Post Harvest Quality of *Agaricus bisporus* Mushrooms

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Abstract: Postharvest growth of mushrooms leads to development of open cups and long stipes. At the same time, mushroom browning also occurs. In the present study, we found that commercial mushrooms strains differ in their postharvest growth rate. Postharvest growth was retarded by application of the cytokinins: benzyladenin and m-topolin. Postharvest growth was slower and the retarding effect of cytokinins on it was stronger during winter and spring, as compared to the summer and fall. Cytokinins had a growth retarding effect on mushrooms only when they were applied immediately after harvest. This response to the applied growth regulators indicates a potential role of endogenous growth regulators in postharvest growth. Postharvest immersion of the mushrooms in H_2O_2 reduced browning and the surface bacterial counts. This indication a role for bacteria in the browning process.

Key words: Postharvest; *Agaricus bisporus* cytokinins hydrogen peroxide

1 Introduction

Agaricus bisporus known as "White button mushroom" or "Champignon" is the most common mushroom, cultivated in Europe and the USA. Current production in those countries is estimated to be 1.25 billion tons annually. These mushrooms are consumed either fresh or in processed form (e.g. canned, frozen, sterilised, in brine) The trend in recent years is towards a decreased market share for processed mushrooms and an increase in the consumption ratio of fresh mushrooms. From a quality point of view, fresh and processed mushrooms are considered as two different products. Processed mushrooms are handled by the industry almost directly after picking. On the other hand, fresh mushrooms are channelled through fresh product (fruit and vegetables) marketing networks. The competition in these markets is forcing the producers to deliver high quality mushrooms with extended shelf life. An important quality parameter is mushroom appearance, in particular its morphology. The desired morphology is evenly round closed "cups" with a short stipe. Colour is also an important quality component, since bright white mushrooms are most desirable, while there is some demand also for mushrooms of strains with 'off-white' and brown colour. The shelf life of high quality fresh mushrooms is shorter than that of most common vegetables. Harvested mushrooms continue to grow, as part of the ageing process. This postharvest morphogenesis includes cap opening and stipe elongation that occur simultaneously. Browning of mushrooms also takes place at the same time. This phenomenon occurs as a result of two different mechanisms of phenol oxidation: a) enzymatic activation of tyrosinase, an enzyme that belongs to the polyphenoloxdases (PPO) family; b) spontaneous oxidation.[1] There is also evidence of a role for bacteria in the browning process.[2] Treatments for browning retardation of mushrooms that were studied in the past included the use of H_2O_2 and citric acid for this purpose.[3] The current knowledge on postharvest physiology and mechanisms that affect postharvest morphology of *A. bisporus* mushrooms is limited compared to that of common fruits and vegetables. Mushroom endogenous growth regulators that have been reported to influence mushroom morphology are limited to octadecanoic acid (ODA) in *A. bisporus,*[4] and an undefined active preparation claimed to effect stipe bending of *Coprinus* spp.[5] Evidence for the existence of a growth regulator for mushrooms was also reported from studies on *Flammulina velutipes.*[6] Reports exist too on the possible occurrence of a cytokinin-like substance,

to which no physiological effect has so far been assigned.[7, 8] There is preliminary data on the role of plant growth-regulators on cap opening in *A. bisporus*. Cap opening was suppressed in the presence of the plant hormones. The cytokinin, benzyladenin, showed considerable delaying effects on cup opening.[9]
The present study deals with postharvest quality of hand-picked mushrooms for the fresh produce market. In this context, the effect of cytokinins on mushroom postharvest morphogenesis was determined. The study was aimed at evaluating the effect of these growth regulators under real mushroom cultivation practices. Since browning is one of the main quality problems of postharvest storage and marketing of mushrooms, retardation of browning was also the aim of the present study.

2 Materials and Methods

2.1 Strains

The following strains of *A. bisporus* included in the research were received from major spawn companies: strain 2200 (Amycell, U.S.A); strains 512, U1, U3, 608, A15 (Sylvan, The Netherlands); and strains X6, X13, X25 (Le Lion, France).

2.2 Inhibitory compounds

The following cytokinins were tested at concentrations of 0.1-0.001mM for their effect on postharvest morphogenesis: benzyladenin, kinetin, zeatin, m-topolin, m-topolin riboside

2.3 Harvesting of fruit bodies

Mushrooms for cap opening studies were harvested at commercial farms. Fruit bodies were harvested for testing preferably immediately after a flush peak. At this stage, fruit bodies are very prone to cap opening and most sensitive for assay. The mushrooms were stored at 18°C during experiments, which is the room temperature regularly maintained in the marketing channels (e.g. supermarkets)

2.4 Application of test compounds

Potential inhibitors were applied to fruit bodies placed in a suitable container (usually a Petri dish), with the freshly cut stipe immersed in the liquid containing the test compound. Fruit bodies were exposed to test solutions until all liquid had been absorbed.

2.5 Criteria for cap-opening

Two, basically similar, methods to assess the degree of cap-opening were used:
a. The degree of breakage of the veils was taken as a measure for cap opening.
b. Alternatively, the developmental stage was determined according to the system of Hammond and Nichols.[10]
The effectiveness of any specific treatment to delay cap opening was determined by the delay, in days, resulting from the treatment as compared to an untreated control. The difference between the treatment and the control was measured when both samples reached 50% cap opening (Figure 1).

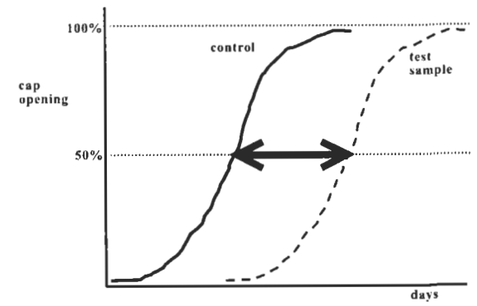

Figure 1. Measure of treatment effectiveness

2.6 Stipe growth

Stipe growth was measured and expressed in millimetres

2.7 Colour measurement

Colour was measured with a Minolta or a Hunter colour meter and expressed as degree of brightness (*L*-value).

2.8 Hydrogen peroxide (H_2O_2) treatment

The inhibitory effect on browning during storage was tested using mushrooms immersed in 5% H_2O_2 or in water (control) for 10 min. after drying, the mushrooms were packed in high-density polyethylene bags and stored at 4°C for 10 days. During the storage period, changes in the mushrooms cap colour (L) were measured.

2.9 CFU count

The microbial population of intact mushrooms was determined by taking three 7 mm diameter disks from each of three replicate mushrooms. The disks were vortexed vigorously for 1 min in a test tube with 5mL PBS buffer and a liquots of the solutions were plated on to Petri dishes containing nutrient agar medium, incubated at 25°C and counted after 2 days.

3 Results

The results are presented in the three categories of mushroom postharvest growth and quality:
(i) cap-opening, (ii) mushroom colour and (iii) stipe elongation.

3.1 Cap-opening

The main commercial mushroom strains were divided into three groups according to their cap-opening rate (Figure 2). Strains X-13, U-3 and 2200 were designated fast cap-opening strains: 100% of the caps were open 3 days after harvest. Strains X-6, 512 and 608 were designated medium cap-opening strains: 50% of the caps were open 3 days after harvest. Strains U-1, X-25 and A-15 were designated slow cap-opening strains: only 25% of the caps were open 3 days after harvest. Differences were also observed in the cap-opening rate of mushrooms from different flushes. For example, cap-opening in strain U-3 mushrooms was slowest with mushrooms from the third flush.

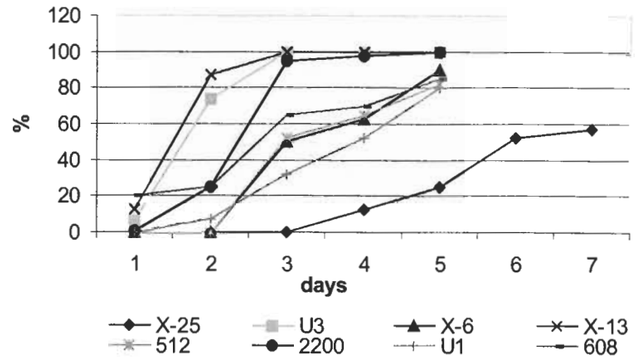

Figure 2. Cap-opening rates of test strains

3.2 The effect of different cytokinins on cap-opening

The effect of different cytokinins on cap-opening was studied using strain U-1 and the results are presented in Figure 3. Benzyladenin and M-topolin had the most noticeable delaying effect on cap-opening; over a period of five days following harvest, less than 10% of the treated mushrooms were open compared to 80% in controls.

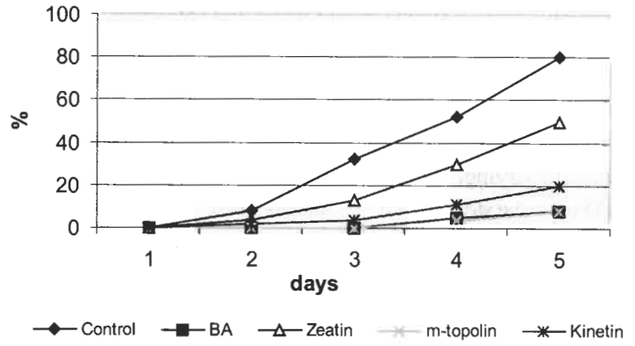

Figure 3. The effect of cytokinin on cap-opening rate of mushrooms (%)

3.3 The effect of different cytokinin concentrations on cap-opening

Two different concentrations (0.1 and 0.01 mM) of the most effective cytokinins (benzylafenin and M-topolin) retarded cap-opening to essentially the same degree (Figure 4).

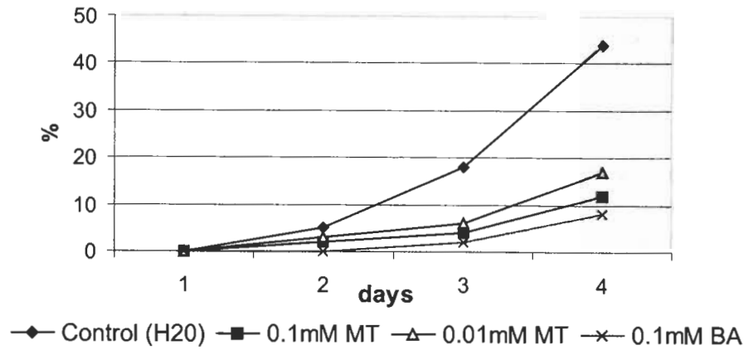

Figure 4. Effect of cytokinin concentration on cap-opening rate (%)

3.4 Seasonal effect

The cap-opening rate and the effect of cap-opening retardant were studied over a two year period. It was found that opening rate was slower, and the effect of cap-opening retardant was
stronger, during the winter and spring. Conversely, faster cap opening and a weaker cap-opening retardant effect was observed during the summer and the fall. Results for strain U-1 mushrooms treated with 0.1mM benzyladenin as cap-opening retardant are presented in Figure 5.

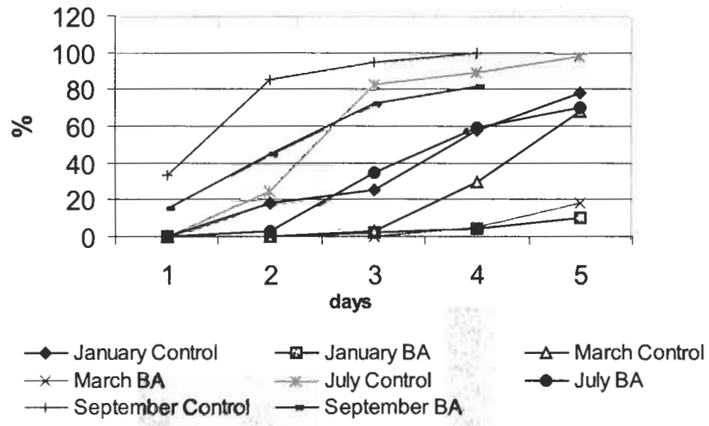

Figure 5. Seasonal effect on cap-opening (%)

3.5 Effect of time of application of retardant treatment

The timing of the retardation treatment is also important. Delay in treating mushrooms with retardant reduced the retardation effect. Mushrooms of strain U-1 were treated with distilled water or 0.1mM benzyladenin immediately after harvest or two hours later (Figure 6).
During this period, the mushrooms were kept at 20°C. Treatment with distilled water or benzyladenin after two hours had similarly no effect on cap-opening. However, when these treatments were given immediately following harvest, a retardation effect was observed and was stronger with benzyladenin.

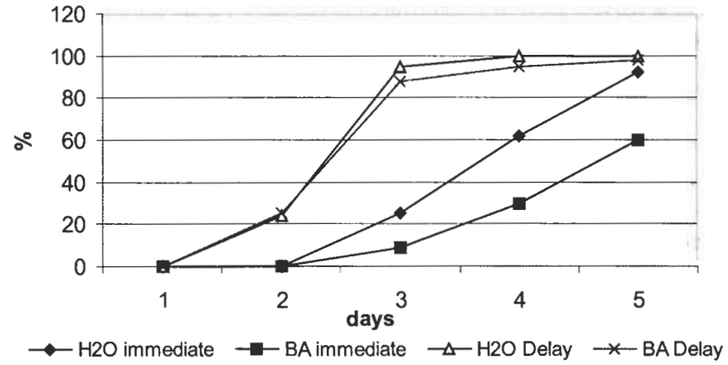

Figure 6. The effect of treatment timing on cap-opening inhibition (%)

3.6 Mushroom browning

The effects of H_2O_2 application as a mushroom browning retardant are shown in Figure 7 (a,b). There were two

measurable results due to H_2O_2 application on the mushrooms. The microbial count, measured as colony forming units (CFU), on the mushrooms was reduced 10^4-fold immediately after application (from 491 to 0.39 cfu/cm^2). After storage for a further 10 days, microbial counts on treated mushrooms reached 244 cfu/cm^2, and 11,186 cfu/cm^2 on the untreated controls. Hydrogen peroxide treatment delayed mushroom browning, especially during the first 4-8 storage days. The untreated mushrooms reached the lowest brightness allowed for fresh mushrooms ("L"=87) after 8 storage days while the treated mushrooms reached this brightness level two days later.

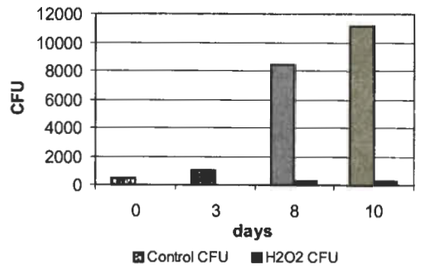

Figure 7a. The effect of H_2O_2 on CFU on mushrooms

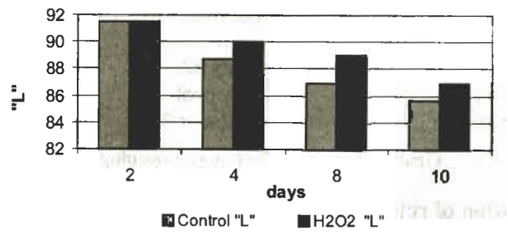

Figure 7b. The effect of H_2O_2 on mushrooms brightness

3.7 Stipe elongation

Treatments with cap-opening retardants also had a retarding effect on stipe elongation. The tested retardants in these experiments were novel products that are under advanced stages of development. Untreated mushrooms stipes 2.0 mm in length at harvest grew to 16.0mm after seven days. At the same time, stipes of treated mushrooms reached only 8-10mm.

4 Discussion

The present study deals with quality enhancement of hand picked mushrooms, produced for the fresh market. The main objective was to find ways to retard the effects of the postharvest morphogenesis of the mushrooms. These include continued growth of the harvested mushrooms observed as cap-opening and stipe elongation. At the same time, browning of the mushrooms also takes place. The experiments in this study were done under conditions (storage temperature of 18°C) that represent the actual situation in the marketing chains.

Postharvest morphogenesis of different mushroom strains was studied by following their cap-opening rate. It was demonstrated that the commercial strains could be classified to three main groups, based on their postharvest growth rate, since there are fast, medium and slow cap-opening strains (Figure 2). This fact should be taken into consideration in selecting a strain for cultivation, since it has an effect on the value of the mushrooms in the market. Differences in cap opening rate were also found between mushrooms of the same strains, harvested on different flushes. Such phenomena were previously reported.[11]

Cytokinins were tested for their impact on post harvest growth. Application of the cytokinins benzyladenin and m-topolin delayed cap-opening. Several aspects of this impact of cytokinins were studied including timing of application and seasonal effects. It was found that application timing is most important. A delay of two hours in application resulted in the treatment having almost no impact, while immediate application successfully retarded postharvest growth. Seasonal effects on postharvest morphogenesis were also recorded. Postharvest growth was slower and was retarded by cytokinins more effectively during winter and spring as compared to the summer and fall.

The results described above provide an indication of possible involvement of endogenous mushroom growth regulators in postharvest growth. The fact that postharvest morphogenesis, and the impact on it of cytokinins, is season-dependant, and that immediate application of cytokinins is necessary for their effectiveness, lead to this hypothesis. Our findings also support earlier reports on the potential role of growth regulators in mushroom morphogenesis.[4-9] It is anticipated that endogenous growth regulators involved in morphogenesis of mushroom ageing are affected by mushrooms harvest since, until harvest, there is continuous flow of metabolites between the fungal mycelium and the fruit bodies. Preliminary studies by our group have revealed the presence of plant growth regulators in harvested mushrooms, (Wichers et al. unpublished data). Further studies on the presence and mode of action of such endogenous growth regulators are needed in order to obtain a better understanding of their role in postharvest growth of mushrooms. Such knowledge could be the basis for the development of strategies to control this undesired postharvest development.

The present study dealt also with postharvest mushroom browning. It was demonstrated that browning was effectively retarded for ten days under cold storage conditions (4°C) by H_2O_2 treatment. The results in this study are for intact fresh mushrooms, while previous reports on such treatment dealt with sliced mushrooms.[3] The presence of bacteria on the mushroom surface was drastically reduced by H_2O_2 treatment. Certain bacteria species (especially *Pseudomonas* spp.) are known to induce mushroom browning during cultivation and after harvest.[2] Therefore, the elimination of such harmful bacteria is positively affecting mushroom quality. Another by-product of H_2O_2 treatment could be the elimination of other harmful (pathogenic) and undesirable bacteria.

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