

PhD dissertation - thesis

The effect of UV radiation on the vitamin D level, bioactive matter content and organileptic properties of cultivated button and oyster mushrooms

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1. Introduction

In the past few years, numerous surveys have been conducted to evaluate the vitamin D status of the fast growing population of the Earth. It has become clear that vitamin D deficiency is a common condition, which effects men and women in different ages and social statuses (Ginde et al, 2009). The reason of the high ratio of vitamin D deficiency is the limited number and availability of this vitamin (Holick, 2007). One type of vitamin D is vitamin D₃, which is formed in the human skin by sunlight. The access to this form of the vitamin is influenced by the age of the individual (and the quality of the skin), the season (the access to sunlight), and the geographical location (northern regions are prone to be deficient in sunlight) (Webb et al., 1988; Tangpricha et al., 2002; Hanley & Davison, 2005; Park & Johnson, 2005; Schwartz & Hanchette, 2006; Holick, 2007). In the other hand, people living in great poverty, in overpopulated regions, or people with dietary restrictions (e.g. vegetarians) get less vitamin D₂ (the type of vitamin D found in foodstuffs) through their diet (Chen et al., 2007; Holick, 2007).

Since the majority of the Earth's population is affected by vitamin D deficiency, in the end of the 20th century, a number of studies aimed to produce some form of vitamin D artificially in large quantities, to make vitamin D accessible widely in the form of supplents or supplemented food. Thanks to these efforts, nowadays different products (e.g. pills, drops) are available on the market (Holick et al., 1992; Tangpricha et al., 2003). For vitamin supplementation, conscious consumers prefer natural sources. That is why the focus of numerous reseraches was to develop a natural, vitamin D enhanced product.

Since mushrooms have a low energy level and high nutrient content, they are conscidered to be an important element of a healthy diet. Also, they are one of the few foddstuffs with natural vitamin D content (Mattila et al., 1994). Besides vitamin D, mushrooms have a quite consciderable level of ergosterol (Mattila et al., 2002; Jasinghe & Perera, 2005), which is converted into vitamin D by UV radiation (Jasinghe et al., 2007). Various studies are aiming to achieve the same reaction (observed in the nature with wild grown mushroom and sunlight) in case of cultivated mushrooms in the growing houses with artificial UV irradiation (Mau et al., 1998; Perera et al., 2003; Kalaras et al., 2012; Jasinghe & Perera, 2006; Teichmann et al., 2007; Ko et al., 2008; Simon et al., 2011; Phillips & Rasor, 2013). These research works differ in many elements: whether the treatments are pre- or post-harvest or whole or sliced samples are used; which mushroom species and UV wavelength is applied; how long the treatments are etc. Most references on such studies are about white

button mushrooms or oyster mushrooms and post-harvest treatments. Only one publication is available on the effect of pre-harvest UV irradiation of biologically active mushroom cultures (Kristensen et al., 2012).

Since UV radiation is a stress factor, besides enhancing the vitamin D levels, it could impact other materials, e.g. the antioxidants or polyphenoles in the fruitbody. The appearance of the mushroom could be affected as well, or UV radiation could also have a negative effect on the yields.

The evaluation of such vitamin D enhanced mushroom product can only be done in a complex approach, by taking the nutritional value, consumer demands and organoleptic features into consideration. Sensory analysis (analytical evaluation) performed by trained panelists provide analytical data on the sensory features of a product. Whether a new product is suitable for the market is determined by many factors: e.g. price, origin, presentation, packaging, added value (enhanced vitamin D content) etc. A product is usually judged by the consumer, as they are the ones to decide whether it is worth buying or not. Consumers and members of a naive panel choose a product (with a given combination of factors) based on preference. That is why it is important to include them in the evaluation process of a new product. With conjoint analysis we can identify the relative importance and utility function of the decision factors, and to find the ideal combination as a final step of product optimization.

2. Objectives

Only a few sources are available in the topic of pre-harvest UV treatments of cultivated mushrooms. There is only a limited amount of information about the changes UV treatments cause in the ergosterol and vitamin D content and in the organoleptic features of the mushrooms.

The main objective of my thesis was to determine the effect of pre-harvest UV treatments applied on biologically active, developing mushroom cultures; to find out what changes do different UV radiation methods cause in the ergosterol and vitamin D content of white and brown button mushrooms (*Agaricus bisporus*) and of oyster mushroom (*Pleurotus ostreatus*). Another goal was to study the effect of UV radiation on the yield and on the critical organoleptic parameters (e.g. color).

Naive and trained panelists evaluated the vitamin D enhanced mushroom products, which is the first step of product development. Triangle tests performed by naive panelists

provide crucial data for product optimization based on consumer demand. Trained panelists prepared the sensory evaluation and conjoint analysis.

Since international studies have proved that UVA radiation is much less effective in enhancing the vitamin D level of mushrooms than UVB and UVC (Jasinghe & Perera, 2006), only the latter two were used for the purposes of my experiments.

1. Determining the changes in **yield**, **vitamin D** and **ergosterol** content and in **color** was in the focus of the analysis of pre-harvest UV treated button mushrooms and oyster mushroom.

- 1.1. Do the treatments applied during the growing period have an impact on the yield?
- 1.2. How do different time period and different wavelength UV treatments affect the vitamin D content?
- 1.3. How do different time period and different wavelength UV treatments affect the ergosterol content?
- 1.4. Do UV treatments cause any other change in the appearance of the mushrooms?
- 2. The **antioxidant** capacity and **polyphenol** content were determined by laboratory analysis as well. The following goals were set:
 - 2.1. What significant differences can be proven in the impact of UVB and UVC treatments on the antioxidant capacity and polyphenol content?
 - 2.2. Do the 6 types of irradiation lengths (15 to 90 minutes) have an effect on antioxidant and polyphenol levels?
 - 2.3. Is there any difference in these levels between the different mushrooms examined?

In case of a new product, complex assessment is necessary. Besides laboratory analysis, it is crucial to conduct evaluation by using naive and trained panelists.

Sensory analysis (analytical evaluation) was performed by an expert panel. The 9-15 trained panel members were selected by product specific tests. Their task is different from that of the naive panelists, who are average consumers. Trained panelists provide analytical data on the sensory features of a product; their decisions are not influenced by feelings or preference.

- 3. The tasks of the **expert panel** were the following:
 - 3.1. Which expressions are suitable for the description of the different mushrooms?
 - 3.2. How does the sensory profile of the mushrooms look like?
 - 3.3. In which organoleptic feature occurs any difference between the various treatments?

Whether a new product is suitable for the market is determined by many factors: e.g. price, origin, presentation, packaging, added value (enhanced vitamin D content) etc. A product is usually judged by the consumer, as they are the ones to decide whether a product is worth buying or not. Consumers and members of a naive panel choose a product (with a given combination of factors) based on preference. We assume that if a combination is preferable for the consumers, it is more likely that they will choose and buy it again. That is why it is important to start product optimization by learning the opinion and needs of consumers.

- 4. The following points were included in the **consumer based optimization** survey:
 - 4.1. Which factors influence the decision making process of mushroom consumers?
 - 4.2. What is the relative importance of the decision factors?
 - 4.3. What is the ideal combination of factors for the consumers participating in the study?
 - 4.4. What are the utility functions of the decision factors for the consumers?

The relevance of such studies is that functional foods are getting a higher level of recognition these days. Conscious consumers prefer and continuously choose healthy foods with enhanced antioxidant and vitamin levels.

With my results, I would like to contribute to the international initializations that are aiming to find new methods and equipment, which can be used to produce vitamin D enhanced mushrooms. The ultimate objective is to develop vitamin D rich mushroom products that can help us fight health issues deriving from vitamin D deficiency. As a result of such initializations, another functional food will become available for those consumers, who prefer natural sources of supplementation. Vitamin D enhanced cultivated mushrooms are not only healthy, but also provide a pure source of vitamin D for us.

3. Material and methods

3.1. The button and oyster mushrooms used in the study

The white and brown button muhrooms and oyster mushroom used for the purposes of the study were cultivated in the experimental growing chamber of the Department of Vegetable and Mushroom Growing (Faculty of Horticulture, Corvinus University of Budapest).

The fist white button mushroom experiments started in 2009, the second in 2011. The first brown button and oyster mushroom experiments were conducted in 2010, the secon ones in 2012.

3.2. The method of the UV treatments

Vilbert Lourmat-115M type UV lamps were used with the following parameters. UVB lamp: 312 nm, $2.1 \text{ W} (0.1023 \text{ mW/cm}^2)$; UVC lamp: 245 nm, 0.6 W (0.5995 mW/cm²). The lamps were placed 30-32 cm from the surface of the mushroom blocks. Treatments started when the fruitbodies were pea-sized, and continued on 3 consecutive days until the mushrooms reached 4-6 cm in diameter.

| Length (minute) | | 0 | 15 | 30 | 45 | 60 | 75 | 90 |
|-----------------|---------------------------|---------|------|------|------|------|------|------|
| UVB | Dose (J/cm ²) | 0 | 0,54 | 1,08 | 1,62 | 2,16 | 2,7 | 3,24 |
| | Name | control | B15 | B30 | B45 | B60 | B75 | B90 |
| UVC | Dose (J/cm ²) | 0 | 0,09 | 0,18 | 0,28 | 0,37 | 0,46 | 0,55 |
| | Name | control | C15 | C30 | C45 | C60 | C75 | C90 |

Table 1. The length, dose and name of the pre-harvest UV treatments applied on the mushroom cultures

3.3. Measuring yields

UV radiation has a negative effect on the cells, which means that UV treatments of developing mushrooms could affect the mycelia and also the yield. That is why the yields were measured by digital scale in case of each treatment and mushroom species.

The data were than calculated to 100 kg compost. The average of the two experiments were taken into account. For statistical analysis Kruskal-Wallis test were used, by calculating the exact p-value on 95% significance level. If case of significant difference, Dunn's post-hoc test was applied as well.

3.4. Determination of dry matter content

5 g fresh sample was taken in case of each treatment (UVB, UVC; 15, 30, 45, 60, 75, 90 min), from each mushroom species in 5 repeats. The average of the two experimental years is shown.

3.5. Determination of vitamin D and ergosterol contents

Analysis was done in the laboratories of the Departments of Applied Chemistry (Faculty of Food Science, Corvinus University of Budapest).

Sample preparation procedure for mushroom samples was based on the EN 12821:2000 European Norm with some modifications. Briefly, 500 g fresh mushroom sample

was lyophilized prior to pulverization. An amount of 0.5 g of the homogenized powder was weighed into glass flask and 50 ml ethanol along with 4 ml 1M sodium-ascorbate, 10 ml 50 % (m/v) potassium-hydroxide and 50 μ l of 200 μ g/g cholecalciferol (vitamin D₃) reference solution as a surrogate standard were added. Saponification was carried out at 80°C for 1 h. The supernatant was transferred to a separation funnel and the sediment was washed with 15 ml ethanol, followed by 50 ml n-pentane and finally 2×100 ml water. The pooled two-phase solution was extracted manually and after separating and collecting the solvent phase the aqueous phase was extracted repeatedly with 2×20 ml n-pentane. The pooled solvent phase was re-extracted three times with 3×50 ml aqueous solution containing 3 % (m/v) KOH and 5 % (v/v) MeOH. The separated solvent phase was then sequentially washed with water until the pH of the washing medium reached 7. Finally, the solvent phase is transferred to a rotary evaporator. The funnel is washed with an additional 5-10 ml n-pentane, and the pooled solvent phase is evaporated near to dryness at 30°C. The residue is reconstituted in ethanol to 5 ml and filtered through a 0.45 µm PTFE filter before HPLC analysis.

Prepared samples were analyzed by reversed phase high performance liquid chromatography (RP-HPLC) coupled to an ESI-MS/MS system. Experiments were performed with an Agilent (Agilent Technologies, Waldbronn, Germany) 1100 HPLC system coupled to an Applied Biosystems (Foster City, CA, USA) 3200 Q-Trap hybrid triple quadruple/linear ion trap MS/MS instrument equipped with a Turbo-V ESI ion source. Separation was carried out on a YMC Hydrosphere C18 100 × 2.0mm, 2μ m C18 RP-HPLC column (YMC Europe, Dinslaken, Germany) within 6 minutes applying isocratic elution at 0.3 ml/min flow rate with methanol containing 0.1 % (v/v) formic acid. Quantitation of ergocalciferol was carried out by the standard addition calibration method using a reference substance. Multiple reaction monitoring (MRM) was used in positive ion mode for data acquisition and the transitions of 397/105 and 397/159 were monitored for ergocalciferol, while in the case of the internal standard (cholecalciferol) transitions of 385/105 and 385/159 were used.

3.6. Colorimetry

The objective color of foodstuffs can be determined by the tristimulus method recommended by the Commission Internationale de L'Éclairage (CIE). Fort measuring the CIELab L*a*b* color coordinates a Chromameter CR-400 equipment was used.

The color of the fruitbodies was measured for three consecutive days, from the first treatment until after the third, in 24 repeats. This determined the color difference on a scale

that shows if the color change is visible for the human eye. The color differences were determined by Pythagoras theorem: $\Delta E_{ab*} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The average human eye detects the following values: 0.0-0.5 non-detectable, 0.5-1.5 barely detectable, 1.5-3.0 detectable, 3.0-6.0 visible, 6.0-12.0 highly visible (Wenczel, 2013).

3.7. Determination of antioxidant capacity and polyphenol content

Sample preparation was followed by the methods detailed below.

ABTS-assay

ABTS-assay was measured as described by Huang et al (2005). Reaction mixture contained 10µl of sample; 20µl of 3.50mg/ml myoglobin in 50 mM, pH 7.4, 9% NaCl and 1% glucose containing potassium-phosphate buffer;150µl of 1mg ABTS and 25µl 3% H₂O₂ in 0.1 M pH5 citrate buffer. This mixture was shaked for 5 minutes at 37°C, than alkaline stop solution was added, and measured at λ =405nm against trolox calibration curve.

CUPRAC-assay

CUPRAC assay was measured by the method of Apak and co-workers. 1ml 10^{-2} M CuCl₂, 1ml 7.5*10⁻³ M neocuproine solution, 1 ml pH 7.4 1 M NH₄Ac buffer, 100 µl sample and 1 ml distilled water was mixed, and incubated for 30 minutes in dark at room temperature. After the absorbance reading at λ =450nm, values were calculated to trolox equivalents.

DPPH-assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) elimination assay was performed as described by Brand-Williams et al. 100µl of sample was added to 3.9ml of $6*10^{-5}$ M methanolic DPPH solution, than incubated for 20 minutes in dark. Absorbance readings were performed at λ =517nm, and inhibition % were calculated.

FRAP-assay

Ferric reduction antioxidant power (FRAP) was determined according to Benzie and Strain. 100µl of sample was added to pH3.6 300mM acetate buffer- 10 mmol/liter TPTZ (2,4,6tripyridyl-s-triazine) in 40 mM HCl- 20 mmol/liter FeCl₃* 6H₂O, than after 5 minutes, absorbance was read at λ =593nm. Results were generated in ascorbic acid equivalence.

TPC method

Total Phenolic Compound (TPC) was recorded as described by Singleton and Rossi. 1250ul of 10 fold diluted Folin-Cioalteau reagent, 240 µl methanol, 10µl of sample, and 1ml of 0.7 M

NaCO₃ was mixed, ant kept at 50°C for 5 minutes, than measured at λ =765 nm to calibration curved set up with gallic acid.

All of the measurements were carried out in five replicate. For statistical analysis Kruskal-Wallis test were used, by calculating the exact p-value on 95% significance level. If case of significant difference, Dunn's post-hoc test was applied as well. For the comparison of the results of different assays, the values were normalized (on a scale from 0 to 100) by using XL-Stat program.

3.8. Organoleptic evaluation

Both descriptive and difference testing were used to evaluate UV treated mushrooms. Tests and evaluation were done based on the rules and regulations of international ISO patterns.

3.8.1. Sensory analysis performed by expert panel

Tests were completed at the same time every day (10 AM), which is the time period recommended by ISO regulations. The evaluation was done by 11 trained panelists of the Laboratory of Sensory Evaluation. They have excessive knowledge and experience on softver supported organileptic evaluation.

The evaluation took place in the laboratory of the Department of Postharvest Science and Sensory Evaluation to ensure reproductivity and stable environment. For sample identification and processing data, ProfiSens softver was used.

3.8.2. Triangle test performed by naive assessors

In case of difference testing we are looking for statistically proven differences or similarities between the samples. The aim of the triangle test was to see, whether naive assessors can find any differences between the untreated control and the samples treated for 90 minutes with UVB light, and in case of oyster mushroom, between the control and the 45 and the 90 minutes UVB treated samples. Evaluation of the results was done both on the basis of binomial distribution and sequential analysis. Students were members of the naive panel. Triangle tests took place in the Laboratory of Sensory Evaluation.

3.8.3. Product optimization (focus group and conjoint analysis)

Focus groups aimed to prepare the list of characteristics used later in the conjoint analysis. Members of the focus group were housewives between the age of 25 and 70. They identified the relative importance and utility function of the decision factors, which are important for the consumer during purchasing button or oyster mushrooms.

Conjoint value analysis was then conducted. For this we have prepared combinations of factors, which were then reduced to 32 combinations (or so called card) by the program SPSS 22.0. Each card was then given a random number between 100 and 999. The evaluation of the cards was done through Google document. In the end of the survey, participants were asked to fill in a socio-demographic questionnaire (frequency of mushroom consumption, type of purchased mushroom, net income, highest degree, place of residence etc.). These data were then used to characterize consumer segments. The survey was done by 306 people. The data were evaluated with the conjoint module of the SPSS 22.0. program.

5. Results and discussion

5.1. Yield

Based on the results, none of the UV treatments coused any significant (α =0,05) change in the yields of the button and oyster mushrooms.

5.2. Vitamin D content

60 minute long UVB treatment is proved to be most effective in case of white button mushrooms, as the resulting vitamin D level was seven times as high as that of the control samples. 100 g of untreated, fresh white button mushroom contains 115% of the recommended minimum 400 IU vitamin D and only 31% of the recommended daily intake (RDA = 1500 IU), whereas 60 min UVB treated mushrooms have a vitamin D level of 73.22 μ g/100 g (fresh weight), which is 195% of the RDA (1500 IU), and is still well under the safe maximum value (10 000 IU). In case of UVC treatments the best result was caused by 45 minute long irradiation. 100 g fresh mushroom had enough vitamin D to provide 314% of the daily minimum and 84% of the RDA. **Figure 1.** shows which UVB treatments proved to be efficient enough to provide the RDA value in case of white button mushroom.



Figure 1. Vitamin D levels resulted by different UVB treatments of white button mushrooms in reference of the RDA (1500 IU, green line) and the safe maximum intake (10000 IU, red line) (100 g fresh mushroom)

In case of brown button mushrooms the 45 minute long UVB treatment resulted the highest vitamin D level (control: 6.38 μ g/g dw; UVB45: 10.00 μ g/g dw) and only this irradiation method was effective enough to raise the vitamin D level significantly (α =0,05). 100 g of untreated, fresh brown button mushroom contains 500% of the recommended minimum 400 IU vitamin D and 136% of the recommended daily intake (RDA = 1500 IU), whereas 45 min UVB treated mushrooms have a vitamin D level of 88.17 μ g/100 g (fresh weight), which is 235% of the RDA (1500 IU), and is still well under the safe maximum value (10 000 IU). In case of UVC treatments the best result was caused by 45 minute long irradiation (UVC45: 8.49 μ g/g dw), but still did not cause significant change. 100 g fresh mushroom had enough vitamin D (2580 IU, 64.5 μ g/100 g fw) to provide 645% of the daily minimum and 172% of the RDA. **Figure 2.** shows which UVB treatments proved to be efficient enough to provide the RDA value in case of brown button mushroom.



Figure 2. Vitamin D levels resulted by different UVB treatments of brown button mushrooms in reference of the RDA (1500 IU, green line) and the safe maximum intake (10000 IU, red line) (100 g fresh mushroom)

90 minute long UVB treatment is proved to be most effective in case of oyster mushrooms, as the resulting vitamin D level was nine times as high as that of the control samples (control: 2.97 μ g/g dw; UVB90: 25.66 μ g/g dw). Even the shortest treatment (15 min) was enough to double the vitamin D content (UVB15: 6.24 μ g/g dw). 100 g of untreated, fresh oyster mushroom contains 1317 IU (32.93 μ g/100 g), 329% of the recommended minimum 400 IU vitamin D and 88% of the recommended daily intake (RDA = 1500 IU). The 90 min UVB treated oyster mushrooms have a vitamin D level of 9128 IU (228 μ g/100 g) (fresh weight), which is 609% of the RDA (1500 IU). 109 g of 90 min UVB treated oyster mushroom contains the safe maximum value (10 000 IU). In case of UVC treatments, the 15-60 min resulted in a vitamin D level double (212-229%) of the recommended daily intake. **Figure 3.** shows which UVB treatments proved to be efficient enough to provide the RDA value in case of oyster mushroom.



Figure 2. Vitamin D levels resulted by different UVB treatments of oyster mushrooms in reference of the RDA (1500 IU, green line) and the safe maximum intake (10000 IU, red line) (100 g fresh mushroom)

The results of vitamin D analysis presented in this thesis correspond with the data found in international sources.

5.3. Ergosterol content

The presence of ergosterol, as pre-vitamin D_2 is essential in mushrooms for vitamin D conversion. In case of white button mushroom 30, 45 and 60 minutes of UVB and 75 minutes of UVC treatments caused significantly higher ergosterol levels in the samples. In brown button mushrooms UVB irradiation did not cause any change, but the ergosterol level decreased after 30 and 45 minutes of UVC treatments. A drop in ergosterol content was observed in case of oyster mushroom samples as well due to 15, 30 and 45 minutes UVB and 75 and 90 minutes of UVC irradiation.

5.4. Colorimetry results

Both colorimetry data and sensory analysis proved a color change in case of 90 min UVB treated white button mushroom.

On brown button mushrooms UV treatments caused measurable and visible color change as well. It is important to emphasize that color change was determined by the comparison of treated and untreated fruitbodies, which cannot be done by a consumer while purchasing a mushroom product.

UVB irradiation between 15 and 75 minutes caused visible color change on oyster mushrooms. Just like in case of brown button mushrooms, without the simultaneous comparison of treated and control specimens, the consumer is not likely to detect any

differences. Moreover the color of the fruitbody of oyster mushroom is not constant and could vary from light brown to grey or even chocolate brown.

5.5. Antioxidant capacity and polyphenol content

Although the antioxidant capacity and polyphenol levels were affected by the different wavelength and durations of UV treatments, we were not able to find any tendencies.

5.6. Organoleptic evaluation

5.6.1. Sensory analysis performed by expert panel (profile analysis)

By the total profile analysis of the UV treated mushrooms, sensory evaluation by an expert panel was possible and effective. Results show that the visual features of the 45 and 90 min UVB treated white button mushrooms are significantly different from the control: the cap color became yellower, the color was darker and spots became visible on the caps. This result correlated with the colorimetry data.

The organoleptic profile of brown button mushrooms was similar to that of white button mushroom samples. No significant difference occurred in case of any of the treatments.

In case of two characteristics (color and consistence) were there significant differences in oyster mushroom samples. Control samples were the lightest, while every treated sample proved to be darker.

5.6.2. Triangle test results

The triangle test results of white button mushrooms showed that naive assessors can find differences between the untreated control and the samples treated for 90 minutes with UVB light, and in case of oyster mushroom, between the control and the 45 and the 90 minutes UVB treated samples. The results proved that evaluation on the basis of binomial distribution is more susceptible to differences than the sequential analysis.

5.6.3. Product optimization (focus group and conjoint analysis) results

With conjoint analysis I was able to identify the relative importance and utility function of the decision factors, and to find the ideal combination. By using cluster analysis I characterized the consumer segments and based on their demand I prepared their optimized product.

Result of conjoint analysis show that enhanced vitamin D content has a higher utility and greater importance for the consumers, thus it should be in the focus of communication. Brown button mushroom if preferred by consumers between the age of 20 and 40, who consume mushrooms every other week or once a month, have a net income around 151-300 000 HUF and live in big cities. Vitamin D enhanced oyster mushroom is preferred by the 50+ age group, who eat mushrooms every other week, have a net income around 151-300 000 HUF and live in smaller cities.

6. Results

- I proved that the 60 minute long pre-harvest UVB treatment is the most effective method of enhancing the vitamin D level of biologically active white button mushrooms. In case of brown button mushrooms 60 minutes, and in oyster cultures 90 minutes of UVB irradiation induces the highest vitamin D levels.
- 2. With nonparametric Kruskal-Wallis statistical test I proved that the 15, 30, 45, 60, 75 and 90 minute long UVB and UVC treatments of white and brown button mushrooms and oyster mushroom do not cause significant (α =0,05) change is the yields.
- 3. I was the first to characterize the antioxydant capacity and polyphenol content of UV treated white and brown button mushrooms and oyster mushroom by multiple methods (DPPH, CUPRAC, TPC, FRAP, ABTS), and the significant (α =0,05) effect of the UV treatments in the antioxidant level.
- 4. I was the first to conduct complex sensory evaluation of UV treated white and brown button mushrooms and oyster mushroom, and with a panel of trained experts I have prepared the sensory profile of these mushrooms.
- 5. With triangle test I proved that naive assessors can find significant (α =0,05) differences between the untreated control and the samples treated for 90 minutes with UVB light, and in case of oyster mushroom, between the control and the 45 and the 90 minutes UVB treated samples.
- 6. I identified the relative importance and utility function of the decision factors, and found the ideal combination in case of UV treated, vitamin D enhanced white and brown button mushrooms and oyster mushroom. By using cluster analysis I characterized the consumer segments and based on their demand I prepared their optimized product.

References

- 1. Babbie E. (1995): A társadalomtudományi kutatás gyakorlata. Budapest, Balassi Kiadó. 103-348, 411-473, 494-526. p.
- 2. Chen T. C., Chimeh F., Lu Z., Mathiew J (2007): Factors that influence the cutaneous synthesis and dietary sources of vitamin D. Archives of Biochemistry and Biophysics, 460 (2), 213-217. p.
- 3. Ginde A. A., Liu M. C., Camargo Jr. C. A. (2009): Demographic differences and trends of Vitamin D insufficiency in the US population, 1988-2004. Archives of International Medicine, 158, 531-537. p.
- 4. Hanley D. A., Davidson K. S. (2005): Vitamin D insufficiency in North America. Journal of Nutrition, 135, 332-337. p.
- 5. Holick M. F. (2007): Vitamin D deficiency. American Journal of Clinical Nutrition, 87 (4), 1080S-1086S. p.
- 6. Holick, M. F., Shao Q., Liu, W. W., Chen T. C. (1992): The vitamin content of fortified milk and infant formula. New England Journal of Medicine, 326, 1178-1181. p.
- 7. Jasinghe V. J., Perera C. O. (2006): Ultraviolet irradiation: The generator of vitamin D₂ in edible mushrooms. Food Chemistry. 95, 638-643. p.
- Jasinghe V. J., Perera C. O., Barlow P. J. (2005): Bioavailability of vitamin D₂ from irradiated mushrooms: an in vivo study. British Journal of Nutrition, 93, 951-955.
 p.
- 9. Jasinghe V. J., Perera C. O., Sablani S. S. (2007): Kinetics of the conversion of ergosterol in edible mushrooms. Journal of Food Engineering. 79, 864-869. p.
- Kalaras M. D., Beelman R. B., Elias R. J. (2012): Effects of post-harvest pulsed UV light teratment of white button mushrooms (*Agracius bisporus*) on Vitamin D content and quality attributes. Journal of Agricultural and Food Chemistry, 60, 220-225. p.
- Ko J. A., Lee B. H., Lee J. S., Park H. J. (2008): Effect of UV-B exposure on the concentration of vitamin D2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). Journal of Agriculture and Food Chemistry, 56: 3671-3674. p.
- Kristensen H. L., Rosenqvist E., Jakobsen J. (2012): Increae of Vitamin D₂ by UV-B exposure during the growth phase of white button mushroom (*Agaricus bisporus*). Food & Nutrition Research, 56, 1-7. p.
- 13. Lehota, J. (2001): Marketingkutatás az agrárgazdaságban. Budapest, Mezőgazda Kiadó.
- Mattila P. H., Lampi A.-M., Ronkainen R., Toivo J., Piironen V. (2002): Sterol and vitamin D₂ contents in some wild and cultivated mushrooms. Food Chemistry. 76, 293-298. p.
- Mattila P. H., Piironen V. I., Uusi-Rauva E. J., Koivistoinen P. E. (1994): Vitamin D contents in edible mushrooms. Journal of Agricultural and Food Chemistry. 42, 2449-2453. p.

- Mau J. L., Chen P. R., Yang J. H. (1998): Ultraviolet irradiation increased vitamin D₂ content in edible mushrooms. Journal of Agricultural and Food Chemistry. 46 (12), 5269-5272. p.
- 17. Park S., Johnson M. A. (2005): Living in low attitude regions in the United States does not prevent poor vitamin D status. Nutrition Reviews, 63, 203-209. p.
- 18. Perera C. O., Jasinghe V. J., Ng F. L., Mujumdar A. S. (2003): The effect of moisture content on the conversion of ergosterol to vitamin D in shiitake mushrooms. Drying Technology, 21, 1093-1101. p.
- Phillips K. M., Rasor A. S. (2013): A Nutritionally Meaningful Increase in Vitamin D in Retail Mushrooms is Attainable by Exposure to Sunlight Prior to Consumption. Journal of Nutrition and Food Sciences, 3 (6), 236. p.
- 20. Schwartz G. G., Hanchette C. L. (2006): UV, latitude, and spatial trends in prostate cancer mortality: All sunlight is not the same (United States). Cancer Causes Control, 17, 1091-1101. p.
- 21. Simon R. R., Phillips K. M., Horst R. L., Munro I. O. (2011): Viatmin D mushrooms: Comparison of the composition of button mushrooms (*Agaricus bisporus*) treated postharvest with UVB light or sunlight. Journal of Agricultural and Food Chemistry, 59, 8724-8732. p.
- 22. Tangpricha V., Koutkia P., Rieke S. M., Chen T. C. (2003): Fortificatoion of ornage juice with vitamin D: A novel approach to enhance vitamin D nutritional health. American Journal of Clinical Nutrition, 77, 1478-1483. p.
- 23. Tangpricha V., Pearce E. N., Chen T. C., Holick M. F. (2002): Vitamin D insufficiency among free-living healthy young adults. American Journal of Medicine, 121 (8), 659-662. p.
- Teichmann A., Dutta, P. C., Staffas A., Jägerstad M. (2007): Sterol and vitamin D₂ concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. LWT. 40: 815-822. p.
- 25. Vicsek L. (2006): Fókuszcsoport. Budapest: Osiris Kiadó, 18, 59-64, 166, 228. p.
- 26. Webb A. R., Kline L., Holick M. F. (1988): Influence of season and latitude on the cutaneous synthesis of vitamin D₃: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D synthesis in human skin. Journal of Clinical Endocrinology and Metabolism, 67, 373-378. p.
- 27. Wenczel K. (2013): Színtan. Budapest, Budapesti Műszaki és Gazdaságtudományi Egyetem, Gépészmérnöki Kar, Mechatronika, Optika és Gépészeti Informatika Tanszék. 36-45., 69-70. p.

Publications in the topic of the thesis

Publications in journals with IF

*Szabó, A., Gyepes, A., Nagy, A., Abrankó, L., Győrfi, J. (2012). The effect of UVB radiation on the vitamin D_2 content of white and cream type button mushrooms (*Agaricus bisporus* Lange/Imbach) and oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm). Acta Alimentaria (41) supplement, 119-123. ISSN 0139-3006. Impakt faktor: 0,475

Publications in peer reviewed journals

*Győrfi J., Balázs S., Geösel A., **Szabó A.** (2011). Fehér és barna kalapú csiperkegomba (*Agaricus bisporus* (Lange/Imbach)) D-vitamin tartalmának növelése UVB kezeléssel. Kertgazdaság 43. (1) 3-7.

***Szabó A.**, Geösel A., Kókai Z., Orbán Cs., Töreki K., Szőke A.: Antioxidant activity as indicator of UV radiation and other abiotic stress factors on *Agaricus bisporus* (Lange/Imbach) and *Sedum hybridum* (L.). Acta Universitatis Sapientiae, Alimentaria. *In press*

Other journals

*Győrfi, J., Kovács, A., **Szabó, A.** (2011). Increasing the vitamin D level of oyster mushrooms by UV light. International Journal of Horticultural Science (17) 4-5., 119-123. ISSN 1585-0404

Other scientific journals

*Győrfi J., Geösel A., Szabó A. (2011). D-vitamin a gombákban. Zöldségtermesztés 41 (2) 14-16.

Conference proceedings

*Győrfi J., Geösel A., **Szabó A.** (2010). Az *Agaricus bisporus* (Lange/Imbach) csiperkegomba Dvitamin tartalmának növelése UVB sugárzással. IX. Wellmann Oszkár Nemzetközi Tudományos Konferencia (Hódmezővásárhely, 2010. április 22.) Agrár- és Vidékfejlesztési Szemle 5 (1) supplement: 668-672 (full paper CD-mellékleten). ISSN: 1788-5345.

***Szabó A**., Győrfi J., Geösel A. (2011). Enhancing the vitamin-D concentration in white button mushrooms by UV light. Proceeding of the 45th Croatian & 5th International Symposium on Agriculture (15-19 February 2010, Opatija, Croatia), 599-603. ISBN: 978-953-6331-79-6.

***Szabó A.**, Győrfi J. (2011). Az UV sugárzás hatása a gombatermesztésben károsító patogén gombák micélium-növekedésére. Erdei Ferenc VI. Tudományos Konferencia (Kecskemét, 2011. augusztus 25-26.). Konferencia kiadvány III. kötet: 493-497. (ISBN 978-963-7294-98-3 a teljes kiadvány) ISBN 978-615-5192-01-2. a III. kötet

*A. Szabó, J. Győrfi (2011). The effect of UV radiation on the mycelia growth of white button mushroom and the pathogenic fungi of cultivated mushrooms. The 17th International Symposium on Analytical and Environmental Problems (19 September 2011, Szeged), Proceedings: 116-119. ISBN: 978-963-315-066-5.

*A. Szabó, J. Győrfi (2012). The effect of UV light on the Vitamin D content and mycelial growth of oyster mushroom. X. Wellmann Oszkár Nemzetközi Tudományos Konferencia (Hódmezővásárhely, 2012. május 10.) Review on Agriculture and Rural Development: 428-433 (full paper CD-mellékleten). ISSN 2063-4803.

Conference abstracts

*Győrfi J., Geösel A., **Szabó A.** (2009). A kétspórás csiperkegomba (*Agaricus bisporus* LANGE/SINGER) D-vitamin tartalmának növelése ultraviola sugárzással. Lippay-Ormos-Vas Tudományos Ülésszak (Budapest, 2009. október 28-30.), Abstracts: 322-323. ISBN: 978-963-503-397-3.

*Győrfi J., Geösel A., Szarvas J., **Szabó A.** (2010). Increasing the Vitamin D content of white and cream type *Agaricus bisporus* (Lange/Imbach) mushrooms by UVB irradiation. 2nd International Conference on Horticulture Post-Graduate Study (30-31 August 2010, Lednice, Czech Republic), Book of Abstracts: 16.

*J. Győrfi, A. Geösel, J. Szarvas, **A. Szabó** (2011). Enhancing vitamin D2 concentration in white and cream strains of *Agaricus bisporus* mushrooms by UVB irradiation. The 6th International Medicinal Mushroom Conference (25-29 September 2011, Zagreb, Croatia), Book of Abstracts: 60. ISBN: 978-953-56837-0-4

*J. Győrfi, **A. Szabó** (2011). The effect of UV radiation on the pathogenic fungi of cultivated mushrooms. 7th International Conference on Mushroom Biology and Mushroom Products (4-7 October 2011, Arcachon, France), Book of Abstracts: 126-127.