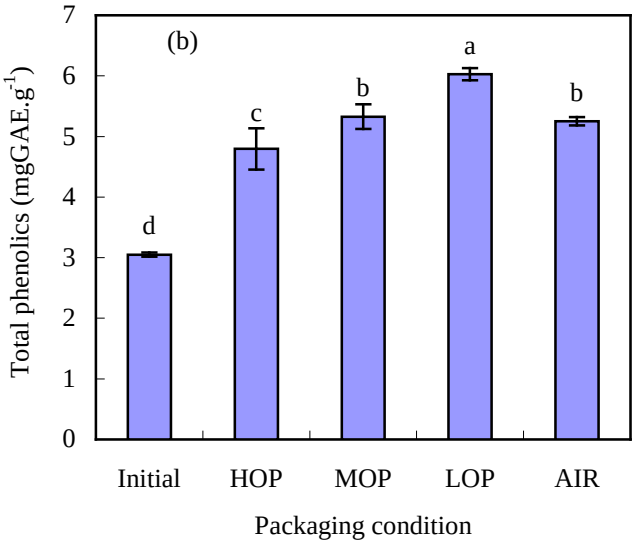


Fig. 3.4 Water-soluble crude polysaccharide content and total phenolic content change between initial samples and samples after storage under different packaging conditions. (a) Water-soluble polysaccharide content, (b) Total phenolics content. Different letters are significantly different ($p \leq 0.05$). Vertical bars represent standard deviation (n=3).

3.4.4 Changes in free amino acid content

Shiitake mushrooms contain various amino acids. Table 3.1 shows the change of amino acid before and after the storage under various packaging conditions. In this study, taurine, aspartic acid (Asp), glutamic acid (Glu), and arginine (Arg) showed highest amounts among all the detected free amino acids in the shiitake mushroom samples, and aspartic and glutamic acid are monosodium jlutamate-like (MSG-like) components, which give the most typical mushroom taste (Tseng and Mau 1999). Compared with the initial samples, total, essential and non-essential free amino acid contents significantly increased after the storage, and showed higher values in the active modified atmosphere packagings (HOP, MOP and LOP) than AIR packaging (control). Most of the detected amino acids of mushrooms after storage showed the different levels of change. Tau, Asp, Glu, b-Ala, Hypro content decreased, but other amino acids increased after the storage compared with the initial content.

The free amino acids increased after the storage mainly attributed to the protease activities increased to degrade the protein into free amino acids which also found by Minamide and Iwata (1986). Tseng and Mau (1999) also indicated a significant increase of free amino acid of *Agaricus bisporus* mushrooms packaged in polystyrene trays with polyvinyl chloride film (16-18 μm) and stored at 12 °C for 12 days.

Table 3.1 Free amino acid content of initial shiitake mushroom samples and samples after storage under different packaging conditions.

	Content (mg·g^{-1} dry wt.)				
Amino acid	Initial	HOP	MOP	LOP	AIR
P-Ser	0.51±0.01	0.79±0.12	0.83±0.09	0.63±0.02	0.65±0.06
Tau	6.01±0.13	6.01±0.16	5.77±0.10	4.37±0.07	5.63±0.34
PEA	nd	nd	nd	nd	nd
Urea	4.20±0.25	3.15±0.55	2.76±2.28	3.07±0.21	2.87±0.87
Asp	1.76±0.02	1.40±0.01	1.11±0.03	0.57±0.01	0.75±0.03
Thr[e]	0.70±0.01	2.49±0.02	2.80±0.13	2.08±0.06	2.23±0.08
Ser	0.52±0.01	1.31±0.02	1.80±0.06	1.53±0.02	1.51±0.07
Asn	1.37±0.03	2.22±0.02	2.59±0.09	2.97±0.06	2.28±0.10
Glu	3.50±0.03	1.42±0.02	2.00±0.07	2.16±0.03	2.03±0.04
Gln	1.13±0.13	2.82±0.33	2.94±0.31	2.17±0.03	2.73±0.22
Sar	nd	0.01±0.01	0.06±0.03	0.04±0.03	0.06±0.01
AAA	0.17±0.01	0.32±0.02	0.10±0.03	0.09±0.06	0.04±0.04
Gly	0.26±0.00	2.63±0.03	2.10±0.09	1.41±0.04	1.57±0.06
Ala	0.76±0.01	4.04±0.04	3.25±0.12	4.67±0.08	3.40±0.10
Cit	0.02±0.02	0.08±0.00	0.07±0.02	0.08±0.01	0.06±0.01
a-ABA	0.07±0.00	0.15±0.00	0.18±0.01	0.10±0.07	0.15±0.00
Val[e]	0.36±0.01	2.19±0.02	2.35±0.08	1.74±0.04	1.79±0.08
Cys	0.21±0.00	0.36±0.30	0.57±0.03	0.89±0.02	0.57±0.02
Met[e]	0.01±0.01	0.09±0.00	0.10±0.01	0.12±0.00	0.11±0.01
Cysta	0.21±0.01	1.20±0.02	0.85±0.04	0.35±0.03	0.68±0.02
Ile[e]	0.17±0.04	1.10±0.01	2.17±0.07	2.03±0.04	1.73±0.18
Leu[e]	0.37±0.01	1.83±0.04	2.37±0.06	2.26±0.04	2.05±0.06
Tyr	0.43±0.01	0.36±0.01	1.06±0.05	1.52±0.05	1.17±0.04
b-Ala	0.23±0.35	0.05±0.03	0.06±0.03	nd	0.01±0.01
Phe[e]	0.25±0.01	0.80±0.01	1.36±0.06	1.31±0.02	1.17±0.06
b-ABA	0.05±0.00	0.05±0.02	0.13±0.05	0.15±0.01	0.04±0.03
GABA	0.13±0.01	0.36±0.02	0.83±0.03	4.01±0.22	1.35±0.08
MEA	0.02±0.01	0.03±0.01	0.07±0.02	0.10±0.05	0.08±0.01
NH3	0.22±0.01	0.54±0.03	0.41±0.06	0.39±0.01	0.39±0.01
Hylys-1	nd	nd	nd	nd	nd
Orn	1.73±0.00	6.17±0.14	6.31±0.13	3.96±0.09	5.50±0.01
1M-His	nd	nd	nd	nd	nd

(*continued*)

His[e]	0.38±0.03	0.81±0.02	0.96±0.05	0.91±0.02	0.86±0.03
Lys[e]	0.89±0.06	2.28±0.02	2.70±0.14	2.04±0.04	2.17±0.05
3M-His	0.05±0.05	0.30±0.01	0.39±0.06	0.18±0.02	0.24±0.01
Trp[e]	0.03±0.03	0.14±0.07	0.26±0.09	0.31±0.15	0.33±0.10
Ans	0.60±0.06	0.86±0.11	1.23±0.01	1.02±0.15	0.88±0.20
Car	nd	0.03±0.03	nd	nd	nd
Arg[e]	2.18±0.04	3.69±0.19	2.45±0.36	3.38±0.10	2.61±0.09
Hypro	1.01±0.03	0.77±0.04	0.65±0.03	0.27±0.00	0.40±0.03
Pro	0.16±0.05	0.62±0.05	1.00±0.07	1.11±0.03	0.87±0.04
Tot.	30.68±0.75 [c]	53.47±0.72 [ab]	56.65±3.49 [a]	53.99±1.00 [ab]	50.96±1.94 [b]
Essen.	5.33±0.06 [d]	15.41±0.27 [bc]	17.53±0.79 [a]	16.17±0.18 [b]	15.04±0.52 [c]
Non-essen.	25.35±0.70 [c]	38.05±0.46 [ab]	39.12±2.70 [a]	37.83±0.82 [ab]	35.92±1.52 [b]

Tot.: Total amino acid; Essen (e).: Essential amino acid; Non-essen.: Non-essential amino acid.
Values represent mean ± SD based on three experiments and reported in $mg \cdot g^{-1}$ dry weight.
Different letters are significantly different (n=3, $p \leq 0.05$).
nd: not detected.

3.5 Conclusion

In this study, active modified atmosphere packaging with various initial O_2 concentrations was applied on the storage of postharvest shiitake mushrooms. LOP with the initial 3 % O_2 /5 % CO_2 and AIR treatment induced the anaerobic fermentation immediately after packaging and the high accumulation of ethanol concentration during the storage. Ethanol release was well retarded in MOA with 50 % O_2 initially and HOP with 100% O_2 initially. Shiitake mushrooms' tissue was affected significantly by LOP for a high value of electrolyte leakage after the storage and a consequent high total phenolics content. No packaging could prevent the polysaccharide content decreasing in this research. And the various packaging methods did not show the big difference of decrease of polysaccharide content. The modified atmosphere packaging had the significant effect on the increase of free amino acid content. For the fresh shiitake mushrooms which have high respiration rate, the higher oxygen packaging is better than low oxygen packaging, because the low oxygen packaging induced the anaerobic respiration immediately, and the damage to membrane caused by the low oxygen or the fermentation gases, although the polysaccharide and amino acid did not show the significant difference between the LOP and HOP after the storage. In addition to the physiology and nutritive constituents change, the sensory quality should also be considered in the further study to judge the effect of HOP, MOP and LOP on the shelf life of shiitake mushrooms.

CHAPTER 4

Effect of High-oxygen Packaging on Respiratory Physiology and Sensorial Qualities of Fresh Shiitake Mushrooms (*Lentinus edodes*)

4.1 Introduction

Shiitake mushroom (*Lentinus edodes*) is one of the most common edible mushrooms, and its cultivation and consumption have grown continuously (Ares et al. 2006, ÇaglarIrmak 2007). Shiitake mushrooms have a high nutritional value and contain several nutritive compounds, including polysaccharides, antioxidants, dietary fiber, ergosterol, minerals, vitamin B1, B2 and C (Beluhan and Ranogajec 2011, ÇaglarIrmak 2007, Jiang et al. 2010a). However, fresh shiitake mushrooms are very easy to get deteriorate and the short shelf life of mushrooms becomes an impediment to the distribution and marketing of the fresh produce (Parentelli et al. 2007, Antmann et al. 2008). Therefore, the appropriate packaging technology to preserve their quality and prolong the shelf life becomes very important.

At present, fresh shiitake mushrooms packaged in modified atmosphere packaging (MAP) are widely researched (Phillips 1996, Church and Parsons 1995). Traditional MAP provided oxygen with low concentration or air as initial atmospheres to reduce the respiration rate of produce, but if the oxygen inside the package is consumed fast by some produce with high respiration rate, the anaerobic metabolism is soon induced and some fermentation off-odors such as acetaldehyde and ethanol will be released. The accumulation of these off-odors formed through anaerobic respiration may have detrimental effect for storage (Francis M 1996, Oms-Oliu et al. 2008). High oxygen packaging (HOP) as an alternative to traditional MAP has been researched to be effective to reduce the anaerobic fermentation, inhibit the microbiology growth and discoloration on

some fruits and vegetables. Pérez and Sanz (2001) found that when strawberries were treated by high oxygen atmospheres (80% O_2/20% CO_2 and 90% O_2/ 10% CO_2) and low oxygen atmosphere (5% O_2, 20% CO_2), fungal growth could be prevented and strawberry firmness was enhanced under both high oxygen and low oxygen atmospheres, but color, titrable acidity, sugars and aroma were only mildly affected by high oxygen level and more affected by low oxygen atmosphere. Besides, when sliced mushrooms (*Agaricus bisporus*) packaged in high oxygen atmosphere, the shelf life was prolonged compared with low oxygen atmosphere. However, there are also some researches reporting that exposure to high oxygen may simulated, have no effect or reduce the respiration rate of produce depending on the commodity, maturity and ripeness stage, storage temperature and time (Jacxsens et al. 2001). Respiration rate of grapefruit was stimulated under 80 kPa O_2 atmosphere at 14°C, however exposure of “Bartlett” pear slices to 40, 60, or 80 kPa O_2 decreased their respiration rates during 4 days at 10°C (Kader and Ben-Yehoshua 2000). According to the results of previous study, high-oxygen packaging with initial 100% O_2 could retard the anaerobic respiration and do less damage to the tissue membrane of fresh shiitake mushrooms, and showed the potential to maintain the nutritive compounds. However, the respiratory physiology and sensorial quality of shiitake mushrooms under high-oxygen packaging, and optimal oxygen concentration for high-oxygen packaging still call for the further research.

The objective of the study in this chapter was to investigate the effect of the high oxygen packaging (HOP) with different initial oxygen concentrations on the respiratory physiology and sensorial qualities of fresh shiitake mushrooms (*Lentinus edodes*) during the storage.

4.2 Materials and methods

4.2.1 Samples preparation, packaging and storage conditions

Fresh shiitake mushrooms were harvested from Ibaraki Prefecture in Japan, and transported to the lab within 24 hours of harvest. The mushrooms were stored before packaging at 10°C with 90% relative humidity (RH) for 2 hours until the core temperature was the same as the storage chamber.

Shiitake mushrooms (70±5 g) were selected for uniform size and color, and packaged by Low- Density Polyethylene (LDPE) packages of 25cm×15cm×100μm. Gas transmission rate of this film was 753.27 $ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$ for O_2 and 3475.54 $ml \cdot m^{-2} \cdot 24h^{-1} \cdot atm^{-1}$ for CO_2. Then different initial gases were injected into the packages after which they were heat sealed and vacuumized: (1) High oxygen packaging 1 (HOP1): 80% O_2 balanced by N_2, (2) High oxygen packaging 2 (HOP2): 100% O_2, (3) Control: air. Then all the samples were stored at 10°C with RH 90% for 9 days. Then the headspace gas composition, respiration rate, hardness, TSS concentration, color were determined, and sensory evaluation was also carried out on the day 0, 2, 4, 7, 9 (Fig. 4. 1).

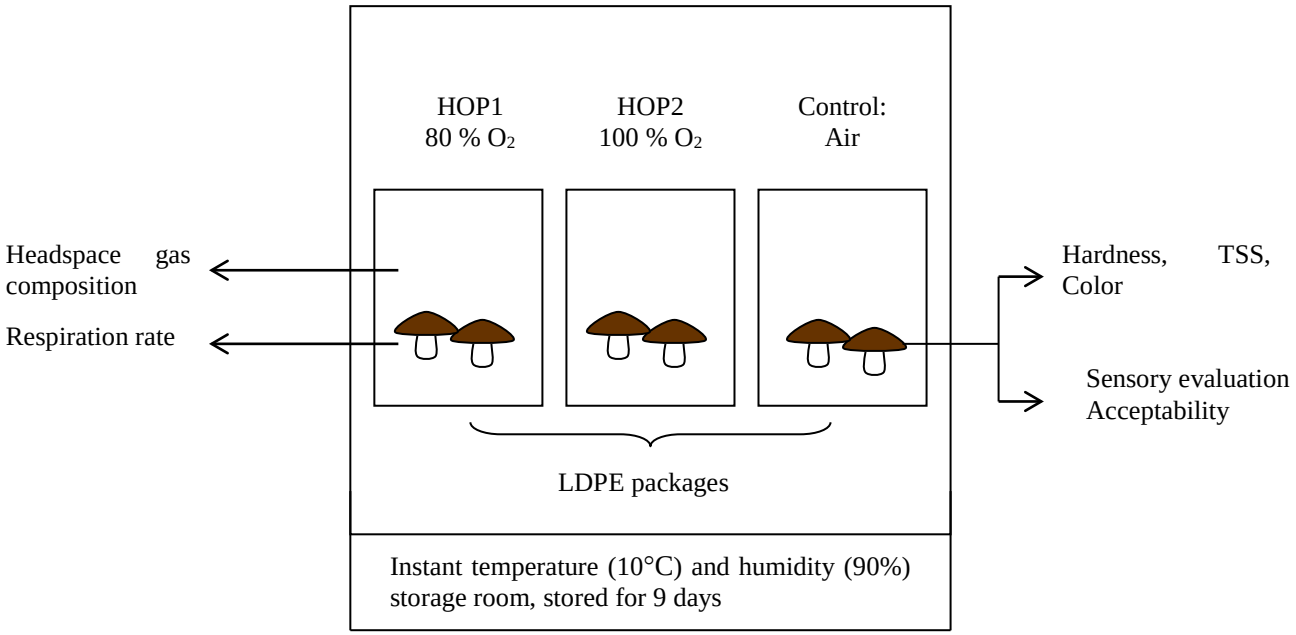


Fig. 4.1 Illustration of packaging and storage conditions

4.2.2 Gas composition analysis

The headspace gas composition (O_2, CO_2, ethanol and acetaldehyde) under all packaging conditions was measured at 0, 2, 4, 7, 9 days of storage. CO_2 and O_2 concentrations in the packages were determined by withdrawing a gas sample (1 ml) from the package headspace and injecting into gas chromatograph (GC-8A, Shimadzu, Japan) with a thermal conductivity detector (TCD) and the packed column ZY-2 consisting of Molecular Sieve 5A column, Porapak Q column and Shimalite Q column. The carrier gas was helium with 0.67 $ml \cdot s^{-1}$ flow rate. Column temperature was 75 °C; injector and detector temperature was 80°C.

Ethanol and acetaldehyde concentrations were detected by injecting gas samples (1 ml) into a gas chromatograph (GC-14B, Shimadzu, Japan) with a flame ionization detector (FID) and PEG 20M column, and the carrier gases were helium with 0.89 $ml \cdot s^{-1}$ flow rate, hydrogen with 0.67 $ml \cdot s^{-1}$ flow rate and air with 8.17 $ml \cdot s^{-1}$ flow rate. Column temperature was 65°C, injector temperature was 250°C and detector temperature was 275 °C.

4.2.3 Respiration rate determination

The respiration rate of fresh produce can be expressed as O_2 consumption rate or CO_2 production rate (Fonseca et al. 2002b). The enclosed system was used to determine the respiration rate (Iqbal et al. 2009a). In this experiment, the CO_2 production rate was taken to be the respiration rate.

The vegetables were enclosed in 1 L airtight organic glass containers with a rubber stopper for gas sampling. Gas samples of 1ml were withdrawn from the container and injected into the gas chromatography (Shimadzu GC-8A, Japan) with a TCD after 0 hour, 1 hour, and 2 hours. CO_2 production rate (R_{CO_2}) was calculated as the slope of the CO_2 concentration percent versus time curve shown as the equations (1) (Parentelli et al. 2007,

Iqbal et al. 2009b). It was expressed as $mg \cdot kg^{-1} \cdot h^{-1}$.

$$R_{CO_2} = (K_{CO_2} \times V_f) / m \quad (1)$$

Where R_{CO_2} is carbon dioxide production rate which can be expressed as $mg \cdot kg^{-1} \cdot h^{-1}$, K_{CO_2} is the slope of carbon dioxide concentration percent versus time curve which can be expressed as $\% \cdot h^{-1}$. V_f is the free volume of container that subtracts the sample's volume from the container volume.

4.2.4 Hardness, TSS and color determination

Hardness, total soluble solids (TSS) concentration and color were measured after the 0, 2, 4, 7, 9 days of the storage. Hardness was assessed in four symmetrical places on the mushroom cap using a hardness tester (Hardmatic Type E, Mitutoyo, Kawasaki, Japan), and mean values were generated. TSS was expressed as the Brix% of the mushroom juice using a digital refractometer (PR-201α, Atago, Tokyo, Japan). Color determination was performed by assessing three equidistant points on the mushroom cap using a Chroma Meter (CR 300, Minolta, Osaka, Japan), and mean L^* (lightness), a^* (red-greenness) and b^* (yellow-blueness) were generated.

4.2.5 Sensory evaluation and acceptability

The sensory quality characteristics of shiitake mushrooms were evaluated by a panel of 3 trained assessors. The sensory quality parameters were discussed by panelists and referred to other publications and decided to score from 1 to 9 points, and scoring method could be described as: (1) Aroma, which was determined by smelling the whole mushroom, and the 9= full shiitake mushroom typical scent or aroma, 7= moderate full aroma, 5= moderate and slight alcohol fermentation smell, 3= slight aroma and obvious smelly odor, 1= severely and unpleasantly smelly odor. (2) Texture was determined by pressing the cap surface with fingers. 9= very firm and resilient, 7= firm, 5= moderately firm, 3= soft and

less resilient, 1= very soft and no resilient. (3) Cap color was observed visually, 9= light brown, 7= brown, 5= dark brown, 3= dark brown with black spots, 1= light black. (4) Gill color was also observed gill of shiitake mushrooms visually, in which 9= white, 7= light yellow, 5= yellow, 3= dark yellow, 1= light brown. Then the assessors made the decision that whether they would accept the shiitake mushroom to buy or not.

4.3 Result and discussion

4.3.1 Changes in headspace gas composition and respiration rate

Changes of O_2 and CO_2 concentrations in the packages were depicted in Fig. 4.2. Both O_2 and CO_2 concentration changed significantly over time in all packaging conditions. O_2 concentration in initial air packages decreased fast and kept < 1 % from the 2nd day of storage. Till the 4th day, O_2 concentration in initial 80% O_2 packages also decreased to lower than 1 %, which would induce the anaerobic metabolism. Initial 100% O_2 packaging maintained the oxygen concentration ＞10% till the 4 days of storage although after 7 days decreased lower than 1 %. CO_2 concentration in all the high oxygen packages increased above 40% till the 4th day and then fell down till the end of the storage. CO_2 concentration in air packages increased to about 20% and then decreased mildly to the end of the storage.

Respiration rate under all the packaging conditions (showed in Fig. 4.3) reached the climacteric peak on the second day at 10 °C, after that it decreased gradually till the end of the storage. It was also obvious that the respiration rates under 80% and 100% O_2 packaging conditions were significantly higher than air conditions. It could be manifested that respiration rate of fresh shiitake mushroom could be accelerated by increasing oxygen concentration inside the package, which also induced the higher concentration of CO_2 in

100 % and 80 % O_2 packages.

Elevated O_2 atmospheres may influence the production and accumulation of some volatile compounds associated with respiratory metabolism such as ethanol and acetaldehyde shown in Fig. 4.4. Ethanol gas could be detected in all high oxygen packages after 4 days when the oxygen concentration decreased to 0 %, while in air packages the ethanol concentration has reached beyond 80 $\mu l \cdot l^{-1}$ with an oxygen concentration of 0 %. With the storage period prolonged, ethanol and acetaldehyde concentration rose up quickly under 80 % O_2 packaging and higher than air condition on Day 7, and ethanol concentration showed a decrease tendency after that. Although the fermentation off-odors could also be detected in 100 % O_2 packages, the ethanol and acetaldehyde levels are much lower than 80 % O_2 and air packages. Therefore low O_2 atmosphere in the package could promote the production of anaerobic metabolites, and high oxygen packaging could not inhibit the fermentation metabolism completely, but HOP with initial 100 % O_2 concentration reduced the ethanol and acetaldehyde concentration. This result is also in agreement with the hypothesis of Day (1996) which declared that under high oxygen atmospheres there would be less fermentative metabolites than under high CO_2 atmosphere.

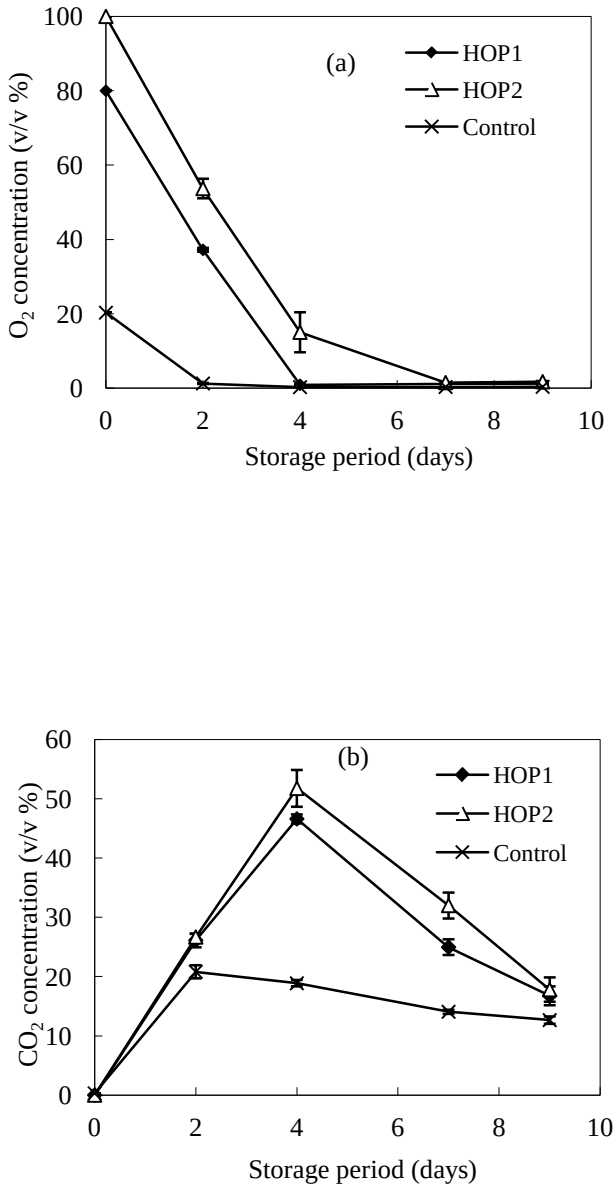


Fig. 4.2 O_2 (a) and CO_2 (b) concentrations in packaged shiitake mushrooms stored at 10°C. Vertical bars represent standard deviation (n=3).

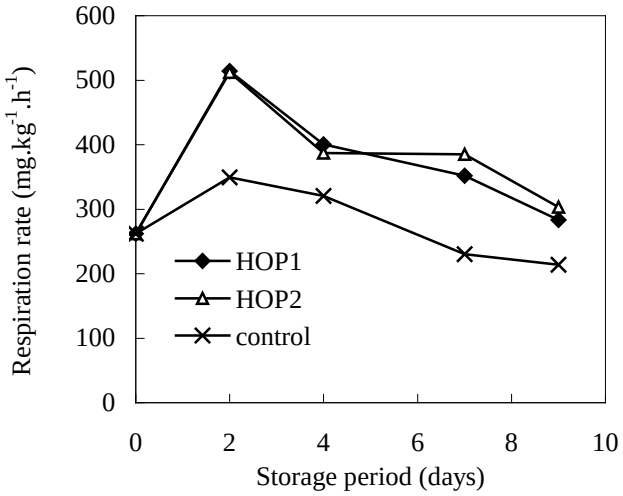


Fig. 4.3 The changes of respiration rate of fresh shiitake mushrooms in packages with different initial oxygen concentrations

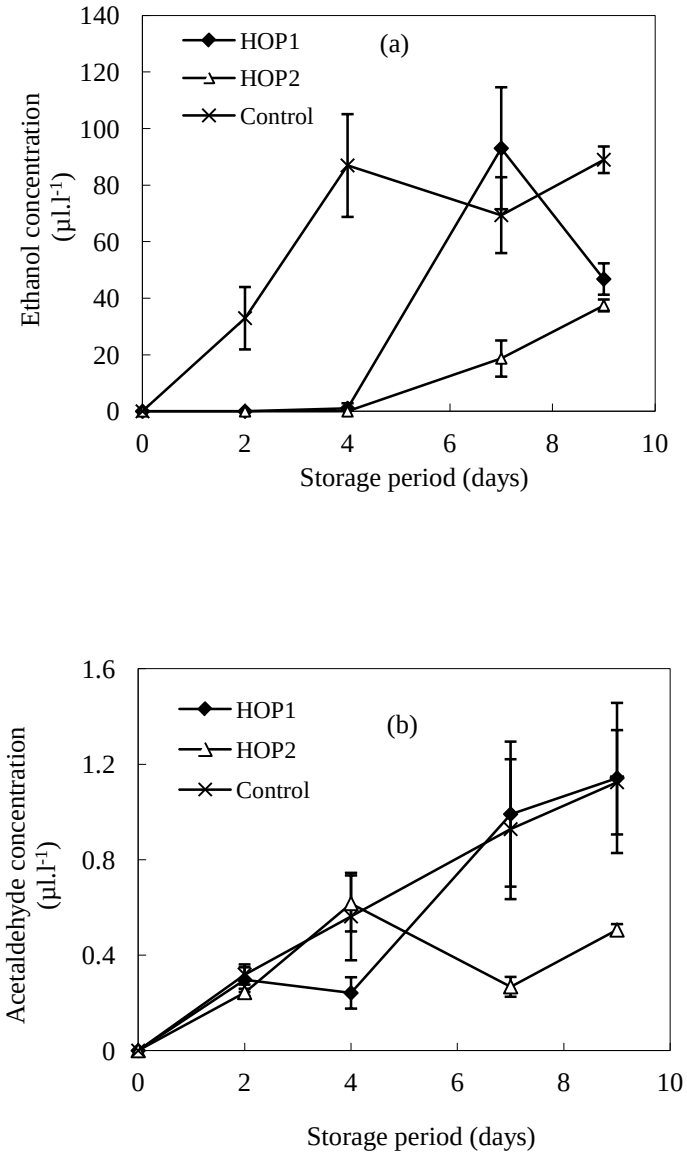


Fig. 4.4 Ethanol and acetaldehyde concentration in packaged shiitake mushrooms stored at 10°C. Vertical bars represent standard deviation (n=3). (a) Ethanol concentration, (b) Acetaldehyde concentration

4.3.2 Changes in hardness, TSS and color during the storage

Changes in hardness, total soluble solid (TSS) concentration, color parameters (L^*, a^* and b^*) of fresh shiitake mushrooms under all the high oxygen and AIR packaging conditions at 10 °C throughout 9 days' storage were depicted in Table 4.1. Samples under all the three packaging conditions decreased in hardness during the storage, but did not have any significant difference between treatments. This result was different with the high oxygen packaging applied on strawberries which showed that HOP exhibited lower hardness compared to low O_2 MA (Van der Steen et al. 2002). TSS content of shiitake mushrooms did not show any obvious change during the storage at 10 °C, and the different packaging conditions also did not contribute any advantages for TSS content. Colors in all the color parameters did not change dramatically until the 4 days of storage, but on the Day 7, significant higher L^* was observed in 80% O_2 packages and a^* was observed in 100 % O_2 packages. At the end of the storage, HOP got the significantly higher L^* and a^* than AIR packaging, which manifested that HOP could prevent the discoloration better than AIR packaging.

Table 4.1 Changes of hardness, TSS and color (L^*, a^* and b^*) in packaged shiitake mushrooms

	Hardness (N)	TSS (Brix%)	L^*	a^*	b^*
Initial	14.78±1.65	2.28±0.43	42.54±1.86	12.68±0.32	28.55±2.06
Day 2					
HOP1 (80% O_2)	14.03±3.89 [a]	1.78±0.21 [a]	38.32±2.98 [a]	12.11±0.72 [a]	22.65±2.26 [a]
HOP2 (100% O_2)	19.70±6.81 [a]	2.03±0.24 [a]	39.91±5.78 [a]	12.32±0.99 [a]	23.33±2.31 [a]
Control(Air)	14.03±2.93 [a]	1.95±0.65 [a]	41.20±5.25 [a]	12.33±1.39 [a]	24.73±1.62 [a]
Day 4					
HOP1 (80% O_2)	11.63±4.01 [a]	1.70±0.29 [a]	42.68±2.63 [a]	12.30±0.43 [a]	25.39±1.77 [a]
HOP2 (100% O_2)	12.03±3.92 [a]	1.58±0.41 [a]	36.59±9.80 [a]	12.09±0.64 [a]	20.23±5.95 [a]
Control (Air)	14.58±5.42 [a]	1.93±0.40 [a]	41.98±8.63 [a]	11.65±0.31 [a]	26.20±6.00 [a]
Day 7					
HOP1 (80% O_2)	10.00±2.87 [a]	3.00±0.47 [a]	46.60±5.49 [a]	10.59±2.04 [ab]	28.71±5.08 [a]
HOP2 (100% O_2)	7.58±4.18 [a]	2.20±0.27 [a]	35.06±3.02 [b]	13.36±0.72 [a]	23.38±3.28 [a]
Control (Air)	9.63±3.37 [a]	2.55±0.75 [a]	39.99±2.59 [b]	12.13±0.98 [b]	26.41±2.21 [a]
Day 9					
HOP1 (80% O_2)	9.18±2.28 [a]	2.98±0.13 [a]	45.73±3.72 [a]	11.32±1.18 [a]	30.16±2.84 [a]
HOP2 (100% O_2)	7.38±3.42 [a]	2.90±0.41 [a]	43.09±5.09 [ab]	11.76±0.72 [a]	27.32±3.13 [ab]
Control (Air)	7.15±1.38 [a]	3.05±0.26 [a]	37.87±2.17 [b]	10.72±0.98 [a]	24.23±2.55 [b]

Storage temperature: 10°C
Mean values with different letters are significantly different (n=3, $p \leq 0.05$).

4.3.3 Changes in sensorial quality and acceptability

Sensorial quality of shiitake mushrooms stored at 10 °C was determined during the storage. On the second day, aroma under all the HOP conditions was significant higher than that under AIR condition. HOP with 100 % O_2 concentration initially showed higher values in texture, cap color and gill color than other packages after 2 days of storage, but didn't show any significant difference. From the Day 4 to Day 9, aroma scores for mushroom under AIR condition decreased significantly compared with other packaging conditions. However, texture scores under air condition were higher than other conditions and showed significant difference compared with 100 % O_2 condition. There were not obvious changes in cap color and gill color during the whole storage period, and significant differences cannot be detected between all the packaging conditions. Therefore, the result indicated that high oxygen packaging could maintain the aroma of fresh shiitake mushrooms, but could not keep the texture better compared with the air atmosphere.

Acceptability has a highest correlation (R^2=0.66) with aroma among the four sensory parameters. Acceptability kept stable 100 % in all high oxygen packages till the 4 days of storage. But about 50 % samples were rejected from the 2nd day under air packaging condition. After 4 days, more samples could not be accepted and 0 % acceptability was showed up in AIR condition, but about 50 % in HOP till the end of the storage. Therefore, the in-package atmosphere of air accelerated the decreasing rate of acceptability of shiitake mushrooms and decreased their shelf life, when compared to mushrooms packaged under high oxygen condition during the 9 days' storage period.

Table 4.2 Sensory scores of packaged fresh shiitake mushrooms

	Aroma	Texture	Cap color	Gill color
Initial	8.77±0.20	8.22±0.19	7.44±0.51	7.22±1.92
Day 2				
HOP1 (80% O_2)	8.00±1.15 ^{a}	7.25±0.96 ^{a}	7.00±0.00 ^{a}	6.50±1.29 ^{a}
HOP2 (100% O_2)	8.00±1.15 ^{a}	8.00±1.15 ^{a}	7.50±1.00 ^{a}	7.50±1.00 ^{a}
Control(Air)	2.50±1.00 ^{b}	7.50±1.00 ^{a}	6.50±1.00 ^{a}	7.50±1.00 ^{a}
Day 4				
HOP1 (80% O_2)	7.50±1.00 ^{a}	6.50±1.00 ^{a}	6.50±1.00 ^{a}	6.00±1.15 ^{a}
HOP2 (100% O_2)	7.00±0.00 ^{a}	6.50±1.00 ^{a}	6.00±1.15 ^{a}	6.50±1.00 ^{a}
Control (Air)	2.50±1.91 ^{b}	7.00±1.63 ^{a}	6.00±1.15 ^{a}	7.25±0.50 ^{a}
Day 7				
HOP1 (80% O_2)	4.50±1.00 ^{a}	4.50±1.91 ^{a}	6.00±1.15 ^{a}	6.00±1.15 ^{a}
HOP2 (100% O_2)	3.50±1.00 ^{ab}	4.50±1.00 ^{a}	5.00±0.00 ^{a}	6.50±1.00 ^{a}
Control (Air)	2.50±1.91 ^{b}	6.00±1.15 ^{a}	6.00±1.15 ^{a}	6.50±1.00 ^{a}
Day 9				
HOP1 (80% O_2)	2.00±1.15 ^{ab}	4.50±1.00 ^{ab}	6.00±1.15 ^{a}	6.00±1.15 ^{a}
HOP2 (100% O_2)	3.00±1.63 ^{a}	3.50±1.00 ^{b}	5.00±0.00 ^{a}	6.00±1.00 ^{a}
Control (Air)	1.00±0.00 ^{b}	5.00±0.00 ^{a}	5.50±1.00 ^{a}	5.50±1.00 ^{a}

Storage temperature: 10 ℃
Mean values with different letters are significantly different (n=3, $p \leq 0.05$)

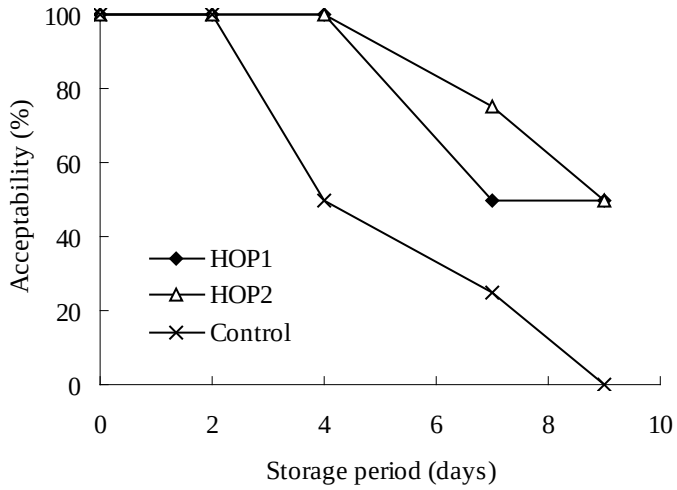


Fig. 4.5 Acceptability of packaged shiitake mushrooms stored at 10°C.

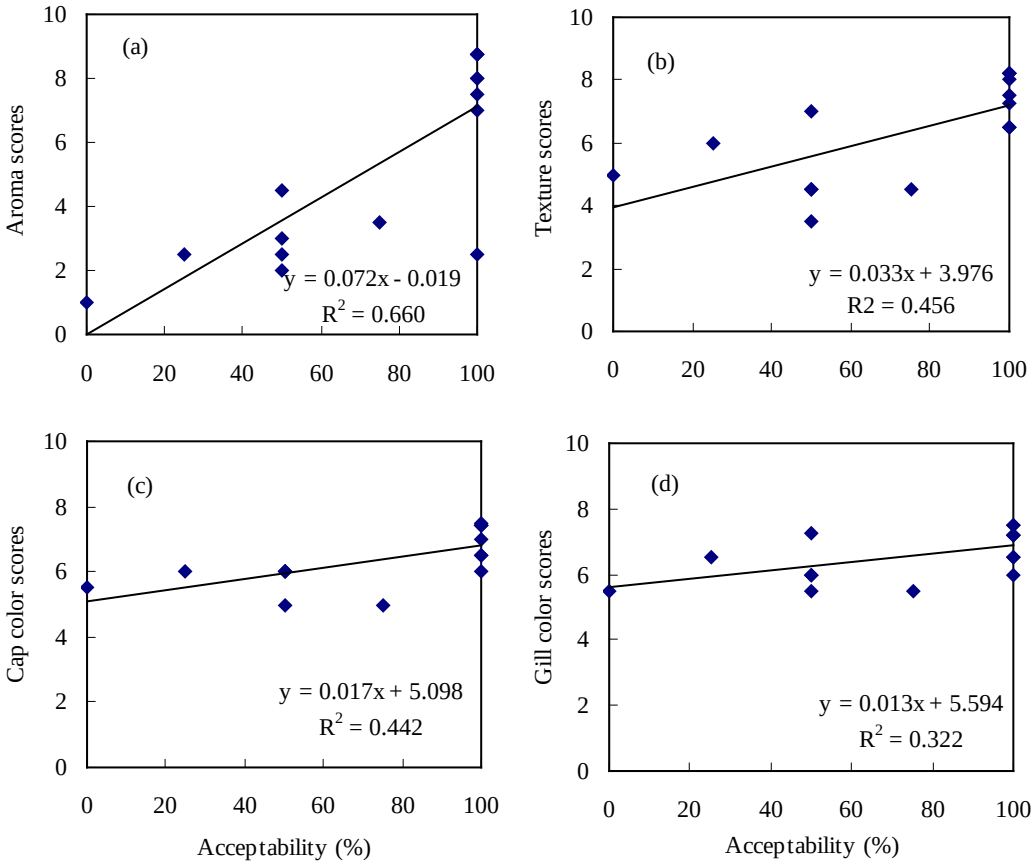


Fig. 4.6 Correlation (R^2) between acceptability and sensory parameters. (a) Aroma, (b) Texture, (c)Cap color, (d) Gill color.

4.4 Conclusion

High oxygen packaging with initial 100 % O_2 and 80 % O_2 had beneficial effect on the sensorial quality of fresh shiitake mushrooms by retarding the anaerobic metabolism occurrence and contributing the better aroma scores compared with air packaging. HOP can not reduce the respiration rate or prevent the fermentation metabolism of shiitake mushrooms completely, but HOP with initial 100 % O_2 could retard the ethanol and acetaldehyde accumulation. Mushroom's hardness, color parameters of L^* and a^* got significant higher values in HOP than in air packaging after the storage, but TSS content did not show any significant difference between treatments. Aroma played an important role when judging the sensorial quality of fresh shiitake mushrooms, and aroma scores in HOP samples were significantly higher than AIR samples. During the storage, samples stored under HOP condition especially 100 % O_2 atmosphere, showed a lower deterioration rate and higher sensory quality than those stored under air condition.

CHAPTER 5

Effect of High-oxygen Packaging Compared with Perforation-mediated Modified Atmosphere Packaging on the Qualities of Fresh Shiitake Mushrooms (*Lentinus edodes*)

5.1 Introduction

Shiitake mushrooms (*Lentinus edodes*) are one of the most widely cultivated mushrooms (Antmann et al. 2008), and production has quickly increased in recent years due to their distinctive taste and abundant nutrient compounds, including polysaccharides, proteins, and dietary fiber (Jiang et al. 2010b). However, shiitake mushrooms are highly perishable and deteriorate rapidly due to their fast respiration (Ares et al. 2006, Parentelli et al. 2007). The high rate of respiration results in rapid oxygen consumption, inducing an anaerobic atmosphere in the packaging and consequent fermentation odors and tissue deterioration.

The application of modified atmosphere packaging (MAP) to a wide variety of vegetables and fruits has been extensively researched. In MAP, the composition of gases inside the packaging is manipulated to provide an optimal atmosphere, reducing the respiration rate and resulting in prolonged product shelf life. However, the fast respiration rate of fresh shiitake mushrooms resulted in shorter shelf life in passive MAP without perforation than macro perforated packaging (Ares et al. 2006). Therefore, the use of perforation-mediated MAP (PM-MAP) could increase permeability (González et al. 2008, Ozdemir, Monnet and Gouble 2005), modifying gas exchange and retarding anaerobic respiration, thereby prolonging the shelf life of produce.

High oxygen packaging (HOP) has been extensively researched as an alternative to traditional MAP. A high oxygen environment has been reported to decrease the release of

fermentation odor, inhibit typical spoilage microbial growth and prolong the shelf life of some produce. Jacxsens et al. (2011) demonstrated that sliced mushrooms (*Agaricus bisporus*) packaged under a high O_2 concentration were rejected after the 6th day of storage, while under a low O_2 atmosphere, they were rejected after only 3 days. It also has been hypothesized that high O_2 levels may result in substrate inhibition of the enzyme polyphenoloxidase (PPO), or alternatively, that high levels of subsequently formed colorless quinones may cause feedback inhibition of PPO. Therefore the enzymatic browning discoloration caused by PPO could be inhibited accordingly (Day. 1996, 2000). However, exposure to high O_2 levels was observed to induce deleterious effects in some fruits. It was reported that production of farnesene and trienol, related to the development of storage scald, increased in apples kept in 100 kPa O_2 atmosphere at 0 °C for up to 3 months. “Granny Smith” apples stored under the above condition were completely bronzed after 3 months and contained high ethanol concentrations (Kader and Ben-Yehoshua 2000). Besides, the high oxygen may have the possibility of accelerating the respiration rate of product and induce the high deterioration. It is very necessary to clearly know whether a high oxygen atmosphere could prolong the shelf life or induce injury to fresh shiitake mushrooms. Therefore, while both HOP and PM-MAP could be utilized to prolong the shelf life of shiitake mushrooms, whether a high oxygen atmosphere would be deleterious to shiitake mushrooms or maintain quality compared with PM-MAP requires investigation.

The objective of the research in this chapter was to compare the effects of HOP and PM-MAP on the qualities of fresh shiitake mushrooms.

5.2 Material and methods

5.2.1 Sample preparation, packaging and storage conditions

Fresh shiitake mushrooms were harvested from Ibaraki Prefecture in Japan, and transported to the lab within 24 hours of harvest. The mushrooms were stored before

packaging at 10 °C with 90 % relative humidity (RH) for 2 hours until the core temperature was the same as the storage chamber.

Shiitake mushrooms (70±5 g) were randomly selected and packaged under each of the following conditions: (1) HOP: aluminum-plastic composite packaging film (O_2 and CO_2 transmission rates under 10 °C are about 0 $mol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) of 18 cm × 18 cm and 60 μm thickness was selected to prevent oxygen transmission to the outside of the package. O_2 (100%) was then injected into the package after it was heat sealed and vacuumized. The initial gas volume was 2.7 L. (2) PM-MAP: 4, 8 and 20 micro perforations (P4, P8 and P20) were utilized to generate different equilibrium gas compositions, and the ratio of total perforations' area to package area was 0.00025 %, 0.0005 % or 0.00125 % respectively. The PM-MAP was perforated with the needle manually. The microperforation size was determined using a hybrid microscope (SH-4500, Hirox, Tokyo, Japan) equipped with a mid-range (200×) rotary zoom lens (SX-5030Z, Hirox, Tokyo, Japan). An image of the microperforation sample is shown in Fig. 5.1. The average diameter of the microperforation for all the PM-MAP was 234±26μm. These packages were sealed with 2.7 L of normal air inside initially. (3) Macro perforation packaging was employed as the control treatment. Four macro perforations (6 mm in diameter) were made in each package and the packages were subsequently sealed. This maintained the air composition within the package and limited dehydration. The packaging material employed for the micro and macro perforation MAP was polyethylene film with 21.5 cm × 15 cm in area and 100 μm in thickness. The O_2 permeance was 976 $ml \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$ (5.04×10^{-12} $mol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) for P4, 1948 $ml \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$ (1.01×10^{-11} $mol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) for P8 and 4863 $ml \cdot m^{-2} \cdot day^{-1} \cdot atm^{-1}$ (2.51×10^{-11} $mol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) for P20. The samples were stored at 10 °C and 90 % RH for 8 days. Three replicates were evaluated per each packaging condition.

5.2.2 Gas compositions analysis

The gas composition (O_2, CO_2 and ethanol) under all packaging conditions was measured at 0, 2, 4, 6, 8 days of storage. CO_2 and O_2 concentrations in the packages were determined by withdrawing a gas sample (1 ml) from the package headspace and injecting into a gas chromatograph (GC-8A, Shimadzu, Japan) with a thermal conductivity detector (TCD) and the packed column ZY-2 consisting of Molecular Sieve 5A column, Porapak Q column and Shimalite Q column. The carrier gas was helium with 0.67 $ml \cdot s^{-1}$ flow rate. Column temperature was 75 °C; injector and detector temperature was 80 °C.

Ethanol concentrations were detected by injecting gas samples (1 ml) into a gas chromatograph (GC-14B, Shimadzu, Japan) with a flame ionization detector (FID) and PEG 20M column, and the carrier gases were helium with 0.89 $ml \cdot s^{-1}$ flow rate, hydrogen with 0.67 $ml \cdot s^{-1}$ flow rate and air with 8.17 $ml \cdot s^{-1}$ flow rate. Column temperature was 65°C, injector temperature was 250 °C and detector temperature was 275 °C.

5.2.3 Color, hardness, TSS and mass loss determination

Color, hardness, total soluble solids (TSS) concentration and mass loss were determined before and after storage. Color determination was performed by assessing three equidistant points on the mushroom cap using a Chroma Meter (CR 300, Minolta, Osaka, Japan), and mean L^*-values (lightness) were generated. Hardness was assessed in four symmetrical places on the mushroom cap using a hardness tester (Hardmatic Type E, Mitutoyo, Kawasaki, Japan), and mean values were generated. TSS was expressed as the Brix% of the mushroom juice using a digital refractometer (PR-201α, Atago, Tokyo, Japan).

5.2.4 Sensory evaluation

The sensory quality parameters were discussed and selected by 3 trained panelists from

a preliminary test and in reference to other publications (Villaescusa and Gil 2003, Jiang et al. 2010a). In preliminary experiment, the packaging and storage condition were totally same as this experiment, the assessors wrote down the main parameters which can reflect the main sensory quality change of shiitake mushrooms. The assessors discussed the parameters and agreed on the parameters which could best differentiate the shiitake mushrooms quality during the storage. The selected parameters were: overall visual quality, aroma, texture, cap color, gill color and gill integrity. The scoring method was also discussed and described as follows:

A score from 1 to 9 points was utilized: (1) Overall visual quality. The whole intact mushroom including the cap, gill and stipe was evaluated visually, in which 9 = excellent, 7= very good, 5= good and can be sold, 3= just edible, 1= poor and not edible. (2) Aroma was determined by smelling the whole mushroom, in which 9= full shiitake mushroom typical scent or aroma, 7= moderate full aroma, 5= moderate and slight alcohol fermentation smell, 3= slight aroma and obvious smelly odor, 1= severe and unpleasant smelly odor. (3) Texture was determined by pressing the cap surface with the fingers, in which 9= very firm and resilient, 7= firm, 5= moderately firm, 3= soft and less resilient, 1= very soft and not resilient. (4) Cap color was observed visually, in which 9= light brown, 7= brown, 5= dark brown, 3= dark brown with black spots, 1= light black. (5) Gill color was observed visually, in which 9= white, 7= light yellow, 5= yellow, 3= dark yellow, 1= light brown. (6) Gill integrity was visually observed and scored as follows, 9= intact and no damage, 7= intact but minor damage, 5= not very intact and some damage, 3= half of gills damaged, 1= totally damaged. The sensory quality of shiitake mushrooms were evaluated on 0, 4th, and 8th day.

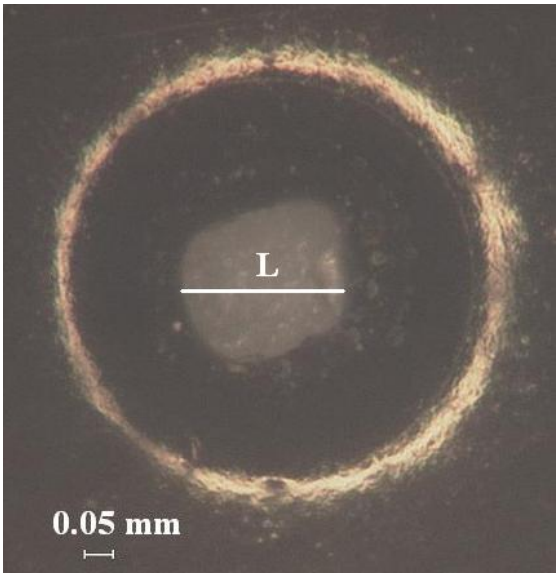


Fig. 5.1 Microscopic imaging of microperforation.
(L: the effective diameter of the microperforation)

5.2.5 Statistical analysis

All the results were expressed as mean values± standard deviation (SD). All data were analyzed by analysis of variance (ANOVA). Differences between treatments were analyzed by LSD tests and differences at $p \leq 0.05$ were considered to be significantly different.

5.3 Results and discussion

5.3.1 Changes in headspace gas composition

The results of O_2 and CO_2 concentrations are presented as a percentage of the total in-package atmosphere. O_2 concentration decreased and CO_2 concentration increased as expected during storage at 10 °C (Fig. 5.2). Although the O_2 concentration in the HOP decreased during the first 4 storage days, a relatively high level was maintained (>20%). The O_2 concentration in the P4 packaging decreased, and from the 6th day onward was <1%. In P20 and macro perforation packages, the O_2 level maintained near equilibrium to the end of storage, at about 17% and 20% O_2, respectively. The accumulation of CO_2 in HOP was continuous and obviously higher than under the other conditions. CO_2 concentrations in P4, P8 and P20 increased rapidly in the first 2 days, and then increased only slightly thereafter. During the storage period, O_2 and CO_2 concentrations were stable in the control treatment (macro perforation MAP).

The ethanol concentration of the packages reflects the effect of an anaerobic atmosphere on fermentation metabolism. Ethanol accumulation under various packaging conditions is shown in Fig. 5.3. Ethanol accumulation was observed in P4 and HOP from the 4th day. At the end of the storage period, the ethanol concentration was 18 $\mu l \cdot l^{-1}$ in HOP and 31 $\mu l \cdot l^{-1}$ in P4, whereas in P8, P20 and macro perforation packages, ethanol accumulation was not detected.

5.3.2 Changes in color, hardness, TSS and mass loss under various packaging conditions

The results of L-value, hardness, TSS and mass loss ratio are shown in Table 5.1. The L^* (lightness) is an index that is representative of the color change in shiitake mushrooms. After 8 days of storage at 10 °C, a significantly higher L^* was detected in 100% O_2 packaging as compared to PM-MAP and control. A decrease in hardness was observed under all packaging conditions. However, hardness in HOP (13.33 N) was significantly higher than in other treatments after 8 days of storage, while hardness in P4 and P8 were significantly higher than in P20 and control. TSS content was observed to be significantly lower in P20 on the 8th day, whereas no significant differences were detected in other treatments.

As expected, a significant and severe mass loss (10.9 % on the 8th day) occurred only in the macro perforation packaging, and was judged to be unacceptable.

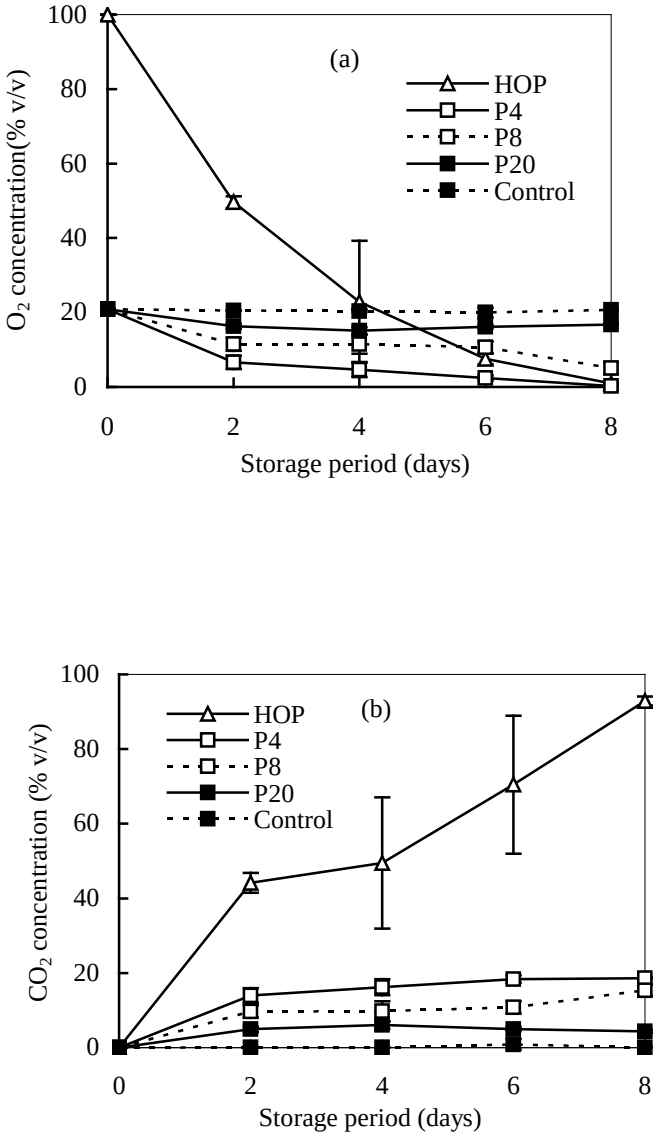


Fig. 5.2 O_2 (a) and CO_2 (b) concentrations in packaged shiitake mushrooms stored at 10 °C. Vertical bars represent standard deviation (n=3).

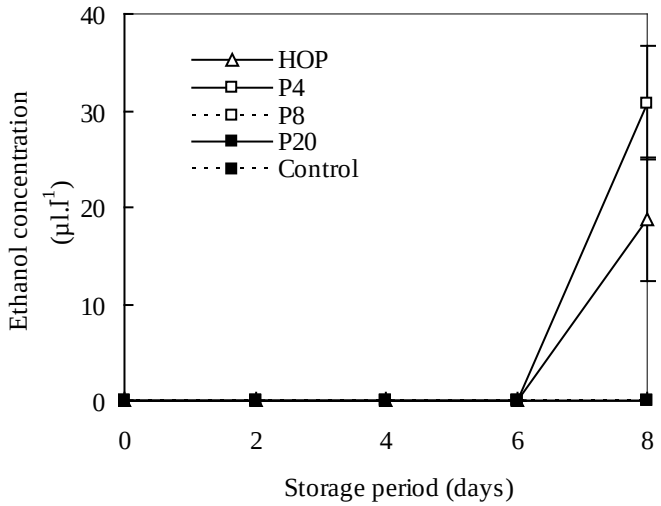


Fig. 5.3 Ethanol concentration in packaged shiitake mushrooms stored at 10 °C. Vertical bars represent standard deviation (n=3).

Table 5.1 L-value (L^*), hardness, TSS and mass loss in packaged shiitake mushrooms

		L^*	Hardness (N)	TSS (Brix%)	Mass loss ratio (%)
Day 0		46.22±0.65	20.11±1.21	4.70±0.58	nd
Day 8	HOP	34.59±3.66 ^{a}	13.33±2.38 ^{a}	3.41±0.30 ^{a}	1.49±0.17 ^{b}
	P4	28.13±1.67 ^{b}	5.72±1.22 ^{b}	3.10±0.25 ^{a}	1.49±0.35 ^{b}
	P8	27.04±1.80 ^{b}	5.26±0.64 ^{b}	3.16±0.40 ^{a}	1.82±0.23 ^{b}
	P20	22.07±0.87 ^{d}	3.83±0.67 ^{c}	2.87±0.27 ^{b}	1.51±0.20 ^{b}
	Control	24.27±2.31 ^{cd}	3.22±1.01 ^{c}	3.56±0.10 ^{a}	10.91±1.39 ^{a}

Storage temperature: 10°C
Mean values with different letters are significantly different ($p \leq 0.05$, n=3).
nd: not detected

5.3.3 Changes in sensory quality

The change and the significance of sensory quality after 0, 4 and 8 days was shown in Fig. 5.4. The F-values and *p*-values of ANOVA of every sensory parameter under every packaging condition between 0, 4 and 8 storage days were listed in Table 5.2. The factor of ANOVA was storage days (levels: 0, 4 and 8 days). From the data in Table 5.2, it could be known that the F-value of cap color in HOP was 2.94 which was lower than referenced $F_{0.05\,(2,\,6)}$ with 5.14, and the *p*-value was 0.1286 which was higher than 0.05. So it could be known that the result of cap color in HOP was not significantly different between 0, 4 and 8 days. But other F-values were higher than referenced $F_{0.05\,(2,\,6)}$ and *p*-values were lower than 0.05, which means other sensory parameters under various packaging conditions showed the significant differences between 0, 4 and 8 days. And from the multiple comparison results shown in Fig 5.4 it could be known that all the sensory parameters in HOP didn't have significant decrease between Day 0 and Day 4, while other packaging conditions showed the significant decrease between 0 and 4 days. Cap color in HOP did not change significantly between 0, 4 and 8 days. Meanwhile, overall visual quality, cap color and gill color decreased significantly in P4 and P8 after 4 days, and in P20, only aroma was not significantly different after 4 days. Shiitake mushrooms in macro perforation packaging (control) showed a significant decrease in all the sensory parameters. When prolonging the storage time from 4 to 8 days, cap color scores in HOP did not decrease significantly and other sensory parameters were also higher than under other packaging conditions. Aroma scores for mushrooms under all packaging conditions decreased during storage. After the storage, aroma score of samples was 4.48 in HOP and 4.30 in P8, which were higher than others, and the aroma score in P4 was only 3.37 at the end of the storage. Both the ethanol accumulation and deterioration of shiitake mushrooms induced the aroma decreasing. The decrease of aroma scores in P4 and HOP is mainly due

to the alcohol smell induced by anaerobic respiration. And the aroma score in HOP was higher than in P4, which was in accordance with the ethanol concentration result of 18 $\mu l \cdot l^{-1}$ in HOP and 31 $\mu l \cdot l^{-1}$ in P4. However, the decrease of aroma scores in P8 and P20 is associated with the deterioration smell but not the alcohol smell. So P20 and macro-perforated packages induced the severe deterioration of shiitake mushrooms with low aroma scores although the ethanol accumulation was not detected in them. Therefore, only the HOP shiitake mushrooms showed the lowest aroma deterioration, which was preferable to the others at the end of the storage. While severe texture losses occurred under all PM-MAP conditions, texture was maintained in HOP (score of 6.59 on the 8th day). Browning was assessed by scoring of the cap and gill. The images before and after the storage were shown in Fig. 5.5. Severe cap and gill browning was observed in P20 and macro perforation packages and the mushrooms were considered inedible. The gills showed much less integrity in P20 and macro perforation packaging, whereas minor damage occurred in samples under HOP.

The correlation between L^*, TSS, mass loss ratio and sensory parameters is shown in Table 5.3. Overall visual quality was highly correlated with texture, cap color and gill color. It was demonstrated that texture, cap color and gill color played more important roles when judging the sensory quality of shiitake mushrooms.

Table 5.2 ANOVA results (F-value and *p*-value) of the sensory parameters of shiitake mushrooms between storage days (0, 4 and 8)

F-value (df_1=2, df_2=6, $F_{0.05\ (2,\ 6)}$=5.14)					
	HOP	P4	P8	P20	Control
overall visual quality	21.49	166.90	236.34	92.75	61.88
Aroma	14.77	13.02	18.34	19.10	23.50
Texture	5.84	32.86	31.35	30.70	51.27
Cap color	2.94	140.01	167.62	83.65	56.63
Gill color	12.05	319.48	213.93	76.95	48.22
Gill color	18.66	14.09	6.50	31.88	52.96

p-value					
	HOP	P4	P8	P20	Control
overall visual quality	0.0018	0.0001	0.0001	0.0001	0.0001
Aroma	0.0048	0.0066	0.0028	0.0025	0.0015
Texture	0.0391	0.0006	0.0007	0.0007	0.0002
Cap color	0.1286	0.0001	0.0001	0.0001	0.0001
Gill color	0.0079	0.0001	0.0001	0.0001	0.0002
Gill color	0.0027	0.0054	0.0315	0.0006	0.0002

Storage temperature: 10°C
Factor of ANOVA: storage days (level: 0, 4 and 8 days)
$F_{0.05\ (2,\ 6)}$: referenced F-value under 0.05 significance level and degrees of freedom 2 and 6.

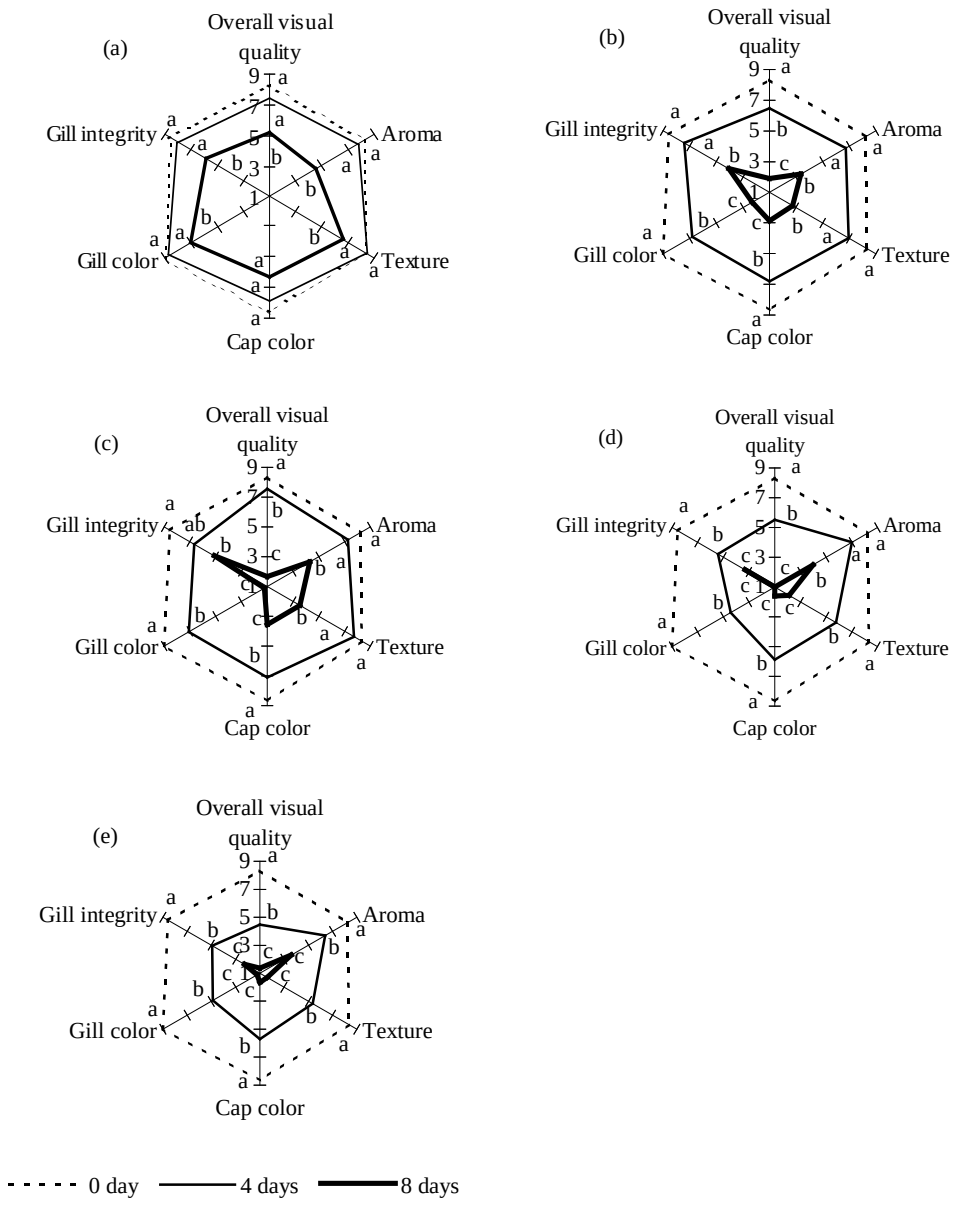
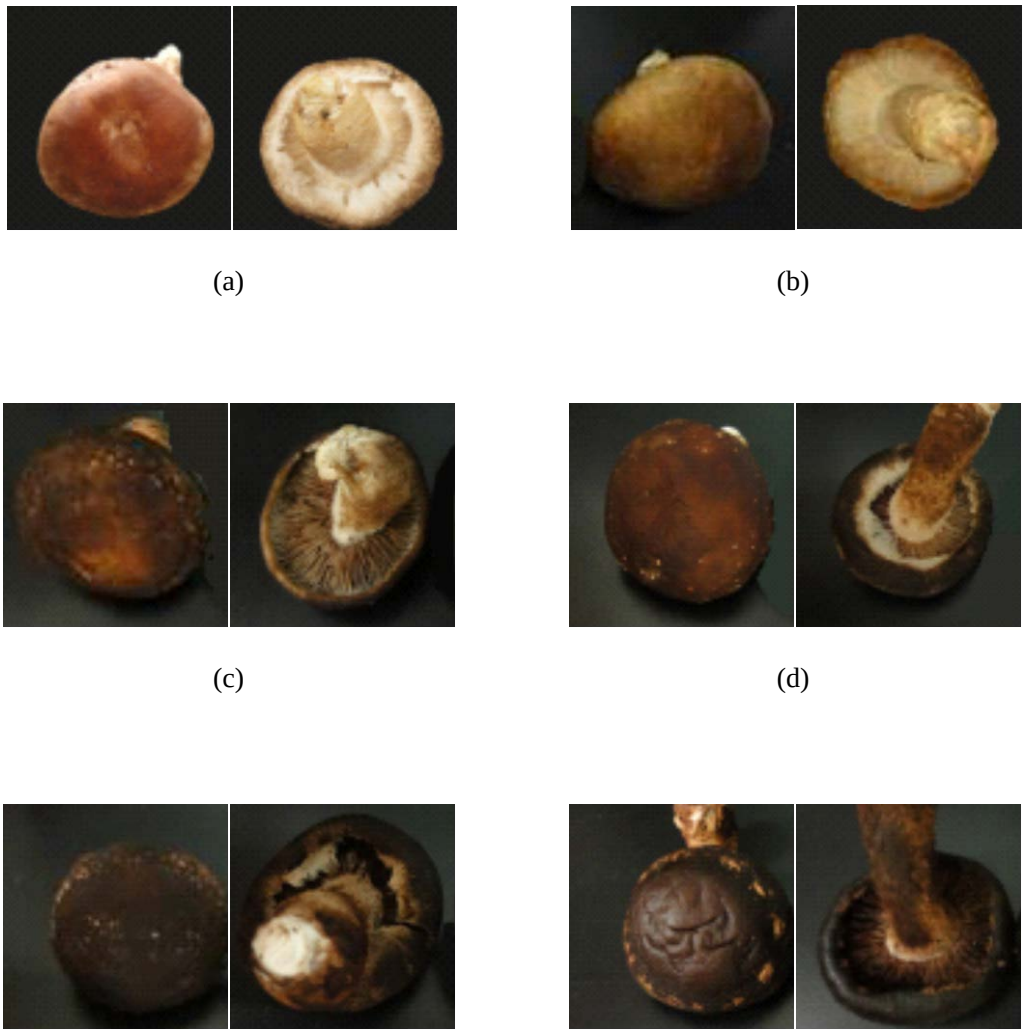


Fig. 5.4 Sensory evaluation of packaged fresh shiitake mushrooms stored at 10 °C. Vertical bars represent standard deviation (n=3). (a) HOP, (b) P4, (c) P8, (d) P20 and (e) Control. Mean values with different letters are significantly different ($p \leq 0.05$).



(a) (b)

(c) (d)

(e) (f)

Fig. 5.5 Images of shiitake mushrooms under different packaging conditions before and after the storage.
(a) Sample before storage, (b) HOP sample after storage, (c) P4 sample after storage, (d) P8 sample after storage, (e) P20 sample after storage, (f) Control sample after storage.

Table 5.3 Correlation (expressed by coefficient of correlation: r) between L-value (L^*), TSS, hardness, mass loss ratio, and sensory parameters of packaged shiitake mushrooms

	L^*	TSS	Hardness	Mass loss ratio	Overall visual quality	Aroma	Texture	Cap color	Gill color	Gill integrity
L^*	1.00									
TSS	0.55	1.00								
Hardness	0.93	0.76	1.00							
Mass loss ratio	-0.53	-0.35	-0.61	1.00						
Overall visual Quality	0.98	0.54	0.93	-0.57	1.00					
Aroma	0.96	0.53	0.87	-0.52	0.95	1.00				
Texture	0.97	0.47	0.92	-0.58	0.98	0.92	1.00			
Cap color	0.98	0.56	0.94	-0.6	0.98	0.94	0.99	1.00		
Gill color	0.96	0.57	0.95	-0.56	0.98	0.89	0.98	0.98	1.00	
Gill integrity	0.94	0.58	0.94	-0.7	0.94	0.91	0.96	0.96	0.94	1.00

Storage temperature: 10 °C

From the results above it could be known that fresh shiitake mushrooms have a very fast rate of respiration and are highly perishable. Mushrooms in HOP showed rapid oxygen consumption and carbon dioxide accumulation rate at 10 °C. The accelerated rate of respiration might be a result of the high oxygen environment, similar to the observations of Jacxsens et al. (2001) in sliced *A. bisporus* mushrooms at 4 °C and Kader and Ben-Yehoshua (Kader and Ben-Yehoshua 2000) in grapefruits under 14 °C. Ethanol concentration on the final day of storage was assessed, and it was demonstrated that a high barrier package with high oxygen atmosphere did not completely inhibit the accumulation of ethanol, although the ethanol concentration was lower than in P4 on the 8th day.

The observed L^* values and cap and gill color scores reflected that HOP samples maintained a superior color to PM-MAP, as reported in fresh *A. bisporus* mushrooms stored under a controlled high oxygen atmosphere (Liu et al. 2010). Severe browning of shiitake mushrooms occurred in all the perforation mediated packages, especially in P20 and macro perforation mediated packaging. While enzymatic browning inhibition has been observed in high oxygen barrier packaging material (Agudelo Laverde et al. 2011, Jiang and Fu 1999), in this study not only did PM-MAP not prevent browning, the severity of browning increased with the increase in perforations. Samples in P20 showed a severe discoloration and softening while the gas concentration did not show any obvious change during the storage. Samples in P4 got to anaerobic fermentation， as well as a significant color and hardness change, so they could not be accepted from Day 6. The total soluble solids (TSS) concentration of samples in PM-MAP with 20 perforations was significantly lower than in other packaging. The total soluble solids (TSS) concentration of samples in PM-MAP with 20 perforations was significantly lower than in other packaging conditions. This could be explained that the soluble solid content was highly consumed in the instant air atmosphere. Although the control treatment was also kept in the instant air atmosphere,

the high moisture loss induced the shiitake mushroom getting dry, and total soluble solid content value did not decrease dramatically. Both PM-MAP and HOP effectively maintained all sensory parameters until the 4th day; however, at the end of the storage period, only HOP was obviously able to maintain shiitake mushroom freshness. HOP samples were texturally intact; however, the high oxygen condition was unable to maintain the TSS concentration in fresh shiitake mushrooms at the end of the storage. The mass loss ratio result showed that both HOP and PM-MAP could reduce mass loss, which mainly occurs through moisture loss, more effectively than macro perforation packaging (control).

5.4 Conclusion

In this study, P4 and P8 created the low oxygen and high carbon dioxide atmospheres and effectively maintained shiitake mushrooms quality within 4 days at 10 °C. However, with extended storage, severe anaerobic atmosphere with a high ethanol concentration happened in P4 and obvious browning happened in P8. So P4 and P8 did not show the effect on keeping freshness of shiitake mushrooms at the end of the storage. However, HOP with 100 % initial O_2 concentration maintained a significantly higher L^*, hardness and sensory quality than PM-MAP. Therefore, it could be concluded that HOP showed the potential to maintain the quality of shiitake mushrooms when prolonging the storage period more than 8 days.

Future study will focus on the effect of various packaging materials on alterations in fermentation aroma and shiitake mushroom quality. Furthermore, the effect of a high oxygen environment on nutrients and enzyme activity in shiitake mushrooms, as well as any detrimental effects of varying oxygen concentrations, will be assessed.

CHAPTER 6

Overall Conclusions

Modified atmosphere packaging (MAP) has been researched on many product. The respiration rate of fresh fruits and vegetables could be inhibited and the quality could be maintained by modifying the inner gas atmosphere. Temperature as a critical factor to affect the respiration rate of fresh produce needs to be taken into consideration when designing the MAP. Shiitake mushroom as a produce with affluent nutrients and typical taste is very perishable and easy to get brown. MAP on fresh shiitake mushrooms isn't widely researched and applied, which requires further and deeper research to obtain the optimum packaging materials and gas compositions. Therefore, in order to clarify the effect of gas composition on respiring product, the change of physiology and nutrient components, and to prolong the shelf life. Based on these backgrounds, the experiments were conducted as follows: (1) Analysis the commercial packages of 7 vegetables (Shiitake mushroom, Maitake mushroom, Enokitake mushroom, Long bean, Green hot pepper, Edamame and Baby leaf) in present supermarket in Japan. According to the comparison of package transmission rate and respiration rate of vegetables, the applicability of the package films of these vegetables was analyzed and the suggestive improvement for the improper packages was also proposed. (2) Determination of the physiology change, polysaccharide, total phenolics and free amino acid content of shiitake mushrooms packaged in various atmospheres with different oxygen levels. (3) Analysis of respiration rate and sensorial qualities change under high oxygen modified atmosphere packaging with 80 % O_2 and 100 % O_2. (4) Comparison of the effect of high-oxygen packaging and perforation mediated atmosphere packaging on the qualities of fresh shiitake mushrooms. The overall conclusions could be depicted as follows:

1) In chapter 1, the general information of modified atmosphere packaging and the physiology, nutrients and storage characteristics after postharvest of shiitake mushrooms were introduced. In addition, researches about the modified atmosphere packaging application on some fruits and vegetables, as well as the storage and packaging methods of fresh shiitake mushrooms were reviewed.

2) In chapter 2, seven selected vegetables with various package types were investigated, and the effect of temperature on O_2 consumption rate (RO_2), CO_2 production rates (RCO_2) and respiratory quotient (RQ) of seven selected vegetables were also studied. Temperature greatly influenced the O_2 consumption rate (RO_2) of Shiitake mushroom, Enokitake mushroom and Green hot pepper, and significantly influenced CO_2 production rates (RCO_2) of all these seven kinds of vegetables. It was also found that RQ was dependent on the temperature, and it became high as the temperature increasing from 10 to 20°C. And the gas transmission rate change of all the films with reciprocal of Kelvin temperature well fitted the exponential regularity. Among the packages of 7 vegetables, in-package oxygen concentrations were extremely low of Shiitake, Maitake and Enokitake mushrooms, for the reason of high oxygen consumption by respiration and low oxygen transmission from film. The suggestive solution is to improve the film gas transmission rate or increase the initial oxygen concentration. Decreasing the mass of produce is not suggested because of the waste of package materials. For Long bean, Green hot pepper and Baby leaf, the MA atmosphere was not created for the too high oxygen concentration inside the packages, and the respiration won't be inhibited, and the suggestions for these three vegetables are to select the package film with lower gas permeability or increase the mass of being packaged produce. For Edamame, perforation-mediated method was suggested, but the number of perforations should be decreased.

3) In chapter 3, active modified atmosphere packaging with different initial O_2

concentrations (HOP, MOP, LOP and AIR) were applied on the storage of postharvest shiitake mushrooms. LOP with the initial 3 % O_2 and AIR treatment induced the anaerobic fermentation immediately after packaging and the high accumulation of ethanol concentration during the storage. Ethanol release was well retarded in MOA with 50 % O_2 initially and HOP with 100 % O_2 initially. Shiitake mushrooms' tissue was affected significantly by LOP for a high value of electrolyte leakage after the storage and a consequent high total phenolic content. No packaging could prevent the polysaccharide content decreasing in this research. And the various packaging methods did not show the big difference of decrease of polysaccharide content. The active modified atmosphere packaging had the significant effect on the increase of free amino acid content increased after storage under all the packaging conditions. Therefore, all the active modified atmosphere packaging showed the advantageous effect on the free amino acid increase, but the LOP had the detrimental effect on shiitake mushrooms.

4) In chapter 4, high oxygen atmosphere with 100 % O_2 and 80% O_2 were applied on preserving shiitake mushrooms with polyethylene packages. The result showed that both 100 % O_2 and 80 % O_2 had beneficial effect on the sensorial quality of fresh shiitake mushrooms by retarding the anaerobic metabolism occurrence and contributing the better aroma scores compared with air packaging. HOP can not reduce the respiration rate or prevent the fermentation metabolism of shiitake mushrooms completely, but HOP with initial 100 % O_2 could retard the ethanol and acetaldehyde accumulation. Mushroom's hardness, color parameters of L^* and a^* got significant higher values in HOP than in air packaging after the storage, but TSS content did not show any significant difference between treatments. Aroma played an important role when judging the sensorial quality of fresh shiitake mushrooms, and aroma scores in HOP samples were significantly higher than AIR samples. During the storage, samples stored under HOP condition especially

100% O_2 atmosphere, showed a lower deterioration rate and higher acceptable quality than those stored under air condition.

5) In chapter 5, the change in qualities using high oxygen packaging (HOP), with an initial 100 % O_2 concentration, was investigated at 10 °C and 90% RH and compared to perforation-mediated modified atmosphere packaging (PM-MAP) with 4 (P4), 8 (P8) and 20 (P20) perforations (Diameter = 234 ± 26 μm). Gas composition, color, hardness, TSS concentration, mass loss and sensory evaluation were determined during the 8 days' storage. The result showed that P4 and P8 created the low oxygen and high carbon dioxide atmospheres and effectively maintained shiitake mushrooms quality within 4 days at 10 °C. However, with extended storage, severe fermentation atmosphere with a high ethanol concentration happened in P4 and obvious browning happened in P8. So P4 and P8 did not show the effect on keeping freshness of shiitake mushrooms at the end of the storage. However, HOP with 100 % initial O_2 concentration maintained a significantly higher L^*, hardness and sensory quality than PM-MAP. Therefore, it could be concluded that HOP showed the potential to maintain the quality of shiitake mushrooms when prolonging the storage period more than 8 days.

In this study, the respiration rates of some selected vegetables' dependence on temperature were researched and the package film transmission rate change with temperature also was studied, which are critical for MAP designing. And the modified atmosphere packaging with different oxygen concentrations initially was also applied on fresh shiitake mushrooms. In addition, the MAP with high oxygen concentration was researched to preserve the fresh shiitake mushrooms compared with the perforation-mediated modified atmosphere packaging, and the high oxygen packaging indicated the better effect to alleviate browning and retard the anaerobic respiration. The results showed the potential of research on the modeling of respiration rate and enzyme

activity change during the physiology change under different packaging conditions. Therefore, in the further study, some researches should be developed with more details. Firstly, the modeling of effect of O_2 and CO_2 concentration on the respiration rates of selected vegetables with the storage period prolonged should be researched in the future study. In this study, only the effect of temperature on the respiration of some vegetables was studied. In the subsequent study, the effect of O_2 and CO_2 concentration, as well as the storage period on respiration rate should also be focused and modeled, which will provide more information to find the optimum conditions for MAP designing. Secondly, the further study should also continue to research on the enzyme activity change during the storage under different packaging conditions. In this study, high oxygen packaging (HOP) was found to be effective inhibit the browning of shiitake mushroom, which may be induced by the polyphenol oxidase (PPO). So the further study should also focus on the PPO activity change under different packaging conditions. Thirdly, the further study should also consider about the effect of packaging film with different permeabilities on the shiitake mushroom’s quality.

REFERENCES

Alasalvar, C., M. Al-Farsi, P. C. Quantick, F. Shahidi & R. Wiktorowicz (2005) Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots, *Food Chemistry*, 89, 69-76.

Amanatidou, A., R. A. Slump, L. G. M. Gorris & E. J. Smid (2000) High Oxygen and High Carbon Dioxide Modified Atmospheres for Shelf-life Extension of Minimally Processed Carrots, *Journal of Food Science*, 65, 61-66.

Antmann, G., G. Ares, P. Lema & C. Lareo (2008) Influence of modified atmosphere packaging on sensory quality of shiitake mushrooms, *Postharvest Biology and Technology*, 49, 164-170.

Ares, G., C. Parentelli, A. Gámbaro, C. Lareo & P. Lema (2006) Sensory shelf life of shiitake mushrooms stored under passive modified atmosphere, *Postharvest Biology and Technology*, 41, 191-197.

Beaudry, R. M., A. C. Cameron, A. Shirazi & D. L. Dostal-Lange (1992) Modified-atmosphere Packaging of Blueberry Fruit: Effect of Temperature on Package O_2 and CO_2, *Journal of the American Society for Horticultural Science*, 117, 436-441.

Beluhan, S. & A. Ranogajec (2011) Chemical composition and non-volatile components of Croatian wild edible mushrooms, *Food Chemistry*, 124, 1076-1082.

Bower, J. H., J. J. Jobling, B. D. Patterson & D. J. Ryan (1998) A method for measuring the respiration rate and respiratory quotient of detached plant tissues, *Postharvest Biology and Technology*, 13, 263-270.

ÇaglarIrmak, N. (2007) The nutrients of exotic mushrooms (*Lentinula edodes* and *Pleurotus* species) and an estimated approach to the volatile compounds, *Food*

Chemistry, 105, 1188-1194.

Caleb, O. J., P. V. Mahajan, U. L. Opara & C. R. Witthuhn (2012) Modelling the respiration rates of pomegranate fruit and arils, *Postharvest Biology and Technology*, 64, 49-54.

Charles, F., C. Guillaume & N. Gontard (2008) Effect of passive and active modified atmosphere packaging on quality changes of fresh endives, *Postharvest Biology and Technology*, 48, 22-29.

Cliffe-Byrnes, V., C. P. Mc Laughlin & D. O'Beirne (2003) The effects of packaging film and storage temperature on the quality of a dry coleslaw mix packaged in a modified atmosphere, *International Journal of Food Science & Technology*, 38,187-199.

Costa, C., A. Lucera, A. Conte, M. Mastromatteo, B. Speranza, A. Antonacci & M. A. Del Nobile (2011) Effects of passive and active modified atmosphere packaging conditions on ready-to-eat table grape. *Journal of Food Engineering*, 102, 115-121.

Dubost, N. J., B. Ou & R. B. Beelman (2007) Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity, *Food Chemistry*, 105, 727-735.

Exama, A., J. Arul, R. W. Lencki, L. Z. Lee & C. Toupin (1993) Suitability of Plastic Films for Modified Atmosphere Packaging of Fruits and Vegetables, *Journal of Food Science*, 58, 1365-1370.

Fonseca, S. C., F. A. R. Oliveira & J. K. Brecht (2002a) Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review, *Journal of Food Engineering*, 52, 99-119.

Fonseca, S. C., F. A. R. Oliveira, J. M. Frias, J. K. Brecht & K. V. Chau (2002b) Modelling respiration rate of shredded Galega kale for development of modified atmosphere

packaging, *Journal of Food Engineering*, 54, 299-307.

Francis M, M. (1996) Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide, *Postharvest Biology and Technology*, 9, 247-264.

Gómez, P. A. & F. Artés (2005) Improved keeping quality of minimally fresh processed celery sticks by modified atmosphere packaging, *LWT - Food Science and Technology*, 38, 323-329.

González, J., A. Ferrer, R. Oria & M. L. Salvador (2008) Determination of O_2 and CO_2 transmission rates through microperforated films for modified atmosphere packaging of fresh fruits and vegetables, *Journal of Food Engineering*, 86, 194-201.

Han, X.Q., X.M. Wu, X.Y. Chai, D. Chen, H. Dai, H.L. Dong, Z.Z. Ma, X.M. Gao & P.F. Tu (2011) Isolation, characterization and immunological activity of a polysaccharide from the fruit bodies of an edible mushroom, Sarcodon aspratus (Berk.) S. Ito, *Food Research International*, 44, 489-493.

Hobbs, C. (2000) Medicinal Value of (*Lentinus edodes*) Sing. (*Agaricomycetideae*), A Literature Review. 2, 16.

Horev, B., S. Sela, Y. Vinokur, E. Gorbatsevich, R. Pinto & V. Rodov (2012) The effects of active and passive modified atmosphere packaging on the survival of Salmonella enterica serotype Typhimurium on washed romaine lettuce leaves, *Food Research International*, 45, 1129-1132.

Iqbal, T., F. A. S. Rodrigues, P. V. Mahajan & J. P. Kerry (2009a) - Mathematical modelling of O_2 consumption and CO_2 production rates of whole mushrooms accounting for the effect of temperature and gas composition, 44, 1414.

Iqbal, T., F. A. S. Rodrigues, P. V. Mahajan & J. P. Kerry (2009b) Mathematical modelling of O_2 consumption and CO_2 production rates of whole mushrooms accounting for

the effect of temperature and gas composition, *International Journal of Food Science & Technology*, 44, 1408-1414.

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

Jiang, T., L. Feng & J. Li (2012) Changes in microbial and postharvest quality of shiitake mushroom (*Lentinus edodes*) treated with chitosan–glucose complex coating under cold storage, *Food Chemistry*, 131, 780-786.

Jiang, T., S. Luo, Q. Chen, L. Shen & T. Ying (2010a) Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*), *Food Chemistry*, 122, 761-767.

Jiang, T., Q. Wang, S. Xu, M. M. Jahangir & T. Ying (2010b) Structure and composition changes in the cell wall in relation to texture of shiitake mushrooms (*Lentinula edodes*) stored in modified atmosphere packaging, *Journal of the Science of Food and Agriculture*, 90, 742-749.

Jiang, Y., T. Shiina, N. Nakamura & A. Nakahara (2001) Electrical Conductivity Evaluation of Postharvest Strawberry Damage, *Journal of Food Science*, 66, 1392-1395.

Joles, D. W., A. C. Cameron, A. Shirazi, P. D. Petracek & R. M. Beaudry (1994) Modified-atmosphere Packaging of `Heritage' Red Raspberry Fruit: Respiratory Response to Reduced Oxygen, Enhanced Carbon Dioxide, and Temperature, *Journal of the American Society for Horticultural Science*, 119, 40-545.

Kader, A. A. & S. Ben-Yehoshua (2000) Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables, *Postharvest*

Biology and Technology, 20, 1-13.

Kader, A. A., D. Zagory, E. L. Kerbel & C. Y. Wang (1989) Modified atmosphere packaging of fruits and vegetables, *Critical Reviews in Food Science and Nutrition*, 28, 1-30.

Kim, M.Y., I.M. Chung, S.J. Lee, J.K. Ahn, E.H. Kim, M.J. Kim, S.L. Kim, H.I. Moon, H.M. Ro, E.Y. Kang, S.H. Seo & H.K. Song (2009) Comparison of free amino acid, carbohydrates concentrations in Korean edible and medicinal mushrooms, *Food Chemistry*, 113, 386-393.

Lee, L., J. Arul, R. Lencki & F. Castaigne (1996) A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: Physiological basis and practical aspects—part II, *Packaging Technology and Science*, 9, 1-17.

Liu, Z., X. Wang, J. Zhu & J. Wang (2010) Effect of high oxygen modified atmosphere on post-harvest physiology and sensorial qualities of mushroom, *International Journal of Food Science & Technology*, 45, 1097-1103.

Mahajan, P. V., F. A. R. Oliveira, J. C. Montanez & J. Frias (2007) Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce, *Innovative Food Science & Emerging Technologies*, 8, 84-92.

Manolopoulou, H., G. Xanthopoulos, N. Douros & G. Lambrinos (2010) Modified atmosphere packaging storage of green bell peppers: Quality criteria, *Biosystems Engineering*, 106, 535-543.

Marangoni, A. G., T. Palma & D. W. Stanley (1996) Membrane effects in postharvest physiology, *Postharvest Biology and Technology*, 7, 193-217.

McLaughlin, C. P. & D. O'Beirne (1999) Respiration Rate of a Dry Coleslaw Mix Affected by Storage Temperature and Respiratory Gas Concentrations. *Journal of Food Science*, 64**,** 116-119.

Mizuno, T. (1995) Shiitake, *lentinus edodes*: Functional properties for medicinal and food purposes, *Food Reviews International*, 11, 109-128.

Oms-Oliu, G., R. Soliva-Fortuny & O. Martín-Belloso (2008) 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 Biology and Technology*, 48, 295-301.

Ozdemir, I., F. Monnet & B. Gouble (2005) Simple determination of the O_2 and CO_2 permeances of microperforated pouches for modified atmosphere packaging of respiring foods, *Postharvest Biology and Technology*, 36, 209-213.

Pérez, A. G. & C. Sanz (2001) Effect of High-Oxygen and High-Carbon-Dioxide Atmospheres on Strawberry Flavor and Other Quality Traits, *Journal of Agricultural and Food Chemistry*, 49, 2370-2375.

Parentelli, C., G. Ares, M. Corona, C. Lareo, A. Gámbaro, M. Soubes & P. Lema (2007) Sensory and microbiological quality of shiitake mushrooms in modified-atmosphere packages, *Journal of the Science of Food and Agriculture*, 87, 1645-1652.

Rennie, T. J. & S. Tavoularis (2009) Perforation-mediated modified atmosphere packaging: Part I. Development of a mathematical model, *Postharvest Biology and Technology*, 51, 1-9.

Sandhya (2010) Modified atmosphere packaging of fresh produce: Current status and future needs, *LWT - Food Science and Technology*, 43, 381-392.

Simón, A., E. González-Fandos & V. Tobar (2005) The sensory and microbiological quality of fresh sliced mushroom (*Agaricus bisporus*) packaged in modified atmospheres, *International Journal of Food Science & Technology*, 40, 943-952.

Tseng, Y.H. & J.L. Mau (1999) Contents of sugars, free amino acids and free 5′-nucleotides

in mushrooms, *Agaricus bisporus*, during post-harvest storage, *Journal of the Science of Food and Agriculture*, 79, 1519-1523.

Van der Steen, C., L. Jacxsens, F. Devlieghere & J. Debevere (2002) Combining high oxygen atmospheres with low oxygen modified atmosphere packaging to improve the keeping quality of strawberries and raspberries, *Postharvest Biology and Technology*, 26, 49-58.

Villaescusa, R. & M. I. Gil (2003) Quality improvement of Pleurotus mushrooms by modified atmosphere packaging and moisture absorbers, *Postharvest Biology and Technology*, 28, 169-179.

XuJie, H. & C. Wei (2008) Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology, *Carbohydrate Polymers*, 72, 67-74.

Zhang, M., S. W. Cui, P. C. K. Cheung & Q. Wang (2007) Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity, *Trends in Food Science & Technology*, 18, 4-19.

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