Part III

POST-HARVEST PROCESSING AND QUALITY CONTROL

CHAPTER 18

MUSHROOM SENESCENCE: ITS MECHANISM AND CONTROL

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1. INTRODUCTION

The mushroom is a high value food product with a short storage life. To ensure that mushrooms are acceptable and nutritious to the consumer at the time of purchase, it is necessary to delay or prevent senescence. Technologies such as cooling and modified atmosphere packaging can be used to delay the rate of senescence, while preservative technologies such as canning, drying, pickling, freezing and γ-irradiation arrest biological function to prevent senescence. In the U.K., 91% of mushrooms grown are purchased fresh (Anon, 1993). In other European countries and in North America, an increasing proportion of mushrooms are bought fresh as part of the trend for eating healthy food. Consequently, research into quality and storage-life of *Agaricus* spp. has concentrated on non-preservative technologies to delay senescence and the regulation of mushroom senescence. This paper describes research into postharvest biology of *Agaricus* mushrooms and the mechanism of action of modified atmosphere packaging.

Sporophore growth prior to harvest is sustained by nutrients and water from the compost, and casing material. When the sporophore is harvested and the nutrient and water supply are cut off, its metabolism adapts to sustain gill growth and spore production. There have been relatively few areas of investigation into mushroom postharvest physiology, two of which, carbon and nitrogen metabolism are concerned with nutrients and their redistribution.

2. CARBON CATABOLISM

Non-structural carbohydrate can account for 30-50% of the sporophore dry weight. This is mainly in the form of mannitol (which is considered to be the major osmoticum for cell expansion), trehalose and glycogen (Hammond & Nichols, 1975; Hammond, 1979). During 4 days storage at 18°C mannitol levels in intact sporophores fall from 24.5 to 4% and glycogen levels fall from 11.8 to 4.5% (Hammond & Nichols, 1975; Hammond, 1979). These compositional changes are not uniform throughout the sporophore. While the mannitol level in the pileus and cap falls during

postharvest storage, a peak in the mannitol concentration occurs in the stipe after 2 days followed by a decline. Levels of trehalose and glucose within the stipe of stored mushrooms also peak at the same time as mannitol (Hammond & Nichols, 1975).

Mannitol is catabolised by the enzyme NADP-dependent mannitol dehydrogenase producing fructose and NADPH. Mushrooms growing in contact with their mycelium oxidise a relatively large proportion of their hexoses via the pentose phosphate pathway. While the flux through both the pentose phosphate pathway and glycolysis declines after harvest, a greater decline was reported to occur in the pentose phosphate pathway (Hammond 1978). This change facilitates mannitol oxidation by reducing NADPH production. An increase in NADPH oxidase activity also occurred after harvest (Hammond, 1978). Glucosephosphate isomerase activity was shown to increase for the first two days of storage at 18°C followed by a rapid decline. Hammond (1978) speculated that this increased activity was to facilitate hexose transfer from the breakdown of trehalose and glycogen to glycolysis.

3. NITROGEN METABOLISM

Protein is the main source of nitrogen for redistribution after harvest. The level of soluble protein in sporophore tissues decreases during postharvest storage of *A. bisporus* and *A. bitorquis* (Burton, 1988a; Burton *et al.*, 1993a). After 5 days storage at 18°C, the soluble protein level falls to 30-70% of that in freshly harvested sporophores. Levels of chitin, urea and cell wall associated protein were shown to increase after harvest (Hammond, 1979). Urea, which is 46.6% nitrogen, increased 9-fold over 4 days storage which is equivalent to approximately 2.4 mg protein g^{-1} (dry weight) (Hammond, 1979). The function of urea is probably as a replacement osmoticum for mannitol. Moore (1984) has shown that urea is the major osmoticum in the developing fruitbodies of *Coprinus cinereus*.

Hammond (1979) speculated that nucleic acid breakdown could provide another nitrogen source. However, both DNA and RNA levels in whole sporophores have been shown to increase postharvest (Minamide & Iwata, 1986). Care should be taken in interpreting this result, as major tissue differences in the nucleic acid levels of pre-harvest developing mushrooms have been observed (Minamide & Hammond, 1985). Nucleic acid levels in the gill tissues have been shown to increase during development while those in the stipe and pileus tissues generally fall.

Proteinase (or protease) activity increases during sporophore senescence (Murr and Morris, 1975a) and this increase has been shown to be a harvest-induced event (Burton, 1988a). The protein level starts to fall prior to the induction of proteinase activity. This is probably due to a distorted protein turnover system, i.e. synthesis is reduced by nutrient limitation but degradation rates remain unchanged. Inhibitor studies have revealed that the proteinases from senescent sporophores of *A. bisporus* and *A. bitorquis* are predominantly metallo- and serine-proteinases (Burton *et al.*, 1993a).

A serine-proteinase from senescent sporophores of *A. bisporus* has recently been purified and characterized (Burton *et al.*, 1993b). The enzyme has a broad pH optimum, 6.5-11.5 and a narrow substrate specificity requiring both a hydrophobic amino acid on the amino-terminal side of the scissile bond and a minimum peptide chain length. The molecular mass of the proteinase is 27 kDa (when determined by SDS-PAGE) and its isoelectric point is 9.0. The N-terminal amino sequence of the serine proteinase has 60% identity with that of proteinase K from the fungus, *Tritirachium album*. The serine proteinase of *A. bisporus* accounts for approximately 3% of the soluble protein in the senescent mushroom stipe at the 5 day postharvest stage. For complete degradation of proteins

to amino acids, additional activities of as yet uncharacterised proteinases and peptidases may be required because the serine proteinase has such a narrow substrate specificity.

4. TISSUE BROWNING

Mushroom sporophores are normally white when harvested and develop brown discolouration during storage. The browning reaction is a normal feature of postharvest ageing, but can be increased by mechanical damage or bacterial infection by *Pseudomonas tolaasii*. The enzyme which initiates browning is tyrosinase, which catalyses the oxidation of phenols. This is followed by a series of chemical reactions which convert the oxidation products to the brown polymer, melanin. Tyrosinase is also responsible for melanogenesis during spore maturation (Hegnauer *et al.*, 1985) but in non-reproductive tissues, the most plausible function is as a defensive mechanism against invading micro-organisms. This is supported by the higher tyrosinase activity and phenol concentration found in skin of the pileus (*pilei pellis*) than in the underlying pileus flesh (*pileus trama*) of both *A. bisporus* and *A. bitorquis* (Burton *et al.*, 1993a).

Tyrosinase is a copper-containing oxidase and has been described as a tetramer consisting of two heavy (43 kDa) and two light (13 kDa) subunits (Strothkamp *et al.*, 1976). Tyrosinase antibodies have been used to immunoprecipitate *in vitro* translation products of total purified m-RNA. The two proteins so identified have molecular weights of 41 kDa and 15 kDa (Podila & Flurkey, 1986). Two research groups using PCR techniques have recently identified putative sequences containing the *Agaricus* tyrosinase gene (Khush *et al.*, 1991; Wichers *et al.*, 1993). A stated aim of several groups world-wide is to remove or reduce tyrosinase activity by molecular genetical techniques and so enhance the colour characteristics of the mushroom. If this aim is achieved, it will be interesting to observe not only changes in any quality characteristics but also possible changes in resistance to bacterial attack.

Tyrosinase from *A. bisporus* has been shown to be present in two forms, active and latent (Yamaguchi *et al.*, 1970). The enzyme can be activated *in vitro* by the detergent sodium dodecyl sulphate, SDS (Yamaguchi *et al.*, 1970) or by proteolytic action (Burton, 1988b). During postharvest storage of sporophores, when cap browning develops in a linear manner (Smith *et al.*, 1993), no increases have been observed in the tyrosinase activity or phenol level of the sporophore skin in both *A. bisporus* and *A. bitorquis* (Burton *et al.*, 1993). However, the *in vitro* activated tyrosinase activity (by SDS or trypsin) declines with increasing storage duration possibly indicating *in vivo* activation during storage. The whiteness of different strains **at the time of harvest** is consistent with their low phenol levels and tyrosinase activities but no correlation between these factors and **postharvest** discolouration could be observed (Burton *et al.*, 1993a). It has been hypothesized that tyrosinase and phenols may be confined to separate compartments in the cell and during postharvest ageing the intracellular membranes may break down (Burton, 1986).

5. CELL STRUCTURE AND TISSUE PARTITIONING

The membrane components have been examined during postharvest storage (Braaksma & Schaap, 1991; Braaksma *et al.*, 1993). Phosphatidic acid was reported to increase in the stipe and cap during ageing, which may be indicative of increased activity of phospholipase D. Membrane protein content showed a marked decrease during storage. The overall conclusion of these reports,

however, is that membrane integrity is not lost during storage.

Expansion of the pileus by growth of gills and elongation of the stipe postharvest is supported by increased cell wall chitin and protein (Hammond, 1979). Chitin synthase can be activated by proteinases (Hänsler *et al.*, 1983). Recently, a compound 10-oxo-*trans*-8-decanoic acid (ODA) has been isolated from mushrooms and shown to stimulate mycelial growth and stipe elongation (Mau *et al.*, 1992). ODA is produced when the fruitbody tissues are damaged (e.g. harvested). Hammond and Nichols (1975) have shown that during ageing, there is a major re-distribution of dry weight between tissues with gill dry weight increasing during storage while that of the pileus and stipe diminishing. The rate of cap opening depends on the stipe length (the longer the stipe, the greater the expansion), indicating that the stipe is acting as a major nutrient source for the expanding gill tissue (Ajlouni *et al.*, 1992).

6. MODIFIED ATMOSPHERE PACKAGING

Modified atmosphere packaging (MAP) has been shown experimentally to delay senescence by delaying cap browning, retarding development and microbial spoilage (Burton, 1991 and references therein). However, this technology is currently not widely used largely due to economic factors. Under MAP mushroom sporophores are subjected to a raised carbon dioxide level, a reduced oxygen level and a higher relative humidity. Tyrosinase has a Km for oxygen equivalent to water saturated in 12% oxygen (Duckworth & Coleman, 1970) with catechol as a co-substrate. Therefore, a reduction in oxygen concentration as found in MAP would be expected to reduce tyrosinase activity and tissue discolouration. However, Briones *et al.* (1992) found that carbon dioxide rather than oxygen has the major influence on cap discolouration. Murr and Morris (1975b) showed that oxygen levels reduced to 2% or carbon dioxide levels raised to 5% had no effect on the induction of postharvest proteases. Modified atmosphere inhibits respiration and mannitol loss but the sporophore can adapt by increasing its rate of respiration (measured in air) particularly in response to increased carbon dioxide concentrations (Briones *et al.*, 1992). Carbon dioxide was also shown by Briones *et al.* (1992) to be the major factor influencing texture and cap opening. This is consistent with the widely described role of carbon dioxide as a regulator for mycelial growth and mushroom morphogenesis (Hammond & Wood, 1985).

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