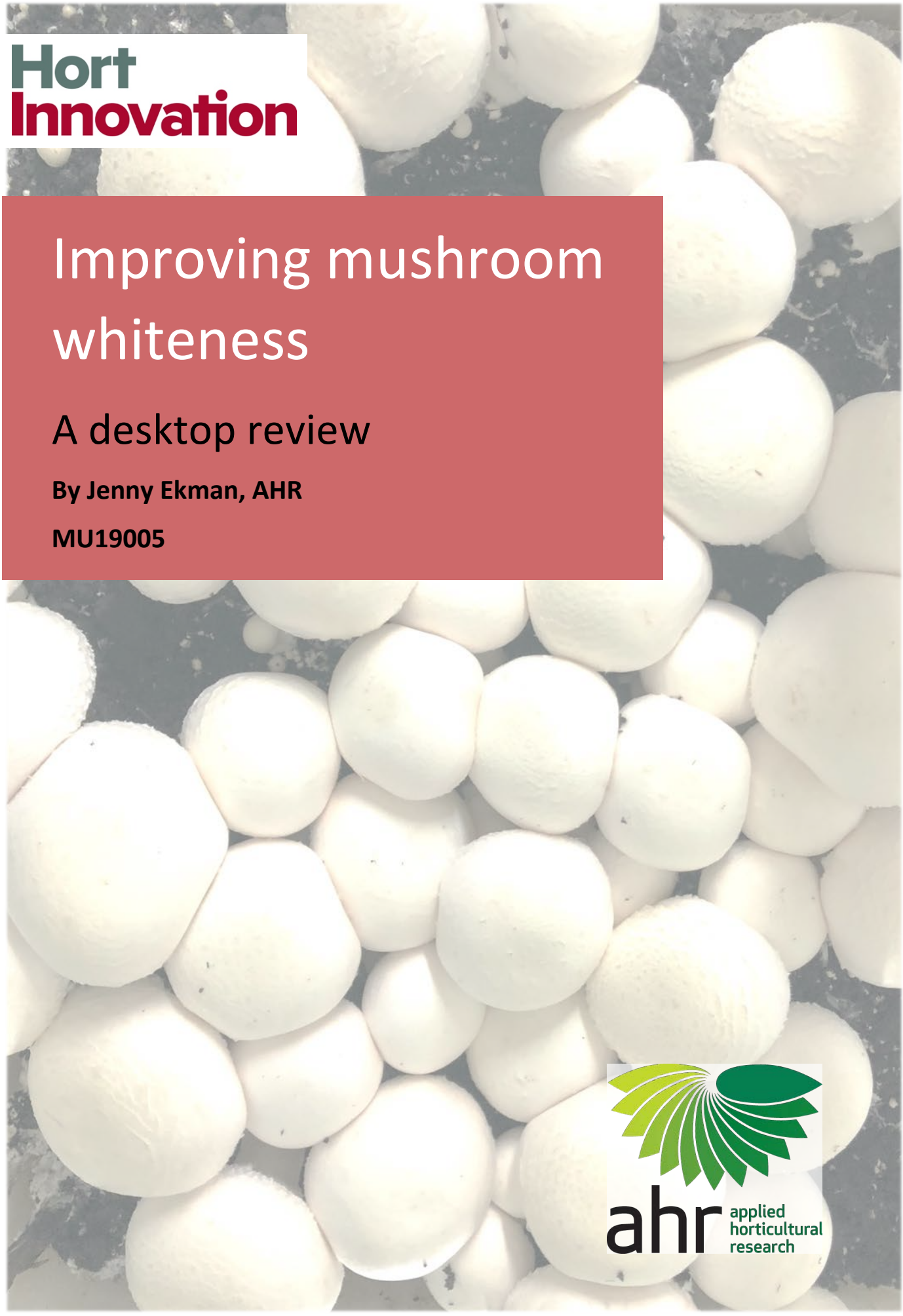
Hort Innovation

Improving mushroom whiteness

A desktop review

By Jenny Ekman, AHR

MU19005



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Improving mushroom whiteness – a desktop review
Report by Dr Jenny Ekman for project MU19005
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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 *Pseudomanas 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.

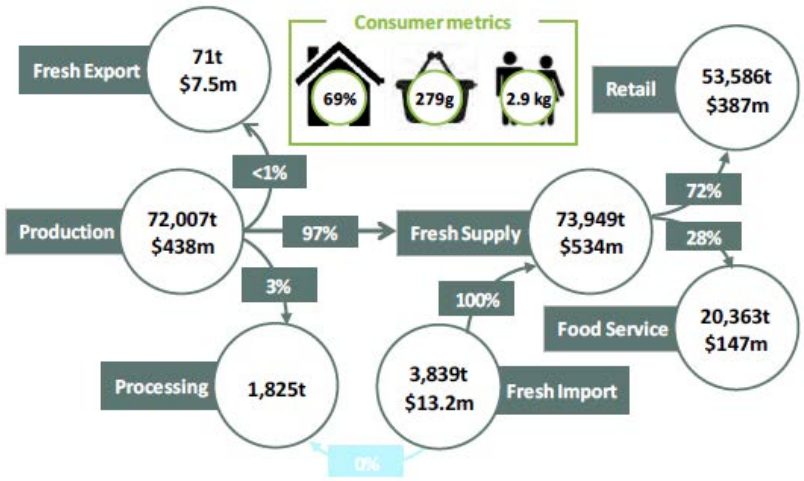

Figure 1. Australian mushroom production by volume, value and supply chain. Data from the Australian Horticulture Statistics Handbook 2018/19.

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.

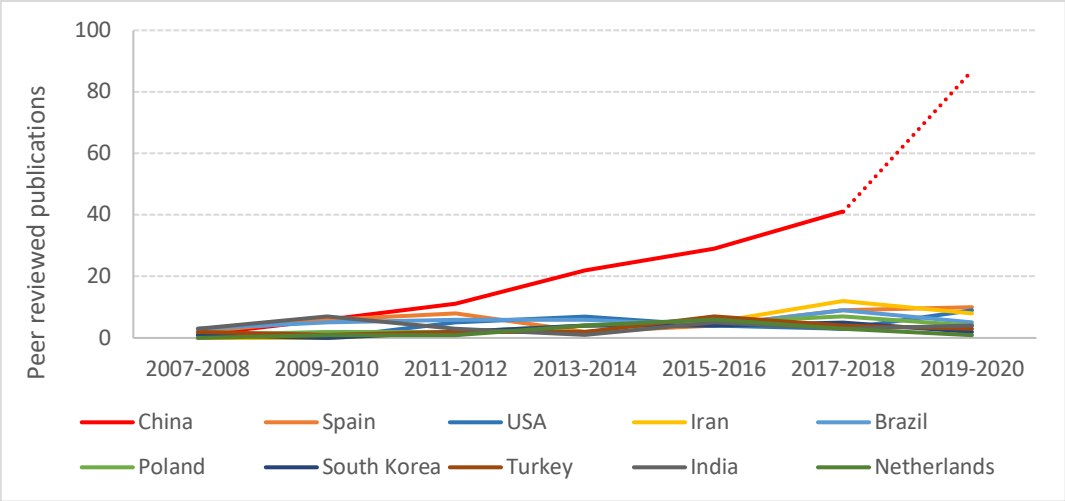

Figure 3. Total peer reviewed publications from the top 10 mushroom research countries, retrieved using a search for "Topic = *Agaricus* + quality", analysed by two year period + country of origin. Only the top 10 countries are included.

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'Sullivan[3], L values indicate;

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

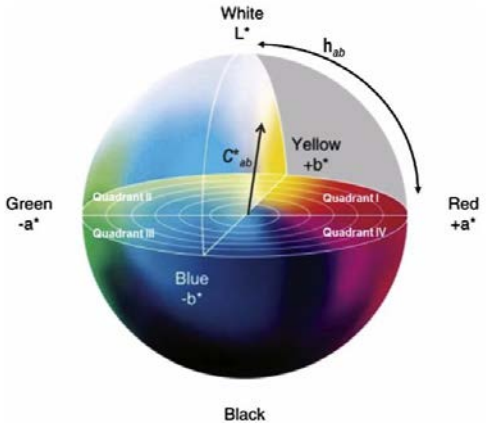

Figure 4. The CIE L*a*b* colour space.

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 cap[7].
    - 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 storage[9].
- 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. agaraci* has been identified as a frequent cause of browning in Europe[8]. 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.

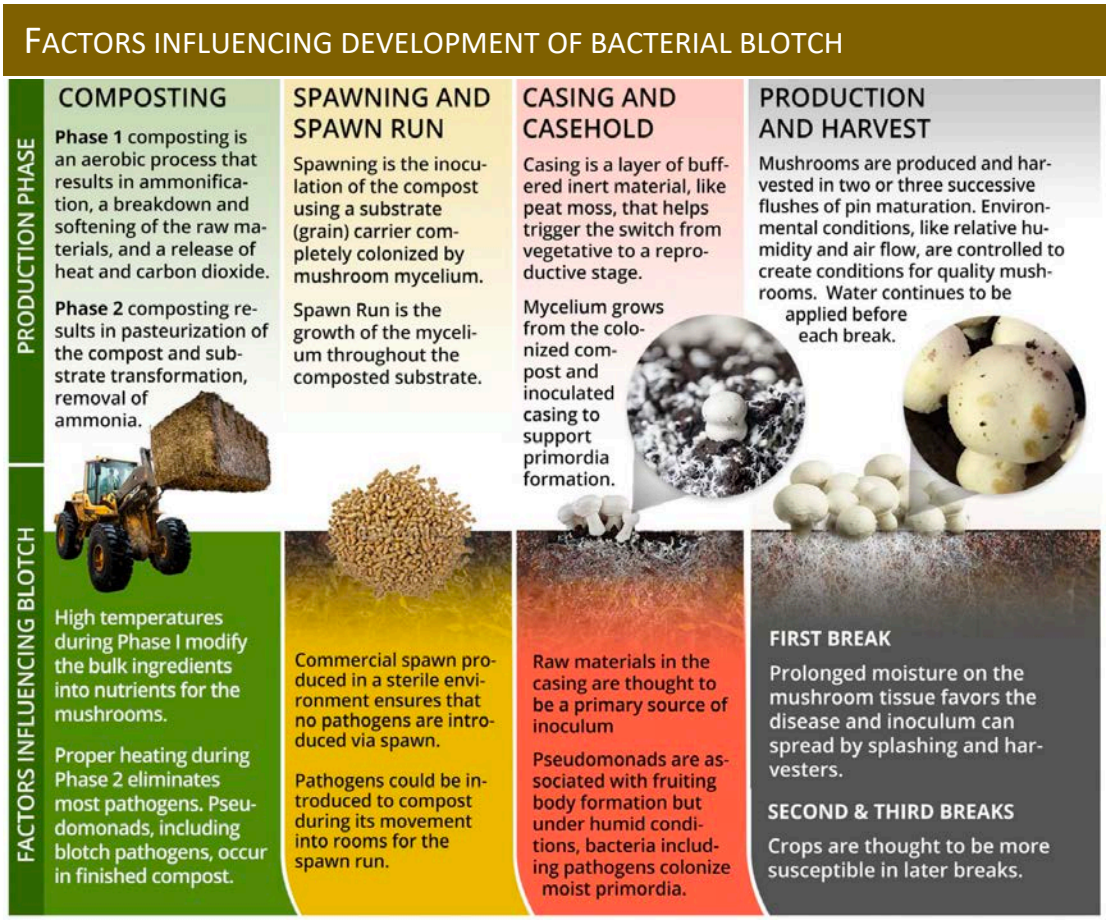

Figure 6. Factors influencing the development of bacterial blotch at different stages of mushroom production. From Osdaghi et al, 2019.

- 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 mycelia[9].
- 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 present[10] (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 melanin[11]. For the mushroom, melanin acts as a chemical barrier, limiting spread of the bacteria into neighbouring cells[7]. However, melanin is also the cause of mushroom browning.
- It was recently found[8] 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.

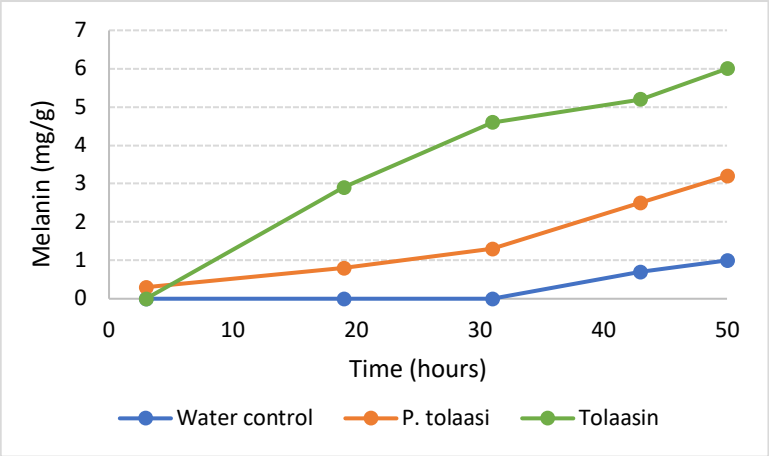

Figure 7. Formation of melanin in mushroom caps following application of water, a suspension of *P. tolaasii*, or partially purified tolaasin extract. Derived from Soler-Rivas et al., 1999.

- *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 caps[10].
    - 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 metabolites[10]
    - 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 equipment[12].
- 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 irrigation[9]
    - Casing has become resistant to water infiltration, especially as splashing water spreads bacteria to pins, which then stay wet for longer[9]
    - 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 caps[13].
    - 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 <u>before</u> irrigation to reduce humidity, then keeping temperatures stable as the mushrooms dry[12]
- 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 development[9].

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 responsible[14].
- Symptoms of La France disease include elongated stalks, small caps and browning mushrooms. Originally identified in Pennsylvania[15], 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 browning[16].

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 al[17], 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 disease[18].

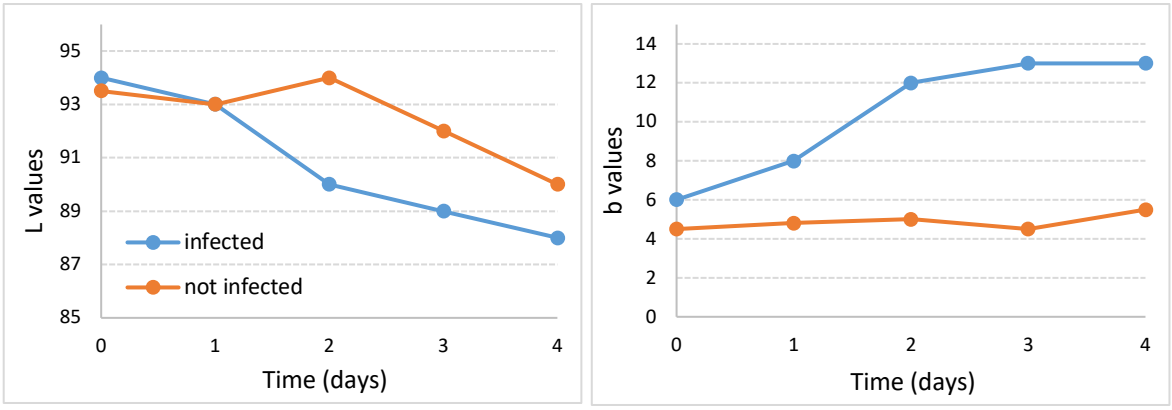

Figure 9. Values for "L" (lightness) and "b" (yellowness) of developing mushrooms that were infected or not with MVX. The non-infected mushrooms appeared off white to the human eye. Derived from Eastwood et al., 2015.

- 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 products[17].

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 melanin[20]. 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 substrates[20].

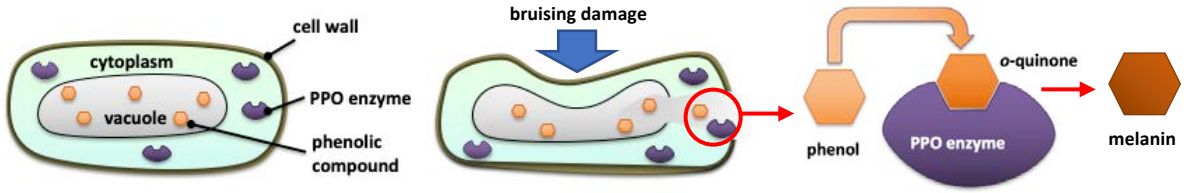

Figure 10. Process by which physical damage causes mushroom browning. Damage to cell membranes allows phenolic compounds to mix with enzymes in the cell cytoplasm. These catalyse a series of reactions culminating in production of the brown pigment melanin.

- 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 slicing[21].
- 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 structure[22]. 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 (Figure 11).

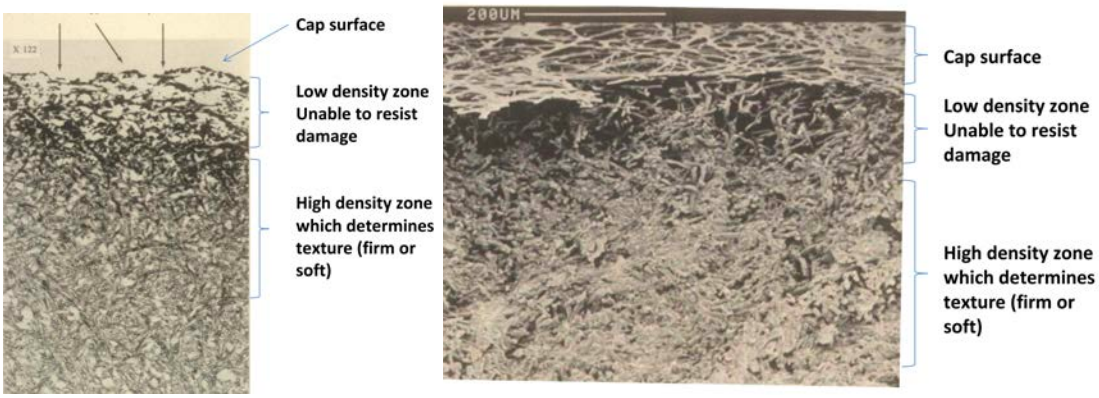

Figure 11. Mushroom cellular structure and density is a factor in bruising susceptibility. From Burton, 2011.

- 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 employees[23]. 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, 2012[20]. 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 <u>less</u> 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 <u>less</u> 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.

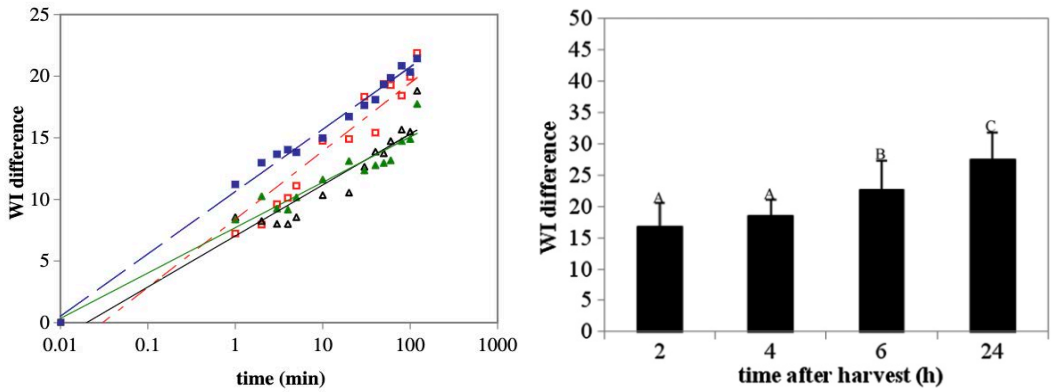

Figure 12. Bruise development of four mushroom varieties over time (left) and the effect of the time interval between harvest and damage (right). Bruising was measured in terms of the whiteness index (WI), which measures the difference between the bruised and non-bruised parts of the mushroom cap. From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

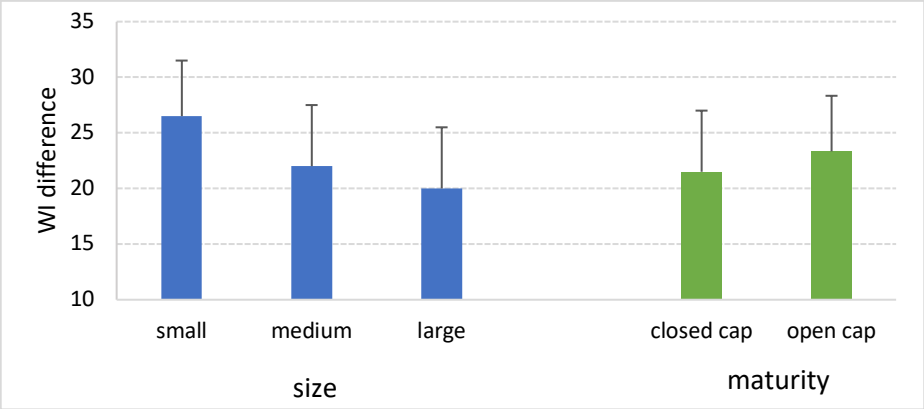

Figure 13. Average bruise development according to mushroom size and maturity. Bars indicate the approximate std. deviation of each mean value. Derived from Weijn et al., 2012.

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

Other physical causes of browning

- **Low relative humidity**; low RH degrades texture and structure and may increase enzyme activity[1], with the result mushrooms can become scaly. Low RH (85%) also increases susceptibility to bruising compared to higher RH (92%) particularly for early flushes[24].
- **Casing wetness**; dry casing can increase susceptibility to browning and bruising[24].
- **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 surface[25].
- **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 browning[26].
    - Rapid airflow physically damages the mushroom tissue, stimulating formation of melanin[27].
    - Managing CO_2 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 storage[27].
- **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.

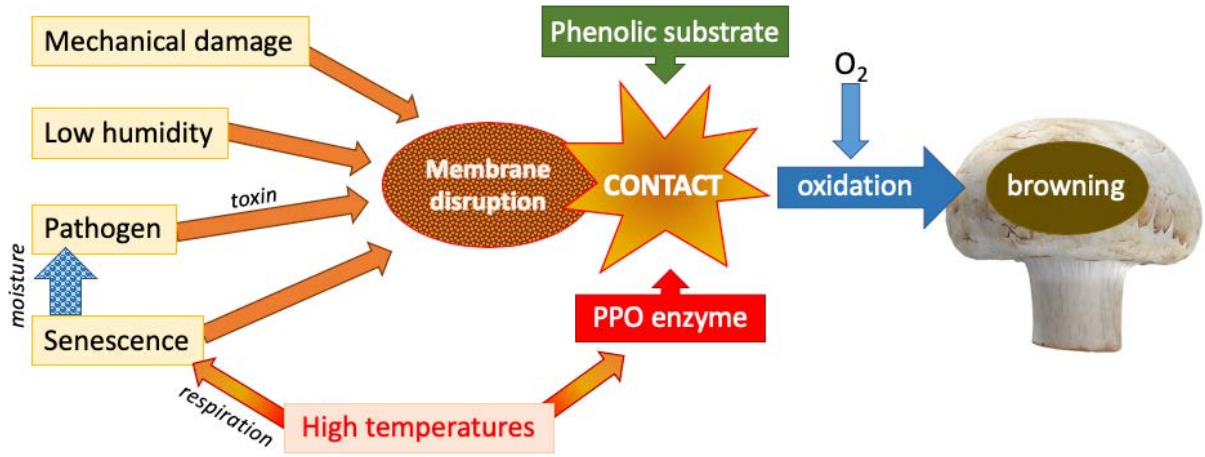

Figure 14. Causes and contributors to browning of mushrooms.

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/m^3;
    - The highest rate of SBL raised pH from 7.3 – 7.5, increased EC and decreased volumetric water content of the casing[28].
    - Mushrooms were whitest with the lowest rate of SBL of 50 kg/m^3. 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/m^3 of SBL with <0.25mm particle size to casing.
- Conversely, Burton[24] 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 Burton[24] 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 al[29] 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 al[30], 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 al[31] 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 paper[32] 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 produced[31].
- 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 casing[33].

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 CO_2 levels in the growing environment is essential; dropping CO_2 quickly can result in over-pinning, whereas dropping CO_2 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 bruised[2].
- 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 CO_2 levels (e.g. from 5,000 ppm to less than 1,000 ppm).
- According to Eastwood et al[34], CO_2 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, CO_2 determines the number of mushrooms that form.
    - Ideally, casing material should be approximately 50% colonized by mycelium (T. Adlington pers. com.); gradually reducing CO_2 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 pinning[35].

- 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 used[38]. 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 1960s[39]. 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' effects[40].
- 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 pistachios[41].
- 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 substrate[42]
    - Controlling increases in temperature triggered by addition of the supplement, especially as these can increase the incidence of fungal competitors[43]
    - 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-cased[44]. 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 trials[40] 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.

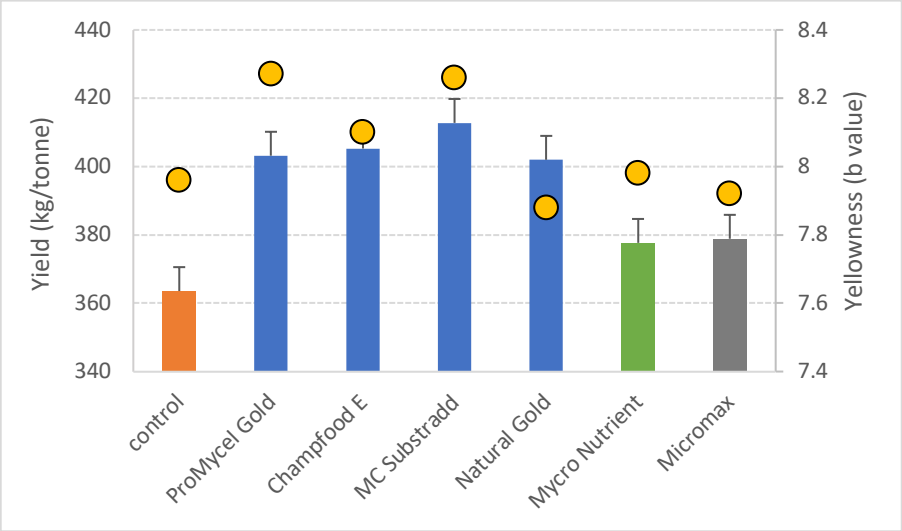

Figure 15. Effect of supplements on yield (columns) and yellowness (yellow dots) of white mushrooms. Products were based on protein or protein plus lipids (blue columns); carboxylic acid (green column) or micronutrients (grey column). From Burton and Noble (2015).

- 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 2012[45] 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 meal[41] or 15g/kg defatted almond meal[46] failed to significantly increase yield or improve whiteness compared to non-supplemented controls.
  - Later work[47] 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 parameters[48].

- 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.

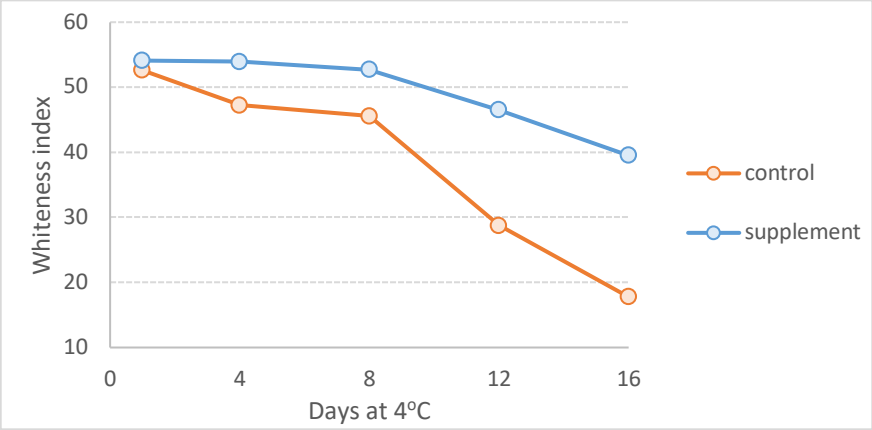

Figure 16. Effect of supplementing casing with 10g/kg corn or soybean meal (average of both treatments) on the whiteness index of stored mushrooms. Whiteness index calculated from presented data using the formula WI = L – 2b + 3a. Derived from Adibian and Mami, 2015.

- 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 visible[50].

- 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 surface[30].
- 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 caps[51].
- 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 Kalberer[52], 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 al[51] 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 relectometry (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 CO_2 gradients between the compost and casing[34].
- 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 decline[53].
- 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 out[54];
    - 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 result[30].

- If a heavy first flush results in dry casing for the second flush, these mushrooms will be less white and susceptible to premature opening[26].
- Dry casing can result in water stress; mushrooms may feel soft and damp and have increased susceptibility to browning and bruising[26].
- 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 attributes[55].
  - 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 m^3 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/m^3 sugar beet lime respectively[53].

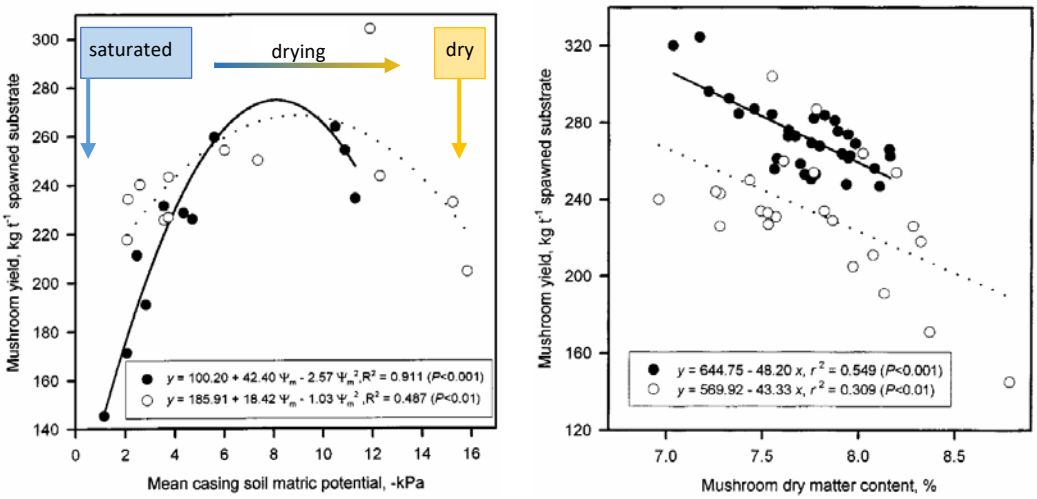

Figure 17. Effect of moisture content of casing on yield of mushrooms (left) and relationship between yield and dry matter (right). Data from two separate experiments (● and ○), reported in Noble et al., 1998.

- Burton[24] 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 al[56] 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 levels[55]. Moreover both whiteness and DM are maximised in first flush mushrooms[57].

- High DM can also be associated with ***reduced*** whiteness[30], or increased yellowness[29] 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 measured[29].

- 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.

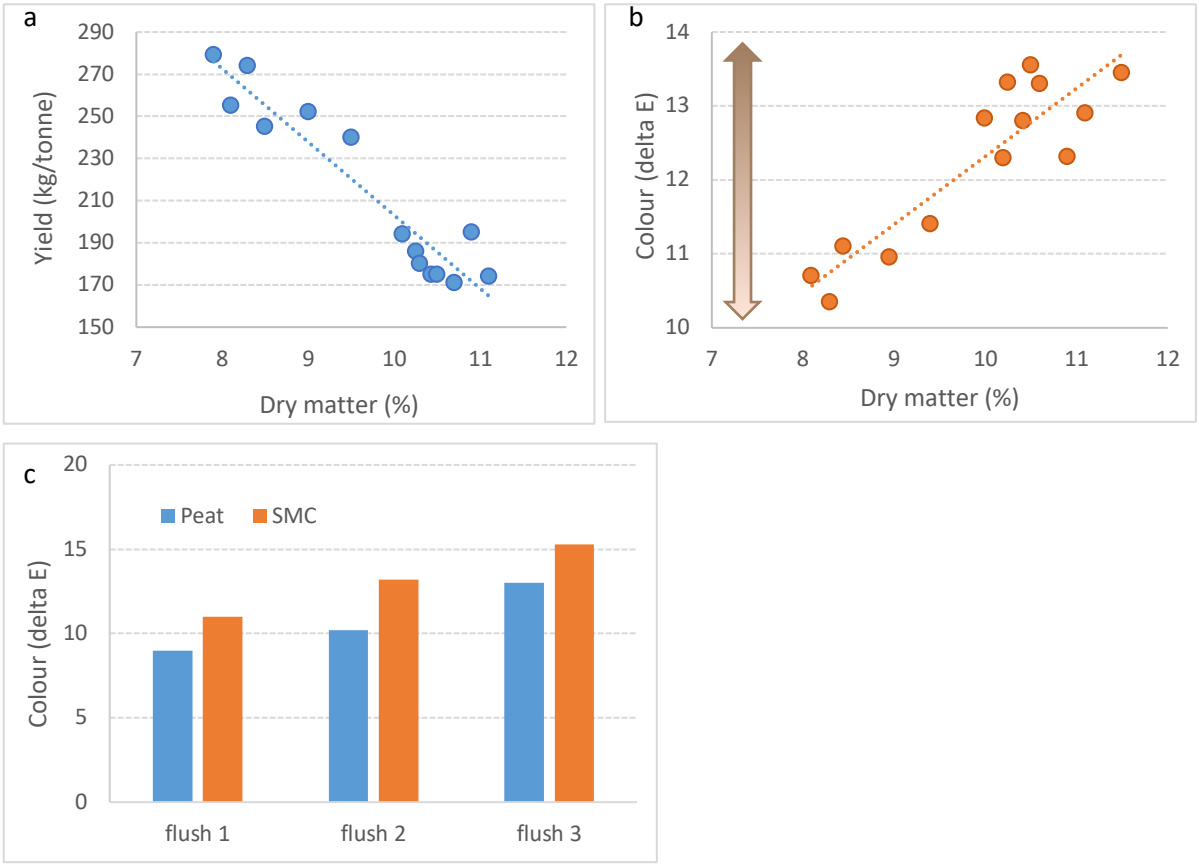

Figure 18. Relationships between mushroom dry matter and yield (a); mushroom dry matter and colour (ΔE) (b); flush and colour when mushrooms were cased using peat + sugar beet lime or spent mushroom compost + vermiculite (c). Derived from Barry et al., 2016.

- 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 storage[32].

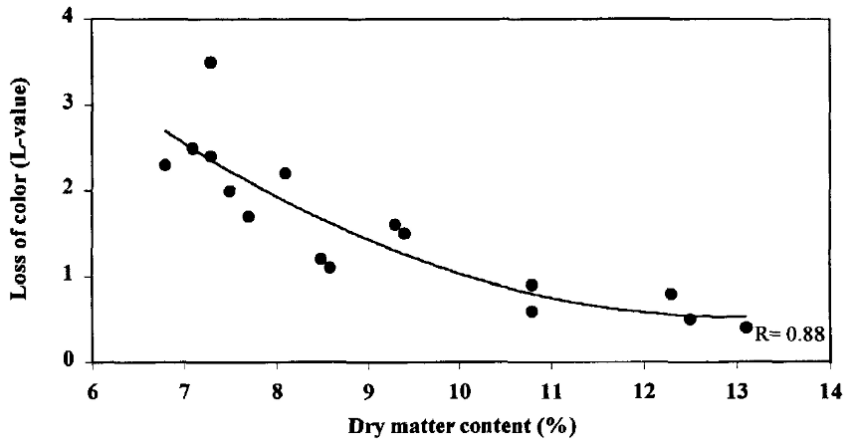

Figure 19. Change in L values (whiteness) after 7 days storage at 8°C as a function of dry matter. From van Loon et al, 2000.

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 blotch[58]. 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 disease[59].
- Geels et al[58] 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 storage[27].
- Like all other chlorine products, stabilized 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 inappropriately[58].
- 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 time[61].
        - 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.)

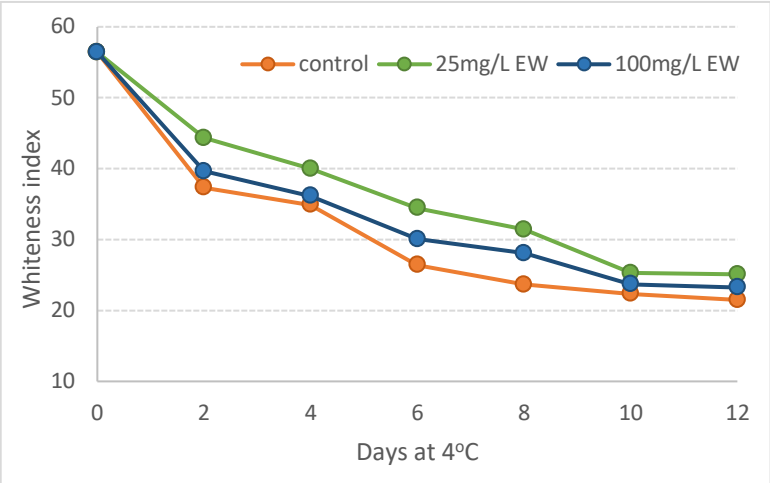

Figure 20. Changes in the whiteness index (calculated from L, a and b values) of mushrooms washed for 3 minutes in a solution containing electrolysed water, then stored at 4°C. Derived from Aday, 2016.

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 pinning[62].
    - Numerous authors report that irrigation with ≥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 eye[22].
    - This treatment approximately doubles calcium content of the mushrooms compared to untreated controls, e.g. Beelman and Simons[63] – 8.5 to 17.5µg/g tissue; Beelman et al[64] – 11.1 to 26.6µg/g tissue, with the effects frequently greater in the later flushes e.g. Kukura et al[65] 14 to 29µg/g tissue second flush.
    - This accumulation occurs in all tissues *except* the outer skin of the cap[62]; 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).

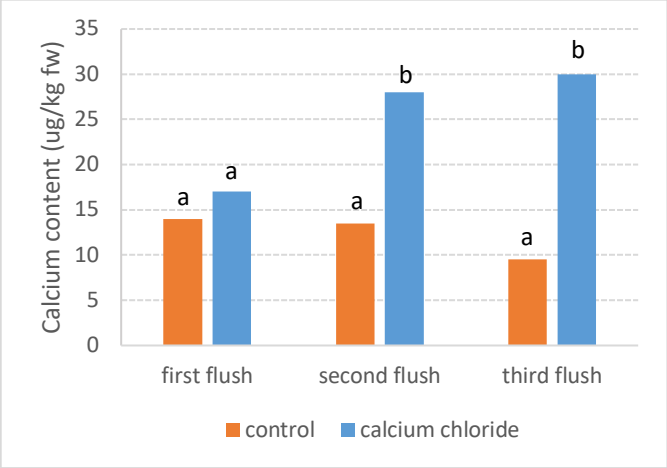

Figure 21. Calcium content of mushrooms irrigated with 0.3% CaCl2. Derived from Kukura et al., 1998.

- While $CaCl_2$ improves whiteness at harvest, greater differences emerge during storage between treated and untreated mushrooms in terms of both browning[66] and development of bruises[65] (Figure 22).

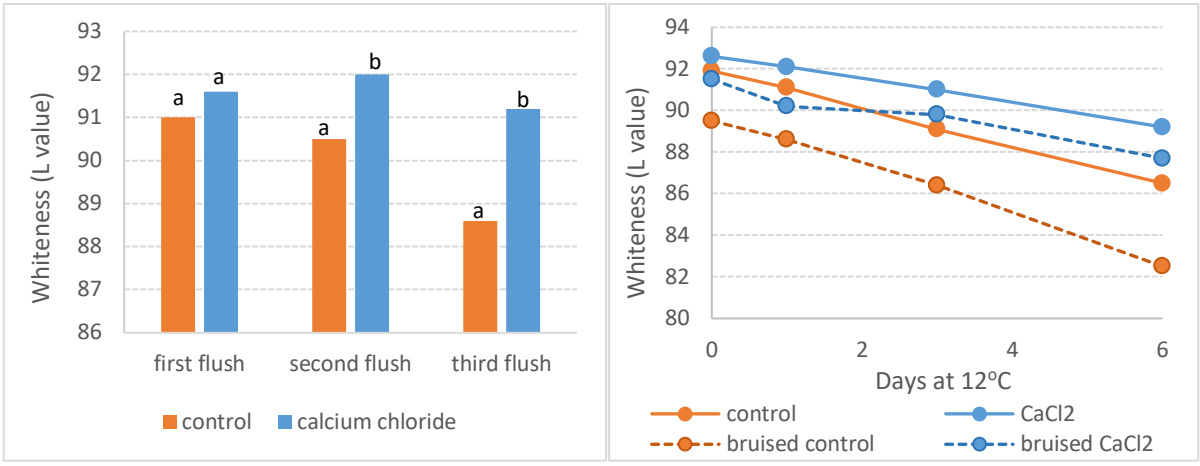

Figure 22. Effect of irrigation with 0.3% $CaCl_2$ on initial colour at harvest (left) and browning of bruised and unbruised mushrooms during storage at 12°C (right). Derived from Kukura et al., 1998.

- A recent trial at the Marsh Lawson Mushroom Research Unit (MLMRU) compared mushroom quality following irrigation with 0.3% CaCl2, tap water or reverse osmosis filtered water. If "white index = 50" is used to indicate the end of saleable life, then irrigation with CaCl2 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 matter[67], as did addition of table salt (NaCl) to casing, suggesting that changes are primarily due to increased osmolarity in the casing soil and water[56].
    - 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 al[67] 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 al[68] found no effect on yield of 0.1% $CaCl_2$. Overall, addition of 0.3% $CaCl_2$ to water appears to have no consistent effect on yield[63].

- It is unclear whether $CaCl_2$ can legally be added to irrigation water without registration through the APVMA. Although $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$[56].
    - 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 $CaCl_2$ and EDTA were applied[64].
- Calcium is associated with increased strength in cell walls in many fruit and vegetables. Mushrooms treated with $CaCl_2$ 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 $CaCl_2$ before bruising.
    - However, these compounds are absent from cells of the bruised controls, which instead contain a large number of small, empty vacuoles.

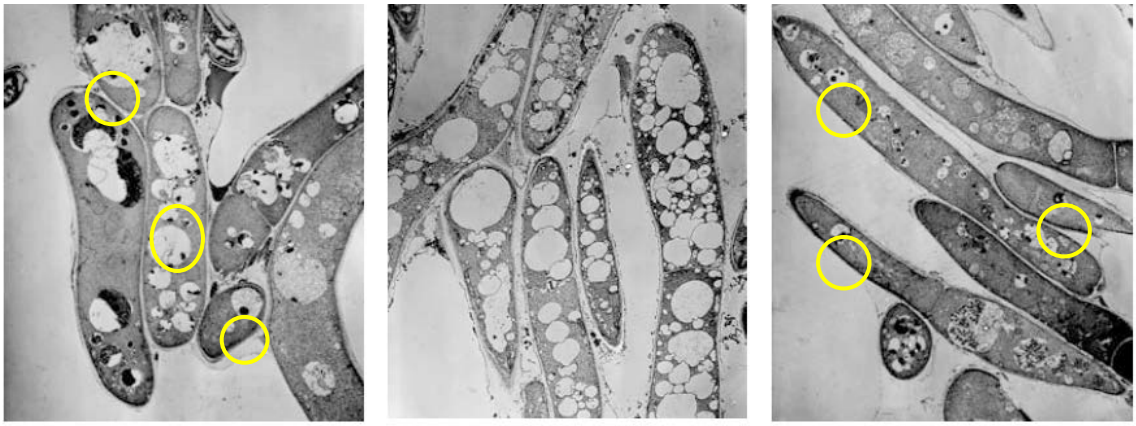

Figure 24. Electron micrographs of undamaged cells (left), bruised cells (centre) and bruised cells of mushrooms irrigated with 0.3% $CaCl_2$ (right). The dark areas (circled) within the vacuoles are phenolic compounds. These are no longer visible in the bruised cells at centre, but are still present 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 researchers[69] 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.

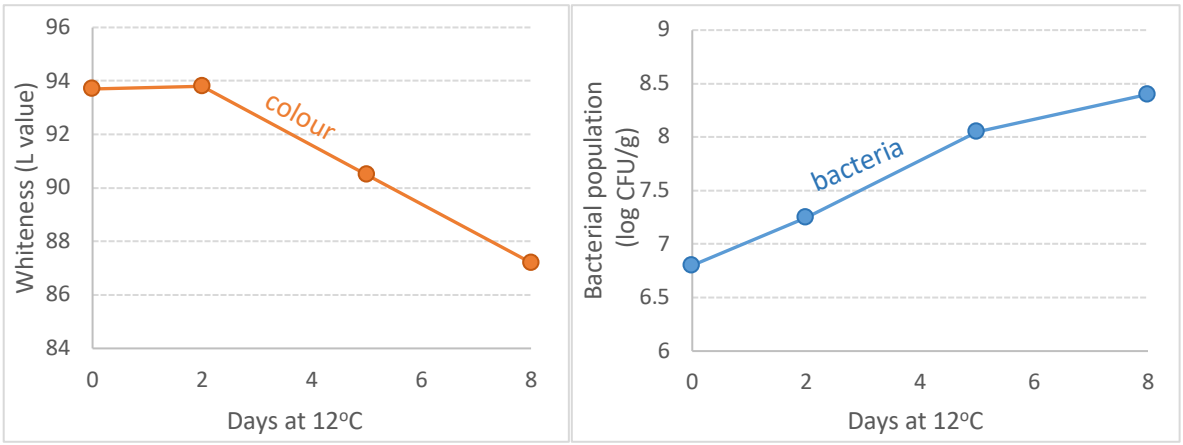

Figure 25. Changes in colour of mushrooms and bacterial populations on caps during storage at 12°C. Derived from Chikthimmah et al., 2006.

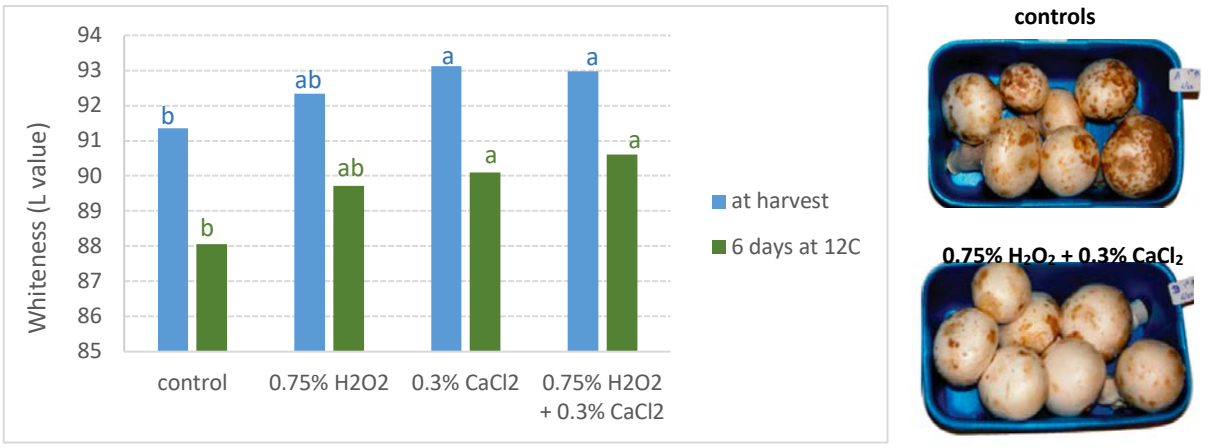

Figure 26. Effect of irrigation with 0.3% calcium chloride and/or 0.75% hydrogen peroxide on whiteness at harvest and then after 6 days at 12°C (left); Appearance of mushrooms irrigated with 0.3% calcium chloride and 0.75% hydrogen peroxide compared to controls after 6 days at 12°C (right). Letters indicate 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 Beelman[70] 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 $CaCl_2$ and/or sodium selenite (Na_2SeO_3). Irrigation with the selenite commenced at casing. Once pins appeared, the amendment was changed to 0.3% $CaCl_2$, 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, $CaCl_2$ only and combination treatment all increased mushroom whiteness at harvest and during subsequent storage.

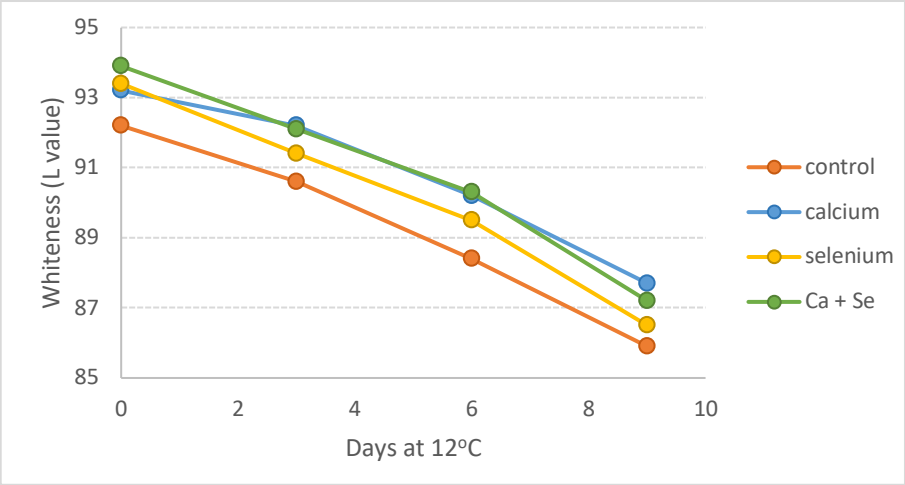

Figure 27. Effect of irrigation with 0.3% calcium chloride, 0.05g/L sodium selenite or both on mushroom colour at harvest and during storage at 12°C. Derived from Hartman et al., 2000.

- 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 treatment[71].

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 al[72] 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.

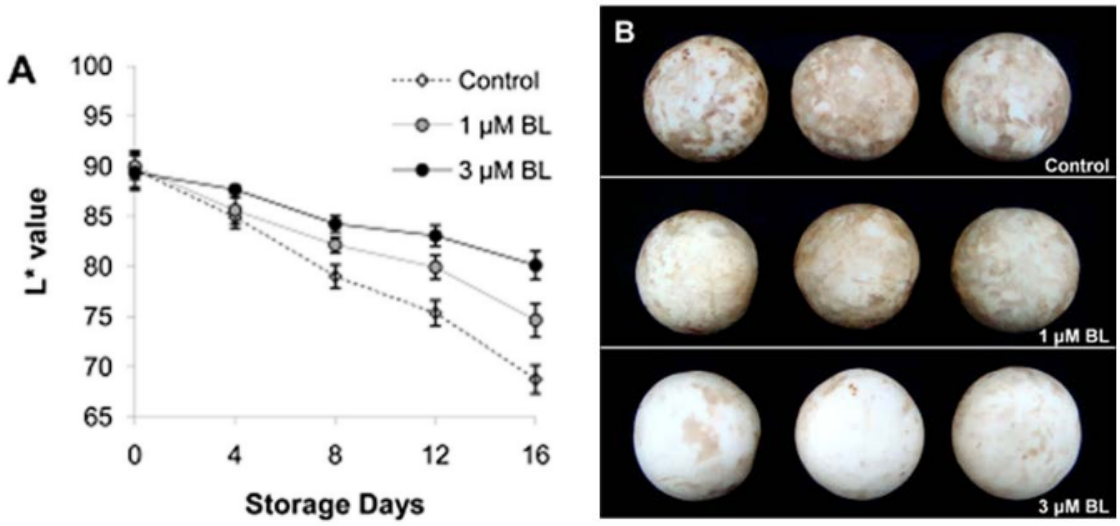

Figure 29. Changes in colour (A) and appearance (B) of mushrooms dipped for one minute in 1 or 3 µM solutions of brassinolide. From Ding et al, 2016.

- 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 browning[73].
- Postharvest browning was inhibited in mushrooms washed for 10 minutes in 40g/L citric acid, differences developing after 2 weeks storage at 4°C[74].
- 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 appearance[76].
- Other acids which have been used experimentally to reduce browning of mushrooms include cinnamic acid, pyruvic acid, propanoic acid, methoxysalicyclic acid, benzoic acid and many others[77]. 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 browning[78].
- 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).

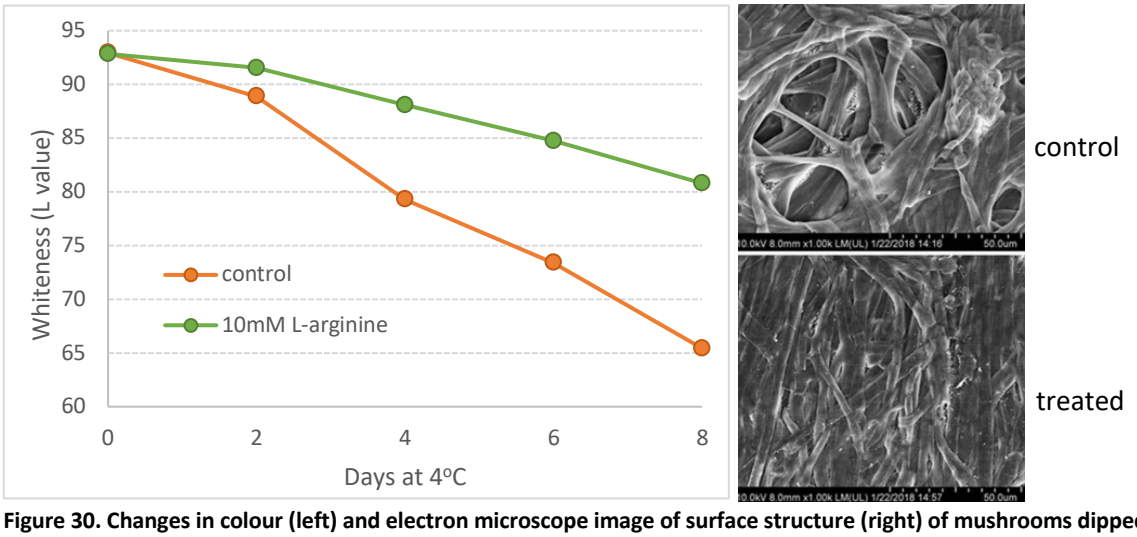

Figure 30. Changes in colour (left) and electron microscope image of surface structure (right) of mushrooms dipped in 10mM L-arginine or water. From Li et al, 2019.

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 Amsing[80] 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.

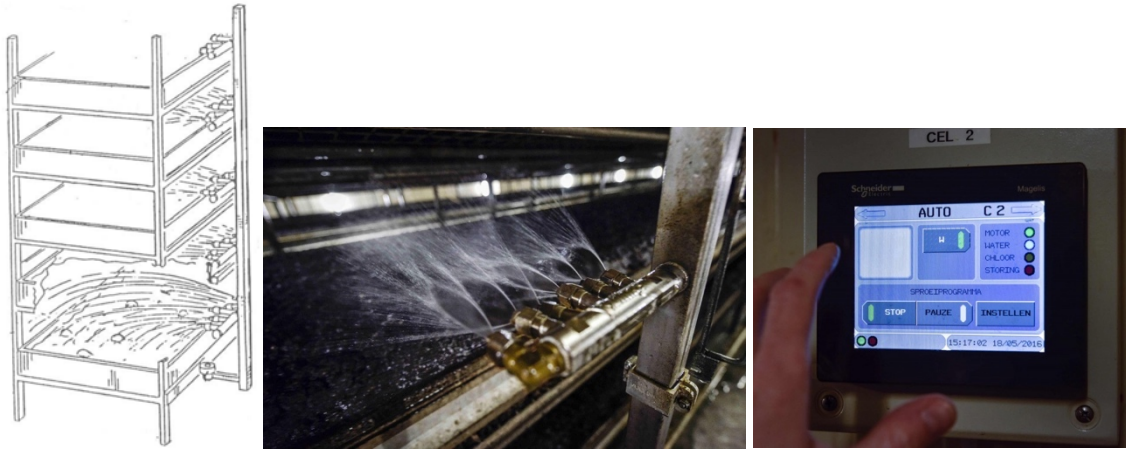

Figure 31. The Vullings water supply system for mushroom production.

- 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 systems[81].
- 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 disease[82].
- Drip irrigation reduces potential dissemination of fungal spores e.g. *Verticillium*, etc.

- Danay et al[83] 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.

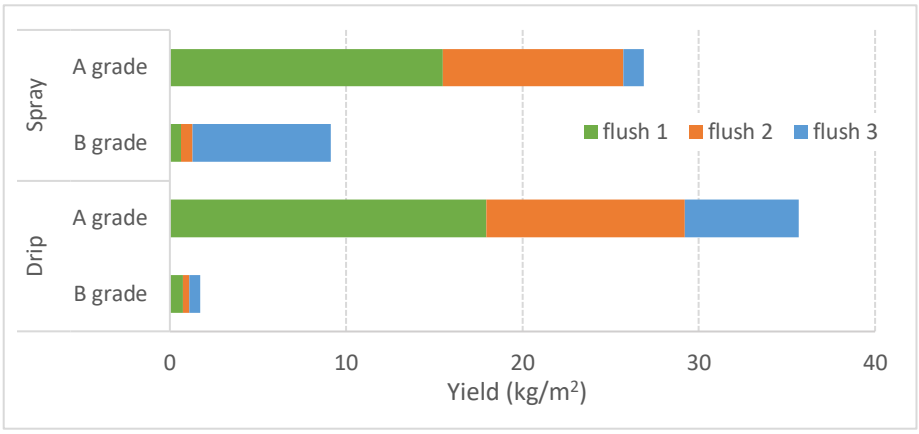

Figure 33. Yield and quality grade of mushrooms grown with normal or drip irrigation. Derived from Danay et al., 2016.

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 sale[84]. 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 al[85] 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).

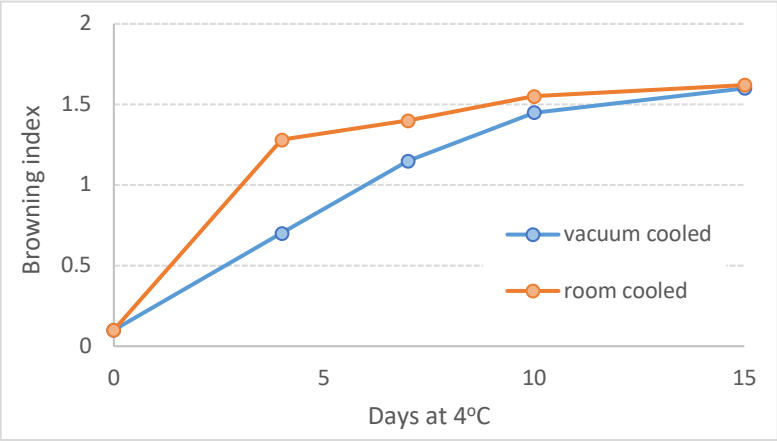

Figure 34. Increases in browning during storage at 4°C of vacuum cooled or room cooled mushroom. Derived from Tao et al., 2007.

- 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 mushrooms[85]. 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 picking[25].
- 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 structures[86].
    - A model measuring absorbance at wavelengths 1090nm, 1188nm and 1384nm plus scaling from 1454nm was able to correctly identify damaged mushrooms[87].
    - NIR spectra can also be used to estimate moisture content and firmness[88].

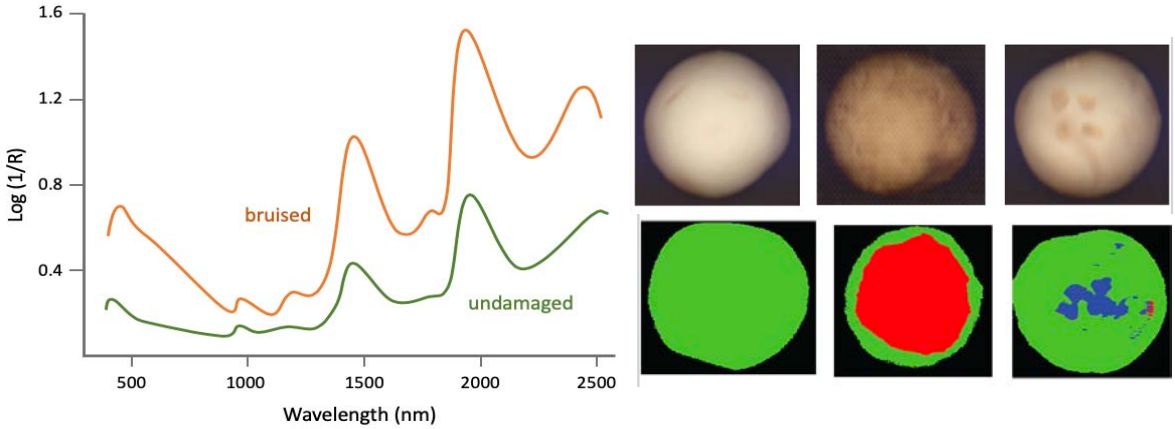

Figure 35. Average raw spectra of damaged and undamaged mushrooms, derived from Esquerre et al., 2009 (left) and original and hyperspectral images of undamaged, damaged and bacterial blotch infected mushrooms, from Gaston et al., 2010.

- 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 al[32] 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 stipe[32]. According to work by Mau et al (1993), combining short stipes with $CaCl_2$ 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 Beelman[70] 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 al[74]. An initial pre-wash with 0.5 to 1% hydrogen peroxide (H_2O_2) was followed by a 30 second spray with 5% H_2O_2 + 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.

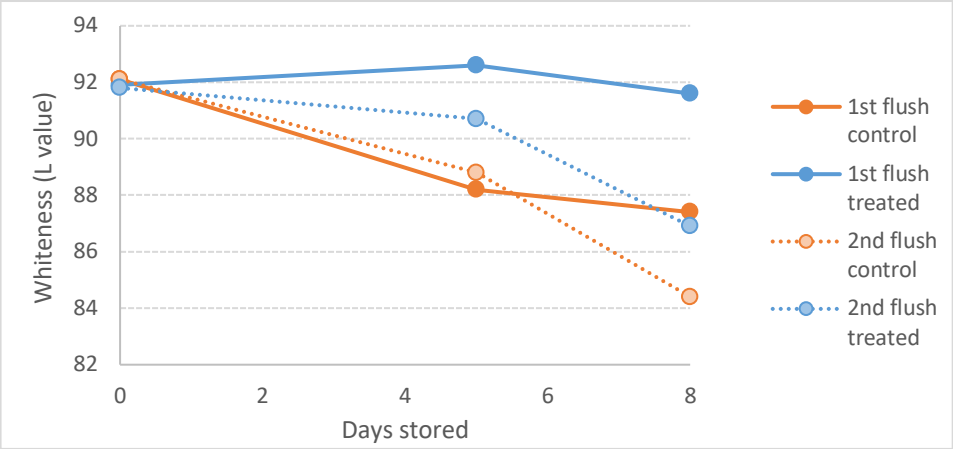

Figure 36. Colour changes of mushrooms pre-washed with hydrogen peroxide (H_2O_2) for approx. 13s then washed in a mixture of H_2O_2, sodium erythorbate and sodium chloride for approx. 30s. Derived from Sapers et al, 2001.

- 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% $CaCl_2$ solution[90]. 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.
    - Eissa[91] 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 colour[92].
    - Other authors[93] 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 presented[94] or may even be negative[95].

- Coating mushrooms with a 50% aloe vera solution before storage reduced surface browning and inhibited weight loss[96]. 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 study[97] 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 al[98] 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 al[99], 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.

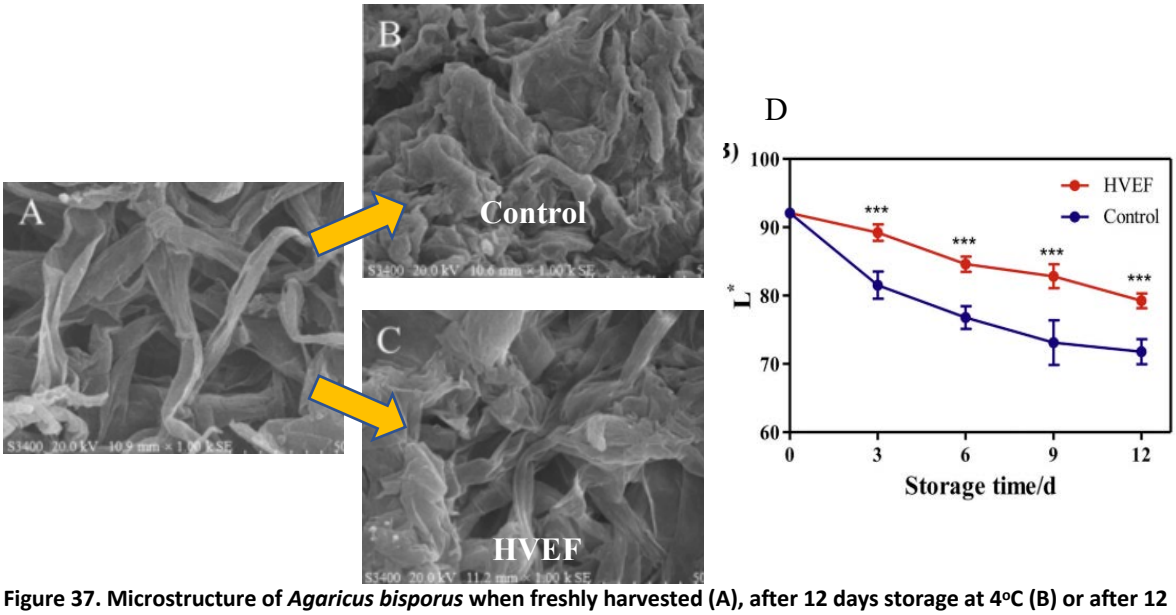

Figure 37. Microstructure of *Agaricus bisporus* when freshly harvested (A), after 12 days storage at 4°C (B) or after 12 days storage at 4°C in the presence of HVEF (C), and the effects of HVEF on mushroom whiteness (L value) (D). From Yan et al., 2020.

- 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 storage[100].
    - A similar method was used for bitter orange[101] and cumin[102] 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 al[103] 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 al[104] 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.

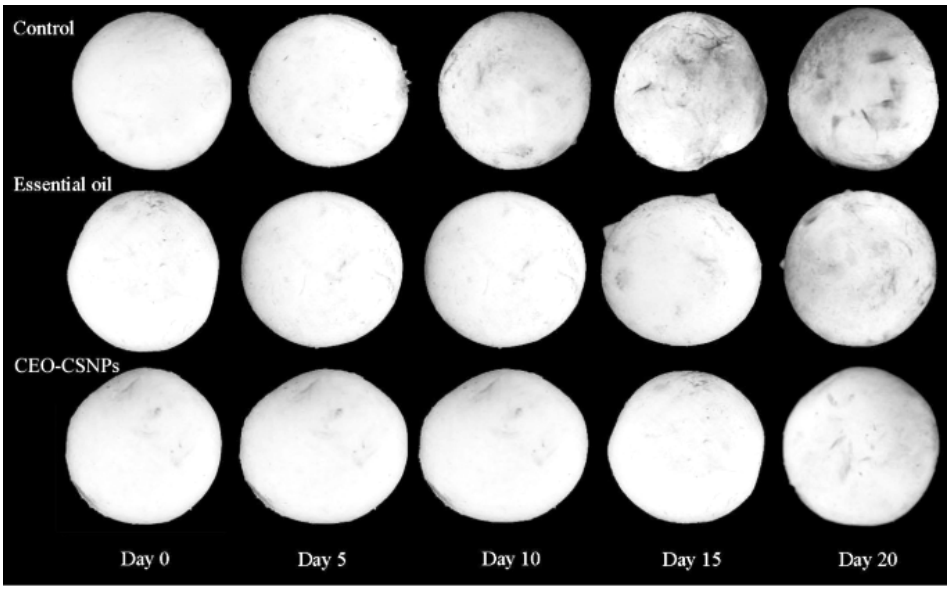

Figure 38. Appearance of mushrooms packed with water only, cumin essential oil (CEO) or CEO microencapsulated into chitosan nanoparticles (CEO-CSNPs). From Karimad et al., 2019.

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 darken[105].
    - Similar results were reported by Beaulieu[106], 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 al[107] 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 units[108].
    - 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 D_2; a serving of mushrooms treated this way can supply the body's daily requirement, providing a natural supplement for the many people deficient in D_2. 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 product[109].

Packaging

Key points

Modified atmosphere packaging (MAP) has been widely investigated for use with mushrooms. However, mushroom respiration is relatively unaffected by low O_2 concentrations, while accumulation of CO_2 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 (O_2) and increase carbon dioxide (CO_2) inside a sealed package. The atmosphere that develops is a function of respiration rate, surface area relative to volume, and film permeability[110].
- MAP can increase storage life of fresh produce by inhibiting ripening, reducing rot development, retaining chlorophyll and reducing the rate of O_2 consumption and, therefore, senescence. Of these, only the last appears relevant to mushrooms. However, mushrooms are highly gas-permeable, so O_2 needs to fall below at least 2%[110] and more likely below 1%[111] to significantly reduce respiration rate.

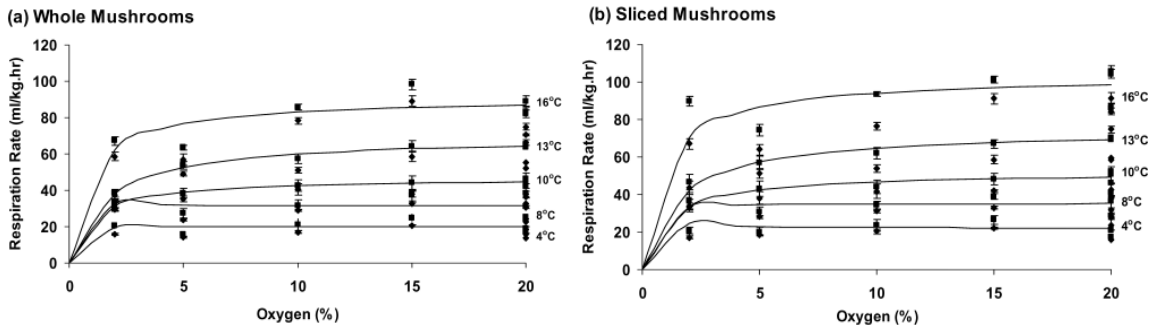

Figure 39. The effect of oxygen concentration on respiration rates of whole and sliced mushrooms. From Cliffe-Byrnes and O'Beirne, 2007.

- 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% CO_2 and 2-10% O_2 can improve storage life of mushrooms[112]. However, these values can be difficult to obtain because they require a much higher film permeability to CO_2 than O_2.
- Moreover, there is evidence that any accumulation of CO_2 inside a package can reduce mushroom whiteness; Lopez-Briones et al[113] found a linear relationship between CO_2 concentration inside packages and mushroom cap colour (Figure 40).
- This is supported by Zalewska et al[114], who conducted a complicated trial using two film types flushed with one of four gas combinations.
    - Although the concentrations of O_2 and CO_2 in the packages during storage were not measured, it is clear that packages flushed with 10% or 20% CO_2 yellowed significantly more than mushrooms simply overwrapped with PVC film.
    - The whitest mushrooms were obtained using a film highly permeable to CO_2 flushed with 20% O_2 + nitrogen (essentially air). This package reduced veil opening and weight loss without accumulating excessive CO_2.
- 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% O_2 + 3.5% CO_2 provided moderate improvements in whiteness and quality.
    - However, it was noted that high RH combined with low levels of CO_2 increased stipe elongation, while high levels of CO_2 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 al[111] 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.

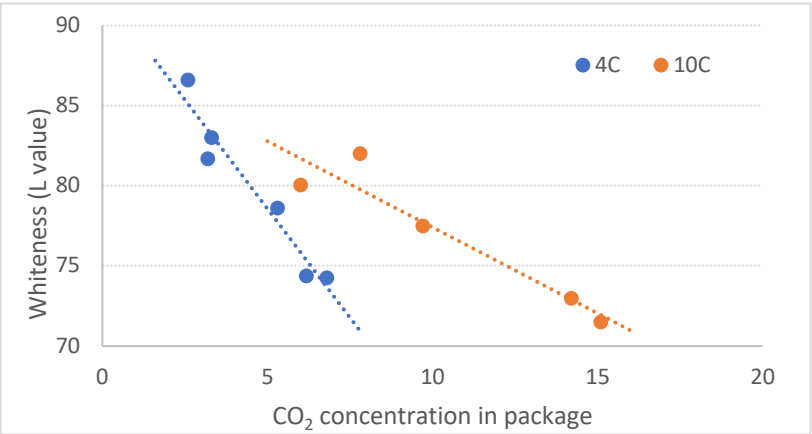

Figure 40. The effect of CO_2 concentrations inside the package on mushroom cap whiteness following 8 days at 4°C or 10°C. Derived from Lopez-Briones et al., 1993.

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 acid[116] 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-tocopherol[117]. The (edible) film stimulated enzymes involved in anti-oxidant defence and inhibited bacterial blotch, reducing browning during storage.
    - Ban et al[95] and Gholami et al[93] 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 al[118] compared wheat gluten (WG) coated paper with uncoated paper and stretchable PVC film. Although CO_2 increased to 9% in the WG paper, the paper had the benefit of avoiding free moisture while increasing RH around the mushrooms.
- Singh et al[119] 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 browning[120].
- 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 lifecycle[121].
    - *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 heterokarytotic 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.

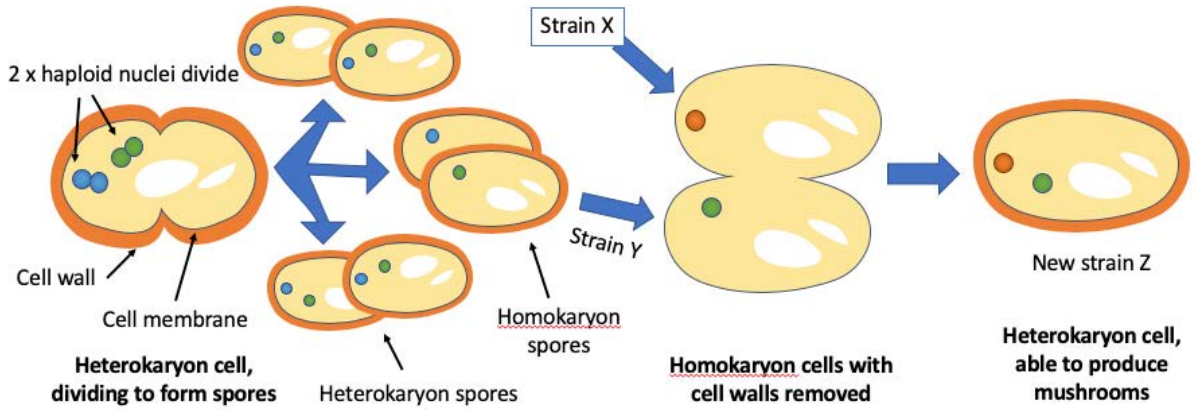

Figure 41. The process by which new varieties of *Agaricus* can be produced. See text above for explanatory notes.

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 characteristics[123].
- 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).

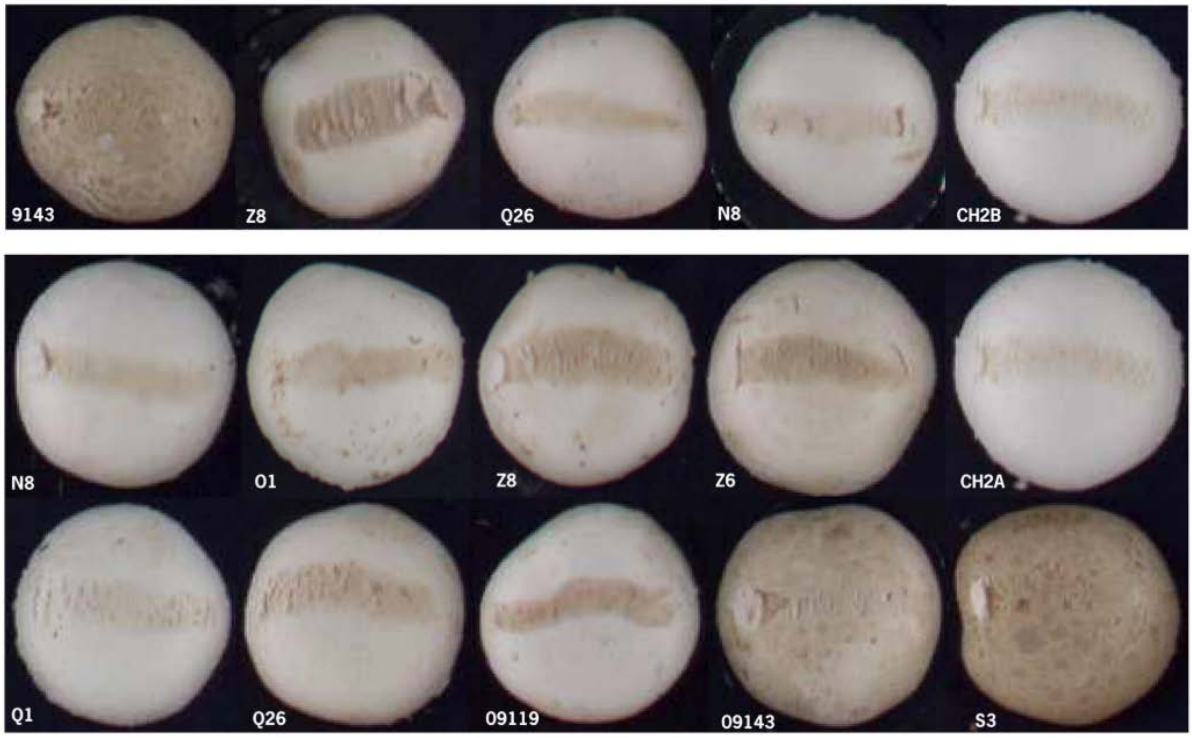

Figure 42. Bruised first flush hybrid mushrooms with strain CH2A (top) or CH2B (bottom) as one parental line. All mushrooms photographed 60 minutes after bruising. From Gao et al., 2013.

- 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 chromosome[124].

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 alone[125]
    - 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 health[126]. 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 commercialisation[127].
    - 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-gbrowning 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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