

Final Report

New innovations to improve mushroom whiteness and shelf life

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Applied Horticultural Research

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New innovations to improve mushroom whiteness and shelf life MU19005

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Summary

A detailed desktop study has been conducted on mushroom whiteness. As well as detailing the causes of browning, the study has thoroughly reviewed pre-harvest, harvest and postharvest strategies to improve whiteness at harvest and retain colour through the supply chain.

Peer reviewed literature on mushroom quality has increased immensely in the last few years. This almost entirely due to increased focus on this topic by Chinese researchers. There is now substantial literature on the cellular and metabolic process involved in browning of mushrooms, as well as a wide range of methods to retard this process. While many papers describe treatments or technologies not readily adopted commercially in Australia, such work still identifies some promising areas for future investigation.

The review therefore describes the various causes of mushroom browning, including microbial, physical and senescence effects. This is followed by chapters on mushroom breeding, crop production and nutrition, irrigation methods and additives, harvest and postharvest handling. While some of the techniques may be relatively old (~20 years) they are still relevant to current commercial practice. Others are new, such as the application of electrolysed water, automated subsurface irrigation, novel packaging with antimicrobial or antioxidant effects and fumigants such as ozone or 1-MCP. The review includes key points from each section, as well as pictures, diagrams and graphs where appropriate. It is written in an easy to follow format, with significant findings dot pointed and discussed to enable the reader to focus on the areas of greatest interest.

The review conclusions have been summarised in a “Best Bets” guide to improving mushroom whiteness and storage life (to be published in the AMGA magazine as well as made available online) and will be presented to industry members as a short webinar.

Based on the most promising opportunities, a number of research gaps have been identified. These include effects of supplements on postharvest quality, additives to irrigation water, validation of new irrigation technology, fumigation with 1-MCP and the effects of ozone in the storage environment. There are also promising developments overseas, particularly in the area of mushroom breeding, where the industry may wish to keep a watching brief.

Keywords

Mushroom; *Agaricus bisporus*; postharvest; browning; bruising; bacterial blotch; colour; calcium

Introduction

Presenting clean, white mushrooms to consumers at retail is a proven method of increasing sales. Conversely, browning mushrooms lack freshness, appearing damaged or near the end of storage life. Finding ways to improve and maintain mushroom whiteness therefore has the potential to boost sales and reduce waste.

Mushroom browning is primarily an enzymatic reaction, the speed and intensity of which depends on the concentrations of enzymes such as polyphenol oxidase (PPO) and phenolic compounds present, as well as pH, temperature, water activity and availability of oxygen. Bruising from handling and impacts and disease, usually bacterial blotch (*Pseudomonas tolaasii*) are the most common causes of browning. Browning can also be initiated by dehydration or simply age and senescence.

International research into improving mushroom quality and whiteness has accelerated significantly since 2012, a change driven almost entirely by China. However, many published papers are focused on postharvest treatments such as modified atmosphere packaging, dips, washes and coatings, which have limited relevance to the Australian industry. Nevertheless, they can provide insight into strategies to slow mushroom browning processes.

This project has undertaken a detailed review of pre-harvest, harvest and postharvest techniques and technologies reported to improve mushroom whiteness. Promising areas requiring further work to achieve commercialisation have been identified, along with proven methods that industry could adopt right now. The results of the review and industry consultation have been compiled into a grower focused “Best Bets” guide to improving mushroom whiteness.

Methodology

Desktop review

This project has undertaken a detailed review of pre-harvest, harvest and postharvest techniques and technologies reported to improve mushroom whiteness. More than 120 peer reviewed publications were accessed. These describe the effects on mushroom whiteness of crop production and nutrition methods, irrigation practices, a wide range of additives to irrigation water, harvest practices, postharvest handling including novel packaging treatments, coatings and packaging and finally mushroom breeding.

Information is presented as a series of dot points, with a 'key points' block at the start of each section. This summarises the main conclusions derived from the literature, without the reader needing to necessarily read the entire section. Where appropriate, graphs, diagrams and photographs have been included so as to clarify research findings. In some cases these are copied directly from published research, in other cases they have been created based on presented information.

The review is written in a plain English style, suitable for the lay reader. Although more than 60 pages long, the review is intended to provide a comprehensive, yet succinct summary of information on this topic. The review is attached to this report as **Appendix 1**.

Preliminary trial – irrigation water

A small scale trial was conducted examining one of the most promising methods for increasing and maintaining mushroom whiteness (irrigation with 0.3% calcium chloride) as well as testing an industry members observation that filtered water produced whiter mushrooms than tap water. Nine tubs of 7kg mushroom compost plus casing were assembled at the March Lawson Mushroom Research Unit. All were watered with tap water until pins appeared, after which they were irrigated with tap water, reverse osmosis filtered water or a 0.3% CaCl₂ solution.

Yield from each replicate tub was recorded, and one 500g punnet of mushrooms overwrapped and stored at 3°C. Colour was measured initially then at intervals until the mushrooms were no longer acceptable. The L, a and b values were used to calculate the "whiteness index" and "browning index" according to a published methodology.

A report on the trial is attached as **Appendix 2**.

Extension and communication

The results of the review and industry consultation have been compiled into a grower focused "Best Bets" guide to improving mushroom whiteness (attached as **Appendix 3**). While this will be available as a standalone document, it will also be included as an article on the project in the summer edition of the AMGA journal, and presented in a webinar scheduled for October 2020. The webinar will be available afterwards for industry members unable to participate at the time.

Research recommendations

Based on the results of the review, the most promising areas requiring further work to achieve commercialisation were identified. These are described and ranked in **Appendix 4**.

Outputs

- Comprehensive desktop review of the factors that may increase / reduce mushroom whiteness, initially and during postharvest storage (Appendix 1)
- Writeup of small irrigation trial results (Appendix 2)
- Best bets document for publication in the AMGA journal (Appendix 3)
- Recommendations for future research investment and development (Appendix 4)
- Webinar for presentation to the industry during October 2020, accessible live and later online.

Outcomes

A thorough review has been conducted on the factors affecting mushroom quality and whiteness. The industry has access to this information, both as a full report and as a summarised “Best Bets” document available in the Mushroom Journal magazine and online.

A preliminary trial has demonstrated the benefits of irrigation with 0.3% CaCl₂ in improving mushroom quality at harvest and during storage. The CaCl₂ treatment doubled storage life for first and third flush mushrooms compared to irrigation with tap water alone, with a 20% improvement for second flush mushrooms. There was no effect of irrigating with reverse osmosis filtered water.

Key technologies and techniques which could benefit the industry, but need some further work to achieve commercialisation, have been identified and proposed as projects or watching briefs.

Monitoring and evaluation

As this project was a short (6 months) desktop review exercise, with a single milestone report, no M&E Plan was developed.

The project has met all key objectives, including consultation with industry members, a detailed in-depth review and creation of a range of outputs.

Recommendations

A number of investment opportunities have been identified and ranked. While these are described in detail in **Appendix 4**, they are summarised as follows:

1. Additives to irrigation water

- A.** Investigate the costs and benefits of commercial application of CaCl₂, Se and possibly brassinolide and L-arginine in irrigation water, including the potential to make health claims based on increased content of calcium / selenium.
- B.** Examine whether the CALCLEAR system increases calcium uptake by mushrooms from moderately hard tap water.
- C.** Determine whether irrigation with electrolysed water (EW) can reduce infection by bacterial blotch and improve mushroom whiteness.

2. Mushroom supplements

Examine the effects of different commercial mushroom supplements on postharvest quality of mushrooms. This should include assessment of the effects of supplements on both susceptibility to bruising and senescent browning during storage.

3. Irrigation methods and moisture monitoring

- A.** Compare the accuracy and responsiveness of different sensors that could be used to monitor moisture levels in mushroom casing and compost and evaluate how these could add value in a commercial setting. This should include the sensors used in the Vullings and Netafim systems.
- B.** Compare the whiteness and quality of mushrooms grown using sub-surface drip irrigation with those irrigated conventionally, when managed using the most effective sensor as identified in Part A.

4. Postharvest treatment with 1-MCP

A small trial (e.g. student honours project) to examine the effects of 1-MCP on whiteness of stored mushrooms with regard to appearance at retail sale.

5. Postharvest treatment with ozone

A small trial (e.g. student honours project) to examine the effects of ozone on whiteness of stored mushrooms with and without inoculation with bacterial blotch.

6. Breeding whiter mushrooms

It is not proposed to develop a breeding program in Australia. However, a watching brief should be maintained on international progress in this rapidly progressing field.

Refereed scientific publications

Not applicable

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

Acknowledgements

The project team acknowledge the valuable input of Tim Adlington, Judy Allan, Warwick Gill and Graham Price into this project, particularly their “ground-truthing” of reported research results, adapting them to the needs of the Australian mushroom industry.

Appendices

Appendix 1 – Desktop review

Appendix 2 – Irrigation water additives report

Appendix 3 – Best Bets document, for publication

Appendix 4 – Research needs and Recommendations

Improving mushroom whiteness

A desktop review

By Jenny Ekman, AHR

MU19005

Improving mushroom whiteness – a desktop review

Report by Dr Jenny Ekman for project MU19005

August 2020

Applied Horticultural Research

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1 Introduction

White mushrooms are quality mushrooms

Surveys of consumers consistently indicate that they prefer mushrooms to have white colour, firm texture, consistent maturity and good flavour. Of these, colour is clearly the top priority, and often the only one that can be easily considered at retail. Presenting clean, white mushrooms to consumers at retail is a proven method of increasing sales. For mushrooms, whiteness signals quality. It may also be assumed to indicate storage life, flavour and freshness.

Conversely, browning on mushrooms is a negative for consumers. Browning may be due to disease, bruising, dehydration or simply age and senescence. Browning mushrooms are more likely to be soft, slimy and/or with poor flavour and texture, as expected near the end of storage life.

Mushroom browning is primarily an enzymatic reaction, the speed and intensity of which depends on the concentrations of polyphenol oxidase (PPO) and phenolic compounds present, as well as pH, temperature, water activity and availability of oxygen. Browning can be induced by rough handling, senescence or bacterial infection, particularly by *Pseudomonas tolaasii*¹.

Quality increases sales

Improving and maintaining mushroom whiteness can potentially boost sales and reduce waste. This is particularly important at the current time; mushroom consumption has remained static at around 2.8 to 2.9kg per person/year since at least 2017, while production has increased slightly and exports fallen. The result has been downward pressure on prices at the same time as costs for energy and raw materials (peat, wheat straw) have increased dramatically.

Moreover, as for other horticultural industries, COVID-19 has had significant impacts on mushroom purchasing behavior. According to market research organisation [fiftyfive-5](#), almost half of all grocery shoppers believe that packaging is important to prevent spread of the virus. This suggests that pre-packed mushrooms may improve their share of the retail market. The crisis has also influenced how people cook, with more consumers cooking from scratch and making complex meals at home.

Conversely, demand for produce by restaurants, cafes and other food service has plunged due to COVID-19. A significant percentage – 28% – of mushroom sales are to food service, which is more than for many other horticultural products. For example, 20% of all vegetables and only 14% of fruit are sent to food service ([Hort Innovation, 2020](#)). The mushroom industry is therefore strongly exposed to fluctuations in demand from cafes and restaurants.

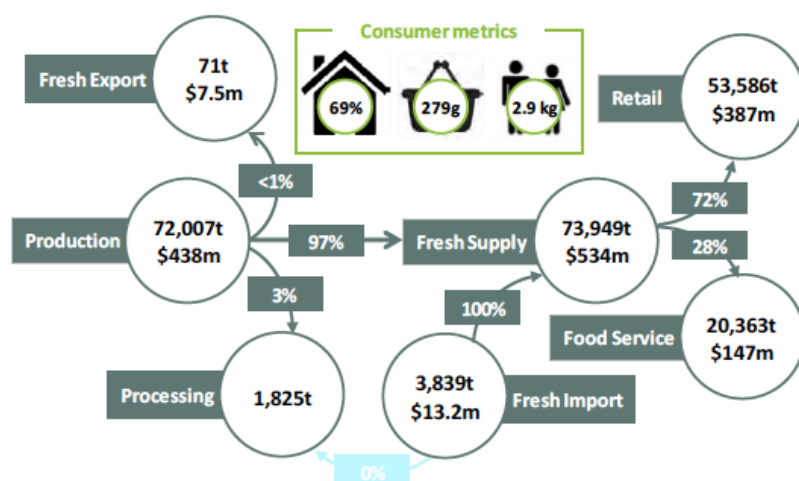


Figure 1. Australian mushroom production by volume, value and supply chain. Data from the [Australian Horticulture Statistics Handbook 2018/19](#).

International research on mushroom quality

A search for “Topic = *Agaricus* + quality” in the peer reviewed literature using the CAB abstract search engine reveals a huge surge in the number of publications on this topic. While many publications may only tangentially relate to improving mushroom quality and whiteness, this does demonstrate the international focus on this issue.



Figure 2. Number of peer reviewed publications retrieved using a search for "Topic = *Agaricus* + quality" using CAB abstracts.

On further examining this phenomenon, it becomes clear that this is entirely driven by increased research conducted in China. Peer reviewed publications from Chinese institutions have increased from only one in the two-year period from 2007-2008 to 41 in 2017-2018. There have been a staggering 66 papers already published from 2019-2020, even though at the time of writing there is still six months of the period to run.

At least half these papers describe postharvest treatments to improve storage life and quality. Examples include modified atmosphere packaging, dips and coatings. While the treatments described may not be directly applicable in the Australian commercial environment, they can provide further insight as to the mechanisms of mushroom browning, and strategies to prevent or slow these processes.

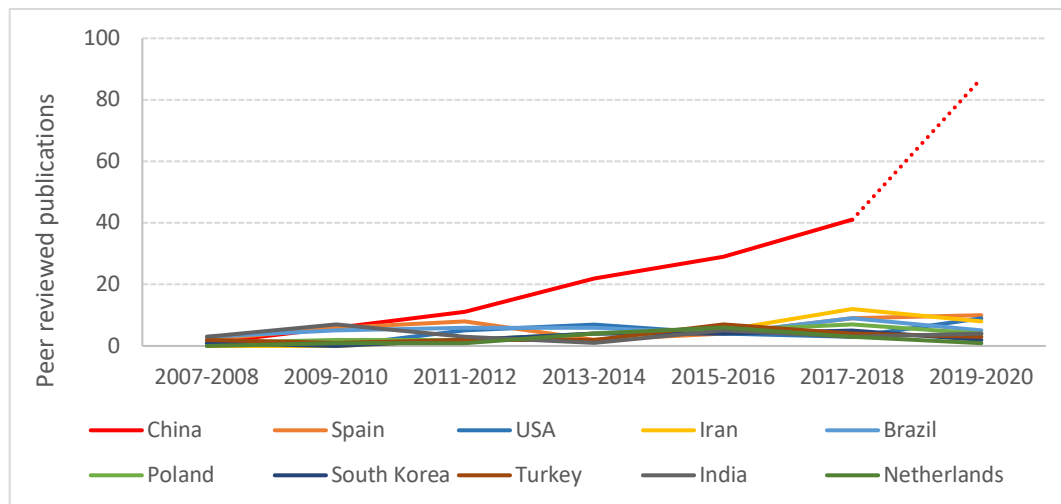


Figure 3. Total peer reviewed publications from the top 10 mushroom research countries, retrieved using a search for "Topic = *Agaricus* + quality", analysed by two year period + country of origin. Only the top 10 countries are included.

Measuring whiteness of mushrooms

The most common way to report mushroom colour is the CIE $L^*a^*b^*$ colour scale, usually measured with a Minolta chromameter. The L value corresponds to whiteness, as it indicates a degree of reflectance from 0 (black) to a maximum of 100 (white). The "b" value is also sometimes used, as it indicates an increase in yellow (+ve) or blue (-ve) tones. As mushrooms are white, values for a and b are generally low. Good quality mushrooms should have an a value 0-1 (no redness) and b of 6-10 (indicating very slight yellowness)².

According to Gormley and O'Sullivan³, L values indicate;

L value	Grade
>93	Excellent
90 to 93	Very good
86 to 89	Good
80 to 85	Reasonable
<80	Poor

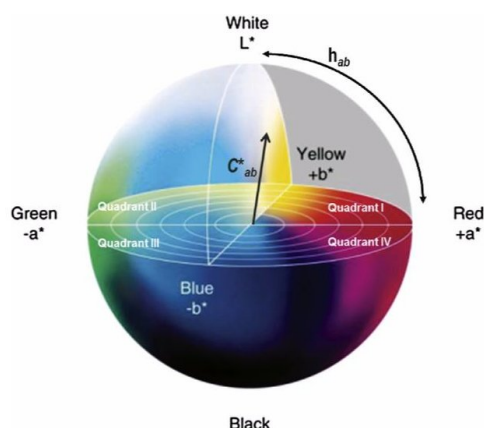


Figure 4. The CIE $L^*a^*b^*$ colour space.

Some researchers cite the " ΔE " value. Delta E indicates the degree of change - browning - from initial values or from an "ideal" white mushroom. A number of formulas may be used, some weighting the different colour parameters according to how they are perceived by the human eye. Delta E values can provide information on aggregate colour or, more often, changes from initial

values during storage. While ΔE values are useful within trials, L values are the most consistent for comparing results from different studies.

Although a and b values are small, they can have a strong impact on perceived mushroom whiteness. As a result, some authors calculate values which weight a and b values against L^4 . For example,

Browning index (BI) = $(100(x - 0.31))/(0.17)$ where $x = (a + 1.75L)/(5.645L + a - 3.012b)$
(reported by M. Maskan, 2001, in work on colour change of kiwifruits)

Whiteness index (WI) = $L - 3b + 3a$
(Paper whiteness standard developed by the Technical Association of the Pulp and Paper industry)

These calculations are much more sensitive to changes in mushroom whiteness than simply citing the “L” value as they can detect significant colour changes which are at the limit of what is perceptible by the human eye.

2 Causes of mushroom browning

Microbial

Bacteria

Key point

Bacterial blotch, usually caused by *Pseudomonas tolaasii*, is the main bacterial disease that can cause browning of mushrooms. *P. tolaasii* is commonly present in compost and can cause disease even if initial populations are low. Symptoms are primarily due to production of the toxin **tolaasin**, which breaks down cell membranes, catalyzing formation of the brown pigment melanin. The bacteria switches between pathogenic and non-pathogenic forms in response to environmental stimuli. Wetness on mushroom caps is key to development of disease, with later flushes and poorly nourished crops generally more susceptible.

- Bacterial blotch of mushrooms is a complex disease, characterized by light to dark brown sunken lesions on the mushroom caps.
 - It can be caused by a number of Pseudomonad bacteria including *Pseudomonas tolaasii*, *P. 'reactans'*, *P. costantinii*, *P. gingerii*⁵, *P. fluorescens*⁶ and several others.
 - *Agaricus* mushrooms can also be affected by cavity disease, caused by *Burkholderia gladioli*. Symptoms range from mild blotching to deep, sunken cavities extending through the cap⁷.
 - Other bacterial diseases include *Janthinobacterium agaricidamnosum*, which causes soft rot, and *P. agarici*⁸ which causes drippy gill or yellow blotch.
- Bacterial blotch is considered the most important disease affecting quality due to discolouration of the mushroom caps. *P. tolaasii* is the main cause of bacterial blotch symptoms. While it can devastate crops pre-harvest, growth and symptoms more commonly occur during postharvest storage⁹.
- The *P. tolaasii* bacteria can easily spread on pests (e.g. flies), equipment and staff, as well as being present in compost. Factors that increase the risk of bacterial blotch developing can occur during composting, spawn run, casing production and at harvest (Figure 6). In many cases, the pathogen can be detectable without any apparent symptoms on mushrooms.
- *P. agarici* has been identified as a frequent cause of browning in Europe⁸. The key symptoms of infection were previously considered to be bacterial exudates from the gills and stipe (hence 'drippy gill'). However, recent reports from Europe and South Korea have described symptoms similar to mild brown blotch (W. Gill, pers. com.) (Figure 5).



Figure 5. Sunken brown lesions caused by *P. tolaasii* (left) and more superficial lesions caused by *P. agarici* (right). From Milijasevic-Marcic et al, 2016.

FACTORS INFLUENCING DEVELOPMENT OF BACTERIAL BLOTCH

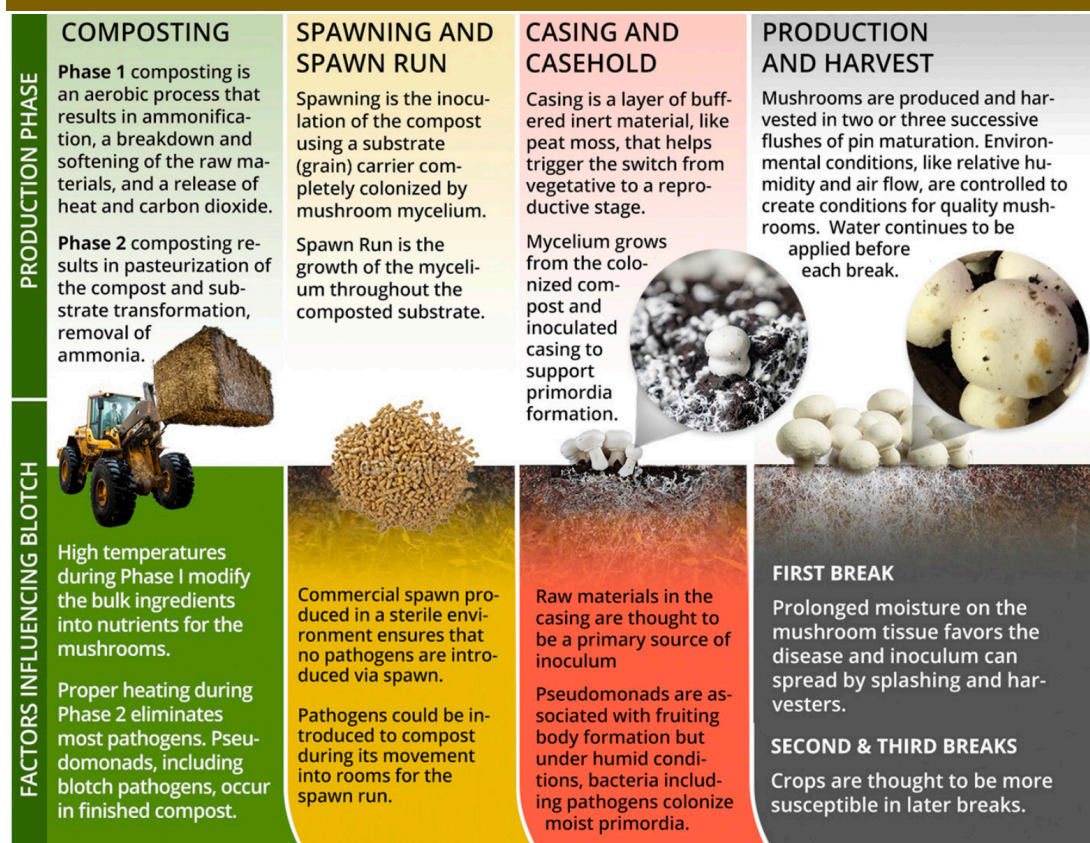


Figure 6. Factors influencing the development of bacterial blotch at different stages of mushroom production. From Osdaghi et al, 2019.

- Bacteria in compost are attracted to the growing *Agaricus* mycelium, particularly to the young hyphae. They use their flagella (tails) to move through the wet compost or casing and then adhere to the outside of the mycelia⁹.
- Browning on infected mushrooms is due to the toxin **tolaasin**, which is only produced by the pathogenic variant of *P. tolaasii*. Dropping tolaasin onto the mushroom caps can replicate symptoms of the disease without any bacteria present¹⁰ (Figure 7).
- Tolaasin breaks down the cell membranes that normally separate tyrosinases and their substrates. This in turn leads to formation of the brown pigment melanin¹¹. For the mushroom, melanin acts as a chemical barrier, limiting spread of the bacteria into neighbouring cells⁷. However, melanin is also the cause of mushroom browning.
- It was recently found⁸ that bacteria from the genus *Mycetocola* (*M. tolaasinivorans* and *M. lacteus*) detoxify tolaasin, as well as inhibit spread of *P. tolaasii*. While the 'helper' bacteria prevented bacterial blotch symptoms on mushrooms, infection by *M. lacteus* alone also led to slight browning of mushroom tissue. Despite this, the authors suggest that understanding the mechanism of inhibition could allow development of biocontrol strategies for commercial use.

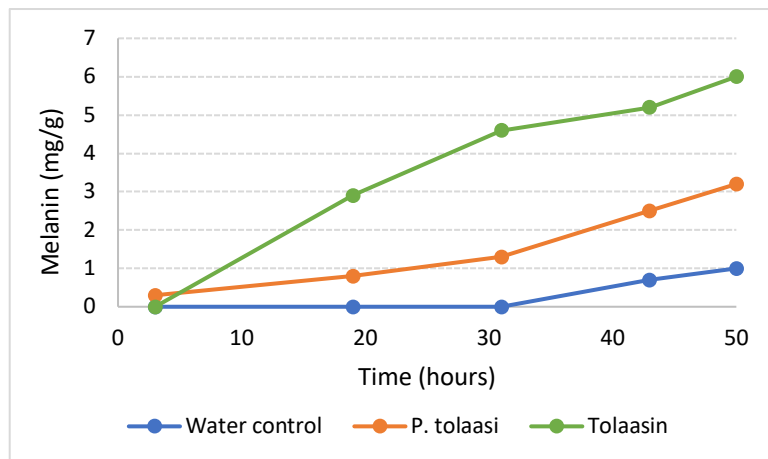


Figure 7. Formation of melanin in mushroom caps following application of water, a suspension of *P. tolaasii*, or partially purified tolaasin extract. Derived from Soler-Rivas et al., 1999.

- *P. tolaasii* bacteria present two different appearances when cultured; a wild-type or “smooth” form which is pathogenic to mushrooms, and a variant “rough” type which is non-pathogenic. *P. tolaasii* switches between the two in response to environmental signals, particularly high relative humidity and free water on mushroom caps¹⁰.
 - Populations of the non-pathogenic “rough” form tend to increase in culture as the bacterial populations multiply, which is thought to be due to depletion of nutrients and accumulation of secondary metabolites¹⁰
 - The ability of *P. tolaasii* to switch between pathogenic (smooth) and saprophytic (rough) forms enables it to colonise many different substrates and environments
 - The bacteria can be present in a wide range of organic materials, including compost and peat and spreads easily on pests, workers and equipment¹².
- Growth of blotch is associated with free water on the mushrooms, which is more likely if:
 - Bed temperatures are low, resulting in slower evaporation rates following irrigation⁹
 - Casing has become resistant to water infiltration, especially as splashing water spreads bacteria to pins, which then stay wet for longer⁹
 - Mushrooms are cooler than the room air; If relative humidity (RH) is high within the grow room, then fluctuations in temperature can lead to condensation on the mushroom caps¹³.
 - Fans used to dry mushrooms after irrigation increase the air temperature inside the room, leading to ‘sweating’ by the cooler mushrooms.
 - The issue may be overcome by increasing temperature before irrigation to reduce humidity, then keeping temperatures stable as the mushrooms dry¹²
- There is no clear population threshold at which *P. tolaasii* can cause blotch, with the populations reported as being sufficient to cause symptoms ranging from 200,000 CFU/cap to 100,000,000 CFU/cap. This suggests that even relatively low populations of bacteria can cause disease, if conditions are conducive. As *P. tolaasii* is widely present in compost and casing, this emphasises the importance of avoiding environmental situations which facilitate its development⁹.

Fungal diseases

- The main fungal disease of mushroom in Australia include dry bubble, cobweb, green mould and wet bubble. All these diseases significantly reduce mushroom yields and quality and can cause browning.
- However, the damage caused by fungal diseases is usually catastrophic to infected mushrooms. These mushrooms are unlikely to be marketable, due deformity and rapid breakdown. While browning may occur, it is unlikely to develop postharvest in packed mushrooms.
- Moreover, the management of fungal disease in mushrooms is reasonably well understood, and there are recognised experts in Australia who work actively in the industry to help growers manage disease.
- *Fungal diseases are therefore not considered further in this review.*

Viruses

Key point

The two main viruses that can increase mushroom browning are mushroom virus X (MVX), or Brown cap mushroom disease, and La France disease. La France affected mushrooms are malformed as well as browned, so unlikely to be marketable. Symptoms of MVX are sporadically expressed within crops and frequently subtle, resulting in slight off white colour rather than obviously brown caps. The simplest method of detecting the virus is by objective colour measurement, with confirmation by PCR techniques. MVX is not currently confirmed as present in Australia.

- While a number of viruses can infect mushrooms, only a very few cause disease symptoms. Viruses are difficult to study, and it can take some time between observation of symptoms and association with a virus. For example, La France disease was described in 1950, but it took more than 10 years to isolate the virus responsible¹⁴.
- Symptoms of La France disease include elongated stalks, small caps and browning mushrooms. Originally identified in Pennsylvania¹⁵, it is a significant issue on mushroom farms around the world including Europe, Turkey and Australia (W. Gill, pers. com).
- Mushroom virus X (MVX), also known as Brown Cap Mushroom Disease (BCMD) was first described in the 1990s. The disease is related to a complex of virus strains, which cause a range of symptoms that include developmental delay, malformed caps, bare patches in the bed and cap browning¹⁶.



Figure 8. Pale brown mushroom emerging from a BCMD infected crop (left) from Eastwood et al., 2015; La France affected mushroom with small tilted cap and discoloured cap and stipe (right) from Fletcher, 2003.

- The effects of MVX can be subtle; infected mushrooms may become brown or simply appear slightly 'off white' to the human eye. However, objective colour measurement using a Minolta chromameter can detect a difference between infected and non-infected mushrooms using the L (lightness) and b (yellowness) values on the L*a*b* colour scale (Figure 9).
- Similar results were reported by Fleming-Archibald et al¹⁷, who used ΔE values to assess browning; the closer to 0, the whiter the mushroom. Infected mushrooms frequently returned ΔE values >10 but were only visibly browned if ΔE was >15 . Perhaps surprisingly, symptoms were worse in the first than the second flush.
- Virus particles have been shown to be concentrated in the growing edges of the mycelium, and may be present at low levels in crops which show no symptoms of the disease¹⁸.

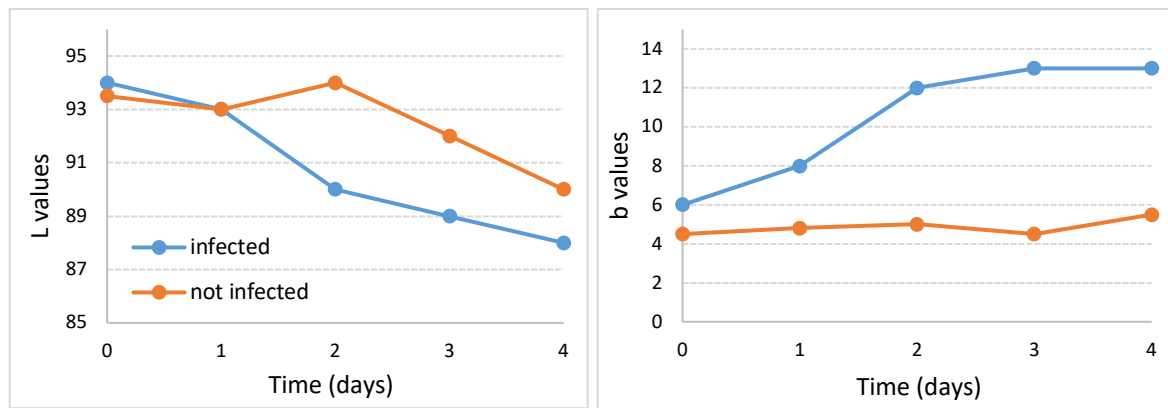


Figure 9. Values for "L" (lightness) and "b" (yellowness) of developing mushrooms that were infected or not with MVX. The non-infected mushrooms appeared off white to the human eye. Derived from Eastwood et al., 2015.

- MVX is not known to be currently present in Australia (W. Gill, pers. com.)
- There are currently no cures for virus diseases of mushrooms or known resistant varieties. Maintaining a high level of biosecurity to exclude the virus from production areas is the most effective method of control. Positive pressure inside growing rooms can stop air-borne virus particles entering, particularly during critical operations. The only other control option is elimination through effective cookout to kill host mycelia, followed by strict hygiene and disposal of waste products¹⁷.

Physical – senescence, handling and bruising

Key point

Browning in mushrooms is due to reactions between enzymes (polyphenol oxidases) and phenolic compounds held in the cell vacuoles. Disruption of the cell membranes due to physical damage, or simply ageing and senescence, allows the enzyme and substrate to mix, forming the brown pigment melanin. Varieties that have reduced levels of substrate or enzymes are likely to be less susceptible to bruising and browning in general. Susceptibility to browning also differs between varieties, over time and by size, maturity and flush.

What turns mushrooms brown?

- Mushroom browning is primarily catalysed by six different forms of tyrosinase, and to a slightly lesser extent by laccase laccases and peroxidases. These enzymes oxidise phenolic compounds, so are generally referred to as polyphenol oxidases (PPOs)¹⁹.
- Normally, phenolic compounds are kept separated from PPO enzymes. The enzymes are contained within the cell vacuoles, whereas phenolics are present in the cytoplasm. However, senescence or physical damage, as well as microbial infections, can break down these barriers, allowing the substrates to mix (Figure 10).
- The initial reaction forms quinones, which then undergo further reactions to form the brown pigment melanin²⁰. This is the brown colour observable on old or bruised mushrooms.
- Browning is therefore governed by factors that include the amount of tyrosinase present, its activity and presence of phenolic substrates²⁰.

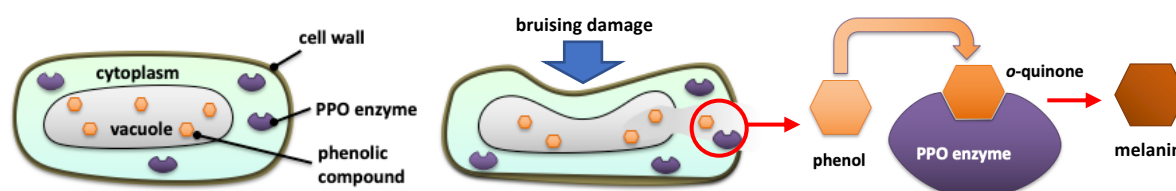


Figure 10. Process by which physical damage causes mushroom browning. Damage to cell membranes allows phenolic compounds to mix with enzymes in the cell cytoplasm. These catalyse a series of reactions culminating in production of the brown pigment melanin.

- Researchers in India have developed two hybrid mushroom strains with low levels of tyrosinase and laccase, enzymes that are involved in browning. These strains have reduced reaction to mechanical injury, with no browning observed on cut surfaces two hours after slicing²¹.
- The potential to reduce browning through breeding new varieties is discussed in more detail in Section 6 of this report.

Bruising

- One of the reasons mushrooms bruise so easily relates to their structure²². The cap surface overlays a relatively low cellular density zone with limited resistance to crush damage, compared to the higher density core tissue which determines texture (firm or soft).

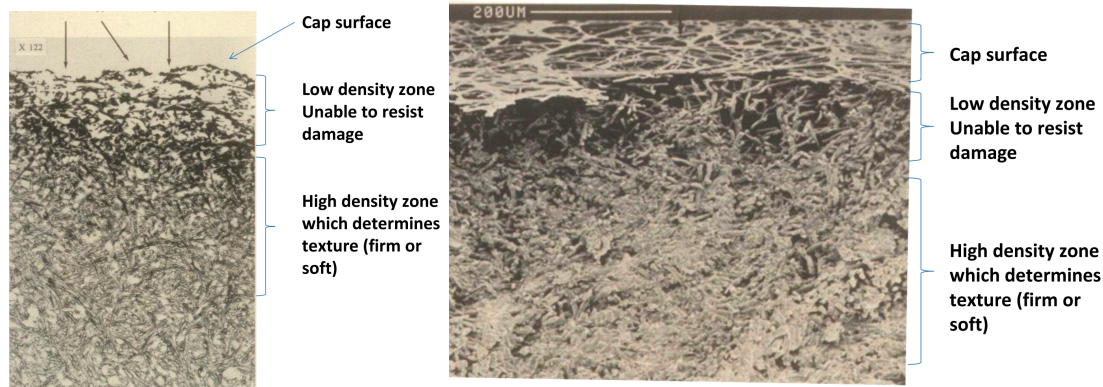


Figure 11. Mushroom cellular structure and density is a factor in bruising susceptibility. From Burton, 2011.

- Mushrooms are easily bruised during picking, particularly by shear forces that rub across the mushroom cap. Avoiding damage during harvest requires good supervision, effective training and well-motivated employees²³. Some growers have found that paying hourly rates results in better quality than paying by weight picked.
- Bruising was examined in some detail by Weijn et al, 2012²⁰. The factors that increased bruise development included;
 - Variety**; The variety Somycel X135 developed less severe bruising than varieties Darlington 735 and Horst U1 (Figure 12)
 - Time**; bruises continue to develop and darken for >2 hours after damage has occurred (Figure 12)
 - Storage interval**; as the time between harvest and damage increased, so did the degree of bruising, with mushrooms significantly more susceptible to bruising 24 hours after harvest than 2-4 hours after harvest (Figure 12)
 - Size and maturity**; a trend was noted to increased bruising susceptibility in small mushrooms (25-35mm) compared to larger ones (55-70mm).
 - Small mushrooms with open caps were the most susceptible to bruising. However, large mushrooms with open caps were less susceptible to bruising than those with closed caps.
 - As a result, differences due to maturity were not significant overall (Figure 13).
 - It should be noted this is a different result to earlier researchers, who found that mushrooms harvested at earlier developmental stages are less susceptible to browning, presumed due to their lower levels of enzymes.
 - Flush**; although first flush mushrooms were more sensitive to bruising than those from the second flush, the third flush was the most easily bruised.

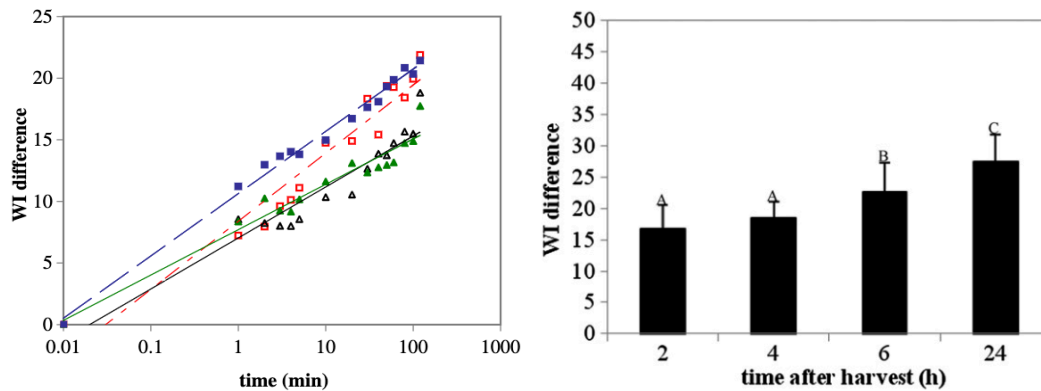


Figure 12. Bruise development of four mushroom varieties over time (left) and the effect of the time interval between harvest and damage (right). Bruising was measured in terms of the whiteness index (WI), which measures the difference between the bruised and non-bruised parts of the mushroom cap. From Weijn et al., 2012.

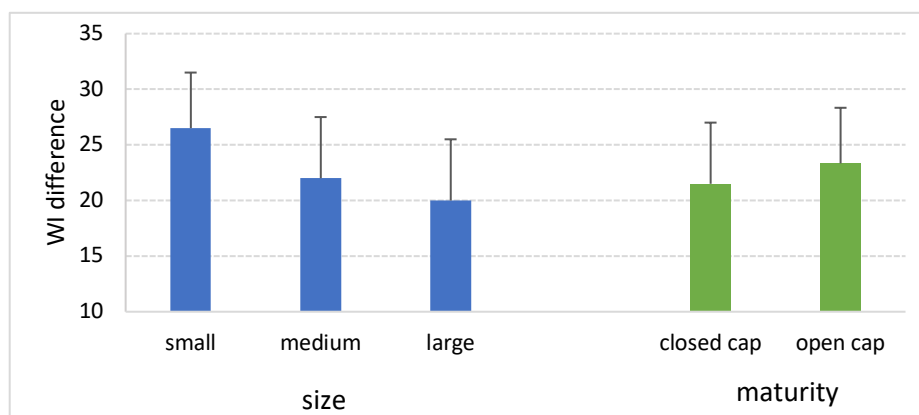


Figure 13. Average bruise development according to mushroom size and maturity. Bars indicate the approximate std. deviation of each mean value. Derived from Weijn et al., 2012.

- Two compounds (GHB, GDHB) are associated with bruising sensitivity. These compounds are 15x to 20x higher in bruising sensitive strains compared to bruise tolerant strains²⁰

Other physical causes of browning

- **Low relative humidity;** low RH degrades texture and structure and may increase enzyme activity¹, with the result mushrooms can become scaly. Low RH (85%) also increases susceptibility to bruising compared to higher RH (92%) particularly for early flushes²⁴.
- **Casing wetness;** dry casing can increase susceptibility to browning and bruising²⁴.
- **High temperatures;** high temperatures stimulate respiration and increase the rate of senescence, as well as activating tyrosinase and potentially increasing growth of bacteria on the mushroom surface²⁵.
- **Rapid airflow;** While it is important to dry mushrooms quickly, if the air velocity is too high mushroom caps are likely to develop dry scales and browning²⁶.
 - Rapid airflow physically damages the mushroom tissue, stimulating formation of melanin²⁷.
 - Managing CO₂ accurately and uniformly within the room is difficult without adequate airflow. Air-trainers, such as netting diffusers and cones linked to airlines, can be used to soften or increase speed, guiding ventilation to where it is needed.

- Airflow needs to be balanced against the metabolic heat produced by the crop. So, for example, rates of air exchange may need to be higher approaching flush 1 than during flush 3.
- **Flush;** In general, first flush mushrooms are whitest, with colour declining thereafter. Third flush mushrooms may also brown more quickly than first flush mushrooms during storage²⁷.
- **High moisture;** Mushroom caps can contain tiny dimples, which allow water to pool. Even if bacterial blotch is not present, these can result in 'watermarks' on the cap surfaces.

Summary

The causes of browning are summarised in Figure 14. Whether caused by physiological changes, external damage or microbial activity, browning of mushrooms is essentially the result of oxidative processes that form the pigment melanin.

Avoiding damage and minimising microbial activity are obvious ways to reduce mushroom browning. In addition to this, many strategies for enhancing mushroom whiteness are focused on inhibiting the reactions that form melanin. Treatments may aim to enhance the strength of cell membranes, prevent oxidative reactions, or create genetic changes that reduce enzyme activity. Strategies to improve whiteness can be employed at all stages of production, from casing to harvest, packing and storage. The following sections of this review will discuss the many different methods proposed to enhance mushroom whiteness.

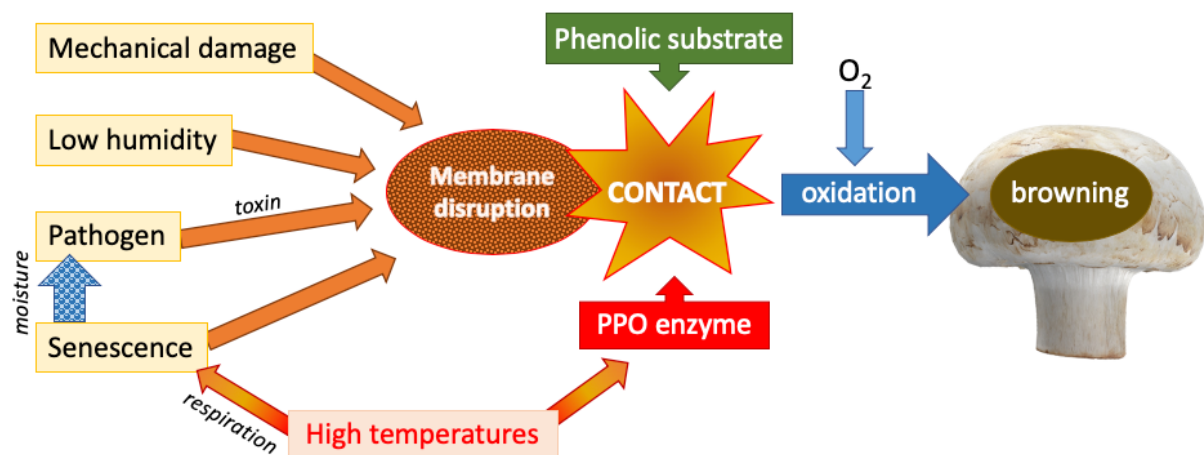


Figure 14. Causes and contributors to browning of mushrooms.

3 Crop production and nutrition

Casing materials

Key point

Adding different amounts of lime to casing has mixed effects on colour, but small particle sizes may be beneficial. Casing made from black peat plus lime generally results in whiter mushrooms than alternative casing materials. However, a percentage of the peat can be replaced with other materials (e.g. SMC) without impacting mushroom colour.

Casing attributes

- Casing commonly consists of peat, plus lime which is added to neutralize pH. Sugar beet lime (SBL) is a waste product commonly used in Ireland, the UK and North America. Trials tested the effect on mushroom colour of different grades of SBL, added to peat at rates of 50 to 125 kg/m³;
 - The highest rate of SBL raised pH from 7.3 – 7.5, increased EC and decreased volumetric water content of the casing²⁸.
 - Mushrooms were whitest with the lowest rate of SBL of 50 kg/m³. However, the researchers note that peat is more prone to waterlogging at this rate of lime addition, with the result that yield (and dry matter) was reduced.
 - Mushroom whiteness was maximised when SBL was finely graded, reducing particle sizes from up to 4.75mm to <0.25mm.
 - It is suggested that optimum yield and colour is obtained by adding 75 kg/m³ of SBL with <0.25mm particle size to casing.
- Conversely, Burton²⁴ found that increasing the SBL content of casing from 9% to 30% – a rate likely significantly higher than the maximum volume added in the study above – reduced susceptibility to bruising in first flush mushrooms without affecting total yield.
- Although Burton²⁴ reported that shallow casing (25mm) produced mushrooms less susceptible to bruising than deep casing (50mm), this difference occurred only in the second flush and is a result from a single trial.

Casing material

- Pardo et al²⁹ tested a number of different casing materials, including mixtures of soil, black peat, brown sphagnum peat and limestone quarry gravel. The black peat generally resulted in whiter mushrooms than the other materials, although the difference was small.
- Similar results were reported by Barry et al³⁰, who found that casing with 70% peat or 100% peat produced whiter mushrooms than casing materials made from blends of spent mushroom substrate and vermiculite.
- Spent mushroom compost (SMC) has been widely investigated as a partial replacement for peat in casing. An investigation of a range of blends of SMC with peat by Pardo-Gimenez et al³¹ found that blends with up to 60% SMC did not affect colour. However, increasing the SMC to 80 or 100% significantly reduced whiteness.

- The same paper³² also compared sphagnum peat plus SBL (which produces a material with similar structure to black peat) to a local casing made of mineral soil plus coconut fibre. There was no significant difference in colour, dry matter or biological efficiency between the two.
- Addition of 25% coal tailings to either brown or black peat did not affect cleanliness (or yield) of the mushrooms produced³¹.
- In South Africa, the only local source of peat is reed-sedge 'topogenous' peat. While yields are satisfactory, this material often dirties the mushrooms, so is not a preferred casing³³.

Stimulation of pinning

Key point

Over-pinning on mushroom beds increases bruising both as the mushrooms grow and when they are picked. Careful management of CO₂ levels in the growing environment is essential; dropping CO₂ quickly can result in over-pinning, whereas dropping CO₂ too slowly will reduce yield. Reductions in temperature, the microbiota present of the substrate and the moisture content and granulation of casing also influence the number of pins that form.

- One of the key factors in producing white mushrooms is avoiding over-pinning on the beds.
 - Mushrooms that press against each other as they develop will be bruised even before harvest.
 - Tightly clustered mushrooms are difficult to pick, so are likely to be further damaged simply by the process of harvesting from the beds (T. Adlington, pers. com.).
 - Over-pinned crops are likely to be over-mature and soft, so are easily bruised².
- Pinning is stimulated in response to removal of accumulated eight-carbon volatiles (such as 1-octen-3-ol) which are produced by the mycelia. Pinning is also stimulated by drops in temperature (e.g. from 25°C to 18°C) and reductions in CO₂ levels (e.g. from 5,000 ppm to less than 1,000 ppm).
- According to Eastwood et al³⁴, CO₂ is the most important factor determining the number of fruiting bodies that develop.
 - As *A. bisporus* does not respond to either light or gravity during fruiting, it is thought that changes in the composition of the air allow the emerging mushrooms to detect cracks in the substrate and proximity to the surface.
 - Whereas the presence/absence of 1-octen-3-ol and temperature changes are control switches for mushroom formation, CO₂ determines the number of mushrooms that form.
 - Ideally, casing material should be approximately 50% colonized by mycelium (T. Adlington pers. com.); gradually reducing CO₂ can stagger pin set, known as "choking" the beds. However, if airing is delayed too much, especially with only very gradual reductions in temperature, then more mycelium will colonise the casing material and yield will be reduced.
- The removal of inhibitory 8 carbon volatiles can be achieved through airing or inclusion of activated carbon. More commonly, they are metabolized by microbiota in the casing material, particularly various *Pseudomonas* species including *P. putida*. Monitoring levels of 8 carbon

volatiles in casing, and addition of certain *Pseudomonas* isolates, could theoretically allow better control over pinning³⁵.

- The number of pins that develop also depend on the way casing material is applied; granulated, moderately moist material will result in more pins than clumping, wet material.

Microbial inoculants

Key point

A number of supplements claimed to improve mushroom yield and quality are composed of living bacteria – ‘bioinoculants’. Results are mixed, possibly depending on whether these microbes are already present in the substrate. No information was found on the effects of bioinoculants on colour.

- Mushroom production may be supported using “bioinoculants” – living organisms which can stimulate development. A range of bacteria have been suggested as potential alternatives to chemical/nutritional supplements, with the aim of increasing yield and quality. Examples include *Bacillus subtilis*, *B. megaterium*, and *Pseudomonas putida*³⁶.
- Much recent interest has focused on the fungus *Mycothermus thermophilus* (*Scytalidium thermophilum*). This organism reduces the concentration of ammonia and increases the degradation of celluloses and other materials in the compost.
 - Yield has been shown to be increased in the presence of *M. thermophilus*³⁷.
 - However, commercial products based on this species have had limited success at increasing yield and are not generally used³⁸. This may be because it is already present in compost.

Nutritional supplements

Key points

Supplements may be added at spawning or prior to casing. A wide range of commercial formulations and agricultural by-products have been tested and are available, and development of microbial inoculants seems likely in the future. While increases in yield exceeding 20% have been reported, results are highly variable. This is likely due to interactions between supplement and compost. Many studies report minimal or no effect.

There is little evidence that supplements improve mushroom quality or whiteness, and some may even have negative effects on colour. However, not all researchers measure quality attributes, and those who do rarely include postharvest assessments. This is unfortunate, as there is (limited) evidence that some supplements can significantly increase storage life by slowing postharvest browning, even though they may appear similar at harvest.

- The practice of adding nutritional supplements to compost during either spawn run or casing has been practiced since the 1960s³⁹. While the key objective has been to increase yield, it is frequently suggested that these treatments also improve quality.
- Supplements are commonly manufactured products with high protein content, such as soybean meal or cereal bran. They may be further enriched by addition of minerals or nutrients. Some are designed to correct nutrient deficiencies in compost, whereas others are reported to stimulate growth through ‘hormonal’ effects⁴⁰.
- A wide range of commercial supplements are available. Examples include;
 - ProMycel Gold, Champfood E, MCSubstradd – mainly soy protein based
 - Natural Gold – a blend of lipids and protein
 - MycroNutrient – Carboxylic acid (casing supplement)
 - Micromax – mineral micronutrients
- Low cost agricultural by-products have also been investigated, including products such as olive mill waste, cottonseed meal, grape pomace and defatted nut meals, such as from peanuts or pistachios⁴¹.
- The effectiveness of supplements depends on factors that include;
 - Delaying the release of nutrients added during spawning to ensure they are available to the *Agaricus* mycelium once it has colonised the substrate⁴²
 - Controlling increases in temperature triggered by addition of the supplement, especially as these can increase the incidence of fungal competitors⁴³
 - Matching the nutrients required with the attributes of the compost
- Addition of supplements can enable re-use of “spent” mushroom compost; after removal of the casing, second break compost is fragmented by passing through a turner with rotating drum, mixed with supplement and then re-cased⁴⁴. Unfortunately, mushroom quality is not reported.
- In 2015, Burton and Noble reviewed supplement use in Europe. They found that >90% of phase 3 compost is supplemented, usually with a protein-based product. It was widely

believed that supplements increase quality as well as yield. The products were usually applied during spawning at a rate of 1.2 – 1.5%.

- It is likely that the majority (>90%) of Australian growers also supplement compost (T. Adlington, pers. com.).
- Subsequent trials⁴⁰ examined the effect of supplements adding during spawning;
 - All of the tested protein-based supplements significantly increased yield (11.5%), while Promycel Gold and Champfood E significantly increased mushroom density.
 - Although 'L' values (whiteness) were not affected by the supplements, both ProMycel Gold and MC Substradd increased 'b' values (yellowness) (Figure 15). While this is clearly undesirable, the authors suggest this difference may not be detectable by consumers. It is further noted that 'b' values vary between flushes and between compost based on wheat straw compared to stable manure.
 - Although non-protein supplements had little effect in this trial, they had previously been reported as improving yield and quality in the US. This suggests the latter composts may be deficient in the elements these contain.

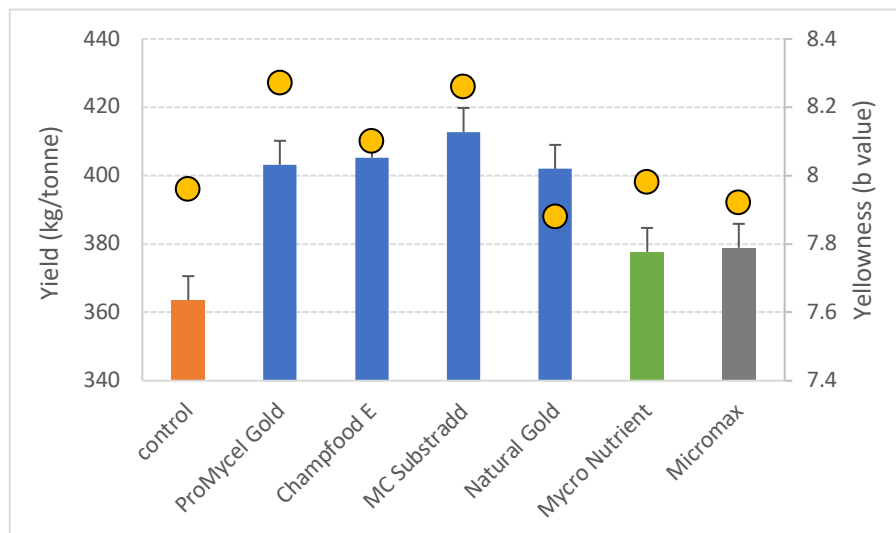


Figure 15. Effect of supplements on yield (columns) and yellowness (yellow dots) of white mushrooms. Products were based on protein or protein plus lipids (blue columns); carboxylic acid (green column) or micronutrients (grey column). From Burton and Noble (2015).

- While Spanish researchers have published prolifically on the effects of both commercial and low-cost supplements on yield and quality, effects are generally small;
 - Work published in 2012⁴⁵ did not find significant differences in either yield or colour between mushrooms grown with ProMycel Gold, Champfood S, Calprozime or various grapeseed extracts compared to non-supplemented controls.
 - ProMycel 600, up to 15g/kg defatted pistachio meal⁴¹ or 15g/kg defatted almond meal⁴⁶ failed to significantly increase yield or improve whiteness compared to non-supplemented controls.
 - Later work⁴⁷ found a 10% to 22% increase in yield when compost was supplemented with 0.8% ProMycel 480. Again, there were no significant or consistent effects on either 'L' or 'b' values, suggesting colour was unaffected.

- The trials with ProMycel 480 were repeated in Brazil. Yield was increased by 10 to 16%, with no significant effects on mushroom colour or other quality parameters⁴⁸.
- An alternative approach, but not widely used commercially, involves supplementation of casing. An Iranian study by Adibian and Mami (2015)⁴⁹ examined the effect of supplementing casing (peat) with ground corn or soybean meal.
 - While all mushrooms appeared similar at harvest, differences emerged during storage at 4°C. Adding 51g of either material to 5kg of peat significantly reduced browning during storage (Figure 16). Adding 17g or 34g had intermediate effects.
 - The 51g/5kg soybean meal treatment also significantly increased protein content.

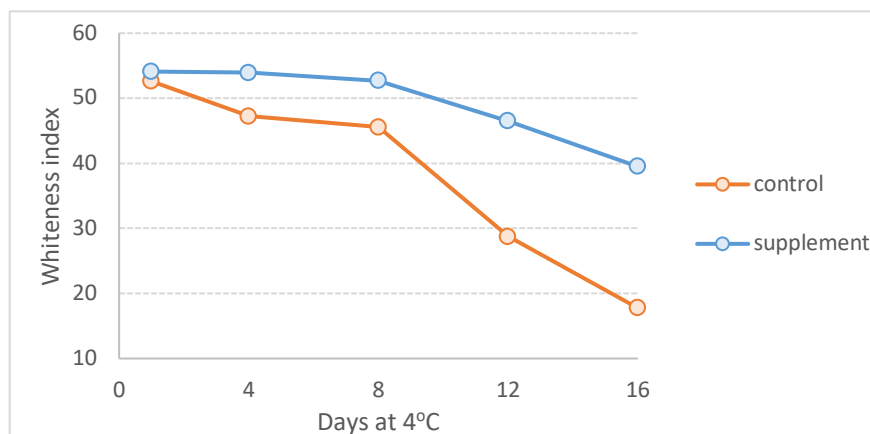


Figure 16. Effect of supplementing casing with 10g/kg corn or soybean meal (average of both treatments) on the whiteness index of stored mushrooms. Whiteness index calculated from presented data using the formula $WI = L - 2b + 3a$. Derived from Adibian and Mami, 2015.

- This effect is consistent with a report from 1991, which found that although mushrooms supplemented with 300ml safflower oil/tray at casing were similar to controls at harvest, they stayed significantly whiter during storage at 12°C. After 6 days treated and control mushrooms had L values of 82.8 and 79.5 respectively, a difference which would be clearly visible⁵⁰.
- These studies demonstrate that even if mushrooms appear the same colour at harvest, differences can develop during storage. It is therefore surprising that so few studies have examined the effects of supplements on postharvest quality of mushrooms.

4 Irrigation

Irrigation during production – current practice

Key point

While too little irrigation can result in mushrooms being scaly and dry, too much encourages growth of bacterial blotch. Water availability is the sum of matric potential (water bound to the soil) and osmotic potential (water bound to dissolved solutes). If osmotic potential is high, it is difficult for the mycelium to extract sufficient water, even if the substrate is wet (high matric potential).

At times, beds may need to be watered daily or even several times in a day, as they cannot be irrigated during harvest. Irrigation may also be used to prevent beds overheating. However, because wetness increases the risk of developing bacterial blotch, mushrooms need to dry within four hours of irrigation. This can be achieved dropping room temperature and RH before irrigating, then increasing temperature and fan speeds after irrigating.

- Mushrooms are 90 to 95% water, so the quality of water used, and when and how it is applied, will strongly influence mushroom quality and storage life.
- Low humidity, high airflow and water stress all reduce whiteness of the mushroom cap, which can become scaly and dry. If casing dries out it can become somewhat hydrophobic, a problem made worse by increased mycelial overlay on the surface³⁰.
- Conversely, excessive moisture increases the development of bacterial blotch and other diseases. It can also kill mycelia due to waterlogging and decreases whiteness of mushroom caps⁵¹.
- The availability of water in the substrate depends on water potential. Water potential is the sum of osmotic potential (water bound to dissolved solutes) and matric potential (water bound to soil particles).
 - According to Kalberer⁵², the osmotic potential in substrate can decrease from -1.4MPa at casing to -2.4MPa after 4th flush, while matric potentials range from 0 (fully wet) to only -0.3MPa.
 - Even if the substrate is relatively dry, matric potential still represents a relatively small component of total water potential. Despite this, Beecher et al⁵¹ found that matric potential had the most effect on mushroom growth and quality.
 - On the other hand, if the osmotic potential is higher in the substrate than inside the growing mycelium, then it is difficult for developing mushrooms to draw up sufficient water for good quality; osmotic pressure will pull water back into the substrate. As a result, although solutes in the water include nutrients for growth, it may be necessary to limit their concentration to reduce osmotic potential, helping water move into the mushrooms.
- Irrigation is usually applied using either fixed or manual water fountains and is a major daily activity on mushroom farms;
 - Peat (plus lime, and other materials if required) is usually supplied partially dried. While dried peat never recovers its original moisture holding capacity, mixing with water before casing ensures it is initially well hydrated.

- After casing, the crop is watered to runoff to ensure it is at full moisture holding capacity. The beds are then watered intermittently, cycling between air-drying and additional irrigation.
- Crops are commonly watered once thumbnail size pins appear, with a final irrigation just before mushrooms reach harvestable size.
- No water can be applied during harvest, as mushrooms must be picked dry.
- Once the first flush is removed, intensive watering is conducted to return boxes to full capacity, readying them for the appearance of the second break.
- Irrigation may also be managed in response to temperature; if bed temperatures are rising, more water may be needed, whereas irrigation needs to be reduced if bed temperatures are declining.
- Even though crops must be kept fully hydrated, it is essential that the mushrooms themselves do not stay wet. The aim is to dry the crop within four hours of irrigation. This can be achieved by:
 - *Before irrigating* – Dropping the air temperature to reduce absolute humidity (RH).
 - *After irrigating* – Raising the room temperature, and increasing fan speed by approximately 5-10%.
 - *After irrigating* – Introducing fresh, dry air to the grow room, if conditions are conducive.
- Evaporation rates also increase if there is a larger temperature differential between the bed and the air; increased fill rates and higher dry matter in compost can raise bed temperatures,
- increasing this temperature gradient.

Moisture levels in casing

Key point

Managing moisture levels in the casing is essential to maximise yield, with trials indicating that -8kPa soil matric potential is optimal. Maintaining constant moisture levels in the casing can improve yield and quality. However, this is difficult to achieve due to the large draw down that occurs during cropping.

The relationship between matric potential and volumetric moisture content depends on peat type, depth and lime content. Once the moisture-holding characteristics of the casing are known, more commonly available systems for measuring soil moisture directly – such as time domain reflectometry (TDR) – can be used. As a guide, maintaining casing at 58-60% volumetric water content resulted in significantly whiter mushrooms than 46-48% v/v, with casing at 52-54% v/v providing intermediate results.

Although increased availability of moisture in casing can improve yield, dry matter is likely to be reduced. The effects of dry matter on whiteness are unclear; high dry matter has been associated with both reduced and increased whiteness. However, high dry matter mushrooms generally retain whiteness for longer during storage compared to mushrooms with low dry matter. This is likely due to their increased energy reserves.

- The key purpose of the casing layer is to initiate formation of mushrooms. This is believed to be triggered by destruction/-absorption of volatile compounds produced by the *Agaricus* mycelia (primarily 1-octen-3-ol), combined with a drop in temperature and generation of CO₂ gradients between the compost and casing³⁴.
- Noble et al (1999)⁵³ has a good explanation of how to measure casing matric potential and how this relates to volumetric soil moisture content. This work also notes a number of other functions of the casing layer:
 - To supply water for growth and development of the mycelium and mushrooms
 - To protect the compost from drying out
 - To resist breakdown of the compost due to repeated watering
 - To provide support for developing mushrooms
- Peat that has been partially dried (milled) never recovers the same water holding potential as material that has remained continually wet (bulk extracted). This is due to the milled peat having a less defined structure. Even though the milled peat can't hold as much water, its' air filled porosity (AFP) is initially similar to bulk peat casing. However, AFP increases during cropping, particularly for the milled product, even though water holding capacity tends to decline⁵³.
- During flushing, the availability of water in the casing material falls dramatically. Short periods of relative dryness have little effect. However, longer term average moisture content has large effects on yield and dry matter. Yield and quality are both optimised when water is applied evenly during production and cropping, instead of allowing casing material to dry out⁵⁴;
 - Maintaining casing at 58-60% volumetric water content resulted in significantly whiter mushrooms than when casing was kept at 46-48% v/v, with casing at 52-54% v/v providing an intermediate result³⁰.

- If a heavy first flush results in dry casing for the second flush, these mushrooms will be less white and susceptible to premature opening²⁶.
- Dry casing can result in water stress; mushrooms may feel soft and damp and have increased susceptibility to browning and bruising²⁶.
- Wet casing overlaying dry compost reduces evaporation from the mushrooms. This can result in browning mushrooms that weep liquid, or water-soaked “windows”²⁶.
- Optimising moisture levels in the casing material is essential to maximise yield. However, a balance is required; even though increasing the water content of casing can increase yield (up to a point), these mushrooms will have reduced dry matter (Figure 17), which in turn affects other quality attributes⁵⁵.
 - Yield was highest with soil matric potential maintained at –6 to –12kPa.
 - This is equivalent to 60-65% moisture content or 70-77% moisture content for milled peat containing 250 or 100kg sugar beet lime per m³ casing soil respectively.
 - Bulk peat has greater water holding capacity than milled peat; to optimise matric potential, bulk peat should contain 75-70% or 77-82% moisture when combined with 250 or 100kg/m³ sugar beet lime respectively⁵³.

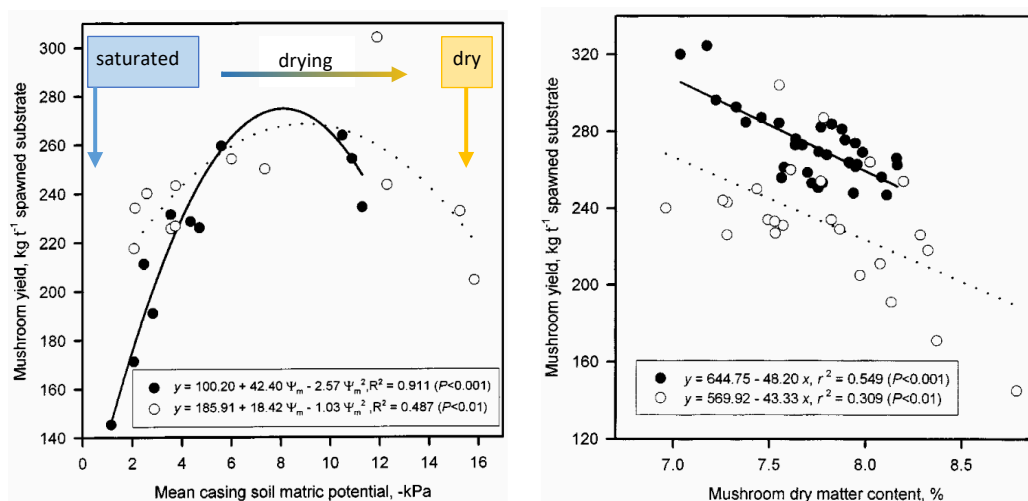


Figure 17. Effect of moisture content of casing on yield of mushrooms (left) and relationship between yield and dry matter (right). Data from two separate experiments (● and ○), reported in Noble et al., 1998.

- Burton²⁴ found that first flush mushrooms grown with wet casing (-4kPa) were less susceptible to bruising than those grown in drier casing (-8 to -12kPa). However, the opposite occurred in the third flush, mushrooms grown in dry casing proving the least susceptible to bruising.
- Although Noble et al (2000) found that matric potential was more important for mushroom development than osmotic potential, van Loon et al⁵⁶ demonstrated that adding mineral salts to casing (thereby increasing the osmotic potential) significantly increased mushroom density and dry matter.
- While levels of dry matter (DM) are strongly influenced by casing attributes and moisture content, the relationship between DM and mushroom colour is variable:
 - Higher levels of DM can be associated with **increased** whiteness at harvest, particularly in association with high flesh calcium levels⁵⁵. Moreover both whiteness and DM are maximised in first flush mushrooms⁵⁷.

- High DM can also be associated with **reduced** whiteness³⁰, or increased yellowness²⁹ at harvest, both yield and whiteness being maximized at lower DM (Figure 18).
- There does not appear to be any strong relationship between DM content and mushroom colour. For example, a large study using three strains and a range of casing materials did not find any relationship between whiteness and other physical and quality attributes measured²⁹.
- High dry matter is frequently associated with decreased yield, as shown in Figure 17 and Figure 18.
- Both whiteness and dry matter often decline with each flush.

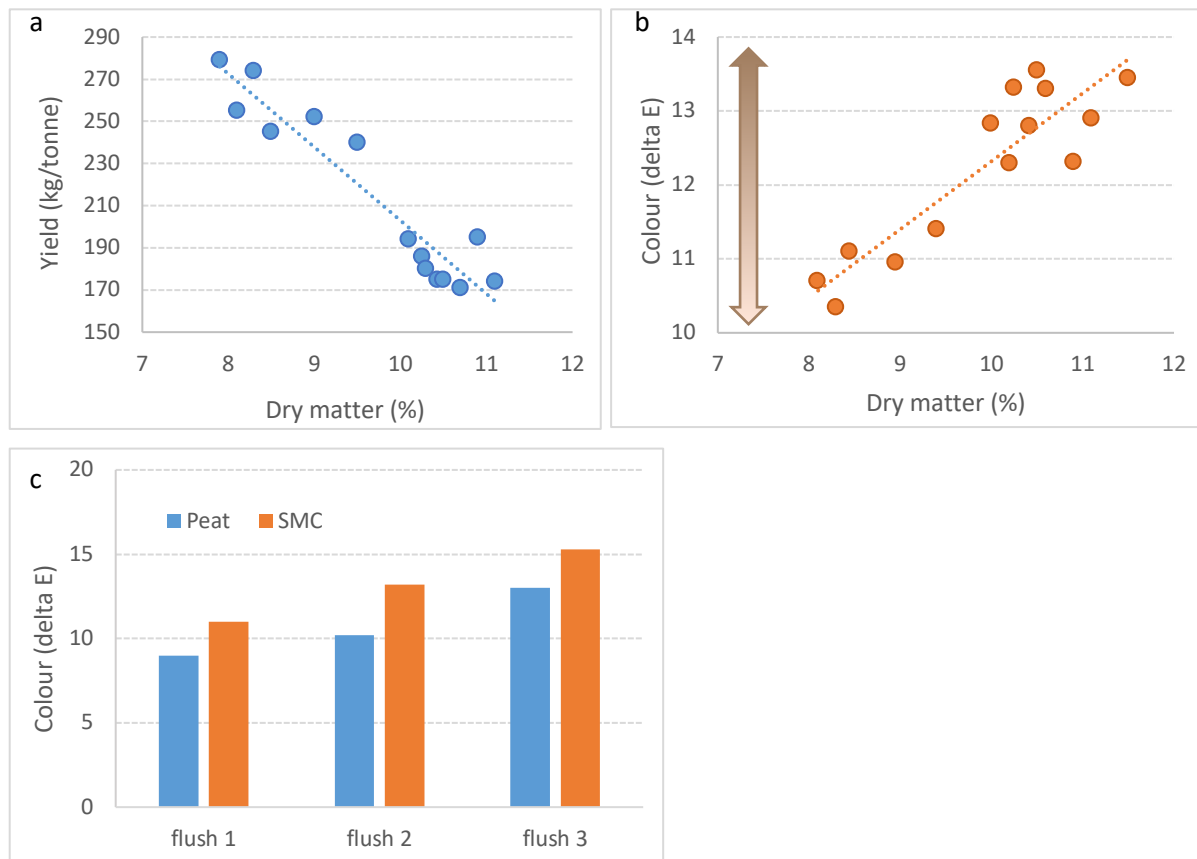


Figure 18. Relationships between mushroom dry matter and yield (a); mushroom dry matter and colour (ΔE) (b); flush and colour when mushrooms were cased using peat + sugar beet lime or spent mushroom compost + vermiculite (c). Derived from Barry et al., 2016.

- Even if mushrooms are less white initially, high DM can improve firmness and slow the rate of browning during storage (Figure 19)⁵⁶.
 - This is likely be due to increased energy reserves in mushrooms with high DM, which may increase storage life.
 - It has been shown that mannitol (the main storage sugar in mushrooms) moves from the stipe into the cap during postharvest storage³².

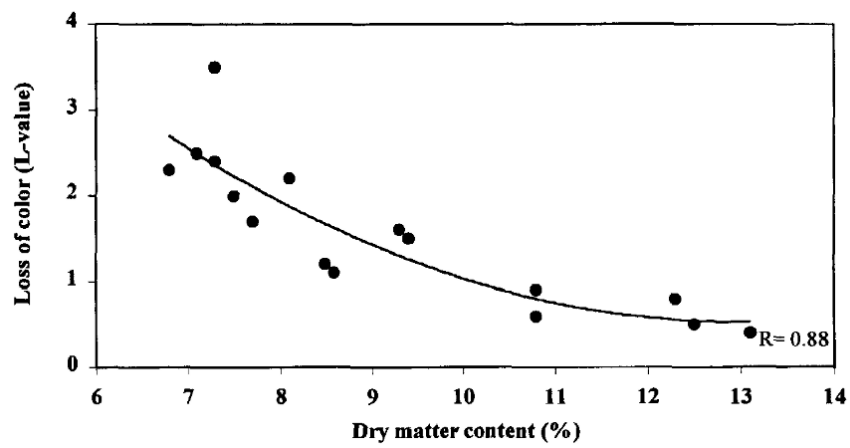


Figure 19. Change in L values (whiteness) after 7 days storage at 8°C as a function of dry matter. From van Loon et al, 2000.

Additives to irrigation water

Key points

Early efforts to control bacterial blotch involved addition of sanitisers to irrigation water. While chlorine products generally had limited effectiveness, stabilised chlorine dioxide provided excellent control. Plasma activated water and electrolysed water are new technologies that may offer future promise as irrigation water sanitation treatments.

Research during the 1980s – 90s demonstrated that mushroom whiteness could be improved by adding 0.3% calcium chloride (CaCl_2) to all irrigation water applied after pinning. Benefits were primarily observed for the second and third flushes, with major differences emerging during storage. Treated mushrooms were heavier, more resistant to bruising, higher in calcium and had increased storage life. Yield was generally unaffected. These results are supported by a recent trial conducted at the MLMRU, which found mushroom storage life could be as much as doubled by irrigation with 0.3% CaCl_2 . The effectiveness of this treatment may be due to calcium strengthening internal cell membranes and/or anti-microbial effects.

There are a number of other compounds that could also potentially improve mushroom whiteness if added to irrigation water. Products that may inhibit browning reactions include selenium, methyl jasmonate, brassinosteroids, citric acid, salicylic acid, L-arginine and an extract of green pistachio hulls. Selenium can also improve nutritional value, potentially providing a marketing advantage.

Irrigation with sanitisers

- Bacterial blotch is a major cause of quality loss of mushrooms, both before and after harvest. In the 1980's, before environmental controls improved, 5-15% of crops in the Netherlands were unharvestable due to blotch⁵⁸. While avoiding excessive or sustained wetness is the best control strategy, addition of sanitisers to irrigation water can also provide some benefits.
- Early trials and commercial practice focused on sodium hypochlorite (bleach). Irrigation with 150ppm chlorine solution, commenced almost immediately after casing, reduced disease incidence from 90% to 39% in inoculated casing. If treatment was delayed until mushrooms started pinning, chlorination had no effect. Even under optimum commercial conditions this treatment did not eliminate the disease⁵⁹.
- Geels et al⁵⁸ therefore proposed the use of stabilised chlorine dioxide (ClO_2).
 - Chlorine dioxide is normally a gas at above 11°C; the stabilised solution combines an acid with buffered sodium chlorite, gradually releasing small amounts of ClO_2 gas.
 - Irrigation with 50ppm ClO_2 was far more effective in controlling blotch than up to 250ppm chlorine in sodium hypochlorite.
 - Stabilised ClO_2 specifically reacts with reduced sulphur compounds, interfering with transport of nutrients across cell walls. This means it is less reactive with organic material than hypochlorite. It is active at pH levels between 4 and 10 and far more effective than chlorine against spores, bacteria and viruses.
 - Stabilised ClO_2 is registered and used as a sanitiser during mushroom production in many different countries.
 - In Australia, stabilised chlorine dioxide is registered for use as a sanitiser in mushroom growing facilities:

- To treat water at 5ppm
- For disinfecting walls, floors, equipment etc. at 100ppm
- The effects of adding stabilized chlorine dioxide to irrigation water was further improved by combining 50ppm stabilised ClO_2 ('Oxine') with 0.75% calcium chloride. This treatment not only reduced bacterial blotch, but also significantly increased whiteness of the harvested mushrooms. The greatest benefits were observed for the third flush mushrooms, differences that increased during postharvest storage²⁷.
- Like all other chlorine products, stabilised ClO_2 should be used with care. The acidified solution releases chlorine gas, gives off heat and can potentially produce chlorite/chlorate residues if used inappropriately⁵⁸.
- More recently, a range of sanitisers have been evaluated as postharvest treatments. These could be tested for similar effects pre-harvest.
 - Hydrogen peroxide is used as an irrigation treatment by some Northern American growers.
 - Electrolysed water (EW) is increasingly used as a low dose chlorine sanitiser; free chlorine and other ions are generated by passing an electrical current through water containing low levels of salts. Mushrooms were washed for 3 minutes in EW containing 5 to 100mg/L free chlorine. EW containing 25mg/L free chlorine was the most effective at retaining whiteness during storage (Figure 20)⁶⁰.
 - Cold plasma, and plasma activated water (PAW), have been shown to kill bacteria on plant surfaces. Plasma treatment of water generates reactive oxygen molecules in solution as well as reducing pH and increasing conductivity.
 - Although dipping mushrooms in PAW reduced bacterial counts by 1.5 log (approx. 97%) browning in storage was not reduced, but actually increased at the longest dip time⁶¹.
 - Initial trials at the Marsh Lawson Mushroom Research Unit suggest that pre-harvest irrigation with PAW could reduce postharvest development of brown blotch and improve quality (Tighe, pers. com.)

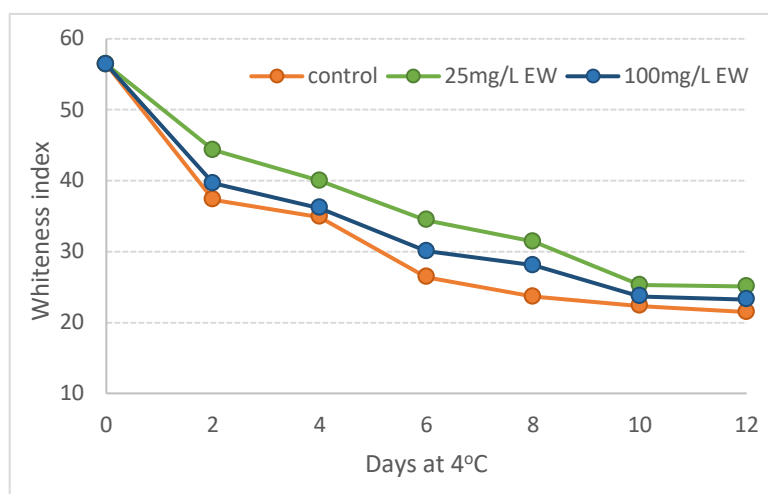


Figure 20. Changes in the whiteness index (calculated from L, a and b values) of mushrooms washed for 3 minutes in a solution containing electrolysed water, then stored at 4°C. Derived from Aday, 2016.

Irrigation with calcium chloride

- During the late 1980s a method to improve mushroom whiteness was developed. This was based on previous results from washing trials as well as the addition of various soluble salts to the casing layer. It involved the addition of calcium chloride (CaCl_2) to all irrigation applied to the beds after pinning⁶².
 - Numerous authors report that irrigation with $\geq 0.3\%$ CaCl_2 significantly improves whiteness at harvest; results for lower concentrations are much more variable.
 - CaCl_2 treated mushrooms are less damaged by deliberate bruising, a difference clearly visible to the human eye²².
 - This treatment approximately doubles calcium content of the mushrooms compared to untreated controls, e.g. Beelman and Simons⁶³ – 8.5 to 17.5 $\mu\text{g/g}$ tissue; Beelman et al⁶⁴ – 11.1 to 26.6 $\mu\text{g/g}$ tissue, with the effects frequently greater in the later flushes e.g. Kukura et al⁶⁵ 14 to 29 $\mu\text{g/g}$ tissue second flush.
 - This accumulation occurs in all tissues *except* the outer skin of the cap⁶²; this suggests that calcium is not absorbed directly through the mushroom cap, but taken up through the mycelia. It further suggests that the effects of CaCl_2 are due to physiological changes, rather than purely superficial factors.
- The major improvements in whiteness, and the increases in calcium, are observed in the second and third flushes, rather than first flush (Figure 21Figure 22).

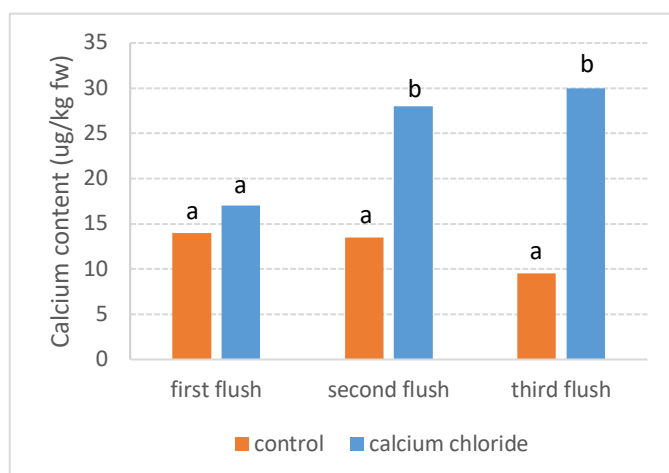


Figure 21. Calcium content of mushrooms irrigated with 0.3% CaCl_2 . Derived from Kukura et al., 1998.

- While CaCl_2 improves whiteness at harvest, greater differences emerge during storage between treated and untreated mushrooms in terms of both browning⁶⁶ and development of bruises⁶⁵ (Figure 22).

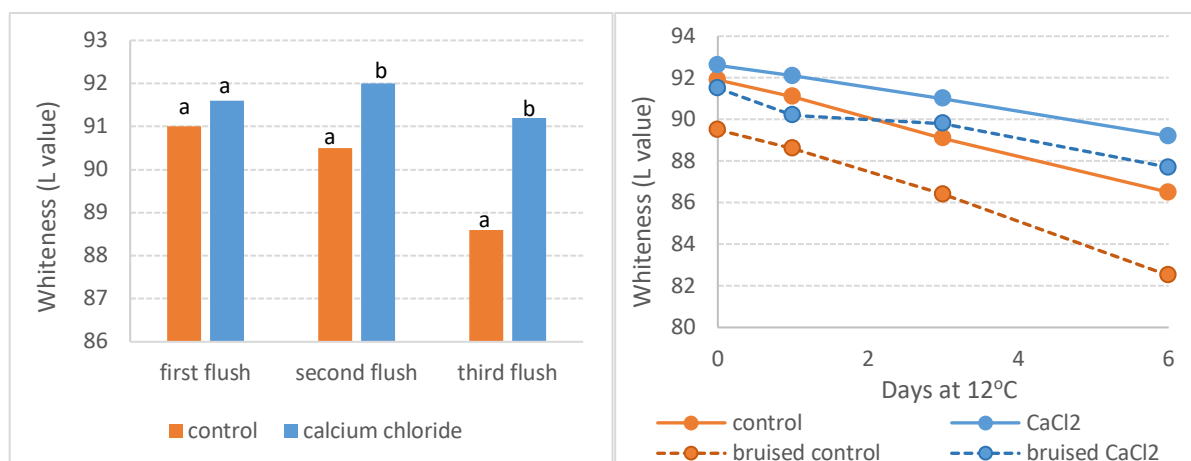


Figure 22. Effect of irrigation with 0.3% CaCl_2 on initial colour at harvest (left) and browning of bruised and unbruised mushrooms during storage at 12°C (right). Derived from Kukura et al., 1998.

- A recent trial at the Marsh Lawson Mushroom Research Unit (MLMRU) compared mushroom quality following irrigation with 0.3% CaCl_2 , tap water or reverse osmosis filtered water. If “white index = 50” is used to indicate the end of saleable life, then irrigation with CaCl_2 increased life from 4 to 9 days (flush 1); 8 to 12 days (flush 2) and 7 to 13 days (flush 3). In the case of flush 3, the effects of the treatment were clearly visible after only a few days of storage (Figure 23).



Figure 23. Third flush mushrooms irrigated with tap water (left) or 0.3% CaCl_2 (right) then stored for 6 days at 3°C.

- The effects of adding CaCl_2 to irrigation water on dry matter are variable.
 - The majority of trials report significant increases in dry matter. For example, trials by Desrumaux et al (2000) found that 0.4% CaCl_2 significantly increased dry matter, but that lower concentrations had less effect. Beelman et al. (2000) and Hartman et al (2000) also reported significant increases in dry matter from irrigating with 0.3% CaCl_2 .
 - Irrigation with 0.6% calcium lactate also increased dry matter⁶⁷, as did addition of table salt (NaCl) to casing, suggesting that changes are primarily due to increased osmolarity in the casing soil and water⁵⁶.
 - However, other researchers e.g. Miklus and Beelman (1996) have found no effect on dry matter.
- The effects on yield are generally minor. Kaluzewicz et al⁶⁷ found that yield was decreased by the addition of 0.6% but not by 0.4% CaCl_2 , with effects varying between strains. Philippoussis

et al⁶⁸ found no effect on yield of 0.1% CaCl₂. Overall, addition of 0.3% CaCl₂ to water appears to have no consistent effect on yield⁶³.

- It is unclear whether CaCl₂ can legally be added to irrigation water without registration through the APVMA. Although CaCl₂ is approved as an ingredient in food, and “Generally Recognised as Safe” (GRAS), it is also an “agricultural chemical” suggesting registration is required.
 - Judy Allen is currently investigating the status of CaCl₂ with regards registration.
 - One alternative could be to make calcium ions already in (hard) water more readily available. The CALCLEAR inline system passes water through an electromagnetic field. This is claimed to reduce clumping of ions, and therefore formation of crystals, in the solution. While the primary aim is to prevent scale forming in irrigation lines, this potentially increases availability of calcium in the water.

Why does calcium chloride affect whiteness?

Various mechanisms have been proposed for the positive effects of CaCl₂ on mushroom colour. These include linking increased calcium to membrane strength, antimicrobial effects and changes in the mushroom surface.

- The activity of enzymes that catalyse browning activity, such as tyrosinase, are not affected by irrigation with CaCl₂⁵⁶.
 - One of the co-factors for tyrosinase is copper. Copper also accumulates in mushrooms with flush, which may be one reason later flushes are often less white than first flush mushrooms.
 - Trials examining the joint effects of calcium and copper showed that copper is negatively correlated with whiteness. Although attempts to block uptake of copper with EDTA (a food chelating agent) were unsuccessful, there was a trend to reduced copper content when both CaCl₂ and EDTA were applied⁶⁴.
- Calcium is associated with increased strength in cell walls in many fruit and vegetables. Mushrooms treated with CaCl₂ do appear to have stronger cell membranes. This potentially reduces leakage of phenolics from the vacuoles into the cytoplasm, where browning reactions occur.
 - As may be observed in Figure 24, dark material inside the vacuoles – thought to be phenolic compounds – is still present in the cells of mushrooms irrigated with CaCl₂ before bruising.
 - However, these compounds are absent from cells of the bruised controls, which instead contain a large number of small, empty vacuoles.

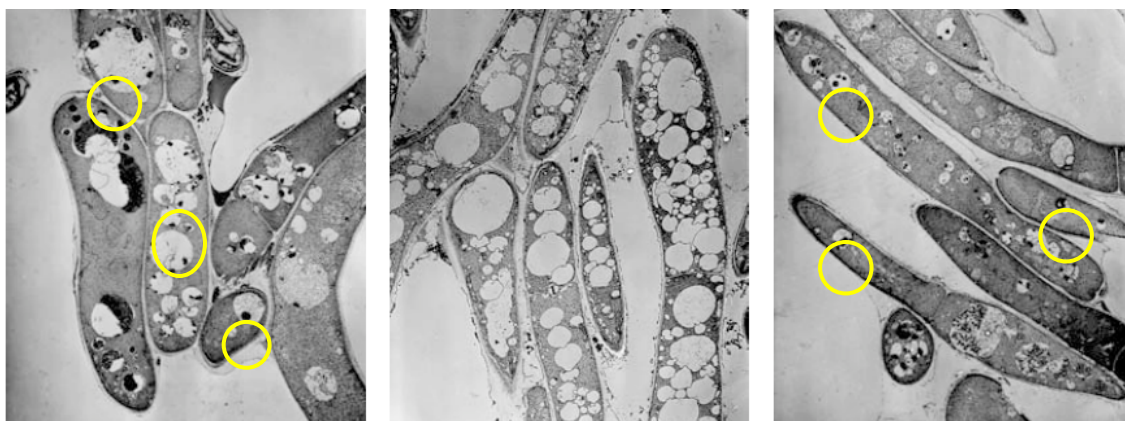


Figure 24. Electron micrographs of undamaged cells (left), bruised cells (centre) and bruised cells of mushrooms irrigated with 0.3% CaCl_2 (right). The dark areas (circled) within the vacuoles are phenolic compounds. These are no longer visible in the bruised cells at centre, but are still present in the bruised cells treated with CaCl_2 (right). From Kukura et al., 1998.

- Despite these findings, the role of calcium in mushroom whiteness remains unclear;
 - Penn State researchers⁶⁹ have observed a steady trend of increasing calcium levels in mushrooms, even though growing conditions are relatively unchanged.
 - Despite increases in calcium levels, whiteness at harvest has remained relatively constant.
 - The researchers therefore suggest the effects of CaCl_2 may be due to its anti-microbial activities and/or more rapid drying of irrigation water from the mushroom surface, rather than increased cell calcium.
- This is supported by Chikthimmah et al (2006)⁵⁷ who showed that “L” values decreased at the same time as bacterial populations on the mushroom caps increased (Figure 25). Irrigation with 0.3% CaCl_2 plus 0.75% hydrogen peroxide, commencing one week before the first harvest, achieved a nearly 2-log reduction in bacterial populations. Treatment with 0.3% CaCl_2 and/or 0.75% hydrogen peroxide also significantly improved both whiteness at harvest and retention of whiteness during storage at 4°C or 12°C.

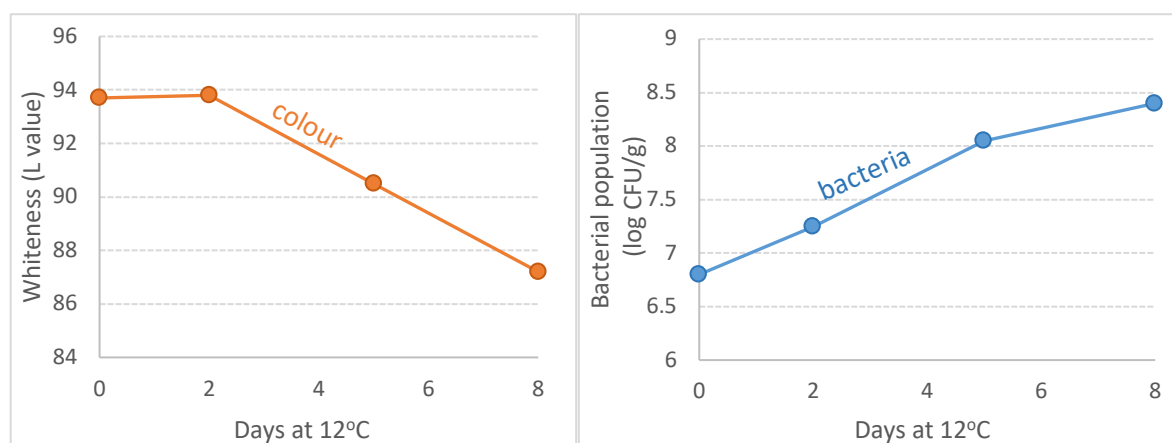


Figure 25. Changes in colour of mushrooms and bacterial populations on caps during storage at 12°C. Derived from Chikthimmah et al., 2006.

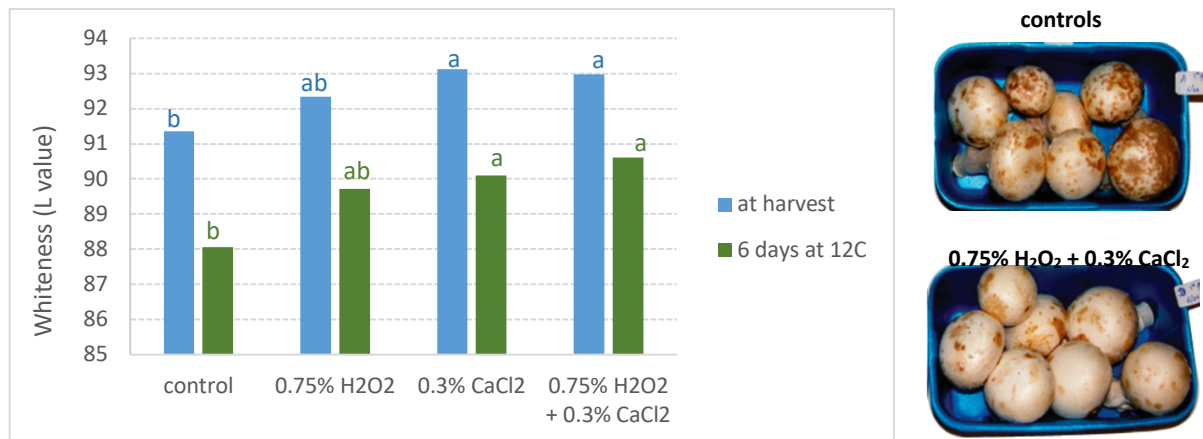


Figure 26. Effect of irrigation with 0.3% calcium chloride and/or 0.75% hydrogen peroxide on whiteness at harvest and then after 6 days at 12°C (left); Appearance of mushrooms irrigated with 0.3% calcium chloride and 0.75% hydrogen peroxide compared to controls after 6 days at 12°C (right). Letters indicate means that are significantly different. Derived from Chikthimma et al., 2006.

Other irrigation water treatments

- An informal trial conducted on-farm observed that mushrooms were whiter when irrigated with water filtered to remove dissolved solutes including chlorine, magnesium and calcium carbonate (limescale).
 - The levels of dissolved minerals in water, particularly calcium carbonate, determine water “hardness”.
 - Desrumaux et al (2000) compared mushroom quality when irrigated with water defined as soft (37mg/L Ca), medium hard (64mg/L Ca) and hard (109mg/L Ca). Water hardness did not affect mushroom colour or yield; the researchers suggest this was due to the relatively small differences between the water types tested.
 - In contrast, Guthrie and Beelman⁷⁰ found that washing mushrooms in hard water (151mg/L Ca) reduced bacterial growth and browning during storage when compared to washing in distilled water (0mg/L Ca). Medium/hard water (74mg/L) was intermediate.
- Addition of selenium to irrigation water may improve both quality and nutritional value of mushrooms. Hartman et al. (2000) tested irrigation with CaCl₂ and/or sodium selenite (Na₂SeO₃). Irrigation with the selenite commenced at casing. Once pins appeared, the amendment was changed to 0.3% CaCl₂, which then continued through to the third flush. This treatment increased selenium content of the casing from 1.5ppm to 18.2ppm dry weight.
 - The selenium only, CaCl₂ only and combination treatment all increased mushroom whiteness at harvest and during subsequent storage.

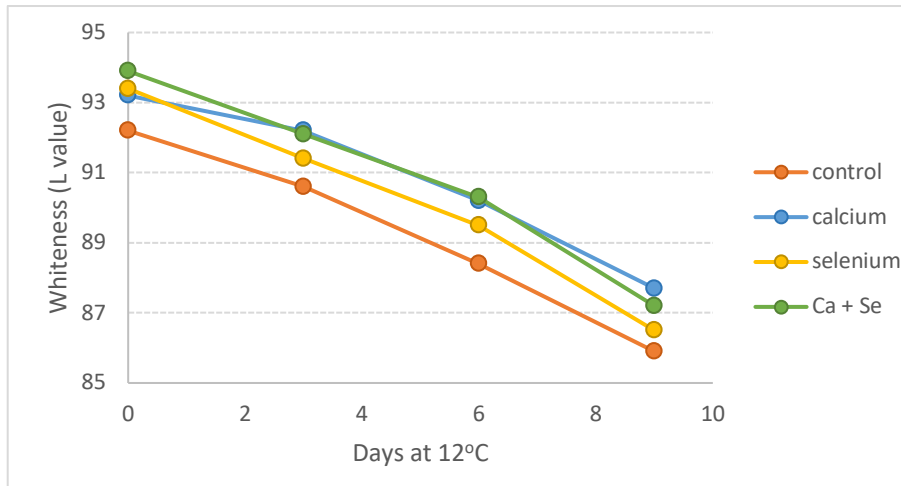


Figure 27. Effect of irrigation with 0.3% calcium chloride, 0.05g/L sodium selenite or both on mushroom colour at harvest and during storage at 12°C. Derived from Hartman et al., 2000.

- Addition of selenium to the irrigation water increased Se in the mushrooms to approximately 60 µg per 100g serving of mushrooms. This meets the recommended daily intake of 60-70 µg/day for Australian adults.
 - Selenium is an antioxidant and essential nutrient which has been strongly associated with reduced rates of certain cancers.
 - In Australia, approximately 40% of women and 25% of men are somewhat deficient in selenium.
 - Estimated levels of selenium in food, as measured by FSANZ, fell by approximately 20% between 2003 and 2008, a trend which has also been noted in other parts of the world. The effect has been attributed to changing agricultural practices and soil degradation. (<https://www.foodstandards.gov.au/publications/documents/ATDS.pdf>)
 - In 2009 Irish farmer Tom Keogh developed selenium enriched potatoes as a “functional food”, sold with a 20% price premium. The Selena potatoes provide 14 µg per 100g serving. (<https://www.potatogrower.com/2009/04/selenium-enriched-potatoes-released-in-ireland>)



Figure 28. Selenium enriched potatoes, developed in Ireland.

- The plant growth regulator methyl jasmonate (MeJA) has been widely associated with improved plant defences.

- According to Jahangir et al., (2011), application of MeJA as a postharvest dip reduces browning due to inhibition of oxidative enzymes.
- Chinese researchers tested the effects of spraying developing pins (approx. 12mm) with 10, 100 or 200µm of MeJA. Although mushrooms were similar whiteness at harvest, all three levels of MeJA inhibited browning during storage at 4°C, with significant effects after only two days. The best results were achieved with the 100µm treatment⁷¹.

Postharvest dips that could be adapted to pre-harvest sprays

A significant number of recent papers describe the effects of novel postharvest dips and washes. Many are claimed to reduce browning due to de-activation of enzymes such as polyphenol oxidase or tyrosinase.

Australian mushroom farms are highly unlikely to adopt dips or washes, particularly for whole mushrooms. However, these products may have potential as part of a pre-harvest spray or irrigation program; it seems likely that a pre-harvest spray that remains on the mushrooms for up to four hours could be even more effective than a short postharvest dip.

Examples include:

- Brassinosteroids are a group of natural plant hormones that may improve membrane stability and increase antioxidant activity. Ding et al⁷² tested the effect of one-minute dips in 1 or 3 µM solutions of brassinolide (BL). The 3µm solution halved the rate of browning in storage (Figure 29). The effect was associated with reduced PPO activity, electrolyte leakage and weight loss. The authors suggest that this is because BL protects cell membranes from oxidative damage, reducing enzymatic browning reactions.

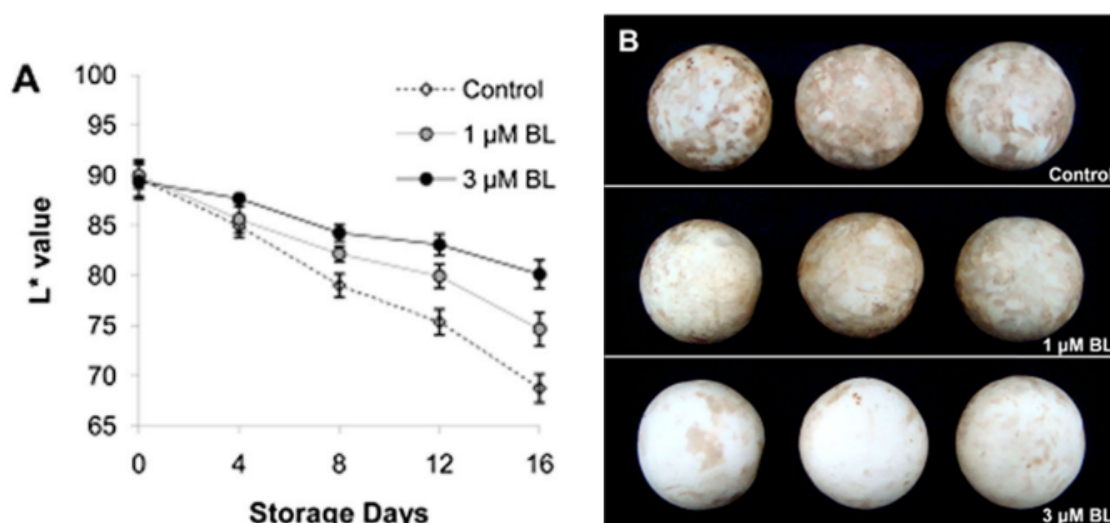


Figure 29. Changes in colour (A) and appearance (B) of mushrooms dipped for one minute in 1 or 3 µM solutions of brassinolide. From Ding et al, 2016.

- Anyone who has squeezed a lemon over an avocado to stop it browning understands that acids reduce browning reactions. Organic acids reduce pH and thereby activity of PPO, which is key to enzymatic browning. However, published results are highly variable;

- A 60 second dip in 250µm salicylic acid significantly reduced postharvest browning. A lower concentration (50µm) was less effective, while higher concentrations (500µm, 1,000µm) increased browning⁷³.
- Postharvest browning was inhibited in mushrooms washed for 10 minutes in 40g/L citric acid, differences developing after 2 weeks storage at 4°C⁷⁴.
- Singla et al.⁷⁵, found no effect of citric acid and a negative effect of acetic acid. However, a 5 minute dip in a 4% solution of malic acid significantly improved whiteness retention.
- In contrast, a mixture of 1g/L ascorbic acid and 0.5g/L citric acid had negative effects on mushroom quality when applied by 15 minutes of vacuum infiltration; although the L values of these mushrooms appeared to be higher than the controls, the mushrooms had a discoloured, water-soaked appearance⁷⁶.
- Other acids which have been used experimentally to reduce browning of mushrooms include cinnamic acid, pyruvic acid, propanoic acid, methoxysalicylic acid, benzoic acid and many others⁷⁷. However, most such studies have focused on the chemical processes that are occurring, rather than practical methods to reduce mushroom browning.
- Hydrogen peroxide has been demonstrated to inactivate mushroom tyrosinase (Andrawis and Kahn, 1985). However, although a 10-minute dip in 50ml/L hydrogen peroxide significantly reduced the number of bacteria on fresh mushrooms, the treatment was less effective than citric acid in preventing browning⁷⁸.
- Fattahifar et al.⁷⁶ also examined treatments to reduce tyrosinase activity. An extract from green pistachio hulls, applied under vacuum, reduced mushroom browning. This was thought due to its phenolic content and antioxidant activity.
- The amino acid L-arginine also affects enzyme activity, thereby reducing browning reactions. Li et al.⁷⁹ found optimal effects dipping mushrooms in 10mM L-arginine for 10 minutes. This treatment increased antioxidants and reduced PPO activity. It also significantly reduced weight loss, possibly due to its effects on the mushroom surface. Surface cells in the control were damaged and cracked; those in the mushrooms treated with L-arginine remained dense and connected (Figure 30).

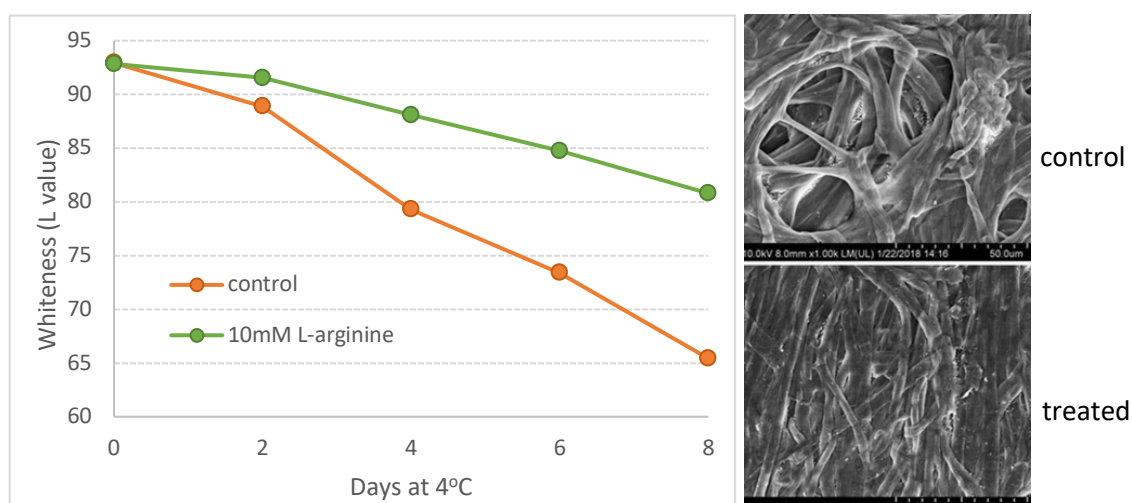


Figure 30. Changes in colour (left) and electron microscope image of surface structure (right) of mushrooms dipped in 10mM L-arginine or water. From Li et al, 2019.

Irrigation method

Key points

Optimising irrigation is essential for good quality mushrooms. While irrigation of fruit and vegetable crops is frequently managed with soil moisture probes, mushrooms are usually irrigated using manually controlled sprinkler systems or hand wands. Improvements in soil moisture measurement mean that systems are now available which can irrigate in response to substrate water deficits. New subsurface drip irrigation systems can keep substrate moisture content at optimal levels throughout cropping. This system could have major benefits in terms of maximizing whiteness and reducing bacterial blotch.

- Mushroom production requires large amounts of water. Irrigation may be applied manually or through installed sprinkler systems. While soil moisture probes are commonly used to manage irrigation in field crops, this is not commonly done for mushrooms. Instead, a skilled human operator judges how much water to apply, a decision based on experience and observation.
- One reason for this may have been the lack of accurate moisture sensors suitable for casing and substrate materials.
 - For example, Hermans and Amsing⁸⁰ tested the Decagon Devices GS3 capacitance sensor, which estimates volumetric moisture. The sensor is recommended for use in peat, coir and other soilless substrates. The sensor underestimated volumetric water content by up to 25%.
 - Despite this, the authors note that there was a good correlation between readings and actual values, so suggest that this sensor could still be used to manage irrigation.
- The Vullings water supply system (<http://www.vullings-systemen.com>) uses spray jets mounted on a moving pulley system to provide even water distribution (Figure 31). The jets can be linked to moisture measurements from sensors in the bed, to fully automate irrigation.

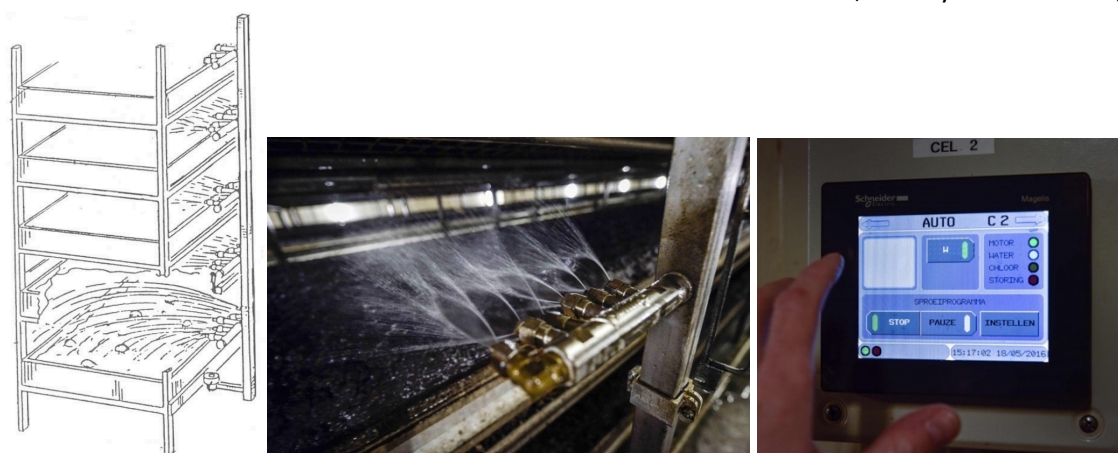


Figure 31. The Vullings water supply system for mushroom production.

- Netafim, together with Vullings and researchers at the Galilee Research Institute, have developed the “Mushroom Master” irrigation system (<https://www.netafim.com/en/crop-knowledge/mushroom/>) (Figure 32). Irrigation is provided by subsurface (3cm under casing) non-leaking flow drippers within the beds. This means that irrigation can continue during harvest, maintaining casing soil at constant moisture levels throughout the cropping cycle. Irrigation can also be completely automated, with water supplied in small pulses in direct response to changes in water potential.

- This method can reduce casing material requirements by 30% as well as improving quality attributes (e.g. firmness) and density.
- The dripper lines are recovered after cropping and can be re-installed several times.
- Dripline insertion does not change the regular filler speed of 7 to 11m/minute for shelf systems⁸¹.
- Nutrients can be added through the dripper lines to fertigate the crop.
- No bacterial blotch occurred with drip irrigation, while 6% of mushrooms grown with spray irrigation were affected by this disease⁸².
- Drip irrigation reduces potential dissemination of fungal spores e.g. *Verticillium*, etc.
- Danay et al⁸³ found that drip irrigation slightly increased yield, but that the main effects were on mushroom quality, particularly for third flush (Figure 33). The authors note also note that there was decreased incidence of bacterial blotch on drip irrigated mushrooms, although no figures are presented.



Figure 32. The Netafim "Mushroom Master" drip irrigation system (left) and the insertion unit on a Thilot head filler. From Raz et al., 2016.

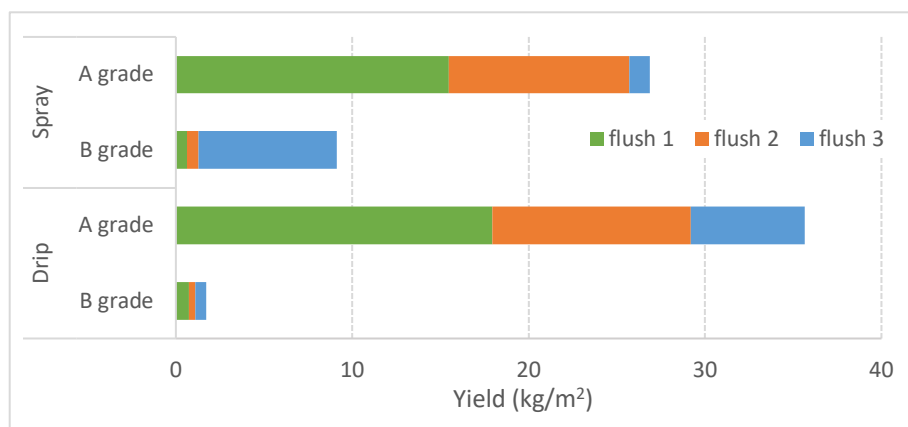


Figure 33. Yield and quality grade of mushrooms grown with normal or drip irrigation. Derived from Danay et al., 2016.

5 Harvest and postharvest handling

Temperature management

Key point

Cooling mushrooms as quickly as possible after harvest is essential to retain whiteness and quality. Although capital intensive, vacuum cooling is fast and efficient. Mushrooms that have been vacuum cooled remain whiter for longer than mushrooms simply room cooled. Vacuum cooling appears to increase activity of antioxidants and decrease activity of PPO, thereby inhibiting browning. Once cold, maintaining uniform temperatures avoids condensation, thereby maintaining whiteness.

Cooling

- Temperature is the most important factor determining storage life and quality of fresh produce. Harvested mushrooms are cut off from their source of water and nutrients. The faster they are cooled, the less moisture and quality they will lose. Cooling slows metabolic processes, reducing the rate at which mushrooms develop, senesce and use up their storage reserves. Cold temperatures also limit growth of bacterial blotch, extending storage life.
- It is recommended that mushrooms are cooled below 4°C within one hour of harvest to maximise storage life and minimise loss of whiteness.
- Vacuum cooling is capital intensive, but also fast and energy efficient. The mushrooms are cooled by changing liquid water inside the mushrooms into vapour, a process that absorbs heat energy. Naturally, this results in some weight loss; Burton et al.⁸⁴ reported that mushrooms lost 1.7% of their weight during vacuum cooling. Newer 'hydrovac' systems provide misting, which can minimise this effect.
- Mushrooms that have been vacuum cooled stay whiter longer than those that have been cooled more slowly using conventional methods.
 - Although mushrooms that were cooled by different methods appeared similar during storage at 5°C, significant differences emerged after transfer to 18°C for retail sale⁸⁴. The authors estimated vacuum cooling provided an additional 24 hours life after 4.5 days storage, a difference not explained by the total time taken to cool.
 - Tao et al⁸⁵ reported that mushrooms that were room cooled were twice as brown as those that were vacuum cooled after 4 days at 4°C, a significant difference that continued for up to a week (Figure 34).

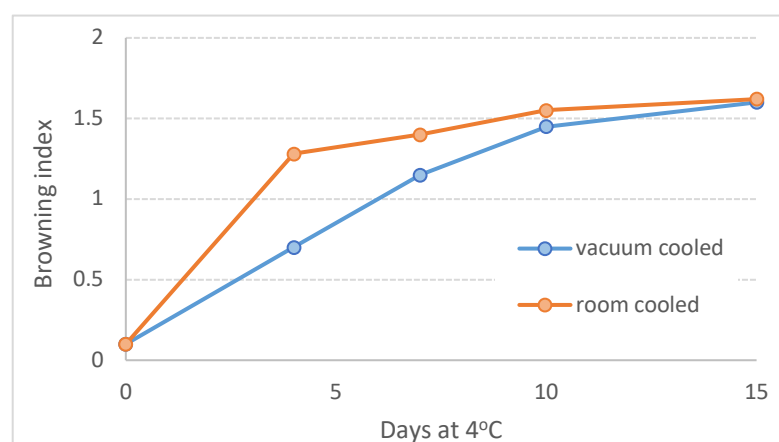


Figure 34. Increases in browning during storage at 4°C of vacuum cooled or room cooled mushroom. Derived from Tao et al., 2007.

- The effectiveness of vacuum cooling for mushrooms is not simply due to its speed, but to effects on enzyme activity. The activity of polyphenol oxidase was inhibited, while activity of a range of antioxidant enzymes is increased in vacuum cooled mushrooms⁸⁵. This may account for observed benefits in retention of whiteness even after transfer to warmer temperatures.

Storage

- As mushrooms are mostly water, their freezing point is close to 0°C. Storage life is maximized at 0-2°C with 90-95% RH.
- Minimising temperature fluctuations during storage and transport prevents condensation forming on either the mushrooms or their packaging. Condensation on packaging indicates moisture loss from mushrooms, while condensation on mushrooms leads to bacterial blotch. Both processes reduce whiteness. Uniform temperature control can be achieved by:
 - Reducing the gap between the high and low temperature setpoints (when the compressor turns on/off) for the coolroom.
 - Minimising the frequency with which doors are opened and using an air curtain to reduce ingress of warm air.
 - Ensuring cold rooms are well insulated, and that insulating materials are sealed against moisture.
 - Loading directly from cold rooms into pre-cooled trucks.

Detecting browning

Key point

Imaging systems have been developed which can assess colour and presence of bacteria, with the stated aim of facilitating automatic commercial grading. However, none of the described systems appears to have been tested in a commercial environment. It is also difficult to see how this technology could be applied with existing equipment, as packing lines do not sort individually.

- Mushrooms are easily damaged during harvest due to bruising or abrasion. This limits the potential for automating harvest. In Europe and Asia, mechanically harvested mushrooms are only used for canning or processing as they brown within an hour of picking²⁵.
- A number of researchers have developed imaging systems to detect bruising on harvested mushrooms, generally with the aim of developing an automatic grading system:
 - Changes in colour are indicated by differences in the visible light spectrum (400 to 750nm); brown mushrooms have higher absorbance due to melanin formation.
 - The average spectrum of damaged mushrooms is more absorbent than that of non-damaged mushrooms, especially in wavelengths absorbed by water molecules. This indicates that damage has released water from internal structures⁸⁶.
 - A model measuring absorbance at wavelengths 1090nm, 1188nm and 1384nm plus scaling from 1454nm was able to correctly identify damaged mushrooms⁸⁷.
 - NIR spectra can also be used to estimate moisture content and firmness⁸⁸.

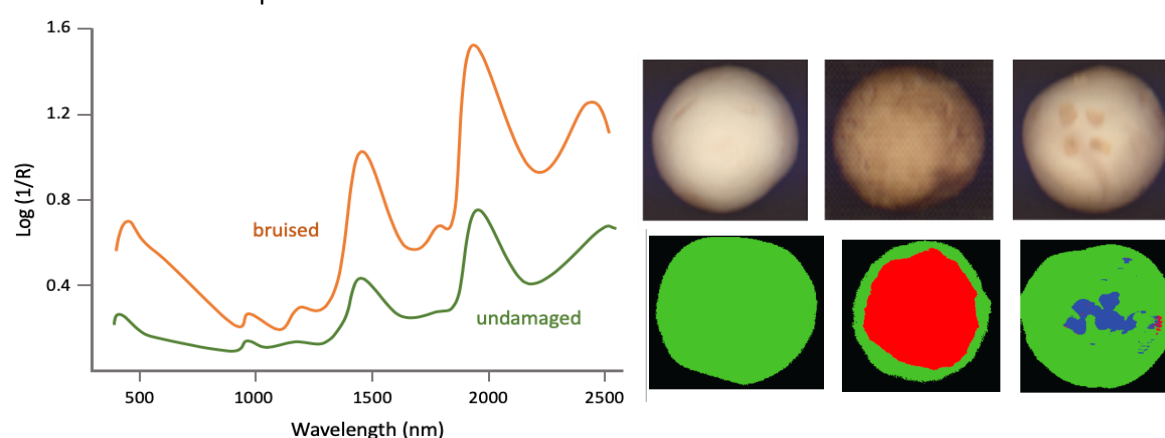


Figure 35. Average raw spectra of damaged and undamaged mushrooms, derived from Esquerre et al., 2009 (left) and original and hyperspectral images of undamaged, damaged and bacterial blotch infected mushrooms, from Gaston et al., 2010.

- Gaston et al ⁸⁹ took a slightly different approach, focusing on the use of hyperspectral imaging to discriminate between mushrooms that were undamaged, mechanically damaged or infected with brown blotch bacteria. Hyperspectral images, known as “hypercubes” are essentially three-dimensional blocks of data, describing the location spectrum of each image pixel. The model could classify deliberately damaged or inoculated mushrooms with an accuracy of 95%.
- However, neither the NIR or hyperspectral models appear to have been tested against naturally damaged or browned mushrooms; in all cases damage was artificially applied, being more severe than would be expected to occur in a commercial situation.

Postharvest treatments

Key points

Mushrooms harvested while relatively immature and trimmed to a short stipe are less susceptible to browning during storage. In the USA, mushrooms may be washed postharvest. This cleans the mushrooms and can potentially improve retention of whiteness during storage. Researchers have also tested a range of coatings, including chitosan, essential oils and even aloe vera, with mixed results.

A better way to apply essential oils is as an encapsulated, low dose fumigant added to packaged mushrooms. Positive results have been reported, however cost and technical difficulty remain unaddressed.

The ethylene antagonist 1-MCP, which is widely used to extend storage life of apples, pears and other crops, may have potential to improve appearance of mushrooms on retail displays. Limited work suggests that ozone may also have antimicrobial effects.

Low dose irradiation has also been proposed as a way to inhibit browning reactions. While this process is safe and chemical free, cost and consumer resistance are likely to inhibit adoption.

Trimming and maturity

- Mushrooms harvested early, while still relatively small and immature (veils intact and tight) brown more slowly during storage. They are also less likely to continue to develop. Although Beelman et al³² found that total yield was slightly increased by this practice, labour costs would likely be higher.
- Trimming the stipe to 5mm immediately after harvest reduces browning during storage compared to leaving the stipe long (30-35mm). It is believed that this is due to inhibition of further physiological development, which is otherwise fueled by the stipe³². According to work by Mau et al (1993), combining short stipes with CaCl₂ irrigation dramatically increased storage life.

Washing

- Considerable research effort in the US has focused on developing washing treatments for mushrooms. These aim to remove casing materials and sanitise mushrooms without increasing development of bacterial blotch or browning.
 - Initially mushrooms were washed with 1,000 mg/L sodium sulfite.
 - After use of sulfites was banned (FDA, 1986), Guthrie and Beelman⁷⁰ developed a wash involving stabilized chlorine dioxide, calcium chloride and sodium erythorbate. This combination significantly reduced the number of viable bacteria on mushrooms and delayed browning during storage.
 - This method was refined into a two stage process by Sapers et al⁷⁴. An initial pre-wash with 0.5 to 1% hydrogen peroxide (H₂O₂) was followed by a 30 second spray with 5% H₂O₂ + 4% sodium erythorbate + 0.1% sodium chloride. The method was scaled up to a commercial size, and developed into a continuous treatment system. This treatment reduced both development of brown blotch and browning during storage (Figure 36, but was more effective for first grade than processing grade mushrooms).

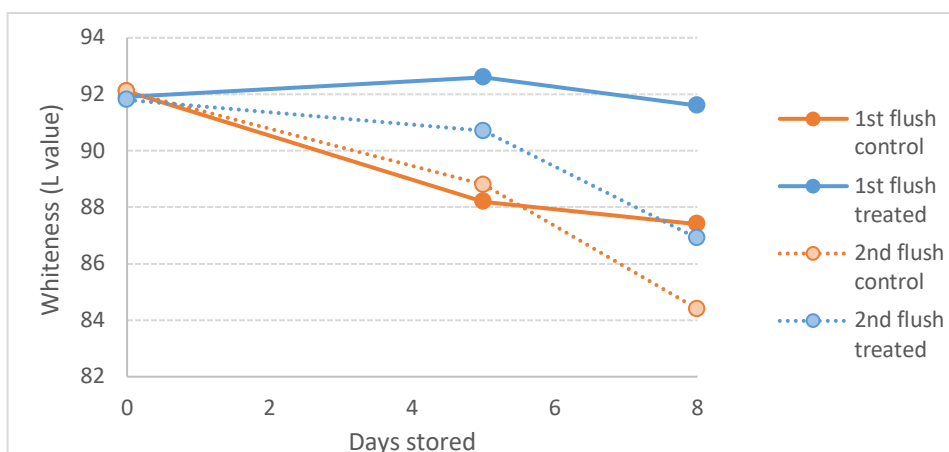


Figure 36. Colour changes of mushrooms pre-washed with hydrogen peroxide (H_2O_2) for approx. 13s then washed in a mixture of H_2O_2 , sodium erythorbate and sodium chloride for approx. 30s. Derived from Sapers et al, 2001.

- A range of other postharvest wash treatments have been trialed. These involve products such as calcium chloride, organic acids, hydrogen peroxide, amino acids and various other anti-oxidants and sanitisers.
 - Unlike the wash system noted above, none of these appear to have yet found commercial adoption, possibly because of the relatively long dip times and subsequent drying required.
 - It may also be noted that the good results achieved by irrigating with calcium carbonate (as detailed previously in this document), led to the proposal of washing with a 1-2% $CaCl_2$ solution⁹⁰. In this case colour was not measured, but firmness was reportedly retained.
 - The Australian industry is unlikely to adopt washing as a postharvest treatment for whole mushrooms, so these treatments are not considered further here. However, a number of these options are included in the section on potential additions to irrigation water.

Coatings

- Chitosan, produced from shellfish waste, has anti-bacterial properties that may inhibit growth of bacteria.
 - Eissa⁹¹ reported positive effects from dipping mushrooms in a chitosan solution due to reductions in bacterial growth.
 - However, when this work was repeated with better quality mushrooms, chitosan had negative effects on mushroom colour⁹².
 - Other authors⁹³ have also found negative results from direct application of chitosan to mushrooms.
- Essential oils are regarded as natural antimicrobials. A number of studies have examined the effects of coatings that include essential oils, with gums or other materials used as carriers. While such materials have been shown to affect enzyme activity and, in some cases, microbial growth, the effects on mushroom whiteness are not always presented⁹⁴ or may even be negative⁹⁵.

- Coating mushrooms with a 50% aloe vera solution before storage reduced surface browning and inhibited weight loss⁹⁶. The effects on eating quality or how this could be applied are not discussed.

Novel treatments – fumigants

- The fumigant 1-methylcyclopropene (1-MCP) is widely used to inhibit the effects of ethylene. It is used commercially to extend the storage life of apples, pear, persimmons and other fruit, and is effective at preventing yellowing of broccoli. While mushrooms are very low emitters of ethylene, it has been suggested ethylene exposure increases browning during storage.
 - A recent study⁹⁷ combined 1-MCP fumigation with various modified atmosphere packaging materials. While 1-MCP reduced mushroom respiration, thereby changing the atmospheres that developed inside the packages, the effects on colour were mixed (Table 1).
 - After 4 days storage, all of the 1-MCP treated mushrooms were whiter than the controls.
 - Mushrooms packed in low permeability film developed a yellow tinge after 8 days.
 - Mushrooms treated with 1-MCP then packed in high permeability film remained externally whiter than the controls. However, after 8 days the internal tissue started to break down.
 - The best results were gained by treating with 1-MCP then packing in medium permeability film.

Table 1. Condition of mushrooms treated for 12 hours with 5µl/L 1-MCP then packed in films with varying permeability, following storage for 4 or 15 days at 5°C. From Sun et al., 2020.

Film permeability		Control			1-MCP		
After 4 days	Low						
	Med						
	High						
After 15 days	Low						
	Med						
	High						

- Ozone can be used as a sanitiser, and to control mould in storage environments.
 - Exposure to ozone at rates of 2.8 or 5.3mg/L was demonstrated to achieve greater than 2 log (99%) reductions in bacteria inoculated onto the mushroom surface. Although Akata et al⁹⁸ were studying food safety pathogens, similar results may be

achieved for bacterial blotch. Unfortunately, the condition of the mushrooms was not reported.

- According to Yan et al⁹⁹, high voltage electric fields (HVEF) ionize the air, producing a range of active substances, including ozone. Ozone is thought to reduce the activity of PPO, thereby inhibiting formation of melanin.
 - Browning was significantly reduced when mushrooms were stored in the presence of a HVEF (Figure 37).
 - There were also clear differences in integrity of the mushroom structure, the HVEF reducing deterioration of the mycelium after 12 days at 4°C.
- While the HVEF treatment was clearly non-commercial, it could be useful to investigate whether the observed effects were due to the presence of low levels of ozone.

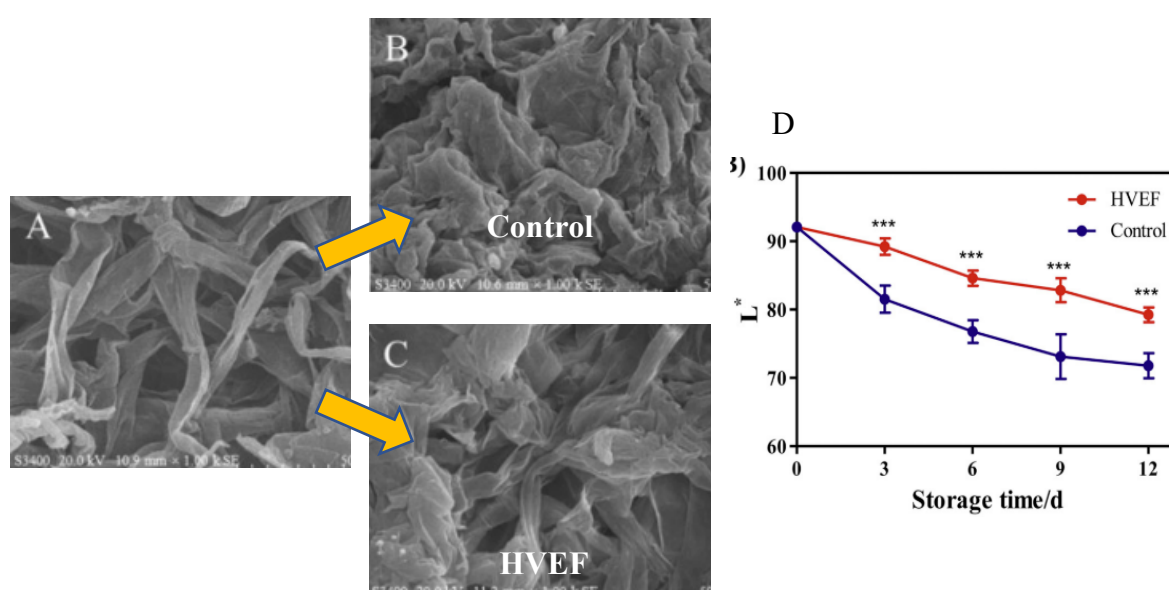


Figure 37. Microstructure of *Agaricus bisporus* when freshly harvested (A), after 12 days storage at 4°C (B) or after 12 days storage at 4°C in the presence of HVEF (C), and the effects of HVEF on mushroom whiteness (L value) (D). From Yan et al., 2020.

- Essential oils, extracted from plants, have been widely investigated for their antimicrobial effects and anti-oxidant activity. Essential oils can be applied as low dose fumigants, either formulated to provide sustained release during storage or as a single postharvest fumigation.
 - Rosemary and thyme oil were microencapsulated then spotted onto filter paper included inside polyethylene packages of mushrooms, inhibiting browning during storage¹⁰⁰.
 - A similar method was used for bitter orange¹⁰¹ and cumin¹⁰² essential oils. The oils were microencapsulated with chitosan then spotted onto filter paper added to packaged mushrooms. Vapour from the oils suppressed microbial growth and the activity of browning enzymes, the cumin oil proving most effective at retaining whiteness during storage (Figure 38).
 - Moradian et al¹⁰³ also tested rosemary oil, as well as extracts of green tea and pomegranate peels. Instead of filter paper, the compounds were incorporated into a biodegradable bacterial-cellulose based material. All three extracts reduced

browning and weight loss. The authors suggest that this was due to their antimicrobial and antioxidant properties.

- Qu et al¹⁰⁴ took a different approach, fumigating mushrooms with peppermint oil for 24 hours at 20°C before re-packing and storing at 4°C. The fumigation delayed browning as well as reducing weight loss and softening.

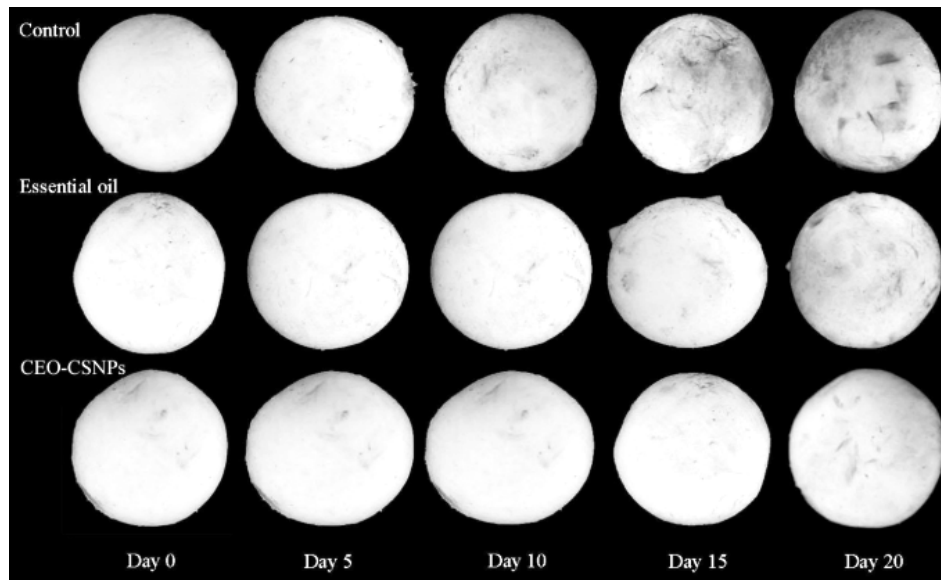


Figure 38. Appearance of mushrooms packed with water only, cumin essential oil (CEO) or CEO microencapsulated into chitosan nanoparticles (CEO-CSNPs). From Karimad et al., 2019.

Novel treatments – Irradiation

- Low dose irradiation can alter the expression of enzymes and other biochemical processes. While irradiation has traditionally been conducted using radioactive materials e.g. Cobalt 60 to produce gamma irradiation, irradiation can also be achieved with x-rays or high energy electrons. For example, the new Steritech facility in Melbourne is focused on electronic irradiation methods.
 - Exposure to up to 2.5 kGy gamma irradiation increased PPO activity and initially had little effect on colour change. However, after 4 days, the treated mushrooms showed little further change, whereas the controls continued to darken¹⁰⁵.
 - Similar results were reported by Beaulieu¹⁰⁶, who found that the best results were achieved using a slower rate (4.5 kGy/h) to achieve a total of 2 kGy exposure.
 - Mami et al¹⁰⁷ confirmed that generating the 2 kGy exposure using an electron beam accelerator (instead of a radioactive source) improved whiteness and retention of protein during storage at 4°C.
 - Mushrooms that were irradiated with 2 kGy then packaged in an antimicrobial film impregnated with silver nanoparticles remained virtually unchanged during two weeks storage, whereas L* values of controls had declined by up to 20 units¹⁰⁸.
 - While irradiation is safe, chemical free and can be achieved without using a radioactive source, there is likely to be considerable consumer resistance to such technology. It also adds significant cost and technical difficulty.

- Mushrooms may be exposed to UV-C radiation to trigger formation of vitamin D₂; a serving of mushrooms treated this way can supply the body's daily requirement, providing a natural supplement for the many people deficient in D₂. While UV-C treatment initially reduces whiteness, further browning during storage is inhibited. After a 2 weeks or more, treated mushrooms may be similar to or whiter than untreated product¹⁰⁹.

Packaging

Key points

Modified atmosphere packaging (MAP) has been widely investigated for use with mushrooms. However, mushroom respiration is relatively unaffected by low O₂ concentrations, while accumulation of CO₂ inside packages can increase yellowing and maturation/stipe elongation. MAP appears unlikely to be a practical method to improve mushroom whiteness, with risks outweighing benefits.

A number of novel, biodegradable films have recently been tested. These can incorporate antioxidants and anti-bacterial compounds which inhibit browning and/or bacterial growth. This appears a particularly promising field for future commercial development as long as costs and consumer appeal are suitable.

Modified atmosphere packaging (MAP)

- MAP uses the respiration of the product to reduce oxygen (O₂) and increase carbon dioxide (CO₂) inside a sealed package. The atmosphere that develops is a function of respiration rate, surface area relative to volume, and film permeability¹¹⁰.
- MAP can increase storage life of fresh produce by inhibiting ripening, reducing rot development, retaining chlorophyll and reducing the rate of O₂ consumption and, therefore, senescence. Of these, only the last appears relevant to mushrooms. However, mushrooms are highly gas-permeable, so O₂ needs to fall below at least 2%¹¹⁰ and more likely below 1%¹¹¹ to significantly reduce respiration rate.

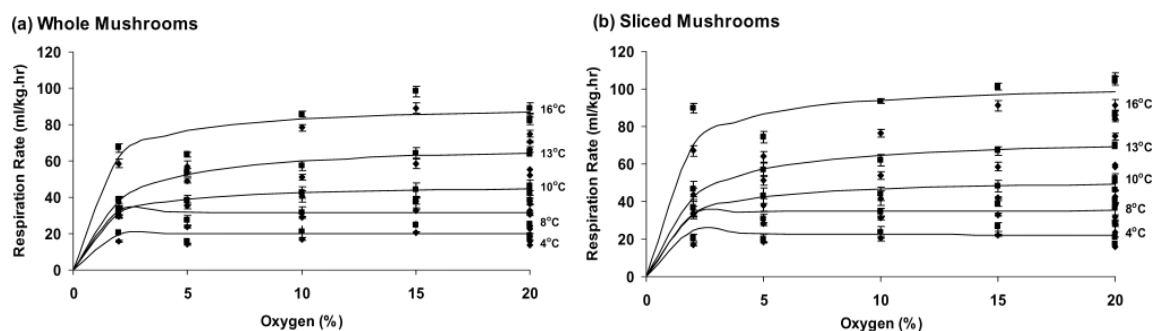


Figure 39. The effect of oxygen concentration on respiration rates of whole and sliced mushrooms. From Cliffe-Byrnes and O'Beirne, 2007.

- As changes in temperature have a greater effect on respiration rate than film permeability, packages need to be designed for very specific conditions; storage at lower than target temperatures may result in little atmospheric modification, whereas at higher temperatures packages can become anaerobic.
- There have been many studies of MAP for mushrooms.

- It is frequently stated that an atmosphere containing 2.5 to 5% CO₂ and 2-10% O₂ can improve storage life of mushrooms¹¹². However, these values can be difficult to obtain because they require a much higher film permeability to CO₂ than O₂.
- Moreover, there is evidence that any accumulation of CO₂ inside a package can reduce mushroom whiteness; Lopez-Briones et al¹¹³ found a linear relationship between CO₂ concentration inside packages and mushroom cap colour (Figure 40).
- This is supported by Zalewska et al¹¹⁴, who conducted a complicated trial using two film types flushed with one of four gas combinations.
 - Although the concentrations of O₂ and CO₂ in the packages during storage were not measured, it is clear that packages flushed with 10% or 20% CO₂ yellowed significantly more than mushrooms simply overwrapped with PVC film.
 - The whitest mushrooms were obtained using a film highly permeable to CO₂ flushed with 20% O₂ + nitrogen (essentially air). This package reduced veil opening and weight loss without accumulating excessive CO₂.
- A Horticulture Australia project conducted at the CSIRO Active Packaging unit North Ryde in 1997 (Bower J, Jobling J & Patterson BD) examined the effects of a range of atmospheres and packaging materials on mushroom quality.
 - An atmosphere containing 5% O₂ + 3.5% CO₂ provided moderate improvements in whiteness and quality.
 - However, it was noted that high RH combined with low levels of CO₂ increased stipe elongation, while high levels of CO₂ increased yellowing.
 - Moreover, all of the MAP treatments deteriorated rapidly after transfer to room temperature. It was concluded that MAP was unlikely to be cost effective for mushrooms.
- Based on studies of respiration, Varoquaux et al¹¹¹ concluded that no extension of mushroom storage life was possible using modified atmosphere packaging. Instead, controlling RH and free moisture was considered far more likely to be effective.

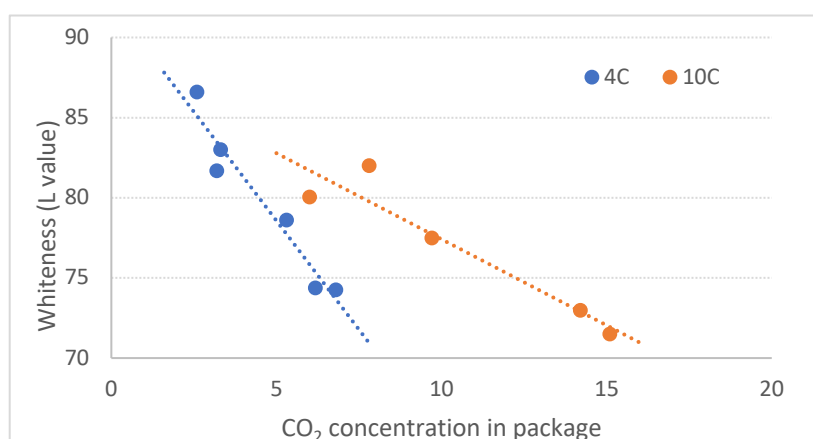


Figure 40. The effect of CO₂ concentrations inside the package on mushroom cap whiteness following 8 days at 4°C or 10°C. Derived from Lopez-Briones et al., 1993.

Packaging – general

- Other researchers have examined novel packaging products which have different modes of action for improving storage life.
- Many recent papers have explored uses for chitosan, a product commonly extracted from shellfish waste. Chitosan can stimulate plant defences, has anti-microbial properties and can be manufactured into many different products. As previously discussed, chitosan be used to encapsulate essential oils^{102,101}, or be used as a coating. It can also be made into biodegradable film.
 - Mushrooms packaged using films made by blending 1:1 chitosan and zein (maize protein)¹¹⁵ or chitosan and gallic acid¹¹⁶ retained whiteness better than mushrooms packaged in standard PVC film during storage at 4°C.
 - The chitosan – zein result was further improved by adding the anti-oxidant alpha-tocopherol¹¹⁷. The (edible) film stimulated enzymes involved in anti-oxidant defence and inhibited bacterial blotch, reducing browning during storage.
 - Ban et al⁹⁵ and Gholami et al⁹³ took a different approach, coating mushrooms with chitosan before packaging with various films. In both cases the chitosan coating increased, rather than reduced, browning. Although the authors state that the MAP increased storage life, none of the treatments appeared to have significant benefits for mushroom whiteness.
- Guillaume et al¹¹⁸ compared wheat gluten (WG) coated paper with uncoated paper and stretchable PVC film. Although CO₂ increased to 9% in the WG paper, the paper had the benefit of avoiding free moisture while increasing RH around the mushrooms.
- Singh et al¹¹⁹ developed packaging to help keep mushrooms cold during transport, potentially including for home-delivery. Paraffin was encapsulated in melamine powder, then used as a coating for a poly-textile. This was used to line cardboard cartons. As paraffin oil melts it absorbs heat energy, keeping the contents cool. While this is certainly a novel and interesting development, cost would seem likely to be a barrier to use.

6 The future – breeding whiter mushrooms

Key points

As mushroom browning occurs through the relatively narrow physiological pathway that ends with formation of melanin, it should be possible to breed mushroom varieties resistant to browning reactions. Unfortunately, mushroom breeding is technically difficult due to the *Agaricus bisporus* lifecycle, which limits opportunities for re-combination of different strains.

New molecular techniques are overcoming these issues and have successfully identified areas on the mushroom genome responsible for both white cap colour and resistance to bruising. This progress should facilitate faster development of new varieties with improved quality attributes.

One of the other factors hampering progress has been the difficulty of protecting new varieties; it is relatively easy to produce a new mushroom culture from the sold product, and difficult to identify varieties simply from their morphology. However, awareness of this issue, together with new sequencing tools, are hoped to overcome this issue in the future.

Creating new varieties

- Browning of mushrooms, whether due to physical damage or microbial infection, occurs by oxidation of phenolic compounds and formation of melanin. Discolouration therefore depends on the presence and concentration of a number of enzymes and substrates as well as the strength of the cells' internal membranes. This effectively means there is a strong genetic basis in susceptibility to browning¹²⁰.
- Developing strains of *Agaricus bisporus* with reduced levels of either phenols or enzymes would be a clear way to produce whiter, less bruise-sensitive mushrooms. According to Gao et. al. (2013), this has been considered a particular priority in Europe as it would allow mechanical harvesting for the fresh market, with major cost savings as a result.
- Breeding of fungi presents distinct challenges, as reproduction may be sexual or asexual. The main reason for the lack of new cultivars of *Agaricus* relates to the difficulties posed by its lifecycle¹²¹.
 - *Agaricus bisporus* is a member of the homobasidiomycetes. One of the characteristics of this family is that each cell contains two different haploid nuclei, each of which contains only half of the fungal chromosomes. These stay apart in the cell, resulting in a fertile "heterokaryote", capable of developing and forming new mushrooms.
 - During production of spores by the basidia, the nuclei divide into four, with one of each pair distributed to each of the two (heterokaryon) spores produced.
 - About 10-15% of basidia will form three or four spores instead of only two, the majority of which contain only a single haploid nuclei. These homokaryotic cells can grow vegetatively, but are unable to form mushroom fruiting bodies. However, importantly, they can be used for breeding.
 - To identify homokaryons, cultures are grown from single cells. These differ from the original culture in growth habit and appearance, a difference that can be confirmed using molecular techniques.

- The homokaryons are then “protoplasted”, removing the cell wall.
- Two different protoplasts are grown together on agar plates seeded with compost. Where the mycelia cross, the cells can merge.
- These crossings can be identified through close examination of the hyphal connections and confirmed through molecular methods.
- The crossing forms a new heterokaryotic cell with two separate nuclei; effectively, a potential new mushroom variety.
- The difficulty in identifying homokaryon cells for breeding is noted by Manju et al (2016)¹²², who note that of 1,642 single spore isolates cultured from parental strains, only 36 were homokaryons.

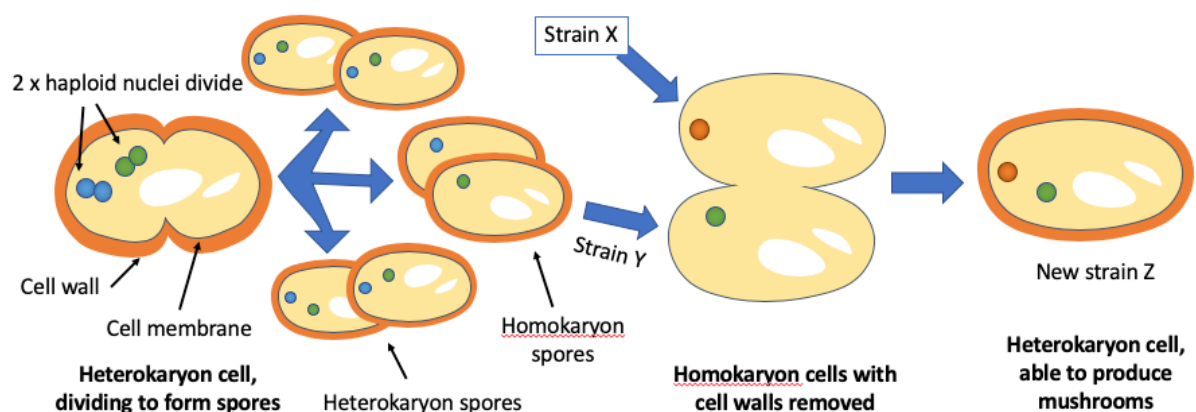


Figure 41. The process by which new varieties of *Agaricus* can be produced. See text above for explanatory notes.

Identifying genes for cap colour and bruise susceptibility

- Quantitative trait locus (QTL) analysis is used to identify the genetic regions that are responsible for particular traits. This is done by crossing and re-crossing strains with/without the trait of interest, then comparing the genomes of those crosses using molecular markers.
- Up to 90% of variation in cap colour is explained through a single major location (gene) – the PPC1 locus – together with two additional minor loci. Whiteness is therefore highly heritable and stable. As cap whiteness is at a different location on the genome to loci that relate to yield and disease tolerance, it should be possible to develop mushroom varieties with all of these desirable characteristics¹²³.
- In a large study of genetic variation in bruising susceptibility, Gao et al (2013) developed a number of hybrid strains. These had widely differing cap colours as well as susceptibility to bruising, allowing selection for whiteness at harvest and following packing and storage (Figure 42).

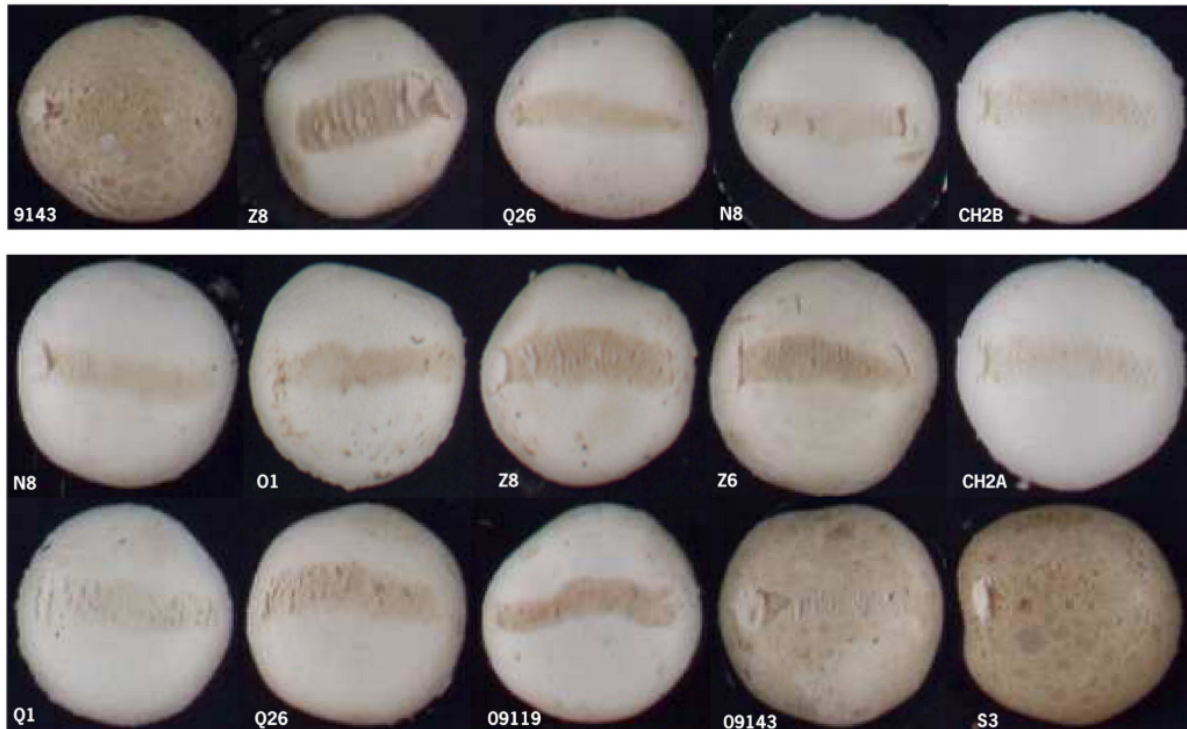


Figure 42. Bruised first flush hybrid mushrooms with strain CH2A (top) or CH2B (bottom) as one parental line. All mushrooms photographed 60 minutes after bruising. From Gao et al., 2013.

- Bruising susceptibility is controlled by a number of different genes. So, for example, up to 54% of bruising sensitivity is explained by changes in two chromosomes, with up to 31% of bruising in flush 2 mushrooms associated with an area on a third chromosome¹²⁴.

So why don't we have new, whiter varieties already?

- Breeding techniques using molecular methods have successfully identified differences between strains of mushrooms as well as the genetic basis of desirable traits such as whiteness and resistance to bruising. A substantial collection of strains is now available for research purposes. However, this improvement has so far not translated into large numbers of new varieties. Reasons for this include:
 - The technical difficulty of generating new cultivars
 - Lack of good breeding stock as starting points; breeders are often forced to use wild germplasm to develop new varieties
 - The difficulty of protecting new varieties (even with plant variety rights), as it is relatively easy to generate new cultures from marketed mushrooms or commercial spawn, and difficult to identify varieties from their morphology alone¹²⁵
 - According to Sonnenburg et al (2017), this last issue is the most important, but may be resolved through (global) industry wide awareness, legal changes and enforcement through present-day sequencing tools as these can prove derivation.
- Gene editing offers a more efficient method for selective breeding than traditional techniques. Gene editing allows breeding programs to precisely target and delete parts of the genome that are desirable to change.
 - Site Directed Nuclease (SDN-1) techniques are used to cut DNA at a specific location, preventing gene expression.

- In April 2020 the Australian Office of the Gene Technology Regulator (OGTR) decided not to regulate this form of gene editing, at least for research purposes. It was considered that these changes are no different from those that can occur naturally, so do not pose additional risks to the environment and human health¹²⁶. The [exclusions](#) apply only if:
 - No nucleic acid template was added to cells to guide genome repair
 - The organism has no other new traits from the gene technology eg an expressed protein or cas9 transgene
- A new, browning resistant mushroom variety has been produced in the USA using CRISPR-Cas9, and is believed suitable for commercialisation¹²⁷.
 - CRISPR stands for “clustered regularly interspaced short palindromic repeats”. The technique is highly targeted at specific genes, using the DNA cutting enzyme “Cas9” to delete targeted sections of DNA.
 - In 2016 Dr Yinong Yang from Penn State used this technique to delete enzymes involved in mushroom browning.
 - The US Food and Drug Administration has ruled that the strain is **not** a genetically modified organism (GMO), so can be commercialized.
- The development of the non-browning mushroom has brought this technology into focus, with the decision on whether non-browning mushrooms are “GMO” remaining controversial. While the USA has completely excluded gene-edited plants from regulatory oversight, the European Union has ruled that these crops should be treated as genetically modified organisms, making them subject to stringent regulation. There also appears to be potential consumer resistance to such techniques. For example;
 - The German institute Testbiotech has questioned why a full risk assessment has not been conducted to examine the mushroom for other physiological changes. They have created a [YouTube video](#) suggesting that there could be unintended effects from genome editing, such as diarrhea due to changed digestability.
 - Anti-genetic modification groups such as “Natural Society” actively campaign to force products created with CRISPR technology – including Dr Yangs non-browning mushrooms – to undergo the same regulatory procedures (e.g. mandatory labelling) as GMOs.
- FSANZ is currently (April 2020) [reviewing](#) how the Food Standards code should apply to food derived using new breeding techniques (NBTs), such as CRISPR. They note that there are no NBT foods in the Australian food supply and only one that has been commercialized overseas (soybean) (foodstandards.gov.au).

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Appendix 2

Effect of irrigation with calcium chloride, tap water or distilled (RO) water on mushroom whiteness during refrigerated storage

A preliminary trial

Background

One of the key findings from the desktop review of “Improving mushroom whiteness” was the large body of research indicating that irrigation with 0.3% calcium chloride (CaCl_2) improves mushroom whiteness. The effects become more significant with flush and, while observable at harvest, become increasingly obvious during storage. The effects are believed to be due to strengthening of the cell walls, increased dry matter and potential antimicrobial effects on *Pseudomonas tolaasii*, the bacteria responsible for brown blotch.

Even though much of this research was conducted more than 20 years ago, there has been limited adoption by industry. This may be due to concern over the legality of adding CaCl_2 to irrigation water, or lack of local data to support this technique.

Another technique that emerged during discussion with mushroom growers was the use of Brita filtered irrigation water to improve whiteness. Brita filters are designed to reduce chlorine, metal salts and calcium ions from water. In this case, results were observational, and nothing supportive could be found in the literature.

It was therefore decided to conduct a small, preliminary trial examining comparing the effects of irrigation with tap water, 0.3% CaCl_2 or reverse osmosis (RO) filtered water. Reverse osmosis filtration removes the same dissolved solutes as a normal water filter, as well as volatile organic compounds and fluoride. The effects on mushroom colour at harvest and during storage in a normal domestic refrigerator were recorded.

Method

Holes were drilled in the bases of nine plastic tubs (3 replicates/treatment), then each tub was packed with 7kg of Phase 3 mushroom compost. A casing layer (approx. 5cm thick) of black peat plus lime was added. The tubs were placed on the top layer of racks inside the March Lawson Mushroom in a randomised block design and watered to runoff with normal tap water. Irrigation with 0.3% CaCl_2 solution or RO water commenced once the first pins appeared. All subsequent irrigations used these solutions.

Mushrooms were harvested at the cup stage, then weighed so as to record total yield/tub. One 500g punnet was packed with mushrooms from each tub from flush 1 and 2; as yield from flush 3 was small, a single punnet per treatment was prepared using equal numbers of mushrooms from each replicate. Each punnet was overwrapped with stretchable plastic film.

Colour was recorded using a Nix Pro colour scanner (nixsensor.com) on six mushrooms per replicate (18 measurements/punnet for flush 3). Measurements were taken at harvest, then at intervals during 3°C storage in a domestic refrigerator. The L, a and b values were used to calculate the white index (WI) and browning index (BI) according to the methods described by Aday et al., 2016.

$$WI = L - (3a + 3b)$$

$$BI = 100 \times ((x - 0.31) / 0.17) \text{ where } x = (a + (1.75 \times L)) / ((5.645 \times L) + a - (3.012 \times b))$$

The results were analysed using CoStat statistical software. Flush, treatment and replicate were used as factors, with differences in whiteness and browning index analysed at harvest, then following 8/9 days of refrigerated storage. Following ANOVA, mean values were compared using the Student-Newman-Keuls test for least significant difference, with means analysed at the 95% confidence interval.

Results

Total yield was similar for all three treatments for the first two flushes, with a small increase in yield in tubs irrigated with RO water compared to those irrigated with tap water in flush 3. It is unclear why this may have occurred.

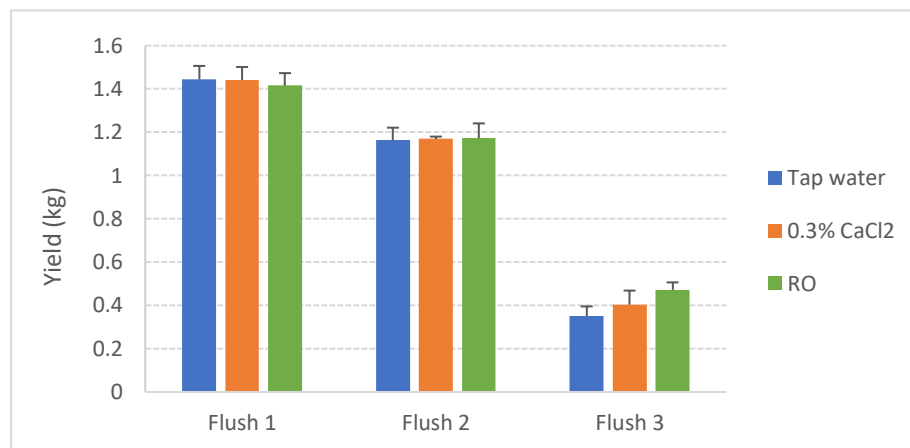


Figure 1. Average yield / replicate tub of mushrooms irrigated with tap water, a solution of 0.3% CaCl₂ or reverse osmosis filtered water. Error bars indicate the standard error of each mean value (n=3).

Unusually, average whiteness at harvest increased between flush 1 and flush 3. This may be partly due to the increased scaling that was observed for flush 1 mushrooms, a condition likely caused by insufficient humidification of the growing room.

Both the whiteness and browning indexes proved effective at discriminating between the irrigation treatments. In both flush 1 and flush 3, the mushrooms irrigated with CaCl₂ stayed whiter, and appeared to be less susceptible to bruising than the other two treatments. Although the initial difference was small, it increased during storage. For flush 2, the differences between treatments were smaller. However, the calcium treatment still provided some benefit.

The difference between treatments was greatest for flush 3, where there was a clear visual difference in the incidence of bacterial blotch, as well as expression of bruising for the CaCl₂ treated mushrooms.

If it is considered that saleable life is ended at WI = 50, then CaCl₂ treatment increased estimated saleable life from 4 days to 9 days (flush 1); 8 days to 12 days (flush 2); 7 days to 13 days (flush 3) when compared to irrigation with tap water alone.

These results are consistent with those reported in the literature on the effects of irrigation with CaCl₂.

There was no significant or consistent effect of irrigation with RO filtered water compared to tap water, in this trial. This may reflect the fact that Sydney water from the Potts Hill delivery system contains only 47 to 56 mg CaCO₃/L which means it is considered 'soft'. The tap water also contains a very low level of chlorine (1.12 mg/L) chlorine and is slightly acidic (pH = approx. 7.8). While the RO water should contain close to zero CaCl₂ and chlorine, the differences between these two solutions may have been too small to significantly impact mushroom quality.

Statistical analysis of the data indicated that differences due to replicate and flush were not significant. However, there were small, but significant differences in browning (p=0.015) and whiteness (p=0.031) among the treatments at harvest, with these increasing (p<0.000) during refrigerated storage.

Table 1. Mean whiteness and browning indices of mushrooms irrigated with tap water, reverse osmosis filtered water, or water containing 0.3% CaCl₂, at harvest and following 8 (flush 1 and 2) or 9 (flush 3) days of refrigerated storage. Letters indicate values that are significantly different (p<0.05). Values combined from all three flushes.

	At harvest		After 8/9 days storage	
	Whiteness index	Browning index	Whiteness index	Browning index
Tap water	59.2 b	12.2 a	48.1 b	15.9 a
RO water	59.9 b	12.0 a	49.2 b	15.3 a
0.3% CaCl ₂	62.8 a	10.9 b	55.5 a	13.3 b

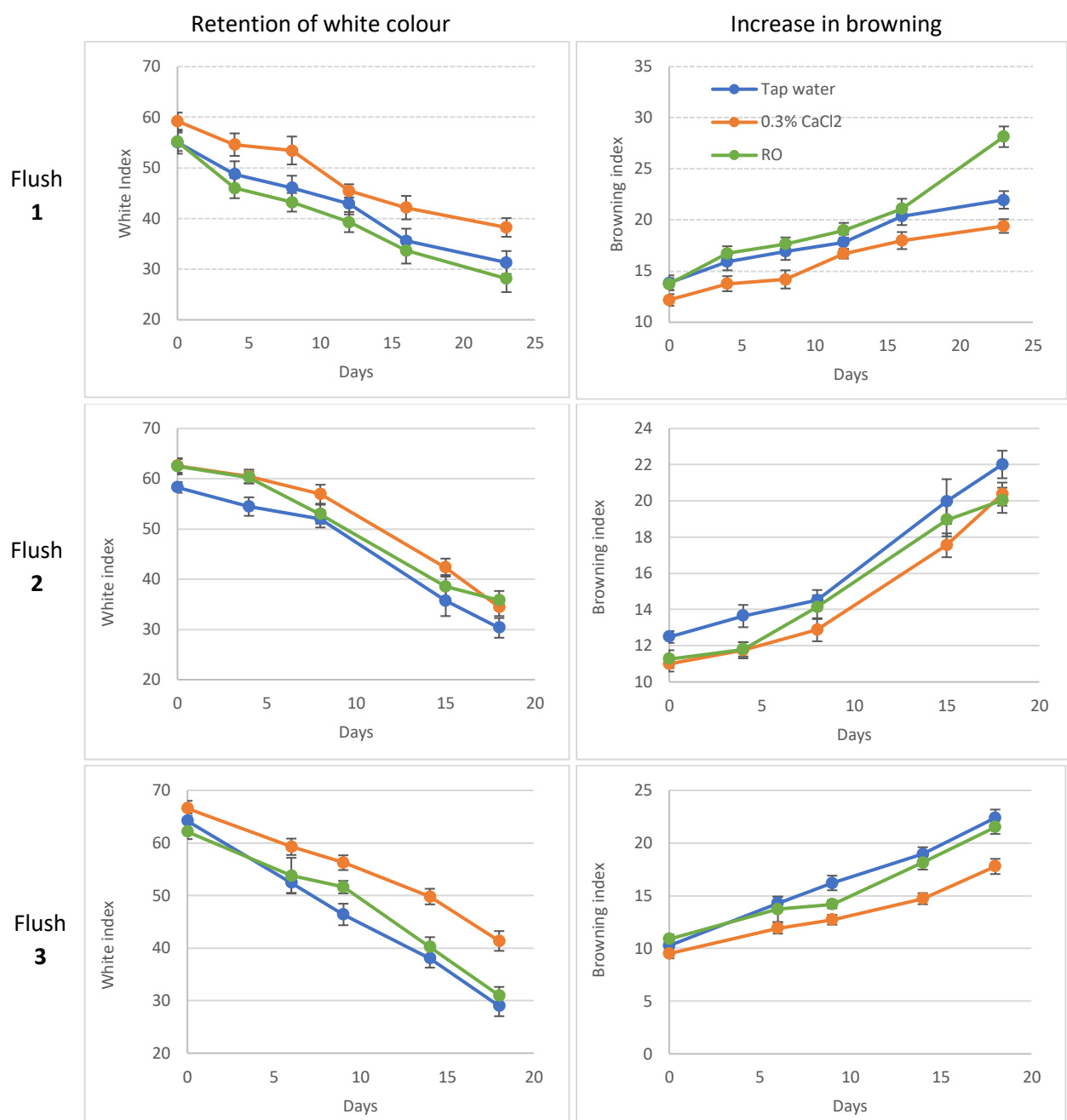


Figure 2. Changes in whiteness (left) and browning (right) of mushrooms irrigated with tap water, a solution of 0.3% CaCl₂ or reverse osmosis filtered water during storage at 3°C. Bars indicate the standard error of each mean value (n=18).

Table 2. Appearance of samples during storage of mushrooms irrigated with tap water only, 0.3% CaCl₂ or RO water.

	Tap water	CaCl ₂	RO water
Flush 1 <i>pre-harvest</i>			
<i>day 23</i>			
Flush 2 <i>day 15</i>			
Flush 3 <i>day 0</i>			
<i>day 6</i>			
<i>day 14</i>			

Conclusions

Irrigation with 0.3% CaCl_2 had significant benefits for mushroom quality. Mushrooms irrigated with this solution were whiter and had increased storage life. They also appeared to be firmer and heavier, an observation that could be confirmed by measurements of texture and density in a later trial. The treatment approximately doubled estimated storage life of flush 1 and flush 3 mushrooms, with a 50% extension for flush 2 mushrooms, when compared to irrigation with tap water alone.

Calcium chloride is generally recognised as safe by the US FDA and approved as a food ingredient in Europe. It is used as an ingredient in a number of processed foods as well as isotonic sports drinks. Moreover, research has shown that mushrooms irrigated with CaCl_2 have increased levels of calcium, suggesting there could be a potential benefit to health.

It is strongly recommended that the industry further investigate the use of calcium chloride in irrigation water.

Improving mushroom whiteness – Best Bets

White mushrooms are quality mushrooms

Presenting clean, white mushrooms to consumers at retail is a proven method of increasing sales. For mushrooms, whiteness signals quality. It may also be assumed to indicate storage life, flavour and freshness.

Conversely, browning on mushrooms is a negative. Browning may be due to disease, bruising, dehydration or simply age and senescence. Browning mushrooms are more likely to be soft, slimy and/or with poor flavour and texture, as expected near the end of storage life.

Mushrooms turn brown due to a reaction between phenolics normally kept inside the cell vacuoles and enzymes in the cell cytoplasm. When these mix, they oxidise, producing the brown pigment melanin.

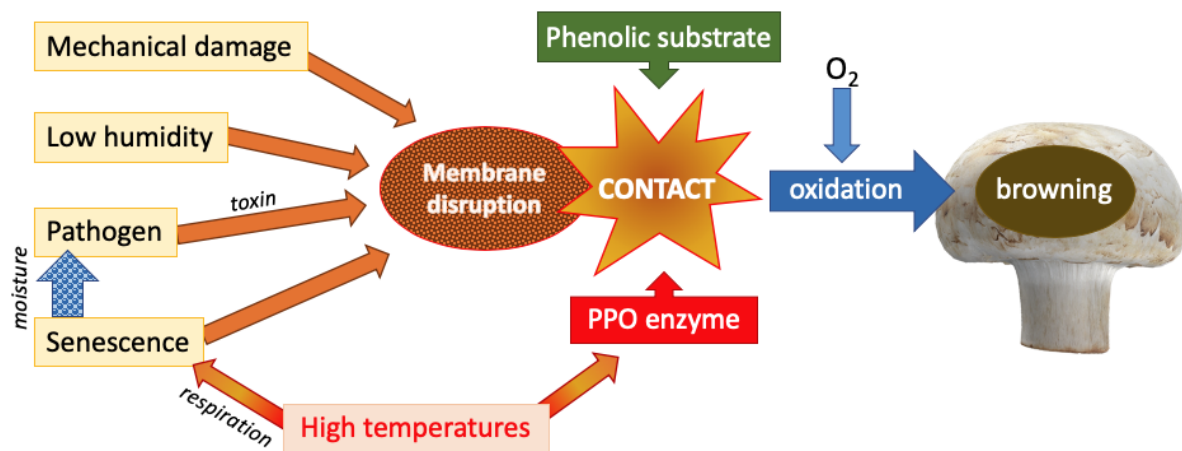


Figure 1. Mechanisms of mushroom browning.

Many of the strategies used to improve mushroom storage life and whiteness are focused on inhibiting the reactions that form melanin. Treatments may aim to enhance the strength of cell membranes, prevent oxidative reactions, or create genetic changes that reduce enzyme activity. Methods to improve whiteness can be employed at all stages of production, from casing to harvest, packing and storage.

This document summarises the “Best Bets” for enhancing mushroom whiteness at each stage of the production process.

Crop production and nutrition

Using healthy, well-balanced compost is the first step to producing high quality white mushrooms.

Nutritional supplements are commonly added to compost to increase yield. They may also be added to casing, either to increase yield or improve pinning. While most supplements are protein based, they can include mineral micronutrients, lipids, acids and activated carbon. Their effectiveness depends on:

- Delaying release of nutrients to ensure they are available through the cropping cycle
- Controlling temperature increases triggered by adding the supplement
- Matching the minerals and nutrients required with the attributes of the compost

Although most supplements have little or no effect on colour at harvest, differences may develop postharvest. Limited research has found that mushrooms produced with supplements brown more slowly during storage. This could be partly linked to dry matter, which can be increased by supplements. Mushrooms with high dry matter have larger carbohydrate reserves, which helps to sustain them during storage.

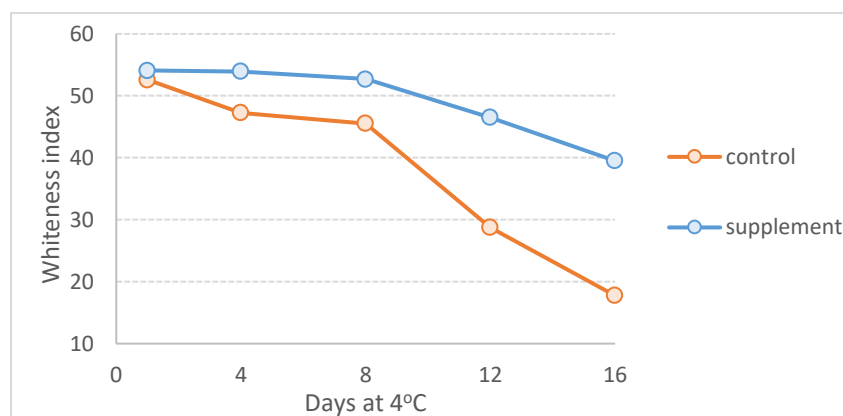


Figure 2. Effect of supplementing casing with 10g/kg corn or soybean meal (average of both treatments) on the whiteness index of stored mushrooms. Derived from Adibian and Mami, 2015.

Even though mushrooms with high dry matter can sometimes be **less white** at harvest they often **stay whiter** during storage when compared to mushrooms with low dry matter.

Casing

The whitest mushrooms are generally produced using black peat. However, blends of peat with up to 25% coal tailings, or 60% spent mushroom compost, did not impact colour. Local trials have also found little difference in colour when peat based casings were blended with up to 50% recycled organics. Although some trials have found that mushrooms were less white when cased with only 0 - 20% peat, a Spanish study found that a local blend of mineral soil with coconut fibre produced high quality white mushrooms.

Pinning

Pinning is stimulated by bacterial activity in the casing (which removes volatiles produced by the mycelia), dropping temperatures and changes in CO₂ levels in the room air. Of these, reductions in CO₂ are the key factor determining the number of mushrooms that develop.

Avoiding over-pinning on mushroom beds is essential to prevent bruising, both during development and at harvest. Over-pinned crops are likely to become over-mature and soft, further reducing whiteness.

Gradually reducing CO₂ can stagger pin set, known as 'choking the beds'. However, it is important not to delay airing too long, or yield will be reduced.

Managing the room environment

While it is important to keep relative humidity (RH) high inside the room, it is clearly essential to avoid free water sitting on the mushrooms themselves. Accurate control of temperature, manipulation of CO₂ to increase/decrease pinning and avoiding excessively high or low airflow are all essential in order to produce high yields of white, smooth, high quality mushrooms.

Irrigation is a balance between the mushrooms being too dry – which can result in the caps being scaly and dry – and too wet, which allows bacterial blotch to develop. Mushrooms are 90 to 95% water, so the quality of water used and when and how it is applied will strongly influence quality and storage life.

It is essential that mushrooms dry within four hours of irrigation:

- *Before irrigating* – Dropping the air temperature to reduce absolute humidity (total moisture content of the air)
- *After irrigating* – Raising the room temperature and increasing fan speed by approximately 5-10%.
- *After irrigating* – Introducing fresh, dry air to the grow room, if conditions are conducive.

The temperature difference between the bed and the room air also drives evaporation; beds are likely to be warmer after addition of supplements, during first flush and when the compost volume to surface area ratio is high.

A new, automated drip irrigation system is available from Netafim which is designed to maintain constant moisture (and nutrient) levels in the substrate throughout the cropping cycle. It is claimed that bacterial blotch is reduced and quality improved, due to avoiding irrigation water contacting the mushrooms. The company also states that casing material requirements are reduced by 30%, a significant cost saving.



Figure 3. The Netafim "Mushroom Master" drip irrigation system (left) and the insertion unit on a Thilot head filler. From Raz et al., 2016.

Airflow needs to balance the metabolic heat produced by the crop. So, for example, higher rates of air exchange may be needed approaching flush 1 than for flush 3. Good airflow is also essential in order to manage CO₂ levels and to help mushrooms dry after irrigation.

However, if air velocity is too high the mushrooms will be scaly and discoloured. High airflow can physically damage the delicate caps, increasing browning. This can be avoided using air-trainers, such as netting diffusers and cones linked to airlines, to soften or increase speed, guiding ventilation to where it is needed.

Relative humidity affects mushroom texture and structure. Low RH results in the mushroom caps developing a scaly, off white appearance as well as increasing enzyme activity. This in turn increases susceptibility to bruising, an effect that is particularly pronounced in the early flushes. However high RH reduces drying, so can increase development of blotch. Room RH should be held as constant as possible, ideally between 82-90%.

Casing moisture levels should be maintained at approximately 60% water content by volume. Dry casing results in mushrooms that are soft, susceptible to premature opening and less white overall. However, wet casing reduces yield and evaporation, resulting in mushrooms that are low dry matter, may have water-soaked 'windows' and are prone to browning. Keeping casing well hydrated can be difficult if there is a heavy first flush, as this removes a lot of water from the substrate. It is essential casing does not dry out, as it never regains its previous moisture holding capacity.

Additives to irrigation water

There is a long history of including sanitisers in irrigation water. While chlorine (in the form hypochlorous acid) can control human pathogens in water, high concentrations are needed to reduce bacterial blotch on mushrooms.

Stabilised chlorine dioxide has a more specific mode of action than hypochlorous acid and is relatively unaffected by pH and organic matter. A concentration of 50ppm has been shown to be effective against bacterial blotch.

Stabilised chlorine dioxide is registered for treating water (5ppm) and disinfecting equipment (100ppm) in mushroom facilities. Like all chlorine products, stabilised chlorine dioxide should be used with care, according to label directions.

Calcium chloride (CaCl₂) has been widely demonstrated to improve whiteness at harvest, with effects increasing during postharvest storage. Application of a 0.3% CaCl₂ solution from pinning increases cell wall integrity, improving firmness and reducing susceptibility to bruising and browning. Calcium chloride is used as an ingredient in a range of foods and beverages and is "generally recognised as safe" by the US FDA.

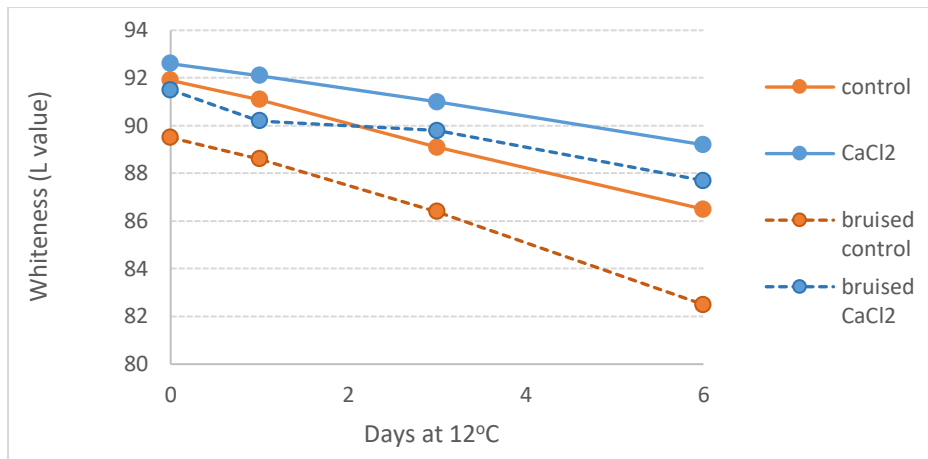


Figure 4. Effect of irrigation with 0.3% CaCl₂ on whiteness of intact and bruised mushrooms during storage at 12°C. Derived from Kukura et al., 1998.



Figure 5. Mushrooms irrigated with tap water (left) or 0.3% CaCl₂ (right) following 6 days storage at 3°C.

The legal status of adding CaCl₂ to irrigation water for mushrooms is currently being investigated by the AMGA.

A large number of other additives to irrigation water have been tested, including hydrogen peroxide, citric acid, Vitamin C, plant growth regulators and selenium. While many of these had minor or mixed effects on quality, selenium improved both whiteness and nutritional value, so appears worthy of further investigation.

Harvest and storage

Temperature is the most important factor affecting mushroom storage life. The faster mushrooms are cooled, the less moisture and quality they will lose. To maximise storage life, mushrooms should be cooled below 4°C within one hour of harvest. The best way to do this is by vacuum cooling.

The benefits of vacuum cooling are not just due to the speed of cooling, but to effects on enzyme activity. Vacuum cooling has been shown to inhibit polyphenol oxidase and increase anti-oxidant activity, thereby inhibiting browning reactions.

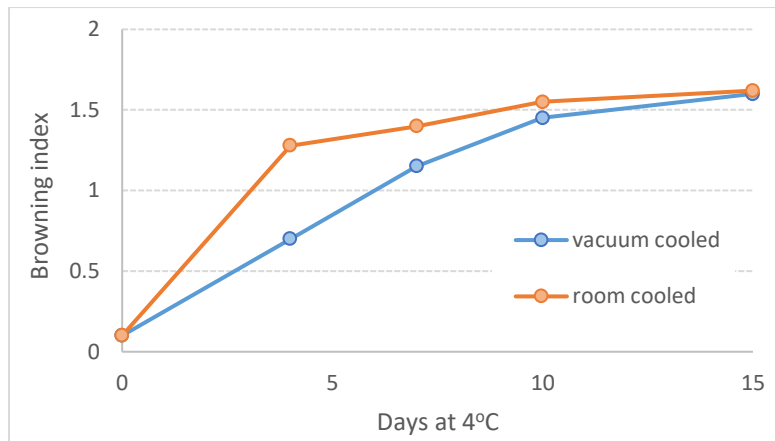


Figure 6. Increases in browning during storage at 4°C of vacuum cooled or room cooled mushroom. Derived from Tao et al., 2007.

Once mushrooms are cold, minimising fluctuations in temperature is the best way to avoid condensation and, therefore, development of bacterial blotch as well as moisture loss. Uniform temperatures can be achieved by

- Reducing the gap between the coolrooms' high and low temperature setpoints (when the compressor turns on/off).
- Minimising the frequency with which doors are opened, and using an air curtain to reduce ingress of warm air.
- Ensuring cold rooms are well insulated, and that insulating materials are sealed against moisture.
- Loading directly from cold rooms into pre-cooled trucks.

A lot of new research (especially from China) has examined the application of various dips, washes, coatings, fumigants, packaging and other novel treatments to extend mushroom storage life. Examples include films made from biodegradable materials or impregnated with anti-bacterial compounds such as essential oil or chitosan. Although none of these are currently commercially available, this is certainly a fast-developing area to watch.

The future – Mushroom breeding

As mushroom browning occurs through the relatively narrow physiological pathway that ends with formation of melanin, it should be possible to breed mushroom varieties resistant to browning reactions.

New molecular techniques have successfully identified three genes which are responsible for up to 90% of variation in mushroom colour. This progress should facilitate faster development of varieties with improved quality attributes.



Figure 7. Differences in bruise susceptibility of a range of first flush hybrid mushrooms, photographed 60 minutes after bruising. From Gao et al., 2013.

A new, browning resistant mushroom has already been produced in the USA using CRISPR-Cas9. This technology allows deletion of specific, targeted parts of the genome – such as the genes responsible for browning. While the resulting organism is not officially considered “transgenic” (no genes were added), there is still public resistance to use of this technique. FSANZ is currently reviewing how the food standards code should apply to this type of product.

The development of new, whiter mushroom strains is an area of significant international effort, with major potential future benefits.

Research Needs and Recommendations

1. Additions to irrigation water

Calcium

Addition of calcium chloride (CaCl_2) to irrigation water, particularly when combined with stabilised chlorine dioxide (ClO_2), has been demonstrated to improve mushroom whiteness and reduce susceptibility to bruising. The effects are most pronounced in 2nd and 3rd flushes. While small improvements may be observed at harvest, major differences develop during storage. Treated mushrooms stay firmer and contain up to 3x more calcium compared to untreated mushrooms.

These effects were confirmed by a small trial at the MLMRU, conducted as part of this project. Mushrooms irrigated with water containing 0.3% CaCl_2 remained whiter during storage at 2-3°C than mushrooms irrigated with tap water or RO filtered water. As observed in the published literature, the effects were greatest for flush 3 mushrooms. It is possible that combining filtration with CaCl_2 could further enhance the results.

Calcium chloride is already permitted as a food additive in the EU and is generally recognised as safe (GRAS) by the US FDA. It is found in canned vegetables, tofu, pickles, and sports drinks. This makes it unclear whether it needs to be registered with APVMA. Judy Allen is currently investigating the legal status of CaCl_2 as an additive to irrigation water, which (if positive) could potentially clear the way to widespread commercial use.

Alternatively, the CALCLEAR inline treatment system claims to reduce clumping of calcium ions, and therefore formation of crystals, in irrigation water. The main objective is to prevent scale forming in irrigation lines and blocking drippers (in hydroponic systems for example). The company also claims that the CalcClear system increases uptake of calcium by plants. Improved calcium uptake by mushrooms could potentially produce similar results to irrigation with CaCl_2 .

Other additives

One paper reviewed examined the effect of including sodium selenite (Se) in irrigation water. Irrigation with both Se and CaCl_2 retained whiteness and increased the Se content in the mushrooms to 60 µg per 100g. This could allow mushrooms to be sold as a functional food, targeted at the 40% of women and 25% of men who are somewhat deficient in this important nutrient.

The literature review also revealed a large number of compounds applied as dips and washes. Compounds with significant effects that are unlikely to require registration include the brassinosteroid brassinolide (a natural plant extract), the amino acid L-arginine (GRAS at intakes of up to 20g/day) and electrolysed water (EW). Electrolysed water is produced by passing an electric current through unfiltered water, generating ultra-low dose chlorine and other ions in solution.

Project proposed:

- A. Investigate the costs and benefits of commercial application of CaCl_2 , Se and possibly brassinolide and L-arginine in filtered / unfiltered irrigation water.
- B. Examine whether the CALCLEAR system increases calcium uptake by mushrooms from moderately hard tap water.
- C. Determine whether irrigation with electrolysed water (EW) can reduce infection by bacterial blotch and improve mushroom whiteness.

In all cases quantify improvements in colour, postharvest quality and storage life. Include consideration of potential food safety issues and regulation as well as whether these products would affect existing irrigation infrastructure. The marketing benefits of calcium and/or selenium enhanced mushrooms could also be investigated.

Crop nutrition

In Europe, protein based supplements are widely used pre-casing, while mineral based supplements have proven beneficial in the USA. The majority of Australian producers also use supplements, such as soybean meal, to increase yield. While effects can be variable, supplements allow re-use (or partial re-use) of spent compost and can correct protein and mineral deficiencies where these exist.

Several studies have indicated that supplements have little or no effect on colour at harvest. However, limited (two) studies also examined postharvest storage attributes. These suggest that major differences emerge during storage, the supplemented mushrooms retaining whiteness and quality significantly longer than the untreated controls. However, such effects may vary greatly between the different supplements available. Testing the effects of supplements on postharvest quality could allow growers to choose supplements that provide the greatest benefit in terms of quality rather than simply yield.

Project proposed:

Independently verify the effects of different commercial mushroom supplements on harvest and postharvest quality of mushrooms. This should include assessment of the effects of supplements on both susceptibility to bruising and senescent browning during storage.

Irrigation method

Even though good management of irrigation is essential for high quality mushrooms, this has generally relied on experience and skill of the operator. However, a large number of moisture sensors are available. These could be used as tools to accurately control irrigation, improving quality while avoiding excess water use.

There are also commercial products available; the Vullings automated water supply system and the Netafim "Mushroom Master" sub-surface drip system. The latter claims to significantly improve mushroom quality by reducing bacterial blotch and maintaining even moisture levels in the casing during flushing. At least one Australian farm is believed to have installed this technology.

Project proposed:

- A. Compare the accuracy and responsiveness of different sensors that could be used to monitor moisture levels in mushroom casing and compost and evaluate how these could add value in a commercial setting. This should include the sensors used in the Vullings and Netafim systems.
- B. Compare the whiteness and quality of mushrooms grown using 1. Sub-surface drip irrigation which maintains a constant moisture level during cropping; 2. Conventional, manual irrigation 3. Conventional but managed (triggered) using the best moisture sensor identified in Part A.

Postharvest treatments

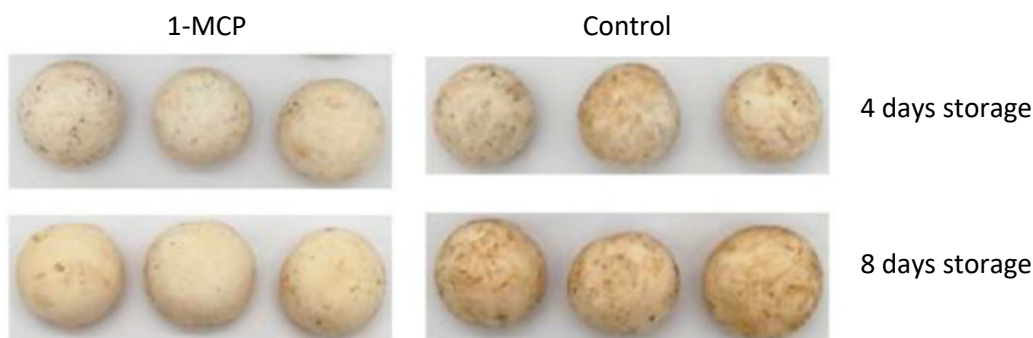
More research has been conducted on postharvest methods to improve storage life of mushrooms than any other part of the process. Treatments include washes, coatings, fumigants, essential oils (incorporated into packaging and direct), irradiation, modified atmosphere packaging and active packaging with various modes of action.

While mushroom washes are used in the USA, Australian growers seem unlikely to adopt this method. While essential oil encapsulation / release systems in packaging appears promising, none of the published studies reported effects on flavour and odour of the treated mushrooms. Moreover, costs may be significant. Many of the other proposed methods and materials (e.g. biodegradable films, films with encapsulated anti-oxidants etc) appear to be a long way from commercialisation.

1-MCP (*Smartfresh*)

One of the options that is already commercialised is fumigation with the ethylene blocker 1-MCP. The majority of apples worldwide are treated with 1-MCP, and it is registered for a range of other crops including pears, persimmons and broccoli. New delivery mechanisms are making it easier to apply, while the release of the compound from patent means that several companies can now supply this product. Extremely low concentrations are effective, and there are no detectable residues after treatment.

While mushrooms are not usually considered ethylene sensitive, recently reported results (Sun et al., 2020) suggest that treatment with 1-MCP can significantly improve mushroom quality during storage and, therefore, appearance at retail, as shown below:



Ozone

Another option that is already available is treatment with ozone during storage. Ozone is thought to reduce browning by inhibiting PPO activity, and can potentially kill bacteria on the mushroom

surface. It would be a simple task to test the effects of low levels of ozone on stored mushrooms using existing storage facilities.

As a potential spinoff, it would be possible to investigate using ozone to control airborne spores in the growing environment, particularly those from “weed moulds”. New ozone generation and monitoring systems can treat the room with higher levels overnight, then flush the residual to safe levels before allowing entry to the room by workers.

Projects proposed:

- A.** A small trial (e.g. student honours project) to examine the effect of 1-MCP fumigation on whiteness of stored mushrooms. This could be conducted in co-operation with AgroFresh, as the company is supportive of research exploring new potentially commercial applications for 1-MCP.
- B.** A small trial (e.g. student honours project) to examine the effects of ozone on whiteness of stored mushrooms with and without inoculation with bacterial blotch. Again, there are many companies interested in this type of application, who may be able to contribute to such a trial.
- C.** *If* there are no negative effects of ozone on mushrooms, a second trial could examine application of ozone in the growing environment to destroy airborne spores of weed moulds. Measurements to include whiteness and quality of mushrooms, as well as potential for spread and persistence of weed moulds using spore traps.

Breeding

Mushroom breeding programs are difficult, time consuming, labour intensive and costly. Moreover, the resulting varieties are difficult to protect through IP. However, new understanding of the genetic factors associated with susceptibility to bruising and browning mean that there are major potential gains in development of new varieties.

New mushroom varieties with reduced susceptibility to bruising / browning may originate in Europe, where there is pressure to automate mushroom harvesting. There also appears to be major research interest in this field in China, while the application of CRISPR to produce a browning resistant variety in the USA is a major – if controversial – innovation.

It is not proposed to develop a breeding program in Australia. However, a watching brief should be maintained on international progress in this rapidly progressing field.