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White button mushroom (*Agaricus bisporus*) cultivation technique and farm project for a developing country

Bachelor Thesis

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Declaration

I, Dennis Kamelin, declare that the Bachelor Thesis “White button mushroom (Agaricus bisporus) cultivation technique and farm project” is my own work and all the sources I cited in it are listed in Bibliography.

Göttingen, 18.04.2019 Signature

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Summary

Mushrooms represent a small branch in the evolution of the fungal kingdom Eumycota and are commonly known as the "fleshy fungi". Mankind has collected wild mushrooms for thousands and thousands of years. The White button mushroom, *Agaricus bisporus*, was domesticated in France. The first descriptions date from 1707. Cultivation of *A. bisporus* rapidly spread after the second World War when reliable spawn became commonly available in a number of countries.

Mushrooms are the fruit of the mushroom plant, the mycelium. A mycelium is a vast network of interconnected cells that permeates the ground and lives perennially. During few weeks of fruiting, the mycelium is in a frenzied state of growth, amassing nutrients and forming dense ball-like masses called primordia that eventually enlarge into the towering mushroom structure, which is the main consumption product.

The starting culture can be made from a fresh and healthy fruit body or obtained from a type collection. Most growers start a culture from spores. Tissue culture is another assured method of preserving the exact genetic character of a living mushroom. Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilized substrates under aseptic conditions. All the stages of mushroom production after spawn is ready have to be carefully planned because these steps determine the farm layout. Because the project farm includes all the production phases it is important to decide the number of rooms and if various stages of growing will be done in the same room with changing conditions (substrate doesn’t need to be transported between rooms) or in different rooms with stabile conditions suitable for a single phase (transport of substrate between rooms).

Project farm is located in Kazakhstan as an example of a developing country with very low mushroom production, location is chosen with respect to market distance and infrastructure costs. Investments are kept as low as possible.

Key words: Mushroom cultivation, Agaricus bisporus, developing country, horticulture, project farm.

1 Introduction to mushroom production

Mankind has collected wild mushrooms for thousands and thousands of years. They have been used for religion purposes and as food. Even in present times more wild species are being collected for consumption, then are cultivated. For centuries, the edible mushroom defied cultivation. In the 16th century, some European scientists discovered the function of the spores, but their knowledge did not spread much for centuries. But the first mention of mushroom cultivation was mentioned as early as 1313 in a Chinese document describing cultivation of Shiitake on wood logs. The White button mushroom, *Agaricus bisporus*, was domesticated in France. The first descriptions date from 1707. Cultivation of *A. bisporus* rapidly spread after the second World War when reliable spawn became commonly available in a number of countries.

Neither plant-like nor animal-like, mushrooms have a texture, appearance and manner of growth all their own. Mushrooms represent a small branch in the evolution of the fungal kingdom Eumycota and are commonly known as the "fleshy fungi". The primary role of fungi in the ecosystem is decomposition, one organism in a succession of microbes that break down dead organic matter.

Mushroom production has many advantages: no arable land is needed, agricultural waste is converted into fertilizers and soil conditioners, it is income-generating and mushrooms provide an extra source of protein and minerals and valuable vitamins (BEETZ, KUSTUDIA, 2004, p. 1).

Kazakhstan as location for the projected farm was chosen partly regarding origin of author, but mainly on basis of very low volume of mushroom production and market in the named country. More specific data further below.

2 Objectives of the work

The main goals of this thesis can be divided into several parts which follow step by step in progression. As basis, principles of industrial cultivation of *Agaricus bisporus* should be studied. After obtaining basic knowledge about all production steps, layout decision should be made prior to establishment of a business plan of the projected farm. Additionally, some practical skills and knowledge in mycology were gathered that are directly related to the topic and specifically to first few steps of production, which will be shown in a separate chapter.

3 *Agaricus bisporus* cultivation

Having a basic understanding of the mushroom life cycle greatly aids the learning of techniques essential to cultivation. Mushrooms are the fruit of the mushroom plant, the mycelium. A mycelium is a vast network of interconnected cells that permeates the ground and lives perennially. This resident mycelium only produces fruitbodies, what are commonly called mushrooms, under optimum conditions of temperature, humidity and nutrition. For the most part, the parent mycelium has but one recourse for insuring the survival of the species: to release enormous numbers of spores. This is accomplished through the generation of mushrooms. In the life cycle of the mushroom plant, the fruitbody occurs briefly. The mycelial network can sit dormant for months, sometimes years and may only produce a single flush of mushrooms. During those few weeks of fruiting, the mycelium is in a frenzied state of growth, amassing nutrients and forming dense ball-like masses called primordia that eventually enlarge into the towering mushroom structure. The gills first develop from the tissue on the underside of the cap, appearing as folds, then becoming blunt ridges and eventually extending into flat, vertically aligned plates. These efficiently arranged symmetrical gills are populated with spore producing cells called basidia. From a structural point of view, the mushroom is an efficient reproductive body. The cap acts as a domed shield protecting the underlying gills from the damaging effects of rain, wind and sun. Covering the gills in many species is a well developed layer of tissue called the partial veil which extends from the cap margin to the stem. Spores start falling from the gills just before the

partial veil tears. After the partial veil has fallen, spores are projected from the gills in ever increasing numbers. The cap is supported by a pillar-like stem that elevates the gills above ground where the spores can be carried off by the slightest wind currents. Every part of the mushroom fruitbody is designed to give the spores the best opportunity to mature and spread in an external environment that is often harsh and drastically fluctuating. As the mushroom matures, spore production slows and eventually stops. At this time mushrooms are in their last hours of life. Soon decay from bacteria and other fungi sets in, reducing the once majestic mushroom into a soggy mass of fetid tissue that melts into the ground from which it sprung (STAMETS, CHILTON, 1983, pp. 4-6).

Since mushrooms lack chlorophyll they cannot, like green plants, get their energy from the sun radiation through photosynthesis. Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate. The degraded compounds are then absorbed by the hyphae and the mycelium enlarges usually laterally, and in some cases growing several meters in diameter with the substrate. Partially understood environmental factors stimulate the second or reproductive growth stage. Cells of one mycelial strain fuse with cells of the opposite type to form a mycelium that contains both types of nuclei. The new mycelium continues to grow and eventually develops into a mature fruiting body, the gills of which are lined with spore bearing cells basidii. Various mechanisms trigger the dispersal of spores, which in turn lodge in a substrate, become hyphae and begin the cycle anew.

Cultivation of the White button mushroom can include several different stages depending on supply with culture, spawn, premade or already inoculated substrate. This project farm will include the complete growing cycle in one facility due to local issues that will be described in following sections. The main challenge is that due to low development of mushroom sector in the country there is a very little chance that a good quality spawn is produced regularly in Kazakhstan and can be purchased. The same situation is on the market of substrates. As a farm in a developing country should be not of high establishment costs a decision is made not to import substrate from

other countries, also because of the huge distances and related transportation costs. Therefore, spawn and substrate should be produced on the growing facility.

3.1 Obtaining culture

The very first step in every mushroom production is obtaining a starting culture. The starting culture can be made from a fresh and healthy fruit body or obtained from a type collection. It is problematic due to fact that Kazakhstan legislation prohibits sending of any part of mushroom via post. Young and vigorous mycelium can be obtained from a young fruit body (preferably in button stage), but this is related to risk of growing not an optimal strain as there could be a significant variability in characteristics inside one strain. That's why the best option is to obtain a culture from some local universities with microbiology or mycology laboratories. It guarantees a good quality of culture for experiments and model farm. A fresh starting culture should be obtained every 3 months to exclude the possibility of strain degradation which can happen already after 4 cycles of spawn production (APCAEM, 2019, p. 37).

A short experiment with the aim to obtain fresh culture from wild *Agaricus* mushrooms was undertaken, description follows in separate chapter.

3.2 Producing spawn

A mushroom culture can be started in one of two ways. Most growers start a culture from spores. The advantage of using spores is that they are viable for weeks to months after the mushroom has decomposed. The other way of obtaining a culture is to cut a piece of interior tissue from a live specimen, in effect a clone. Tissue cultures must be taken within a day or two from the time the mushroom has been picked, after which a healthy clone becomes increasingly difficult to establish.

To collect spores, the cap from the stem of a fresh, well cleaned mushroom is severed and placed gills down on a piece of clean white paper or a clean glass surface such as a microscope slide. If a specimen is partially dried, a drop or two of water is added to the cap surface to aid in the release

of spores. To lessen evaporation and disturbance from air currents, a cup or glass is placed over the mushroom cap. After a few hours, the spores will have fallen according to the radiating symmetry of the gills. If the spore print has been taken on paper, it should be cut out, folded in half, seal in an airtight container and label the print with the date, species and collection number. When using microscope slides, the spores can be sandwiched between two pieces of glass and taped along the edges to prevent the entry of contaminant spores. A spore print carelessly taken or stored can easily become contaminated, decreasing the chance of acquiring a pure culture. Once a spore print is obtained, mushroom culture can begin. Sterilize an inoculating loop or scalpel by holding it over the flame of an alcohol lamp or butane torch for five or ten seconds until it is red hot. (If a butane torch is used, turn it down to the lowest possible setting to minimize air disturbance). Cool the tip by inserting it into the sterile media in a petri dish and scrape some spores off the print. Transfer the spores by streaking the tip of the transfer tool across the agar surface. A similar method calls for scraping the spore print above an opened petri dish and allowing them to freefall onto the medium. When starting a new culture from spores, it is best to inoculate at least three media dishes to improve the chances of getting a successful germination. Mycelium started in this manner is called a multispore culture.

Tissue culture is another assured method of preserving the exact genetic character of a living mushroom. In tissue culture a living specimen is cloned whereas in multispore culture new strains are created. Tissue cultures must be taken from mushrooms within twenty-four to forty-eight hours of being picked. If the specimens are several days old, too dry or too mature, a pure culture will be difficult to isolate. Spores, on the other hand, can be saved over long periods of time (STAMETS, CHILTON, 1983, pp. 27-29).

Basically, spawn production is nothing more than putting mycelium of the desired mushroom in suitable sterilized substrates under aseptic conditions (OEI, 2016, p. 105). From this starting culture another agar cultures are made. These serve to inoculate larger containers (like bottles) with spawn and these can be used to inoculate the final spawn substrate. The cap, the upper region of the stem and/or the area where the gill plate joins the underside of the cap are the best

locations for excising clean tissue. Transfer the tissue fragment to the center of the nutrient filled petri dish as quickly as possible, exposing the tissue and agar to the open air for a minimal time. Repeat this technique into at least three, preferably five more dishes. Label each dish with the species, date, type of culture (tissue) and kind of agar medium. If successful, mycelial growth will be evident in three to seven days .

The minimal requirements for a spawn production unit are:

- a sterilization unit (pressure cooker, autoclave),
- sterile environment: inoculation box, laminar flow cabinet or clean room,
- incubation rooms,
- laboratory equipment like petri dishes, test tubes, scales, alcohol, flame.

Raw materials required:

- elements for media preparation,
- substrate material (grain, sawdust etc.),
- spawn containers.

Sterilization unit serves to sterilize substrate materials. For example, a simple grain kernel may contain an estimated 100.000 bacteria, 25.000 fungi and more than 400.000 actinomycetes. Most spores of fungi are effectively killed at temperatures around 100 ^{o}C. Bacteria are more resistant to heat, especially the endospore-forming bacteria. Theoretically, 15 minutes at 121 ^{o}C should be sufficient to kill all organisms.

The best choice for a medium scale production of spawn is a laboratory autoclave. They can process 100 to 300 liters of substrate in one batch. Vertical autoclaves are more comfortable, because they can be filled easier. The best option is to have an autoclave with two doors. In that case, the autoclave is filled at the ‘dirty’ side, and the opposite door is opened in a clean room after sterilization. Still, one door autoclaves can perform well if hygienic conditions are considered carefully. A good choice is a used hospital autoclave, because a new one is rather expensive because it can operate under higher pressure than needed for mushroom cultivation.

A clean environment is absolutely essential to spawn production. Especially the stages at which the containers with sterilized media are opened have to be performed under aseptic conditions. For medium-scale production mostly suitable is a laminar flow cabinet. It consists of a fan, a duct, filters and the laminar flow hood. The advantage is that contaminants can spread in only one direction. HEPA (High Efficiency Particulate Air) filters are sufficient as they are rated 99,99% for 0,3 micron particles, which means only one out of every 10000 particles of that size will pass the filter. The lifetime of the HEPA filter is generally as long as that of the complete clean room (OEI, 2016, p. 110).

The inoculated spawn substrates have to be incubated for about two weeks for grain spawn. The interior of an incubation room should consist of non-biodegradable materials that are easy to clean. Spawn can be placed on shelves made of galvanized iron or Formica. Around 60 kilos (90 liters) of spawn can be placed per m^2. 1800 liters of grain substrate can be put in the same incubation room each week. Not big containers will be used (less than 5 L) so temperature of the room must be sustained on approximately the same level as substrate temperature due to mycelium growth and heat radiation. There should be some ventilation to avoid ambient CO_2 levels exceeding 1000 ppm. Humidity of the room is not affecting the growth so much as the humidity level of the substrate. Humidity inside the spawn containers should be high enough to prevent the top layer of substrate from drying out. Room humidity should be low (30-50% relative humidity) to avoid moist conditions, in which spores of contaminants could germinate (OEI, 2016, p. 115).

Many media have been developed to which support mycelial growth of fungi. Due to the fact that Kazakhstan agriculture specializes on wheat production, wheat agar will be used as growing media. Ingredients: 32 g wheat kernels, 1 liter water, 20 g agar. Wheat is to boil in 1 liter of water for 2 hours. The broth is to be filtered after 25 hours and water is to be added to exactly 1 liter. Than agar is added to solidify the broth. After that test tubes or Petri dishes can be filled and sterilized for 30 minutes at 15 psi. There is no need for sterile laboratory agar, common consumption agar is suitable and much cheaper. For small-scale laboratory simple pressure

cookers are used. For controlling bacteria, 0.10 grams of 60-80% pure gentamycin sulfate can be added to each liter of media prior to sterilization. Water quality—its pH and mineral content—varies from region to region. If living in an area of questionable water purity, the use of distilled water is advisable. For all practical purposes, however, tap water can be used without harm to the mushroom mycelium. The pH of media can be altered by adding a drop at a time of 1 molar concentration of hydrochloric acid (HCL) or sodium hydroxide (NaOH). The medium is thoroughly mixed and then measured using a pH meter or pH papers. One molar HCL has a pH of 0; one molar NaOH has a pH of 1 2; and distilled water has a pH of 7 (STAMETS, CHILTON, 1983, p. 20).

Once a pure culture is obtained it has to be multiplied. The originally isolated culture is labeled T1, next tubes T2 (isolated from T1) and so on. It is advisable not to transfer more than eight times (T8).

Spawn containers should be made out of resistant material, mostly glass and polypropylene. Wide-mouthed jars, milk bottles and dextrose bottles can be used. Dextrose bottles are ideal, because they can be obtained for free from hospitals and they have air outlets that can easily be plugged with cotton wool.

In simple laboratories, grain mother spawn should not be used to inoculate another generation of grain mother spawn because the risk of contamination and degeneration will become too great.

The final spawn substrate is specific for different mushroom species. For *Agaricus bisporus* and *A. bitorquis* grain substrate with cultivation method on fermented substrate are used most frequently. Before inoculation of the final spawn it is necessary to check the mother spawn again for contamination. The grain spawn has to be broken in the bottles by slamming them against the palm of the hand or shaking them against a bald tire. The risk of contaminant dropping in is lowered when the containers are placed horizontally, and a spoon is used to inoculate.

The main advantage of grain is that it is very nutritious for fungi and forms kernels easily. The kernels can easily be dispersed in the substrate. It is also cheap in mostly cereals focused agriculture in Kazakhstan. The main disadvantage is that it provides an optimal substrate for other

organisms, too. A good quality of the grain is very important. It should contain only few broken kernels, few bacterial endospores, little extraneous debris and be recently harvested. Moisture content should be around 50%. Two-hour long sterilization is usually sufficient for 500 g containers; 3 kg bags have to be sterilized for about three to four hours. After the temperature in the center of the container has dropped to below the maximum mycelial growth temperature, the spawn containers can be inoculated, At least one (for 250 ml bottles) or two (for bigger bottles) squares of 10 x 10 mm2 from the full-grown agar of the mother culture for each bottle. Two weeks of incubation are necessary. The spawn must be kept in the refrigerator and only taken out when needed. Grain spawn can spoil in one night at temperatures above 25 ^{o}C (OEI, 2016, p. 121).

Refrigerated spawn can be kept for up to six months after complete colonization of the spawn substrate.

However, spawn production is rather complicated and at least one year is needed to obtain expertise in production of good quality spawn. In that time a research facility could be running. Government and developing mushroom industry may jointly finance such facility. The following fields should be profitable for investigation: development of substrate production techniques, development or conservation of strains and species, testing different substrates, determining optimal growing conditions, optimizing climate control systems, pest and disease control, performing regular cost/benefit analyses for the sector.

3.3 Producing substrate. Layout decision

All the stages of mushroom production after spawn is ready have to be carefully planned because these steps determine the farm layout. Because the project farm includes all the production phases it is important to decide the number of rooms and if various stages of growing will be done in the same room with changing conditions (substrate doesn’t need to be transported between rooms) or in different rooms with stabile conditions suitable for a single phase (transport of substrate between rooms). Theoretically transport should be kept to a minimum to avoid labor

and risk of contamination. On the other hand, the erection of rooms purposely built for only one stage of the process has proven to give the best results (OEI, 2016, p. 200). A very important factor is standardization of growing rooms because it will be easier to control conditions in uniform rooms, and appropriate technic is developed for standardized dimensions of the rooms.

For the substrate preparation several rooms are needed. At first, dry substrate has to be stored. It must be kept dry and shielded from outside conditions like rain and wind, which can consist contaminating ingredients. Size depend on the amount of materials that are going to be stored.

Second room is designed for composting (fermentation). It must have concrete floor that is inclined (0,5 cm per meter) to collect percolate water. Plenty of water should be available. Compost is formed into piles by pile formers: wooden planks, one for each side of heap, 1.20 to 1.50 m high and 2.5 m long. Pitch forks with long handles are used to turn the compost heap. A sprinkler or hose with spray nozzle serves for water distribution. Long-stemmed thermometers are used to control temperature inside the piles. The size of the floor depends on the dimensions of the heaps. For *Agaricus*: the heaps are 2 meters wide with a variable length. Between the heaps at least 1 meter is necessary for turning by machines. If turned by hand, one heap should have additional space of 4 meters on one side. One meter of the heaps will result in about 1.5 tons of compost. Mixing and moistening of substrate ingredients can be done with substrate mixers. About 21 m^2 is needed to mix three tons of substrate (STAMETS, 1993, p. 455).

In the projected farm short composting technique will be used. It lasts around 20 days and consists of adding straw, horse manure and water. Straw provides a compost with carbohydrates, the basic food stuffs of mushroom nutrition.

Wheat straw is 36% cellulose, 25% pentosan and 1 6% lignin. Cellulose and pentosan are carbohydrates which upon break down yield simple sugars. These sugars supply the energy for microbial growth. Lignin, a highly resistant material also found in the heartwood of trees, is changed during composting to a "Nitrogen-rich-lignin-humus-complex", a source of protein. In essence, straw is a material with the structural and chemical properties ideal for making a mushroom compost. After first turning gypsum is also added. The compost prepared this way is

ready for filling when the color has changed to a uniform deep brown, there is no excessive water, pH is 8.0 to 8.5, uniformly distributed white flecks of Actinomycetes can be seen, and the C/N ratio has been reduced to 20. It may still have a strong smell of ammonia. Horse manure content, if rich on wheat straw, is cheaper than straw, because farmers have to pay for the horse manure disposal, therefore it could be a good possibility for Kazakhstani mushroom growers. When cereal straw is gathered from horse stables, it is called "horse manure". Although cultivators call it by this name, the material is actually 90% straw and 10% manure. This "horse manure" includes the droppings, urine and straw that has been bedding material. The quality of this material depends on the proportions of urine and droppings present, the essential elements nitrogen, phosphorous and potassium being contained therein. The reason horse manure is favored for making compost is the fact that fully 30-40% of the droppings are comprised of living microorganisms. These microorganisms accelerate the composting process, thereby giving horse manure a decided advantage over other raw materials (STAMETS, CHILTON, 1983, pp. 78-79).

After composting, the compost must be put on the shelves for pasteurization. In case of projected farm, the substrate will be placed in trays which can be easily moved. For better outcome movable shelves on wheels or carts, further mechanization with conveyor belt could be implemented.

The functions of pasteurization are to make the substrate more selective for *Agaricus* (by eliminating of ammonia NH_3 and creating a suitable climate for specific micro-organisms) and to eliminate eggs and larvae of harmful insects, as well as nematodes (OEI, 2016, p. 207).

For the pasteurization (peak heating) and subsequent conditioning phase a steam boiler is needed. It can proceed in the same room if it is well-insulated. Compost for White button mushroom compost in poorly insulated mushroom houses is difficult to pasteurize – the heat leaks away and the compost doesn't reach the necessary high temperature. This room may have been dug in the ground. Pasteurization by steam proceeds in beds with a steam boiler of sufficient capacity (for wheat straw substrate: 1.1 kW per ton). 1 m^3 of substrate can contain about 400-500 kg of substrate. Steam is blown into the mushroom house to reach the desired temperature of 60 ^oC. It is possible to add wheat bran one day before filling or even during filling. The

microorganisms in the compost will use these nutrients very quickly, thus raising the temperature of the compost. This will speed up and lengthen pasteurization process, thus saving on energy costs during peak heating.

It has been shown that the quality of the compost for *Agaricus* is positively correlated with the amount of biomass of a thermo-tolerant fungus, *Scytalidium thermophilum*. This is one of the reasons why *Agaricus* compost preparation is time-consuming. All commercially viable techniques consist of pre-wetting stage, a fermentation stage and a heat treatment. An additional treatment (conditioning) can further increase the quality of compost.

Next step is spawning of pasteurized substrate, it should run preferably in a closed room with overpressure and filtered air, which can be the same room. For the spawn run, however, a shielded from insects and airborne spores room with constant temperature is needed. Substrates can be stacked more densely (up to twice the amount) during spawn run than during cropping. A lot of heat is generated during mycelium growth, so demand on cooling can depend on the used technique, outside temperatures and how much substrate is packed into the room.

Grain spawn is most commonly used in *Agaricus* cultivation. It is mixed through the compost with a small fork. Approximately 1.2 to 2 liters of spawn are used per m^2 when 100 to 140 kg/m^2 compost are filled. If the compost cools down below 20 ^oC the yields will suffer (STAMETS, 1993, p. 457).

3.4 Growing fruit bodies and harvesting

The most common indoor cultivation method is the shelf system. In this system, shelves form a platform upon which the mushroom growing substrate is placed. The development of the tray system in Agaricus culture is largely due to the work of Dr. James Sinden. In direct contrast to anchored static shelves, trays are individual cropping units that have the distinct advantage of being mobile. This mobility has made mechanization of commercial cultivation possible. Automated tray lines are capable of filling, spawning and casing in less time, with fewer people and with better quality management.

Whereas in the shelf system all stages of the cultural cycle occur in the same room, the tray system utilizes a separate room for Phase II composting. On a commercial tray farm only the Phase II room is equipped for steaming and high velocity air movement. The real advantage of the tray system is the ability to fill, spawn and case single units in an unrestricted environment outside the actual growing room. The tray system also gives the cultivator more control over hygiene and improves the efficiency of the operation. Moving trays from room to room does present contamination possibilities; therefore, the operations room must also be clean and fly tight for spawning and casing. Because there is no fixed framework in the growing room, it is easily cleaned and disinfected. The tray method has many distinct advantages over the mason jar method for home cultivators preferring to fruit mushrooms on sterilized grain. These advantages are (STAMETS, CHILTON, 1983, pp. 65-66):

- fewer necessary spawn containers;
- fewer aborts due to uncontrolled primordia formation between the glass/grain interface;
- ease of picking and watering;
- better ratio of surface area to grain depth; and comparatively higher yields on the first and second flushes.

Present day Agaricus farms integrate heating, cooling and humidification equipment into the air handling system and in this way are able to achieve balanced conditions throughout the growing room.

Filtered fresh air enters the room at the mixing box where it is proportionally regulated with recirculated air by a single damper. To prevent leakage during spawn running and pre-pinning, the damper fits tightly against the fresh air inlet. This allows full recirculation of room air to maintain even conditions, thereby counteracting temperature and CO2 stratification. When fresh air is required, the damper can be adjusted to any setting, including complete closure of the recirculation inlet. As fresh air is introduced, room air is displaced and evacuated through an exhaust vent or cracks around the door. Because fresh air is generally at a different temperature

than the one required for the growing room, it must be used judiciously in order to avoid disrupting the growing room environment or overworking the heating, cooling and humidification systems. By properly mixing the fresh outside air and the room air, a balance can be achieved and optimum conditions for mushroom growth prevail.

Fresh air serves many important functions in mushroom culture, primarily by supplying oxygen to the growing mushrooms and carrying away CO2. Fresh air also facilitates moisture evaporation from the cropping surface. To determine the exact amount of air needed in a given situation, a knowledge of the CO2 requirements for the species being grown is necessary. The fresh air can also be measured in terms of air changes per hour, a common way mushroom growers size the fan in the growing room. Axial flow and centrifugal fans are the two most commonly used in mushroom houses. Both fans operate well against high static pressure, which is a measure of the resistance to forced air. Static pressure is measured in inches of water gauge—the height in inches to which the pressure lifts a column of water—and is caused by filters, heating and cooling coils or other obstructions to the free flow of air. crop. Their function is to screen out atmospheric dust particles like smoke, silica, soot and decayed biological matter. Atmospheric dust also contains spores, bacteria and plant pollen, some of which are detrimental to mushroom culture. Furthermore, spores and microorganisms originating within the cropping room can also be spread by air movement. To counteract this danger, some mushroom farms filter recirculated air as well. Agaricus growers commonly use high efficiency, extended surface, dry filters. These filters are of pleated or deep fold design which gives them much more surface area than their frame opening. They filter out 0.3 micron particles with 90-95.% efficiency and 5.0 micron particles with an efficiency of 99% at an initial resistance of 0.10 to 0.50 inches of static pressure.

High efficiency particulate air (HEPA) filters are even more efficient than those just described and are cost effective for the home cultivator. They screen out particulates down to 0.1 -0.3 microns with a rated 99.96% to 99.99% efficiency and have a resistance of 0,75-1,0 inches of static pressure.

HEPA filters are made of a variety of materials, depending on their intended application. Most HEPA filters operate in environments of up to 80% humidity without disintegration. Special "waterproof" filters operable in 1 00% humidify environments can also be purchased at little or no extra expense. These "waterproof" filters are especially appropriate for use with systems that push recirculated air through the filter. (STAMETS, CHILTON, 1983, pp. 68-72).

Heating systems for cropping rooms can be based on either dry heat or live steam. Dry heat refers to a heating source that lowers the moisture content of the air as it raises the temperature. These systems utilize either hot water or steam circulating through a closed system of pipes or radiator coils. Heating systems can also be simple resistance coils or baseboard electric heaters. Heat supplied by live steam has the advantage of keeping the humidity high while raising the temperature of the room. If regulated correctly, steam can maintain the temperature and relative humidity within the required ranges without drawing upon other sources. Nevertheless, a backup heat source is advantageous in the event humidity levels become too high.

Few organisms are as sensitive to fluctuations in the environment as mushrooms. A matter of a few degrees in temperature or humidity can dramatically influence the progression of fruiting and affect overall yields. To adequately monitor the growing environment, quality equipment is essential for accurate readings. This equipment should include maximum-minimum thermometers to gauge temperature fluctuations and a hygrometer or a sling psychrometer for measuring humidity. Hygrometers should be periodically calibrated with a sling psychrometer to insure accuracy. Thermometers also should be checked as there are occasional irregularities. Other more advanced, expensive but not absolutely essential equipment helpful to mushroom growers include: CO2 detectors; moisture meters; anemometers; and light measuring devices (KASHANGURA, 2004, p. 25).

After the mycelium has grown through the compost, a casing soil must be applied. Fully grown *Agaricus* compost hardly gives rise to any fruit body formation if there is no casing soil (a top

layer with a different composition than the compost) applied to the full-grown compost. Casing soil fulfils the following functions:

- to supply water for the growth of both mycelium and fruit bodies,
- buffering climatic conditions in the growing room,
- protect the compost layer from drying out,
- provide an environment suitable for the stimulation of fruit bodies and their development.

Casing soil formula can variate depending on locally available ingredients:

- farmyard manure + loamy soil (1 : 1)
- coarse peat + limestone flour + limestone grit (4 : 1 : 0,5)

When farmyard manure is used, it is necessary to sterilize the casing soil with steam.

The casing soil is laid on top of the fully-grown compost in a layer of 1-5 cm. It depends on the compactness and water holding capacity and temperature in the growing room. If higher yields are expected (15-25 kg/m^2), the thickness should be 5 cm of high water holding capacity soil. If no peat is used and soil is rather compact, only 2,5 – 3 cm casing soil is applied. Before primordia initiation the Agaricus mycelium has to colonize the casing soil. This is promoted by maintaining a high CO_2 level in the growing room by minimizing fresh air input (<1000 ppm during pinning/cropping). Temperature for spawn run (including case-run) has to be maintained at level of 20-28 ^oC. Fruiting temperature is 10-20 ^oC, and relative humidity for cropping is 85-92%. At the moment of casing, the moisture content of the casing soil is between 68 and 76%. The EC (electricity conductivity) of the casing soil, with added salt, is between 0,5 and 1 mS/cm. The optimal pH for mycelium grow is around 7. The pH of the casing soil is between 6,8 and 7,5.

When the mycelium can be seen running over half the surface of the casing soil, fresh air should be introduced in the growing room to stop mycelial growth and promote pinhead production. Ideally the temperature should drop now. It can be done cheap by ventilating with fresh air in

the night. The casing soil must remain wet all the time as each kilo of mushrooms evaporate three to four liters of water.

Fruit body formation takes place at a CO2 content of 600-2000 ppm, and relative humidity above 92% and lower as 96% is required.

Harvest the mushrooms by gently turning them out of the soil. Only a little soil should stick to the stalk, otherwise the soil is too dry, or the technique is too crude.

Yields from 3 to 30 kg/m^2 can be harvested, depending on the quality of the compost, the strains involved and how well the climate can be regulated.

Bacteria in casing soil have an influence on fruit body formation. As mycelium incubates in the casing soil, selection takes place in flavor of these bacteria that are essential for fruit body formation. One of these bacteria, *Pseudomonas putida* has been identified. *Pseudomonas putida* absorbs the excess of metabolic products content (acetone, acetaldehyde, ethylene, ethylacinal and ethyl acetate), which repress fruit body formation.

Continuous production is only possible when rooms are regularly filled with new substrate. A 12-week schedule would be safer to account for longer periods between flushes due to fluctuating climate in the growing rooms. With good climate control activity or process per week (10-week growing cycle period) is as follows (OEI, 2016, p. 212):

Filling and spawning	1
Spawn run	1-2
Casing and caccing	3
First flush	5
Second flush	6
Third flush	7
Fourth flush	8
Fifth flush	9

Emptying	10

Caccing is a technique to mix some full-grown compost through the casing soil to speed up casing soil colonization.

3.5 Protection against important pests and diseases

There are five primary sources of contamination in mushroom culture work (STAMETS, CHILTON, 1983, p. 16):

- The immediate external environment
- The culture medium
- The culturing equipment
- The cultivator and his or her clothes
- The mushroom spores or the mycelium

Integrated pest management (IPM) is a least toxic approach for managing any pest. IPM views pests as a natural part of the farm environment. The integrated management of a pest is accomplished by altering the environment to the disadvantage of that pest.

Some examples of non-chemical methods used to control typical pests in the production of white button mushrooms are:

- Mushroom flies, a common pest among many cultivated mushrooms, are attracted to the smell of decaying vegetation such as mushroom substrates. Screening the mushroom house ventilation system will keep adult flies out.
- Double doors and positive atmospheric pressure within the structure also prevent flies from entering.
- Since adult fungus flies are drawn to standing pools of water on benches, walks, or floors, places where water can collect should be eliminated.

- Biocontrol is another option for several mushroom pests, the sciarid fly among them. A predatory nematode attacks this fly in its larval form. Therefore, this nematode can be added to the composting substrate to prevent infestation. (BEETZ, KUSTUDIA, 2004, p. 11)

Quite a large number of pests can be encouraged when cultivating white button mushrooms. Especially if the heat treatment has been performed poorly, all kinds of pests can multiply after the compost mass has cooled down. A common disease is caused by a bacterium called *Pseudomonas tolaasii*. Under high humidity it can develop very quickly and form brown stains in the mushrooms. The remedy is to keep mushrooms dry. If they remain wet for two to three hours after watering an infection may have occurred. Temperatures above 20 ^{o}C and an air humidity above 85% promote bacterial blotch, especially with little air movement. Additional fresh air and air movement can ensure that the mushrooms will dry within two to three hours. Some growers use to spray concentrations of 125 ml chlorinated water (10%) per 100 liters of water per 100 m^2. If more water is applied, the chlorine should be added to the last 100 liters (OEI, 2016, p.212).

The pH of the casing soil must never drop below 6,8, as this will greatly increase the risk of Trichoderma (competitor/green mould). With casing there is always a risk that spores or parasitic moulds enter the growing room with casing soil. To destroy as many of these spores as possible, a formalin solution can be used to disinfect the casing soil. After casing, the casing soil is sprinkled with 2% formalin solution, which means 2 litres of formalin (40%) in 100 liters of water per 100m^2 growing surface.

After casing, an ideal compost temperature of between 25 and 27 ^{o}C is aimed for. With a green mould infection it is better to stay in the lower part of this range as green moulds grow faster at high temperatures. The first four days after casing, cooling is possible with outside air. Afterwards it is better to use internal cooling to prevent prepinners because if low CO_2.

4 General characterization: Site, Economics and Climate environment

The best way to describe main characteristics of an agricultural enterprise is to start with it's location. Various aspects will be shown why this particular location is chosen, including benefits and possible threats.

The described farm is calculated in circumstances of legislative, economic and climatic environment of Kazakhstan. Main beneficious factors affecting the placement decision are:

- Low domestic production of mushrooms overall and White Button Mushroom among others – good chance of coming into market and getting a share of it with little competition, as the practically first mushroom farm (also *Agaricus*) was established only in 2010 (CAREV, 2011).
- State subsidies, cheap credits, infrastructure development, service support: Fund of Entrepreneurship Development "Damu" as a part of "Business Development Roadmap 2020" includes mushroom and truffles production to the list of prioritized economical spheres (AKHMETOV, 2015).
- As wheat production is main focus of agriculture, wheat straw for the substrate should be always affordable and cheap. Alternatively, horse manure rich with straw could be also cheap and accessible, as Kazakhs have a strong tradition in horse breeding and horse milk and meat consumption.
- Cheaper labor cost comparing to Europe, but same or higher price on mushrooms.
- No strict regulations about contaminations and odor by mushroom production.

Also, some threats are to mention:

- Low experience in mushroom production can lead to insufficient qualification of workers – learning must be involved.
- Possibly no inland substrate and/or spawn production.
- Sharp continental climate: difference between summer and winter temperature can be up to 70 ^{o}C, which postpones high investment in building and/or indoor climatic control.

- Variety of wild mushrooms collected in forests is a seasonal competitor.

As no arable land is needed, location of the farm should be chosen by availability of infrastructure and existence of resellers / end consumers of the harvested mushrooms. Kazakhstan has a low population density, so the enterprise should stand not too far from bigger cities to avoid high transportation costs. Flat relief of Kazakh land causes no problems in altitude fluctuation resulting in different relative air humidity. Availability of clean water must be obtained. Small distance from energy producers can lower costs, but the fact that most energy is produced by burning coal must be taken in consideration (ppm in to the facility incoming air). Also, no sources which pollute the air with biological contaminants (e.g. spores of green molds) such as waste dumps, compost piles, sawing mills etc. should be near the mushroom farm. There should also be a possibility to enlarge the farm in the future.

Keeping in mind all these factors some site in north Kazakhstan can be chosen to be able to deliver mushrooms to the market in big cities like the capital city Nur-Sultan (former Astana) or big cities in the south of central Russia.

5 Marketing and Logistics of harvest and postharvest operations

The goal of marketing is to sell products at the best price. The price that customers are willing to pay, depends on several factors: while price and reliable delivery are very important for professional buyers (wholesalers, restaurants, food industry), for consumer convenience may prevail. Small-scale growers may deliver directly to restaurants and greengrocers, and receive a higher price in return for time they spend on marketing and distributing. For larger growers this strategy doesn't work: the need distributers to assure them all their mushrooms are sold.

Agaricus bisporus mushrooms are sold fresh, in brine, canned and freeze-dried. Investment in production of end product another than fresh mushrooms, for example sliced mushrooms or ready to fry convenience product may increase the value and give the possibility to store the product longer, making the life cycle of the product longer.

Distribution to private consumers takes considerable time and is suitable for only small-scale producers (less then 25 kg a day). However, it can be interesting to sell a part of the production at farmers markets, because relatively high prices are paid for premium fresh products, Moreover, selling at these kinds of markets is promotion for the mushrooms and the grower gets in contact in contact with the consumers.

Distribution to professional users in the neighborhood is feasible and less time-consuming compared to selling to private households. With 40-50 restaurants or greengrocers with a weekly demand of 5 kg mushrooms on average small mushroom farms can sell their complete production locally. It does at least one day a week to administrate, deliver and keep in touch with the professional users.

The added value of wholesalers is their distribution network and their ability to match supply and demand. The best is when the marketing organization is owned by the growers and the wholesalers' profits return to the individual growers.

Mushrooms and mushroom products can be exported if the quality and price meet the requirements of the buyers. Exporting mushrooms from a developing country can earn foreign currencies, while this agricultural product does not claim any arable land. Most likely an export license is required, and to obtain and keep it following strict sanitary rules is required. In case of exporting fresh mushrooms, the conditions during the transport have to be controlled carefully to keep the quality of the product. The quantities should be not less than 500 kg per shipment and preferably higher to cover incurring costs. Only production nearby international airports or close to borders with a good road infrastructure can export fresh mushrooms, as the time between picking and delivery should preferably be less than two days. Canned mushrooms are often shipped in sea containers, dried mushrooms could be sent in smaller crates. (BEETZ, KUSTUDIA, 2004, p. 13)

From north of Kazakhstan mushrooms can be easily exported to nearest big cities of Russia like Ekaterinburg, Omsk and Chelyabinsk, they present far more concentrated markets for mushrooms.

A common way of promoting mushrooms to the general public is to provide recipes on the packaging. Extra value can be added when nutritional value and medical aspects are printed on the packaging.

6 Costs

The costs depend on the chosen technology and what kind of equipment us necessary to produce the substrate.

It is important to calculate the depreciation costs per year by dividing the cost per item by their expected depreciation period (linear depreciation). For example, for autoclaves and tanks for the heat treatment, steam boiler, clean room depreciation period is 10 years.

6.1 Operating costs of substrate production

Operating costs of substrate production depend on different factors, the most difficult to predict of them is labor. Different people will accomplish the same job in different periods, which may range from 50% (very efficient) to 200% of the average.

Factors	Remarks
Depreciation of substrate production facilities	
Frequency and amount of substrate production	The more used, the more efficient. Depreciation should be adjusted for increased wear.
Substrate ingredients	The exact formulation has to be defined after trials; include the transportation costs

Substrate containers	Prices of trays vary enormously. Durability has to be checked.
Spawn	Production facility labor and materials.
Energy	For heat treatment and optionally for climate control.
Labour	Mixing and moistening substrate ingredients Filling substrate in containers Spawning Monitoring spawn run Transport

The result of operating costs calculations should be the cost price per ton of substrate. Figures can be estimated by a pilot scale plant or simulating the work flow.

6.2 Investments in growing rooms

The capacity of growing rooms ranges from e few m^2 to 500 m^2. Large rooms are easier to climate control but require higher initial investment and a small number of rooms fluctuates in time, depending on the flushes. All rooms are similarly dimensioned.

Factors:

- building costs
- permit and consultancy
- climate control
- shelves
- installation of electricity, steam etc.

- number of growing rooms.

Result is the necessary investment and the yearly depreciation of the growing rooms.

For the projected farm a flexible investment plan can be implemented. There will be several waves of investments:

1. spawn production – 1 room (takes 1 year, possible research and building activities in that time)
2. compost production – 2 rooms
3. spawn run – 1 room
4. growing rooms – 4 rooms
5. mushrooms processing and storage

This makes the project farm more attractive for the investors. Profitability can be increased by selling semi-products after each wave of investment: spawn, compost, substrate, inoculated substrate.

6.3 Operating costs of mushroom growing

- depreciation on investment in growing rooms
- cost of substrate
- energy and water
- labour
- pesticides
- packaging
- transport to the market

7 Supposed exercise price

To determine the desired production volume is necessary. For the project assume production volume of 1000 kg of mushrooms every week (52 weeks = 52000 kg per year) for the first period before expansion.

- Each growing room has a growing surface of 200 m^2
- 100 kg of substrate per m^2
- 15 kg per 100 kg of substrate (or m^2) harvest
- 12 weeks growing cycle

Each room produces 3000 kg in 12 weeks or 13000 per year. 4 rooms produce 52000 kg mushrooms per year.

Mean price for white button mushrooms in Kazakhstan is around 4€, wich makes a brutto revenue of around 208.000€ per year. Real income depends on practical prices of construction or renovation and equipment. Mean salary of a worker in Kazakhstan in 2018 is 500.000 tenge, according to the data of the Statistical Ministry, which is approximately 1100€, can be a good sum for the most expensive technical process involving labor – hand picking of fruit bodies. Raw calculation to follow in the presentation.

8 Practical investigation

In order to gain an inside to the white button mushroom production processes, several actions were undertaken:

- With assistance of scientific supervisor in Prague, a forest trip was made to find and harvest *Agaricus* sp. mushrooms. For that purpose, author joined a very experienced Czech mushroom hunter known as Peter the "Forrest Runner" in a trip to National nature reserve in Příbram District on the 29th of July roughly a week after rains in that region. This area is protected and entrance is forbidden for most people except bee keepers, what was the case with Mr. Forrest Runner. After several hours many different mushrooms have

been picked, like *Armillaria mellea*, *Cantharēllus cibārius*, *Rússula*, *Boletus edulis*, but this species are not related to the topic. More important was finding of three fruiting bodies of *Bovista dermoxantha*, another representative of the class Agaricomycetes (location 49.7975975N, 14.2204606E) and especially *Agaricus arvensis* which is very similar to *Agaricus bisporus* and is of the same genus (location 49.7017111N, 13.9348161E). *Agaricus arvensis* is a perfect culinary mushroom with high potential of cultivation thank to clearly bigger fruiting bodies than *A. bisporus*. *Bovista dermoxantha*, also called puffball or puffmushroom, is perfectly edible before the maturation, where white and soft gleba (internal flesh) turns yellowish to mature brown powdery or dusty spores massive.

- Next process in the production chain is isolation of the tissue to Petri dish with potato dextrose and/or agar medium. This action was undertaken in the mycological laboratory of CULS under guidance of Prof. Dr. Jablonský. Unfortunately, only one of three founded fruiting bodies of *Bovista dermoxantha* were not damaged and suitable for isolation, and the only part of *Agaricus arvensis* that was found is the cap. The next working steps were done in the laminar flow cabinet, where the fruiting body was teared apart and very fast newly opened flesh was taken with a lancet or scalpel, and then immediately transferred to the medium. Although all possible sterilization was done, all the probes were contaminated with more competitive species or bacteria. Despite this fact, it was a precious experience that was implemented and improved during a Basics of Mycology course in GAU Göttingen. During the training *Agaricus bisporus* fruiting body was observed under a microscope to distinguish the bisporous structures and also mother spawn production steps with exception of grain inoculation (due to absence of high culture).
- Also a part in CULS excursion to a mushroom production facility was taken. It is the "Münzner Roland Champignonzucht" farm situated in German town Reitzenhain on the border to Czechia. It is a beautiful industrial facility producing many edible and medicinal mushrooms with own post-harvest processing and packaging line, among which *A. bisporu*s mushrooms play a significant. This rare insight to mushroom production and contact to producers was highly appreciated.

9 Conclusion

Mushroom production in Kazakhstan is attractive as there is low competition on the market and governmental programs can stimulate rapid growth after production process is calibrated. White button mushrooms are well-accepted in this country and have a good potential for marketing issues. Huge possibilities for extension of the farm in the future by mechanization of different stages of production and extra growing room make this project interesting for investors and local government. Spreading of knowledge can forward to selling of substrates to new host farm that can sell grown mushrooms back to the project farm and implemented in own distribution system.

More investigation and planning needed as prices of equipment and raw materials vary.

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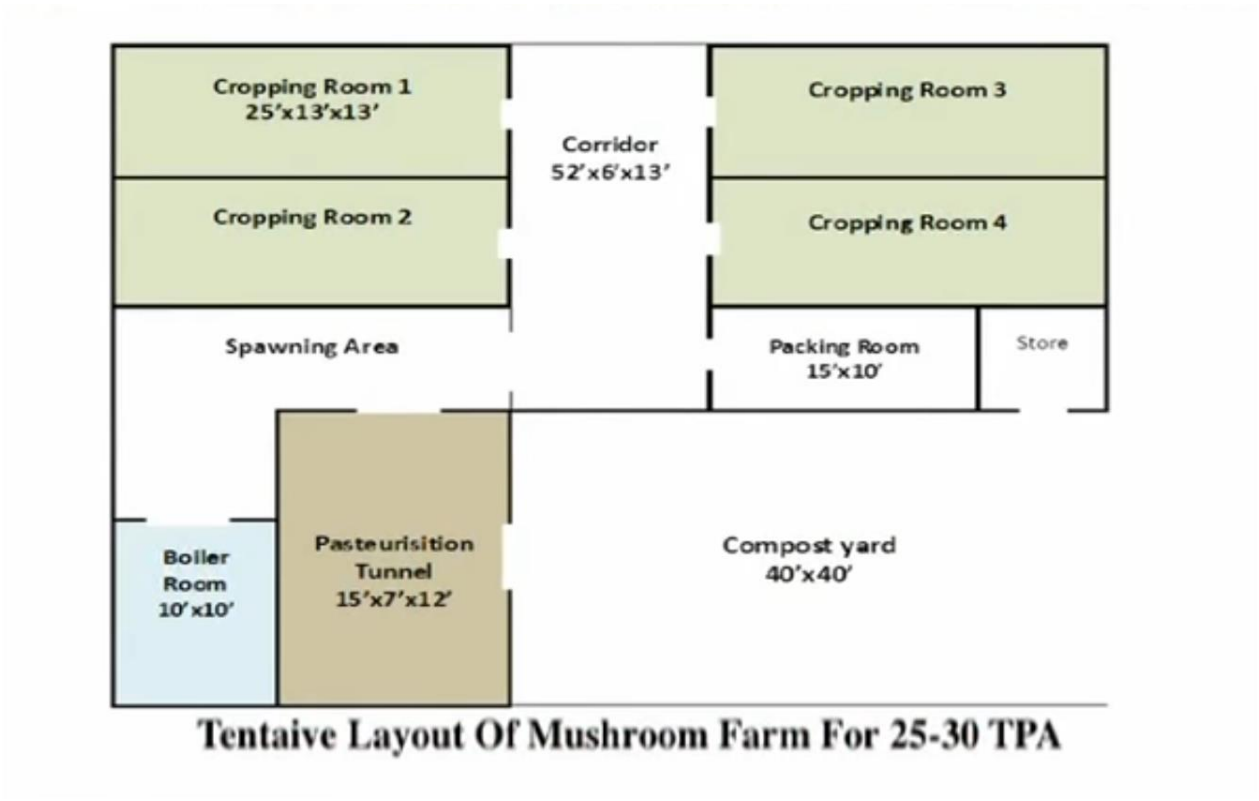
Enclosures



Tentaive Layout Of Mushroom Farm For 25-30 TPA