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Modified Atmosphere Packaging for Postharvest Storage of Mushrooms. A Review

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ABSTRACT

Production and consumption of edible mushrooms have grown continuously in the last fifteen years, particularly due to interest in their nutritional and health benefits. The three most cultivated mushrooms worldwide are *Agaricus bisporus* (common mushrooms), *Lentinus edodes* (shiitake) and *Pleurotus* spp. (oyster mushroom). Mushrooms are highly perishable. They tend to lose quality right after harvest, mainly because of their high respiration rate and the fact that they have no barrier to protect them from water loss. Mushrooms' shelf-life is limited to a few days under normal refrigeration conditions, which is a constraint on the distribution and marketing of fresh product, making extension of mushroom's shelf life a constant quest. Modified atmosphere packaging has been used for postharvest storage and commercialization of mushrooms. This technology alters the normal composition of air to provide an appropriate atmosphere in order to decrease product's respiration rate, microbial growth and physiological disorders, which leads to preservation of product's quality and an increased shelf life. However, modified atmosphere packaging conditions should be carefully designed. Inappropriate modified atmosphere conditions may be ineffective or even shorten the shelf life of the product due to damage of tissues. Reported results for modified atmosphere packaging of mushrooms depend on the mushroom species considered. In this paper, the use of modified atmosphere packaging for mushrooms, specifically for *Agaricus*, *Lentinus edodes* and *Pleurotus*, is reviewed.

Keywords: *Agaricus bisporus*, *Lentinus edodes*, minimally processed mushrooms, *Pleurotus* spp., shiitake

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INTRODUCTION

According to Chang and Miles (1992) mushrooms can be described as 'macrofungus with a distinctive fruiting body, large enough to be seen with the naked eye and to be picked by hand'. These visible structures are generically referred to as "fruiting bodies". From a taxonomic point of view, mainly Basidiomycetes but also some species of Ascomycetes belong to mushrooms (Lindequist *et al*. 2005). Mushrooms constitute at least 14,000 species, of which approximately 2,000 are edible (Chang 1999a).

Edible mushrooms have been collected and consumed by people for thousands of years. Archaeological record reveals edible species associated with people living 13,000 years ago in Chile, but it was in China where consumption of wild fungi was first reliably noted several hundred years A.C. (Boa 2004). However, their production and consumption have grown continuously in the last fifteen years (Royse 2001; Boa 2004). Total commercial mushroom production worldwide has increased more than 21 times in 35 years, from about 350,000 tons in 1965 to about 7.5 million tons in 2000 (Boa 2004). The world production value of edible mushrooms in 2001 was estimated at around US$ 23 billion, which exceeds the value of many agricultural products (Chang 1999b). Out of 14,000 known mushroom species, the most consumed edible mushrooms are *Agaricus bisporus* (white button or common mushroom), *Lentinus edodes* (shiitake mushroom), *Pleurotus* spp. (oyster mushroom), *Volvarella volvacea* (paddy straw mushroom), *Flammulna velutipis* (winter mushroom) and *Auricularia polytricha* (Jew's ear mushroom) (Chang 1999b; Singh *et al*. 1999; Boa 2004). In 1997 the most cultivated edible mushroom was *Agaricus bisporus*, comprising 32.8% of the worldwide mushroom production, followed by *Lentinus edodes* with 25.4% and *Pleurotus* spp. with 14.2% (Chang 1999b; Boa 2004).

Received: 28 February, 2007. Accepted: 9 April, 2007.

Invited Review

Considering the increased interest of consumers in health and well being, the demand and markets for edible mushrooms might increase if they are promoted as functional foods. A functional food can be considered as a food that has value beyond its nutritional value because it promotes specific aspects of human health. In this context, many health benefits have been ascribed to mushrooms and compounds isolated from them, including bioactive polysaccharides (β-glucans, such as lentinan), antioxidants, dietary fiber, ergosterol, vitamin B1, B2 and C, folates, niacin, and minerals (Mattila *et al.* 2000; Lindequist *et al.* 2005). Concentration of these substances varied with mushrooms species considered. According to Manzi and Pizzoferrato (2000) β-glucan concentration in *Agaricus bisporus*, *Pleurotus* spp. and *Lentinus edodes* ranges from 0.21 to 0.53 g/100 g on a dry basis. Lentinan is a β-glucan present in shiitake mushrooms in concentrations of approximately 3.4 mg lentinan g^{-1} (fresh weight) (Mizuno *et al*. 1996), which has been reported to show antitumour activity (Ikekawa *et al.* 1969). Moreover, shiitake mushrooms have been reported to be a good source of dietary fiber (3.3 g/100 g fresh weight); while *Agaricus bisporus* and *Pleurotus* spp. contained 1.5-2.4 g/100 g fresh weight (Mattila *et al.* 2002). Edible mushrooms are good sources of vitamin B2, niacin, and folates, with contents varying in the ranges 1.8-5.1, 31-65, and 0.30-0.64 mg/100 g dry weight (dw) respectively (Mattila *et al.* 2001). According to Mattila *et al.* (2001) vitamin C content in mushrooms vary from 17 mg/100 g dw for *A. bisporus* to 25 mg/100 g dw in *L. edodes*. The contents of vitamin B1 (thiamin) in mushrooms (0.6-0.9 mg/100 g dw) are similar to those generally found in vegetables (Mattila *et al.* 2001). Compared with vegetables, mushrooms are a good source of many mineral elements. The contents of K, P, Zn, and Cu varied in the ranges 26.7-47.3 g/kg, 8.7-13.9 g/kg, 47-92 mg/kg, and 5.2-35 mg/kg dw, respectively. *Agaricus bisporus* contain large amounts of Se (3.2 mg/kg dw), while Cd content in shiitake mushrooms in quite high (1.2 mg/kg dw) (Mattila *et al.* 2001). Numerous studies have reported mushrooms having medicinal attributes including anti-tumor (Ikekawa *et al*. 1969; Suzuki *et al.* 1994; Mizuno 1999; Cheung 2003; Lindequist *et al*. 2005; Choi *et al*. 2006); antimicrobial (Lindequist *et al*. 2005); liver function improving (Lindequist *et al*. 2005); enhancement of macrophage function and host resistance to many bacterial, viral, fungal and parasitic infections (Lindequist *et al.* 2005); activation of a non-specific immune stimulation (Yoshino *et al.* 2000; Lindequist *et al.* 2005); and reduction of blood cholesterol and blood glucose levels (Fukushima *et al*. 2001; Lindequist *et al*. 2005). *In vitro* studies, *in vivo* studies in mice as well as clinical trials have been carried out to evaluate antitumour activity of mushrooms (Zhang *et al.* 2001; Hashimoto *et al.* 2002; Lindequist *et al.* 2005; Surenjav *et al.* 2006). In these studies mushrooms extracts have proved to have antitumour activity against sarcoma, carcinoma MM-46, carcinoma IMC, colon cancer, colorectal cancer, and stomach cancer (Zhang *et al.* 2001; Hashimoto *et al.* 2002; Lindequist *et al.* 2005). Moreover, mushrooms extracts have been reported to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) in human cells *in vitro* (Jong and Birmingham 1993; Hatvani 2001; Lindequist *et al.* 2005). Antimicrobial effect of mushrooms extracts has been studied against methicillin-resistant *Staphylococcus aureus* and microorganisms responsible for skin problems (*Pityrosporum ovale*, *Staphylococcus epidermidis*, *Paramecium caudatum*) (Lindequist *et al.* 2005).

For these reasons, mushrooms appear as an attractive food product with an interesting potential market.

Moreover, fresh vegetables have become highly desirable foods because consumers perceive them as healthy, tasty, convenient, and fresh. The fresh fruit and vegetable industry has experienced an important growth over the past 10 years as illustrated by increasing consumption and sales data and increasing space devoted to these products in supermarkets and on restaurant menus (Garret *et al*. 2003). Worldwide per capita annual consumption of fresh produce increased from 129 kg in 1987 to 145 kg in 1997, whereas total fresh produce market reached U$S 70.8 billion in retail and foodservice sales in 1997, compared to U$S 34.6 billion in 1987 (Garret *et al*. 2003).

Mushrooms are highly perishable and start deteriorating immediately within a day after harvest (Burton *et al*. 1987; López-Briones *et al*. 1992, 1993; Roy *et al*. 1996; Tano *et al*. 1999), causing difficulties in their distribution and marketing as fresh produce. For this reason extension of postharvest storage is a constant quest. One alternative to extend mushrooms' shelf life during postharvest storage and commercialization is modified atmosphere packaging and cold storage.

The purpose of the present paper is to review the use of modified atmosphere packaging to fresh mushroom preservation, specifically for *Agaricus bisporus*, *Lentinus edodes*, and *Pleurotus* spp.

MUSHROOM DETERIORATION

Mushrooms are composed of densely packed fine threads known as hyphae, which together form a mycelium. Specialized hyphae, called sporophores, produce spores that are dispersed in a number of ways (Boa 2004). A mature hypha forms fructifications which, in most cases, protrude from the surface of the substratum. Mushroom fructifications are composed of two basic parts: the pileus and the stipe (or cap), which can take various shapes, size and colour (Boa 2004). **Fig. 1** shows the basic parts of a mushroom.

Fresh mushrooms' shelf life is limited to 1-3 days at ambient temperature, and to 4-7 days at 4ºC (Burton and Twyning 1989; López-Briones *et al*. 1992, 1993; Villaescusa and Gil 2003). The main processes responsible for mushrooms' sensory quality loss are browning and texture changes (López-Briones *et al*. 1992, 1993; Varoquaux *et al*. 1999; Villaescusa and Gil 2003; Ares *et al*. 2006). Mushrooms' quick deterioration is mainly caused by their high metabolic activity, respiration rate and dehydration (Burton and Noble 1993; Braaksma *et al*. 1994; Burton *et al*. 1995; Jolivet *et al*. 1995).

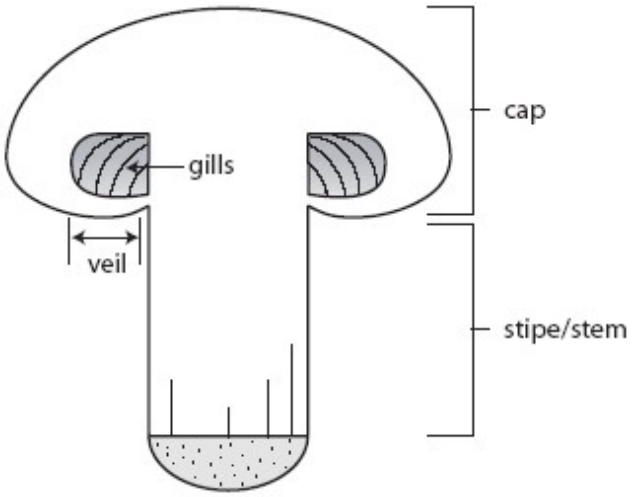

Fig. 1 Basic parts of a mushroom (redrawn from Hill 2003).

Respiration rate

Fresh fruits, vegetables and mushrooms are metabolically active for long periods after harvesting. Respiration is a metabolic process that provides the energy for biochemical processes. Aerobic respiration consists of oxidative breakdown of organic reserves (mainly carbohydrates, lipids, and organic acids) to simpler molecules, including CO_2 and water, with release of energy; consuming O_2 in a series of enzymatic reactions (Fonseca *et al*. 2002). Taking into account that respiration determines the demand of organic

resources, respiration rate can be regarded as a measure of the metabolic activity of a fresh fruit or vegetable (Fonseca *et al*. 2002). In the case of mushrooms, the harvested sporophore of the cultivated mushroom undergoes a course of development and senescence very similar to that of the growing fruit body (Hammond and Nichols 1975). However, substrates of mycelial origin are no longer available for cut sporophore which is therefore supported only by organic reserves (Banner *et al*. 1956). Hence, respiration rate is important in determining deterioration rate and onset of senescence in cultivated mushroom. Therefore, respiration rate is proportional to product deterioration rate and inversely proportional to its shelf life (Farber *et al*. 2003).

Respiration rate of fresh produce is usually expressed as O_2 consumption rate or CO_2 production rate. The ratio of CO_2 produced to O_2 consumed, known as the respiratory quotient (RQ), is indicative of the type of metabolism in the produce. An RQ near to 1.0 is indicative of aerobic respiration, while a RQ greater than 1.0 might indicate anaerobic metabolism (Fonseca *et al*. 2002).

Mushroom respiration rate has been determined using closed systems (López-Briones *et al*. 1993; Varoquaux *et al*. 1999; Villaescusa and Gil 2003; Ares *et al*. 2006; Parentelli *et al*. 2007). Mushrooms are placed in closed gastight containers and stored in a temperature controlled room. Atmosphere within the jars is modified as a consequence of mushrooms respiration rate. Therefore, atmosphere composition is periodically determined and respiration rate is calculated considering the change in O_2 and CO_2 concentration with storage time.

Mushrooms show a high respiration rate compared to other fruits and vegetables (Farber *et al*. 2003; Fonseca *et al*. 2002). Respiration rate of fresh mushrooms under air at 10ºC ranges from 17.8 to 178 CO_2 kg^{-1} s^{-1}, depending on mushroom species considered (López-Briones *et al*. 1993; Varoquaux *et al*. 1999; Villaescusa and Gil 2003; Ares *et al*. 2006; Parentelli *et al*. 2007).

Varoquaux *et al*. (1999) reported an oxygen consumption rate of 17.8 μg O_2 kg^{-1} s^{-1} for *Agaricus bisporus* under air at 10ºC, decreasing to 3.7 μg O_2 kg^{-1} s^{-1} at 0ºC. According to these authors respiration rate increased 2.9 times when increasing 10ºC storage temperature in the range comprised between 0 and 10ºC. They found that respiration rate was unaffected by atmosphere composition in the ranges between 0-10 kPa CO_2 and 1-20 kPa O_2 at 5 to 20ºC. In these atmosphere compositions RQ remained constant at values near to 1.0, even at 1 kPa, indicating that mushrooms metabolism was likely to be aerobic even at low O_2 partial pressures.

Very few studies have been found reporting respiration rate measure of *Pleurotus* spp. and *Lentinus edodes* (shiitake). Villaescusa and Gil (2003) reported a CO_2 production rate of 20.0 μgCO_2 kg^{-1} s^{-1}, 39.6 mgCO_2 kg^{-1} s^{-1} and 49.2 μgCO_2 kg^{-1} s^{-1} for *Pleurotus ostreatus* stored under air at 0, 4 and 7ºC respectively. Furthermore, shiitake mushrooms seemed to have a high respiration rate compared to other mushrooms species, making them particularly perishable. According to Ares *et al*. (2006) and Parentelli *et al*. (2007) respiration rate of shiitake mushrooms under air at 10ºC was 178 μgCO_2 kg^{-1} s^{-1}, nearly 10 times higher than for *Agaricus bisporus* at 10ºC (Varoquax *et al*. 1999) or for *Pleurotus* at 0ºC (Villaescusa and Gil 2003).

These results suggest that mushroom respiration rate could not be generalized and that respiration rate measurements for each mushroom species are necessary.

Dehydration

Mushrooms are only protected by a thin and porous epidermal structure, lacking the specialized epidermal structure of higher plant tissues. This epidermal layer does not avoid a quick superficial dehydration that causes important quality losses (Singer 1986). San Antonio and Flegg (1964) reported that water loss from growing mushrooms was comparable to that from a free water surface. Since 90% of mushrooms weight at harvest, is water, Nichols (1985) hypothesized that freshly harvested mushroom transpires at the same rate as the fruiting sporophore. Dehydration causes economic losses to mushrooms' producers and also influences their deterioration rate.

Browning

Mushroom browning is an important cause of loss of quality during postharvest storage (López-Briones *et al*. 1993; Braaksma *et al*. 1994; Villaescusa and Gil 2003; Ares *et al*. 2006; Parentelli *et al*. 2007). Browning occurs as a result of two distinct mechanisms of phenol oxidation: activation of tyrosinase, an enzyme belonging to the polyphenoloxidase family; and spontaneous oxidation (Jolivet *et al*. 1998). Tyrosinase oxidizes some monophenols to *o*-diphenols and then the former are oxidized to quinines, which spontaneously polymerize to form brown, black or red pigments (Jolivet *et al*. 1998; Nerya *et al*. 2006). As a result of senescence, cell membranes are disrupted and compartmentalization is lost, allowing enzymes and substrates to mix, accelerating browning (Jolivet *et al*. 1998).

Texture changes

Texture is an important quality parameter for fresh mushrooms (López-Briones *et al*. 1992). One of the main changes associated with mushrooms deterioration are changes in their texture (López-Briones *et al*. 1992; Villaescusa and Gil 2003; Ares *et al*. 2006; Parentelli *et al*. 2007). Post-harvest senescence in a variety of horticultural commodities is accompanied by changes in cell membrane characteristics, which leads to loss of barrier function and loss of turgor (Mazliak 1987). Mushrooms softening or loss of firmness during postharvest storage has been ascribed to changes in membrane (Beelman *et al*. 1987). On the other hand, according to Zivanovic *et al*. (2000) these texture changes are also related to protein and polysaccharide degradation, hyphae shrinkage, central vacuole disruption and expansion of intercellular space at the pilei surface (Zivanovic *et al*. 2000). Also, an increase in cohesiveness with storage time has been observed (Zivanovic *et al*. 2000; Parentelli *et al*. 2007). This trend has been explained by an increase in chitin content and formation of covalent bonds between chitin and R-glucan, increasing rigidity of the hyphal wall (Zivanovic *et al*. 2000).

MODIFIED ATMOSPHERE PACKAGING

Modified atmosphere can be defined as an atmosphere that is created by altering normal air composition, in order to provide an appropriate atmosphere surrounding the product for decreasing its deterioration rate and increasing its shelf life (Moleyar and Narasimham 1994; Phillips 1996; Farber *et al*. 2003).

The use of modified atmosphere includes two kinds of storage: controlled atmosphere storage and modified atmosphere packaging. When controlled atmosphere storage (CAS) is used, the product is stored in cold storage rooms under an atmospheric composition that is maintained constant throughout storage. On the other hand, during modified atmosphere packaging (MAP), fresh produce is generally packaged in polymeric films bags; being the atmosphere inside the package modified due to two processes: respiration of the product and diffusion of gases through the packaging film (Fonseca *et al*. 2002; Farber *et al*. 2003). Active or passive modified atmosphere packaging can be employed. Active modification occurs by changing initial air at packaging by a desired mixture of gases, while passive modification occurs when the product is packaged using air as initial package atmosphere composition (Farber *et al*. 2003). In MAP, package atmosphere composition might change during storage. It might reach a constant equilibrium composition if respiration rate equals gas diffusion rate after a certain storage period (Fishman *et al*. 1995). Although con-

Table 1 Permeability of films commonly used for modified atmosphere packaging

Film	Permeability (cm^3/m^2 d atm) for 25 µm film at 25ºC		Water transmission (g/m^2 d) for 25 µm film at 38ºC 90% RH
	Oxygen	Carbon dioxide	
Low density Polyethylene (LDPE)	3,900 – 13,000	7,700 – 77,000	6 – 23
Medium density Polyethylene (MDPE)	2,600 – 8,300	7,700 – 38,750	8 – 15
High density Polypropylene (HDPE)	520 – 4,000	3,900 – 10,000	4 – 10
Polypropylene (PP)	1,300 – 6,400	7,700 – 21,000	4 – 11
Polyvinyl chloride (PVC)	150 – 2,200	450 – 8000	30 – 40
Plasticized Polyvinyl chloride (PVC)	77 – 7,500	770 – 55,000	15 – 40
Polystyrene (PS)	2,000 – 7,700	10,000 – 26,000	100 – 150
Polyurethane	800 – 1,500	7,000 – 25,000	400 – 600
Polyamide (Nylon)	40	150 – 190	84 – 3,100
Microperforated	> 15,000	–	–

Data from Greengrass 1995; Schlimme and Rooney 1997; Farber *et al*. 2003

trolled atmosphere storage and modified atmosphere packaging use the same principles for prolonging shelf life of products, modified atmosphere storage is used on smaller quantities of produce than controlled atmosphere packaging (Parry 1993). Passive modified atmosphere is also applied to larger quantities when product is packaged in pallet bags and paperboard containers during its transportation and storage (Lee *et al*. 1996).

Modified atmospheres richer in CO_2 and poorer in O_2 than air, can potentially reduce respiration rate, ethylene production and sensitivity, decay and physiological changes (Kader 1986; Saltveit 1997; Gorris and Tauscher 1999; Farber *et al*. 2003). Shelf life extension due to modified atmosphere can be mainly attributed to low O_2 and high CO_2 concentration in the atmosphere that surrounds the product, which causes a decrease in respiration rate and also inhibits microbial growth (Solomos 1997; Farber *et al*. 2003).

Respiration is slowed down by decreasing O_2 concentration. This reduction in respiration rate in response to low O_2 levels is the result of a decrease in the activity of oxidases, such as polyphenoloxidase, ascorbic acid oxidase and glycolic acid oxidase (Kader 1986). Decrease in respiration delays the oxidative breakdown of the complex substrates which make up the product, prolonging its shelf life. Usually, in modified atmosphere packages the concentration of O_2 is kept low (1-5%) (Fonseca *et al*. 2002; Farber *et al*. 2003). However, at extremely low O_2 levels (<1%), anaerobic respiration can occur, resulting in tissue destruction and production of substances that contribute to off-flavors and off-odours (Lee *et al*. 1995; Austin *et al*. 1998). It also generates a potential risk for the growth of anaerobic foodborne pathogens, such as *Clostridium botulinum* (Austin *et al*. 1998; Farber *et al*. 2003).

Elevated O_2 atmospheres have been suggested as alternative to traditional MA treatments that use reduced O_2 (Day 1996). This treatment is referred to as "oxygen shock", "gas shock" uses superatmospheric O_2 conditions (pressures higher than 40 kPa). This treatment has been found to be effective in inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions, and inhibiting aerobic and anaerobic microbial growth (Day 1996; Kader and Ben-Yehoshua 2000; Wszelaki and Mitcham 2000). No studies have been found reporting the use of superatmospheric conditions in mushrooms packaging.

The influence of CO_2 in respiration rate depends on the product, its developmental stage, CO_2 concentration and time of exposure (Fonseca *et al*. 2002). CO_2 concentration has been reported to have an inhibitory effect on respiration rate of mushrooms (Fonseca *et al*. 2002). This inhibition has been explained considering two different hypotheses: simple feedback inhibition (Wolfe 1980; Herner 1987) and elevated CO_2 concentrations which might affect the Krebs cycle intermediates and enzymes (Kader 1989). In modified atmosphere packages the excessive accumulation of CO_2 can cause cell membrane damage and physiological injuries to the product, such as severe enzymatic browning and loss of firmness (Burton *et al*. 1987; López- Briones *et al*. 1992; Varoquaux *et al*. 1999).

CO_2 is the only gas used in MAP that has significant and direct antimicrobial activity. Different theories have been suggested to explain this antimicrobial effect. In general, CO_2 in MAP results in an increased lag phase and generation time during logarithmic phase of growth of microorganisms (Phillips 1996). Theories to explain the antimicrobial action of CO_2 have been summarized by Farber (1991) and include: alteration of cell membrane function including effects on nutrient uptake and absorption; direct inhibition of enzymes or decreases in the rate of enzyme reactions; penetration of bacterial membranes leading to intracellular pH changes; direct changes to physicochemical properties of proteins. Aerobic bacteria, such as *Pseudomonas*, are inhibited by moderate to high levels of CO_2 (10-20%), whereas microorganisms such as lactic acid bacteria can be stimulated by high CO_2 concentrations (Farber 1991; Lee *et al*. 1995; Farber *et al*. 2003).

Also, nitrogen is used in MAP in order to displace O_2 during active modified atmosphere packaging and is used as a filler to maintain package conformity (Parry 1993).

Films used for modified atmosphere packaging

The design of MAP involves careful selection of the film, package type and size for each specific product (Farber *et al*. 2003). Permeability to O_2 and CO_2 and water vapor transmission rate are the most important factors to be considered when selecting a film for MAP. These permeabilities are key factors in determining package atmosphere composition and humidity inside the packages, and therefore might influence product's deterioration rate. Several films have been used for MAP, being the most commonly used low and high density polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC). O_2 and CO_2 transmission, as well as water vapor permeation rate, of these and other films are shown in **Table 1**. Permeability to O_2 and CO_2 are usually determined at 25ºC, while water vapor transmission rate is determined at 38ºC (Greengrass 1995; Schlimme and Rooney 1997; Farber *et al*. 2003). However, little information is found in literature related to film permeability at other temperatures. Thus, research is needed to gather information regarding film permeability at common storage temperatures (0-10ºC) and relative humidities (75-95%), in order to properly design MAP.

Mushrooms, being products with high respiration rate, require packaging films with high O_2 and CO_2 permeability. Permeabilities of the commonly used polymeric films are not sufficiently high as required and anaerobic conditions might occur, as well as physiological damages due to high CO_2 concentrations. In order to overcome this problem, microperforated and macroperforated films have been developed (Farber *et al*. 2003). Because of their high O_2, CO_2 and water vapor permeability, these films precludes the possibility of developing an adequate modified atmosphere for packaging high respiring products. The permeability of these films depends on the type of film, its thickness and the number, size and shape of the perforations (Zanderighi 2001).

When selecting packaging films, in addition to their permeability, the following aspects also have to be taken into account: provided protection, strength, sealability, clarity, machineability, and ability to label (Zagory 1995).

Recently, smart packaging systems have been developed for modified atmosphere packaging (Phillips 1996; Rooney 2000). Some intelligent systems alter package oxygen and/or carbon dioxide permeability by sensing and responding to changes in temperature. Other smart films incorporate chemicals into packets placed in the packaging system, with no contact with the product. Examples of this type of packaging systems would be the use of iron or ascorbic acid to reduce O_2 concentration, calcium hydroxide or activated carbon as CO_2 scavengers (Parry 1993; Farber *et al*. 2003).

Factors affecting shelf life during modified atmosphere packaging

Two of the most important factors in determining deterioration rate during modified atmosphere storage are temperature and gas composition.

Decreasing storage temperature causes a reduction in biochemical reaction rates of horticultural products, and thus on respiration rate (Kader 1986). Biological reactions are reported to increase two or three times for every 10ºC rise in temperature within the temperature range usually used during distribution and marketing chain (Fonseca *et al*. 2002).

As previously mentioned respiration is widely assumed to be slowed down by decreasing available O_2 and increasing CO_2. Furthermore, if O_2 concentrations are too low or CO_2 too high physiological damages might occur to the product. Therefore modified atmosphere packages should be carefully designed since a system incorrectly designed may be ineffective or even shorten the shelf life of the product. Effective MAP of produce requires consideration of the optimal gas concentration, product respiration rate, gas diffusion through the film, as well as the optimal storage temperature in order to achieve the most benefit for the product and consumer.

In general, those products with increased wounding, as in the case of fresh-cut produce, will have a high degree of perishability (Fonseca *et al*. 2002). Cutting and slicing induce mechanical injury in the tissue changing its physiology. Cutting ruptures the cells, which then decompartmentalizes and releases cell contents leading to biochemical reactions (Delaquis *et al*. 1999). The main physiological manifestations that appear due to wounding include increased respiration and ethylene production and accumulation of secondary metabolites (Farber 1991; Lee *et al*. 1995; Varoquaux and Wiley 1999; Fonseca *et al*. 2002). Wounding causes a gradual increase in respiration rate with storage time, until a maximum is reached and then start decreasing to either the value before the wounding or to a higher one (Fonseca *et al*. 2002). Cutting can lead to a 2- to 3-fold increase in respiration rate when compared to that of the whole product (Fonseca *et al*. 2002).

MODIFIED ATMOSPHERE PACKAGING OF *AGARICUS BISPORUS*

Postharvest physiology of *Agaricus bisporus* has been directly related to development of viable spores (Braaksma *et al*. 1994). During postharvest storage, the main processes related to mushrooms deterioration have been related to the development of the sporophore, such as breaking of the veil, elongation of the stipe, opening of the pileus, expansion of gill-tissue and spore formation (López-Briones 1992; Braaksma *et al*. 1994; Eastwood and Burton 2002). These phenomena, together with browning of the cap and gills and a general loss of appearance, have been considered negative quality characteristics and limit the shelf life of this type of mushroom.

Considering physiological changes during postharvest storage, maturity indexes have been developed for common mushrooms. Hammond and Nichols (1975) classified mushrooms in 7 arbitrary stages of development based on cap opening (c.f. **Table 2**). On the other hand, Guthrie (1984) also assigned a maturity index to common mushrooms using a 7 point-scale ((1) veilintact tightly; (2) veil intact stretched; (3) veil partially broken (≤half); (4) veil partially broken (>half); (5) veil completely broken; (6) cap open and gills well exposed; and (7) cap open and gill surface flat). These indexes have been used to evaluate the influence of storage time and storage conditions on quality of common mushrooms (Nichols and Hammond 1973; Burton *et al*. 1987; López-Briones *et al*. 1992, 1993; Roy *et al*. 1995).

Table 2 Maturity index based on cap opening for *Agaricus bisporus* developed by Hammond and Nichols (1975).

Stage	Approx. diameter of the cap (mm)	Description
1	≤5	Velum not differentiated
2	20-30	Velum visible and intact but not stretched
3	30-40	Velum stretched but still intact
4	30-40	Velum starting to tear
5	30-50	Velum torn, cap still cup-shaped, gills clearly visible
6	40-60	Upper surface of cap convex, gill surface flat or slightly concave
7	50-70	Gill surface curving upwards

Although modified atmosphere packaging of *Agaricus bisporus* has been studied for more than 30 years, there is little published consensus on the optimum atmosphere composition for controlled atmosphere storage of this type of mushrooms.

According to Varoquaux *et al*. (1999), mushroom catabolism is not dependent on O_2 partial pressure from 0.1 to 20 kPa or CO_2 partial pressure from 0 to 20 kPa. Therefore, they suggested that CAS or MAP would reduce neither respiration rate nor metabolite consumption in mushrooms. However it has been demonstrated that CO_2, at partial pressures from 5 to 20 kPa, prevented cap opening, probably due to repression of aerobic metabolism (Burton *et al*. 1987), inhibition of endogeneous growth regulators (López-Briones *et al*. 1992), or the effect of CO_2 as regulator for mycelial growth and mushroom morphogenesis (Roy *et al*. 1995). According to Sveine *et al*. (1967) high CO_2 and low O_2 concentrations prevented cap opening. These authors reported that optimum atmosphere for achieving maximum shelf life was 0.1% O_2, and 5% CO_2. On the contrary, Murr and Morris (1974) reported that 0% O_2 retarded pileus expansion and stipe growth, while 5% O_2 promoted pileus expansion and stipe growth after 7 days at 10°C. Besides, CO_2 at 5% stimulated stipe elongation but suppressed cap growth. On the other hand, according to López-Briones (1992) optimum O_2 concentration for controlled atmosphere storage might be 2.5-5% CO_2 and 5-10% O_2, oxygen concentration being less important than CO_2 concentration. According to these authors, up to 5% CO_2 seems to retard mushrooms maturity, browning and to preserve texture loss. However, CO_2 concentrations higher than 5% might induce both internal and external yellowing of the cap.

Gormley and MacCanna (1967) reported that shelf-life of *Agaricus bisporus* mushrooms could be increased by overwrapping the product with PVC films. Nichols and Hammond (1973) studied the influence of package atmosphere composition on the quality of common mushrooms in passive modified atmosphere packages stored at 2 and 18°C. Packages with CO_2 concentration of 10-12% and O_2 of l-2% stored at 18°C resulted in mushrooms with slowest opening of the pileus and colour deterioration. At 2°C CO_2 and O_2 concentrations came to equilibrium at about 4-10% and l-

17%, respectively. Roy *et al*. (1995) studied the effect of O_2 concentration in an atmosphere containing 2-6% CO_2, on shelf-life of fresh common mushrooms packaged in polyethylene pouches. Optimum in-package O_2 atmosphere was 6% to reduce cap development. According to these authors maturity index decreased with O_2 concentration and increased with CO_2 concentration. Reduced O_2 concentration inside the packages during storage did not influence enzymatic browning of mushrooms.

Nichols and Hammond (1973) packaged mushrooms with either PVC film or its combination with a microporous film. Steady state was reached at low O_2 concentration (<10%) and high CO_2, (>10%) concentration. Oxygen at 5% was optimum for retardation of cap opening of conventional mushrooms. Further reduction of O_2 concentration did not reduce the maturity index. Burton *et al*. (1987) studied packaging of common mushrooms in styrene punnets over-wrapped with oriented polypropylene (permeability 240 mL O_2 m^{-2} d^{-1}) combined with different areas (0, 40, 80 and 240 mm^2) of a microporous film (permeability 3.6 x 10^8 mL O_2 m^{-2} d^{-1}), stored at 18ºC for 4 days. As expected, increasing areas of microporous films led to a higher O_2 concentration, preventing anaerobic conditions. Increasing microporous film area also resulted in higher maturity index as a consequence of higher metabolical activity due to higher O_2 and lower CO_2 concentrations.

Microperforated films have been evaluated to prevent excessive CO_2 accumulation and O_2 depletion within packages. López-Briones *et al*. (1993) evaluated the use of microperforated films (permeabilities ranging from 50,000 to 200,000 mL O_2 m^{-2} d^{-1}), as well as PVC (permeability 25,000 mL O_2 m^{-2} d^{-1}) for passive modified atmosphere of common mushrooms at 4 and 10ºC. Less permeable films (PVC and those with oxygen permeability lower than 72,000 mL O_2 m^{-2} d^{-1}) generated atmospheres that delayed mushroom maturation, retarding breaking of the veil (5% and 15% CO_2 at 4 and 10ºC respectively) but promoted internal and external browning, probably due to CO_2 damage. According these authors, shelf life of common mushrooms stored at 4ºC was limited to a week at 4ºC when packaged in the less permeable films.

MODIFIED ATMOSPHERE PACKAGING OF *PLEUROTUS* SPP.

Pleurotus spp. occurs throughout the hardwood forests of the world that include the most diverse climates (Gunde-Cimerman 1999; Rosado *et al*. 2002). *Pleurotus* mushrooms are a delicate variety of mushroom, requiring a growing temperature range between 5 and 22ºC, depending on the cultivar, as well as good ventilation and high relative humidity (Villaescusa and Gil 2003).

According to Villaescusa and Gil (2003) the main changes associated with *Pleurotus* deterioration during postharvest storage are changes in colour, caused by enzymatic browning, and the occurrence of soft and spongy texture, due to cell growth and water migration. They reported no significant changes in texture or colour of *Pleurotus* mushrooms after 7 days of storage at 0ºC. However, high texture losses and an important discoloration to yellowish colours occurred after 7 days of storage at 4 and 7ºC.

There is little information published in current journals concerning modified atmosphere packaging of *Pleurotus* mushrooms and the effect of gas composition on their quality during storage. Popa *et al*. (1999) determined the most efficient MAP composition for storage as 1 kPa O_2 /5 kPa CO_2 at 4ºC for 14 days. Henze (1989) studied storage of modified atmosphere packaging of *Pleurotus* with different CO_2 and O_2 concentrations, stored at 1°C and 94% relative humidity. Sensory quality was higher when mushrooms were stored under high CO_2 concentrations. 30% CO_2 gave better results than 20% or 10% CO_2. Regarding O_2 concentration, the best results were obtained when it was lowered to 1% in combination with 30% CO_2. In these conditions, the quality of mushrooms was acceptable for 10 days. Authors also reported good results when mushrooms were stored under high CO_2 concentrations (50%) with subsequent equilibration to normal atmospheric conditions.

Villaescusa and Gil (2003) evaluated passive modified atmosphere packaging of *Pleurotus ostreatus* stored at 4ºC in bags of LDPE, PVC and a microperforated film (MPP1). After 7 days of storage, the better visual quality was obtained for mushrooms in PVC packages. However, off-odours were detected for samples stored in PVC and LDPE packages. Off-odour development might have been caused by fermentation, induced by O_2 levels which decreased to 2 kPa within packages. Mushrooms in microperforated packages showed a decrease in their visual appearance due to high water content on the tissue, caused by high condensation inside the package. Therefore, the tested MA packages resulted in either anoxia (PVC, LDPE) or CO_2 stress (LDPE) or condensation (MPP1), suggesting that more permeable films would be necessary to extend mushrooms shelf life. These authors increased the perforation area of MPP to test a microperforated film with higher permeability (MPP2). In this film equilibrium atmosphere (12-15 kPa O_2 /5 kPa CO_2) was developed and was found beneficial for maintaining quality of *Pleurotus* mushrooms for 7 days.

However, other package atmosphere compositions could be explored; being the optimum MAP for stored *Pleurotus* is therefore still to be determined.

MODIFIED ATMOSPHERE PACKAGING OF *LENTINUS EDODES*

Lentinus edodes, or shiitake mushroom, is one of most common edible mushrooms traditionally cultivated in Japan. It is cultivated both on natural and artificial logs (Chang 1999b; Nóra and Mécs 2001).

Parentelli *et al*. (2007) reported that shelf life of shiitake mushrooms, both in modified atmosphere and macroperforated packages, was limited by changes in its sensory characteristics, due to mushrooms' metabolic activity, and not by microbial growth. Aerobic mesophilic bacteria counts, as well as yeast and molds, decreased at the beginning of the storage period and afterwards remained in low counts in all the studied storage conditions.

According to Minamide *et al*. (1980a, 1980b) quality reduction of shiitake mushrooms during postharvest storage is closely related to its browning. Moreover, other authors reported that as shiitake mushrooms deteriorate, their gills become browner and less uniform, their cap becomes less firm, and its surface less uniform with increasing dark zones (Ares *et al*. 2006; Parentelli *et al*. 2007). These attributes have been evaluated by a trained assessors panel, using unstructured 10 cm intensity scales, in order to evaluate the influence of modified atmosphere packaging on shiitake sensory quality decay (Ares *et al*. 2006). To estimate sensory shelf life of shiitake mushrooms Ares *et al*. (2006) determined limits for the evaluated sensory attributes based on consumers' rejection percentage of the product. On the other hand, Parentelli *et al*. (2007) developed a structured 9-point descriptive quality scale to evaluate shiitake colour, shape and texture quality. In this scale shiitake typical characteristics were described in the 7 to 9 rank, atypical or strange characteristics indicative of the beginning of deterioration, being the food still eatable were described in the 4 to 6 rank, and those strange components that deteriorate the product, rendering it non-eatable, were included in the 1 to 3 rank.

Some studies about modified atmosphere packaging of shiitake mushrooms report results that may be contradictory.

According to Gong *et al*. (1993), browning of shiitake mushrooms was inhibited by packaging in passive modified atmosphere in polyethylene bags. Browning inhibition was not attributed to high CO_2 and low O_2 concentrations, but to endogenous ethanol accumulation in shiitake tissue under conditions of high CO_2 and low O_2. Besides, based on respiration rate and ethanol and acetaldehyde production Kim *et al*. (1989) reported that optimum controlled atmosphere

conditions for extending shiitake's shelf life was those containing 2% O_2/2% CO_2. According to Minamide *et al*. (1980a, 1980b) controlled atmosphere storage of shiitake mushrooms with 40% CO_2 and 1% O_2 extended their shelf life 4 times in comparison to storage under air.

On the other hand, Lee *et al*. (1991) measured quality indices of shiitake mushrooms (weight loss, protein content, ethyl alcohol and acetaldehyde concentration) during storage in modified atmosphere conditions using polyethylene film bags of different thickness (20, 40 and 60 μm). They reported that mushrooms stored in the thicker film developed off-odours, which were attributed to ethanol and acetaldehyde production due to low O_2 concentrations inside the packages.

Ares *et al*. (2006) and Parentelli *et al*. (2007) evaluated the use of passive and active (initial atmosphere 5% O_2 / 2.5% CO_2) modified atmosphere packaging in polyethylene and polypropylene bags at 5ºC. As control a macroperforated common polypropylene film was used, in which the atmosphere within the package was maintained at normal air composition. According to these authors, both passive and active modified atmosphere resulted in a significantly increase in sensory deterioration rate of shiitake mushrooms with respect to samples packaged under air in macroperforated bags. Off-odours were developed in PE and PP packages, probably due to low O_2 concentration (1.3%) inside the packages. Although mushrooms in macroperforated packages had a lower sensory deterioration rate than those stored under modified atmosphere, they had a higher respiration rate due to the higher O_2 and lower CO_2 concentrations within packages. Therefore, these authors concluded that, in the studied conditions, mushroom deterioration rate was mainly determined by physiological damage caused by high CO_2 concentrations. Parentelli *et al*. (2007) reported that CO_2 concentrations higher than 5.4% might cause physiological damage. Considering the occurrence of physiological damage due to CO_2 in *Agaricus* and *Pleurotus*, results suggested that shiitake mushrooms might be more susceptible to CO_2 than other mushrooms species. Ares *et al*. (2006) estimated shiitake mushrooms shelf lives as the time at which 25% consumers would reject to consume the product. Using this criterion, shelf lives were estimated as 5±2 days for mushrooms stored in passive modified atmosphere in PE or PP bags, and as 12±2 days for mushrooms stored in macroperforated packages (Ares *et al*. 2006). Therefore, in the studied conditions passive modified atmosphere did not extend the shelf life of mushrooms, when compared with those maintained under atmospheric air composition.

The studied films were not enough permeable as to compensate the high respiration rate of shiitake mushrooms, leading to physiological damage due to low O_2 and high CO_2 concentrations. Therefore, further research is needed to evaluate the influence of using more permeable films and different O_2/CO_2 combinations for active modified atmosphere packages, in order to determine packaging conditions that reduce metabolic activity without secondary effects of CO_2 on quality.

While Ares *et al*. (2006) and Parentelli *et al*. (2007) reported physiological damage at CO_2 concentrations bellow 10%, Minamide *et al*. (1980a) concluded that atmospheres containing 40% CO_2 were optimum for controlled atmosphere storage of shiitake mushrooms. This difference in the results they encountered regarding susceptibility to CO_2 may be due to different developmental stage. Depending of the market, shiitake can be harvested at two developmental stages, in its early stage, when its cap is closed (**Fig. 2A**) or in its late stage when its cap has split along, showing the white inner part (**Fig. 2B**). Developmental stage has been reported to have a great influence on how products' respond to high CO_2 concentrations (Fonseca *et al*. 2002). While shiitake mushrooms in Japan are traditionally harvested in its late developmental stage (Tokimoto 2005), in Uruguay they are harvested in its early stage; which could explain results differences between studies performed in Japan and Korea and those performed in Uruguay. Therefore, further research is needed in order to evaluate the influence of developmental stage on shiitake's CO_2 susceptibility and response to modified atmosphere packaging.

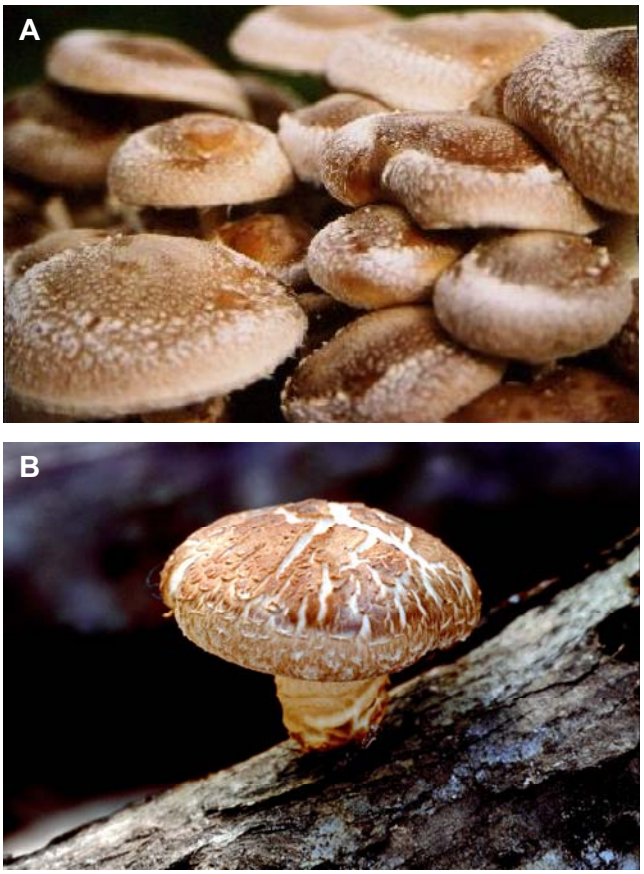

Fig. 2 Typical shiitake mushrooms in two different developmental stages (A) in its early stage, when its cap is closed; and (B) in its late stage when its cap has split lengthwise.

CONCLUSIONS

Mushrooms appear as attractive food products with potential market due to the increase in mushrooms' production and consumption registered in the last fifteen years and consumers' increasing interest in consuming fresh and healthy produce. However, mushrooms' high deterioration rate and short shelf life causes difficulties for their distribution and marketing. Therefore, the use of modified atmosphere packaging and cold storage appear as an alternative to extend mushrooms' shelf life during postharvest storage and commercialization.

Modified atmosphere packaging conditions should be carefully designed since non appropriate modified atmosphere conditions may be ineffective or even shorten the shelf life of the product. Information regarding films permeability and mushrooms' respiration rate is crucial for modified atmosphere packaging design. However, little information is available regarding films permeability at the common storage temperatures, as well as about mushrooms' respiration rate and its dependence with atmospheric composition and storage temperature.

The common polymeric films seemed to be not permeable enough to compensate the high respiration rate of mushrooms. Modified atmosphere packaging in these films leads to an increase in deterioration rate due to physiological damage in response to high CO_2 and low O_2 concentrations. The development of microperforated and macroperforated films make possible to fulfill the oxygen requirement of mushrooms in sealed bags, maintaining package atmospheric composition in values that allow shelf life extension. The use of this type of films has proved to be beneficial for

modified atmosphere packaging of *Agaricus* and *Pleurotus*.

Modified atmosphere packaging, if properly designed, can provide good results for extending mushrooms' shelf life. However, research is needed to provide information in order to find packaging conditions that imply low metabolic activity without secondary effects of CO_2 on quality.

Although many studies have been published about *Agaricus bisporus*, very little research has been reported on modified atmosphere packaging of other mushrooms species, such as *Pleurotus* spp. and shiitake. Therefore, further research is needed to evaluate the use of highly permeable microperforated and macroperforated films, and to study the influence of active modified atmosphere packaging with different O_2/CO_2 combinations.

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