Cultivation of the wood-rotting mushrooms *Lentinula edodes* and *Pleurotus ostreatus* on lignocellulosic substrates containing wheat bran

University of Natural Resources and Life Sciences, Vienna, Austria

Department of Food Science and Technology

Institute of Food Science

Thesis submitted for the award of the title "Diplomingenieurin" (Dipl.Ing.^{in}) by:

TERÉZIA CISÁRIKOVÁ

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Supervised by:

Univ.Prof. Dipl.-Ing. Dr.nat.techn. Wolfgang KNEIFEL

Co-supervised by

Dipl.-Ing. Dr. Silvia APPRICH

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Abstract

The cultivation of mushrooms on agro-industrial by-product is substantially increasing in popularity. It represents an environmentally friendly and economically efficient approach. In this study, we have focused on the cultivation of shiitake mushroom (*Lentinula edodes*) and oyster mushrooms (*Pleurotus ostreatus*). Two different concentrations of wheat bran enrichment were selected for the experimenting. Basic substrate with beech wood chips and beech wood sawdust were enriched with 25% and 50% wheat bran, which is the major by-product of the wheat milling industry. The enrichment was performed with native bran as well as with destarched bran for both types of mushrooms. Bag cultivation has been used in this study. Spawn addition was 5% for each substrate. Samples from every substrate were taken before and after inoculation for determination of dry matter, ash, protein, fat, fiber, cellulose, hemicellulose and lignin content. Based on the obtained results improvement strategies were suggested. Due to water deficiency in our substrate the cultivation was not effective. Dry matter content decreased in every substrate after mycelium growth and the values were lower than expected. This indicates the significant role of water content in the substrate. In comparison with the values determined for each substrate before the inoculation, starch and glucose contents decreased after mycelium growth. An increase in values of ash, protein, fat and fiber were observed. The content of cellulose, hemicellulose and lignin were also determined in this project. However, the obtained results do not show any correlation, thus other methods for determination of these parameters are suggested. Additonally, further possible applications have been identified for substrates overgrown with mushroom mycelium. It has been recognized that mycelia growth enhances nutrient content and therefore it can find use in animal feeding. Another possible application is to use the transformation of the substrate in the food industry for improving cereal products attributes.

Key words: Cultivation*; Lentinula edodes; Pleurotus ostreatus*; Wheat bran; Enrichment; Chemical composition

Zusammenfassung

Die Kultivierung von Pilzen auf Abfall der Agrarindustrie gewinnt substantiell an Popularität. Die Kosten sind niedrig, die Effizienz ist hoch und die Methode ist umweltfreundlich. In dieser Studie wurden Shiitake Pilze (*Lentinula edodes*) und Austernpilze (*Pleurotus ostreatus*) auf Substraten mit zwei unterschiedlichen Weizenkleiekonzentrationen verwendet. Das Basissubstrat aus Buchenholzschnitzeln und Buchensägespänen wurde mit 25% und 50% Weizenkleie, welche ein Beiprodukt der Weizenmüllerei ist, angereichert. Diese Anreicherung erfolgte für beide Pilzarten mit nativer und entstärkter Kleie. Für diese Studie wurden die Substrate in Beuteln unter Zugabe von 5% Körnerbrut kultiviert. Die Probenahme für die Ermittlung von Trockenmasse, Asche, Protein, Fett, Ballaststoffe, Zellulose, Hemizellulose und Lignin erfolgte jeweils vor und nach der Inokulation. Die Resultate wurden verglichen, um daraus Verbesserungsvorschläge abzuleiten. Die Kultivierung war infolge einer zu niedrigen Feuchtigkeit im Substrat nicht effektiv. Die Trockenmasse nahm in jedem Substrat nach dem Wachstum von Myzel in höherem Ausmaß als erwartet ab, was auf eine Schlüsselrolle des Wassergehaltes im Substrat schließen lässt. Im Vergleich mit den Werten vor der Inokulierung nahmen die Werte für Stärke und Glukose in jedem Substrat nach dem Wachstum des Myzels ab. Es wurde eine Zunahme im Asche-, Protein-, Fett- und Ballaststoffgehalt beobachtetet. Darüber hinaus wurde auch der Gehalt an Zellulose, Hemizellulose und Lignin bestimmt, jedoch waren die Resultate nicht aussagekräftig, weshalb andere Methoden für deren Ermittlung vorgeschlagen werden. In diesem Projekt wurden weitere Anwendungen für myzelierte Substrate identifiziert. Es wurde beobachtet, dass Myzelwachstum den Nährstoffgehalt im Substrat erhöht, weshalb es Anwendung im Tierfutterbereich finden könnte. Eine weitere Anwendungsmöglichkeit wäre in der Lebensmittelindustrie, um die Attribute von Getreideprodukten zu verbessern.

Schlüsselwörter: Kultivierung; *Lentinula edodes*; *Pleurotus ostreatus*; Weizenkleie; Anreicherung; Chemische Zusammensetzung

Content

1 INTRODUCTION

1.1 Fungi

The history of fungi is as long as the history of mankind. The first written reference about fungi is found in Egyptian hieroglyphs. Egyptians believed that fungi are life prolonging sacral food. The first written report about using fungi for healing processes is a recipe from India dated around the year 3000 BC. Ancient civilizations in Greece, Middle America, China and Siberia connected the usage of fungi with ritual ceremony. Nowadays the fungi are part of the human diet and they are considered as health beneficial **(Lepšová, 2005).**

Fungi are a unique group of organisms. They probably played a significant role in the transition of plant from aquatic environment to dry land. Previously and at the moment roots of the overwhelming majority of higher plants create a myccorrhizal symbiosis with fungi. A mutual predecessor of fungi and animal was probably unicellular and motile **(Bačkor, 2007).**

The kingdom Fungi includes higher fungi and also lower fungi (filamentous fungi, yeasts). Their reproduction was described in 1970 by P. A. Micheli. Based on the place of spore origin, there are two recognized categories: *Acomycetes* und *Basidiomycetes* **(Smotlacha, Erhartovci, 2004).** Fungi are heterotrophic organisms, their body constitutes from fungal filaments – hyphae. Hyphae can be unicellular – they are not separated by septa (lower fungi) or multicellular – septate (higher fungi). The cell wall consists of the biopolymer chitin, in the cells of mushrooms there are no plastids. The storage of energy is glycogen **(Križan, 2002; Ševlikova et al., 2007).** The vegetative thallus of most fungi is mycelium. The mycelium may endure in the substrate until the fruiting bodies are composed under suitable conditions. Fruiting bodies are reproductive structures on which the spore-producing structures are formed. For an ascomycete it is anascocarp, for a basidiomycete it is basidiocarp. Basidiocarps are arranged in the hymen. Hymen can cover the whole surface of fruiting body or just its distinctive parts – a hymenophore. There are four different types of hymenophore: smooth, tubular, gill and teeth **(Evans and Kibby, 2007)**.

If the spores are in a suitable environment regarding biological and ecological conditions, germination occurs and the mycelium is formed. From the spores, distinct primary mycelium is formed physiologically. After a fusion of two physiologically distinct primary mycelia of the same kind of fungi, the fruiting body is created. The fruiting body then ensures the

reproduction. The spores are transmitted by wind, insect or animals **(Bielli, 2001)**. Reproduction through spores is asexual **(Križan, 2002)**.

The nutrition of fungi can be considered as either parasitic or saprotrophic nutrition. Saprotrophic fungi decay dead bodies of animals, plants and organic matter and it makes them an irreplaceable organism in the natural ecosystems. Parasitic fungi gain the organic nutrients from living organisms and they are usually specified on a certain kind of host. They can be obligate parasites or they overcome the parasitic phase in their evolutionary cycle and become saprotrophs. There are also know facultative parasites – they live as saphrotrophs but under certain conditions the parasitism can appear. There is also recognized symbiosis of fungi with cyanobacteria which can lead to a formation of morphologically and ecologically independent organisms. This kind of relation is called lichenism. Another kind of symbiosis is a mycorrhiza. The fungi filaments colonize the surface of xyloid plants and thereby their absorption of water, minerals and biologically active compounds is improved. It is also called a mutualistic relationship because the plant provides to the fungi the components needed for the spore formation **(Križan, 2002; Ševlikova et al, 2006).**

Based on knowledge, fungi can be delineated by the following characteristics:

1. Nutrition: Lack of photosynthesis, absorption is more likely than ingestion.
2. Cell wall: The present cell wall is typically formed from chitin and glucans.
3. Reproduction: Both sexual and asexual reproductive events are recognized.
4. Life cycle: usually more complex than simple.
5. Sporocarps: Characteristic shapes.
6. Habitat: In terrestrial and freshwater environment ubiquitous, less typical for marine habitat.
7. Ecology: Important part of ecological systems due to parasitism, symbiosis and saprotrophism.
8. Distribution: Worldwide **(Webster und Weber, 2007).**

1.2 Mushrooms - definition

Four phylums belonging to the kingdom *Fungi* are considered as the real fungi – *Chytridiomycota, Zygomycota, Ascomycota* and *Basiomycota* (**Bačkor, 2007)**. Recent estimation suggests that there exist around 5.1 million fungal species **(Blackwell, 2011).**

For a long time there was no unique definition for mushrooms. In the literature, there are over six different theories for the definition of mushrooms. The first appeared definition

characterized mushrooms as a part of the group basidiomycetes without any distinction between mushroom and toadstool. Another found definition classified mushrooms to basidiomycetes or rarely to ascomycetes. Another definition distinguishes between edible and poisonous unpalatable mushrooms while using the term edible as a counterpart to the term toadstool. Mushrooms were specified as epigeous and hypogeous fruiting bodies of macroscopic fungi **(Okhuoya, 2011).**

In the year 1992, Chang and Miles came up with a definition that is now generally accepted. "A mushroom is defined as a macrofungus with a distinctive fruiting body which can be hypogeous or epigeous, large enough to be seen with the eye and to be picked by hand"**(Moon, Lo, 2013).**

1.3 Mushrooms – nomenclature

For the identification of mushrooms a system of scientific names was developed. The same fungus was named in various countries differently although some of the names have been accepted more widely. The aim of the system is to remove doubts and to make the description clear. The genus is the first name followed by the name of the species. Local names are usually given according to the importance and distinctive properties for the people, as shape or taste. A scientific name provides proof that the knowledge about a certain specimen is reliable. It is also a good way of finding out whether a species is poisonous or edible, or if it provides information about other important attributes. The scientific names are not permanent and are readily changeable. As a result we can meet with scientific names of fungi that are "preferred" rather than "correct". The changes are usually not dramatic. Shiitake is one example. Instead of *Lentinus edodes* the preferred name is *Lentinula edodes* now. The preferred name for *Auricularia auricula – judae* may appear as *Hirneola auricula – judae* or *Auricularia auricula.* There is a standard reference – *The Dictionary of the fungi* that reconsiders the names to avoid any doubt **(Boa, 2004).**

1.4 Mushrooms – Toxicity

Around 2000 species of mushrooms are considered as edible. There is also a group of poisonous species which bring risks to human health if ingested. Some mushroom species are known for their toxicity and every year many cases of mushroom poisoning occur. Mushroom intoxications in France are expected around 8000 - 10 000 people per year. It is caused due to incorrect identification based on empirical and traditional knowledge. There is no rule for easy distinction between edible and poisonous mushroom. The golden rule endures: if you doubt, throw it out. There are mushrooms with dangerous toxins and they represent a real

hazard for human health even after a consumption of small doses. The toxins that are found in mushrooms are categorized according to the part of the body they affect and the time of symptom onset. The toxins affect liver, intestinal mucosa, kidneys and also the central nervous system. Abdominal pain, dehydration, vomiting, jaundice, diarrhoea, tachycardia are the typical symptoms of an intoxication. If a species such as *Amanita* or *Psilocybe* is ingested, then the consequences may be very severe or even fatal. The symptoms may appear after 6 to 48 hours. Most of the toxins found in mushrooms cause gastrointestinal irritations. There are also identified toxins that cause problems only when they are mixed with alcohol. Proper identification is not the only important part. Mushrooms, as any other vegetable, are considered as perishable food. Precise harvest, transport, storage, preparation and selection of fitting food and drinks are other important factors **(Hall et al, 2003; Lima et al, 2012; McKnights, 1987)**.

Commonly consumed mushrooms show also certain toxicity. They contain substances causing harm to health depending on their dose. Therefore it is necessary to observe the magnitude of any adverse health effects to avoid accidents. The effects of the protein ostreolysin isolated from the genus *Pleurotus* was observed in rats. The consequences of this protein caused higher blood pressure, tachycardia, and cardiac ischemia. A potential carcinogen was classified also in the most common consumed mushroom *Agaricus bisporus*. The carcinogenicity is caused by substantial amounts of aromatic hydrazines that are classified as direct-acting chemical carcinogens. *Tricholoma equestre* ingestion was fatal in 3 human cases in France; although this wild mushroom is considered as a delicacy in Europe. The toxic substance was not pinpointed. The toxicity was established for fresh mushrooms in the range from 100 to 400 grams at 3 to 9 subsequent meals. Due to the potential adverse health effects of commonly consumed mushrooms, toxicological and medical assays have to be conducted to minimize the risks **(Lima et al, 2012).**

1.5 The nutritional attributes of edible mushrooms

Mushrooms are considered as a delicacy all around the world. Palatability may be judged according to taste, colour, texture and flavour. Nowadays attention is paid to their pharmacological, nutritional and sensory attributes. The nutritional attributes may vary due to the genetic makeup, environment and the nature of substratum **(Valverde et al, 2014; Chang and Miles, 2004).** Mushrooms are typically low in energy and fat content and contain remarkable amounts of vitamins, minerals, dietary fibre and protein **(Cheung, 2010).** Genereal nutritional value of edible mushrooms is summarized in the Table 1.

Table 1 General nutritional values of edible mushrooms

Constituent	Amount [% DW]	Source
Protein	15-35	Change and Milles, 2004
Lipids	2-6	Kalač, 2009
Carbohydrates	50-60	Kalač, 2009
Soluble fiber	4-9	Kalač, 2009
Insoluble fiber	22-30	Kalač, 2009
Mineral constituents	5-13	Bernas et al., 2006

1.5.1 Protein

Generally the amount of protein in edible mushrooms varies; the range is from 15% to 35% of dry weight. In comparison, rice has a protein content of around 7.3%, wheat 13.2%, soybean 39.1% and milk 25.2% of dry weight **(Chang and Miles, 2004)**. The protein content of their fresh weight has an average value of 3.5% to 4%. The quality of mushroom protein is considered higher than the protein quality of most plants.

The crude protein content depends on the species, variety and the stage of development of the fruiting body. There was no significant difference in the amount of crude protein between cultivated and wild mushrooms. The amount of essential amino acids varies from 30g to 50g per 100g protein DW, with a few exceptions. Commonly cultivated mushrooms contain all essential amino acids. Lysine is the most abundant essential amino acid, the lowest levels of essential amino acids are found for tryptophan and methionine. There were reported also less common amino acids and related nitrogenous compounds such as cystic acid, sulfoxides, hydroxyprolines, aminoadipic acid, phosphoserine, canavanine, citrulline, ornithine, glucosamine and ethanolamine. Cereal protein is characterized by a low level of isoleucine, therefore the consumption of cereals together with mushrooms is highly recommended for balancing the level of essential amino acids in the diet. Free amino acids together with 5´-nucleotides are similar to monosodium glutamate and this fact contributes to the unique taste of mushrooms **(Chang and Milles, 2004; Cheung, 2010; Bernas et al, 2006).**

1.5.2 Lipids

The fat content in mushrooms is generally low. The range of crude fat (total lipids) varies from 2% to 6% of dry matter. All classes of lipid compounds were found in mushrooms. Three fatty acids are significantly dominant: polyunsaturated linoleic acid, monounsaturated

oleic acid and saturated palmitic acid. The presence of trans- isomer of oleic acid, elaidic acid, was confirmed at levels of < 0.1% to 0.3% of total fatty acids. The content of linolenic acid is low, but it contributes to the smell of mushrooms. Linolenic acid is the precursor to 1-octen-3-ol, which is the major aromatic compound. **(Kalač, 2009; Cheung, 2010; Chang and Milles, 2004; Valverde et al, 2014).**

1.5.3 Carbohydrates

The main components of dry matter in mushrooms are carbohydrates. The dry matter comprises usually about 50-60% of carbohydrates. The sugar content differs depending on the species. Cooking, drying and freezing are important factors that influence the sugar content. All of the processes decrease the amount of sugars. Some of reported sugars are glucose, arabinose, maltose and melezitose.

The content of polysaccharide glycogen in dry matter is 5-10%. Chitin is the main component of the cell wall. This nitrogen containing polysaccharide represents 80-90 % of dry matter in mushroom cell wall. The difference between chitin content in wild and cultivated mushrooms is unknown. Cooking decreases the chitin content. For humans, chitin is indigestible and it influences the digestibility of other mushrooms components **(Kalač, 2009)**.

Polysaccharides such as β-glucans are present in fungal cell wall mass. They are very important because they influence anticarcinogenic, antioxidant, neuroprotective and immunomodulating activities of many edible mushrooms. β-Glucans are capable of treating several diseases. They induce innate and adaptive immune responses. They are not synthesized by humans and our immune systems do not recognize them as self-molecules. A mushrooms β-glucans provide many benefits to humans. They have a stimulating effect on the immune system, they protect from pathogenic microbes, environmental toxins and carcinogens. A variation of their amount in mushrooms is dependent on the species and it varies from 0.21 to 0.53 g/100 g dry basis **(Valverde et al, 2014)**. Nowadays, great attention is paid to the water-soluble polysaccharides that are present in mushroom fruiting bodies, because they are able to inhibit the growth of tumours. Strong antitumor activity is ascribed to a major fraction of the acidic polysaccharide constructed as H51. The structure of this component contains a skeleton of β(1,3)-linked glucose residues, that probably has branches of mannose and galactose residues and possibly also acidic sugars **(Chang and Milles, 2004)**.

Dietary fiber content differs and related information is limited. The range is around 4-9% and 22-30% for soluble and insoluble fiber, respectively. The literature mentions the presence of

hemicelluloses and pectic substances. There is no information available whether there occurs a change in polysaccharide contents during preservations. Further research is required **(Kalač, 2009).**

1.5.4 Mineral constituents

The amount of mineral constituents is dependent on the species, the substratum, on the age of mushrooms and the diameter of the pilei. The variability of ash content for edible fungi is from 5 to 13% dry matter **(Bernas et al, 2006).** Mushrooms are rich in potassium, phosphorus, sodium, calcium, magnesium, which are considered as the major mineral compounds in mushrooms and copper, zinc, iron, manganese, molybdenum and cadmium are recognized as minor mineral constituents **(Chang and Milles, 2004).**

1.5.5 Vitamins

Mushrooms are an important source of vitamins. They are rich in the vitamin group of B, thiamine, riboflavine, piridoxine, pantotene acid, nicotinic acid, nicotinamid, folic acid, cobalamin. There are also other vitamins present in mushrooms such as biotin, phytochinon and tocopherols **(Bernaset et al., 2006).**

The content of ascorbic acid is reported as 20-30 mg 100 g^{-1}fresh matter, but the values can vary. It is assumed that the content of ascorbic acid (vitamin C), thiamin (vitamin B1) and riboflavin (vitamin B2) decreases during soaking or branching.This effect was observed in *Boletus edulis* and in *Pleurotus ostreatus*. Total tocopherol content is reported 0.05 – 0.25 mg 100 g^{-1} fresh matter. The dominant isomer was β-tocopherol **(Kalač, 2009)**.

1.5.6 Phenolic compounds

Mushrooms were evaluated regarding the phenolic compounds, which show a great antioxidant activity. There is a conclusion that the content of phenolics in dry mushrooms is 1-6 mg g^{-1} and the flavonoid concentration varies between 0.9 and 3.0 mg g^{-1} of dry matter. Myricetin and catechin were the main flavonoids found in mushrooms **(Valverde et al, 2014).**

1.5.7 Flavour and taste components

The component contributing to the mushrooms taste are soluble sugars, polyols, organic acids, free amino acids, and 5´-nucleotides.The umami taste is the main flavour of mushrooms. This flavour, sometimes called the “palatable taste´” or the “perception of satisfaction” is caused by monosodium glutamate (MSG). Free amino acids are found in mushrooms in high

amounts, they contribute to the food taste with a smooth feeling. Due to this property, the free amino acids are able to soften a sharp taste from some substances. It was discovered, that taste-active amino acids in mushrooms are alanine, glycine and threonine and MSG-like components (aspartic and glutamic acid). There are 6 5´-nucleotides recognized in mushrooms: 5´-adenosine monophosphate, 5´-cytosine monophosphate, 5´-uridine monophosphate, 5´-guanosine monophosphate, 5´-xanthosine monophosphate and 5´-inosine monophosphate **(Mau, 2005).**

1.6 Mushroom production

The number of edible and medicinal fungi is around 2000 species and over 200 genera of mushrooms are used around the world. Most of the widely cultivated mushrooms have also trivial names which are stated in the Table 2 below.

Table 2 The most cultivated mushrooms

Species	Trivial name	Source
Agaricus bisporus	White Button mushroom	Çağlarirmak, 2011; Chakravarty, 2011
Lentinula edodes	Shiitake mushroom	
Pleurotus ostreatus	Oyster mushroom	
Flammulina velutipes	Winter mushroom	
Volvariella volvacaea	Straw mushroom	
Auricularia auricular	Ear mushroom	
Hericium spp	Lion´s head mushroom	
Ganoderma spp	Reishi mushroom	
Grifola frondosa	Maitake mushroom	
Pholiota spp	Nameko mushroom	
Coprinus spp	Shaggy Mane mushroom	

Worldwide mushroom production is estimated at around 3.5 million tons per year. The leaders in mushroom production are China, USA, The Netherlands, Poland, Spain, France, Italy, Ireland, Canada and UK. About half of the production is cultivated in China. The USA, China and The Netherlands produce together more than 60% of the world production. White button mushroom is the most common cultivated species in the USA and in Europe. In Asia the situation is different and other species are cultivated for commercial use. 95% of mushroom production in China is consumed locally. This relates to around 3 times more than that in Europe and the USA. The biggest mushroom exporters are Poland, The Netherlands, Ireland, China, Belgium, Canada and the USA. The countries such as Russia, Japan, Germany and France import the mushrooms from the countries above **(Wakchaure, 2011)**. The European Union's mushroom production was 1324 thousand tonnes per year in the period 2011-2013.

Just four countries, the Netherlands, Italy, Poland and Spain, contributed to a total production with 69%. 5.3% of its production was exported outside the EU. The export to Russia represented 3.7% of production. Export to Russia is shared by Poland (75%) and Lithuania (25%). There has been a slight decrease of EU exports to Russia, since 2011. Despite this, exports from Poland have increased **(European Commission, 2014)**.

1.7 Genus *Pleurotus*

Efficient growth, propitious organoleptic and medical properties and undemanding cultivation conditions are characteristic for the genus *Pleurotus* **(Gregori et al., 2007).** This genus is lignicolous fungi with specific characteristic features as flabelliform carpophores with flabella form carpophores, short lateral stipe, decurrent lamellae, and presence of versiform shaped cheilocystidia and monomitic hyphal tissue. This genus represents 20 species. Distinguishing attributes are external morphology and internal details **(Kumari et al., 2012).**

After nucleic acids breakdown these fungi were assigned to the family *Pleurotaceae* belonging to order *Agaricales*. The most common fungus from this family is *Pleurotus ostreatus*. Other common *Pleurotus* fungi are *Pleurotus pulmonarius, Pleurotus eryngii, Pleurotus cornucopiae* and *Pleurotus dryinus* **(Lepšová, 2005).**

The *Pleurotus* mushrooms are distributed all over the world. *Pleurotus pulmonarius* and *Pleurotus cystidiosus* are distributed in the subtropical and tropical regions. *Pleurotus eryngii* is found in Asia, Europe and Africa. In Korea and Japan there is no evidence of wild *Pleurotus,* however, they cultivate the mushroom commercially. *Pleurotus ostreatus* is the most commercially produced and important mushroom of the genus *Pleurotus*. Temperate areas provide good growing conditions for *Pleurotus ostreatus*, which is adaptable to a spectrum of climates and substrate materials. Following the button mushroom, *Pleurotus* genus is the second most common mushroom produced worldwide **(Kong, 2004).** This may be caused by a quick mycelium growth and fruiting, the cultivation is low of costs, they are affected by diseases just marginally, their adaptation and maintenance is easy and therefore they require minimal monitoring of the cultivation environment **(Fernandes et al., 2015).**

Wood is their natural substrate. From wood they utilize nutrients and energy required for their growth and formation of fruiting body. Additional nitrogen is gained from snared nematodes. They are non-segmented tiny microorganisms protected by a transparent cuticle. *Pleurotus* mycelium secretes toxic drops that immobilize the nematodes. The mycelium fibre penetrates the nematodes, overgrows them and digests them **(Lepšová, 2005 and Barron, 2003).**

1.7.1 *Pleurotus ostreatus* – morphology

Regarding **OECD (2005)** the morphological similarities and possible environmental effects make species identification within the genus *Pleurotus* difficult.

1.7.2 Macroscopic features of *Pleurotus ostreatus*

- Pileus is of oyster-shape, 40 – 250 mm wide. In early stages it is spatulate to lingulate, later convex and becoming concate to flabellate. Surface is smooth. Pileus is very diverse in colour: young pileus is coloured grey lilac, violet-brown to lilac blackish and these colours can alter to cream-beige. It is margin smooth when young, later we can observe somewhat undulating and striate.
- Context is white to grey-white, fleshy and radially fibrous. The odour is fungoid and it tastes mild.
- Lamella is long and decurrent. The edge is smooth when young, later undulating.
- Stipe is approximately 10-20x10-25 mm, usually lateral, rudimentary. Surface is longitudinally striate with solid context **(OECD, 2005).**

1.7.3 Microscopic features of *Pleurotus ostreatus*

- Spores vary from 6.5 - 9 x 2.8 - 3.5µm, they are cylindrical-ellipsoidal, hyaline, smooth and with vacuoles.
- Basidia are 23.6 - 27 x 5 - 7.5 µm, slenderly becoming wide from the base to the distal end
- Hymenophoraltrama is regular to irregular, monomitic.
- Cystidia are absent or rarely seen, cystioid.
- Pileipellis is composed of densely interwoven, irregular, flexuous and branched hyphae with a brown pigment.
- Habit & Habitat: *Pleurotus ostreatus* are usually gregarious, they cluster on the dead hardwood in park, and they are rarely on conifers. They originally came from Korea and they are now distributed in Europe, Asia, North Africa and America. Their season begins in spring and ends in autumn **(OECD, 2005).**

1.7.4 *Pleurotus ostreatus* – nutritional properties

The species *Pleurotus ostreatus* has specific flavour and aromatic properties. It is acknowledged to be rich in fiber, protein and carbohydrates. In the table 3 below, there is the

summary of nutritional composition of *Pleurotus ostreatus*. It has high amounts of minerals and vitamins while the fat content is low **(Hernandez et al., 2003; Kalmis et al., 2008).** Cultivated species of *Pleurotus ostreatus* are considered as dietary food. A medium caloric value is 151 J in 100 g of edible part **(Manzi et al. 2001).**

Table 3 Nutritional composition of *Pleurotus ostreatus*

Constituent	Amount [g/100g DW]	Source
Protein	17-42	Khan, 2010
Lipids	0.5-5	
Carbohydrates	37-48	
Fiber	24-31	
Minerals	4-10	
Moisture	85-87 %	

The content of nutritional components in wild types mushroom is similar to commercially cultivated mushrooms. However, the chemical composition of *Pleurotus ostreatus* may differ qualitatively and quantitatively depending on the origin, strain, extraction process and cultivation conditions **(Wang et al., 2001).**

Protein content in *P. ostreatus* varies. It differs depending on strains, differences in growing medium, substrate composition, harvest time and size of the pileus. Some ssential fatty acids are present in mushrooms; however, not in significant amounts. It has been observed that the following lipids are present: oleic acid, linoleic acid, arachidonic acid **(Deepalakshmi and Mirunalini, 2014)**.

Carbohydrates are the predominant components of the mushroom dry matter. Polysaccharides presented in *P. ostreatus* are represented by glycogen, dietary fiber, chitin, cellulose and hemicelluloses such as mannans, galactans and xylans.

P. ostreatus is richer in vitamin B1, vitamin B3 and folic acid in comparison with other mushroom species but the content of B12 vitamin is lower.

Mineral content is strongly influenced by substratum and the pilei diameter. High contents of copper, potassium, iron, phosphorus and magnesium are stated for the pilei of *P. ostreatus*. However, the bioavailability of them is doubtful and further tests in animals and humans should be performed. The ash content in 100g is stated as 0.8g **(Deepalakshmi and Mirunalini, 2014)**.

Various enzymes can be found in the composition of mushrooms. These enzymes present in mushrooms are rarely or never found in other organisms. Enzymatic activity varies with species and *P. ostreatus* is characterized with higher activity of polyphenoloxidase. This enzyme causes a rapid darkening of harvested mushrooms and reduction of their sensory and nutritive properties. It decreases their quality and their market value.

Pleurotus ostreatus mushrooms show also numerous medicinal effects due to components of some polysaccharides, lectins, polysaccharide-peptides and protein complex. Some of the described health effects are; anticarcinogenic, antioxidative, antitumor, antiviral, antibacterial, antidiabetic, antihypercholesterolic, and anti-arthritic as well as eye health supporting effect **(Deepalakshmi and Mirunalini, 2014).**

1.7.5 *Pleuratus ostreatus* – cultivation methods

The first cultivation of *Pleuratus ostratus* in laboratory conditions was attempted by German scientist Falck, who published his results in year 1917. After the First World War the cultivation declined and the expansion started first in the sixties in Japan. From Asia the mushrooms were expanded to Europe, especially to Hungary. The cultivation on artificial substrates is dated back to 1958 when an American researcher block used sawdust of diverse woody plants for cultivation. In the beginning of the seventies wheat straw was used for the cultivation of *Pleurotus* mushrooms **(Lepšová, 2005).**

1.7.5.1 Log cultivation

There are two basic methods for oyster mushroom cultivation on logs depending on their length. First method is the long log method. The spawn is inoculated into the inoculation holes that are created after cutting trees into one-meter sections and drilling the wood. This method is similar to the traditional cultivation method of shiitake mushrooms. The short log method is more labour intensive and the production is relatively higher. Tree trunks are cut into 20 cm pieces in length and those sections are inoculated.

Wild oyster mushrooms can often be seen on the dead broad-leaf trees such as poplars. These tree species are suitable for their cultivation. Softwood trees contain phenolic resin components which makes these trees unsuitable as a substrate for oyster mushroom cultivation. Conifer wood can be used for cultivation of oyster mushroom but a previous destruction of some phenolic compounds is needed. Commonly used tree species are poplar, elm, alder, willow and beech. Unlike shiitake, the growth of oyster mushrooms on oak tree logs is not efficient **(Kwon and Kang, 2004).**

1.7.5.2 Bag cultivation

This mode is the most common method used for cultivation of mushrooms all around the world. It brings following advantages:

- Initial investment is possibly small
- Crop failure is of much smaller risk
- Can be performed inside houses
- Pest and diseases control is easy
- Quick return of capital
- All year round production

The most commonly used substrates for bag mode are sawdust, cereal straw, cottonseed hull, corncob and other plant fibres with high content of cellulose. Using of agro-wastes for mushroom cultivation is increasing owing to their low cost. Oyster mushroom can grow on various types of agricultural wastes due to their multilateral enzyme system. If necessary, the substrates are supplemented with wheat bran, sorghum, millet or rice, as additional sources of nitrogen. Other supplements are gypsum, chalk or limestone functioning as a buffer and thus controlling the pH value. Depending on the region, various trees are used as source for sawdust. The commonly used tree species include poplar, oak, alder and elm. The moisture content of the substrates is usually adjusted to 65%. Typical composition of a substrate differs among the countries. In America and Bangladesh they use sawdust sources supplemented with nitrogen sources in ration 4:1 **(Kwon and Kang, 2004).**

Primary components for oyster mushroom production are wheat straw and cottonseed hulls. Wheat straw is usually milled to about 2-6 cm. Usage of cottonseed hulls for the production does not require chopping of the hulls. Mixture of wheat straw and cottonseed hulls is commonly practiced because together they have a higher water holding capacity than cottonseed hulls would have alone. The substrates are then pasteurised at 65 °C for 1 hour with aerated steam. After pasteurization the substrates are cooled.

Growers have tried to optimize the amount of spawn for inoculation. The amount of spawn is usually up to 5%. Higher amounts of spawns increase yields. It is caused by higher levels of nutrients providing energy, which leads to a faster substrate colonization. A higher spawn rate decreases the production time by more than 7 days. Shorter crop cycles also minimize the risk of pest infestations. Spawned mixtures are filled into polyethylene bags. The incubation

temperature is 23-25 °C for 12-14 days. After 3 or 4 weeks of spawning, mushrooms can be harvested **(Pennstate, 2003).**

1.7.5.3 Shelf cultivation of oyster mushroom

This unique method comes from Korea. This method was used for button mushrooms at first and later was adapted for other mushrooms. Within this method the substrate is fermented in three steps – pre-fermentation, pasteurization, post-fermentation. Outdoor pre-fermentation is required for 2-3 days; pasteurization for 8-10 hours and post-fermentation is required for 3-4 days at 45-55 °C **(Choi, 2004).**

1.8 Genus *Lentinus*

The common name for *Lentinula edodes* mushroom is formed from 2 words: "shii" is a tree *Castanopsis cuspidate* and "take" is Japanese word for mushroom **(Wasser, 2014).** Shiitake mushroom is a white rot fungus and a saprophyte. Its source of nutrition is dead oak tree species. Each basidium under the gill of a fruiting body produces four basidiospores. Under certain condition they sporulate to become primary mycelium, then secondary mycelium. Shiitake spawn is a medium and it is colonized by the secondary mycelia. Fruiting bodies are formed from the secondary mycelia. Shiitake strains can be classified by fruiting temperature: low-temperature (cold-weather) strains, high-temperature (warm-weather) strains, wide – temperature (all weather, wide-range) strains **(Kang, 2005).**

1.8.1 Nutritional components

Table 4 Nutritional composition of *Lentinula edodes*

Constituent	Amount [g/100g DW]	Source
Protein	2-19	Kwon and Hobbs, 2005
Lipids	0.3-3.1	
Carbohydrates	57-74.4	
Fiber	0.7-7	

Lentinula edodes mushroom has a great value because of its nutritional components. In the Table 4 above, there is summary of nutritional composition of *Lentinula edodes*. Shiitake mushroom is a good provider of D vitamin, because of its high amounts of ergosterol. Sunlight exposure for 3 hours per day increase vitamin D up to 5 times. It also increases the content of free amino acids and it influences the taste in a way that it becomes sweeter and less bitter. Other vitamins present in shiitake are thiamine (B1), riboflavin (B2), niacin (B3), cobalamin (B12) and pantothenic acid. Mineral constituents presented are Cu. Cd, Fe, Mn, K,

Ca, P and Zn. Alcohols and ketones, alkanes, sulfides and fatty acids are major aroma components. 1,2,3,5,6-Pentathiepane is identified as constituent responsible for the characteristic shiitake aroma **(Finimundy et al., 2014; Wasser, 2014; Kwon and Hobbs, 2005)**.

According to **Kwon and Hobbs (2005)** shiitake mushrooms generate beneficial health effects. There are documented antitumor, antiviral, hepatoprotective, antibacterial effects. The main focus is oriented on:

- Lentinan – polysaccharide with high molecular weight, highly purified, extracted from the fruiting bodies or mycelium. It is water soluble, acid stable, heat stable and alkali labile
- LEM – extract of *Lentinula edodes* mycelium harvested before growing of the cap and stem. It contains nucleic acid derivates, B1, B2, ergosterol.
- KS-2 – α-linked mannose containing polysaccharide
- JLS – compound extracted from the mycelium
- Eritadenine – derivated of a nucleic acid

1.8.2 General review of production

In 1976 the world production of *Lentinula edodes* was about 130 000 MT (fresh weight), 3 years later in 1979 it was about 170 000 MT and in 1997 it reached 1 321 600 MT. In 1983 the value of *Lentinula* produced in Japan was $689 million for 158 885 MT fresh weight. If we consider 167 000 growers plus those working in spawn production, marketing and distribution, it is obvious that this production in Japan is significant. In 1983 about 53% of total production was processed to give 12,025 MT of dried shiitake. In the same year, Japan exported 23.2% of total dried mushrooms production. In 1997, the production represented only 55.1% of the production from 1984 and some years later the production had started to decrease. The aging of the Shiitake growers, lack of new growers, and deficiency in low-cost high-quality wood logs for shiitake cultivation as well as rising imports of fresh shiitake mushrooms from China are the reasons for decreasing *Lentinula* production in Japan. In China the production increased from 15.586 MT in 1993 to 26.028 MT in 1997.

Cultivation of Shiitake mushrooms is centuries old, it became an industry after World War II. In the 21st century it can be seen that the product that the product is not limited just to the

traditional fresh and dried mushroom, but it is used canned, pickled, as ingredients in food neutriceuticals and tonics.

Agaricus bisporus is still the most common cultivated mushroom, but there is potential for *Lentinula* to become number 1 in the near future. Over the past three decades, there were millions of dollars spent on developing high advanced technology for *Agaricus bisporus*. On the other hand, cultivation of shiitake does not require sophisticated equipment **(Chang and Milles, 2004)**.

1.8.3 *Lentinula edodes* cultivation

Lentinula mushroom cultivation was performed for many years as log cultivation, but recently it has changed. In the early twentieth century farmers have started to use bags for shiitake cultivation. It brings advantages of shorter production cycle and quicker return of capital. Nevertheless, the traditional log cultivation does not require so much care and labour, because it accepts natural conditions. The logs are protection for shiitake mycelia and due to their low water content attract fewer microorganisms **(Przybylowicz and Donoghue, 1990**). Shiitake produced by log cultivation method have high quality and a fragrant odour. Even though this cultivation requires longer time for the inoculated logs to be colonized and to produce fruiting bodies the method is environmental friendly. There are small differences between the mushroom cultivated on logs and mushroom from sawdust cultivation. Log cultivation breeds mushrooms with higher content of polysaccharides **(Brauer et al., 2002).**

1.8.3.1 Natural log cultivation

According to **Przybylowicz and Donoghue (1990),** one of the most important conditions for the formation of fruiting body preparation is well-colonized logs. This process consists of host tree selection, felling, cutting and spawn run. Polysaccharides present in logs (cellulose, hemicellulose) are decomposed by shiitake mycelium and it provides them with energy. Sugars have significant role for initial mycelial growth. Compared with other agricultural waste, logs have lower amounts of nutrients and it makes them unattractive to other microorganisms.

Trees appropriate for shiitake logs are 5-15 cm in diameter. Trunks of the trees are cut to about 100 cm logs and dried slightly. Before the inoculation there are drilled 30-60 holes into the log, about 2 cm in depth. Shiitake mycelium carrier is then inserted into the holes. Inoculated logs are located to place with suitable humidity, indirect sun and good drainage. Optimal temperature is 22-26 °C, without direct sun. During spawn run the optimal water

content of logs is 35 %. After completing mycelial growth stage there is required a trigger to activate the reproductive fruiting stage. It is reached by sudden change of environmental conditions – higher humidity (65%), lower temperature (5-20 °C) and light. Another important attribute for fruiting body development are physical shocks performed by log beating. More fruiting bodies can be reached by synchronizing beating with watering. Although there is correlation between numbers of days passed from first beating until second one, the mechanism of the effect of physical beating remains unclear **(Tokimoto, 2005)**.

1.8.3.2 Bag cultivation

The most popular ingredients for bag cultivation of *Lentinula* mushroom are sawdust, corncobs or combination of both. The main substrate components are starch-based supplements such as rice bran, millet rye and wheat bran. In combination with other supplements added in less quantity such as calcium carbonate, gypsum or table sugar, they provide nutrients and create an optimal growth medium. The moisture content is increased by mixing with water to about 60 %. The substrate is then filled into the polypropylene bags with special filter enabling breathing. The bags are sterilized for 2 hours at 121 °C, cooled down and inoculated with *Lentinula edodes* spawn, which is propagated on sawdust or cereal grain. The spawn is then mixed through and the bag is sealed. The spawning stage runs for 17-22 days at 21 °C and 4 hours of lights. After finishing this stage the bags are opened. Contains of bags are held together by tiny tendrils. Following stage of browning takes about 4 weeks at 19 °C and requires light watering once or twice a day **(Pennstate, 2009).** There are two possibilities how to stimulate browning phase; browning outside of the bag or browning inside of the back. The crucial factor is timing of bag removal. Browning by oxidation is enhanced by air. After exposure to the air, mycelia colour brown at the surface and they form a protective layer. Fungal metabolism sustains the inner moisture high what secures ideal condition for formation of fruiting bodies **(Chen, 2005).** Soaking the blocks in cool water stimulates the primordial maturation. Mushrooms are ready for harvesting approximately 7-11 days after soaking. After the first harvesting period the blocks are soaked in the water for 12 hours. The third water soaking takes 18 hours in addition to replace lost water. One harvesting period takes about 16 to 20 days **(Pennstate, 2009).**

1.9 Wheat bran as a substrate

Processing the agricultural products generates high amounts of agricultural by-products. They are usually organic matter that could be used in production of biodegradable composites. This can lower the material costs and improve the strength of composites (**Zang et al, 2011**).

Substrates prepared from agricultural by-products have good water absorption and workability (**Panthapulakkal et al, 2006**). Wheat is important cereal grain all around the world and the wheat based industry is a multi-billion dollar market. The result of the wheat milling process is large amount of by-product. During milling, bran and germ are removed while the endosperm is broken down into fine particles. Generally wheat grain is comprised of 14.5% bran, 2.5% germ and about 83% endosperm. Bran is used in bakeries and as fiber supplement in breakfast cereals, this only accounts for 10% of bran, the remaining 90% is used as a low cost animal feed ingredient. Millers often disposed of bran as waste because the transportation costs are higher than the market value of the bran (**Xie et al., 2008**).

Wheat bran consists of protein (12%), carbohydrates (60%), fat (3.5 - 3.9%), minerals (2 - 8.1%), vitamins and bioactive compounds. It also contains compounds such as carotenoids, lignans, phenolic acids, phytosterols, flavonoids, phytic acid and α-tocopherol. Substrates enriched with wheat bran require no addition of nitrogen supplements due to the higher content of nitrogen in the bran (**Javed et al., 2011; Apprich et al., 2014)**.

The agro-industrial by-products are cheap and it makes the mushroom production very attractive and effective. Shiitake has been identified less adaptable to different substrate materials. Sawdust and wood chips are the major substrate material for bag cultivation. However, there have been many trials and success in using alternative materials for shiitake production, for example usage of different coffee residues (**Soccol and Fan, 2005**). Oyster mushrooms utilize various substrate materials better than any other mushroom. It has been reported that oyster mushrooms can be cultivated on almost all kinds of available wastes (**Poppe, 2004**). *Pleurotus* species grow on lignocellulosic materials because they have high saprophytic characteristic. Using substrates containing a high content of protein and nitrogen has been shown to shorten the growth period and increase the biological efficiency and the yield (**Jafarpour et al., 2011**).

2 AIM

The study was conducted to observe the cultivation of wood rottening fungi, *Pleurotus ostreatus* and *Lentinula edodes* on the agroindusrial by-product wheat bran. The aim of this thesis was to apply wheat bran in different amounts as a lignocellulosic substrate, to set optimal cultivation conditions and to analyse changes in the substrate composition after mycelium/mushroom growth. For the substrate determination there were used standardized methods to ascertain the amounts of protein, fat, fiber, ash, starch, hemicellulose, cellulose, lignin and dry matter. Further, the results from substrates determination before and after cultivation were compared.

3 MATERIALS AND METHODS

3.1 Destarching of wheat bran

Principle

Wheat bran starch is monomerized intoD-glucose by the α-amylase and amyloglucosidase activities

(α-amylase + amyloglucosidase)

Starch + H_2O----------> D-glucose

Equipment

- Electrical heater, IKA LABORTECHNIK RcT BASIC
- Analytical balance, SARTORIUS CA 310S
- Basket press
- Dryer, Heraus Instrument, Germany
- Food processor

Chemicals

- Sulphuric acid, 98 %, Roth Germany, Art.-Nr.:2609.2
- α-amylase, Sigma, A3403-500KU
- amyloglucosidase, Sigma, A7095-50ml

Sample

Wheat bran used in the present study was provided by GoodMills Österreich GmbH.

Procedure

For pilot-scale trial 1 kg wheat bran was mixed with 3 kg of water. Another 2 kg of water was added with 0.5 ml α-amylase and mixed before being added to the previous mixture. Finally, 0.5 kg of water was added to the previous mixture. The suspension was mixed for 2 hours at a temperature of 85 °C. After 2 hours, the mixture was cooled down to 55 °C. The starting pH value of the suspension was 6.8. The pH value was set to 5.5 by adding 5 ml 50% sulphuric acid, which was prepared by diluting 59 ml 98% sulphuric acid in 100 mL water. To the

suspension 0.5 mL amyloglucosidase was added. The suspension was mixed and kept in water bath overnight at 55 °C. To remove water from the suspension a basket press was used. The suspension was dried for 3 hours. The destarching process was repeated with another 4 kg of wheat bran.

Substrate preparation and inoculation

Components (1 kg per trial) and composition

- Saw dust (beech): beech wood chips mixed with 8.8% beech wood sawdust fine, Rettenmaier&Söhne (Germany)
- Wheat bran (destarched) and wheat bran (native), GoodMills (Austria)
- Gypsum powder ($CaSO_4$, 1%) and chalk ($CaCO_3$, 1%) as buffering agents and nutrients
- Distilled water, substrate's moisture were calculated and moisture adjusted to 65%
- 5% mushroom spawns (50 g/1 kg substrate)
- PCA agar, Merck KGaA, Germany, Art.-Nr.: 1.05463.0500
- YGC agar, Merck KGaA, Germany, Art.-Nr.: 1.16000.0555
- Peptone water, Merck KGaA, Germany, Art.-Nr.: 1.072280500

Type of mushrooms

- Shiitake mushroom (*Lentinula edodes*), SHI ES 5063, Glückspilze (Austria)
- Oyster mushroom (*Pleurotus ostreatus*), AWR 5063, Glückspilze (Austria)

Substrate sterilization and control sterilization test

The substrates were placed into the sterilisation plastic bags and sealed. All substrates were sterilised in big industrial autoclave Certoclav for 90 min at 121 °C at 100 kPa. After cooling the substrates down, the bags were opened, samples were taken and control sterilisation tests were performed. Below, table 1 and 2 sum up the media preparation and inoculation conditions for the control tests. Pre-mixed media were mixed with water and followed with cooking by constant stirring for 2 minutes. The prepared media were sterilized at 121°C for 15 minutes and stored at 50°C in a water bath to cool down. Preparation of the mediums and the cultivation conditions are summarized in the Table 5 and in the Table 6.

Table 5 Medium preparation

Medium	Water (ml)	Pre-mixed medium (g)
Peptone water	540	36.4
PCA	2500	56.25
YGC	1500	60

Table 6 Inoculation conditions

Parameter	Media	Procedure (Pour/Spread)	Dilution range	Incubation (Aerobic/ Anaerobic)	Incubation	
					Tempt.	Time
Total viable count (TVC)	PCA	Pour plate	10^{-1},10^{-2}, 10^{-3}	Aerobic	30^{o}C	72h
Yeast and molds	YGC	Spread	10^{-1},10^{-2}	Anaerobic	25^{o}C	5d

Inoculation and Incubation

Inoculation of substrates was performed in laminar box to avoid contamination. After inoculation the plastic bags were sealed again and placed in the climate chamber with a temperature of 24℃, 95% of humidity and 2h per day of lightning phase. After 17 and 19 days of mycelium growth phase the bags with *Pleurotus ostreatus* spawns and with *Lentinula edodes* were opened, respectively. The substrates were soaked in the water for 20 minutes and place into the climate chamber. Environmental conditions in the climate chamber were adjusted at 19 ℃, 90% humidity and 24 h of flood-light supplementation. The substrates with mycelium were sprayed with distilled water twice a day to avoid humidity drop. Samples for further analyses were taken from substrates. Following Table 7 explains the preparation of the mixtures. There were prepared 5 different mixtures in 2 trials; one for *Lentinula edodes* and one for *Pleurotus ostreatus*.

Table 7 Mixtures preparation

Mixture 1	Beech wood chips	Beech wood saw dust	Native bran	dest. Bran	gypsum+ chalk	total	Spawn Addition	Total
1 kg	580,94	56,06	0,00	0,00	13,00	650,00		
%	89,38	8,62	0,00	0,00	2,00	100,00		
DM [%]	91,90	91,36			0,00			
Moisture [%]	8,10	8,64						
Moisture in x [g]	47,06	4,84	0,00	0,00		51,90		
H2O Addition [g]	370,60					1020,60	51,03001488	1071,63
Mixture 2	Beech wood chips	Beech wood saw dust	Native bran	dest. Bran	gypsum+ chalk	total		
1 kg	432,74	41,76	0,00	162,50	13,00	650,00		
%	66,58	6,42	0,00	25,00	2,00	100,00		
DM [%]	91,90	91,36		99,39				
Moisture [%]	8,10	8,64		0,61				
Moisture in x [g]	35,05	3,61	0,00	0,99		39,65		
H2O Addition	382,85					1032,85	51,64243838	1084,49
Mixture 3	Beech wood chips	Beech wood saw dust	Native bran	dest. Bran	gypsum+ chalk	total		
1kg	284,54	27,46	0,00	325,00	13,00	650,00		
%	43,78	4,22	0,00	50,00	2,00	100,00		
DM [%]	91,90	91,36		99,39				
Moisture [%]	8,10	8,64		0,61				
Moisture in x [g]	23,05	2,37	0,00	1,98		27,40		
H2O Addition	395,10					1045,10	52,25486188	1097,35
Mixture 4	Beech wood chips	Beech wood saw dust	Native bran	dest. Bran	gypsum+ chalk	total		
1 kg	432,74	41,76	162,50	0,00	13,00	650,00		
%	66,58	6,42	25,00	0,00	2,00	100,00		
DM [%]	91,90	91,36	91,09					
Moisture [%]	8,10	8,64	8,91					
Moisture in x [g]	35,05	3,61	14,48	0		53,14		
H2O Addition	369,36					1019,36	50,96806338	1097,35
Mixture 5	Beech wood chips	Beech wood saw dust	Native bran	dest. Bran	gypsum+ chalk	total		
1kg	284,54	27,46	325,00	0,00	13,00	650,00		
%	43,78	4,22	50,00	0,00	2,00	100,00		
DM [%]	91,9	91,36	91,09					
Moisture [%]	8,10	8,64	8,91					
Moisture in x [g]	23,05	2,37	28,96	0,00		54,38		
H2O Addition	368,12					1018,12	50,90611188	1069,03

3.2 Ash content

Principle

Milled examined substances with the size 0.5 mm are weighed in porcelain crucible. After pre-ashing the substance is annealed in muffle furnace at 650 °C to the constant weight.

Reference: ICC 104/1, AACC 08-01, SOP 1-07

Equipment

- Analytical balance, SARTORIUS BP 10S
- Desiccator
- Muffle furnace
- Porcelain crucible
- Pre – asher
- Crucible tongs

Sample preparation

Wheat bran samples are ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

The empty porcelain crucibles were annealed in a muffle furnace at 650 °C for 30 minutes, cooled down in a desiccator for one hour and weighed. Approximately 2 g of sample were weighed in the crucibles and pre-ashed until there occurred no smoke. Subsequently the pre-ashed porcelain crucibles annealed in the muffle furnace at 650 ± 10 °C for 3 hours to the constant weigh. Porcelain crucibles were weighed after cooling down in desiccator.

Calculation

$$\text{Ash } [\%] = \frac{\text{Final weigh} - \textit{Empty crucible}}{\text{Initial weigh}} * 100$$

$$\text{Ash in dry matter } [\%] = \text{Ash } [\%] * \frac{100}{\text{Dry matter } [\%]}$$

3.3 Dry matter

Principle

The milled sample is weighed into pre-dried Petri dish. The examined sample is dried in an oven at 105± 3 °C to the constant weigh (overnight). The dish is then cooled in the desiccator and weighed.

Reference: ICC 110/1, AACC 44-15, SOP 1-27

Equipment

- Analytical balance, SARTORIUS, Germany, CA 310 S
- Desiccator
- Drying machine, Heraus Instrument, Germany
- Petri dishes
- Crucible tongs

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

Petri dishes were pre-dried in the drying oven at 105 °C for 1 hour and cooled down to room temperature for 1 hour in desiccator and weighed. About 2-3 grams of each sample was weighed in Petri dishes and dried in the oven at 105 °C to constant weight (overnight). The final weight of the Petri dishes was established after cooling in desiccator.

Calculation

$$\text{Dry matter } [\%] = \frac{\text{Final weigh} - \textit{Empty Petri dish}}{\text{Initial weight}} * 100$$

3.4 Determination of total fat

The ground samples are extracted in the Soxhlet extractor with petroleum ether. After separation from the solvent the round bottom flasks are placed into the oven at 105± 3 °C to constant weight (1 hour).

Reference: ICC 136, SOP 1-09

Equipment:

- Analytical balance, SARTORIUS, Germany, CA 310 S
- Heating unit
- Desiccator
- Oven, Germany, Heraus Instrument
- Soxhlet apparatus, FORTUNA optifit, NS29.2132
- Round bottom flasks, Glasspearls, Cotton, Extraction thimbles

Chemicals

- Petroleum ether, ROTH, Germany Art.-Nr.:1173.3
  Usage: 150 ml / flask (reusable)

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

The round bottom flasks with sand stones were pre-dried for one hour in drying oven at 105 °C, then cooled in the desiccator to room temperature and weighed. 2-3 g of ground samples were weighed into the extraction thimbles and closed with cotton. The Soxhlet apparatus was assembled and round bottom flasks were filled with 150 ml of Petroleum ether. After 3h extraction Petroleum ether was poured to the redistillation bottle and round flasks were place into the oven for drying to constant weigh at 105 ± 3 °C. After cooling in desiccator the flasks were weighed, extraction thimbles were dried, cleaned and prepared for another trial.

Calculation

$$\text{Total fat content [\%]} = \frac{\text{Final weigh} - \text{Round flasks}}{\text{Initial weight}} * 100$$

Final weight = Round flask + Sand stones + Fat

Round flasks = Round flask + Sand stones

3.5 Total protein content

Principle

The ground substance is decomposed and oxidized with concentrated sulphuric acid in combination with a catalyst. Formed ammonium sulphate is converted to ammonia by adding a strong base. Ammonia with boric acid creates ammonium triborate that can be determined by back titration with hydrochloric acid. Thus determined nitrogen is converted by using a factor in protein.

Reference: ICC 105/2, SOP 1-06

Equipment

- Analytical balance, SARTORIUS, Germany,
- Kjeldahl apparatus, BÜCHI Speed Digester K-439, BÜCHI Scrubber K-415, BÜCHI Kjelflex K-360
- Dosimat 715, Metrohm
- Kjeldahl flasks, glasspearls, plastic cup, Erlenmeyer flasks, magnets

Chemicals

- Kjeldahl tablets 5g, ROTH, Germany, Art.-Nr.: HN19.1
- Sulphuric acid 96 %, ROTH, Germany, Art.-Nr.:9161.1
- Sodium hydroxide 32 %, ROTH Germany, Art.-Nr.: T197.3
- Boric acid 99,8 %, ROTH, Germany, Art.-Nr.:6943
- Hydrochloric acid 37 %, ROTH Germany, Art.-Nr.: 4625.2

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

Dried and homogenized samples were weighed in plastic cups (0.2g) and transferred into the Kjeldahl tubes. To each tube 3 glass pearls were added along with 1 Kjeldahl tablet and 20 ml of sulphuric acid. Afterwards the tubes were inserted into the Kjeldahl apparatus. There was one blind sample in each row. Apparatus was closed, Scrubber, cooler, ventilation turned on and the temperature was settled slowly up to 420 °C until sulphuric acid boiled. The boiling point was held approximately 2 hours until the digestion solution was clear. The solution was cooled down and the apparatus was turned off. The distillation apparatus was turned on, warmed up and cleaned. Erlenmeyer flasks were filled with 3 drops of Sher indicator. The distillation started with measurement sample and finished with adding HCl from Dosimat to determinate the amount of ammonium. The reaction is observed by green-blue-grey-orange colour change. After finishing all samples the apparatus was cleaned with water. Distillation plant was turned off and the water pipe closed.

Calculation:

$$\text{Total protein content [\%]} = \frac{V\ (HCl)[ml] * c\ (HCl)[mmol\ /ml] * M\ (N)[mg\ /mmol]}{\text{Initial weight [mg]} * 1000} * 100 * \text{Factor}$$

c (HCl) = 0.1 mmol/ml

M (N) = 14.007 mg/mmol

Factor = 5.26

3.6 Total starch

Principle

Principle of this method is based on following chemical reactions:

$$\text{Starch} + H_2O \xrightarrow{\text{α-amylase, pH 7.00 or 5.00, 100 °C}} \text{maltodextrins}$$

Thermostable α-amylase hydrolyses starch into maltodextrines.

AMG

Maltodextrins ----------> D-glucose

Amylogucosidase hydrolyses maltodextrins to D-glucose.

Glucose oxidase

D-glucose + H_2O + O_2 ----------> D-gluconate + H_2O_2

D-glucose is oxidized to D-gluconateand one mole of hydrogen peroxide is released, which is measured in colourimetric reaction catalysed by persoxidase. End product of this reaction is quinoneimine dye.

peroxidase

2 H_2O_2 + p-hydroxybenzoic acid + 4-aminoantipyrine ----------> quinoneimine dye + 4 H_2O_2

Reference: MEGAZYME Starch Kit K-TSTA 09/14

Equipment

- Glass test tubes
- Micro – pipettors
- Centrifuge, EPPENDORF, Centr. 5810
- Analytical balance, SARTORIUS CA 310S
- Spectrophotometer, HITACHI U-1100
- Vortex mixer
- Water bath,
- Bailing water bath, KOTTERMANN 3030
- Stop clock
- Couvettes, MACRO GREINER BIO-ONE

Chemicals

- Thermostable α-amylase, MEGAZYME
- Amyloglucosidase, MEGAZYME

- GOPOD Reagent Buffer, p-hydroxybenzoic acid and sodium azide. MEGAZYME
- GOPOD Reagent Enzymes. Glucose oxidase plus peroxidase, 4-aminoantipyrine. MEGAZYME
- D-Glucoesstandart solution in benzoic acid
- Sodium acetate buffer.
- Glacial acetic acid
- Sodium hydroxide
- Distillated water

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

Total starch assay was performed with MEGAZYME Starch Kit K-TSTA 09/14, p. a, Determination of starch in cereal and food products not containing resistant starch, D-glucose and/or maltodextrins.

100 mM, pH 5.0 sodium acetate buffer was prepared. 5.8 mL of glacial acetic acid was added to 900 mL of distilled water. pH value was adjusted by the addition of 1 M sodium hydroxide solution.

Approximately 100 mg accurately weighed milled samples were added to polyurethane tubes. 0.2 mL of aqueous ethanol (80% v/v) was added to wet each sample. Tubes were stirred on a vortex mixer. 3 mL of thermostable α-amylase was added to each sample. Thermostable α-amylase was prepared by dilution of α-amylase in 100mM sodium acetate buffer in a ration 1:30 (for 6 samples there was prepared 18 mL of α-amylase by diluting 0.6 mL of α-amylase in 17,4 mL of buffer etc.).

The tubes were incubated in a boiling water bath for 12 minutes. The tubes were stirred after 4.8 and 12 minutes. 0.1 mL of amyloglucosidase was added to each sample, stirred on a vortex mixer and placed in a bathat 50 °C for 30 min. The volume of samples was increased with distilled water up to 10 ml. From each sample there was taken 1 mL of solution and added to the Eppendorf tubes. Centrifugation occurred at 3000 rpm for 10 min. 0.1 mL aliquots were taken and put to the bottom of glass test tubes. 3.0 mL of GOPOD Reagent were added to each tube. D-Glucose controls and Reagent Blank solutions were also prepared.

D-Glucose controls consisted of 0.1 mL water and 3.0 mL GOPOD Reagent. Reagent Blank solutions consisted of 0.1 mL of water and 3.0 mL of GOPOD Reagent. All the tubes were placed in the water bath and incubated at 50 °C for 20 min. Spectrophotometer was turned off approximately 30 minutes before measurement and the absorbance of the samples was read at 510 nm against the reagent blank.

Calculation

$$\text{Starch}[\%] = \Delta\text{A} * \text{F} * \frac{FV}{0.1} * \frac{1}{1000} * \frac{1000}{W} * \frac{162}{180}$$

$$= \Delta A * \frac{F}{W} * FV * 0.9$$

Where:

ΔA = absorbance (reaction) read against the reagent blank

F = $\frac{100\ (\mu\text{g of D}-\text{glucose})}{\text{Absorbance for } 100\mu\text{g of glucose}}$ conversion from absorbance to μg

FV = final volume

0.1 = volume of sample analysed

$\frac{1}{1000}$ = conversion from μg to mg

$\frac{100}{W}$ = factor to express starch as a precentage of flour weight

W = the weight in milligrams (as is basis) of the flour

$\frac{162}{180}$ =adjustment from free D-glucose to anhydro D-glucose (as occurs in starch).

Starch % w/w (dry wt.basis):

= Starch [% w/w] (as is) * $\frac{100}{100-\text{moisture content}\left(\%\frac{\text{w}}{\text{w}}\right)}$

3.7 Measurement of Neutral – and Acid Detergent Fibre (NDF, ADF) and Lignin

Principle

The method used for this analysis is Global Standard – Van Soest method. The amount of plant fibre (cellulose, hemicellulose and lignin) is measured by stepwise digestion. There are 3 steps. In the first step proteins, sugars, lipids, starch, pectine and minerals are solved by a neutral detergent solution so that cellulose, hemicellulose and lignin can be weighed. In the second step hemicellulose is solved by an acidic detergent solution, where cellulose and lignin remain unsoluble. In the third step cellulose is solved by 72% sulphuric acid and lignin can be weighed.

Reference: Gerhardt Application: ADF / NDF with Fibrebag; ADL-measurement;

Goering, H. K. and P.J. Van Soest. 1970. Forage Fiber Analysis

USDA Agricultural Handbook No. 379; VDLUFA 6.5.1 – 6.5.3

Equipment

- Analytical balance, SARTORIUS, Germany, CA 310 S
- Heater
- Muffle furnace
- Oven
- Pre-asher
- Desiccator
- Porcelain crucible
- Extraction beaker, Foss
- Capsule holder, Fibertec System, Foss
- Capsules, FiberCap 2021/2023, Foss
- Condensator
- Dishes
- Stopper
- Cooking holder

- Drying stick

Chemicals

- Petroleumether, Roth, Deutschland Art.-Nr.: 1173.3
- Sulphuric acid 95 %, Roth Germany, Art.-Nr: 9316
- Sodium dodecylsulfate> 99%, Roth Germany, Art.-Nr.: 4360
- EDTA, disodiumsalt, dihydrate> 99%, Roth Germany, Art.-Nr.:8043
- Disodiumtetraborat, decahydrat, ACS, Merck Germany, Art.-Nr.: 106308
- Sodiumdihydrogenphosphat* $1H_2O$, >99%, Roth Germany, Art.-Nr.:P030 or Sodiumdihydrogenphosphatwaterfree
- Triethylenglycol
- N-Cetyl-N,N,N-trimethylammoniumbromid, Merck, Deutschland, Art.-Nr.:102342
- Decahydronaphtalin> 98%, Roth, Germany, Art.-Nr.: 4438
- Sulphuric acid 72% (247 mL 95% H_2SO_4 were mixed with 123 mL of water and cooled down)
- Sulphuric acid 0,5 mol/L (Bottle was filled with water and there were added 27.8 mL 95% H_2SO_4 up to 1 L)
- Neutral Detergent Solution: for 1L solution: in 0,4L of distilled water was solved reagents while mixing and heating:
  - 18.6 g EDTA, Disodium, Dihydrate
  - 6.8 g Disodiumtetraborate, Decahydrate

  After cooling it down to 20 °C there were added slowly:

  - 30.0 g sodium dodecylsulfate
  - 10 mL Triethylenglycol

  In another beaker there was solved in 200 mL water with heating

  - 5.25 g Sodiumdihydrogenphosphate (monohydrate)

  And this solution was added to the first one. The pH value was checked and set to 7.0 $\pm$ 0.1 with acetic acid and NaOH.

- Acidic Detergent Solution SD: 20.0 g N-Cetyl-N,N,N-trimethylammoniumbromid was mixed with 0.5 mol/L sulphuric acid.

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

The capsules with lids were weighed empty capsules with lids (W1) and 0.5 g of solid, milled samples (W2). In each trial we used an empty capsule with a lid as a blank sample. The capsules were placed into the carousel and secured with stopper.

Neutral Detergent Fibre

In the beaker, 330 mL of ND-solution was filled and heated to boiling temperature. After reaching the boiling point, the carousel with the capsules and the stopper were placed into the solution. The capsules were wetted completely by rotating and lifting the carousel. The cooler was placed on the extraction vessel and activated. The samples were boiled inside of the beaker for 60 min. After 60 min. the carousel was taken out of the beaker and drained. The ND solution was discarded and the beaker was filled with 100 mL of hot water. The carousel with capsules was washed 3 times. The capsules were then dried in the oven at 105 °C overnight and weighted after cooling in the desiccator (W3). Porcelain crucibles were pre-annealed and weighed (W4). The capsules were put inside, pre-ashed and then put into the muffle furnace at 600 °C for 4 h. The porcelain crucibles were weighed after cooling in desiccator (W5).

Acid Detergent Fibre

Same procedure as for NDF was performed with AD solution instead of ND solution.

Lignin

This procedure was performed the same way as the previous NDF and ADFs method till the washing with boiling water. 330 mL of 72% sulphuric acid were filled in a beaker with handle. The carousel with capsules and the stopper were placed into the acid and wetted by rotating and lifting the carousel and extracted for 3h. After 180 min. the carousel was removed and drained. Sulphuric acid was discarded and the beaker was filled with 100 ml of hot water and washed. The water was changed for 3 times. The capsuled were then dried in the oven at 105 °C overnight and after cooling weighted in the desiccator (W3). Porcelain

crucibles were preannealed and weighed (W4). The capsules were put inside, preashed and then put into the muffle furnace at 600 °C for 4 h. After cooling in desiccator the porcelain crucibles were weighed (W5).

Calculation

$$\text{NDF } [\%] = \frac{(W3 - W1 + C) - (W5 - W4 - D)}{W2} * DM * 100$$

$$\text{ADF } [\%] = \frac{(W3 - W1 + C) - (W5 - W4 - D)}{W2} * DM * 100$$

$$\text{Lignin } [\%] = \frac{(W3 - W1 + C) - (W5 - W4 - D)}{W2} * DM * 100$$

W1	empty capsule [g]	
W2	sample mass [g]	
W3	capsule + rest [g] after detergent treatment	
W4	empty crucible [g]	
W5	crucible + ash [g]	
C	coefficient for solubility of capsule	=W3 of empty capsule / W1
D	ash of empty capsule [g]	
DM	dry matter [%]	

3.8 Dietary fiber

Principle

Dietary fibre analyse is based on chemical reactions and determined gravimetrically following alcohol precipitation. Ash and residual protein are determined on DF residues and substracted.

Reference: MEGAZYME Total Dietary Fiber K-TDFR 130404-1

α-amylase + amyloglucosidase

Starch + H_2O ----------> Glucose

Protease

Protein + H_2O ----------> Peptides

Equipment

- 250 mL bottles
- Filter crucible
- Filtering flasks
- Rubber ring adaptors for use on filtering flasks
- Vacuum pump
- Shaking water bath
- Analytical balance, SARTORIUS, Germany, CA 310 S
- Oven, Germany, Heraus Instrument
- Desiccator
- pH meter
- Pipettors and tips
- Dispensers
- Magnetic stirrer sand stirring bars
- Muffle furnace

Chemicals

- Thermostable α-amylase, MEGAZYME cat. No. E-BLAAM
- Purified protease, MEGAZYME cat. No.
- Purified amyloglucosidase, MEGAZYME cat. No. E-AMGDF
- Celite 545
- Ethanol, 95 % v/v
- Ethanol, 78 %. Into 1 L volumetric flask were placed 179 mL water and diluted to volume with 95 % ethanol and mixed.

- Acetone
- Distilled water
- MES/TRIS buffer, 0.05 M, pH 8.0 at 23 C. 19.52 g 2(N-morpholino) ethanesulfonic acid (MES) (SIGMA) and 14.2 g tris(hydroxymethyl)aminomethane (TRIS) (SIGMA) were disolved in 1.7 L deionised water. pH was adjusted to 8.0 with 6.0 M NaOH. Volume was adjusted up to 2 L.

Sample preparation

Samples were ground with Retsch mill (500 µm – strainer) and homogenised.

Procedure

In this procedure two MEGAZYME kits were used, from the kit Total Dietary Fiber K-TDFR 130404-1 enzymes were taken and according the kit K-ACHDF 141109-4 the analysis was performed.

With each assay, there were two blanks along with samples to measure any contribution from reagents to residue.

Samples were accurately weighed at 1.000 $\pm$ 0.0005 g and then filled into 250 mL bottles. To each sample 40 mL MES-TRIS buffer solution was added. To the bottles a magnetic stirring bars was added and the solutions were stirred until samples were completely dispersed. Heat-stable α-amylase (50 µL) was added to each solution, while stirring at low speed. Bottles were closed and placed in a shaking bath at 80 °C and incubated for 35 min with continuous agitation. After incubation all bottles were removed and the temperature of the water bath was adjusted to 60 °C by draining some of the hot water from the water bath and adding cold water. After cooling the samples in the bottles to about 60 °C there were added 100 µL of protease solution to each bottle at one time. Bottles were incubated in a shaking water bath at 60 $\pm$ 1 °C for 30 min. After incubation they were added with 5 mL of 3 M acetic acid. Amyloglucosidase solution was added (200 µL) to the bottle with continuous stirring of the contents on a magnetic stirrer. The bottles were incubated in a shaking water bath at 60 °C for approx. 30 min. To each bottle there were added 160 mL of 95% (v/v) EtOH pre-heated to 60 °C and mixed well on a magnetic stirrer. The precipitates were formed over night at room temperature.

Crucibles containing celite were pre-annealed in the muffle furnace, cooled down in the desiccator and weighed. The beds of celite were wetted and redistributed in crucibles using

approx. 3 mL of distilled water. Suction was applied to crucibles to draw celite onto glass as an even mat. Sample mixtures were filtered through crucible into a filtration flask. The residues were washed successively with three 15 mL aliquots of 78% (v/v) EtOH, two 15 mL aliquots of 95% (v/v) EtOH and two 15 mL aliquots of acetone. The crucibles which contained the residues were dried overnight at 105 °C. The crucibles were cooled in a desiccator and then weighed.

One residue from each type of fibre was analysed for protein and the second residue of the duplicate was analysed for ash. Protein analysis on residue was performed using Kjeldahl method. For ash analysis, the second residue was incinerated for 5h at 525 °C, cooled in desiccator and then weighed.

Calculation

$$\text{Dietary fiber } \left[\%\frac{\text{w}}{\text{w}}\right] = 1 + \frac{\frac{R1+R2}{2} - p - A - B}{\frac{m1+m2}{2}} * 100$$

Where

R_1 = residue weight 1 from m_1

R_2 = residue weight 2 from m_2

m_1 = sample weight 1

m_2 = samples weight 2

p = protein weight from R_1

A = ash weight from R_2 and

B = blank

= $\frac{BR1+BR2}{2}$ – BP - BA

Where

BR = blank residue

BP = blank protein from BR_1

BA = blank ash from BR_2

4 RESULTS

In this chapter the presented results are supplemented with pictures showing the progress in mushroom cultivation and with the graphs providing results gained from analysed samples. The data are provided in the annex. For the analyses, samples were taken before the inoculation and after the inoculation of substrates with *Lentinula edodes* and *Pleurotus ostreatus* spawns.

The substrates were numbered as follows:

1. control substrate without wheat bran enrichment
2. substrate with 25 % native bran
3. substrate with 50 % native bran
4. substrate with 25 % destarched bran
5. substrate with 50 % destarched bran

4.1 Destarching process

Destarching of wheat bran was performed twice. For the first trial 1 kg of wheat bran was destarched and the second time 3 kg of wheat bran was destarched. The table below (Table 8) summarizes the yield of destarched bran from native bran and the extraction efficiency. From 4 kg of native bran there was gained 2.85 kg destarched bran. Dry matter and total starch content were analysed. The extraction efficiency was 64.42%. The Table 9 summarizes the composition of native and destarched bran.

Table 8 Extraction efficiency

Mass balance	**Native bran**	**Destarched bran**
Fresh weight [g]	4000	2853
DM [g]	3643.6	2835.6
Starch in DM [%]	11.41	5.21
Total starch content [g]	415.58	147.87
Extraction efficiency [%]	64.42	

Table 9 Native vs. destarched bran comparison

Parameter	Native bran [% in DM]	Destarched bran [% in DM]
Moisture	8.91	0.61
Ash	6.14	4.89
Protein	8.11	13.16
Fat	5.49	5.28
Starch	11.41	5.21
Glucose	6.75	29.03
Fiber	47.58	62.57
Hemicellulose	47.43	56.98
Cellulose	13.35	16.30
Lignin	5.18	8.10

Values from Table 9 are compared with literature in the chapter Discussion.

4.2 Substrates

The Figure 1 represents the substrates overgrown with mycelium, held together by tiny tendrils. We marked the substrates and placed them on the shelves.

Figure 1 Substrates in climate chamber

After 23 days from the inoculation we opened the bags inoculated with *Lentinula edodes* spawn and we observed the beginning of fruiting phase on substrate S2/2 and S4/2 what represent the figure 2 and 3. Substrate S2/2 consists of 25% destarched bran. Substrate S4/2 consists of 25% native bran.

Figure 2 Fruiting phase beginning S2/2

Figure 3 Fruiting phase beginning S4/2

The figure 4 represents the substrates during their browning phase.

Figure 4 Browning phase

The figures 5 and 6 represent the final stage – the fruiting phase. Fruiting bodies appeared only on 2 substrates. *Lentinula edodes* grew on substrate S4/2 – 25 % of native bran. *Pleurotus ostreatus* grew on control substrate number P1/2.

Figure 5 Fruting phase - *Pleurotus ostreatus*

Figure 6 Fruiting phase - *Lentinula edodes*

4.3 Substrate composition analyses

Substrates were analysed before inoculation and after the termination of the experiment for their dry matter, ash, protein, fat, total starch, glucose, total dietary fibre, cellulose, hemicellulose and lignin contents.

4.3.1 Dry matter

The first observed parameter was dry matter. In the figure 7 below we can see the values of dry matter for each native substrate, substrates overgrown by shiitake tendrils and substrates inoculated with *Pleurotus ostreatus* spawn. A loss in dry matter was expected. The decrease was highest in substrate 3 inoculated with *Pleurotus ostreatus* spawn, while it was moderate in substrate 4 and 5 inoculated with *P. ostreatus* spawn. Genereally, the decrease was more significant in substrates inoculated with *Lentinula edodes* spawn.

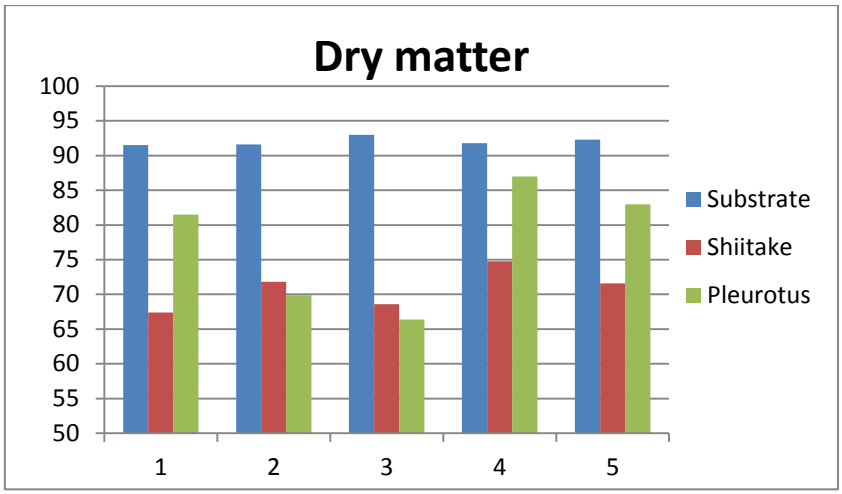

Figure 7 Comparison of dry matter content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Shiitake* spawns

4.3.2 Ash

The following figure 8 shows the results of ash content analyses. A higher content of mineral constituents in the substrates inoculated with spawns can be observed from the figure. Ash content increase is similar for *Lentinula* and *Pleurotus* in each substrate. The highest increase was observed in substrates containing 50% of native bran. This substrate showed an increase of 64.7% after mycelium growing, while *Pleurotus ostreatus* growth showed an increase of 51.4%. In comparison with destarched bran, *Lentinula* and *Pleurotus* mycelium caused in the substrate containing 50% destarched bran an increase of 32.3% *and* 22.7%, respectively.

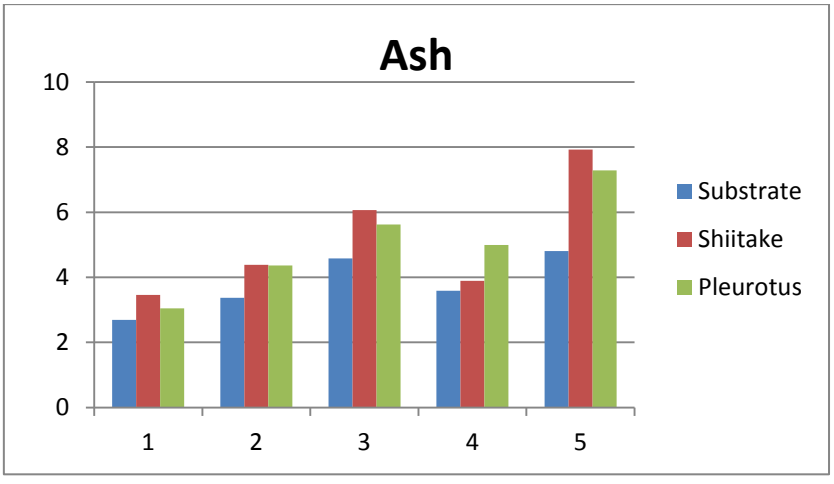

Figure 8 Comparison of ash content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Shiitake* spawns

4.3.3 Protein

From analysed substrates we gained the values for protein content for every substrate. From the figure 9below we can observe protein increase in substrates inoculated with *Lentinula* and *Pleurotus* spawns. The highest increase is observed in the substrate 5 inoculated with *P. ostreatus* spawn and in the substrate 2 inoculated with *L. edodes* spawn. The highest protein content increase was observed in substrate containing 25% destarched bran with shiitake mycelium, i.e., 50.1%.

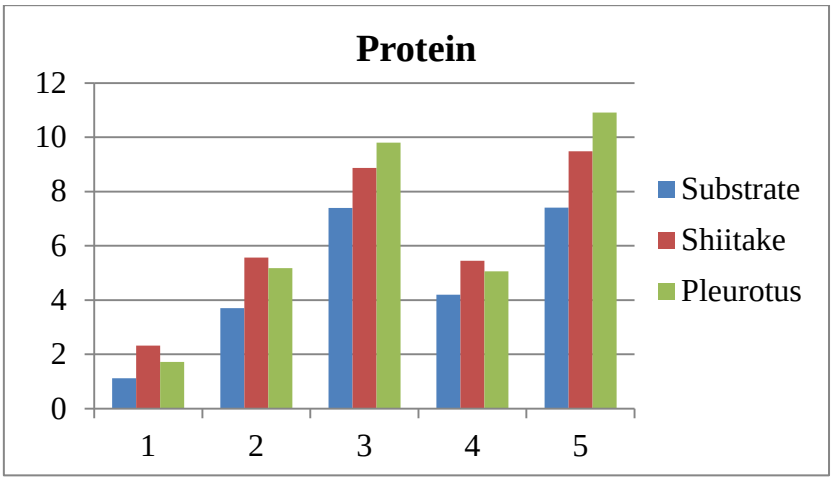

Figure 9 Comparison of protein content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

4.3.4 Fat content

Fat content in the substrates is rather low. Significant increase we can observe in substrates 4 and 5 inoculated with *Pleurotus ostreatus* spawn from the figure 10 below. Beside the substrate 3, substrates inoculates with *Lentinula edodes* did not show any significant increase in values.

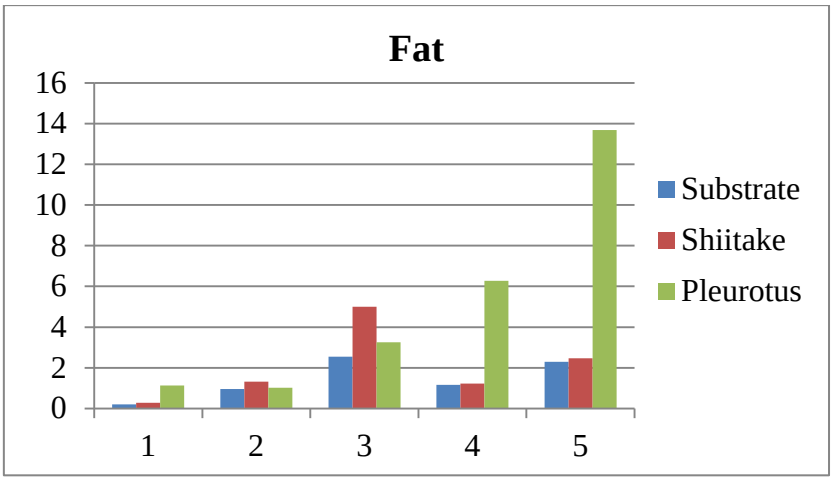

Figure 10 Comparison of fat content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

4.3.5 Starch and glucose content

Starch figure 11 shows higher total starch content in the substrate with native bran. On the other hand, glucose content was high in the substrates with 25% and 50% destarched bran. Both starch content and glucose content decreased after utilization by mycelium. The figures 11 and 12 below demonstrate that *Pleurotus ostreatus* utilized starch and glucose more than shiitake.

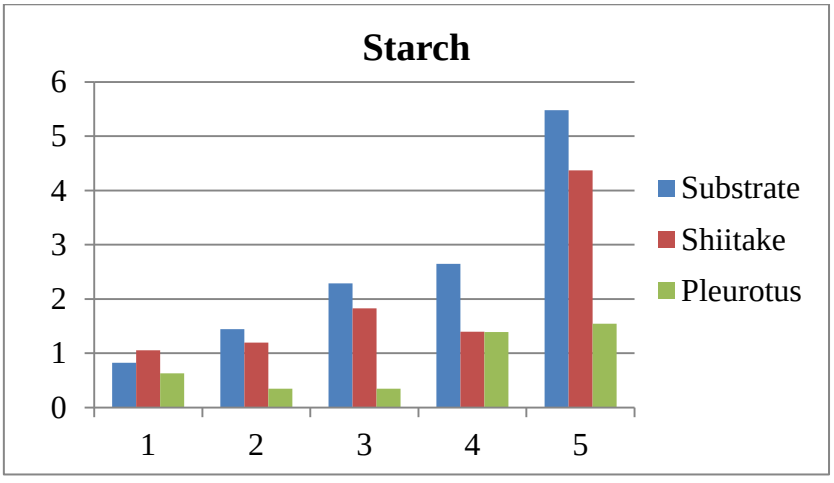

Figure 11 Comparison of starch content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

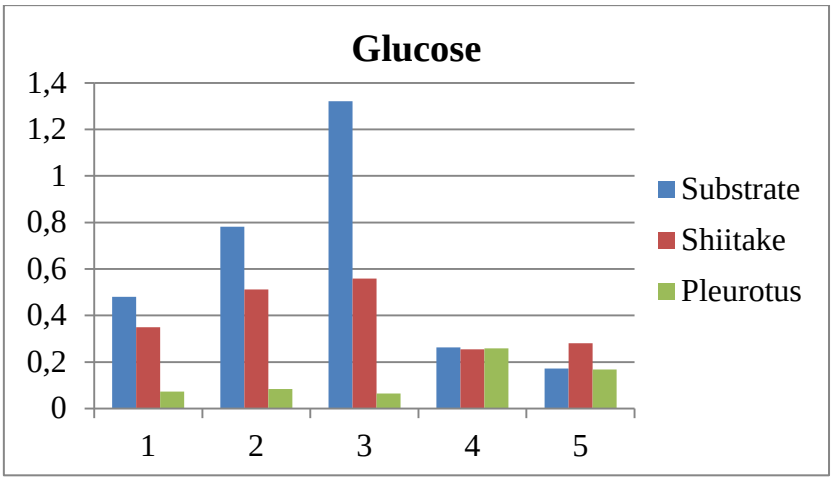

Figure 12 Comparison of glucose content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

4.3.6 Fiber

Fibre content is generally high in all substrates. As the figure 13 shows, *Pleurotus* and *Lentinula* mycelia slightly increased the fibre content in the substrates. The highest increase is observed in the substrate with 50% native bran inoculated with shiitake spawn. The fiber content decreased in native substrate inoculated with *Lentinula edodes* and *Pleurotus ostreatus* spawns.

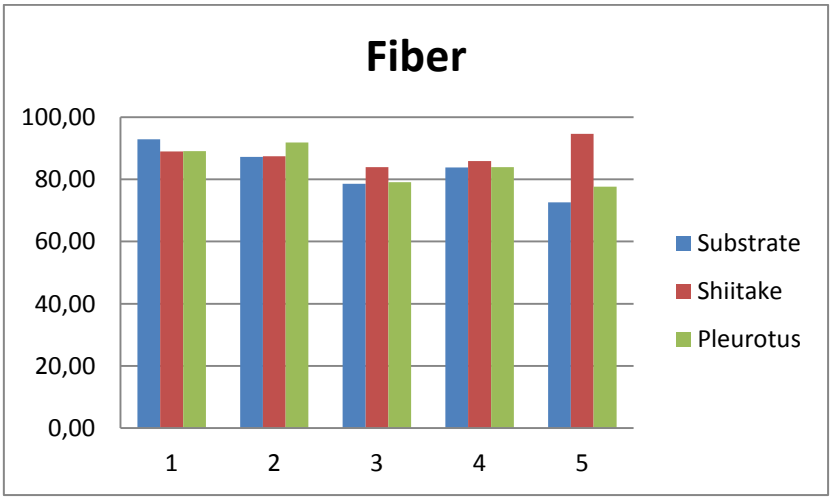

Figure 13 Comparison of fiber content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

4.3.7 Celluloses, hemicelluloses, lignin

The figure 14 shows significant increase in values of celluloses in substrates 1, 2, 3 and 5 inoculated with *Lentinula edodes* spawn. On the other hand the substrate 4 inoculated with the same inoculum shows decrease in the value. Substrates inoculates with *Pleurotus ostreatus* spawn moderate increase in substrate 2 and 3, significant increase in substrate 4 and decrease in substrates 1 and 5.The hemicelluloses values decreased in substrates 2, 3 and 4 inoculated with both spawns. In comparison with control substrate, *L. edodes* decreased the content of hemicelluloses but *P. ostreatus* increased the value. A decrease in lignin content was observed in substrates 2 and 3. In comparison with control substrate, *L. edodes* decreased the content of ligning and *P. ostreatus* spawn caused increase, similarly to the results for hemicellulosis. Results gained from celluloses, hemicelluloses and lignin analyses do not show reliable. The explanation is given further in Discussion chapter.

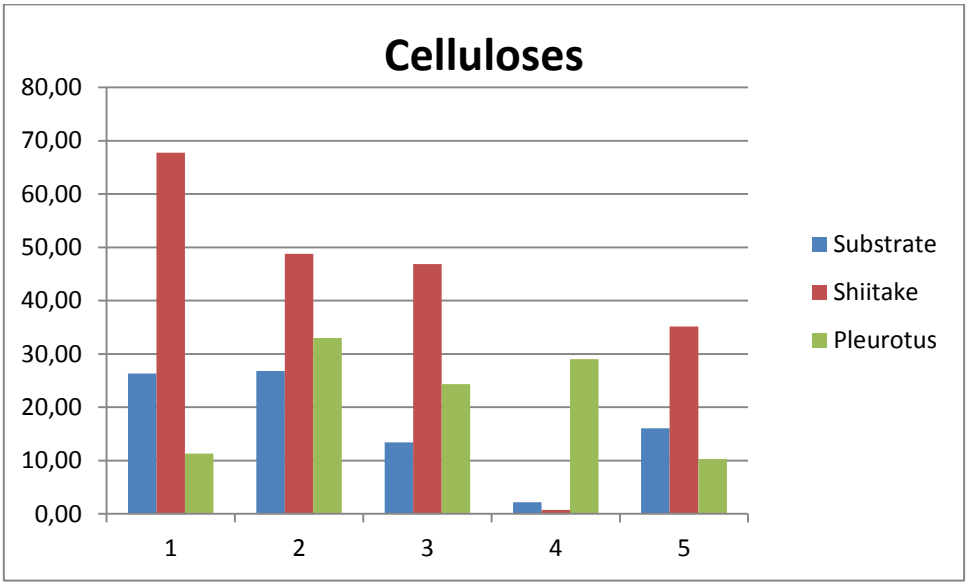

Figure 14 Comparison of cellulose content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

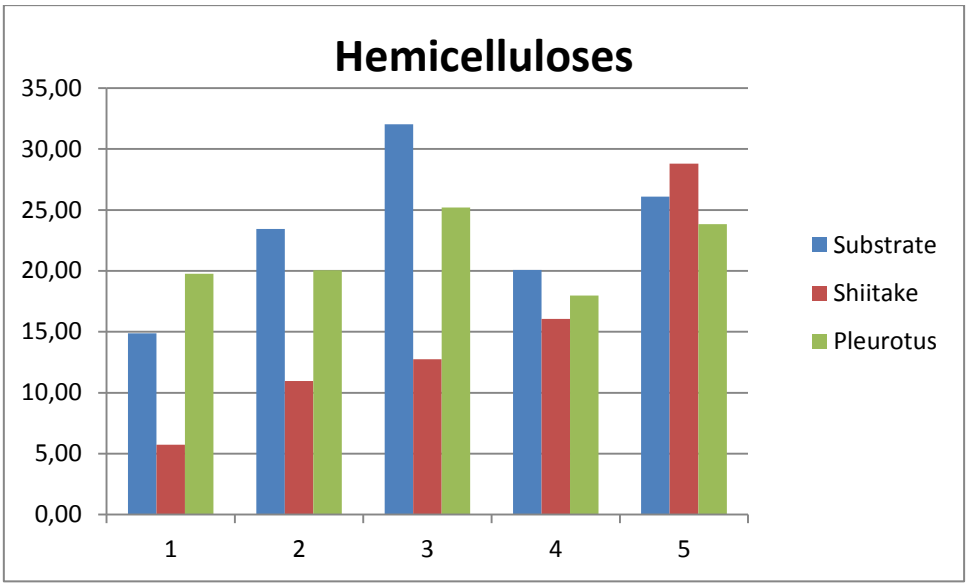

Figure 15 Comparison of hemicelluloses content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

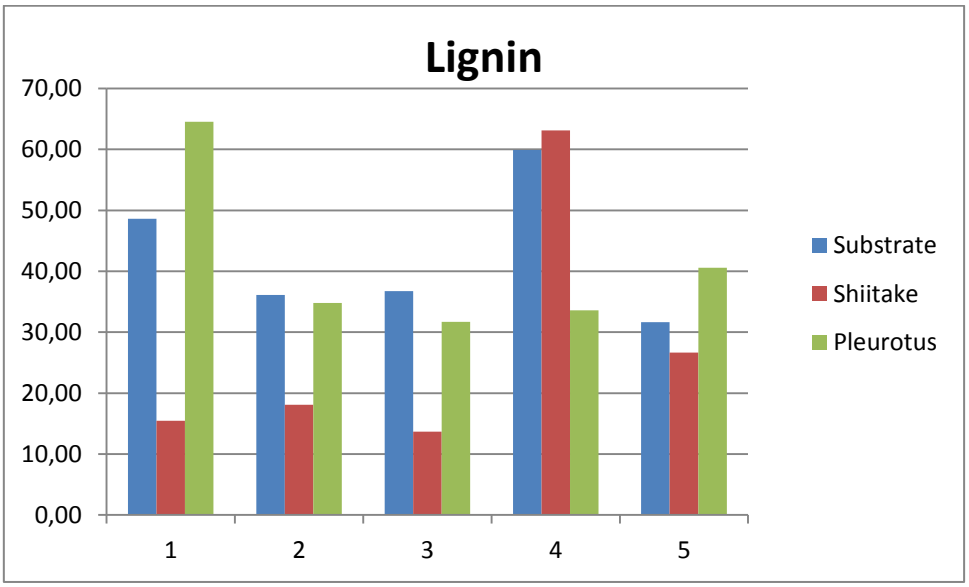

Figure 16 Comparison of lignin content results gained from native substrate without inoculum and substrates inoculated with *Pleurotus* and *Lentinula* spawns

Following table 10, table 11 and table 12 summarize results gained in performed analyses.

Table 10 Summary – Substrates without inoculum

Native substrate	**1** [% in DM]	**2** [% in DM]	**3** [% in DM]	**4** [% in DM]	**5** [% in DM]
DM	91.53	91.59	92.96	91.78	92.27
Ash	2.69	3.37	4.58	3.58	4.81
Protein	1.12	3.71	7.39	4.20	7.41
Fat	0.20	0.96	2.54	1.17	2.30

Starch	0.83	1.45	2.29	2.65	5.48
Glucose	0.48	0.78	1.32	0.26	0.17
Fiber	92.84	87.20	78.60	83.79	72.60
Hemicellulose	14.88	23.44	32.03	20.08	26.10
Cellulose	26.31	26.80	13.39	2.18	16.08
Lignin	48.62	36.12	36.76	59.94	31.65

Table 11 Summary - *Lentinula* substrates

Shiitake substrate	**1** [% in DM]	**2** [% in DM]	**3** [% in DM]	**4** [% in DM]	**5** [% in DM]
DM	67.38	71.80	68.59	74.76	71.57
Ash	3.46	4.38	6.06	3.89	7.92
Protein	2.33	5.57	8.86	5.45	9.48
Fat	0.28	1.31	4.99	1.23	2.47
Starch	1.06	1.20	1.83	1.40	4.37
Glucose	0.35	0.51	0.56	0.25	0.28
Fibre	88.90	87.40	83.88	85.91	94.59
Hemicellulose	5.75	10.95	12.76	16.07	28.80
Cellulose	67.78	48.78	46.87	0.74	35.13
Lignin	15.46	18.10	13.68	63.14	26.65

Table 12 Summary - *Pleurotus* substrates

Pleurotus substrate	**1** [% in DM]	**2** [% in DM]	**3** [% in DM]	**4** [% in DM]	**5** [% in DM]
DM	81.50	69.86	66.39	86.98	82.98
Ash	3.05	4.37	5.62	4.99	7.28
Protein	1.73	5.18	9.80	5.06	10.91
Fat	1.14	1.02	3.26	6.28	13.68
Starch	0.63	0.35	0.35	1.39	1.54
Glucose	0.07	0.08	0.07	0.26	0.17
Fibre	89.05	91.85	79.11	83.90	77.61
Hemicellulose	19.76	20.05	25.20	17.98	23.83
Cellulose	11.29	32.96	24.36	29.05	10.29
Lignin	64.56	34.80	31.72	33.57	40.59

5 DISCUSSION

In this chapter the results are discussed and compared with data from the literature. The possible reasons for inaccuracy are explained and further possibilities for improving are given.

In this thesis, any results related to number of fruiting bodies, dimension of pileus and stalk, biological yield, economical yield or biological efficiency are not provided. Mushrooms cultivation described in this thesis resulted in one fruiting body of *Lentinula edodes* and 2 fruiting bodies of *Pleurotus ostreatus* mushrooms. *Pleurotus ostreatus* mushroom grew on control substrate composed from saw dust and beech wood chips. *Lentinula edodes* grew on substrate containing 25% native bran. After short time period the substrates were contaminated with moulds, which might be one of the reasons for unsuccessful cultivation. In the climate room, the set conditions could not reached due to technical problems that were discovered at the end of the cultivation. The problem with the humidity setting resulted in a high decrease in dry matter content. Before sealing the polypropylene cultivation bags, the water content in the substrates was adjusted to 65% humidity. After opening the bags there was ascertained that the substrates are too dry and they were soaked in the water for 20 minutes. The substrates were sprayed with water regularly twice a day. Deficiency of moisture is a significant fact contributing to poor number of fruiting bodies. It is supported by the fact, that the mushrooms grew on the bottom of the shelfs where the water content was higher. Sprayed water run down to the shelves and the substrates lied in a small puddle of water.

Dry matter graphs show that the dry matter content decreased after cultivation in comparison with native substrate without water adjustment. The drop in dry matter was not as significant as expected. Dry matter was higher in substrates inoculated with *Pleurotus ostreatus* spawns. **Ogha (1999a)** states that water availability is a crucial factor during mushroom cultivation influencing fruiting body production and growth. More authors point to the fact that chemical and physical properties of the substrates influence the mushroom production character and they influence mycelium growth **(Philippoussis et al., 2001, 2002; Rossi et al., 2003; Obodai et al., 2003).** The optimal range for moisture content in substrates is suggested from 55 % to 70 % **(Royse, 1985; Miller and Jong, 1987; Przybylowicz and Donoghue, 1988)**.

As the figure 17 shows, another adverse effect was undesired mould growth on our substrates. According to **Ashrafuzzaman et al. (2009)** the water used for humidification must contain sodium hypochlorite. We used distilled water without any disinfectants for humidification.

Figure 17 Undesired contamination

Another factor influencing the mushroom yield is the length of a spawning run. In this project the length of mycelium growth was 17 days for *Pleurotus ostreatus* and 19 days for *Lentinula edodes. Pleurotus ostreatus* mycelium growth runs faster than mycelium growth of *Lentinula edodes*. **Ashrafuzzaman et al**. **(2009)** used different substrates for the cultivation of *Lentinula edodes* mushrooms. In their project they used mixtures of sawdust of timber plants babla, garzon, champe, jackfruit, ipil-ipil, segun, shimul, mango, rain tree, shisoo, rice straw and sawdust mixtures. In their study there is stated that the number of days for mycelium growth completeness varied in each type of substrate significantly. The range of the days varied from 43 to 61.8. The fastest mycelium growth was observed on jackfruit sawdust. The longest time was required on rice straw. **Bhattiet al. (1987)** explains that the variation in mycelium running rate might be caused by variations in the chemical composition of the substrates and the C:N ratio. Generally it is suggested that a limiting step in the wood components utilization is the low amount of available nitrogen in the lignocellulosic substrates. Addition of nitrogen stimulates mineralization of carbon from plant constituents, whereas lignin and lignified material decomposition is reduced **(Fog, 1988)**. There is an optimal concentration of supplements that can increase or support mycelium growth **(Han et al. 1981)**. On the other hand, extreme amounts of supplements may decrease this effect.

According to **Fasidiand Kadiri (1993)**, supplementation of 30% rice bran to the shiitake mushroom substrate can increase the productivity. This statement is supported by a report of **Rossi et al. (2003)**. In their report they also claim that any amount of supplements added to substrates resulted in better mushroom quality. **Moonmoon et al. (2010)** observed more biological yield and efficiency on wheat bran than on rice bran. In their study supplementation of 25% wheat bran resulted in higher number of fruiting bodies than supplementation with rice bran. The study also shows that shiitake mushroom quality is not always dependent on the supplementation level. However, 40% wheat bran supplementation to saw dust resulted in better quality mushrooms.

Another factor that may improve mycelium growth is substrate composting. **Philippoussis et al. (2001)** observed an effect of substrate composting on colonization rate. In their study they cultivated *Pleurotus spp., Agrocybeae gerita* and *Volvariella volvacea* mushrooms. As substrates they used three different types of agricultural waste – wheat straw, peanut shells and cotton waste. Composting process was performed in capacity rotating drum composting system with a size 2 m^3. 250 kg of waste material was filled inside, moistened to 65% and exposed to a 12 day composting process. Temperature was kept at 65-70 °C for the first 3 days, thereafter it was decreased to 50-65 °C for another 6 days and by the completion of the process the temperature was dropped to 40 °C. Non-composted substrates were supplemented with wheat bran at a 9:1 ratio. It is reported that non-composted substrates resulted in faster mycelium growth for *Pleurotus spp*. than composted substrates. However, **Scrase (1996)** reported successful application of composted substrates for the cultivation *Pleurotus spp*.

One of the improvements that could be performed for future mushrooms cultivation is a cold shock. **Philippoussis et al. (2003)** performed the cold shock after transferring the substrates to the fruiting room. During the spawning, the room temperature was kept at 25 ± 1 °C. Principle of the cold shock was based on a temperature drop to 15 °C, 90% relative humidityand 24 hours illumination per day for 3 days.

Yang et al. (2013) observed a difference between cultivation of *Pleurotus ostreatus* on sterilized and non-sterilized substrate. They searched for a cost effective substrate and they performed cultivation on rice straw substrate, cotton seed hull substrate, wheat straw substrate and rice or wheat straw enriched with cotton seed hull. Mycelium growth was faster on non-sterilized substrates and they also observed shorter time to primordial formation and shorter total colonization period. Cultivation on non-sterilized substrate brought also undesirable characteristics, such as relatively long mushroom stipe length and smaller mushroom cap,

which were also observed by other authors. Therefore it is necessary to perform further research on improving mushrooms quality cultivated on non-sterilized substrate.

5.1 Substrate evaluation

The following table summarizes the results for the nutritional parameters of wheat bran. The results of the samples analysed in this project are within the ranges found in the literature besides the protein that is lower than the values found in the literature.

Table 13 Wheat bran. Comparison of literature values and analysed sample values.

Parameter	Content per 100 g [g]	Source	Analysed sample Content per 100 g [g]
Moisture	7.9 – 12.8	Prückler et al., 2014; NFI DTU, 2009; Bertrand et al., 1980;	8.91
Carbohydrates	56 – 66.4	Prückler et al., 2014; NFI DTU, 2009;	
Fat	3.5 – 6.6	Prückler et al., 2014; NFI DTU, 2009; Bertrand et al., 1980; Bataillon et al., 1998;	5.49
Protein	13 – 18	Prückler et al., 2014; NFI DTU, 2009; Bertrand et al., 1980; Bataillon et al., 1998;	8.11
Ash	4 – 7	NFI DTU, 2009; Bertrand et al., 1980; Bataillon et al., 1998;	6.14
Fiber	36.5 – 52.4	NFI DTU, 2009; Stevenson et al., 2012;	47.58

Aguedo et al. (2013) destarched wheat bran in their project and compared the composition of native wheat bran with destarched wheat bran. After the destarching procedure the starch content dropped to 0.20%. Total glucose content in the native bran was ascertained 10.23% DM and after the enzymatic destarching process the glucose content decreased to 3.08% DM. In our project the starch value drop was not as significant as in reported studies. After we destarched the bran, glucose value raised. Similar to the **Aguedo et al. (2013)** study, the fat content in our analysed native and destarched bran stayed the same. Hemicellulose, cellulose and lignin values vary in literature but in all above mentioned studies there were observed increases in the content. The content of hemicelluloses after destarching was 56.98% which is

comparable with the hemicellulose content of 57.9% in destarched bran reported by **Bertrand et al. (1980)**.

In our native substrate that was composed only from beech wood chips and beech wood sawdust, the content of hemicellulose was 14.88%, cellulose 26.31%, and lignin 48.62%. **Bodîrlău et al. (2008)** reported in their study chemical composition of beech wood as following: Content of cellulose 47.66% and content of lignin 25.53%. Ash content was determined as 0.3%. In their study they used a different method for cellulose determination. They determined cellulose content gravimetrically after chemical reaction with a 1:4 v/v mixture of concentrated nitric acid and ethyl alcohol. Lignin content was determined by using the Klason lignin method and TAPPI T 222 om-88 gravimetrically, after 72% sulphuric acid hydrolysis. **Le Floch et al. (2015)** reported macromolecule composition of oak wood. According to their study, hemicellulose, cellulose and lignin proportions are 26.35%, 41% and 25.71%, respectively. According to **Pöhler-Rotach (2000)** birch wood is composed of 40% cellulose, 30-35% hemicellulose and 20-25% lignin.

The values for cellulose, hemicellulose and lignin content in our study differ significally from the literature. The method used in our study is Global Standard Van Soest method, it requires many weighing steps. Many mistakes can appear. The most problematic step was to determine lignin content. After samples treatment in 72% sulphuric acid and the following drying we could not establish the weight properly, because the acid etched the capsules. We tried to lower the concentration, but 70% sulphuric acid had the same effect on the capsules. We did not lower the concentration more, since this method works just if you follow the description and chemicals preparation properly. For future accurate determination of these components we would suggest to use alternative methods.

Hernández-Gaitán et al. (2011) observed biochemical changes in lignocellulosic residues composition during *Lentinula edodes* vegetative growth. As substrates they used barley straw, vineyard pruning and wheat straw. Hemicellulose concentration in barley straw and vineyard pruning decreased from 25.5% to 15.6% and from 15.8% to 12.3%, respectively. Hemicellulose in wheat straw decreased from 27.2% to 9.5%. Lignin content broke down continuously. In barley straw it decreased with 31.8% and in wheat straw with 34.4%. Lignin degradation was higher than cellulose degradation. Vegetative growth of shiitake increased the ash content steadily. Other authors **(Chantaraj, 2000; Sanchéz et al., 2002)** also confirm an increase in ash content. They proposed that mineral content increase is one of the substrate changes that are undergone during enzymatic breakdown. Ash content increase was observed

in our study. **Leatham and Stahmann (1998)** stated that Shiitake growth and development are strongly influenced by mineral concentration. **Bari et al. (2015)** studied degradation capabilities of *Pleurotus ostreatus* in beech wood. After 120 days of wood exposure to this fungus they observed utilization of hemicellulose, celullose and lignin. The most degraded polymer was hemicellulose. There is no correlation among our results for degradation of hemicellulose, cellulose and fiber because of above mentioned reasons. **Di Lena et al. (1997)** studied improving the nutritional value of wheat bran by cultivation of *Lentinula edodes*. They reported an increase of protein and total dietary fiber content after fungal growth. In our study we also observed an increase in protein and total dietary fiber content.

Inoculation of substrates with fungal spawn can bring other advantages. Denver based food company MycoTechnology created a technology called MycoSmooth. Together with grain companies they explore the influence of mushroom mycelium that could create new values in the cereals market due to nutritional profile transformation of wheat and other grains. Their patent brought process that can eliminate or reduce gluten, decrease glycaemic load of wheat and increase fiber content. The MycoSmooth process is best known for improving taste in foods such as cocoa and coffee **(Watson, 2015)**. Another patent taking advantage of agricultural materials inoculated with fungal spawns is **US20140295023 (2014)**. This patent describes how beneficial it can be to use myceliated agricultural materials in animal feeding. Regarding this patent the agricultural products or by-products are biologically converted by fungi into a valuable feedstuff material with content of protein, carbohydrates and lipids. Additionally, it brings physiological and biochemical advantages such as increased feed conversion rates, immune enhancement for resistance to disease, antibiotic and antimicrobial substitute properties. An unsuitable agricultural material is converted into suitable feedstuff with increased biological activity.

6 CONCLUSION

The outcome presented in this Master thesis is a knowledge expansion in the topics related to mushrooms cultivation on agro-industrial wastes. Two different mushroom strains were used in this study, *Lentinula* and *Pleurotus*. The cultivation of these mushrooms on beech wood chips enriched with natural and destarched wheat bran was tested. The results in this thesis react to the following questions:

Is it beneficial to enrich the substrate with wheat bran?

- Wheat bran enrichment of substrates designated for mushroom cultivation is proven to be beneficial. Many studies state successful cultivation with using wheat bran. Using wheat bran in mushroom cultivation is efficient, cheap and environmentally friendly.

What is the best proportion for enrichment?

- In our project we decided for 25% and 50% substrate enrichment. Unfortunately we cannot state which enrichment is considered to be more efficient, because we met technical problems during our project and due to this technical matters we did not observe mushroom growth. There was observed a growth of fruiting body of shiitake mushroom on the substrate enriched with 25% wheat bran.

What factors are the most important for the successful growth?

- For successful mushroom growth, a number of crucial factors were identified: water content, nutrient availability and environmental conditions. In our project we experienced the importance of humidity in the environment and in the substrate. The lower humidity in environment resulting in a water deficiency in substrates has impeded a successful cultivation.

What changes were determined in the substrate composition after mycelial growth?

- In this project were determined changes in all observed parameters. The mycelial growth resulted in a decrease in dry matter, starch and glucose contents and an increase in ash, protein, fat and fiber contents. There were changes observed in the content of cellulose, hemicellulose and lignin, but from the determined results the relation between the native substrate and substrate with mycelium could not be established.

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Appendix

Table 14 Results for ash in native substrates

Substrate	Mean	StD	rel.StD [%]
Mixture 1	2,692557	0,0309104	1,147993
Mixture 2	3,366961	0,0249935	0,742316
Mixture 3	4,576727	0,0383468	0,837864
Mixture 4	3,582829	0,0042751	0,119323
Mixture 5	4,810101	0,1006315	2,092088

Table 15 Ash analysis - Shiitake substrate

Sub. Shiitake	Mean	STABW	STABW [%]
1A	3,459213	0,0671417	1,940953
1B			
2A	4,38098	0,0664687	1,517211
2B			
3A	6,064712	0,0565525	0,932484
3B			
4A	3,893883	0,1850648	4,752707
4B			
5A	7,922793	0,3237726	4,086597
5B			

Table 16 Ash analysis - *Pleurotus* substrate

Sub. Pleurotus	Mean	STABW	STABW [%]
1A	3,045596	0,189935	6,236382
1B			
2A	4,367583	0,050367	1,153201
2B			
3A	5,62289	0,0528101	0,939199
3B			
4A	4,994059	0,0210133	0,420766
4B			
5A	7,283629	0,0584326	0,802246
5B			

Table 17 Fat analysis - native substrate

Substrate	Mean	STABW	STABW [%]
1A	0,1996044	0,0148005	7,414928958
1B			
2A	0,9591918	0,014482	1,509808655
2B			
3A	2,5416955	0,2098322	8,255598569
3B			
4A	1,1674507	0,0922186	7,899141728
4B			
5A	2,2962035	0,0858436	3,73850253
5B			

Table 18 Fat analysis - Shiitake substrate

Sub. Shiitake	Mean	STABW	STABW [%]
1A	0,2782829	0,0217101	7,80144107
1B			
2A	1,3135288	0,0758081	5,771329323
2B			
3A	4,9927698	0,1107341	2,217888822
3B			
4A	1,2279655	0,1095197	8,918796059
4B			
5A	2,4706066	0,2366523	9,578711425
5B			

Table 19 Fat analysis - *Pleurotus* substrate

Sub. Pleurotus	Mean	STABW	STABW [%]
1A	1,1361043	0,0988968	8,704905227
1B			
2A	1,0221245	0,0106069	1,037733082
2B			
3A	3,2570802	0,0196539	0,603419962
3B			
4A	6,2792465	0,1272328	2,026243023
4B			
5A	13,675638	0,4176035	3,053630663
5B			

Table 20 Protein analysis - native substrate

Substrate	Mean	STDW	STDW [%]
1A	1,1209269	0,0114664	1,0229393
1B			
2A	3,7089082	0,3329906	8,9781314
2B			
3A	7,3897584	0,5019589	6,7926301
3B			
4A	4,2002748	0,0396525	0,944046
4B			
5A	7,4121881	0,3032055	4,0906348
5B			

Table 21 Protein analysis - *Lentinula* substrate

Sub. Shiitake	Mean	STDW	STDW [%]
1A	2,3273885	0,126773	5,4470074
1B			
2A	5,5695523	0,549214	9,861008
2B			
3A	8,8632988	0,1003786	1,1325193
3B			
4A	5,4468524	0,099378	1,8245041
4B			
5	9,4840584	0,1232876	1,2999453

Table 22 Protein analysis - *Pleurotus* substrate

Sub. Pleurotus	Mean	STDW	STDW [%]
1A	1,7259299	0,1419089	8,2221719
1B			
2A	5,1783417	0,1153993	2,2284997
2B			
3A	9,8049822	0,6228129	6,3520046
3B			
4A	5,0583155	0,0399911	0,7906019
4B			
5A	10,912498	0,5141881	4,7119195
5B			

Table 23 Starch and glucose analysis - native substrate

Mean	STABW	STABW %
0,8281979	0,0251827	3,0406658
2,648371	0,0628374	2,3726806
5,4810176	0,3765308	6,8697244
0,4796404	0,0184701	3,8508242
0,262856	0,0069109	2,6291642
0,1719176	0,0106336	6,1852845

Table 24 Starch analysis - *Lentinula* substrate

	Mean	STABW	STABW %
S1	1,06	0,03	3,16
S2	1,20	0,05	4,43
S3	1,83	0,12	6,83
S4	1,40	0,03	2,27
S5	4,37	0,01	0,23
G1	0,35	0,02	5,72
G2	0,51	0,03	5,68
G3	0,56	0,02	2,87
G4	0,25	0,01	3,89
G5	0,28	0,02	6,52

Table 25 Starch analysis - *Pleurotus* substrate

Mean	STABW	STABW %
0,63	0,05	7,45
0,35	0,00	0,77
0,35	0,03	8,46
1,39	0,10	6,84
1,54	0,06	3,62
0,07	0,01	8,12
0,08	0,01	8,76
0,07	0,01	9,94
0,26	0,01	5,47
0,17	0,01	4,18

Table 26 Dry matter - native substrate

Substrate	Mean [%]	STABW	STABW [%]
1A	91,52545595	0,0722744	0,0789665
1B			
2A	91,58617649	0,2494593	0,2723765
2B			
3A	92,96037873	0,0666106	0,0716912
3B			
4A	91,77676203	0,03827	0,041699
4B			
5A	92,27209963	0,0407952	0,0442119
5B			

Table 27 Dry matter - *Lentinula* substrate

Sub. Shiitake	Mean [%]	STABW	STABW [%]
1A			
1B	67,37898331	1,516803	2,2511515
2A			
2B	71,79996455	0,2167481	0,3018777
3A			
3B	68,59453365	0,0203092	0,0296076
4A			
4B	74,75706279	0,17862	0,238934
5A			
5B	71,57140607	0,1957264	0,2734701

Table 28 Dry matter - *Pleurotus* substrate

Sub. Pleurotus	Mean [%]	STABW	STABW [%]
1A			
1B	81,5003723	0,0933553	0,1145458
2A			
2B	69,86216739	0,7247526	1,0374036
3A			
3B	66,39129508	0,071026	0,1069809
4A			
4B	86,9778961	0,9434752	1,0847298
5A			
5B	82,97534041	0,0579651	0,0698583

Table 29 NDF, ADF, ADL - native substrate

NDF, ADF, ADL				
NDF	1A	Lignin+Cellulose+Hemic	SD	VC
	1B	82,198189	0,4951314	0,6023629
	2A			
	2B	79,09407	0,7026791	0,8884094
	3A			
	3B	76,390277	0,8084062	1,0582579
	4A			
	4B	75,449186	1,0254515	1,3591286
	5A			
	5B	68,117117	0,7842237	1,1512873
ADF	1A	Lignin+Cellulose		
	1B	68,580791	1,0500472	1,5311098
	2A			
	2B	57,623023	0,5558685	0,9646639
	3A			
	3B	46,615633	0,0861697	0,1848515
	4A			
	4B	57,017188	2,0901453	3,6658162
	5A			
	5B	44,036465	0,7334415	1,6655323
ADL	1A	Lignin		
	1B	44,498066	0,4149094	0,9324211
	2A			
	2B	33,077193	1,4420682	4,3597056
	3A			
	3B	34,171954	0,7644163	2,2369699
	4A			
	4B	55,014413	2,6249933	4,7714647
	5A			
	5B	29,200237	0,1241689	0,4252325

Table 30 NDF, ADF, ADL - *Lentinula* substrate

NDF, ADF, ADL				
NDF	1A	Lignin+Cellulose+Hemic	SD	VC
	1B	59,963515	0,6530119	1,0890154
	2A			
	2B	55,884044	0,6315189	1,1300523
	3A			
	3B	50,289077	0,8603612	1,7108312
	4A			
	4B	59,768717	1,8775673	3,141388
	5A			
	5B	64,829666	2,8023558	4,3226441
ADF	1A	Lignin+Cellulose		
	1B	56,092188	0,1463349	0,2608829
	2A			
	2B	48,018968	2,2391185	4,6629875
	3A			
	3B	41,539762	0,2027043	0,4879766
	4A			
	4B	47,753296	0,0065698	0,0137577
	5A			
	5B	44,21679	0,0473012	0,1069756
ADL	1A	Lignin		
	1B	10,419377	0,133009	1,2765545
	2A			
	2B	12,996136	0,1090263	0,8389134
	3A			
	3B	9,3870557	0,645137	6,872624
	4A			
	4B	47,200384	3,2561536	6,8985745
	5A			
	5B	19,071476	0,5540823	2,9052933

Table 31 NDF, ADF, ADL - *Pleurotus* substrate

NDF, ADF, ADL				
NDF	1A	Lignin+Cellulose+Hemic	SD	VC
	1B	77,918204	0,3197289	0,4103391
	2A			
	2B	61,346222	0,0817296	0,1332267
	3A			
	3B	53,962892	0,4150214	0,7690866
	4A			
	4B	70,104792	0,0426567	0,060847
	5A			
	5B	61,992798	0,1219386	0,1966981
ADF	1A	Lignin+Cellulose		
	1B	61,817721	2,1460995	3,4716575
	2A			
	2B	47,340194	1,6007113	3,3812943
	3A			
	3B	37,230369	0,7168116	1,925341
	4A			
	4B	54,465634	0,8263522	1,5171994
	5A			
	5B	42,217753	1,4356135	3,4004973
ADL	1A	Lignin		
	1B	52,613655	6,2766453	11,929689
	2A			
	2B	24,312942	0,6214047	2,5558595
	3A			
	3B	21,058577	2,181776	10,36051
	4A			
	4B	29,201104	0,0445027	0,1524008
	5A			
	5B	33,677965	3,5584455	10,566094

Table 32 NDF, ADF, ADL - native and destarched bran

ADL	1,1	Lignin	SD	VC
	1,2	4,7	0,18	3,8335746
	2,1			
	2,2	8,1	0,76	9,4430103
ADF	3,1	Lignin+Cellulose		
	3,2	16,9	0,26	1,562246
	4,1			
	4,2	24,3	0,07	0,2976975
NDF	5,1	Lignin+Cellulose+Hemi		
	5,2	60,1	0,74	1,2233814
	6,1			
	6,2	80,9	0,15	0,1875314

Table 33 Summary of all substrates

	NDF	ADF	ADL
Bran nativ	65,95628892	18,53077632	5,175881289
Bran destarched	81,38755173	24,40785122	8,103603242
Substrate 1	89,80910101	74,93083805	48,6182403
Substrate 2	86,36027045	62,91672517	36,11592355
Substrate 3	82,17509245	50,14570074	36,75969715
Substrate 4	82,20946645	62,12595302	59,94372896
Substrate 5	73,82200839	47,72457232	31,64579184
Shiitake 1	88,99438929	83,24879025	15,46383834
Shiitake 2	77,83296871	66,87881886	18,10047681
Shiitake 3	73,31353482	60,55841423	13,68484515
Shiitake 4	79,9505959	63,87797258	63,13836069
Shiitake 5	90,5804005	61,77996598	26,64678175
Pleurotus 1	95,60472204	75,84961784	64,55633736
Pleurotus 2	87,81036218	67,76227548	34,80129966
Pleurotus 3	81,28007091	56,07718516	31,71888275
Pleurotus 4	80,60069852	62,62008625	33,57301735
Pleurotus 5	74,71231495	50,87987938	40,58792049

Table 34 Hemicellulose, cellulose and lignin

	Hemicellulose NDF-ADF	Cellulose ADF-ADL	Lignin
Bran nativ	47,43	13,35	5,18
Bran destarched	56,98	16,30	8,10
Substrate 1	14,88	26,31	48,62
Substrate 2	23,44	26,80	36,12
Substrate 3	32,03	13,39	36,76
Substrate 4	20,08	2,18	59,94
Substrate 5	26,10	16,08	31,65
Shiitake 1	5,75	67,78	15,46
Shiitake 2	10,95	48,78	18,10
Shiitake 3	12,76	46,87	13,68
Shiitake 4	16,07	0,74	63,14
Shiitake 5	28,80	35,13	26,65
Pleurotus 1	19,76	11,29	64,56
Pleurotus 2	20,05	32,96	34,80
Pleurotus 3	25,20	24,36	31,72
Pleurotus 4	17,98	29,05	33,57
Pleurotus 5	23,83	10,29	40,59

Table 35 Fiber analysis - *Lentinula* substrate 1

	[%]	[% i. d. DM]
1	59,80	88,75
2	60,00	89,05
Mean	59,90	**88,90**
Standard deviation	0,14	0,21
Variability c.	0,239144115	0,239144115

Table 36 Fiber analysis - *Lentinula* substrate 2

	[%]	[% i. d. DM]
1	62,74	87,38
2	62,77	87,42
Mean	62,75	**87,40**
Standard d.	0,02	0,03
Variability c.	0,032816835	0,032816835

Table 37 Fiber analysis - *Lentinula* substrate 3

	[%]	[% i. d. DM]
1	58,22	84,88
2	56,86	82,89
Mean	57,54	**83,88**
Standard d.	0,97	1,41
Variability c.	1,678457007	1,678457007

Table 38 Fiber analysis - *Lentinula* substrate 4

	[%]	[% i. d. DM]
1	64,20	85,88
2	64,25	85,94
Mean	64,23	**85,91**
Standard d.	0,03	0,04
Variability c.	0,048427196	0,048427196

Table 39 Fiber analysis - *Lentinula* substrate 5

	[%]	[% i. d. DM]
1	67,74	94,65
2	67,66	94,54
Mean	67,70	**94,59**
Standard d.	0,06	0,08
Variability c.	0,082594035	0,082594035

Table 40 Fiber analysis - *Pleurotus* substrate 1

	[%]	[% i. d. DM]
1	72,14	88,51
2	73,01	89,59
Mean	72,57	**89,05**
Standard d.	0,62	0,76
Variability c.	0,856048577	0,856048577

Table 41 Fiber analysis - *Pleurotus* substrate 2

Ansatz	[%]	[% i. d. DM]
1	63,90	91,47
2	64,43	92,22
Mittelwert	64,17	**91,85**
Standardabw.	0,37	0,53
VC	0,57651261	0,57651261

Table 42 Fiber analyse - *Pleurotus* substrate 3

	[%]	[% i. d. DM]
1	52,60	79,22
2	52,45	79,00
Mean	52,52	**79,11**
Standard d.	0,10	0,16
Variability c.	0,199208389	0,199208389

Table 43 Fiber analysis - *Pleurotus* substrate 4

	[%]	[% i. d. DM]
1	72,90	83,81
2	73,05	83,99
Mean	72,98	**83,90**
Standard d.	0,11	0,13
Variability c.	0,152398343	0,152398343

Table 44 Fiber analysis - *Pleurotus* substrate 5

	[%]	[% i. d. DM]
1	64,27	77,45
2	64,52	77,76
Mean	64,39	**77,61**
Standard d.	0,18	0,21
Variability c.	0,27670743	0,27670743

Table 45 Fiber analysis - pure substrate 1

	[%]	[% i. d. DM]
1	84,55	92,38
2	85,39	93,30
Mean	84,97	**92,84**
Standard d.	0,60	0,65
Variability c.	0,70191787	0,70191787

Table 46 Fiber analysis - pure substrate 2

	[%]	[% i. d. DM]
1	80,17	87,53
2	79,56	86,87
Mean	79,86	**87,20**
Standard d.	0,43	0,47
Variability c.	0,539871751	0,539871751

Table 47 Fiber analysis - pure substrate 3

	[%]	[% i. d. DM]
1	72,55	78,04
2	73,58	79,15
Mean	73,06	**78,60**
Standard d.	0,73	0,79
Variability c.	1,001057573	1,001057573

Table 48 Fiber analysis - pure substrate 4

	[%]	[% i. d. DM]
1	77,45	84,39
2	76,35	83,20
Mean	76,90	**83,79**
Standard d.	0,77	0,84
Variability c.	1,003973625	1,003973625

Table 49 Fiber analysis - pure substrate 5

	[%]	[% i. d. DM]
1	66,71	72,30
2	67,26	72,90
Mean	66,99	**72,60**
Standard d.	0,39	0,42
VC	0,58008909	0,58008909

Table 50 Fiber analysis - native bran

	[%]	[% i. d. DM]
1	46,58	51,13
2	40,10	44,02
Mean	43,34	**47,58**
Standard d.	4,58	5,03
VC	10,5728005	10,5728005

Table 51 Fiber analysis - destarched bran

	[%]	[% i. d. DM]
1	62,60	62,98
2	61,77	62,15
Mean	62,18	**62,57**
Standard d.	0,58	0,59
VC	0,935532465	0,935532465

Table 52 Fat analysis - Native/ Destarched bran

	Mean [%]	STDEV	VC
Native bran	5,491167	0,411363	7,491365
Destarched bran	5,281998	0,435457	8,244163

Table 53 Protein analysis native/ destarched bran

	Mean [%]	SDTEV	VC
Native bran	8,11	0,11889	1,466198
Destarched bran	13,16026	0,087499	0,664874

Table 54 Ash analysis - Native/ destarched bran

	Mean	STDEV	SVC [%]
NB1			
NB2	6,14316	0,136104	2,21553
DB1			
DB2	4,893971	0,056055	1,145399

Table 55 Starch analysis - native/ destarched bran

	Mean	STABW	VC
Starch			
	10,7	0,43	4,006456
	35,1	3,45	9,810957
Glucose			
	6,8	0,63	9,32826
	29,0	2,79	9,607075
Crtl Starch			
	76,1	0,83	1,086337

Table 56 Dry matter analysis - native/destarched bran

Sample	Mean	STDEV	VC
Native bran A			
Native bran B	91,09	0,01578079	0,01732411
Dest. Bran dried overnight A			
Dest. Bran dried overnight B	99,3900944	0,15574798	0,15670372