The cultivation of the button mushroom, *Agaricus bisporus*, in The Netherlands: a successful industry

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El cultivo del champiñón, *Agaricus bisporus*, en Holanda: una industria exitosa

Resumen. Se describe el desarrollo de la industria del champiñón (*Agaricus bisporus*) en Holanda y los incentivos que han causado el rápido crecimiento de esta, i.e. La organización cooperativa de la misma y su dirección por el ministerio nacional de agricultura. Esto ha llevado a Holanda a ocupar la tercera posición como productor de champiñón a nivel mundial y el primero como exportador. En los últimos 30 años esta industria se ha incrementado 10 veces y actualmente la producción anual es de 270,000 toneladas. Se describe también el desarrollo en la tecnología del substrato y los requerimientos para preparar el compost-indoor. Se discute el desarrollo en la producción de spawn y en la prevención de la degeneración de las cepas. Se discuten finalmente los requerimientos de calidad en los materiales de base, i.e. compost, casing e inóculo y se dan recomendaciones para el manejo de las enfermedades, disminución de los costos de mano de obra y las respuestas al mercado y a las demandas del consumidor.
Palabras clave: *Agaricus bisporus*, Holanda, historia, cooperativas, compost, inóculo, degeneración, mercado, industria de hongos.

Abstract. The publication describes the development of the Netherlands mushroom (*Agaricus bisporus*) industry . It gives the incentives that have been causative for its rapid growth, i.e. the cooperative organisation of the industry itself and its guidance by the national ministry of agriculture. This has led to its position of being the third producer and the first exporter of the white button mushroom in the world. In the past thirty years the industry has grown tenfold, to its present production volume of 270.000 tonnes annually. Further the article also describes the developments in substrate technology and the requirements for making indoor-compost. Also developments in spawn production and prevention of "degeneration"are discussed. The article ends with a discussion of the quality requirements of basic materials, i.e. compost, casing and spawn and gives recommendations for disease management, decrease of labour costs and the responses to market and consumer demands.
Key words: *Agaricus bisporus*, The Netherlands, history, cooperatives, compost, spawn, degeneration, marketing, mushroom industry.

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Introduction

The cultivation of the button mushrooms, *Agaricus bisporus* Lange (Imb), goes back in history to around the year 1700. Originally an outdoor crop on hotbeds, mushroom growing moved into caves, where the climate was more protected and from there to houses. At the beginning of the 20th century mushroom growers in Pennsylvania adopted a standard shed for growing. It contained vertical tiers with six-high shelves. Shelf growing became the standard system in the USA and later in The Netherlands. Tray growing became the main

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production system in England, Germany, and France; it was characterized by a two zone system where trays of compost were peak-heated in one room and grown in another. Mechanical handling equipment was developed for both shelves and trays, and has led to considerable saving of labour.

Sterile mushroom spawn was developed in 1894 and kept secret untill 1902 [4, 6]. Compost was made from horse dung by a process of outdoor fermentation. The product was often of poor quality and usually full of pathogens for mushrooms. The goal of the process - that is, producing a specific substrate for the growth of mushrooms - was often not reached. In 1915Therefore, pasteurization (peak heating or phase 2) of compost was introduced as a means of preventing pests and diseases.

A strong effort was put into studying nutritional requirements of mushrooms and into different ways of preparing compost. This led in 1950 to Sinden and Hauser's method of 'short composting' which has been introduced almost all over the world [19].

The development in the 1970-ies of special fermentation rooms, i.e. tunnels, made the phase 2 process in bulk possible.

Table 1. Production of *Agaricus bisporus* in 10^3 tonnes (stipes cut) in 2001 and in 1970. *

Year	2001	1970
Europe		
The Netherlands	270	30
France	172	68
Italy	110	20
Spain	110	4
Poland	105	5
UK + N. Ireland	86	40
Ireland	66	2
Germany	63	21
Belgium	45	5
Hungary	16	2
Denmark	8	7
Others	20	15
North-America		
USA	380	88
Canada	87	12
Latin America	58	2
Australia/New Zealand	55	6
Africa	35	2
Asia		
China	637	2
India	50	0
Indonesia	28	0
S. Korea	23	6
Taiwan	7	39
Others	10	
World wide production	2,441	382

* Data from Delclaire [2]

Table 2. The composition of an average horse manure compost.

1000 kg horse manure (63 % moisture)
100 kg broiler chicken manure (40% moisture)
30 kg gypsum (20% moisture)
300-900 litres water
Assuming a total matter loss of 30 % during phase I composting, this yields 1,138 kg fresh compost with 72 % moisture content

The compost could then be spawned and mycelial growth could take place in bulk. This started a new way of mushroom growing and of farm management and turned out to be very successful. It also rendered growing of mushrooms on compost filled polythene bags technically and economically viable.

World wide cultivation of button mushrooms has prolifically grown in the past thirty years from 3.82×10^5 tonnes in 1970 to 2.4 million tonnes in 2001, a six fold increase as can be deducted from table 1. European production which was about two third of the world production in 1970 has decreased to about half of the world's production, due to the upcoming of Asian countries.

It is interesting to analyze what have been the main drives behind this impressive growth. Developments in the Netherlands, where production in 2001 was almost 10-fold the level of 1970 may be exemplary for what could happen in other countries with a rapid increase in production.

In our opinion several incentives have been causative for the development of mushroom growing in the Netherlands:

a) The industry was already from the early beginning in the 1950-ies cooperatively organized, which led to the cooperative production of casing soil and compost of good quality and relatively low price. Cooperative auctions and cooperative canning facilities took care of sales of fresh produce respectively of surplusses and in conjunction kept price making forces reasonably stable. The growers' cooperation also resulted in standardization of buildings, equipment and cultivation technique, further reducing the costs of production.

b) The Ministry of Agriculture supported further growth of the industry untill the early 1980-ies through its coherent research, extension and educational program. In return this program was partly financed by the industry and because of the joint interest communication channels between the two were kept wide open.

c) The driving power of the political decisions of the 1980-ies concerning public health and environmental control, including the prevention of emission of harmful and odorous substances from compost, led to advanced methods and materials for compost preparation and to a more reliable production of mushrooms.

d) The harsh attitude of large national and international chain stores in the nineties towards control of quality and price has led the way to optimal quality/price ratio and to the present strong market position of the Dutch industry.

Further development of the Dutch industry will now be influenced by harmonization of European law and the entrance of new EC partners in the coming years.

The strong position of Dutch agriculture and mushroom growing will in our view change. In the coming years, shortness of labour and very strict (national) legislation, concerning environmental issues, will increase the price of the Netherlands mushroom and as a results its market position will be negatively influenced.

Table 3. The composition of an average synthetic compost.

1000 kg wheat straw (15% moisture)
800 kg broiler chicken manure (40% moisture)
85 kg gypsum (20% moisture)
appr. 5000 litres of water.

Straw is first mixed with 500 kg manure and wetted with 3000 to 4000 litres water. After 7-10 days the mixture is used by adding 100 kg manure to each wet tonne and treated in ricks. This formula yields appr. 3100 kg compost (72% moisture).

Developments in substrate technology

The substrate for the cultivation of mushrooms is horse manure compost, which consists of a mixture of horse manure, some broiler chicken manure, and water, to which gypsum is added for structural stability and for stabilizing pH. (Table 2). Alternatively a synthetic compost can be made which is based on wheat straw and broiler chicken manure (Table 3).

When carried out in the open air, phase I of composting is done in long narrow stacks between 1.5 and 2.0 metres in width and height (wind-rows). In these stacks a mixed microbial flora of bacteria, actinomycetes, and fungi initiates the fermentation process, during which the temperature rises to values between 50 and 70°C. Because of increased activity of thermophilic organisms the temperature in the stack rises even higher after a day or two to a maximum of 80°C. To obtain uniform degradation of the mixture, stacks are turned twice a week. The turning also replenishes air, but natural ventilation is the most important source of oxygen.

During the composting process the most readily utilized compounds, such as soluble carbohydrates, are utilized first and carbon is lost as carbon dioxide and a low amount of methane or incorporated into microbial biomass. Some nitrogen is released as ammonia and also odorous organic sulfides escape that may be of nuisance to neighbouring areas [3]. At the end of the process, usually 7-8 days after setting up the stacks, one ton of horse manure will have yielded just over one ton of compost.

The actual yield depends largely on the moisture content: the ideal moisture content is somewhere between 70 and 75 per cent. This fresh compost (phase I compost), which still smells strongly of ammonia, is then subjected to phase II treatment before actual cultivation can start. Phase II (Table 4) composting consists of a pasteurization process followed by further high-temperature fermentation. The pasteurization is meant to rid the substrate and room or container of mushroom pests and diseases. This is done by raising the air temperature to 56 °C and keeping it there for approximately 5 to 6 hours. Compost temperature then increases to slightly higher values, and this is maintained for at least five more hours. Thereafter the compost is kept at a temperature of approximately 45 °C for 4-5 days until all ammonia has evaporated. The process involves a further utilization of soluble carbohydrates combined with incorporation of ammonia and, most importantly, the evolution of a thermophilic micropopulation e.g. of the fungus Scytalidium thermophilum that is important for the later productivity of mushrooms [24].

After phase II the compost is free of pests and diseases and has become selective for the growth of mushroom mycelium. At this stage the compost is practically odourless; it has turned black and in no way resembles the original manure mixture from which it was prepared.

Indoor compost

One of the most important developments in mushroom

Table 4. Phase II of compost preparation.

- Increase compost temperature to max. 60 °C by limiting the air temperature to 57 °C. Keep for 5-6 hrs.
- Decrease then compost temperature to 45-50 °C by lowering the air temperature and keep until NH_3 has disappeared (4-6 days)
- Cool down to ambient temperature.

Phase I compost is well mixed and filled in trays or in bulk in tunnels. The compost temperature is then equilibrated after which heating may start. When phaseII is finished, the compost does not longer smell of ammonia but has an earth-like odour instead. It is then ready for inoculation i.e. spawning

growing of recent years has been the introduction of bulk processes in composting technology. Both phase I and phase II of the composting process can take place in bulk in special fermentation rooms called tunnels. When that process has been completed the compost can be spawned and mycelium can develop in either the same or another tunnel. Phase III then yields fully grown compost, ready for production. In most mushroom producing countries in Europe the practice of indoor composting is now required by governmental regulation, because it prevents environmental pollution due to release of ammonia and odorous substances. Like any other more-zone systems the process requires precise management. Strict hygiene is extremely important. All the trouble is, next to the environmental effects, well worthwhile, because the use of grown compost reduces the growing cycle in mushroom houses by three weeks.

Better process regulation through indoor composting

The first and basic function of phase I composting is the preparation of a medium which is chemically and physically homogeneous. Indoor processes with only less than 1 day for mixing allow no further adjustment of nitrogen and moisture content . It is therefore of major importance to develop a process that allows to achieve the right moisture content. Gerrits *et al*. [11] have studied the relationship between initial moisture content (X) of the compost mixture and the moisture content at spawning (Y) and after spawn run (Z). They found two regression equations:

$Y = 1.0622 X - 9.66$ ($r=0.71$)

$Z = 1.4336 X - 43.98$ ($r=0.61$)

Best yield of mushrooms was obtained at moisture content of 66 - 69 % after spawn run. Culturing practices as watering and putting plastic sheets underneath compost can prevent desiccation [1]. As phase I in tunnels is characterized by temperatures between 70 and 80 °C for 2-3 days, part of the microbial population viz. Scytalidium thermophilum, the presence of which is positively correlated with the yield of mushrooms, appears at risk. Eventual deleterious effects could be cured by inoculation with finished phase II compost; alternatively the indoor phase I was changed by the admission of cool air creating a vertical zone along tunnel walls where micro-organisms survived [24].

The nitrogen content of compost is another decisive argument for yield; its concentration should be 2.2 % in phase II compost. It can be influenced by supplementation of treated proteinaceous products [10, 20].

Economic effects of indoor composting

Growers using fully grown compost obtain now not 4 1/2 full crops each year but 7 to 8. This has led to a more efficient utilization of assets and has reduced the cost price of mushrooms. Further efficiency increase has come about by reducing the number of flushes to two rendering possible 10 annual crops. Also the average productivity of compost increased during two consecutive years after the introduction

Table 5. The annual average productivity in the Netherlands of compost for *Agaricus (Kg/* tonne of fresh compost). It should be noted that the average reflects all grades of mushrooms of all strains grown for both fresh market and for canning. Since 1995, all outdoor production of compost phase I has been replaced by indoor processes.

Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Yield (kg/tonne)	197	213	216	231	232	236	248	258	246	233	219	235

of bulk produced spawn grown compost and then decreased again. This is shown in table 5. We have no easy explanation for the productivity decrease from 1997 till 1999.

Developments in spawn production and breeding

Spawn production

Gerda Fritsche [8] used the traditional white and off-white cultivars for a breeding program which led to the first real cross-bred strains of Agaricus in the world (the hybrids Horst® Ul and Horst® U3). They showed a better productivity and better quality than many of their predecessors. Large spawn suppliers as Amycel and Sylvan have played an important role in the quality management of the Horst ® strains. However the strains have are becoming old; due to the ban on the use of chemicals in the production of crops the industry now require strains that carry resistancies against the major fungal pathogens, i.e. Verticillium fungicola, Trichoderma sp. and Mycogone perniciosa.

Instability of spawn strains

The cell lines used in mushroom cultivation are normally vegetatively propagated on nutritionally rich media and repeatedly subcultured. The inoculating material for the commercial production of mushrooms, i.e. spawn, usually consists of sterilized rye or millet which is colonized by a monoculture of the line/strain and species that is to be cultivated.

In spite of extensive quality control procedures by the commercial spawn companies, the mushroom industry world-wide has been pestered repeatedly by spawn related incidents of malformations in their crop in conjunction with a decrease of yield and quality loss [14, 23, 27, 28] *Agaricus bisporus* has shown "open veil" or "hard gill" [17], "broad stipe" [16], "frilly gill" [5], excessive "stroma formation" combined with severe production loss and malformation of basidiocarps [15, 27] and more recently "cluster" formation [21, 22, 25]. All those incidents were generally termed "degeneration" or "strain instability" although only few were proven to be linked to genetic changes. "Degeneration" *sensu stricto* is a stable genetic change of a cell line leading to a loss of what is generally considered "good quality"; it describes a decline or deterioration in qualities or reversion to a less organized or simpler type. Given the high frequency of phenotypic change the term "strain instability" describes the process better than "degeneration". In some strains and species the spontaneous phenotypic change could be considered a normal part of the developmental repertoire rather than as a degeneration to an inferior form [12]. "Degeneration" has occurred over the years in pure white strains, in off-white strains and in the modern hybrid strains. One should therefore be very careful in the management of storage of the collection of strains.

Cultures can be stored in different ways: Most importantly, cultures are inoculated on a specially prepared compost and kept at 4 °C for a period of up to 1 year. After transfer, subcultures are made on 3 different media and growth rate and morphology is examined. There is always some variation in these characteristics and this seems to be inherent to most fungal species [12]. These small variations never had a detectable effect on yield or quality of mushrooms. All commercial spawn producers select strains for a constant growth rate and colony morphology. Strains which show severe sectoring and/or excessive aerial hyphal growth are to be discarded since these symptoms are usually followed by stroma formation.

A good storage substrate consists of a regular horse and broiler chicken manure based phase II compost which is dried and milled to 5 mm. particles. Three hundred dry grams of this material are wetted with 1 liter of tap water and washed 3 times with hot water. It is finally boiled until the excess water had evaporated, filled in culture tubes and sterilized by autoclaving at 121 °C for 2 hours and stored cold untill further use [9].

A shadow collection of cultures can be kept in the gaseous phase above liquid N_2 at - 196 °C. The cultures on small agar plugs are slowly frozen in straws filled with a cryoprotectant i.e. a sterile aqeous solution of 10 % glycerol. When cultures are to be used they are slowly thawed and directly plated on petri dishes with wheat extract agar. All cultures have to be tested on normal compost using the normal cultivation technique [26]. The resulting mushrooms must be studied for the yield, flushing pattern, and the various quality characteristics originally observed in the mother culture. There is no indication that storage in this way leads to change in traits or to "degeneration".

Where are we standing ?

In the year 2001 we were in the Netherlands at a one time high in the production of Agaricus bisporus, with an almost ten-fold increase of production in a period of 30 years. The quality was not different from mushrooms produced and canned in other countries; its price was comparable because we compensated the high costs of labour in the western world by high efficiency and advanced methods. The basic materials spawn, compost and casing were relatively inexpensive and of generally high quality. However, when we want to remain at that position some things have to be changed.

a) Compost should be better defined and more stable in its properties than at present. This will require more knowledge of the feeding requirements of Agaricus. Casing is also a source of uncertainties. Although it harbours the key for fruiting [7] and supplies an excellent water buffer, it also contains many pathological micro-organisms. It is certainly imaginable to manipulate the casing ecosystem to protect the growing mushroom against a variety of diseases and malformation. The study of the casing ecosystem is in my opinion of major importance more so than now setting up HACCP Not define before! (Food quality law procedure described as Hazard Analysis of Critical Control Points) requirements for casing components i.e. for materials that we presently do not know the purpose of.

b) The quality of spawn is excellent but new strains need to be developed i.e. resistance against fungal disease should replace chemical treatment

c) About diseases we can be short: the prevention of pests and diseases is in my our view Idem a management problem. Even when the use of chemicals is not allowed, damage by diseases can generally be prevented by careful farm management and extreme hygiene. It should here be mentioned that mushroom pests require special attention. They are not only harmful in their own right, but phorids and sciarids both are excellent vectors for transfer of fungal and bacterial diseases [29]. When prevention does not work sciarids can be biologically controlled by the application of the nematode *Steinernema feltiae* [18]. Phorids may be controlled biologically by *Bacillus thuringiensis* var. *israeliensis* [13], but in our hands it did not work. The era of application of pesticides is however over.

d) Cultivation systems and farm design may have to be changed to decrease labour costs.

e) Market and consumer demand are playing roles of more and more importance. After a number of incidents consumers have lost part of their confidence in food safety. Dioxin in meat, CCC Not defined beforein pesticides in pears, BSEMad Cow Disease Idem and FMD IdemFoot and Mouth Disease of cattle are a few examples. A number of large European supermarkets have formed the European Retail Platform (EUREP) and have jointly drawn up good agricultural practice guidelines (EUREP/GAP) describing a number of minimum requirements for sustainable production which growers should comply with. Together with the HACCP (Hazard Analysis of Critical Control Points) requirements, quality and control of the whole production chain will be defined. Compliance with those standards will determine the market position of the product of mushroom growers in the coming years.

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