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A new method to apply and quantify bruising sensitivity of button mushrooms

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ABSTRACT

Mushrooms are prone to develop brown discolouration due to bruising caused by mechanical damage during harvest, which leads to reduced quality of the mushrooms. In order to study the mechanism behind discolouration and to breed for bruise-related browning resistant strains, a high throughput bruise application method and reliable quantification of browning sensitivity is essential. In this study a new bruising device was developed to bruise mushrooms in a fast and reproducible way. The bruising device can apply damage to the cap tissue of button mushrooms by a slip-shear sliding process. The severity of the bruise was quantified with a computer imaging system combined with a newly developed software programme. A protocol was developed to obtain the most reliable and reproducible method to compare browning sensitivity of different *Agaricus bisporus* strains. For this purpose, the extent of damage applied, time between harvest and bruising, developmental stage of the mushrooms and flushes were taken into account. The new method of bruising and image based quantification was subsequently applied to a collection of wild, commercial and hybrid *A. bisporus* strains and a distinction could be made between bruise-related browning sensitive mushrooms and tolerant mushrooms.

1. Introduction

Agaricus bisporus (*A. bisporus*) button mushroom quality is determined by colour, texture, cleanliness, maturity, flush number and flavour. Of these, colour is the most important parameter because it is first perceived by consumers and discolouration decreases the commercial value (Burton, 2004). Mushrooms are prone to develop brown discolouration due to bruising of the mushrooms as a result of mechanical damage during harvest, through fruit senescence or by microbial infections (Jolivet, Arpin, Wichers, & Pellon, 1998). Contact-based discolouration, or bruising, is caused by a mechanical process known as 'slip-shear' (Burton, 2004), a downwards force and a sideways movement, which can occur during picking by hand or by robotic picking equipment with a suction cup device.

Mechanical harvesting is more cost efficient than picking by hand, but cannot be applied yet to serve the fresh market, as commercial strains are too sensitive to bruising. It has been shown that enzymatic browning of mushrooms is caused by polyphenol oxidases (PPOs: tyrosinases and laccases) and peroxidases through an enzyme-catalysed oxidation of phenolic substrates into quinones (Jolivet et al., 1998). These products undergo subsequent reactions leading to the formation of the brown pigment melanin. In order to breed for browning-tolerant lines and to study the molecular and biochemical processes in depth it will be necessary to determine the bruise-related browning sensitivity in a reproducible way. Burton (2004) has developed a device to apply a slip-shear stress on mushroom slices. However, the damage applied was much stronger than in practice occurs and the method was too laborious to apply to a large number of samples.

Flush number is a factor considered to have a major influence on quality. Bartley, Beelman, and Winnett (1991) found that mushrooms prior to harvest and during postharvest storage of the second flush are in general whiter than first flush mushrooms. These first flush mushrooms in turn discolour more slowly than third flush mushrooms. Burton and Noble (1993) concluded the same for mushrooms stored at 5 °C, which were bruised in a polystyrene shaking box which oscillated horizontally through a distance of 40 mm at a frequency of 2 Hz.

In this article a newly developed bruising device and image analysis system to quantify bruising sensitivity is described In order to develop a reliable and reproducible method, several parameters were studied such as the influence of flush, the effects of the

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developmental stage of the mushrooms, the time between harvest and applying the bruise, and the time between bruising and analysing discolouration. Finally, a collection of *A. bisporus* strains was screened for their bruising sensitivity in order to analyse the phenotypic variation among strains. This method identified the genetic variation of bruising sensitivity among strains and can now support unravelling the molecular and biochemical basis for this trait.

2. Material and methods

2.1. Mushroom strains

For the first experiment 50 different *A. bisporus* strains were grown in 2 replicates. In the second experiment, 10 *A. bisporus* strains (Table 1) were grown in 10 replicates. In the third experiment 46 different *A. bisporus* strains were grown in 2 replicates (Tables 2 and 3). In each experiment the strains were randomly distributed over the growing room. Strains used in this research originated either from the department of Plant Breeding at Wageningen UR (old and present-day cultivars) or from the ARP culture collection (wild collected strains, Kerrigan, 1996).

2.2. Mushroom growth

Spawn was prepared by boiling sorghum grain (*Sorghum bicolor*) for 20 min in water. After draining of water, gypsum (2.4% w/w) and chalk (0.7% w/w) were added before sterilising. After sterilising and cooling, grains were transferred to "full-gas microboxes" (Combiness, Gent Belgium) and inoculated with a pure culture of an *A. bisporus* strain grown on agar (1% malt extract w/w, 0.5% mycological peptone w/w, 5 mM MOPS pH 7). The colonisation was completed in approximately 2 weeks with occasional shaking of the boxes to distribute colonised grains. Cultivation was performed in boxes ($56 \times 36 \times 20$ cm) filled with 16 kg of phase II compost (van Gils, 1988). Each box was inoculated with 110 ml of spawn. After a spawn run period of 14 days (air temperature at 21–23 °C; RH 95%; 3500 ppm CO_2) casing soil was applied. After colonisation of the casing soil for 10 days at 21–23 °C, the casing layer was ruffled. 3 Days after ruffling the boxes were vented at a rate of 0.075 °C/h towards 18 °C air temperature. At the same time CO_2 was lowered at 35 ppm/h to a value of 1000 ppm and RH was set at 90–92%. Depending on the strain, pins appeared between 3 and 10 days after onset of venting. Harvest of the fastest strains started 7 days after venting and majority of the strains produced 12–13 days after venting.

2.3. Bruising device

A new mushroom bruising device was constructed, which is able to apply damage to the cap tissue by a slip-shear sliding process using a spatula on a moving wheel (Fig. 1). 10 Mushrooms were fixed on a tray and bruised in series. The force with which the spatula presses on the mushroom caps can be adjusted by the weights placed on the horizontal bar on top of the machine. After initial experiments the total weight of the spatula was set to 40 g. Mushrooms were kept at 20 °C at all time after harvest. After a recorded period of time, the mushrooms were placed into an illuminated cupboard (4 Philips Fluotone TLD on each side of the tray) equipped with a photo-camera (JVC KY-F30E colour video camera with a JVC TV UM lens) and pictures were taken.

Table 1
10 White *A. bisporus* strains used to optimise the browning sensitivity method.

Number	Strain	Classification
1	Sinden A61	Sensitive
2	Somycel X135	Sensitive
3	Bisp 051	Sensitive
4	Claron A3.2	Moderate
5	Les Miz 36	Moderate
6	Royal 21A	Tolerant
7	Royal 23A	Tolerant
8	Le Lion X20	Tolerant
9	Horst U1	Tolerant
10	Darlington 735	Tolerant

Table 2
Result of bruise-related browning of 24 white *A. bisporus* strains (old and present-day cultivars and wild collected strains (ARP)) after 60 min. LSD shown at 0.05 level of significance.

Number	Strain	WI diff	St dev	LSD
1	Commercial hybrid 1	10.81	7.70	a
2	Traditional offwhite strain 1	13.80	4.46	ab
3	Wild white strain 1	14.11	4.97	abc
4	Traditional offwhite strain 2	16.58	6.00	bc
5	Traditional offwhite strain 3	17.07	3.95	bc
6	Commercial hybrid 2	17.08	4.25	bcd
7	Traditional offwhite strain 4	18.54	4.06	cde
8	Traditional white strain 1	18.95	3.41	cde
9	Commercial hybrid 3	19.69	4.40	cde
10	Wild white strain 2	19.75	3.77	cdef
11	Commercial hybrid 4	20.84	3.24	cdefg
12	Wild white strain 3	21.28	3.77	defg
13	Traditional offwhite strain 5	21.56	4.83	defg
14	Wild white strain 4	22.53	5.47	defg
15	Commercial hybrid 5	22.55	4.77	defg
16	Traditional white strain 2	22.69	4.46	defgh
17	Traditional white strain 3	23.12	5.00	efghi
18	Wild white strain 5	23.49	5.25	fghi
19	Traditional offwhite strain 6	24.03	5.60	fghi
20	Traditional white strain 4	25.26	5.36	ghi
21	Wild white strain 6	25.88	6.57	hi
22	Traditional white strain 5	27.90	5.37	i
23	Wild white strain 7	28.23	4.85	j
24	Wild white strain 8	38.32	4.77	k

2.4. Computer image analysis

The developed quantitative image analysis system can measure various parameters from colour images of mushrooms and gives

Table 3
Result of bruise-related browning of 22 brown *A. bisporus* strains (old and present-day cultivars and wild collected strains (ARP)) after 60 min. LSD shown at 0.05 level of significance.

Number	Strain	WI diff	St dev	LSD
1	Wild brown strain 1	−0.10	7.42	a
2	Wild brown strain 2	0.29	5.80	a
3	Wild brown strain 3	1.04	8.43	a
4	Wild brown strain 4	1.06	5.43	a
5	Wild brown strain 5	3.03	4.54	ab
6	Wild brown strain 6	4.87	5.17	abc
7	Wild brown strain 7	5.07	6.20	abc
8	Wild brown strain 8	5.41	6.59	abc
9	Traditional brown strain 1	6.72	5.49	abcd
10	Wild brown strain 9	8.04	5.46	abcd
11	Wild brown strain 10	9.10	3.63	abcde
12	Wild brown strain 11	9.36	6.24	bcde
13	Wild brown strain 12	9.88	7.30	bcde
14	Traditional brown strain 2	10.68	6.08	bcde
15	Wild brown strain 13	11.60	4.11	cde
16	Wild brown strain 14	12.87	7.84	cdef
17	Traditional brown strain 3	13.60	5.07	cdef
18	Traditional brown strain 4	13.62	6.14	def
19	Traditional brown strain 5	17.96	3.54	ef
20	Wild brown strain 15	20.36	7.44	fg
21	Wild brown strain 16	20.61	5.02	fg
22	Wild brown strain 17	24.71	6.41	g