Metabolites contributing to taste in *Agaricus bisporus*

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Summary: During the last 35 years, hardly any breeding has been done in the button mushrooms (*Agaricus bisporus*). The fact that no new varieties are generated directed to trends in the food market has caused a slowly decrease in mushroom consumption in the Netherlands and in Europe. The hurdles for generating new varieties are difficulties in breeding and protection of new varieties. These hurdles are now being tackled and it is time to generate new varieties. One issue that has never been addressed is taste. The collection of Plant Breeding Wageningen UR contains a large number of strains of the button mushroom with a large genetic variation. In previous research this collection has been genotyped and a small selection of genetically different strains has been made to see if there is variation in taste within this collection. In 2014 these strains were cultivated in two different ways that were likely to cause differences in taste. Subsequently the mushroom have been offered to a taste panel for hedonic testing to see if taste differences could be perceived. Because of the positive outcome we decided to grow the strains again in 2015. Three strains (two commercially available and a wild-isolate) were grown both on regular commercially available casing soil and on a casing soil with a high salt content. The mushrooms produced on regular casing soil had a dry matter content of about 77 g/kg fresh weight, while the mushrooms produced on the experimental casing soil had a dry matter content of about 123 g/kg fresh weight. Within one day after harvest fresh mushrooms were conserved into eco-pouches and stored until analysis.
At WUR UR Greenhouse Horticulture a taste panel of 20 people was trained to discriminate between different aspects of mushroom tastes. For this they were provided with fresh mushrooms (white and brown varieties) at different stages of maturation (closed cups, portabella's, flats) and with a subsample of the experimentally grown mushrooms. The training of the expert panel has resulted in a list of attributes describing the taste aspects of the mushrooms (Firmness, Gummi, Fibrous, Juicy, Sweet, Salty, Aroma presence, Aroma type, Mushroom, Mouldy, Earthy, Metallic, Meaty, Nutty, Boiled egg, Bitter and Pungently).
After it's training the taste panel tested the taste of the conserved mushrooms from the experimental crops in two separate sessions (October and December 2015). Results of the two sessions were different. However, statistically significant differences were found in both tests between the mushroom samples for the attributes Firmness, Juiciness and Boiled egg. The differences in Firmness and Juiciness were caused both by differences between the strains and differences in the casing soil that was used. The differences in the scores for the Boiled egg attribute seem to be primarily caused by the type of casing soil that was used.
From the mushrooms that were offered to the taste panel, small subsamples were flash-frozen in liquid nitrogen and analysed for the content of amino acids, 5'-nucleotides, mannitol and the presence of volatiles. Contents of metabolites on fresh weight basis were strongly influenced by the type of casing soil used. For most amino acids and 5'-nucleotides differences were found between the different mushroom strains. In general, higher contents were found in the mushrooms grown on the experimental casing. For a number of amino acids interaction effects were found; i.e. in one out of the three strains tested, the content of the amino acid was not raised in the mushrooms grown on the experimental casing soil.
Attempts were made to link the results from the taste panel to the metabolite concentrations. Even though it is a relatively small dataset, some correlations can be found for the taste attributes Firmness, Gummi and Boiled Egg and for the metabolites Alanine, Arginine and Proline.

Keywords: mushroom, taste, metabolites

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Contents

1 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 flavour 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 flavour of *A.bisporus* is a complex phenomenon in which 1-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 savoury taste resulting from sodium mono glutamate). 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

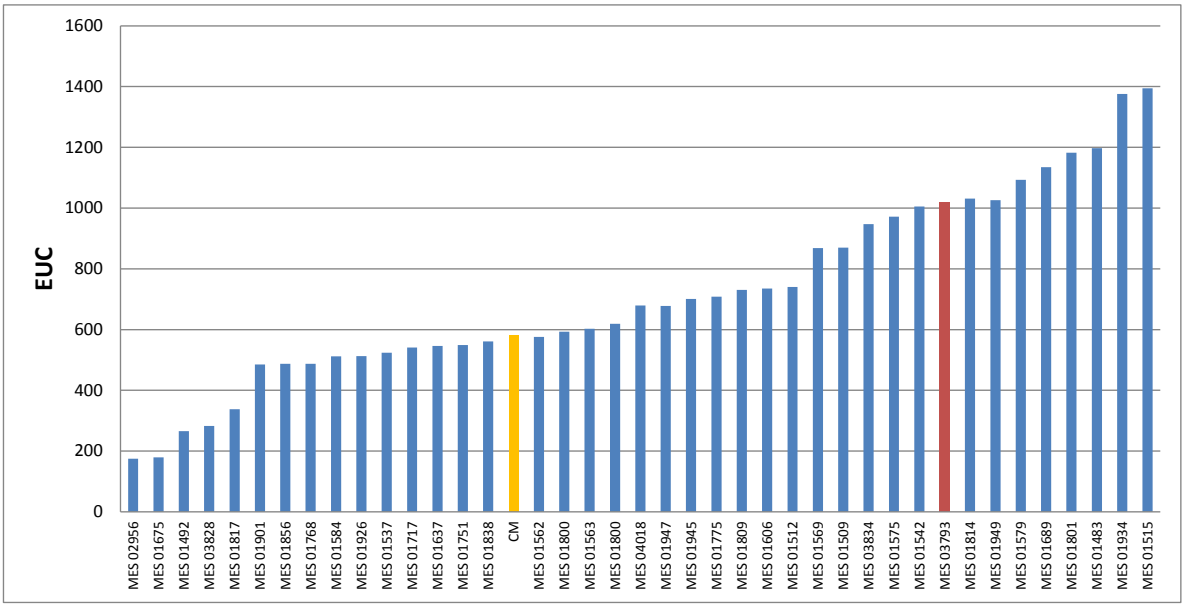

Figure 1. Overview of the equivalent umami concentration of mushroom strains tested, as calculated by the formula designed by Yamaguchi et al. (1971). The yellow bar represents a reference sample. The red bar represents a frequently grown present-day commercial mushroom strain.

very prominent in the mushrooms. The assay technique chosen was semi-quantitative, so accurate absolute 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 test panel. A number of different strains were, therefore, offered to a sensory panel for hedonic testing (Baars et al., 2015). 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 at the company Scelta Essenza 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. Results of the tests by the sensory panel showed statistically significant differences between treatments. The hedonic test panel indeed noticed taste differences between the strains. 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 calcium chloride.

Small samples of the mushrooms grown on regular casing 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.

In order to gain a better understanding of the taste components in *Agaricus bisporus*, a taste panel was trained to recognise the different attributes of *A. bisporus* mushroom taste. After training the taste panel was offered new batches of mushrooms grown in a similar way as for hedonic testing. This report describes the results of experiments in which a small selection of mushroom strains, known to differ in taste from hedonic tests were grown on two different casing soils, conserved and offered to a taste panel for description of their tastes.

2 Material and methods

2.1 Selection of strains.

As shown by Baars et al. (2015) the three strains that were offered for hedonic testing showed differences in taste. Therefore these strains were chosen again for testing by a trained taste panel.

2.2 Cultivation of strains

Spawn was prepared for the selected strains. As shown by Baars et al. (2015) the taste of the mushrooms can be influenced by cultivation technique. Therefore, 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 1. Treatments in cultivation of strains for taste testing.

Treatment	Strain	# replicates	Remarks
1	MES 03793	8	Regular cultivation
2	MES 13488	8	Regular cultivation
3	MES 02956	8	Regular cultivation
4	MES 03793	16	Casing soil with 130 gram $CaCl_2$ per 0.2 m^2 growing surface
5	MES 13488	16	Casing soil with 130 gram $CaCl_2$ per 0.2 m^2 growing surface
6	MES 02956	16	Casing soil with 130 gram $CaCl_2$ per 0.2 m^2 growing surface

In short, strains were inoculated in compost on 19 May 2015. After 15 days of spawn run at 24-25^{o}C, trays were cased (without CAC-ing). Venting started 4 days after casing (Sunday 1 June 2015). 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 2015. Due to the limited size of the project, mushrooms were harvested for one flush and either directly transported to the mushroom processing facility or stored in a cold room until enough mushrooms had been gathered for processing.

2.3 Mushroom processing

For each treatment a small sample of the mushrooms was frozen in liquid nitrogen and stored at -80 ^{o}C for future analysis. Next to this for each treatment about 10 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.

2.4 Testing by sensory panels

Before testing the different batches of mushroom in a taste testing experiment, a description was made of the different attributes of mushroom taste. This list was based on previously published results of taste panels (Leguijt et al., 1996, 1997; Dijkstra & Wikén, 1976; Muresan et al., 1997). Based on this concept list of taste attributes, the members of the sensory expert panel trained on recognition of the attributes on the concept list. At the training fresh mushrooms were tasted from commercially

available white and brown mushroom strains at different stages of development (closed cups and flats). Next to this the conserved mushrooms were tasted. At the end of the training a decision was made on the final list of taste attributes; Firmness, Gummi, Fibrous, Juicy, Sweet, Salty, Aroma presence, Aroma type, Mushroom, Mouldy, Earthy, Metallic, Meaty, Nutty, Boiled egg, Bitter and Pungently.

Mushrooms were offered to the sensory panel on two occasions; 8 October and 4 December 2015. At each occasion the mushrooms were drained from the fluids in the pouches and rinsed under tap water to removed debris of casing soil. Mushrooms were offered in closed cups to the sensory panel lukewarm after heating them for 90 sec at 500 Watt in a microwave oven. At the session on 8 October 2015, 6 treatments were offered to a sensory panel consisting of 20 members of the sensory expert panel of Wageningen UR Greenhouse Horticulture. For each treatment a sample of the (rinsed and heated) mushrooms that were offered to the members of the sensory panel was flash frozen with liquid nitrogen and stored at -80^{o}C until analysis of the amino acids, 5'-nucleotides and the volatiles. At the session on 4 December 2015, only 5 treatments were offered to a sensory panel consisting of 17 members of the sensory expert panel. At the session on 4 December, treatment 4 was not incorporated.

Taste attributes were rated per treatment on a scale of 0 to 100. Each member of the taste panel was offered the treatments in random order. Results were analysed using ANOVA.

2.5 Biochemical analysis of mushrooms

The mushrooms that were offered to the sensory expert panel on 8 October 2015 (drained from fluids and rinsed to remove casing soil particles) were flash frozen in liquid nitrogen and analysed for the amounts of amino acids, 5'-nucleotides and mannitol by PRI Business Unit Bioscience. Analysis of amino acids, 5'-nucleotides and mannitol was performed on an HPLC system. Amounts of amino acids and 5'-nucleotides were measured in nmol/g fresh weight of mushrooms. With respect to the mannitol contents only relative amounts based on peak areas were given.

3 Results

3.1 Cultivation of the mushrooms

Spawn was prepared of sorghum grains for the *Agaricus bisporus* strains that were selected for testing their taste in the period March-April 2015. On 19 May 2015 the strains were inoculated in portions of 16 kg of commercially available compost (CNC Grondstoffen), using 110 ml of spawn. Spawned compost was filled in plastic boxes of 0.2 m^2 growing surface. Colonisation of the compost proceeded for 17 days at a compost temperature of 25°C and a relative humidity of 93-95 %. The CO_2 level in the air was not controlled (>3000 ppm). After spawn run, each tray was covered with 10 L of commercially available casing soil (CNC Grondstoffen basismix nat+). For treatments 4, 5 and 6, calcium chloride (NEDMAG Industries, Veendam 94-97% $CaCl_2$) was dissolved in water (650 g/3 litre). The solution was poured onto the casing soil in two separate portions. During the first 12 days of casing colonization a further 16 litre of water was sprayed per square meter of casing soil surface. After 12 days casing soil was ruffled. Venting was started 3 days after ruffling. During venting the temperature of the air was lowered from 23 till 18°C at a rate of 1 degree per day. Relative humidity was lowered to 90% at a rate of 1.5% per day and the level of carbon dioxide was lowered to 1000 ppm at a rate of 400 ppm per day. First mushrooms were harvested at 23 June 2015. We estimated that about 10 kg of fresh mushrooms were needed per treatment to do all necessary analysis. Hence mushrooms were harvested until that amount was reached or until no more mushrooms were produced. Figure 2 shows the cumulative yield on the consecutive harvesting days. For treatments 1, 2 and 3 (strains grown in a regular casing soil) it took about a week to collect the necessary amount of mushrooms. As compared to treatments 1, 2 and 3, production of treatments 4, 5 and 6 (salty casing soil) was delayed considerably. Next to this, the time needed to collect the necessary amounts of mushrooms was much longer. Strain MES03793 seemed to experience much less problems with the high amount of calcium chloride in the casing soil. Production of mushrooms was delayed by 3-4 days, but once production started the amount of mushrooms was quickly produced. Strain MES02956 experienced much more problems with the high amount of calcium chloride in the casing soil. Again the onset of production is delayed by 3-4 days, but it takes much more time before the 10 kg of mushrooms is produced. Finally strains MES13488 shows to be the most sensitive to the high calcium chloride concentration in the casing soil. Also for this strain we notice a delay of production of about 3 days on high salt casing soil, but the amount of mushrooms needed to perform the experiment was not produced at all. Compared the crop on normal casing soil, production was lowered by about 60%. At the end of cropping, the EC values were checked in the casing soil (Table 2) as a measure for its salt content. During mushroom production the salt content of the casing layer increases which might be due in part by the mycelium leaking salts and partly to uptake of salt by the casing soil from the compost. EC values of fresh casing soils have usually an EC

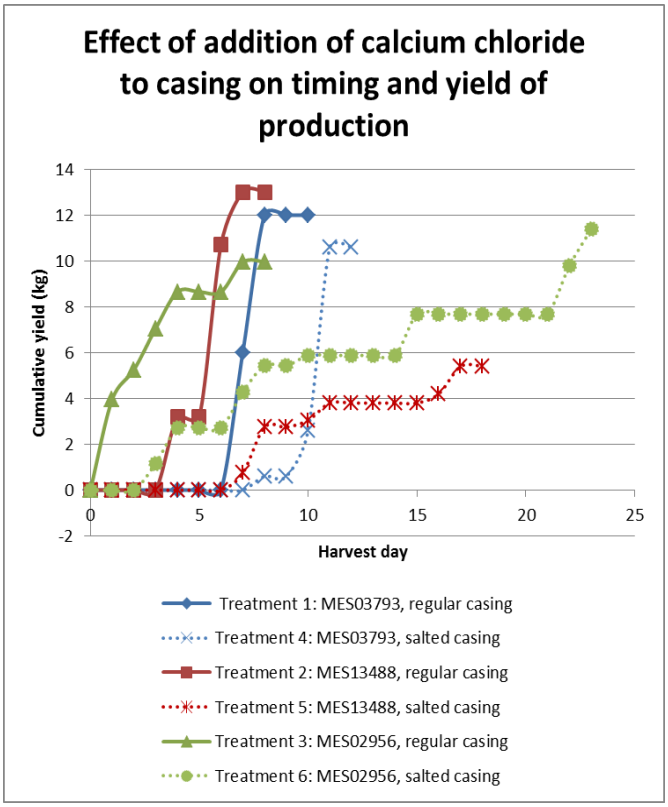

Figure 2. Cumulative production of mushrooms by three different strains on two different types of casing soil (regular and $CaCl_2$ added)

value of less than 1 mS/cm. As can be seen in table 3, EC values of the normal casing soil have increased after mushrooms have been picked and were about 5 times lower in normal casing soil compared to the casing soil with calcium chloride added. The differences in the EC values of the casing soil of treatments 4, 5 and 6 do not explain the low yield of MES13488 on the casing soil with calcium chloride added and suggest that this particular *A. bisporus* strain is more sensitive to a high salt concentration in the casing soil than the other two strains. For treatments 2 and 5 only, also pH of the casing soil was checked and found to be 7.2 for regular casing after cropping and 6.5 for calcium chloride added casing after cropping.

Table 3. EC values of casing soils at the end of cropping.

Treatment	Strain	EC-value
1	MES 03793	8.9
2	MES 13488	7.9
3	MES 02956	10.0
4	MES 03793	52.6
5	MES 13488	54.5
6	MES 02956	45.2

For the mushrooms produced, dry weight was determined (Table 3). As can been seen, addition of calcium chloride to the casing soil increases the dry weight of the mushrooms that are produced. All strains tested behaved rather similar. For strains MES03793 and MES13488, addition of calcium chloride increased the dry weight content of the mushrooms by a factor 1.6. For MES02956 the dry weight content was raised by a factor 1.5.

Table 2. Dry weight of the mushrooms produced.

Treatment	Strain	Dry weight (g/kg FW)
1	MES 03793	71
2	MES 13488	78
3	MES 02956	81
4	MES 03793	118
5	MES 13488	129
6	MES 02956	123

After harvest the 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 were 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. Table 4 gives an overview of the amounts of mushrooms available for testing after conservation in Eco-pouches. Due to the low amount of mushrooms produced, especially for treatment 5 only a limited amount of mushrooms is available for testing.

Table 4. Total amounts of conserved mushrooms available for testing.

Treatment	# Eco-pouches	Total amount (kg) of conserved mushrooms (free fluids included)
1	6	11.9
2	6	12.0
3	5	8.3
4	4	8.2
5	3	3.9
6	7	11.1

3.2 Analysis for taste components

Mushrooms were offered to the sensory panel on two occasions; 8 October and 4 December 2015. At each occasion the mushrooms were drained from the fluids in the pouches. Upon sterilisation, the mushrooms extruded quite a lot of fluid. About 45% of the wet weight enclosed in the Eco-pouches was fluid (mushroom juice). The remaining 55% of the weight were mushroom solids with the remains

of the fluid. These mushrooms were offered to the expert taste panel in 2 sessions with an interval of a few weeks. Results of the analysis of differences in taste between the different treatments are shown in Tables 5 and 6. At the session on 8 October 2015, all 6 treatments were offered to the expert sensory panel, while at the session on 4 December 2015, only 5 treatments were offered.

Table 5. Results of the analysis of differences in taste by an expert taste panel (session of 8 October 2015).

Treatment	Firmness	Gummi	Fibrous	Juicy	Sweet	Salt	Aroma presence	Aroma type	Mushroom	Mouldy	Earthy	Metallic	Meaty	Nutty	Boiled egg	Bitter	Pungently
1	51.72	42.01	30.98	56.95	38.65	27.45	56.02	56.81	48.11	12.11	23.20	4.84	16.75	19.85	7.05	10.98	6.63
2	63.63	51.33	31.61	50.88	39.60	30.88	54.40	52.36	49.52	10.47	15.91	0.75	13.07	29.10	10.57	20.72	9.66
3	53.20	33.61	27.66	37.62	32.70	25.42	59.48	43.12	49.32	25.92	29.39	16.18	13.43	21.10	7.84	21.85	12.71
4	59.64	40.10	27.16	45.28	48.47	33.32	56.38	54.91	45.65	10.97	15.63	7.70	16.15	28.50	12.89	19.84	17.00
5	73.65	51.37	27.70	41.14	35.72	38.14	62.22	47.40	41.81	17.14	21.61	16.57	19.21	27.10	15.29	27.77	14.81
6	65.56	31.78	33.50	39.53	39.41	27.14	57.45	52.30	47.15	11.39	15.76	14.53	11.19	29.45	12.57	20.94	8.54
p	**	*	NS	**	*	*	NS	NS	NS	*	+	*	NS	NS	NS	*	+

Table 6. Results of the analysis of differences in taste by an expert taste panel (session of 4 December 2015).

Treatment	Firmness	Gummi	Fibrous	Juicy	Sweet	Salt	Aroma presence	Aroma type	Mushroom	Mouldy	Earthy	Metallic	Meaty	Nutty	Boiled egg	Bitter	Pungently
1	53.43	45.46	31.93	53.03	41.86	28.12	58.89	59.26	46.92	3.54	5.24	4.70	23.11	18.47	5.29	12.17	7.99
2	60.35	46.41	25.41	50.80	37.42	28.13	52.49	53.24	43.63	13.25	17.41	7.58	15.15	13.72	5.46	10.87	5.28
3	50.09	31.82	31.74	46.84	25.43	27.14	52.41	34.25	28.91	15.89	16.93	13.42	15.16	11.38	13.93	9.54	14.94
4	Not included in the test																
5	71.91	37.55	27.77	49.82	44.07	35.25	60.60	56.73	42.11	15.39	18.39	6.76	27.17	26.51	6.52	14.63	10.77
6	68.17	31.70	38.33	33.75	38.52	29.00	64.85	41.06	42.02	18.23	34.32	11.90	16.06	20.09	9.75	20.74	13.14
p	**	+	*	**	*	NS	NS	**	NS	+	**	NS	NS	NS	NS	NS	+

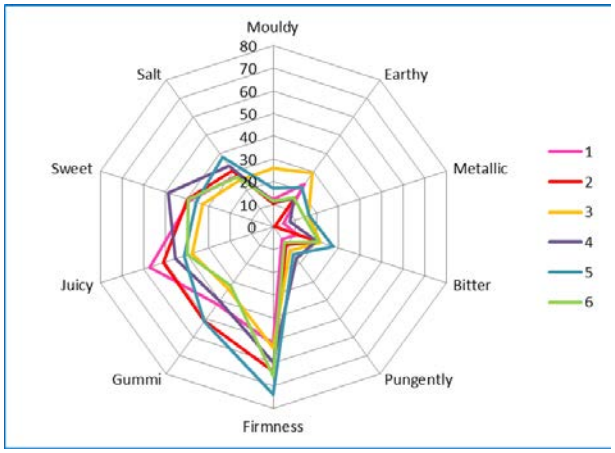

Figure 4. Results of taste analysis on 8 October 2015.

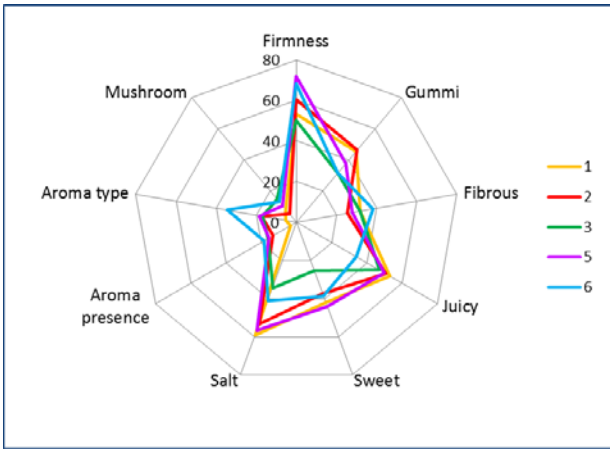

Figure 4. Results of taste analysis on 4 December 2015.

Treatment 4 was not incorporated in this session as the mushrooms of this treatment had gone bad in the last pouch left for analysis. In both sessions statistically significant differences were found in taste aspects between the different treatments (Figures 3 and 4).

On basis of the results obtained on 8 October 2015 it can be concluded that treatment 5 (strain MES 13488 grown on a salty casing soil) obtained high scores for firmness and gummi, was fairly juicy, slightly sweet and scored high with respect to salt taste. Next to this treatment 5 obtained high scores for a metallic aroma and bitterness.
Treatment 1 (MES 03793 grown on a normal casing soil) was the least firm, fairly gummi, the most juicy and not very sweet. Compared to the other treatments, it scored low with respect to bitterness.
Treatment 3 (MES 02956 grown on a normal casing soil) was the least juicy, the least sweet and the least salt but obtained high scores for mouldy and earthy taste tones. Treatment 3 was also fairly bitter.
Treatment 4 (MES 03793 grown on a salty casing soil) had the highest scores for sweet and a fairly high score for salt taste.

Based on the results obtained on the session of 4 December 2015 the taste panel concluded that treatment 6 (MES 02956 grown on a salty casing soil) was less juicy than the other treatments. Next to this it has more earthy taste tones than the other treatments. Also in the session of 4 December 2015 it was concluded that treatment 3 (MES 02956 grown on a normal casing soil) was the least sweet. It was also concluded that it scored low for firmness. Treatment 3 was indicated as the least pleasant aroma (but not less pleasant than treatment 6; same strain grown on a salty casing soil). Furthermore it was concluded that treatment 1 barely has a mouldy aroma, but not significantly less than treatment 2.

From a comparison of Tables 5 and 6, it can be seen that the results of both tasting sessions do not fully coincide. This may be a result from both differences in the composition of the taste panel and differences in the mushrooms offered. In the tasting session of 4 December, the taste panel consisted of 19 members. In the second tasting session there were 17 members. Next to this, in the first tasting session 6 different batches of mushrooms were offered while in the second session one treatment was missing.

3.3 Biochemical analysis.

The mushrooms that were offered to the sensory expert panel on 8 October 2015 (drained from fluids and rinsed to remove casing soil particles) were flash frozen in liquid nitrogen and analysed for the amounts of amino acids, 5'-nucleotides and mannitol.

3.3.1 Mannitol

In the analysis of the mannitol content of the mushrooms, a standard was omitted. Therefore the exact amounts of mannitol in the mushroom tissue cannot be calculated. However, the relative amounts of mannitol in the mushrooms can be compared by comparing peak area's. Mannitol is probably by far the main solute in the mushrooms. For reference, Baars et al. (2015) report for strains MES 02956, MES 03793 and MES 13488 grown on a regular casing in a previous experiment mannitol contents of 73, 117 and 118 g/kg dry weight, respectively.
As can be seen in Figure 5, addition of calcium chloride to the casing soil increases the mannitol content of the mushrooms produced. In MES 02956 the mannitol content was raised by a factor 1.5. In MES 03793 the mannitol content

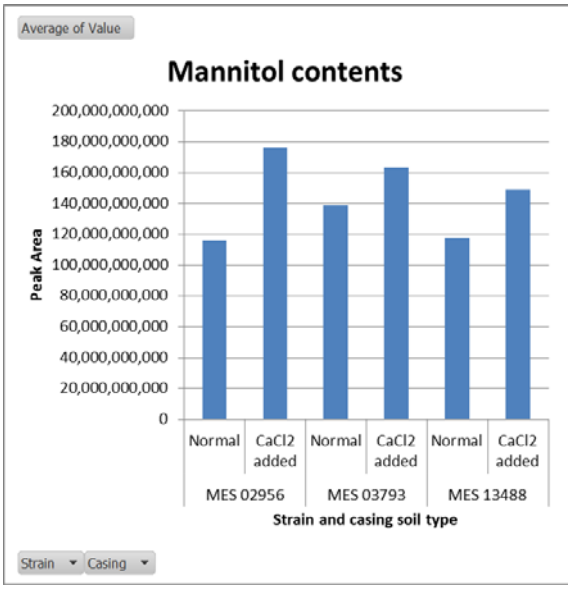

Figure 5. Relative mannitol contents of different combinations of mushroom strains and casing soil treatments.

was raised by a factor 1.2 and in MES 13488 the mannitol content was increased by the calcium chloride treatment of the casing soil by a factor 1.3. All strains tested showed thus an increase in mannitol concentration as a result of the raise in osmotic value of the casing soil.

3.3.2 Amino acids

3.3.2.1 A-ketoglutarate family of amino acids.

The highest levels of amino acids were found for glutamic acid and proline, which both belong to the α-ketoglutarate family of amino acids (glutamate, glutamine, proline and arginine). Their synthesis begins with α-ketoglutarate, an intermediate in the Citric Acid Cycle. Figure 6 shows the contents of glutamate, proline and arginine (glutamine was not measured). As can be seen, levels of the amino acids are higher in mushrooms harvested from a casing soil to which calcium chloride was added. Exception is the level of glutamic acid in strain MES 02956. In the calcium chloride containing casing the level of glutamic acid is lower for this amino acid as compared to the other two mushroom strains.

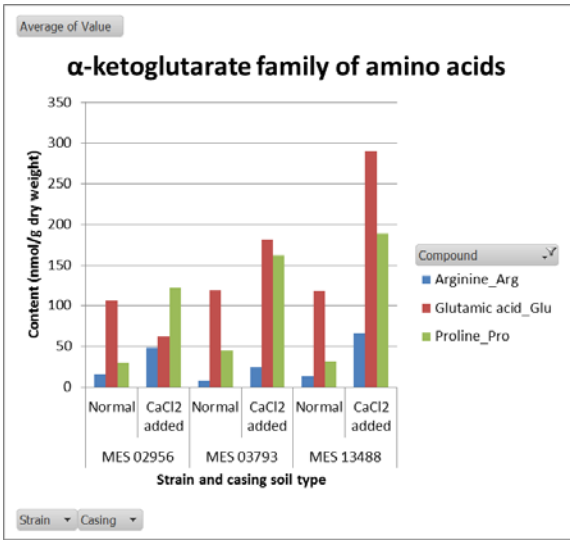

Figure 6. Concentrations of amino acids from the α-ketoglutarate family.

3.3.2.2 Pyruvate family of amino acids

Pyruvate is the end result of glycolysis and can feed into both the TCA cycle and fermentation processes. Reactions beginning with either one or two molecules of pyruvate cause the synthesis of alanine, valine, and leucine. Alanine is present in relatively high concentrations in the mushrooms (Figure 7). Levels of alanine, and to a lesser extent valine and leucine/isoleucine, are influenced by the addition of calcium chloride to the casing soil. Figure 7 shows the sum of leucine and isoleucine. These two amino acids could not be distinguished by the analytical method used. Leucine is a member of the pyruvate family of amino acids while isoleucine is derived from threonine, a member of the oxaloacetate/aspartate family of amino acids.

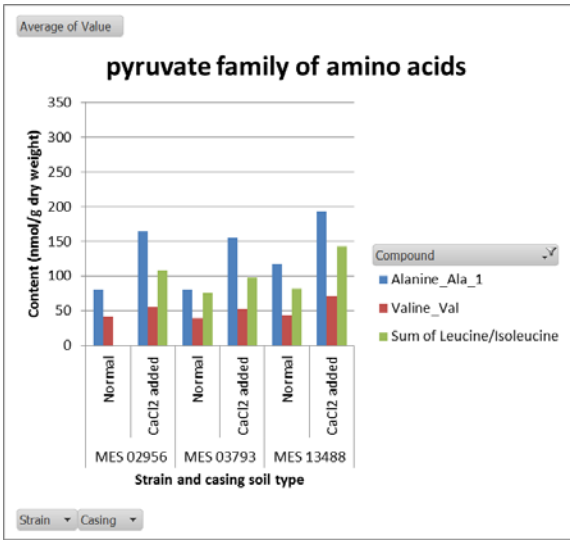

Figure 7. Concentrations of amino acids from the pyruvate family.

3.3.2.3 Oxaloacetate / aspartate family of amino acids

The oxaloacetate/aspartate family of amino acids is composed of lysine, asparagine, methionine, threonine, and isoleucine. Aspartate can be converted into lysine, asparagine, methionine and threonine. Threonine also gives rise to isoleucine. The amino acids from the oxaloacetate/aspartate family are generally present in concentrations lower than 100 nmol/g dry weight (Figure 8). Asparagine and threonine are the most prominent among them. Also for these amino acids levels are generally higher in mushrooms grown in a casing soil treated with calcium chloride.

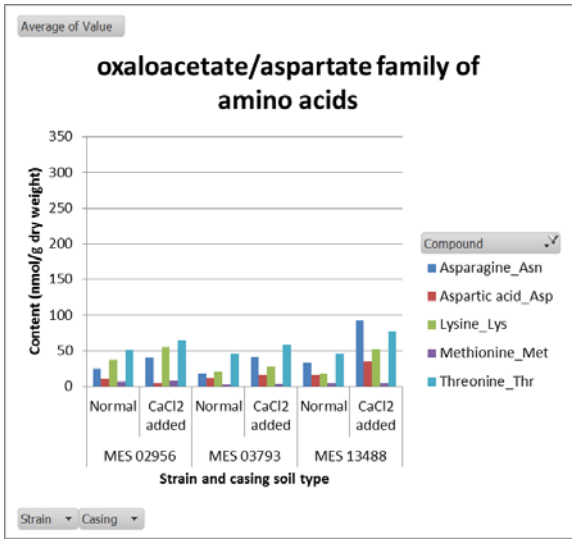

Figure 8. Concentrations of amino acids from the oxaloacetate family.

3.3.2.4 Erythrose 4-phosphate and phosphoenolpyruvate family of amino acids.

Phenylalanine, tyrosine, and tryptophan are known as the aromatic amino acids. The synthesis of all three share a common beginning to their pathways; the formation of chorismate from phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P). As can be seen in Figure 9, levels of these (aromatic) amino acids in the mushrooms are quite low. Nevertheless, in general the levels of these amino acids are higher in mushrooms harvested from a calcium chloride treated casing soil.

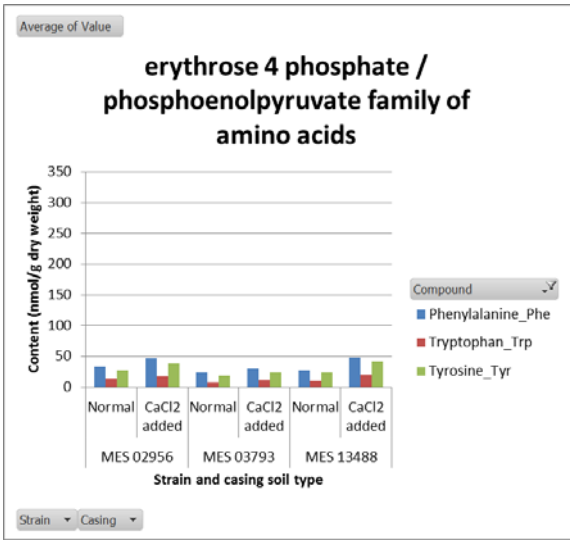

Figure 9. Concentrations of amino acids from the erythrose / phosphoenolpyruvate family.

3.3.2.5 Histidine

Ribose 5-phosphate is the precursor initiating the synthesis of histidine. As can be seen in Figure 10, the levels of histidine are generally around 20-30 nmol/g dry weight and slightly influenced by the addition of calcium chloride to the casing soil.

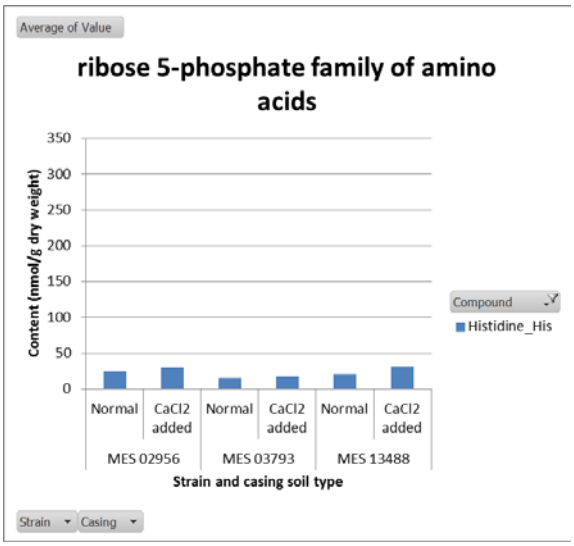

Figure 11. Concentration of histidine

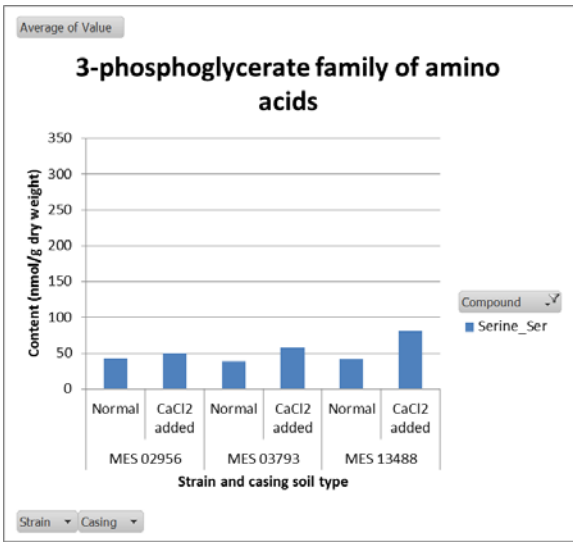

Figure 11. Concentration of serine.

3.3.2.6 3-Phosphoglycerate family

Serine is the first amino acid in this family to be produced; it is then modified to produce both glycine and cysteine. The analytical method used was not able to measure glycine and cysteine. Hence only the levels of serine are shown in Figure 11. Levels of serine were around 50 nmol/g dry weight of mushrooms and are slightly increased by the addition of calcium chloride to the casing soil.

3.3.3 5'-nucleotides

The levels of 5'-nucleotides are shown in Figure 12. 5'-AMP is present in the highest concentrations, followed by 5'-GMP and 5'-IMP. The analytical method used did not allow quantification of 5'-XMP. As can be seen, the

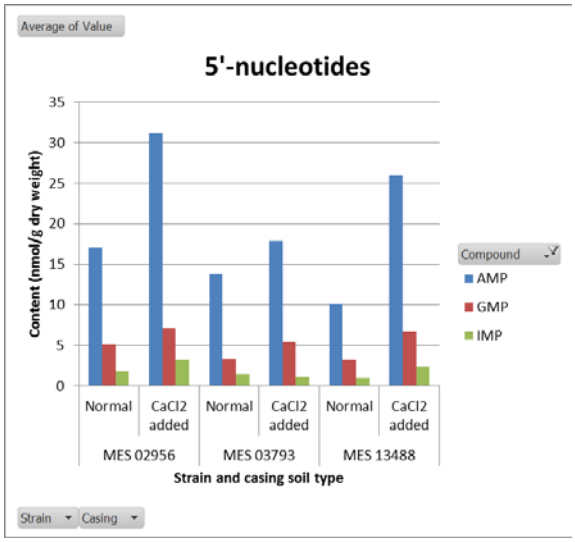

Figure 12. Concentration of 5-nucleotides

addition of calcium chloride to the casing soil has a profound effect on the levels of 5'-AMP (and to a lesser extent on the levels of 5'-GMP and 5'-IMP).

3.4 Attempt to link taste sensations to chemical composition.

An attempt was made by Wageningen UR Biometris to link the two datasets, metabolite levels and taste values, together. The data on taste were used untransformed. The data on metabolites were log-transformed before used in statistical analysis. Correlations between the metabolite and taste panel data are shown in Figure 13. The correlations between results of the first and second session of the taste panels are high (generally above 0.9, see Figure 14), but not higher than the correlations between different samples. It seems that the reproducibility of the taste panel results are not very high. This can have many different causes.
From Figure 13, indications can be derived which metabolites contributed to the taste attributes. For instance, alanine concentrations correlate very well with a sensation of boiled egg as a taste attribute.

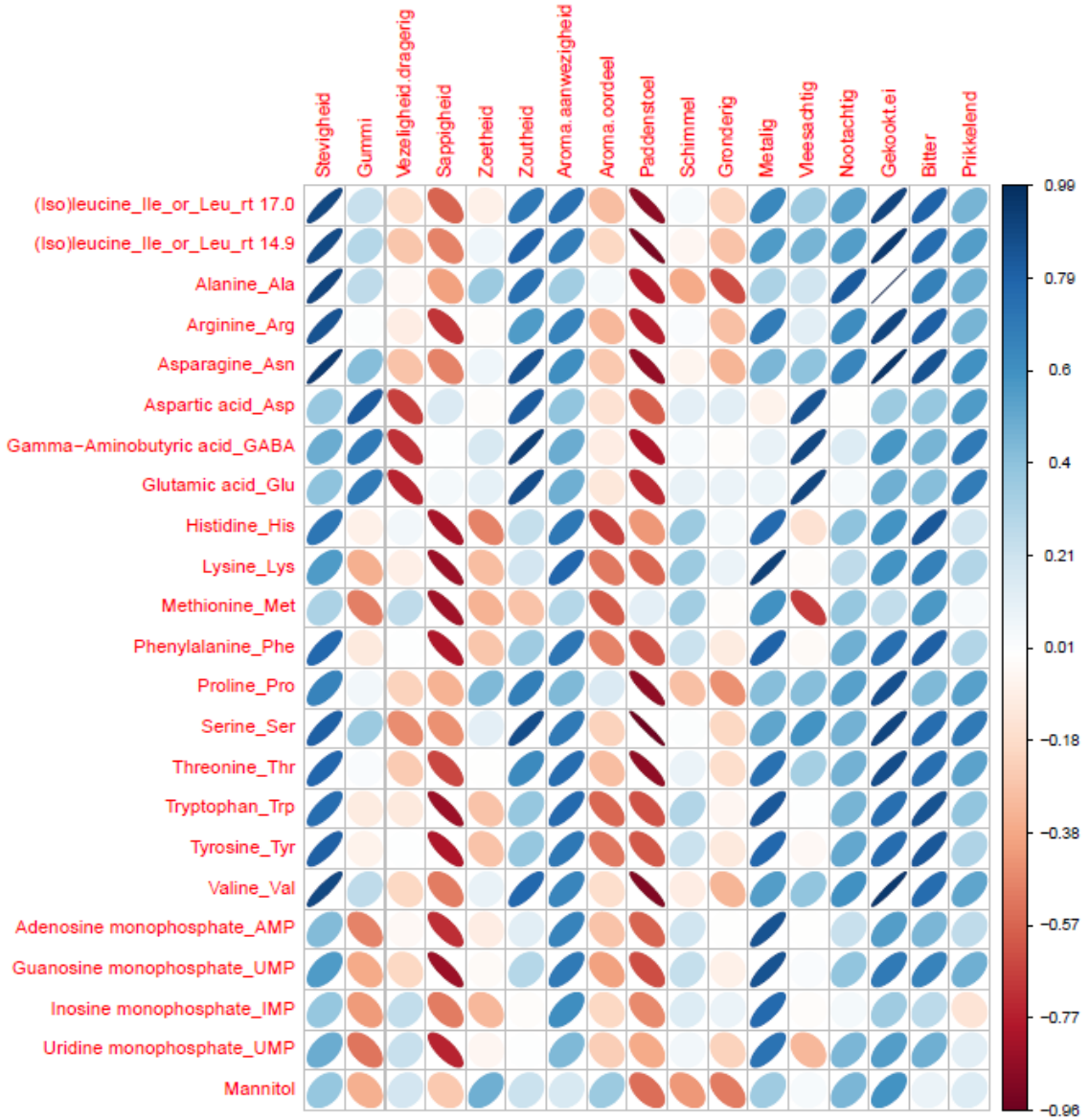

Figure 13. Correlations between the metabolites (vertical axis) and taste attributes (horizontal axis). Only data from the first session of the taste panel were used because that is the only one in which all treatments were tested. The more straight the line, the better the correlation. Blue indicates a positive correlation. Red indicates a negative correlation.

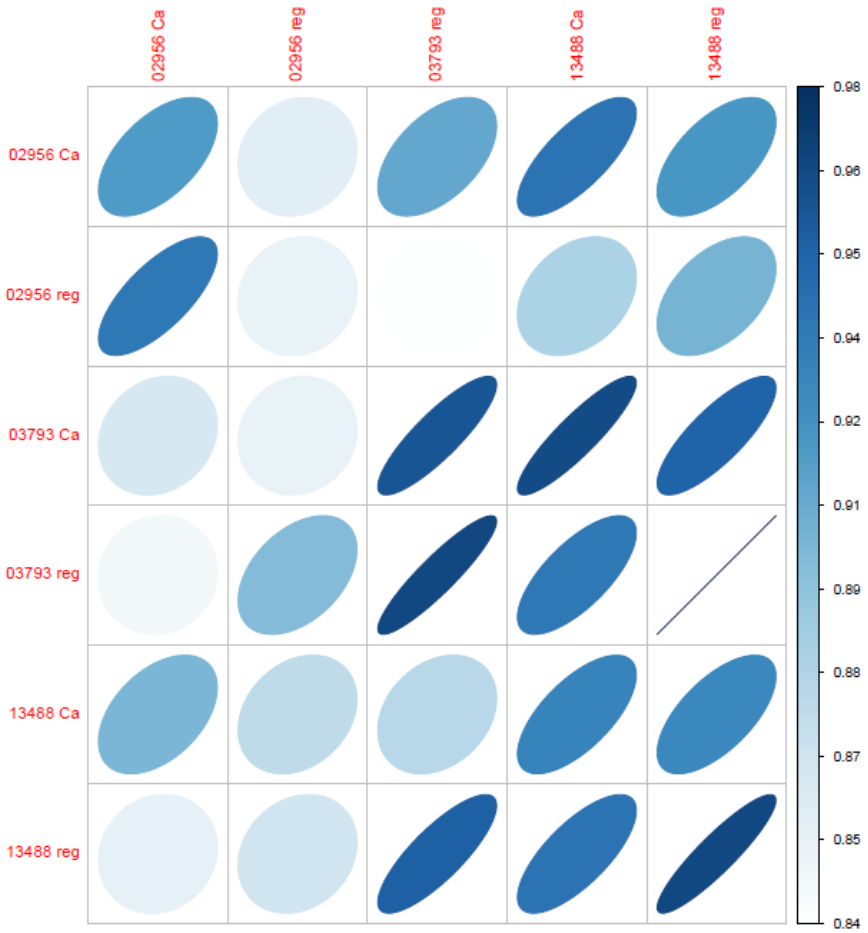

Figure 14. Correlations between the taste results from the first (vertical axis) and the second (horizontal axis) of the taste panels. The darker the color and the less round, the better the correlation.

On the other hand, mannitol (which is a sweet tasting substance) only has a moderate correlation with sweet taste of the mushrooms. A metallic taste seems to correlate quite well with the amount of lysine present in the mushrooms.

Next to this, it seems that some taste attributes can be correlated much better to the metabolites tested than others. For instance, although there are generally positive values in the column, the taste attribute “pungent” (prikkelend) does not show very strong correlation with any of the metabolites tested.

The analysis shown here, is based on a rather small dataset (only 6 treatments) and therefore does not allow firm conclusions. It can best be viewed as an indication of what can be done with a larger and more complete dataset.

4 Discussion and conclusion

The results described in this report, show that it is possible to link attributes of mushroom taste to metabolites present in the mushrooms. However, due to the low number of samples that could be analysed within the project it was not possible to develop a statistical model that could incorporate the different variables. Therefore the results should be seen as a proof of principle.

Nevertheless, it has been shown that the method of cultivation can have a large influence on the concentrations of the metabolites. Making the water in the casing soil less available to the mushroom strains by adding calcium chloride has a profound effect on dry weight and the concentrations of metabolites. Next to this it can be seen that not all mushroom strains appear to react in a similar way to the addition of calcium chloride to the casing soil. As can be seen in Figure 6, addition of calcium chloride to the casing soil increases the level of glutamic acid in strains MES03793 and MESA13488. In strain MES02956 it seems to lower the level of glutamic acid. Also, there are differences in the extent in which concentrations of metabolites are raised. For instance, the concentration of 5'-nucleotides is generally raised in all strains upon addition of calcium chloride to the casing soil. However, the raise is different for the different strains. As seen in Table 7, the increases are highest in strain MES 13488 and lowest in strain MES03793. In this strain the level of IMP even slightly decreases upon addition of calcium chloride in the casing soil.
Glutamic acid and 5'-nucleotides are considered to be important contributors to the umami taste.

Table 7. Increase in the level of 5'-nucleotides in different mushroom strains upon addition of calcium chloride to the casing soil

	AMP	GMP	IMP
MES 02956	80%	40%	80%
MES 03793	30%	60%	-30%
MES 13488	160%	110%	150%

Although the experiments are carried out with only 3 strains under two conditions we can draw some conclusions:

- There are differences between strains in concentrations of metabolites that are related to taste
- Adding salt to the casing soil increases the dry weight of mushrooms and influences the taste significantly
- The concentration of most of the assessed metabolites increases when adding salt to the casing and for some metabolites there are significant differences in increase.

The addition of salt to the casing retard and reduce the production of mushrooms but while for 2 strains this effect is large, for one strain this effect is relatively small. In might thus be interesting screen the collection for sensitivity to salty casing soil and the effect on taste related metabolites as a first improvement of taste.
By growing different mushroom strains on both casing soil with and without calcium chloride we have the possibility to obtain mushroom samples for taste analysis that differ in metabolite concentrations. This appears to be a good model to study which metabolites in mushroom are correlated to specific taste attributes.

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