THE CULTIVATION OPPORTUNITIES AND COMPLEX COMPARISON SURVEY OF *AGARICUS BLAZEI* (MURRILL)

by

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The applicant met the requirements of the Ph.D. regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.
1. INTRODUCTION

The cultivated mushrooms had always a high value in horticultural products. We know them as food with high biological activity, low energy- and sugar content, holding essential amino acids. Their annual production in Hungary was in year 2010 estimated to 14-16 billion HUF, the cultivated amount of mushroom were in the last 2-3 year as an average around 18-20 million kg/year. From growing point of view, the main advantage of mushroom production is that they can be grown in a proper unit all year long, therefore easy to put onto the market as a premium vegetable product. The Hungarian mushroom consumption is quite moderate, the consumed amount is low (1.5 kg mushroom/year/capita) and shows seasonal fluctuation. For example in Germany the consumption is almost three times higher that can be easily targeted in Hungary as well with eating only one portion mushroom per week (100 g).

The mostly cultivated mushrooms in Hungary (button mushroom and oyster) are just a very little part of the promising mushroom. In the last years, the Hungarian mushroom industry sintered significantly and lost market because of the increasing production costs. In addition, the small farms owners do not have enough income to invest into the modern technology and growing units. More intensive competition at the global market can be predicted because our neighbor countries started to promote their own mushroom products. In Poland and Ukraine around 0.5 million euro were donated to increase consumption of inland grown mushrooms. A summer action was announced in UK as well with TV and radio campaign, in the Ireland similar actions were advertised earlier. The targets of all mentioned sales promotion were to increase the inland consumption and achieve larger market for the mushroom. Additionally all the actions were financed by farmers cooperation. At moment it is impossible to imagine a same campaign with Hungarian growers, but there are several other way to increase income of the farmers.

There is a high potential in the mushrooms that has a high biological value and a medicinal effect like *Agaricus blazei* (MURRILL). Many reports in the literature available about its medicinal effect in cancer therapy and I think the cultivation of it can be done in the Carpathian Basin. In 2011 at the international agricultural trade, Fruit Logistica a Dutch company name Prime Champ won innovational award with *Agaricus blazei* products. They got the award because of continuous and successful cultivation and improvement. Following the example, I think there is a potential to produce mushroom with high added-value. Based on the experiences in Hungarian mushroom research and the latest improvement and novelty in the industry (Dutch-type farms, development in compost yards, new oyster hybrids etc.) I
think the new mushroom species may have opportunity. To achieve those targets I tried to do some basic research to adopt the cultivation technology of \textit{A. blazei} to Hungarian conditions.

2. \textbf{THE \textit{AGARICUS BLAZEI} SPECIES}

The \textit{A. blazei} fungus belongs to regnum \textit{Fungi}, into \textit{Eumycota}, \textit{Basidiomycota}, \textit{Agarycomicetes} class, subclass \textit{Agaricomycetidae} and ordo \textit{Agaricales} in family \textit{Agaricaceae} and genus \textit{Agaricus}. W. A. Murrill described the Royal Sun Agaricus or Almond Portobello in 1945. The popularity of this species is increasing rapidly, especially by Japanese, Brazilian and Chinese growers who are exporting dried fruit bodies and fresh mushrooms. The production of this species is raising continually worldwide, due to its very important curative effect, and exotic slightly almond-like taste. In the past decade many reports were published about the positive medicinal effects of different \textit{A. blazei} extracts. Consumers can find this mushroom in variable forms: fresh or dried (pulverized) form, which is supplied in capsules, pills or as an extract produced by hot water. The extract of the mushroom may be useful as additional prophylactic and possibly therapeutic treatment against \textit{Streptococcus pneumoniae} bacteria. Antiviral activity was observed against human and bovine herpes viruses in cell culture, due to the added mushroom extract and the same extract showed antimutagenic effects as well. The extracts of mushroom are rich in $\beta$-glucan that presumably contributes to the observed activity, but other substances are probably involved. The chemical structure and some properties of the immuno-stimulating polysaccharide and glucan-protein complexes were recently studied.

As Stamets found \textit{A. subrufescens} is closely relative of \textit{A. blazei} and differences are between the sizes and shapes of spores. As he underlined, the odour, morphological characteristic, living area is similar to \textit{A. subrufescens} and \textit{A. augustus}. As Stamets reported the shape of spores of \textit{A. blazei} are rounded while \textit{A. subrufescens} have smaller, ellipse spores. \textit{A. augustus} has larger, round spores as \textit{A. blazei}. All of the above mentioned species have yellow discoloration after harvesting or cutting.

Other publication in 2002 found that \textit{A. blazei} ssp. Murrill, \textit{A. blazei} ssp. Heinem. and \textit{A. subrufescens} are clearly distinct species. The samples was collected from cultivation, herbarium and wildly grown sporocarp for analysis. Based on size of spores, shape of them, morphology of basidium, shape of mycelial cell and cheilocystidia the authors found all
species are different. They suggested that the description of \textit{A. subrufescens} seems like two different sporocarps. As Wasser stated, the North-American \textit{A. blazei} ssp. Murrill and \textit{A. blazei} ssp. Heinem are two species and this latter named \textit{A. brasiliensis} as a new species. They found, \textit{A. fiardii}, \textit{A. metieri}, \textit{A. praemagniceps} should be closely relative of the species.

Later Kerrigan using hybridization and ITS sequences data stated that \textit{Agaricus blazei}, \textit{A. subrufescens}, \textit{A. brasiliensis}, \textit{A. rufotegulis} and \textit{Psalliota subrufescens} are still the same species. According to the rules of nomenclature, he suggested the correct name \textit{A. subrufescens} (Peck). He added that the different morphology might be the result of the quite different origin, but they are definitely one species. Kerrigan found the yellowish discoloration of sporocarp is a simple one-gene product. The various spore sizes are coming from monade, diade and triade spores and now a species-related feature.

3. AIMS

In my studies I compared eight different \textit{A. blazei} cultivars that were collected by Júlia Győrfi from gene banks, reserved at Corvinus University of Budapest Department of Vegetable and Mushroom Growing. I wanted to confirm that there could be morphological differences between cultivars and in yield. I would like to find chemical differences and fit the sensory parameters of mushrooms to ground the further researches for comparison of species/varieties. My longer view was to describe the complex cultivation technology of \textit{A. blazei} and adopt it to Hungarian conditions therefore several aims were formulated:

1. To select from the collection the strain with stable and high yield, draw the flushes, determine the earliness of strains and set up the cultivation technology by measuring most important environmental conditions (temperature, humidity, duration, \textit{CO}_2).
2. To describe the cultivars by morphological parameters taking into account the cultivation features.
3. To detect, document and identify the emerging pests and diseases related to cultivation.
4. As chemical analysis to measure the antioxidant and phenolic compounds from \textit{A. blazei} cultivars.
5. To analyze and identify the molecules that are causing the almond-anisum odour reported in literature.
6. To find the general sensory parameters of mushrooms based on the results to create the sensory profile of *A. blazei*, white and cream type button mushroom.

4. MATERIAL AND METHODS

4.1. Cultivation experiments
The researches of cultivation were conducted in years 2008, 2009 and 2010 at CUB. Fermented and pasteurized, commercial Phase II. mushroom compost without any supplier was used as a growing media. Exactly 2 kg from it were filled in plastic bags and spawned with different cultivars. The spawn were prepared at the mushroom laboratory. 8 *A. blazei* cultivars (Marked: 837, 838, 853, 1105, 2603, Brazil, Ma-He and Si-2.2) and *A. bisporus* control (White: ‘A15’-Sylvan Inc.) were cultivated during the experiments. Trial was hold in the experimental mushroom cultivation tunnel, using similar technology (spawn-run, casing, case-run, pinning, etc.) as in *A. bisporus* industry of course with differences in temperature and time.

4.2. Determination of antioxidant and poliphenolic compounds
The mushroom samples were washed, divided by cap and stipe and freeze until measuring. Measuring was carried out at Department of Applied Chemistry. Total antioxidant capacity was evaluated by modified Benzie-Strain method, FRAP rate was determined in spectrophotometer at $\lambda=593$ nm and revealed in ascorbic-acid equivalent. The total phenolic compounds were determined by Folin-Ciocalteu reagent at $\lambda=760$ nm and calculated in gallic-acid equivalent (Singleton- Rossi, 1965). Every measurement was repeated 3 times.

4.3. Determination of the chemical elements
The fruit bodies were cleaned, caps and stipes were separated, cut, dried and milled; the chemical determinations were carried out from this fine powder. 200 mg of dried material was digested in a mixture of 2 cm$^3$ HNO$_3$ and 2 cm$^3$ H$_2$O$_2$ in closed Teflon bombs at 1.56 x 105 Pa (30 min), in three independent replicates of each samples. The digested and filtered materials were diluted to 10 cm$^3$ with bi-distilled water and the contents of elements measured by ICP (inductively coupled plasma spectroscopy).
4.4. Measuring the volatile aromatic components
Immediately after harvest, 200 g from the mushroom samples were cut in small slices and put into a 4000 ml round bottom flask with 180 g of NaCl, 900 ml of distilled water and 150 µl of 0.8 mg/ml concentration undecanol-1 internal standard solution. The simultaneous distillation-extraction process was performed in a modified Likens-Nickerson apparatus. Normal pentane was applied as an organic solvent to extract the volatile fragrance compounds from the mushroom samples. Three consecutive distillations were carried out, and the distillation time was 1 hour in every cases. Following this process, the extract was frozen to remove water traces and it was concentrated to 1 ml, 3×1 µl of what was injected into the GC-MS instrument. The separation and identification of the mushroom volatiles were accomplished with a Hewlett Packard 5890/II gas chromatograph coupled with a 5971A mass selective detector.

4.5. Sensory profiling
The sensory profile analysis method was chosen from many reliable, descriptive methods that are designed to take all of the relevant human senses into account. The method we have chosen can be used to define a production standard and to compare a product with those of similar type already on the market. The sensory profile analysis is one of the most complex food tests with the main advantage being the full description of a food product by rating its characteristics and their relative intensities on a numerical scale. The tests were designed and finished at Sensory Laboratory of CUB, following the ISO 11035:1994 standard.
5. RESULTS AND DISCUSSION

5.1. Cultivation experiments

The results of the yields from years 2008, 2009 and 2010 were statistically analyzed by two-way ANOVA. At 95% significance level, there were differences of the yield between cultivars. It must be added that every crop needs new compost and casing, but the differences may be the result of different potential of the strains. Despite the three independent cultivations, the strain 838, 2603 and MaHe had stable and constant yield that should be underlined from the growing point of view (Fig. 1).

![Figure 1](image.png)

**Figure 1.** Respective yields expressed per 100 kg compost of the different *A. blazei* strains

(same letters mean no significant difference)

High standard deviation were noticed between strain 838, 2603 and MaHe, but based on the results of Tukey probe and more sensitive Duncan test, there were no statistical significance. The large deviation may be the result of little observed sample number. Stable but low yield was got at strain 838 (5.2 kg/100 kg compost) and high yield was observed by MaHe (10.1 kg/100 kg compost). Very low deviation was measured by MaHe in 2009, and yield was stable in all year. The large scale cultivation can be suggest with these three strain because of their reliable yields.
On Figure 2, the strains with significant differences are shown. A whale of difference was calculated by Si2.2 that has different yield in all year. Extreme good yield was noticed in 2009 (13.5 kg/100 kg compost) but can not be repeated in 2010. I think, strain Si2.2 could have a high potential and yield if the technological elements (temperature, irrigation, CO₂) are more understood. Strain Brazil had low, but stable yield in 2008 and 2009, in 2010 it almost doubled the yield (8 kg). Sharp decreasing was observed at strain 837 from 2008 to 2009 without any scientific explanation: the compost parameters did not change that can explain why the yields were less five times. The strain 837 is not suggested for further researches because this feature.

![Figure 2](image-url)

**Figure 2.** Respective yields expressed per 100 kg compost of the different *A. blazei* strains

(different letters mean significant difference between years in strain)

The diameter of cap and length of stipe were measured in every year and averages of those are shown on Figure 3. From the first flush to the second, the mushroom sizes usually decreased and the percentage of decreasing depended on strain. The ratio of cap-stipe is one of the attribute that indicate the form of fresh mushroom. The reason of mushroom’s size reducing can be several: less nutrients in compost, lower moisture in casing or compost could be an answer but the genetically determination of the strain is although an acceptable cause.
Figure 3. The changes of diameter of cap (left) and length of stipe (right) by examined *A. blazei* strains (average of year 2008, 2009 and 2010)

**Morphological description of the strains**

The variety of appearance of the fruit bodies were mentioned in the literature. On the Figure 4. the pictures of a well-developed and representative sporophore can be seen. The shape of cap, length of stipe, pinning time in my experiments were even more related with strain than cultivational conditions. However the temperature and humidity have an influence to the sporophore morphology, but the shape of stipe, cap (round, flat, curve) are distinct parameters and related to strain. No earlier comparision like this were found in the literature between *A. blazei* strains therefore these results are one of the major output of my research.
Figure 4. The morphology of examined *Agaricus blazei* strains
The description of strains is focused mostly on cultivation experiences rather than morphological parameters. The storage conditions were evaluated at +2°C in fridge.

**837:** its cap is hard, suited mostly central to the stipe, but sometime not. Its cap is large, asymmetric and often deformities can be seen on sporocarps. Usually it has late fruiting and long picking period. It can be stored at +2°C for 3-5 days.

**838:** the cap is hard, in the beginning round, later it becomes flatten. It has similar deformity as 837 and it looks similar out. The stalk is uniform thick, late and long picking and 3-5 days long storage has the strain.

**853:** the sporocarps are light, fragile, the cap and stalk are thin. The storage tolerance is poor, fruits quickly and early. Stable and low yield was measured in 2008 and 2009, it climbed in year 2010 almost double.

**1105:** it is similar to strain 853, the fruit bodies are light, fragile, the cap and stalk are thin. It has a high yield and early fruiting, the spores are ripening fast.

**2603:** it has large, heavy and hard sporocarp, its cap is angular and get flat. The stipe at casing is often wider. The fruit bodies are forming one at a time, can storage for long as same as strain Brazil.

**Brazil:** it has a round, hard structural cap, the stalk is thick and hard as well. Late fruiting and sensibility for disease ‘watery stipe’ is related to it. In the second flush, it produces usually much smaller mushrooms. Its big mushrooms can be used for breeding as a partner.

**MaHe:** it has a high and stable yield in the experiments with small but single mushrooms. The cap looked like trapezoid, the stalk is hard and can storage for a while. It fruited early, but for the second flush, the mushrooms turned smaller.

**Si2.2:** its shape is similar to 853 and 1105 with massive production that are small and fruiting in clusters. It produces much spores that are spreading fast therefore picking at least 2 times per day is neccessary.

The pest and disease were recorded during the crops. The cultivations were conducted at spring, summer and winter as well accordingly all major pathogen could appear. The mushroom unit was placed in the arboretum, so high pathogen pressure was assumed. Despite this, no major disease from *A. bisporus* cultivation appeared in the crops. I did not identify neither dry nor wet bubble diseases (*Verticillium fungicola* var. *fungicola* and *Mycogoe perniciosa*) during *A. blazei* growing. The cobweb (*Cladobotryum dendroides*) did not have any serious losses in the cultivation. Mushroom flies (*Sciaridae*) appeared in one year with
low damage. The ‘watery stipe’ disorder was noticed several times especially by strain Brazil, but no causative agent was found, so it seems it is an abiotic disorder as a result of poor watering.

5.2. Results of measuring antioxidants and polyphenols
The data demonstrate differences between cultivars moreover between caps and stipes in antioxidant capacity. The white button mushroom has a similar total antioxidant capacity as the cream type. Both of the examined Agaricus cultivars had a higher level in the caps than the stipes, which was similar in A. blazei cultivars as well. Extremely high differences were measured in antioxidant capacity between cap and stipe in strain 1105. The average amount of total antioxidant capacity showed that A. blazei 853 and 1105 are as similar as white and cream type mushroom, while other A. blazei cultivars lag behind the others. Levels of total polyphenolics were significantly higher in all A. blazei cultivars than in A. bisporus varieties. That is probably caused by molecules that are responsible for the colour in mushrooms as well, because A. blazei has brown cap and yellow colouring stipe to injury. Very high level of total phenolics was measured in A. blazei 853, five times higher than white button mushroom.

5.3. Results of measuring chemical elements
Based on our results the A. blazei cultivars have lower sodium, higher zinc levels, the potassium (as the main macroelement) has lower content, than those of Agaricus bisporus ‘control’ strain. The selenium content is under the detection’s limit, but the cadmium uptake and concentration seems to be higher. I did not find differences in concentration of barium, calcium, magnesium, manganese, nickel and titanium. The quantity of cobalt and vanadium did not attain the statement limit value in the samples of mushrooms. The A. blazei cultivars have significantly lower total mineral content (compared to A. bisporus) the ratio alternate between 56,3 and 87,3 %. The caps of the A. blazei fruit bodies have - in general 11-38 % - higher total element content than of the stipes.

The potassium level in A. bisporus was high (40000 mg/kg) and almost double as amount in A. blazei strains (27-32000 mg/kg). The phosphorus content of A. blazei’s cap are almost the same as in A. bisporus sporopohore, the amount of stipe lags behind this. It can be underline that selenium content of A. blazei was under the detection limit and from this point of view, it has less value. The quantity of sodium was just half or third part in A. blazei than in A. bisporus. It is another advantage for mushroom consumer. A disadvantage from human nutritional point of view that cadmium level was noticeable in A. blazei (2-17 mg/kg) and was
under the limit in *A. bisporus*. The distribution of elements in caps and stipes is characteristic; the majority of beneficial elements have higher contents in caps than in stipes, but some other elements such as Ca, Fe and Na show an inverse proportion.

5.4. Determination of volatile aromatic components
The volatile components of *A. blazei ’1105’* were characterized because that strain fruited enough sporocarp for the measuring. All together 83 aromatic components were separated and 75 from those were recognized at least 70% trustiness ([Fig. 5](#)). 65 compounds were separated from *A. bisporus* and 45 were recognized from the spectra library.

Clearly distinct amount of open chain alcohols, aldehydes and ketones were measured from *A. blazei* (3,59%) and *A. bisporus* (32, 59%). The 8-carbon alcohols and ketons can be found in mushrooms, the amount of those differed as well. The 1-octen-3-ol is responsible for ’mushroom-like odour’ and it had 10 times higher concentration in *A. bisporus*. The less fragrant 3-octanol covered the spectra of *A. bisporus* by 6,12%. A long 17-carbon aldehyde (heptadecenal) was detected in *A. bisporus* and 3-methyl-1-butanol was also found. The S-containing compounds are usually very odour full and their fragrance can be detect in low amount. In both species was detected 3-methylthio-propanal (methional) which is a well-
The terpenes are molecules from isoprene ($C_5H_8$) monomers that can have open and closed chain. They evaporate easily, therefore many essential oils belongs to it. The linalool is a natural terpenic-alcohol was found in a small amount in *A. bisporus* and can be found in several medicinal plants like lavender. In *A. blazei* a sesquiterpene molecule, nerolidole was detected and it has a fresh tree bark odour. It was found in essential oils of ginger, jasmine, levander and in *Melissa*. N-containing compounds were measured in higher amount in *A. blazei* sporocarps. Four different ring contained molecules were found in low concentration in both species in addition the samples contained pyridine as well.

The compounds bearing benzene ring have high concentration in both *Agaricus* species, but *A. blazei* contained almost 50% more. Almost half of the total aromatic compounds was benzaldehyde (42.96%) in *A. blazei* and just one fourth (11.35%) was in *A. bisporus*. The benzaldehyde is the most simple and important aromatic aldehyde where the functional group is related to benzene ring directly and it is part of almond oil. The esterized form of it, benzoic acid-methyl ester had more than 11% of the total amount in *A. blazei* and the niobe oil contains it. The benzoic acid - ethyl ester was found in *A. blazei*, the last mentioned two components are responsible for odour of strawberry. Both mushroom species had high concentration of benzyl alcohol, which has a sleeping effect to human. It is a common molecule in genus *Hyacinthus* and *Balsamina* in esterhized forms. The mixture of different concentration from benzaldehyde and benzyl alcohol could be also smelled as almond or anisum therefore the description of the odour of *A. blazei* is relevant for both. The detected phenylethyl alcohol (A. bisporus: 2.32%, A. blazei: 0.49%) has a nice fragrant, found in essential oil of rose.

5.5. Sensory profiling
In total, 19 descriptive phrases were used in the experiments based on a consensus group decision. The descriptors (Table 1) are some of major results of the thesis, because no complex phrases were found previously in references used for mushrooms. The taste and odour were characterized for both caps and stalks. Only the main attributes are demonstrated which have an influence on mushroom consumption and the industrial food market.
Table 1. The main descriptors and means of achieved scores by mushrooms (scale: 0-100)

<table>
<thead>
<tr>
<th>Attribution</th>
<th>Value (0-100)</th>
<th>White button mushroom</th>
<th>Cream type</th>
<th>A. blazei '1105'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap colour</td>
<td>Dark-bright</td>
<td>90.0</td>
<td>32.78</td>
<td>25.71</td>
</tr>
<tr>
<td>Cap blotchiness</td>
<td>No blotches</td>
<td>0</td>
<td>29.21</td>
<td>81.0</td>
</tr>
<tr>
<td>Cap thickness</td>
<td>Thin-thick</td>
<td>50.0</td>
<td>55.86</td>
<td>21.24</td>
</tr>
<tr>
<td>Cap format</td>
<td>Flat-round</td>
<td>40.0</td>
<td>44.79</td>
<td>70.07</td>
</tr>
<tr>
<td>Stalk colour</td>
<td>Dark-bright</td>
<td>40.0</td>
<td>55.36</td>
<td>81.86</td>
</tr>
<tr>
<td>Stalk length</td>
<td>Short-long</td>
<td>20.0</td>
<td>19.36</td>
<td>85.93</td>
</tr>
<tr>
<td>Stalk thickness</td>
<td>Thin-thick</td>
<td>50.0</td>
<td>65.29</td>
<td>16.93</td>
</tr>
<tr>
<td>Gills colour</td>
<td>Dark brown-white</td>
<td>15.0</td>
<td>33.14</td>
<td>80.57</td>
</tr>
<tr>
<td>Fragility</td>
<td>Firm-fragile</td>
<td>60.0</td>
<td>35.64</td>
<td>73.5</td>
</tr>
<tr>
<td>Hardness</td>
<td>Soft-hard</td>
<td>40.0</td>
<td>74.29</td>
<td>28.72</td>
</tr>
<tr>
<td>Sliminess</td>
<td>Dry-glutinous</td>
<td>70.0</td>
<td>48.43</td>
<td>53.43</td>
</tr>
<tr>
<td>Succulence</td>
<td>Dry-juicy</td>
<td>90.0</td>
<td>42.86</td>
<td>42.86</td>
</tr>
<tr>
<td>Mushroom-like odour</td>
<td>Soft-high</td>
<td>65.0</td>
<td>58.07</td>
<td>40.71</td>
</tr>
<tr>
<td><code>Soil</code> odour</td>
<td>Soft-high</td>
<td>25.0</td>
<td>33.79</td>
<td>61.5</td>
</tr>
<tr>
<td><code>Fresh</code> odour</td>
<td>Unfresh-fresh</td>
<td>15.0</td>
<td>64.36</td>
<td>53.29</td>
</tr>
<tr>
<td>Intesity of 'mushroom-like' taste</td>
<td>Soft-high</td>
<td>60.0</td>
<td>66.29</td>
<td>45.21</td>
</tr>
<tr>
<td>Intesity of 'sweet' taste</td>
<td>Soft-high</td>
<td>0</td>
<td>40.71</td>
<td>59.71</td>
</tr>
<tr>
<td>Intesity of 'fresh' taste</td>
<td>Unfresh-fresh</td>
<td>20.0</td>
<td>67.21</td>
<td>48.36</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>No-strong</td>
<td>40.0</td>
<td>51.79</td>
<td>60.5</td>
</tr>
<tr>
<td>Other taste</td>
<td>No-strong</td>
<td>0</td>
<td>37.93</td>
<td>72.79</td>
</tr>
</tbody>
</table>

The full sensory profiles of the fresh mushrooms assessed are present in Figure 6. The chart demonstrates clear differences between the mushroom varieties tested. The spider chart underlines the fact that the fresh mushrooms assessed have very distinct sensory profiles. A full sensory profile assessment has never been completed for these varieties of mushroom and it may help to mushroom producers, mushroom breeders and for the mushroom industry in general.
Figure 6. The full sensory profile of the tested fresh *Agaricus blazei, Agaricus bisporus* white and cream type button mushrooms.
6. **NEW OR NOVEL SCIENTIFIC RESULTS**

The experiments in three years confirmed that *A. blazei* can be grown in Hungary, using commercial substrate for button mushroom production from inland composters. Based on the results my scientific results are the following:

- I cultivated firstly, successful, for three years *A. blazei* in Hungary.

- I verified that strains of *A. blazei* have significant morphological and cultivating differences and based on results from growing (yield, fruiting time etc.) the cultivars can be selected. For Hungarian conditions from the examined eight strains MaHe can be suggested for cultivation, because its stable and high yield might be suitable for mass production after large-scale tests.

- I affirmed that there are differences in antioxidant and polyphenol concentration in *A. blazei* strains. The caps of *A. blazei* contain more phosphorus and magnesium than stipes and I confirmed *A. blazei* sporocarps accumulate less sodium than *A. bisporus*.

- The volatile components of *A. blazei* were characterized, and the molecules that are responsible for almond-like odour of mushroom were identified (benzaldehyde, benzyl alcohol, methyl-benzoate). The GC-MS application was appropriate to confirm on chemical way the empirical data of literature about odour of anise and almond.

- I prepared and first published the sensory profile of *A. blazei*, white and cream type button mushroom. The sensory attributions were reported first in literature, they are useful to describe fresh mushroom products: cap colour, cap blotchiness, cap thickness, cap format, stalk colour, stalk length, stalk thickness, gills colour, fragility, hardness, sliminess, succulence, mushroom-like odour, ‘soil’ odour, ‘fresh’ odour, intesity of ‘mushroom-like’ taste, intesity of ‘sweet’ taste, intesity of ‘fresh’ taste, aftertaste, other taste.

- I prepared and summarized the technological parameters, that are suggested for cultivation *A. blazei* in Hungary. Those conditions can be used for mass cultivation of the mushroom species (Table 2).
Table 2. Growing conditions for production *A. blazei* mushroom
(based on Stamets 2000, modified and completed)

<table>
<thead>
<tr>
<th></th>
<th>Compost temperature (°C)</th>
<th>Air temperature (°C)</th>
<th>Humidity (%)</th>
<th>Duration (nap)</th>
<th>CO₂ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spawn-run</td>
<td>25-26</td>
<td>22-24</td>
<td>90-95</td>
<td>12-17</td>
<td>5,000-20,000</td>
</tr>
<tr>
<td>Case-run</td>
<td>24-25</td>
<td>21-23</td>
<td>90-92</td>
<td>8-12</td>
<td>5,000-10,000</td>
</tr>
<tr>
<td>Pinning</td>
<td>21-24</td>
<td>19-20</td>
<td>85-90</td>
<td>8-12</td>
<td>500-1,000</td>
</tr>
<tr>
<td>Fruiting</td>
<td>23-25</td>
<td>22-24</td>
<td>85-90</td>
<td>4-8</td>
<td>&lt;2,000</td>
</tr>
</tbody>
</table>
7. SUMMARY
In the past decade a clear tendency can be observed about increased demand in natural food additives and health enhancer food products. The increasing amount of those followed the climbing consumption of medicinal mushrooms as well. My study focused on a less well-known medicinal mushroom, *Agaricus blazei* (syn. *A. subrufescens, A. brasiliensis*) cultivation opportunities in Hungary. I compared in years 2008, 2009 and 2010 eight mushroom strains - originated from genebank - in the point of view cultivation and chemical aspects. I found that the mushroom species can be cultivated successfully on inland substrates, and for the Hungarian conditions strain ‘MaHe’ is the most appropriate because of its high and constant yield. I reported a morphological description about the strains; underline the advantages and disadvantages of them in the point of view cultivation. As the summary of the observations, I prepared the cultivation technology of the mushroom species for Hungarian conditions. I estimated the pests and diseases that may have threatened the cultivation of the mushroom species.

The chemical analysis registered high antioxidant capacity and total phenolic compound in the species *A. blazei*. Those amounts may differ in caps and stipes. By ICP measurement it could appointed that some macro- and microelement concentration in the fruit bodies are different. The cap of *A. blazei* accumulates more phosphorus and magnesium then the stipe. *A. blazei* contain about half amount of sodium than the white button mushroom (*A. bisporus*).

Using gas chromatography–mass spectrometry (GC-MS) the molecules that are responsible for almond-anise odour were separated and identified. Benzaldehyde and benzyl-alcohol were present in high concentration in *A. blazei* therefore the empiric statement of the literature about odour of the species was confirmed by chemical way. By separating the odour component I found, that *A. blazei* contain more volatile odorant than *A. bisporus*.

I published first the sensory profile of *A. blazei*, white button and cream type mushroom. The sensory descriptors were determined that can be used to describe fresh mushroom products. The results of improvement on the cultivation technology and the chemical measurements establish a well basis for Hungarian *A. blazei* cultivation.
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