Review

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Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: a review

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Abstract

Postharvest browning of *Agaricus* mushrooms is a severe problem that reduces the shelf life of harvested mushrooms because of their continued respiration and biochemical activity. There are no simple answers and no single treatment is known to limit overall quality deterioration. However, there are several strategies that are being implemented in order to reduce the rate of respiration for mushrooms. Packaging technology is the common denominator that allows us to implement these strategies and thus is key to quality preservation. In this review, first, the major factors involved in postharvest quality deterioration are discussed and then technological advances/methods used to counteract these hurdles are presented.

Keywords: mushrooms; modified atmosphere packaging; browning; shelf life; microbial growth

INTRODUCTION

Mushrooms are edible fungi of commercial importance and their cultivation has emerged as a promising agro-based land-independent enterprise. Only about 45% of mushrooms produced are consumed in the fresh form. The other 55% are processed, with 5% in dehydrated form and 50% in canned form. This is because their shelf life in the fresh form is very short and hence mushrooms are traded in the world market mostly in the processed form. Fruit bodies (reproductive phase) of *Agaricus* are appreciated not only for texture and flavour but also for their chemical and nutritional characteristics.[1,2]

Fresh produce continues to lose water after harvest, but unlike the growing plant it can no longer replace lost water from the soil and so must use up its water content remaining at harvest. This loss of water from mushrooms after harvest is a serious problem, causing shrinkage and loss of weight. When the harvested produce loses 5–10% of its fresh weight, it begins to wilt and soon becomes unusable. Mushrooms are one of the most perishable products and tend to lose quality right after harvest: usually their shelf life is 1–3 days at ambient temperature under usual shipping and marketing conditions, mainly because they have no cuticle to protect them from physical or microbial attack and water loss;[3] 8 days in modified atmosphere (2–5% O_2 and 3–8% CO_2) at 3 °C;[4] and a maximum of 14 days at 2 °C in controlled atmosphere (5% O_2 and 10% CO_2).[5] They are very sensitive to humidity levels, as high water levels favour microbial growth and discoloration; conversely, low water levels lead to loss of weight (and thus economic value) and undesirable textural changes. Fresh mushrooms respire; they take up of approximately 90% water and give off large amounts of water vapour. The vapour can build up in the package, allowing spoilage bacteria to grow and causing the mushrooms to become brown and spotted.[6] Owing to their thin and porous epidermal structure, the respiration rate of mushrooms is relatively high (200–500 mg kg^{-1} h^{-1} at 20 ± 1 °C) compared to other vegetables and fruits. There are several indicators that determine the quality of mushrooms, such as whiteness, cap development, stipe elongation, and number of ripe spores, respiration rate, mannitol content (28% in lower stipe, 10% in gill and 19% in upper stipe on dry weight basis), weight loss and microbial deterioration.[7]

RESPONSIBLE FACTORS

The impact of relative humidity (RH) on quality, such as appearance and texture, was no doubt ascribed to water loss. In earlier studies on the effects of temperature and RH, the focus was on product appearance (colour, gloss, wrinkling, mass loss, etc.). The development of analytical procedures and heightened safety awareness have expanded the range of studies to consumer quality aspects such as nutritional value and safety.[8]

During the movement of fresh products to market, wholesalers and retailers frequently do not have the facilities to maintain optimum conditions for each commodity. Inventory management and marketing largely determine how a product will be handled. These limitations are especially true for speciality commodities, handled in small quantities.[9] Fresh fruit and vegetables probably receive the greatest temperature abuse at the retail level. Temperature abuse is a function of time and temperature during holding and the relative perishability of a particular commodity in terms of biochemical changes (enzymatic browning). Despite the

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efforts of agricultural production, classification and packaging, one of the main problems in mushroom production is the uncontrollable effect of the natural product variability. From a retailer point of view, different batches of mushrooms arrive at a different stages of maturity and in every batch there is natural product heterogeneity.[10]

Temperature and relative humidity

Psychometric charts give a graphical representation of the relationship between temperature, RH and water vapour pressure (WVP) in moist air.[11] The rate of evaporation from a fresh commodity is dependent on water vapour pressure deficit (WVPD) which is the difference between actual vapour pressure and the saturated vapour pressure at a specific temperature. In many storage studies, temperature is controlled but RH is not. There are practical difficulties in maintaining high RH in large storage rooms within a narrow range. At high RH, a small fluctuation in temperature ($<0.5\,^{\circ}C$) can result in condensation on cool surfaces. Fibreboard and wood absorb water and may decrease RH in a room. High RH will not prevent moisture loss if the product temperature is not close to the air temperature. Newer refrigeration controls, more rugged humidity detectors and humidification technologies have increased the ability to vary both temperature and RH. These controls are now appearing in cold rooms and shipping containers. The nature of the commodity evaporative surface is determined by commodity type and cultivar and both have a major influence on the rate of evaporation.[12]

Water loss or transpiration is an important physiological process that affects the main quality characteristics of fresh mushrooms, such as saleable weight, appearance and texture. A setup was developed to monitor the mass loss of mushrooms under various surrounding temperatures (4, 10, 16 °C) and RH (76%, 86%, 96%). Humidity was the variable with the greatest effect on transpiration rate (range 0.14–2.5 mg cm^{-2} h^{-1}). For distribution and retail temperatures (0–25 °C), low temperature has a positive effect in lowering biochemical reaction rates in mushrooms.[3] Burton and Noble[13] found that weight losses from mushrooms stored in open punnets at either 5 °C (73% RH) or 18 °C (90% RH) were linear, averaging 4% per day at 5 °C and 6% per day at 18 °C. Patel *et al.*[14] calculated transpiration coefficients for mushrooms and found that they decreased with increasing water vapour pressure difference.

Storage temperature is one of the main factors that affect post-ripening and qualities such as respiration, transpiration, senescence and other physiological actions. Temperature fluctuation during storage is another key factor. It can make many kinds of oxidases active and enhance physiological activities, speeding post-ripening of stored mushrooms.[15,16] In practice, it is known that temperature fluctuation causes much damage to mushrooms in storage. To ascertain the optimal storage temperature of *Agaricus bisporus*, the effects of different storage temperatures and temperature fluctuations on postharvest physiology and quality have been studied by Zhu *et al.*,[17] who showed that chilling injury did not appear and quality was maintained at 3 °C. In order to keep the temperature as constant as possible during storage, transfer and sale, frequent high-temperature exposure should be avoided and heat preservation devices of mobile refrigeration must be effective. In addition, RH of the storage environment is one of the main factors affecting postharvest quality. Low RH results in excessive water loss that not only can degrade texture but also may result in cell pressure decline, pore closing, respiration damage and increased enzyme activity, thus accelerating cell disaggregation. Conversely, too high an RH (100%) is apt to maintain microorganism growth at the *Agaricus* surface, finally causing it to decompose. Commonly, during horticulture product storage, RH should be maintained at 90–95%. Zhu *et al.* showed that RH of 95% is appropriate for *Agaricus* storage to avoid excessive water loss. As such, postharvest mushrooms should be stored at stable low temperatures. Because the freezing point of *Agaricus* mushrooms is −0.9 °C and because of its delicate structure lacking a protective coat, a storage temperature below 0 °C will bring about chilling injury.

Zivanovic *et al.*[18] determined ultrastructural and compositional changes in fresh mushrooms associated with adverse changes in texture during 9 days of postharvest storage at 12 °C. They observed that mushroom softening (2.6 to 1.5 N, puncturing force) was consistent with toughening (19.2 to 33.0 N, gumminess). Protein and total carbohydrate content declined, but chitin content increased during mushroom storage. Softening paralleled expansion of the intercellular space at the pileus surface, hyphae shrinkage, central vacuole disruption, and loss of proteins and polysaccharides, while toughening was associated with increased chitin content. Nicholas and Hammond[19] overwrapped *Agaricus bisporus* in styrene pre-packs with one of a range of plastic films for 3–5 days at 2 or 18 °C and concluded that the internal atmosphere of the pre-pack was very rapidly modified at 18 °C by the respiration of the mushrooms and that this, in turn, retards the development and deterioration of the mushrooms. It follows that in developing a suitable package for mushroom marketing the gaseous composition generated inside the pack must be considered. Films which permit an accumulation of CO_2 to about 10–12% and depletion of O_2 to about 2% at 18 °C have proved best experimentally. Greater internal browning of overwrapped mushrooms was observed above and below 10–12% CO_2, although external browning was not much affected by CO_2 concentration at 18 °C. However, at 2 °C, about 10% CO_2 was associated with mushrooms that were externally browner than those in lower concentrations of CO_2.

Storage conditions and natural product variability are both important factors that affect the management of mushrooms, and both can be managed using monitoring systems. Aguirre *et al.*[20] studied the effect of different temperatures and RH on decrease of whiteness and appearance of brown spotting using an image analysis system and showed that the kinetics of colour degradation and spotting followed a logistic pattern, and that the best storage conditions to delay the onset of browning and spotting could be found at high RH (<90%) and refrigeration temperatures as high as 11 °C without a significant reduction in whiteness or development of browning during the first 2 days compared to mushrooms stored at 3 °C. Mushrooms stored at 11 °C for longer than 2 days would show a rapid deterioration in colour.

Respiration rate

Morphological changes, which involve exposure of the gills and sporulation, are supported by substrates which are present in the sporophore at harvest, rather than substrates of mycelial origin, as is the case in the growing sporophore. Thus the substrate expended in postharvest sporophore development, and hence respiration, is an important factor in determining the onset of senescence. Respiration is a metabolic process that provides the energy for plant biochemical processes. The ratio of CO_2 produced to O_2 consumed, known as the respiratory quotient (RQ), is normally assumed to be equal to 1.0 if the metabolic substrates

are carbohydrates.[21] Beit-Halachmy and Mannheim[22] found an RQ of approximately 1 for mushrooms at 20 °C and at O_2 levels greater than 1.5–2%; below this O_2 level, RQ increased rapidly to a value higher than 6. The internal factors affecting respiration are type and maturity stage of the commodity. Even different varieties of the same product can exhibit different respiration rates. Care is necessary when packing in modified atmosphere packaging (MAP) due to alterations in respiration rate over time that are not normally considered in MAP design.[12]

The mushroom sporophore has high soluble carbohydrate content and this appears to be the obvious source of respiratory substrate. In common with other fungi, the cultivated mushroom contains mannitol as a major carbohydrate, being present at about 13% of the dry weight in the sporophore.[23] Mannitol is not thought to function as a respiratory substrate in the growing sporophore; however, it appears to be the only soluble carbohydrate present in sufficient quantity to support postharvest respiration. Trehalose is found at levels of approximately 1% of dry weight in the fruit body; other soluble carbohydrates appear to be present in only small quantities. Glycogen has been found at levels of about 5% of dry weight.[24]

The postharvest respiratory behaviour of the mushroom can be divided into two main phases. First, there is a rapid fall in respiratory rate from the high values prevailing at harvest, which lasts for 5–10 h and in which the respiration may decrease by more than 50%. The magnitude of this fall decreases as the maturity of the sporophore at harvest increases. After this there is a slow decline in respiratory rate which lasts throughout the period of observation. Superimposed on this pattern of decreasing respiration there may be a peak in CO_2 production, the occurrence and timing of which depend on the stage of development of the sporophore at harvest and which roughly coincides with the opening of the mushroom.[25] The overall decline in respiratory activity seen after harvest is due to the exhaustion of substrates and senescence of the tissues. The temporary increase in CO_2 production shown by mushrooms at some developmental stages demonstrates that factors other than a shortage of substrate limit the absolute rate of respiration of the freshly harvested sporophore. The decrease in sporophore trehalose levels indicates that this carbohydrate is also metabolized during storage, and it seems probable that trehalose together with glycogen and perhaps amino acids account for the respiratory CO_2 production which cannot be attributed to mannitol. The relative constancy in glucose levels may be explained as the result of trehalose and glycogen breakdown. The low levels of fructose observed throughout the experiment are in agreement with the supposed catabolism of mannitol, since equilibrium for the mannitol dehydrogenase reaction has been found to be greatly in favour of mannitol.[26]

If the respiration rate of the gill tissue is greater than that of the remaining sporophore tissue it might be expected that mannitol and trehalose levels would fall at a greater rate in the gills than in the pileus or stipe. This does not seem to be the case, and it appears likely that there is transport of substrate from the pileus to the gill. This is indicated both in the transfer of dry matter from pileus to gill, and by the fact that although mannitol and trehalose levels in the gill have fallen to their lowest level after 1 day of storage, gill expansion continues. This continuing growth appears to be supported by mannitol from the pileus, since the mannitol in the pileus decreases at a rapid rate and the respiration of the pileus is relatively low. The initial rise in soluble carbohydrates observed in the stipe tissue is difficult to explain but it may be due to the transport of substrates originating elsewhere in the sporophore. The steady overall decrease observed in sporophore mannitol level appears to indicate that the increase observed in the stipe is due to transport from another part of the sporophore rather than *de novo* synthesis.[27,23]

Temperature has been identified as the most important external factor influencing respiration. Biological reactions generally increase two- or threefold for every 10 °C rise in temperature within the range of temperatures normally encountered in the distribution and marketing chain.[3] At higher temperatures, enzymatic denaturation may occur and reduce respiration rates. If temperatures are too low, physiological injury may occur, which may lead to an increase in respiration rate.[9]

Other external factors are O_2 and CO_2 concentrations. Respiration is widely assumed to be slowed down by decreasing available O_2 as a consequence of reduction of overall metabolic activity.[21] Mushrooms are one such commodity in which CO_2 concentration (0.81–2.6% O_2 plus 0.18–9.7% CO_2) had no influence on respiration rate when exposed for 1–3 days.[28] Different durations of product exposure to the specified atmosphere can cause different results regarding the influence of CO_2 on the commodity. The idea of respiratory inhibition by CO_2 was first supported by simple explanations, i.e., that CO_2 was a product of the respiration process and caused simple feedback inhibition.[29] Another hypothesis considered that CO_2 had a strong controlling effect on mitochondrial activity, including citrate and succinate oxidation. Kader *et al.*[21] considered that elevated CO_2 might affect the Krebs cycle intermediates and enzymes. Others considered that CO_2 might inhibit C_2H_4 production rather than having a direct effect on the respiration process. This would explain, for example, the reported influence of CO_2 only on products producing C_2H_4. The respiration rate increase may be explained in terms of CO_2 injury of tissues with a concomitant increase in C_2H_4 production.[30]

Browning

Mushroom browning is a major biochemical event after harvest. It is one of the main features besides texture and cap opening considered in the 'quality spectrum' defined by Gormley and MacCanna.[31] Particularly in the case of white strains, browning assumes greater importance in determining marketability and consumer acceptability. The most important factors that determine the rate of enzymatic browning are the concentrations of active polyphenol oxidase (PPO) and phenolic compounds present, pH, temperature, water activity and oxygen availability of the tissue.[32] Rough handling, fruiting body senescence and bacterial infection, especially with *Pseudomonas tolaasii*,[33] initiate discoloration reactions.

In the *Agaricus bisporus* Imbach sporophore, the significance of laccases is very limited because of their low levels, whereas tyrosinase plays the most important role. Browning occurs as a result of two distinct mechanisms of phenol oxidation: (a) activation of tyrosinase, an enzyme belonging to the PPO family; and/or (b) spontaneous oxidation.[34,35] The PPO family includes catechol oxidases (EC 1.10.3.1) and laccases (EC 1.10.3.2), which oxidize diphenols to the corresponding quinones at the expense of molecular oxygen.[34] Whereas catechol oxidases (1,2-benzenediol: oxidoreductases) oxidize specifically *o*-phenols ('catecholase' or '*o*-diphenolase' reaction) and can also hydroxylate some monophenols into *o*-diphenols (the so-called 'cresolase' or 'monophenolase' reaction, EC 1.14.1.18), laccases can oxidize both *o*- and *p*-diphenols and are usually inactive against monophenols, although some of them have been reported to occasionally show cresolase activity. This activity is also referred to as 'phenolase' or 'tyrosinase', the latter denomination being commonly used

as a trivial name for catechol oxidases. Catechol oxidases may be devoid of any cresolase activity. When the latter is present, its activity is usually much lower than the catecholase activity; the catecholase/cresolase ratio varies from 40 : 1 to 1 : 1[36] and is regarded as the key step in browning biosynthesis. Tyrosinase was first prepared almost simultaneously by from *Agaricus bisporus* and partially purified by precipitation. Tyrosinase enzyme is located intracellularly and may be partly bound to organelles or membrane structures;[34] crude extracts always contain endogenous phenolic substrates, which are readily oxidized; the resulting quinones can in turn react with proteins, leading to decreased enzyme activity and 'artifactual enzyme forms'.[37]

Several authors[38–40] have described tyrosinase activity in the fruit body of *Agaricus bisporus* during its development. Latent tyrosinase can become active after contact with bacteria or a toxin such as tolaasin. Biochemical changes associated with mushroom browning in *Agaricus bisporus* were studied by Rajarathnam *et al*.[41] They concluded that with increasing storage temperature from 0 to 25 °C there was an increase in phenol oxidase activity up to 15 °C, followed by a decrease at 25 °C in mushrooms. Loss of water content in fresh mushrooms had a direct relationship to phenol oxidase activity, which could be correlated with the visual degree of mushroom browning. *Agaricus bisporus* exhibited intense reactions to tyrosine and catechol. The mushroom skin had more phenolics than the flesh, while the stalks had low phenolics contents.

Role of bacteria in spoilage of mushrooms

Doores *et al*.[42] demonstrated that normal healthy mushrooms have high bacterial populations. Total bacterial numbers ranged from 6.3 to 7.2 log cfu g^{-1} of fresh mushroom tissue. The majority (54%) of bacteria isolated from the mushrooms were identified as fluorescent pseudomonads, with flavobacteria comprising the second largest group (10.0%). Recent experiments by Chikthimmah and Beelman[43] have confirmed this pattern but were also able to isolate the *Chryseobacterium* genus (5.5 log cfu g^{-1}) and the *Coryneform* bacterial genus (5.6 log cfu g^{-1}) from freshly harvested mushrooms. Halami *et al*.[44] isolated lactic acid bacteria belonging to the *Lactobacillus* sp. and *Pediococcus* sp. from fresh mushrooms by incubating *Agaricus* mushrooms in de Man Rogosa and Sharpe (MRS) broth for enrichment of resident lactic acid bacteria. However, the bacterial counts were not enumerated in their study. Mushrooms also contain significant levels of yeasts and moulds. Studies by Chikthimmah and Beelman[43] have shown that freshly harvested mushrooms harbour approximately 3 log cfu of moulds and 6 log cfu of native yeast per gram of fresh tissue.

The presence of high bacterial populations in fresh mushrooms is a major factor that significantly diminishes quality by causing a brown, blotchy appearance. The rate of postharvest deterioration of fresh mushrooms has been directly related to the initial microbial load.[42] It was found that bacterial populations during postharvest storage at 13 °C increased from an initial load of 7 log cfu g^{-1} to almost 11 log cfu g^{-1} over a 10-day storage period. The authors also reported that deterioration of mushroom quality as indicated by maturity and colour measurement appeared to be concomitant with increase in bacterial numbers. *Pseudomonas* spp. and *Flavobacterium* spp. were the two main groups that predominated during *Agaricus* mushroom postharvest storage. Similarly, Chikthimmah and Beelman[43] have observed that bacterial populations tend to increase from 7.3 to 8.4 log cfu g^{-1} during a 1-day storage period at 4 °C. Populations of yeast increased from 6.9 to 8.0 log cfu g^{-1} during the storage period. Population of moulds remained constant (3 log cfu g^{-1}) during the storage period.[45,46] A majority of mushrooms of good quality and colour, harvested and marketed, develop blotches at retail or in consumers' homes, even while kept at refrigeration temperatures. Symptoms of brown blotch disease are sunken, dark and brown spots[47] on the mushroom fruit body surface. *Pseudomonas* is the major spoilage genus associated with blotch formation of fresh mushrooms.[48–50] Paine[51] identified *Pseudomonas tolaasii* as the causative organism of the classical bacterial blotch disease of cultivated mushrooms. Applications of *Pseudomonas tolaasii* cells as low as 20 cfu cm^{-2} of growing beds resulted in blotch formation in mushroom.[52] Symptoms of mushrooms blotch became visible when 5.4×10^6 cfu cm^{-2} were detectable in the mushrooms.[52] When *Pseudomonas tolaasii* was placed directly onto caps, 6×10^7 cfu cm^{-2} were necessary to produce a blotch lesion (though only 3.5×10^6 cfu could be recovered). The researchers of the study[52] concluded that the number of cells of *P. tolaasii* present in the early primordial stages of mushroom growth controls the extent of blotch disease seen at harvesting. It has also been shown that tyrosinase is activated during infection by the bacterium *Pseudomonas tolaasii* or exposure to its toxin, tolaasin, causing brown blotch disease symptoms of fresh mushrooms.[53] Wells *et al*.,[54] by isolating and reinoculating the bacteria on freshly harvested healthy mushrooms, confirmed that postharvest blotch formation and associated discoloration were caused by three phenotypic groups (pathotypes) of fluorescent pseudomonads. Severe infections with darkened or yellowed lesions were caused by strains of pathotype A or B, respectively. Mild infections with superficial discoloration were caused by pathotype C. Based on cellular fatty acid analysis, the authors concluded that each pathotype corresponded to one or several mushroom-related pseudomonads reported in the literature as follows: pathotype A = *Pseudomonas tolaasii*, pathotype B = *Pseudomonas 'gingeri'* and pathotype C = *Pseudomonas 'reactans'*. Isolates from mushroom casing material yielded all three pathotypes.

Fluorescent pseudomonads also produce exopolysaccharides (EPSs) associated with the sliminess accompanying spoilage of mushrooms. Fett *et al*.[55] isolated, partially purified, and characterized acidic EPSs from 63 strains of mushroom-associated fluorescent pseudomonads. The strains were originally isolated from discoloured lesions on mushrooms caps, or from commercial lots of mushroom casing soil. An acidic galactoglucan named marginalan was produced by mucoid strains of the saprophyte *Pseudomonas putida* and the majority of mucoid strains of saprophytic *Pseudomonas fluorescens* isolated from casing medium. Other strains produced EPSs that included alginate, and unique EPSs containing neutral and amino groups and glucuronic acid.

There has been a long and complex association between the fungal genus T*richoderma* and mushroom cultivation since Beach[56] first reported disease symptoms on caps of *Agaricus* mushrooms. In a study by Sharma *et al*.[57] colonization assessment confirmed that *Trichoderma harzianum* biotypes Th1, Th2a, Th2b, and Th3 inoculated into the mushroom substrate became established in the mushroom substrate. The extension rate of two Th2 isolates in the substrate was over 1000 times that of Th1 and Th3. Results confirmed that, while Th1 and Th3 did not significantly affect yield, Th2 could reduce mushroom quality and productivity by as much as 80%. *In vitro* studies by Mumpuni *et al*.[58] suggested that the growth of *Trichoderma harzianum* biotypes could be related to the release of metabolites by *Agaricus bisporus* into the compound substrate. Dilute aqueous solutions of *n*-butanol extracts of *Agaricus bisporus* culture filtrates and fruit bodies

inhibited Th1 and Th3 but stimulated Th2 isolates, suggesting that the active compounds may be constitutive components of the *Agaricus bisporus* species.

METHODS TO EXTEND THE SHELF LIFE OF FRESH MUSHROOMS

In recent years there has been a rapid rise in the sale of fresh mushrooms. It has long been demonstrated that the chill chain reduces the microbial and physiological spoilage of mushrooms, but usual transit and marketing conditions (distribution 1–2 days at 2 °C, marketing 1 day at the store temperature) result in a short shelf life. At 11 °C and 90% RH, mushrooms are saleable for about 3–5 days, but at 13 °C average shelf life is reduced to less than 3 days.[59]

The problem of postharvest browning of mushrooms has been tackled from several aspects. There are many methods to extend the shelf life of mushrooms. The most accepted preservation method for mushrooms is cooling; however, there are other techniques able to complement and strengthen it. Among them, modified (MAP)/controlled atmosphere (CA) stands out, which replaces in-pack atmosphere with an appropriate gas mixture that protects the product against oxidation-caused alterations, microbiological attack and colour and aroma variation,[60–62] coating,[62] refrigeration,[63,64] cultivation with $CaCl_2$ solution,[65] ozone treatment[66] and use of sorbitol.[67] Although CA storage is effective in lowering respiration rate and mannitol content[43] and increases shelf life of fruit and vegetables, it is not appropriate for mushrooms, which have extremely high respiration rates. CA storage is costly and not practical for short-term storage of produce with a short shelf life, such as mushrooms. Any beneficial effects of CA storage are lost as soon as the produce is removed from CA.[67] There is little published consensus on the optimum CA to increase shelf life of mushrooms.

Modified atmosphere packaging

MAP is a technique used to extend the shelf life of fresh produce and has a preservative effect on the colour by slowing down respiration, but it leads to water accumulation at the product surface, promoting microbial growth and sliminess, which impairs the objective of MAP.[68–70] This is especially important for mushrooms, as they have high respiration rates. Hence a careful analysis is required to measure and model the water loss rate of mushrooms in MAP conditions and include this component in MAP engineering design. The steady-state package RH depends on the relative rate of water loss by the packaged produce, water gain by the moisture absorber added to the package and water loss through the polymeric film. For modelling and predicting reliable methods to control RH in modified atmosphere packages, it is desirable to have accurate values of water loss rate as a function of different storage conditions.[71]

In a sealed package, a modified atmosphere is created by respiratory O_2 uptake and CO_2 evolution. Consequently, equilibrium concentrations of O_2 and CO_2 are established so that rates of gas transmission through the packaging material equal the produce respiration rate. The steady-state equilibrium will depend on several parameters, such as temperature, respiration rate, O_2 and CO_2, permeabilities of the packaging material, fill weight, free volume in the package and film surface area. An optimum atmosphere should minimize respiration rate without danger of metabolic damage to the commodity. However, exposure to O_2 levels below the tolerance limit and to CO_2 levels above the tolerance limit will cause anaerobic respiration and physiological disorders, respectively.[29] For short-lived and expensive produce, of which mushrooms are a good example, there is an economic incentive to extend shelf life by using MAP. Sveine *et al*.,[72] while investigating the storage life of mushrooms, reported that high CO_2, low O_2 and low temperature prevented cap opening. In that work, N_2 with 0.1% O_2 and 5% CO_2 in storage was optimal for maximum shelf life. Nichols and Hammond[19] varied in-package gaseous concentrations in pre-packs stored at 2 and 18 °C using different films. Packages with CO_2 of l0–12% 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 1l–17% respectively, depending on film overwrap. At that temperature, mushrooms tended to discolour, which may have been due to the high CO_2. Murr and Morris[38] 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. CO_2 at 5% stimulated stipe elongation but suppressed cap growth. Burton *et al*.[73] used a relatively impermeable microporous film to overwrap mushrooms. They reported a progressive reduction of mushroom development with lowering of O_2 from 14% to 4% and increase in CO_2 from 7% to 20% after 72 h. Briones *et al*.[60] suggested that storage atmosphere should contain 2.5–5% CO_2 and 5–10% O_2. Beit-Halachmy and Mannheim[22] reported that MAP seemed to have a beneficial effect on appearance and inferred that this may be due to a microstatic effect, since MAP did not affect rate of respiration. From their experiments, they concluded that MAP may be beneficial but was not found to be essential. If a certain batch of mushrooms respires faster than predicted, or is exposed to large temperature fluctuations, MAP could have a damaging effect.

In another study,[74] modified atmosphere decreased discolouration of fresh mushrooms in perforated LDPE packages. Spraying of calcium hypochlorite (0.4 g/L) did not influence the colour values; however PVC treatment with two perforations in LDPE packages significantly reduced the microbial counts. Low O_2 concentrations were attained in the PVC treatments, which increased browning. It was concluded by Varoquaux *et al*.[30] that no extension of mushroom shelf life was attainable through MAP. Controlling RH within the package is likely to be more effective.

Coating

Coating vegetables and fruits with semi-permeable film has the beneficial effect of delaying ripening and prolonging the storage life.[75] A suitable coating depends on adjustment of the coating solutions to the structure of the coated object, considering the parameters of viscosity, porosity, surface tension, wettability and roughness, among many others.[76,77] Although much information is available on edible coatings in general,[78,79] less information is available on mushroom coating specifically. Alginate and calcium alginate films can be used as coating materials for mushrooms.[62,77] Coated mushrooms were found to have a better appearance, better colour and a weight advantage in comparison with uncoated ones. An alginate–ergosterol–Tween coating combination was most suitable for maintaining the size and shape of the coated mushroom. A new approach to extend the shelf life and preserve the texture of fresh mushrooms using a hydrocolloid coating was proposed by Nussinovitch and Kampf.[62] Calcium alginate films, applied immediately after harvest to each piece of produce and dried to enrobe the mushroom, reduced transpiration and maintained a modulated atmosphere around

each coated mushroom. Such a coating is also advantageous in achieving better colour and maintaining a lower rate of weight loss in comparison to uncoated mushrooms. The ability of this coating method to sufficiently extend the shelf life of mushrooms, and the effects of the coating itself, were investigated and found to have higher *L* (lightness) values, indicating lighter colour and a better appearance than the uncoated mushrooms, with decreased rate of water evaporation and integrity maintained for a longer period of time. Kim *et al*.[80] determined a suitable commercial MAP film (PVC wrap and polyolefins) and the effect of chitosan coating on the quality of whole and sliced mushrooms (*Agaricus bisporus*), determining CO_2/O_2 concentrations, colour, weight loss and maturity during storage at 12 °C and 80% RH. The extent of darkening was greater in coated whole mushrooms than in sliced ones. The type of coating did not appear to affect maturity index except for the wrap package, where chitosan coating markedly lowered the maturity index of sliced mushrooms. Lee[81] studied the effects of MAP (PVC wrap, PD941, PD961) and coating (chitosan and $CaCl_2$) on the quality of *Agaricus bisporus* at 12 °C and 80% RH for 6 days and concluded that the PD961 package showed the lowest O_2 concentration and lower weight loss. Chitosan coating showed a negative effect on colour change of mushrooms.

Washing

Washing mushrooms has recently gained commercial popularity as a means of removing casing soil particles and for the application of browning and microbial inhibitors. Prior to 1986, aqueous solutions of sulfite, particularly sodium metabisulfite, were used to wash mushrooms for the purpose of removing unwanted particulate matter and to enhance mushroom whiteness. While sulfite treatment yielded mushrooms of excellent initial whiteness and overall quality, it did not inhibit the growth of spoilage bacteria. Therefore the quality improvement brought about by sulfite use was transitory. After 3 days of refrigerated storage, bacterial decay of sulfited mushrooms becomes evident. In 1986 the Food and Drug Administration banned the application of sulfite compounds to fresh mushrooms due to severe allergic reactions to sulfites among certain asthmatics. Following the ban on sulfite compounds for washing fresh mushrooms, there have been several efforts to develop wash solutions for use as a suitable replacement for sulfites.

McConnell[82] conducted a review of potential wash additives for mushrooms, including sodium metabisulfite, hydrogen peroxide, potassium sorbate, and sodium salts of benzoate, ethylenediaminetetraacetic acid (EDTA) and phosphoric acids. The researcher concluded that effective antioxidants in addition to antimicrobial compounds were required to enhance shelf life of fresh mushrooms by washing. A fresh mushroom wash solution containing 10 000 ppm hydrogen peroxide and 1000 ppm calcium disodium EDTA was developed. Hydrogen peroxide present in the wash solution acts as a bacteriocide. Copper is a functional cofactor of the mushroom browning enzyme tyrosinase. EDTA in the wash solution binds copper more readily than tyrosinase, thereby sequestering copper and reducing tyrosinase activity and associated enzymatic browning of mushroom tissue. Beelman and Duncan[83] developed a mushroom wash process (US Patent 5 919 507). The method employed a first-stage high pH (pH of 9.0 or above) antibacterial wash followed by a neutralizing wash containing browning inhibitors. The neutralizing wash contained a buffered solution of erythorbic acid and sodium erythorbate. Other browning inhibitors such as ascorbates, EDTA or calcium chloride were identified as suitable ingredients for addition to the neutralizing solution. The process also helped remove debris and delayed microbial spoilage of fresh mushrooms. Sapers *et al*.[84] developed a two-stage mushroom wash process employing 10 000 ppm (1%) hydrogen peroxide in the first-stage aqueous solution, and 2.25–4.5% sodium erythorbate, 0.2% cysteine-HCl and 500–1000 ppm EDTA in aqueous solution in the second stage. The two-stage washing typically yielded mushrooms nearly as white as sulfited mushrooms initially, and whiteness surpassed that of sulfited mushrooms after 1–2 days of storage at 12 °C.[85,86] The treatment was effective in reducing bacteria in wash water and on mushroom surfaces[87] and had minimal effects on mushroom structure and composition.[88] The process was further modified and optimized[84] to include a prewash step using 0.5% (5000 ppm) to 1% (10 000 ppm) hydrogen peroxide. Mushrooms washed by this process were free of adhering soil, less subject to brown blotch than conventionally washed mushrooms and at least as resistant to enzymatic browning as unwashed mushrooms during storage at 4 °C. However, storage at 10 °C accelerated development of brown blotch and browning.

Refrigeration

Degree of whiteness is one of the most important quality factors associated with mushrooms and generally the whitest mushrooms command the highest price. Much research has been carried out over the last few years on keeping fresh mushrooms white. Gormley[63] carried out tests on the effects of chill storage (1 °C) on whiteness of mushrooms and showed that both time of putting mushrooms into refrigeration after harvest, and time of removal, had an effect on whiteness both at point of removal and during subsequent storage at ambient temperature. In general, the longer the refrigeration time in a given period of days, the whiter the mushrooms. The rate of loss of whiteness at ambient temperature was about the same irrespective of whether mushrooms were stored (1 °C) for 0 or 11 days. Mushrooms were placed in six whiteness categories with the aid of a Hunter colour difference meter (HunterLab, Reston, VA, USA) (from excellent, $L > 93$, to very poor, $L < 69$). Mushrooms with *L* values <80 or <69 were considered unacceptable from a whiteness point of view at wholesale or consumer level, respectively. These categories confer a degree of objectivity to the results in chill storage tests.[43] The experimental data from our laboratory studies (unpublished results) have recently shown that refrigeration at 5 °C significantly lowered the bacterial activity, which in turn had an effect on whiteness (*L* value) of stored mushrooms in perforated ambient atmosphere packs.

Vacuum cooling is a rapid cooling technique extensively used for cooling some agricultural and food products.[89] It is achieved by the evaporation of moisture from the product. The evaporation is encouraged and made more efficient by reducing the pressure to the point where boiling of water takes place at a low temperature.[90] Vacuum cooling is rapid and cools mushrooms uniformly within a stack, but the capital and operating costs are high and weight losses are incurred.[91] Tao *et al*.[92] used vacuum cooling as a rapid cooling method for white mushrooms. In their study, experiments were carried out to evaluate the effects of different storage conditions on weight loss, respiration rate, soluble solid content, membrane permeability and degree of mushroom browning and to investigate the influence of storage conditions on the properties of mushrooms; mushrooms were stored under three different conditions: cold room; hypobaric room; and MAP. Additionally, their cooling processes were also investigated. The results showed that the optimum storage condition was MAP after vacuum

cooling. Also, the chemical and physical properties of mushrooms were shown to be significantly different from those stored in a hypobaric room or cooling room.

Use of humectants

The shelf life of fresh mushrooms (*Agaricus bisporus*) is limited to 1–3 days at ambient temperature.[68] San Antonio and Flegg[93] reported that loss of water from the growing mushrooms was comparable to that from a free water surface. Since >90% of the weight of mushroom at harvest is water, it was hypothesized that freshly harvested mushroom transpires at the same rate as the fruiting sporophore. The low water vapour transmission rate (WVTR), combined with high transpiration rate of mushrooms, bring about a nearly saturated condition in the package in a short time.[12] Gormley and MacCanna,[31] Burton *et al*.[59] and Burton[5] found condensed water on the underside of the plastic film used to overwrap mushrooms, making the package unattractive.

Several methods have been reported to reduce in-package relative humidity (IPRH) while storing fresh produce. Burton[5] used P-Plust films (Courtaulds Packaging, Bristol, UK) with varying WVTR to package mushrooms. They found that water condensation was reduced but could not be completely eliminated. Labuza and Breene[94] described the use of propylene glycol as a moisture absorber sandwiched between sheets of polyvinyl alcohol (PVA) (Showa Denko Co., Tokyo, Japan). They also described the use of a sachet containing 55% ethanol, 35% silicon dioxide and 10% moisture. This allowed slow, controlled release of ethanol into the package atmosphere. Ethanol reduced the water activity and acted as an antimicrobial agent. Gormley and MacCanna[31] reported wrinkling and brown patches on surfaces of uncovered mushrooms due to excessive loss of moisture. Use of sorbitol in conventional packages (with no MAP), resulted in mushrooms with better colour than those packaged without sorbitol during 9 days' storage at 12 °C when moisture loss was <18%.[67] Moisture transfer through MAP occurs only by diffusion through the semi-permeable film. Consequently, condensation is more notable in MAP than in conventional packages. Roy *et al*.[61] reported the optimum in-package O_2 concentration to be 6% for maximum increase in shelf life of mushrooms stored in MAP without creating anaerobic conditions. Roy *et al*.[67] showed that modifying the humidity within a conventional package (without modifying the atmosphere) improved quality and shelf life.

Roy *et al*.[95] experimented on mushrooms and used sorbitol and sodium chloride (NaCl) to modify the IPRH of fresh mushrooms (water irrigated and $CaCl_2$ irrigated) stored in MAP at 12 °C. No differences were observed for maturity index and microbial population between mushrooms stored in MAP with or without moisture absorbers. Lower IPRH was observed in packages containing water-irrigated (normally grown) mushrooms with NaCl but resulted in over-drying of mushrooms and did not improve colour. Normally grown mushrooms with 10 and 15 g sorbitol had the best colour. An IPRH of 87–90% within 9 days' storage was considered optimum. No improvements in quality were found with moisture absorbers with normally grown mushrooms. However, a small amount of sorbitol helped to avoid condensation with $CaCl_2$-irrigated mushrooms. The authors[95] concluded from their experiment that a continued lowering of IPRH was observed in packages containing mushrooms with increasing amounts of moisture absorbers. Mushroom irrigated with $CaCl_2$ showed lower IPRH, lower weight loss and surface moisture content. NaCl resulted in lower RH in the package than did sorbitol. A notable decrease in surface moisture of mushrooms packaged with NaCl occurred during storage. Packages containing 10 and 15g sorbitol resulted in better colour values than those without or with 5 g sorbitol. An IPRH of 87–90%, attained within 9 days' storage, in packages with 10 and 15 g sorbitol, was considered optimal for mushrooms, which corresponded to a surface moisture content of 90.5–91%.

Use of tyrosinase inhibitors

Because of the deleterious effect of enzymatic browning on fruits and vegetables, much work has been devoted to the development of methods for eliminating or at least retarding the process. Browning of fresh sliced mushrooms was inhibited by immersion in citric acid (4%) or hydrogen peroxide (5%).[6] In this case, however, the authors suggested that shelf life extension was the result of antibacterial activity rather than tyrosinase inhibition. Chemical control of enzymatic browning includes chelation of the copper present at the active site of the enzyme and reduction of diquinone to its uncoloured form.[32] McEvilly *et al*.[96] isolated several inhibitors of tyrosinase activity from fig latex (*Ficus carica*), one of them identified as 3-(2,4-dihydroxyphenyl propionic acid) (DPP acid). Shimizu *et al*.[97] examined the biochemical parameters of tyrosinase inhibition by DPP acid and concluded that it is a competitive inhibitor with an IC_{50} (50% inhibitory concentration) of 3.2 μmol L^{-1}.

Mushroom browning occurs mainly as a result of tyrosinase activity, which is known to be a key enzyme in melanin biosynthesis. An ethanolic extract from licorice roots (*Glycyrrhiza glabra*) and DPP acid isolated from fig leaves and fruit has been shown to inhibit tyrosinase activity. Adding these inhibitors to sliced mushrooms had a very strong inhibitory effect on browning, but pre-storage immersion of intact mushroom in the licorice extract did not prevent browning after 8 days' storage at 4 °C. By contrast, treatment with DPP acid at 1 μg mL^{-1} reduced browning by half. Measurement of inhibitor uptake by mass spectrometry and assay of tyrosinase activity indicated that penetration into the mushroom tissue was inadequate for tyrosinase inhibition. Moreover, DPP acid was found to be unstable in the mushroom tissue and within a short time it was, presumably, metabolized.[98]

Theoretically, there are several approaches to the problem, but of greatest current practical significance to the fruit and vegetable industry are methods that depend on the reduction of quinones formed by the oxidation of phenols by PPO or the inhibition or inactivation of PPO. Reducing agents such as ascorbic acid, cysteine and sulfur dioxide reduce the *o*-quinones to their *o*-phenol precursors.[99] However, the effect of such reducing agents is temporary, because they themselves are irreversibly oxidized during the process. The use of reducing agents also can lead to oxidation products with off-flavours. Ascorbic acid is widely used to inhibit enzymatic browning of fruits and vegetables because it is a non-toxic compound at the levels employed. The inhibition of PPO by ascorbic acid is complex. Inhibition of brown colour formation in the reaction of mushroom PPO and *o*-dihydroxyphenols has been demonstrated by Golan-Goldhirsh and Whitaker.[100] The mechanism of ascorbic acid inhibition has generally been attributed to the reduction of *o*-quinone back to the phenolic substrate. Ascorbic acid neither inhibits nor activates the enzyme. A K-type interaction of PPO with ascorbic acid was reported.[100] With this type of inhibition the product of the reactions reacts with the enzyme to form a covalent enzyme derivative which is inactive. Although ascorbic acid is the first choice for anti-browning in fresh fruits and vegetables, its instability led us to investigate further other ascorbic acid derivatives that may have greater stability and

increased effectiveness in preventing browning. Two ascorbic acid (AA) derivatives – AA-2-PO_4, and AA-2-SO_4 – were of most interest, since the time required for enzymatic conversion of AA-2-PO_4, or AA-2-SO_4, to AA may act as a pulse releaser for AA to inhibit PPO. Hsu *et al.*[101] studied the inhibition of mushrooms PPO by AA derivatives, i.e., AA, dehydro-AA, iso-AA, AA-2-phosphate and AA-2-sulfate. Kinetic analysis indicated that AA and iso-AA were more effective than dehydro-AA.

Ozone treatment

During the last few years, ozone has been used in water to replace disinfection.[102] Ozone is a powerful oxidizer that spontaneously decays on air or water, releasing O_2. It has an antimicrobial effect, which depends on growth level, environmental pH, temperature and humidity. Ozone attacks microbial cells' vital parts, and its bactericide action results from a number of processes, among which is enzymatic destruction, unsaturated lipid oxidation and nucleic acid splitting.[103] In the case of mushrooms, studies have exclusively focused on the effect of ozone exposure during growth. Watanabe *et al.*[104] analysed the effects of ozone on mushroom fatty acid composition and observed an increase in the relationship between oleic/linoleic acid. Similarly, in a later work[105] with the mushroom *Pleurotus ostreatus*, the effect of ozone on chemical composition showed an increase in mushroom weight, water content, proteins, Ca, K, Zn, riboflavin and ascorbic acid, and a decrease in carbohydrates, iron and thiamine.

Escriche *et al.*[66] determined the effect of ozone, storage time and temperature on postharvest quality of mushroom (*Agarics bisporus* var. Gurelan 55). Mushrooms treated with ozone (100 mg h^{-1}) for 0, 15 or 25 min were packed in polystyrene packs, overwrapped with PVC plastic film, and stored at 5, 15 or 25 °C for 7 days. Internal and external lightness (L^*) texture properties, maturity index and weight loss were analysed during storage. Ozone treatment on mushrooms prior to packaging caused an increase in external browning rate and a reduction in the internal browning rate. The ozone treatment exhibited no significant differences in terms of texture, maturity index and weight loss of mushrooms. Increased storage time and temperatures meant an increase in browning, senescence and weight loss, as well as a decrease in firmness, following, in all cases, zero-order reaction kinetics. Among all the quality parameters assessed in this research, weight loss was the most affected by temperature increase ($E_a = 17.60$ kcal mol^{-1}). The lowest storage temperature (5 °C) favoured the best quality.

NEED FOR FURTHER RESEARCH

Through this review, the importance of various available techniques can be clearly recognized for the shelf life extension of *Agaricus bisporus* mushrooms. Pre-cooling is in essence the removal of heat or the reduction in temperature of the mushrooms as soon as possible after harvest. Each of these individual techniques also has many variations, leading to a great diversity of perishable produce. Research on fresh mushrooms is still needed to obtain microbiologically safe products, keep its nutritional value and sensory quality. Shelf life has to be enhanced to allow distribution and marketing. Further investigation into the processes that rule the physiology and, therefore, limit the shelf life of cut fruits should be undertaken. In designing CA, MA or MAP systems, it would be prudent to realistically evaluate the time and temperature conditions that the product will likely encounter along the postharvest chain, as well as the likelihood of mixed load conditions. It then will become possible to design systems such as a combination CA/MAP and other available techniques that can maintain optimum atmospheres and product quality throughout the postharvest handling chain. In addition, modelling of the package atmosphere composition, respiration rate and internal atmosphere in the fruit tissue throughout storage are of capital importance in designing appropriate packages. One of the main future goals in this field is the search for new compounds from natural sources that appear to be healthier and permit a better preservation of the mushrooms along with the quest for new methods of packaging such as regulating moisture films. Forthcoming studies on the quality of mushrooms should also take into consideration the prevention of nutritional losses as influenced by processing and storage conditions. Furthermore, a strong approach in the development of the technology required for processing and distribution of fresh mushrooms will solve some of the limitations that mushroom producers and processors find nowadays to maintain stable quality throughout the storage period.

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