Metabolites contributing to taste in *Agaricus bisporus*

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1 WUR Plant Breeding
2 PRI Bioscience
3 WUR Glastuinbouw
4 PRI Biometris

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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 nearly 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. In 2014 these strains were cultivated along two different methods 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. As differences in taste were perceived between the different strains and the different methods of cultivation, the strains were grown again in 2015. Three strains (two commercially available and a wild-isolate) were grown both on regular commerciallt 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 Glastuinbouw 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 amino acid content, 5'-nucleotide content, mannitol content 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.
Atempts 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: goose, deterrence, quantity regulation

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1 Preface

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 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 (-)-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 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

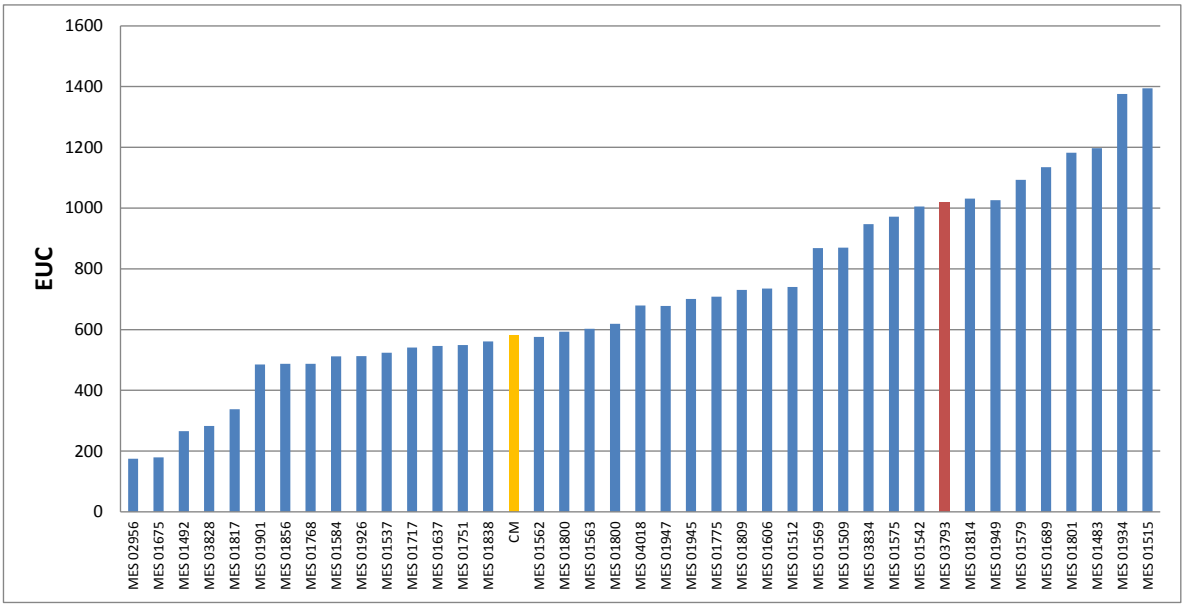

Figure 1. Overview of the equivalent umami concentration of mushroom strains tested, as calculated by the formula designed by Yamaguchi et al. (1971). The yellow bar represents a reference sample. The red bar represents a frequently grown present-day commercial mushroom strain.

very prominent in the mushrooms. The assay technique chosen was semi-quantitative, so accurate 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 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.

3 Material and methods

3.1 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.

3.2 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 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 transported to the mushroom processing facility or stored in a cold room until enough mushrooms had been gathered for processing. Updaten met informative van Ed en misschien nog aan toevoegen hoeveel champignons we geoogst hebben (Patrick vragen om een uitdraai van Ebrida)

3.3 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.

3.4 Testing by sensory panels

Before testing the different batches of mushroom in a taste testing experiment, a description was made of the different attibutes 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 Glastuinbouw. 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.

3.5 Biochemical analysis of mushrooms

The mushrooms that were analysed by the sensory expert panel on 8 October 2015 were analysed for the amounts of amino acids, 5'-nucleotides, mannitol and volatiles by PRI Business Unit Bioscience.
Analysis of amino acids, 5'-nucleotides and mannitol was performed on a ….. 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.
For the analysis of volatiles extraction from the headspace was optimized by either adding or not adding calcium chloride to the macerated tissue…… Volatiles were trapped from the head space using …. And analysed on a ….. system.

3.6 Statistical analysis

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Annex 1 Appendix title

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