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Differences in taste in button mushroom strains (*Agaricus bisporus*).

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1. Summary

This report describes the results of a screening of genetically diverse strains of mushroom *Agaricus bisporus* for differences in taste.

Eight different strains were grown on regular commercial compost and casing soil. Two of these strains were also grown on a casing with calcium chloride added to increase osmotic value. The intension was to increase the dry matter content of the mushrooms that might affect the “bite” sensation of mushrooms.

After harvest, the mushrooms were sterilized without additives, in their own broth. Subsequently they were packed in an Alu-laminated flexible pouch. Mushrooms were kept in these pouches at room temperature until they were offered to a sensory panel for hedonic testing.

Before being offered to the sensory panels, mushroom were heated for 90 sec at 500 Watt in a microwave oven. At each session, 5 strains/treatments were offered to the sensory panel. Treatment 1 was always incorporated as a control. A sensory panel consisted of 50 members of the consumerspanel of Wageningen UR Glastuinbouw. Only 18 members participated in all three sessions. Taste was rated per treatment on a scale of 0 (not agreeable at all) to 100 (very agreeable).

Results of the tests by the sensory panel showed statistically significant differences between treatments. Mushrooms from treatments that were grown on casing soil with added calcium chloride were liked best. One of the treatments grown on a regular casing soil equalled the score of the mushrooms grown on the casing soil with added calciumchloride.

Small samples of the mushrooms grown on regular casing have been have been analysed for their content of mannitol, amino acids and 5’-nucleotides. The content of amino acids and 5’-nucleotides has been used to calculate an equivalent umami concentration (EUC).

Correlations between mannitol content or EUC value and the taste score were tested by linear regression. Variation in the EUC value did not explain the variation in the taste score given by the sensory panel. Variation in the mannitol content explained only 8% of the variation in the taste score. When combined in an equation, EUC value and mannitol content were able to explain 50% of the variation in the taste score. Most of the *Agaricus bisporus* strains tested conformed fairly well to this correlation. One strain did not obey the rules of the equation, indicating that we have no full understanding of the factors contributing to taste yet.

2. Introduction

As part of an ongoing project in which the collection of *Agaricus bisporus* strains at the Mushroom Research Group of Plant Breeding Wageningen UR is analysed for valuable metabolites, a survey was made of metabolites that possibly may contribute to differences in taste (Baars & Sonnenberg, 2014).

Taste in mushrooms is linked both to volatile and non volatile compounds. Mushroom alcohol (1-octen-3-ol), together with the two associated C8 ketones (1-octen-3-one, 3-octanone), constitute the main volatiles and are considered the major contributors to the characteristic mushroom flavor (Cronin and Ward, 1971; Dijkstra & Wikén, 1976; Pyysalo, 1976; Maga, 1981). The chief unsaturated fatty acid of mushroom lipids, linoleic acid, is the precursor of 1-octen-3-ol (Tressl et al., 1982; Wurzenberger and Grosch, 1982; Grosch and Wurzenberger, 1984; Mau et al., 1992). The non-volatile taste components are primarily formed by several small water soluble substances, including 5'-nucleotides, free amino acids and soluble sugars and sugar alcohols (polyols) (Litchfield, 1967).

Dijkstra & Wikén (1976) studied the flavor of button mushrooms by preparing synthetic mushroom extracts and adding or omitting soluble components to these extracts. Effects on taste were tested using a sensory panel. They concluded that the flavor of *A.bisporus* is a complex phenomenon in which (-)-l-octen-3-ol plays a major role. Nucleotides, amino-acids and carbohydrates also contribute significantly. Omission of all amino-acids, except glutamic acid, did not decrease the flavour intensity of the mixture. Omission of all nucleotides, except GMP and AMP, also did not decrease the flavour intensity. However, omission of both amino-acids and nucleotides, except glutamic acid, GMP and AMP, resulted in a decrease in flavour intensity. The other compounds in their synthetic mushroom extract were considered to not have much quantitative influence on the flavour, but they may modify the quality of the flavour.

Yamaguchi et al. (1971) performed sensory analysis on the interaction between amino acids and 5'-nucleotides. These substances contribute heavily to umami taste (the savory taste resulting from sodium monoglutamate). Their research resulted in the development of a formula that can be used to calculate on the basis of the concentrations of amino acids and 5'-nucleotides an equivalent umami concentration EUC (g MSG/100 g). EUC allows comparison of relative umami intensity in taste.

Chen (1986) also conducted a series of sensory evaluations on synthetic mushroom extracts, prepared by omitting and adding soluble components, in order to link chemical groups to taste attributes (sweet, bitter, acid, salt, umami). He found that alanine, glycine, and threonine (sweet), and aspartic and glutamic acids (MSG-like) were taste-active amino acids in common mushrooms, whereas none of the bitter components were found to be taste-active in the overall taste perception. Therefore, MSG-like and sweet components would be responsible for the natural taste of mushrooms. However, contents of MSG-like and sweet components and total soluble sugars and polyols were sufficiently high in mushrooms to suppress and cover the bitter taste arising from the contents of bitter components. Also the presence of soluble sugars and polyols in mushrooms contributes to a sweet taste (Litchfield, 1967). Accordingly, the high amount of sugars and polyols, especially mannitol, would give rise to a sweet perception.

Baars & Sonnenberg (2014) analysed about 60 mushroom strains on content of linoleic acid, amino acids, 5' nucleotides and estimates were made of the content of mannitol. Among the amino acids, alanine was the most abundant one. Among the 60 strains, the lowest value for alanine van 4.1 g/kg dry matter and the highest value was 18.1 g/kg dry matter. The second most abundant amino acid in the mushrooms was glutamic acid, with contents ranging from 0,7 to 13,5 g/kg dry matter. The most abundant 5'-nucleotide was adenosine-monophosphate. Its content ranged from 43 to 2200 mg/kg dry matter. The content of guanosine-monophosphate ranged from 13 to 259 mg/kg dry matter. Levels of inosine-monophosphate were mostly below the detection level of the analysis technique used. The data obtained were used to calculate the equivalent umami concentration for the different mushroom strains (Figure 1). The equivalent umami concentration was found to range from a little less than 200 mg MSG/100 g to 1400 mg MSG/100 g.

As mentioned above, linoleic acid acts as a precursor for the main volatile involved in mushroom taste. On average almost 90% of the fatty acids in *Agaricus bisporus* is linoleic acid. Total amounts of fatty acids ranged from 180 to 5818 mg/kg dry matter in the mushroom strains tested. Mannitol was very prominent in the mushrooms.

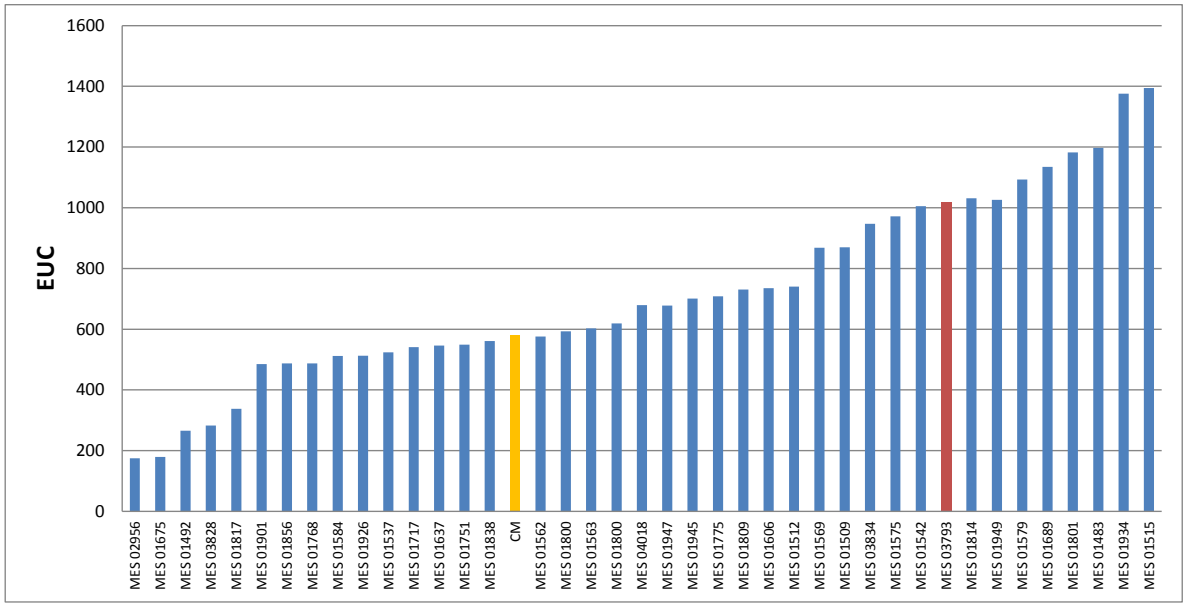

Figure 1. Overview of the equivalent umami concentration of mushroom strains tested, as calculated by the formula designed by Yamaguchi et al. (1971). The yellow bar represents a reference sample. The red bar represents a frequently grown present-day commercial mushroom strain.

The assay technique chosen was semi-quantitative, so accurate amounts cannot be given. Nevertheless it can be stated that there were considerable differences among strains.

The large variation in concentration of taste active compounds in the tested mushroom strains indicates that we might also expect taste differences when offered to a teste panel. A number of different strains were, therefore, offered to a sensory panel for hedonic testing. As sensory panels can only process relatively small numbers of samples, a selection needed to be made from the 60 mushroom strains that were screened on taste related components. This selection was grown on a commercial compost and mushrooms were conserved according to a special procedure in order to maintain their taste as good as possible. The conserved mushrooms were then offered to a sensory panel.

This report describes on what grounds strains were selected, the results of cultivation of these mushroom strains and the results of hedonic testing.

3. Material and methods

3.1 Selection of strains

When selecting strains for testing taste differences, we used the chemical data obtained by Baars & Sonnenberg (2014) on equivalent umami concentration, total content of sweet tasting amino acids and the semi-quantitative estimates for mannitol content.
Next to this we took factors into account that make it more easy to do a segregation analysis of potential taste differences in offspring of crosses of strains with clear differences in taste. This type of analyses reveals genetic regions (Quantitative Trait Loci, QTL) on the genome related to taste. The peculiarities and hurdles that exist with respect this type genetic analysis are explained by Sonnenberg et al. (2011). To be able to obtain an accurate genetic analysis, a sufficiently high genetic recombination in the organism is vital. *Agaricus bisporus* exists in two varieties; the bisporic *A. bisporus* var. *bisporus* and the tetrasporic *A. bisporus* var. *burnettii*. Next to the number of spores per basidium, as the naming suggests, both subspecies also differ in recombination landscape. In the bisporic variety recombination between homologous chromosomes occurs only at the chromosome ends. This results in offspring inheriting mainly parental type chromosomes. In the tetrasporic subspecies, recombination occurs over the entire chromosome. This allows a more accurate mapping of traits on parts of chromosomes. Both varieties are interfertile and the hybrids display a tetrasporic type of recombination (to be published). A selection of strains should therefore include both varieties.
A second prerequisite for genetic analysis is the availability of parental lines. *Agaricus bisporus* is a heterokaryotic multinucleate organism in which the parental nuclei remain separate and only fuse just before spore formation. This provides us with an opportunity to recover both parental nuclear types as homokaryons via protoplasting the heterokaryotic mycelium. When both parental haplotypes can be retrieved as homokaryons, genetic analysis becomes more easy. We are then able to study the relative contribution of each nuclear type to the trait being studied. To enhance the chance on finding differences related to different alleles of genes involved in taste, we selected strains that are genetically distant related, i.e. the selection covers the full width of the genetic diversity within the whole collection.
Last but not least, any strain selected for analysis should yield enough mushrooms. The selection we have made is listed in Table 1. As scientific literature often describes the umami taste as being distinctive for mushrooms, we have ranked the strains on basis of the scores for equivalent umami concentration. We expect taste differences to

Table 1. Strains selected for cultivation and subsequent hedonic taste testing (ranked on equivalent umami concentration (EUC)). [A] Commercial present day white hybrid. [B] Commercial present day brown strain.

Strain.	EUC	Sum of sweet tasting amino acids	Relative Mannitol concentration	Yield in previous test (kg/8 kg compost)	Variety	Parental lines available
MES 01934	1376	45	130.632	1.84	Bisporic	No
MES 03793[A]	1020	33	103.716	2.65	Bisporic	Both
MES 03834	947	28	73.256	2.18	Bisporic	No
MES 01800	606	42	120.745	1.77	Tetrasporic	No
MES 01563	603	22	111.680	2.56	Bisporic	No
MES 01856	487	16	109.579	1.88	Tetrasporic	No
MES 02956	175	15	77.924	2.22	Bisporic	Both
MES 13488[B]	No data	No data	No data	No data	Bisporic	No

originate from an interplay between the umami taste components and the sweet taste components (as suggested by Tsai et al., 2007). For instance MES 01934 is high in umami and high in sweet components. MES 02955 is low in

umami and low in sweet components, etc.. The ranking of the selected strains is also shown in Figure 2. Within the selection we chose a commercial present day white hybrid as a reference. Next to this we chose a commercial brown hybrid (MES 13488) as a reference. For MES 13488 we did not have anay data on chemical composition of the mushrooms.

3.2 Cultivation of strains

Spawn was prepare for the selected strains. In an attempt to influence the concentration of taste components within the mushrooms by cultivation technique, we grew the commercial strains both on a normal casing soil and on a casing soil with a high concentration of calcium chloride. Addition of calcium chloride to the casing soil has been shown to increase the firmness of the mushrooms and their dry matter content (van Loon, 1998, van Loon et al., 2000). The treatment in the cultivation experiment are listed in Table 2

Table 2. Treatments in cultivation of strains for hedonic taste testing.

Treatment	Strain	Remarks
1	MES 03793	Regular cultivation in 6 trays
2	MES 03834	Regular cultivation in 6 trays
3	MES 13488	Regular cultivation in 6 trays
4	MES 01934	Regular cultivation in 6 trays
5	MES 01563	Regular cultivation in 6 trays
6	MES 02956	Regular cultivation in 6 trays
7	MES 01856	Regular cultivation in 6 trays
8	MES 01800	Regular cultivation in 6 trays
9	MES 03793	Casing soil with 130 gram $CaCl_2$ per 0.2 m^2 growing surface (9 trays)
10	MES 13488	Casing soil with 130 gram $CaCl_2$ per 0.2 m^2 growing surface (9 trays)

In short, strains were inoculated in compost on 13 May 2014. After 15 days of spawn run at 24-25°C, trays were cased and CAC-ed. Venting started 4 days after casing (Sunday 1 June 2014). Depending on the strain and cultivation method, mushroom production started between 8 to 14 days after venting. The harvest period lasted from 9 June till 18 June 2014. Due to the limited size of the project, mushrooms were harvested for one flush and either directly tansported to the mushroom processing facility or stored in a cold room until enough mushrooms had been gathered for processing.

3.3 Mushroom processing

For each treatment a small sample of the mushrooms were frozen in liquid nitrogen and stored at -80 °C for future analysis. Next to this for each treatment about 5 kg of mushrooms were transported to Scelta Essenza BV in Broekhuizen (The Netherlands) for processing. Scelta Essenza has facilities to conserve mushrooms in small batches in "Eco-pouches". In short, mushrooms are sterilized without additives in their own broth while being packed in an Alu-laminated flexible pouch which can contain between 2.5 to 6 kg of mushrooms. For this experiment, mushrooms were not washed before sterilization. Therefore flavours of different mushroom strains were not mixed. The immediate sterilization of the mushrooms and storage in pouches eliminates differences that might arise from differences in storage time and allows the presentation of all samples simultaneously to the sensory panel. Samples of mushrooms were collected at Scelta in the last week of June.

3.4 Testing by sensory panels

Mushrooms were offered to sensory panels on three occasions; 8 and 17 July and 26 September 2014. Mushrooms were offered to the sensory panels after heating them for 90 sec at 500 Watt in a microwave oven. At each session, 5 strains/treatments were offered to the sensory panel. Treatment 1 was always incorporated as a control. A sensory panel consisted of 50 members of the consumers panel of Wageningen UR Glastuinbouw. Only 18 members participated in all three sessions.

Figure 3. Example of eco-pouches containing mushrooms.

Taste was rated per treatment on a scale of 0 (not agreeable at all) to 100 (very agreeable). Results were analysed using ANOVA.

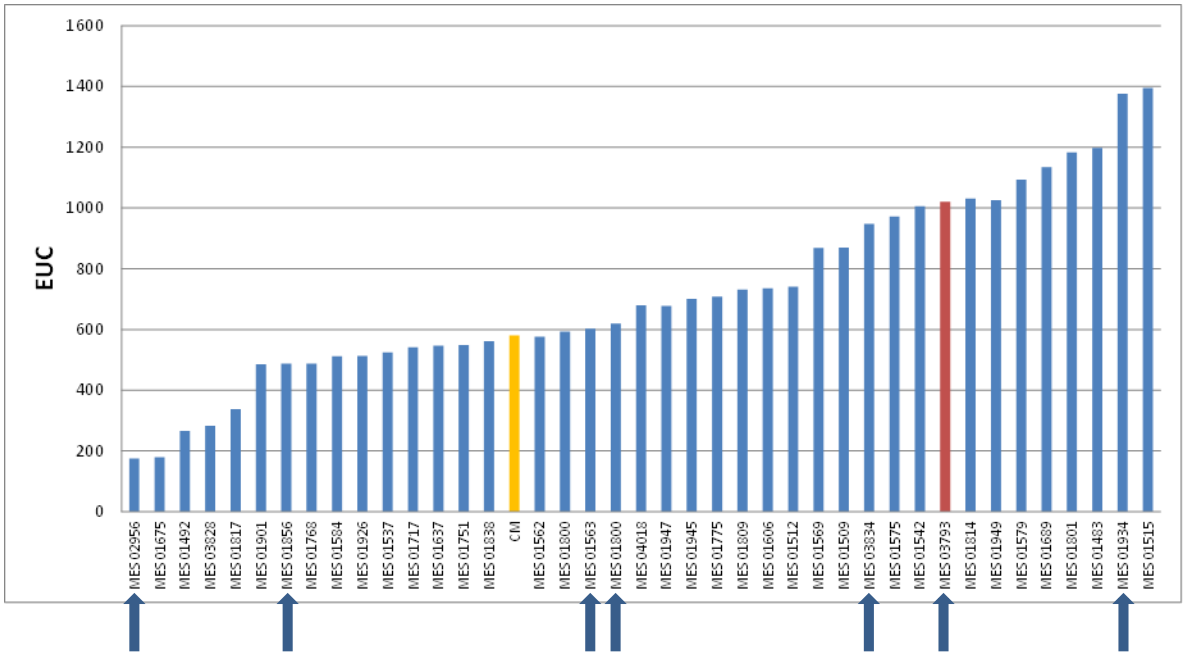

Figure 2. Selection of mushroom strains for hedonic testing, based on equivalent umami concentration (EUC). The yellow bar represents a reference sample. The red bar represents a frequently grown present-day commercial mushroom strain.

4. Results and discussion

4.1 Cultivation of strains.

Table 3 provides an overview of the main cultivation characteristics. Strains MES 03793

Table 3. Some cultivation characteristics of the first flush of the strains selected for analysis by a sensory panel. FDH; First day of harvest (average ± st. dev.). Also yield is given as an average ± st. dev.

Treatment	Strain	# trays	FDH (days)	Yield (g/tray)	Remarks
1	MES 03793	6	10 ± 0	1789 ± 306	Regular cultivation
2	MES 03834	6	10 ± 0	1105 ± 68	Regular cultivation
3	MES 13488	6	10.2 ± 0.4	1388 ± 220	Regular cultivation
4	MES 01934	6	10.2 ± 0.4	755 ± 130	Regular cultivation
5	MES 01563	6	10 ± 0	1943 ± 82	Regular cultivation
6	MES 02956	6	9.2 ± 0.4	2444 ± 134	Regular cultivation
7	MES 01856	6	8 ± 0	2409 ± 161	Regular cultivation
8	MES 01800	6	8 ± 0	1425 ±116	Regular cultivation
9	MES 03793	9	13.9 ± 1.8	1021 ± 148	$CaCl_2$ in casing soil
10	MES 13488	9	13.0 ± 1.4	752 ± 195	$CaCl_2$ in casing soil

and MES 13488 were grown both on a normal casing and on a casing containing 130 gram $CaCl_2$ per 0.2 m^2 growing surface. The effect of addition of $CaCl_2$ to the casing soil is clearly reflected in the first day of harvest (FDH) and average yield in the first flush (no second flush harvested). For strain MES 03793 the first day of harvest was on average delayed by almost 4 days and average yield per tray of the first flush was lowered by 43%. For strain MES 13488, first day of harvest was delayed 3 days and average yield per tray (first flush) was lowered by 46%.
Van Loon (2002) provides a literature overview of the effects of addition of salts on mushroom quality and yield. Literature shows that addition of salt inhibits pinning. In crops of commercial strains, addition of low amounts of salt delay pinning for a day. Addition of high amounts of salt can delay pinning for a

Table 4. Effects of adding salt(s) to casing on yield (fresh weight and dry matter) of mushrooms (van Loon, 1998; van Loon et al., 2000).

Treatment	Moment of application	Osmolarity at pinning (mOsmol/kg)	Dry matter content mushrooms (%)	Yield (kg f.w./m^2)	Yield (kg d.m./m^2)
Control		76	7.0	36.5	2.54
100 g NaCl/m^2	At casing	160	7.8	35.5	2.65
250 g NaCl/m^2	At casing	235	8.4	33.4	2.67
500 g NaCl/m^2	At casing	375	10.4	22.5	2.33
650 g NaCl/m^2	At casing	480	12.9	16.8	2.14
500 g NaCl/m^2	At venting	n.a.	12.2	17.1	2.07
500 g NaCl/m^2	At venting + 5 days later	n.a.	12.1	15.7	1.90
500 g NaCl/m^2	5 days after venting	n.a.	14.4	7.1	0.99
100 g $CaCl_2.2H_2O/m^2$	At casing	126	8.3	36.2	2.72
250 g $CaCl_2.2H_2O/m^2$	At casing	238	8.3	34.5	2.69
500 g $CaCl_2.2H_2O/m^2$	At casing	457	10.3	28.5	2.64
650 g $CaCl_2.2H_2O/m^2$	At casing	406	10.8	24.3	2.57

week. In a few cases, the effect of addition of salt on the quality of the mushrooms was investigated. Addition of salt either had no effect on quality or a positive effect on quality. Van Loon (1998) and van Loon et al. (2000) found that addition of salt in the casing increased firmness, shelf life and conservation efficiency of the mushrooms. This was explained by the higher dry matter content of the mushrooms grown on casing with salt added to it. A summary of their data (Table 4) shows that higher salt concentrations lead to lower yields (as fresh weight). The dry matter content of the mushrooms was increased when salt was added to the casing soil. As a result, the total yield of mushroom dry matter per square meter remains more or less the same. Addition of salt had no effect on color of the mushrooms.

Figure 4. Treatment 1 (MES 03793), harvested in the period between 11 and 13 June 2014

Figure 5. Treatment 2 (MES 03834), harvested in the period between 11 and 17 June 2014

Figure 6. Treatment 3 (MES 13488), harvested in the period between 11 and 16 June 2014

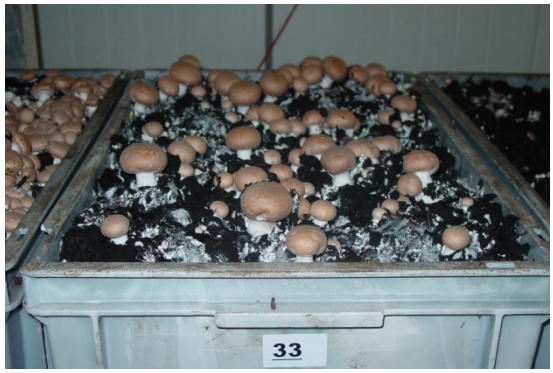

Figure 7. Treatment 4 (MES 01934), harvested in the period between 11 and 16 June 2014

Figure 8. Treatment 5 (MES 01563), harvested in the period between 11 and 17 June 2014

Figure 9. Treatment 6 (MES 02956), harvested in the period between 11 and 13 June 2014

Figure 10. Treatment 7 (MES 01856), harvested in the period between 9 and 17 June 2014

Figure 11. Treatment 8 (MES 01800), harvested in the period between 9 and 17 June 2014

Figure 12. Treatment 9 (MES 03793), $CaCl_2$ added to casing soil, harvested in the period between 13 and 18 June 2014

Figure 13. Treatment 10 (MES 13488), $CaCl_2$ added to casing soil, harvested in the period between 13 and 18 June 2014

In contrast, the color remained well for much longer if salt was added to the casing. Previous research (Stoop & Mooibroek, 1998) has shown that addition of salt to casing soil results in a higher concentration of mannitol in mushrooms and thus will effect taste.Based on these literature data, we believe that the mushrooms of strains MES 03793 and MES 13488 grown on casing soil with $CaCl_2$ added are very likely to have a much higher dry matter content, probably due to an increased mannitol content and likely a different taste. Pictures of the mushrooms of the various treatments are shown in Figures 4 to 13.

4.2 Testing by sensory panels

As sensory panels can only test limited numbers of samples simultaneously, tests were done in three sessions. Treatment 1 (commercial white hybrid) was included as a reference in all sessions. The first session took place on 8 July 2014. Results of this first session are given in Table 5. The scores shown are average values of the scores given by 50 people. Statistical validity of the scores was analysed using ANOVA. Scores are considered to be statistically different at 95% certainty. In the test performed on 8 July differences were found at 98.6% certainty. In this session statistically different tastes were observed by the panel between the batch of mushrooms from strain MES 13488 and those of strains MES 03793 and MES 01934. Next to this, the mushrooms of MES 03834 have a better taste than those of MES 01934.

The second session took place on 17 July 2014. Results of the second session are given in Table 6. In this session no statistically significant differences in taste were found. Scores are considered to be statistically different at 95% certainty. In the test performed on 17July differences were found only at 36.6% certainty.

Table 7. Results of sensory panel session of 8 July 2014

Treatment	Strain	Taste score	Statistical analysis[a]
3	MES 13488	67	a..
2	MES 03834	66	ab.
5	MES 01563	62	abc
1	MES 03793	61	bc
4	MES 01934	58	c
	p	*	
	LSD 5%	5	

A third session took place on 26 September 2014. Results are given in Table 7. Scores are considered to be statistically different at 95% certainty. In the test performed on 26 September differences were found at a very high certainty (> 99.9%). It was concluded that the mushrooms from treatment 10 and 9 taste better than those of treatment 4 and 1. There is no statistically significant difference in taste of the mushrooms of treatment 10, 9 and 3 and there is also no statistically significant difference in taste of the mushrooms of treatment 3, 4 and 1.

Table 7. Results of sensory panel session of 17 July 2014.

Treatment	Strain	Taste score	Statistical analysis[a]
1	MES 03793	67	a
8	MES 01800	63	a
2	MES 03834	63	a
6	MES 02956	62	a
7	MES 01856	61	a
	p	NS	
	LSD 5%	-	

Table 7. Results of sensory panel session of 26 September 2014

Treatment	Strain	Taste score	Statistical analysis[a]
10	MES 13488	68	a.
9	MES 03793	67	a.
3	MES 13488	64	ab
4	MES 01934	60	.b
1	MES 03793	59	.b
p		***	
LSD 5%		5	

[a]If indicated by the same letter, there is no statistically significant difference in taste. Different letters indicate significantly different tastes.

It is interesting to see that enhancing salt concentration in casing soil has not the same effect on the two strains involved in this treatment. The sensory panel noticed a significant difference in taste between the mushrooms of MES 03793 grown on normal casing (treatment 1) and on a casing with $CaCl_2$ added whereas for strain MES 13488 such a difference could not be noticed (compare treatments 10 and 3). This might be due to the fact that in MES 13488, the components related to taste or “bite sensation” are already high.

Some treatments have been tested more often by the sensory panel. Table 8 gives an overview of the scores in the different sessions. There is a considerable difference between the sessions with respect to the score for treatment. One has to keep in mind that sensory testing is very subjective. It is the experience of the people at Wageningen UR Greenhouse Horticulture that the perception of taste of a sample is influenced by the taste of the previous sample. For example, if a sample had a very bad taste, the next sample will usually taste better than when the previous sample had a good taste. .

As treatment 1 was tested in all sessions, it offered the opportunity to combine datasets from the three sessions. In this case, the statistical program estimates the missing values. Results are shown in Table 9. When joined together the data indicate that the mushrooms from treatments 10, 9 and 3 taste better than those of treatments 5, 1, 6, 8 and 4.

Table 8. Overview of the scores received in different sessions of the sensory panels

Treatment	Session		
	7 July	17 July	26 Sept.
1	59	66	59
2	64	62	Not tested
3	65	Not tested	64
4	57	Not tested	60
5	61	Not tested	Not tested
6	Not tested	62	Not tested
7	Not tested	61	Not tested
8	Not tested	63	Not tested
9	Not tested	Not tested	67
10	Not tested	Not tested	69

Table 9. Results obtained when data from the different sessions are combined.

Treatment	Strain	Date	Taste score per session	Statistical analysis	Taste score Sessions joined	Statistical analysis
10	MES 13488	26 sept.	69	a.....	69	a...
9	MES 03793	26 sept.	67	ab....	67	ab..
3	MES 13488	7 juli	65	abcd..	65	abc.
3		26 sept.	64	abcde.		
2	MES 03834	7 juli	64	abcde.	63	.bc.
2		17 juli	62	.bcdef		
7	MES 01856	17 juli	61	..cdef	63	.bc.
5	MES 01563	7 juli	61	..cdef	62	.bcd
1	MES -3793	7 juli	59	....ef		
1		17 juli	66	abc...	61	..cd
1		26 sept.	59	....ef		
6	MES 02956	17 juli	62	.bcdef	61	..cd
8	MES 01800	17 juli	63	abcde.	60	..cd
4	MES 01934	7 juli	57	.....f	57	...d
4		26 sept.	60	...def		
P			***		**	

Although each session of the sensory panel was performed with 50 panel members, not all panel members were involved in all sessions. A group of 18 people participated in all sessions. If data of only these 18 members areused, no missing data have to estimated. Results of this statistical analysis are shown in Table 10. As can be seen, with the scores of only 18 people the statistical power of the analysis is too low to demonstrate the taste differences between strains reliably. This emphasizes that taste panels should be large enough and samples should be tested multiple times in order to reveal significant differences in taste.

Table 10. Statistical analysis of data of sensory panel, based on 18 people that participated in all three sessions.

Treatment	Strain	Taste score 8 July		Taste score 17 July		Taste score 26 Sept.		Combined	
9	MES 03793					70	a	71	a..
10	MES 13488					67	a	67	ab.
3	MES 13488	63	a			66	a	66	ab.
2	MES 03834	64	a	66	a			65	ab.
1	MES -3793	62	a	70	a	61	a	64	ab.
8	MES 01800			66	a			63	abc
5	MES 01563	60	a					62	abc
4	MES 01934	56	a			64	a	61	.bc
7	MES 01856			65	a			60	.bc
6	MES 02956			61	a			57	..c
p		NS		NS		NS		0.118	
LSD 5%		-		-		-		-	

4.3 Analysis of mushrooms for chemical taste components

Remaining mushrooms were lyophilized and analysed for mannitol, 5'-nucleotides and amino acid concentrations. The amino acids glutamic acid and aspartic acid, together with the 5'-nucleotides are reported to contribute to the umami taste of mushrooms. Yamaguchi et al. (1971) developed a mathematical equation to calculate the relative strength of the umami taste of foods (EUC = equivalent umami concentration);

$$Y = \Sigma aibi + 1.218(\Sigma aibi)(\Sigma ajbj)$$

In which Y is the relative strength of the umami taste; a_i is the concentration (g/100 g) of each amino acid which contributes to the umami taste (aspartic acid (asp) or glutamic acid(glu)); a_j is the concentration (g/100 g) of each 5´-nucleotide which contributes to the umami taste (5´-inosin monophosphate (IMP), 5´-guanosin monophosphate (GMP), 5´-xanthosin monophosphate (XMP) or 5´-adenosin monophosphate (AMP)); b_i is the relative umami concentration factor (RUC) for each amino acid which contributes to the umami taste (1 for glutamic acid and 0.077 for aspartic acid); bj is the relative umami concentration factor (RUC) for each 5´-nucleotide which contributes to the umami taste (1 for IMP, 2.3 for GMP, 0.61 for XMP and 0.18 for AMP).

Table 11. Levels of 5'-nucleotides and amino acids measured in dry matter as a measure for relative umami taste.

Treatment	Strain	AMP	GMP	IMP	Aspartic acid	Glutamic acid	EUC (calculated)
		mg/kg	mg/kg	mg/kg	g/kg	g/kg	g/g
1	MES 03793	1580	1780	90	6.9	15.7	2.51
2	MES 03834	2040	3140	168	6.6	15.9	3.19
3	MES 13488	1820	1350	226	7.3	14.8	2.22
4	MES 01934	2350	3080	197	6.9	15.5	3.11
5	MES 01563	1640	1660	32	2.9	6.5	1.01
6	MES 02956	3280	1990	1460	4.3	4.9	0.95
7	MES 01856	1220	1730	109	3.2	8.2	1.29
8	MES 01800	1900	1090	163	3.8	12.5	1.75

The values for EUC for Agaricus bisporus are reported to vary between 207 and 284 g MSG (monosodiumglutamate) per 100 g dry matter (Tsai *et al.*, 2007). Table 11 gives an overview of the levels of 5'-nucleotides and amino acids that were found in the mushrooms offered to the sensory panel.

Table 12. Levels of mannitol present in the mushrooms offered to the sensory panel.

Treatment	Mushroom strain	% D.M.	Mannitol (mass %)
1	MES 03793	97.1	11.7
2	MES 03834	96.6	12.2
3	MES 13488	96.5	11.8
4	MES 01934	95.9	9.5
5	MES 01563	97.9	16.2
6	MES 02956	97.2	7.3
7	MES 01856	97.6	17.8
8	MES 01800	97.1	10.6

As already mentioned, mannitol is a major compound in mushrooms and will probably provide mushrooms with a sweet taste. Higher concentration of mannitol might also increase the dry matter content and influence the bite sensation. Unfortunately, samples from mushrooms grown on casing soil with a higher salt concentration were not analysed since the low production provided only enough mushrooms for the taste panel and not for mannitol analysis. Table 12 provides an overview of the levels of mannitol present in dry matter of the mushrooms tasted by the sensory panel.

4.4 Relationship between EUC value, mannitol content and taste score

As described in the introduction, the taste of mushrooms is believed to be related to the substances responsible for the umami taste and to substances responsible for sweet taste.

Figure 14 shows the relation between the equivalent umami concentration (EUC) and the taste score. As can be seen, EUC is a very poor predictor for taste scores.

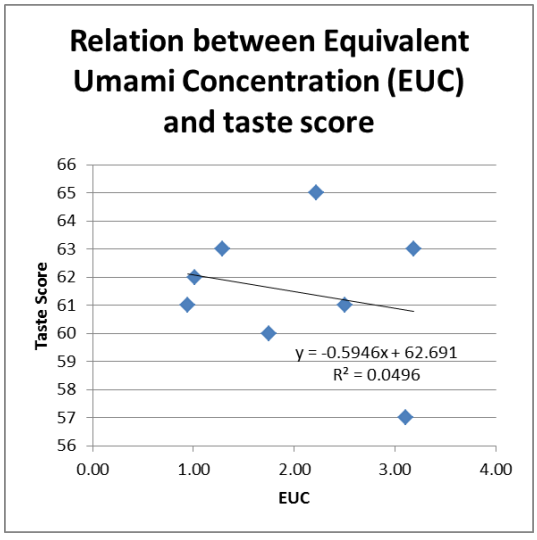

Figure 14. Equivalent umami concentration is not able to predict taste scores.

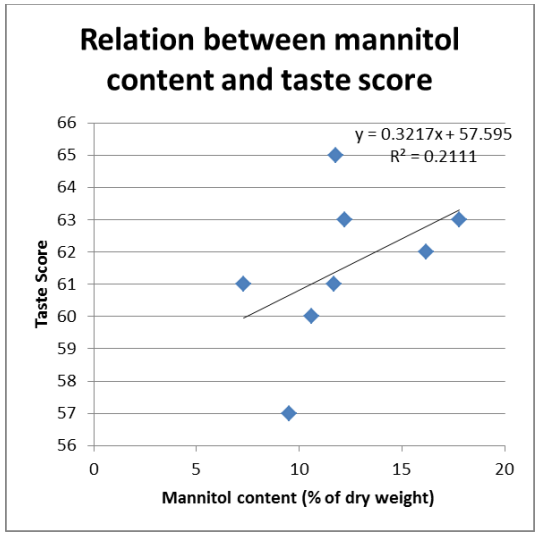

Figure 14. Mannitol content shows some relation with taste scores.

Figure 15 shows the relation between the mannitol content in the mushrooms and the taste score. Although mannitol content is also not a very good predictor of taste scores, it performs better than EUC. Statistical analysis shows that the equation in Figure 15 explains 8 percent of the variance in the taste score.

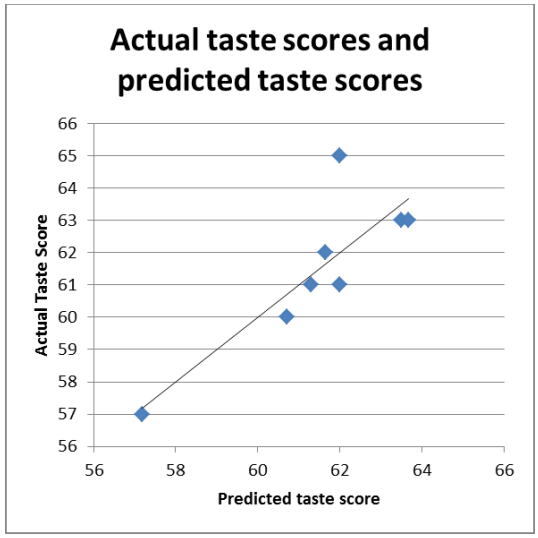

Figure 15. Correlation between actual taste scores and taste scores predicted from the formula 1.

Mannitol content and EUC can be combined in a formula that explains 50% of the variance in the taste score (Formula 1).

Formula 1.

$$Taste\ score = 72.7 + (-1.01\ x\ (mannitol)) + (-12.05\ x\ (EUC) + (1.07\ x\ (mannitol\ x\ EUC)$$

In which (mannitol) is the mannitol content as mass% of the dry matter and EUC.

The values are fitted against the taste values of treatments 1 to 8 in Table 9. Treatments 9 and 10 were omitted from the analysis because the mushrooms were grown in a different way from the other treatments (salt casing soil). As can be seen from Figure 16, most strains (treatments) obey the formula, except treatment 3 which has a taste score of 65. This indicates that at least in this strain other components besides mannitol and EUC might play a role in taste.

5. Discussion

Results have shown that hedonic testing can reveal differences in taste between genetically diverse mushroom strains. Such differences can also be induced by the way in which the mushrooms are cultivated. Compounds contributing to umami taste and to sweet taste are likely to be involved, but there are other, yet unknown, factors that determine whether the taste of a mushroom strain is liked or disliked.

A very basic question would be whether the condition in which the mushrooms are offered to the sensory panel has a large influence of their score. In other words; would it make a difference whether the mushroom are offered as cooked mushrooms (like in the experiments described in this report) or as fried mushrooms.

Ultimate goal of these experiments is to provide a foundation on which breeding of *Agaricus bisporus* for superior taste can be performed. The main item in breeding is the correct assessment of the trait (taste in this case). Testing segregation of taste in a large set of offspring can be done by taste panels but that requires much time and effort. If the main chemical components determining taste are identified and it is shown that concentration of these (combination of) components are directly related to taste, a chemical analysis can replace taste panels and mapping taste on the genome as QTL becomes feasable. We first need to enhance our knowledge on components in button mushrooms related to liking or disliking the mushrooms. Future experiments will therefore be aimed at further dissecting the factors that contribute to taste in *Agaricus bisporus*.

This will involve the use of taste panels that are able to dissect the sensations of tasting mushrooms and put them into words (a trained panel). It will also involve the measurement of traits that are likely to be involved in taste (dry matter content, amino acid content, mannitol content, tissue firmness etc., etc.).

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