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Productivity and Nutritional Composition of *Lentinus strigosus* (Schwinitz) Fries Mushroom from the Amazon Region Cultivated in Sawdust Supplemented with Soy Bran

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1. Introduction

The cultivation of edible mushrooms is a biotechnological process that uses various residues to produce food of high nutritional value. It is an activity of economical importance, in particular, the production of *Agaricus*, *Pleurotus* and *Lentinus* species (Guzmán et al., 1993). Nutraceutical properties of mushrooms are increasing its economic value. The cultivation of mushrooms can be a solution to problems of global importance, such as the lack of protein in developing countries and the possibility of environmental management. The use of organic materials for growing mushrooms is an indication of its extraordinary metabolic activity.

In the Amazon region there are large amounts of wood and agricultural residues whose potential has been underestimated. In the timber industry, raw material waste can be as high as 60%. In the agricultural industry there is no data on how much waste their activities produce. Vianez and Barbosa (2003), suggest several alternatives for the use of wood residues, including the use for the cultivation of edible mushrooms. This activity could contribute to a sustainable regional development. In this way, the objective of this study is to study the feasibility of using sawdust supplemented with soy bran for growing *L. strigosus*, a native mushroom of the Amazon region.

2. Literature review

The fungus proposed in the present study is a wild edible and a wood decomposer (white-rot fungus), whose domestication was sought for the production of mushroom. As there is no cultivation of this fungus with the proposed wild strain, for comparison in the literature review, a parallel association was made with the cultivation of species of edible fungi of related genera that have similar physiology and cultivation conditions, being considered mainly the genera *Lentinus* and *Pleurotus*.

2.1 History

Fossil finds have revealed that fungi exist since the Cretaceous period (approximately 130 million years ago), long before humanity (Chang, 1993). Fungi (mushrooms), also called macromycets, belong to the Fungi Kingdom, being known by man since the most remote period of human history. Edible mushrooms were first collected by man in China and dates from 5000-4000 BC. (Zhanxi and Zhanhua, 2001). It is estimated that the first cultivation of edible mushrooms in China started in the early 7th century, with the species *Auricularia auricula* (Chang and Miles, 1987). China is a country with a long tradition in cultivation and consumption of mushrooms and according to Zhanxi and Zhanhua (2001), it has more than ten species of fungi which are currently cultivated in several countries of the world. That country is a pioneer in the cultivation and consumption of edible and medicinal mushrooms, followed by Japan, Europe and The United States (Urben et al., 2001).

Edible mushrooms were described as the "food of the Gods" and as such, confirmed by Roman gourmets who appreciated them as a kind of spice. The Chinese considered them as the "elixir of life". The Greeks believed that the mushrooms were able to give strength to warriors in battles and the Egyptian pharaohs also nourished themselves on these spices (Chang and Miles, 1984). Mushrooms had a wide acceptance, and some species are considered as "Kings of the dining table" or "kitchen diamonds" (Zhanxi and Zhanhua, 2001).

The Greeks Euripides, Theophrastus and Plinio have described the consumption of edible mushrooms in their time (Guzmán et al., 1993). In some societies, the mushroom was a royal food, probably by its pleasant flavor and texture (Miles and Chang, 1997).

The Romans knew several edible and poisonous fungi. There is a story about the Emperor Julius Caesar who was very fond of *Amanita caesarea* mushroom, whose scientific name was a homage to him and for that reason, it became known as "Mushroom of the Caesars" (Guzmán et al., 1993).

According to Molena (1986), the species *Polyporus tuberoster* (*fungaie stone*) and *Polyporus corilinus* are among the first cultivated mushrooms, collected from the wood of hazels and eucalyptus. These fungi were consumed in 4-5 cm slices, and their production demanded about six months, yielding sometimes one or two mushrooms at a time. There was neither any knowledge about their nutritional requirements, nor about their growth cycle. The only thing that was known was that rubbing a mature mushroom on those woods, and leaving them in a wet environment during a particular period of the year could produce appreciable mushrooms (Molena, 1986).

During the Roman Empire the fungaie stone (stone that produces the mushroom) appeared in Italy, which was composed of a cluster of humus, leaves, twigs and limestone rocks, forming a compact mass, which was cut in blocks in the form of bricks and transported to the royal palaces. They were kept in a damp place and irrigated daily until harvest time to serve the senators and other members of the Roman aristocracy (Molena, 1986). In France, the mushroom cultivation began during the reign of Luis XIV, according to Molena (1986). However, the cultivation of *Agaricus bisporus*, the "Champignon de Paris", the most widely cultivated and commercialized species, has been produced since about 1650 (Delmas, 1978; Chang and Miles, 1984).

With the advances of knowledge and technology of mushroom cultivation, commercial production of dozens of species became viable in several countries in recent decades (Guzmán et al., 1993; Stamets, 1993; 2000; Vedder, 1996; Eira, 2000), reaching a production of approximately 4.3 million tons of edible mushrooms in 1991 (Miles and Chang, 1997). The world current production is around 6.2 million tons (Chang, 2003).

2.2 An overview of the commercial cultivation of edible fungi in the world and in Brazil

After World War II, the edible mushroom industry grew from 350,000 tons in 1965 to 4.3 million tons in 1991, from which 3.4 million tons belong to the six most worldwide important genera: *Agaricus, Pleurotus, Lentinula, Auricularia, Volvariella* and *Flammulina*. The major producers are China, Japan, USA and France (Miles and Chang, 1997). The most cultivated genera are *Agaricus, Pleurotus* and *Lentinula*. This increase was due to several factors, among them: a) the increase in the number of species on a commercial scale; b) the development of cultivation techniques using plastic bags, which allowed many wood decomposers edible fungi to be grown on lignocellulosic residues, preferably the cultivation on logs, reducing considerably the cultivation time; c) due to the marketing techniques highlighting the nutritional merits of mushrooms as an important part of the diet, so they wouldn't be marketed as simple accompaniments or delicacies, but as a food of high nutritional value (Miles and Chang, 1997).

The literature cites approximately 200,000 species of fungi existing in the world, from which, about 2,000 are potentially edible species. However, only 25 of them are commonly used as food, and fewer still are commercially cultivated (Chang and Miles, 1984; Chang, 1980; Bononi, 1999).

In the early 1980s, only *Agaricus bisporus* (Champignon de Paris) and other species of this genus and "shiitake" (*Lentinus edodes*, currently named *Lentinula edodes*) had a modern technology for commercial production, where 70% of the world production was represented by *Agaricus* and 14% by *Lentinula* (Chang and Miles, 1984). However, according to the same authors, the world's attention is turning to the development of new technologies for different species of worldwide known edible mushrooms, especially considering the difficulties of production in tropical and subtropical climates. Special technologies are being developed in several countries allowing the cultivation of: *Volvariella volvaceae* in China, Taiwan, Japan, Philippines and Indonesia; *Kuehneromyces mutabilis*, *Flamulina velutipes*, *Hypholoma capnoides* and *Coprinus comatus* in some countries of Europe and Asia; *Pleurotus ostreatus* in Italy, Hungary, West Germany, Mexico and Brazil (Chang and Miles, 1984; Guzman et al., 1993; Eira and Minhoni, 1997; Bononi et al., 1999; Zhanxi and Zhanhua, 2001; Urben et al., 2001). This way, the overview of the world production has changed suddenly, showing a considerable increase in cultivation and consumption of *Pleurotus* as reported by Eira (1997) adaptated by Fermor (1993).

An adaptation based on Fermor (1993), made by Eira et al. (1997), the world production of cultivated mushrooms in the early 1990s was 1,424,000 tons for *Agaricus bisporus*, 900,000 tons for *Pleurotus* spp, 393,000 tons for *L. edodes* and 887,000 tons for other mushrooms, representing, respectively, 39.51%, 24.98%, 10.91% and 24.61%. The current trend is to increase production.

Concerning the production of mushrooms in Brazil, there is not a precise documentation that could allow us to determine when the cultivation of mushrooms started in the country (Fidalgo and Guimarães, 1985). Its popularization in the Center-South region of Brazil dates back to 50 years ago. Bononi (1999) reports that the cultivation of champignon (*Agaricus*) began in 1953, when the Chinese immigrants settled in Mogi das Cruzes and the Italian Oscar Molena in Atibaia, brought technology and imported strains of their countries. For Molena (1985), mushroom cultivation began in 1953 and developed after the poultry crisis in the period of 1955-1959, when breeders began to use chicken sheds for the cultivation of mushrooms, without proper technical conditions.

The commercial cultivation of edible fungi in Brazil is limited to *Agaricus bisporus* (champignon), *Lentinula edodes* (shiitake) and *Pleurotus* spp, known as oyster mushroom,

giant mushroom or caetetuba (Bononi et al., 1999; Eira, 2000). Varieties or strains of mushrooms of the *Pleurotus* genus gave origin to the "hiratake" (mushrooms with very large basidiocarp, harvested in mature stage with opened basidiocarps, before they turn their edges upwards and with more than 5 cm in diameter) and the "shimeje" (with long stipes, harvested with their basidiocarps very young and dark, smaller than 5 cm, and can be harvested in bunches) (Eira and Minhoni, 1997).

There are few Brazilian research reports about the subject, and the Botanical Institute of São Paulo was one of the pioneers, creating a research center of edible mushrooms in Mogi das Cruzes in 1985 and a teaching, research and extension nucleus was created in the Faculty of Agronomic Sciences/UNESP in Botucatu, in 1986, named Module of Mushrooms (Eira, 2000). Other centers are springing up in many universities and research institutions.

The production of edible mushrooms in Brazil is difficult to be evaluated. Producers give preference (90%) to the cultivation of *A. bisporus*, (Bononi et al., 1999). Among producers, the majority, almost 90%, are from the East of Taiwan, China, Korea, Japan, working in small properties, in a family system, with all family members operating in all stages of cultivation, in a collective way. The region of the city of Mogi das Cruzes, São Paulo, is responsible for approximately 70% of the edible mushrooms commercialized in Brazil. The remainder are produced by other municipalities, most of them also in São Paulo, Ribeirão Pires, Suzano, Cabreúva, Atibaia, Mariporã, Sorocaba (Bononi et al., 1999; Souza, 2011). There are some important producers in Porto Alegre and some producing installations in southern Minas Gerais and Paraná States.

In 1990, the production of Mushrooms was only 3,000 tons according to Eira et al. (1997), being estimated at 10,000 tons per year until 1997. According to the APAN (Natural Agriculture Producers Association), the Brazilian production of shiitake in late 1995 among its associates, was approximately six tons per month. The official data are underestimated, because they include only the mushrooms marketed by CEAGESP (General Supply Center of São Paulo State) and those intended for export, which are recorded by the CACEX-Department of Foreign Trade (Eira et al., 1997; 2004; Bononi et al., 1999). It is known, however, that significant amounts are marketed directly by the producers with restaurants, pizzerias, snack bars and other establishments, as well as street markets.

Brazilian productivity of *Agaricus bisporus* "Champignon de Paris" in Mogi das Cruzes (in São Paulo State) until 2000 was of the order of 5 to 7 kg of fresh mushrooms/100 kg of moist substrate (4 to 6 kg of fresh mushroom/m²) (Eira, 2000). In Europe, however, in countries such as Belgium, Holland, Germany and France, the average productivity of mushroom at that time was 30 kg/100 kg of substrate.

Currently in Brazil, farms with more technology get on average a productivity (substrate conversion in mushrooms) for “Champignon de Paris” ranging from 18 to 24% in 20 to 30 days of the crop cycle. In more rustic crops this conversion varies from 12 to 15% in 70 to 90 days. While the numbers seem to have greatly increased, yet it is little when compared to Asian and European productions, where they manage 30 to 40% of conversion (Souza, 2011).

Even today there are no official data concerning the production of mushrooms in Brazil, but some unofficial sources report that 12,050 tons a year of mushrooms "in natura" (table 1). Since 1995, there is an annual import of 12,000 tons per year, on average, most of it cooked *Agaricus bisporus*, to meet market demand. Therefore, it can be concluded that, Brazilian consumption is much higher than its production, reaching 24,050 tons per year. In this context Brazilian people consume around 130 g per capita (Souza, 2011). The world production is around 6.2 million tons (Chang, 2003).

Agaricus bisporus	8,000 ton
Pleurotus ostreatus	2,000 ton
Lentinula edodes	1,500 ton
Agaricus blazei	500 ton
Other species	50 ton

Table 1. Annual production of mushrooms in Brazil. Source (Souza, 2011).

2.3 The importance of fungi

The importance of fungi is unlimited in the terrestrial ecosystem and consequently in man's life. However, these organisms can be beneficial or not, according to the results of their actions. If we consider the decomposing action of fungi on food and the associated production of toxic substances (mycotoxins), the decomposition of other materials such as wood, pathogenicity caused to plants, animals and man, this is the negative aspect of it. On the other hand, if we consider the important role in the decomposition, which along with other microorganisms, participate in the mineralization of organic matter, as well as the symbiosis with plants in the process of mycorrhizae formation, bioremediation, biological control, food, and medicinal properties, one can see the positive side of these organisms.

In nature, the fungi do not participate only in the role of providing a food source for humans and other animals; they also play an important role in the cycling of carbon and other elements, by breaking the lignocellulosic residues and animal excrements which serve as a substrate for saprophytic fungi. This way, these decomposing agents play a very important environmental role along with other organisms, complementing the cycling of plants and animals. Simultaneously, they produce multiple enzymes that degrade complex substances that allow the absorption of soluble substances used for their own nutrition (Chang, 1993).

Trufem (1999) and Matheus and Okino (1999) highlight the importance of fungi in the context of biotechnology, where they are widely used in the food industry, pharmaceutical industry, bioremediation, in biosorption (removal of heavy and radioactive metals), in agriculture as arbuscular mycorrhizal fungi (AMF), where they are used in techniques that help the development of plants of economic interest, biological control, xenobiotics biodegradation, bioremediation of the soil, treatment of industrial effluents and bioconversion of lignocellulosic residues.

One of the most important processes from an economic point of view is the use of fungi in the conversion of lignocellulosic residues in edible mushrooms by fungus X substrate interaction, enabling the solid fermentation process, through enzymatic system of these microorganisms (Matheus and Okino, 1999).

The cultivation of edible mushrooms has become an increasingly important practice in modern society due to the biotechnological process of bioconversion of various residues in edible mushrooms or in dietary supplements of high nutritional value, enabling a more efficient utilization of materials, besides, it can reduce the volume of waste or accelerate the decomposition process. This way, the residual substrate obtained from the cultivation of edible mushrooms can also be used as soil conditioner, natural fertilizer, or food for animals, closing the exploitation cycle of raw materials (Miles and Chang, 1997), which today is called "zeri" technology, trying to get the maximum use of such material, eliminating the residue of the residue (Chang, 2003).

2.3.1 Nutritional importance

Man has constantly realized the nutritional value of mushrooms, as well as their healthy properties compared to other foods, such as red meat, where mushrooms are more advantageous and important as they are great sources of carbohydrates, proteins, mineral salts, vitamins and essential amino acids, which can help to maintain a good nutritional balance (Crisan and Sands, 1978; Garcia et al., 1993; Miles and Chang, 1997).

Nutritional Analyses of mushrooms have shown their importance. They contain more protein than vegetables. Sources of protein such as meat, chicken, have a high level of cholesterol and fat, which are known to cause increase in weight and cardiovascular diseases. For this reason, the proteins from other sources became more popular in recent years, such as proteins from fungi, algae, bacteria and yeast (Lajolo, 1970; Chang and Haynes, 1978; Urben et al., 2003).

Studies carried out by Lintzel (1941; 1943), according to Crisan and Sands (1978), indicated that approximately 200 g of mushrooms (dry weight) are sufficient to feed a normal human being weighing approximately 70 Kg, providing a good nutritional balance. Nutritionally, these macrofungi are a good food source. The composition of fats, carbohydrates, vitamins, etc., varies according to species, the cultivation method and also with the substrate used in cultivation (Crisan and Sands, 1978; Przybylowicz and Donoguue, 1990; Bononi et al. 1999; Miles and Chang, 1997; Andrade, 2007).

Mushrooms are excellent foods for the diets, because they nourish and do not accumulate fat in the organism. They are sources of all essential and some nonessential amino acids. They contain minerals like calcium, potassium, iodine, phosphorus and vitamins including thiamine, riboflavin, niacin, and ascorbic acid, and others related to the B complex (Molena, 1986; Miles and Chang, 1997 Bononi et al., 1999). They also have a high unsaturated fat content (Miles and Chang, 1997).

Mushrooms with larger nutrition index (based on essential amino acid index) have nutritional value similar to meat and milk, while those with a smaller nutrition index compare to some vegetables such as carrots and tomatoes. The nutritional index of these fungi outperforms those of plants and vegetables, except soy (Crisan and Sands, 1978). In general, the protein content of fresh mushrooms is twice higher than cabbage, four times greater than the content of protein of the orange and twelve times that of the Apple (Chang, 1980).

Research carried out in India by Garcia, et al. (1993), where the authors compared the nutritional levels of *Agaricus* and *Pleutotus*, revealed the importance of the amino acids of these mushrooms for people that are lacking animal protein, for religious reasons, and whose main food source comes from vegetables and grains usually poor in essential amino acids. Food supplementation with mushrooms is of fundamental importance in the diet of this kind of people.

In addition, there is also a great interest in the cultivation of the mycelium in a submerged condition to obtain flavoring and fragrant compounds of great value to the food industry. For this purpose, the mycelium is grown submerged, using a variety of substrates, according to the type of the desired compound. This flavoring property is characteristic of some lignolitic mushrooms, such as the *Pleurotus* genus (Gurtiérrez et al., 1994)

2.4 Factors inherent to the nutritional needs of the mushroom

2.4.1 Carbon

The main source of carbon and energy of a plant tissue, used by fungi for their development, are the polysaccharides and lignin in the cell wall, although other polymeric

compounds such as lipids and proteins can also be used. Approximately 50-60% of the dry weight of wood is made of cellulose; 10-30% of hemicellulose and 20-30% of lignin. Cellulose, which is attacked by both brown-rot fungi as well as white-rot fungi, is made up of glucose molecules. On the other hand, the hemicellulose consists of molecules of arabinnose, galactose, mannose, xylose and uronic acids. The lignin has a more complex structure and has not yet been fully described, being basically units of phenyl-propane with a benzene ring bonded to a hydroxyl group and one or two metoxilic groups. The links in this molecule are highly resistant to chemical degradation. Therefore, there are few microorganisms that can use this substance for their nutrition (Mason, 1980).

In relation to the degradation of wood and other lignocellulosic materials, it is generally known that the most efficient natural decomposers of lignin are the white rot-fungi, which are mostly the basidiomycets. This name comes from the white color that wood acquires in advanced stages of degradation (Capelari, 1996). Such organisms degrade cellulose, hemicellulose and lignin, but the lignin is preferentially attacked and these are the only organisms able to metabolize the molecule of lignin in CO_2 and water (Zadrazil, 1978). The degradation is derived from the excretion of enzymes metabolized through the hyphae of fungi (Miles and Chang, 1997).

As a typical white-rot fungi, with decomposing activities of wood, the fungus studied in this work: *Lentinus strigosus*, grows in nature, in favorable conditions, and produces mushrooms through the degradation of the wood substrate or any substrate containing cellulose. From this degradation, the fungi can absorb the nutrients needed for their development and reproduction. The success of mushroom production depends on the understanding of the biology of the fungus and how the environment can influence its growth and development. The domestication of a strain is not a very easy task, when trying to reproduce in the laboratory the ideal conditions for its development, which requires preliminary tests to try to understand its physiology.

2.4.2 Nitrogen

Although wood is the natural substrate for fungi, this substrate does not have a high nitrogen content, and this is necessary for the synthesis of all nitrogen compounds (proteins, purines, pyrimidines and the cell wall chitin of the fungus). The main sources are: salts of ammonia, nitrate, urea nitrogen, and organic compounds like amino acids (Miles and Chang, 1997). However, the need for nitrogen by wood-rot fungi is not very great.

It should be taken into account that when using a salt as a source of nitrogen, there is the release of the ion that integrates the substrate molecules, and this can change the pH of the medium if it is not metabolized at the same rate as nitrogen, since an accumulation of this ion will take place. The same phenomenon occurs when other salts are used as a supplement. Therefore, the various species and strains may respond differently to the addition of these supplements. Urea, ammonia phosphate, tartarate of ammonia and potassium nitrate, apparently are those with best results according to a research carried out by Maziero (1990). Peptone provides better growth of the fungus when compared with other sources of organic nitrogen.

Some authors (Rangaswami et al., 1975; Ginterová and Lazarová, 1987) cited by Maziero (1990), argued that *Pleurotus* has the ability to fix atmospheric nitrogen into organic compounds, because some experiments conducted with pasteurized substrates showed that the total nitrogen content has increased. Kurtzman (1979), cited by Maziero (1990) however, discussed the improbable ability of an eukaryote organism to fix nitrogen. The author

suggested the hypothesis that the spores of nitrogen fixing bacteria are stimulated to develop during the process of pasteurization of the substrate, generating bacteria responsible for the nitrogen fixation.

Care should be taken to avoid excessive nitrogen supplements, which can inhibit the development of the fungus. Montini (2001) reports that tested substrates with high concentrations of cereal bran inhibited the formation of the mushroom and consequently, the number of cultivated mushrooms *Lentinula edodes* in axenic conditions (cultivation with substrate sterilized and under controlled environmental conditions). In Taiwan, the substrate for cultivation of *Pleurotus* mushroom is prepared with 84% of sawdust, 5% of rice bran, 5% wheat straw, 3% soya bran and 3% calcium oxide (Przybylowicz and Donoghue, 1990).

2.4.3 Mineral salts

In general, the mineral elements necessary for the fructification of the mushroom are the same as those required by any cultivated plant, which are major elements and microelements (Molena, 1986). Phosphorus, potassium, magnesium and sulphur are major nutrients needed for the growth of various fungi (Miles and Chang, 1997). Molena, (1986), cites the calcium as one of these elements. In addition to increased growth of mycelium, some minerals such as sodium chloride, magnesium, and calcium also stimulate the early formation of fruiting bodies (Kurtman and Zadrazil, 1989).

Among the more studied microelements (trace elements) and essential for the growth of many species of fungus are: iron, zinc, aluminium, manganese, copper, chrome and molybdenum (Molena, 1986; Miles and Chang, 1997). Experimentally, it is not easy to determine the required quantity of these elements because the element under test may be present in sufficient quantities in an impure form in any ingredient of the cultivation medium or may have been introduced through the inoculum. These elements are constituents or enzyme activators (Miles and Chang, 1997).

2.4.4 Vitamins

Vitamins play an important role in the metabolism of fungi, acting as coenzymes. Fungi are capable of producing sufficient quantities of most of the vitamins they need (Miles and Chang, 1997).

Maziero (1990), in some studies testing several vitamins (Vitamin C, folic acid, calcium pantothenate, niacin, pyridoxine, riboflavin and thiamine) in relation to the mycelial growth of *Pleurotus*, observed a better growth of the mycelium on all vitamins tested, but the best result was to thiamine. Kurtzman and Zadrazil (1989) say that there is no need for the addition of thiamine or other vitamins in "not sterile" substrates, because the other present organisms will normally synthesize them. Molena, (1986) experimented various combinations of vitamins, but their high cost did not compensate for the increased production of mushrooms. (Eira and Minhoni, 1991), report that the vitamins and other growth factors are normally excreted by many microorganisms that live in synthrofy during composting, pasteurization and incubation of the substrate, therefore there was no need of vitamin supplements.

2.5 Physical factors

The growth and development of the fungus are not affected only by nutritional factors, but also by physical factors such as temperature, humidity, light, aeration and gravity. There is a

range that varies from minimum, maximum, and optimum growth in relation to these physical factors. Certainly these factors are influenced by other factors such as nutrition, medium conditions, genetic characteristics of the strain and mycelial growth stage (Miles and Chang, 1997).

2.5.1 Temperature

The influence of temperature on mycelial growth and production of fruiting bodies is dependent on the species and strains in question, i.e. there is an ideal temperature for the proper development of the metabolism of the fungus, which is a characteristic of each strain. Nonetheless, there is an interval that varies between 10-40° C, which must be respected, because exceeding these limits, it is going to cause the death of the mycelium (Maziero, 1990). The optimum temperature for growth also varies with the purpose of cultivation. So, the ideal temperature to produce the fruiting body (Miles and Chang, 1997) is different from that intended for the production of metabolic products such as those intended for medicinal compounds as polysaccharides/polypeptides immune-regulatory compounds (PSPC). Temperature extremes are important in determining the survival and dispersion of species in nature (Miles and Chang, 1997).

Kaufer (1935), cited by Maziero (1990) cultivated *Pleurotus corticatus* in laboratory and according to their results, the ideal temperature for the growth of mycelium was 27°C. Clock et al., (1959) according to Maziero (1990), obtained a good growth of mycelium of *P. ostreatus* in the range of 22-31° C. At 37° C the mycelium still was able to grow, but abnormally, while at 17° C no growth was observed. The lethal temperature for *P.* "florida", *P. ostreatus* and *P. eringii* is 40° C when exposed to more than 24 hours (Zadrazil, 1978). Maziero (1990) studying different strains of *Pleurorus* observed that the better mycelial growth happened between 25 and 30° C.

In relation to the emergence of primordia, Block et al. (1959) cited by Maziero (1990) report that the strain of *P. ostreatus* fructified at a 26° C, however at 31° C, although the fruiting body continues to develop, there was no emergence of primordia. For Kurtzman and Zadrazil (1989), the authors must have used in their work, a strain of *P.* "*florida*" since the fruiting temperature at 26° C is very high, being more appropriate for *P.* "*florida*".

Eira and Minhoni (1997) report that the control of temperature in a cultivation chamber is decisive for a good harvest. For a good growth of the mycelial mass on substrate cultivation, the ideal temperature for *Pleurotus* spp should be between 24 and 26° C. After that the primordia initiation and growth phases start, when the temperature inside the cultivation chamber must be between 15 and 24^0C, considering that, the lowest are ideal for cultivation of shimeji or *Pleurotus* spp strains that are more demanding and also minimizes the incidence of pests and diseases. According to the same authors, some strains of hiratake usually fructify in hot weather (up to 30° C). For the most demanding strains, temperature and relative humidity control in the chamber of cultivation can be achieved with an automated central air-conditioning associated with a ventilation system, to ensure the ideal climatic conditions for the development of the mushroom.

2.5.2 Moisture

Most fungi require high moisture content. Guzmán et al. (1993) report that fungi have an optimum growth on substrates with 70 to 80% humidity. Urben et al. (2003) cite a good humidity range for *Lentinula edodes* cultivated with Jun-Cao technique between 55-70%. It

has to be taken into consideration, not only the moisture content of the substrate, but also the relative humidity of the air. It should also be taken into account that the mushrooms are composed of approximately 90% water, therefore, water is very important to its development, besides the fact that they do not have special structures to protect themselves against water loss, since they lose water easily to the environment, mainly the vegetative mycelium (Maziero, 1990).

There is an optimum water content, both in the compost and in the air. Low relative air humidity causes the mushroom to lose water to the environment, which can even prevent it from growing properly. The outer layers of the mushroom begin to dry and yellow. This way, there is a loss of quality or a loss of production. Low air humidity also causes the compost to lose moisture to the environment, reducing the availability of water for the formation of the mushroom. In the case of *Pleurotus*, if the superficial mycelium of the compost suffers a very intense dryness, it dies and the primordia are aborted (Eira and Minhoni, 1997; Bononi et al., 1999). The relative humidity of the production room is around 80-90% and can be maintained that way by waterproof walls and by sprinkling water (Eira and Minhoni, 1997). There are highly sophisticated systems of cultivation on a commercial scale in Europe, Canada, United States and Japan, where patterns of moisture, temperature, O_2 and CO_2 are monitored by computers. Currently there are automated systems in South and Southeast of Brazil, but not as much as in those countries. In rustic cultivations in Brazil, it is customary to keep the floor and sides of the cultivation shed damp, so that normal evaporation maintains the relative humidity the air. We consider that, in addition to other factors already mentioned, the humidity is the key factor in the cultivation process of edible mushrooms.

2.5.3 Lighting

Even though it is not a photosynthesizer organism, luminosity is essential to many species of fungi. It can retard the primordia formation in some species while in others, it is essential for fruiting. For *Pleurotus* and *Lentinus* cultivation, as well as for many other edible fungi, there must be some light to induce the formation of primordia and also for the normal development of fruiting bodies. The recommended luminosity for *Pleurotus*, after the incubation period and the opening of the cultivation bags is 2000 lux/hour, 12 hours a day (Bononi et al., 1999). Nevertheless, it can vary according to the mushroom species.

Miles and Chang (1997) mention that ultraviolet light in the range from 200 to 300nm affects the growth of the fungus, it can be lethal or induce mutation, since this wavelength is absorbed by the DNA. The authors report that the effects of ultraviolet light can be reversible by the photo reactivation process, provided that these mycelia are exposed to visible light at a wavelength between 360 and 420nm.

For Przybylowicz and Donoghue (1990), shiitake mushroom needs light in both stages: vegetative growth and fruiting. Light exposure during vegetative growth, according to Ishikawa (1967) cited by Przybylowicz and Donoghue (1990), is a prerequisite for the fruiting stage. The duration is not well defined. However, Przybylowicz and Donoghue (1990) suggest that a brief exposure of 20 minutes per day can be enough. For these authors, the growth of shiitake responds well to a range between 180-940 lux, with an optimum value of 500 lux. Rajarathnan and Bano (1987), cited by Eira and Minhoni (1997), stated that the presence of light is required for the formation of fruiting bodies. However, there may be changes in the color of the pileus, where *Pleurotus* species can change from white to opaque and dark color in the presence of light, due to the release of fenoloxidases that oxidize phenol and form melanoidins.

Urben et al. (2003) report that the light affects the growth of mycelium and spores of *Lentinula edodes*, and therefore, it needs a dark environment for its development. Under a light intensity of 50 to 270 lux and a suitable temperature, the mycelium forms a membranous brown layer for substrates made with Jun-Cao and sawdust. According to the same authors, for the formation of the fruiting body (mushroom), little diffused light is necessary. On the contrary, in a very bright environment the fruiting body becomes pale with a long stipe and a deformed pileus. In very bright environments the authors advise to use plastic bags in the green house to cover the mushrooms during the day.

2.6 Chemical factors

2.6.1 Gaseous exchanges

Requirements during the growth phases of the vegetative mycelium of a fungus are different from those during the fruiting stage. The rate of CO_2 that occurs naturally inside a trunk colonized by *Pleurotus* in the forest will surely be higher than the rate of fruiting. However, it does not cause damage to growth. It is a self-regulated system.

Zadrazil (1975), studied various *pleurotus* species, relating to the effect CO_2, and noted that all studied species grew faster in higher concentrations of CO_2, limited to approximately 22%. The good performance of these strains in high rates of CO_2 demonstrates their significant competitive advantage against other microorganisms which do not grow or do not survive in such conditions, especially if the substrate is colonized in not axenic conditions. On the other hand, high concentrations cause a deformation of the fruiting body, being similar to that which occurs when there is light deficiency in the development of the fruiting body. The stipe grows sharply and the pileus stays reduced, similar to the process of etiolation in plants (Zadrazil, 1978). Oxygen also influences the growth of mycelium. Despite the fact that *Pleurotus* mycelium develops in semi-anaerobic conditions, a certain rate of O_2 is required, otherwise, the growth will be nil (Zadralil, 1978). For the development of fruiting body oxygen is essential.

Adequate ventilation is essential to reduce the carbon dioxide content (generated during the development stages of the fungus) to a desirable level in the mushroom production phase. Concentrations above 2% may cause delays in the mycelial growth and, consequently, decrease productivity (Eira and minhoni, 1997). Concentrations of CO_2 below 0.2% are considered optimum for development. During a peak of growth, the ventilation must be intense and constant, since large quantities of mushrooms in rapid growth give off large amounts of CO_2 (Eira and minhoni, 1997). The same authors reported that in cultivations carried out in The Mushrooms Module of the Faculty of Agricultural Sciences of the "Universidade Estadual Paulista" (FCA/UNESP) it is possible to cultivate strains that usually demand cold weather, provided that climatic chambers for thermal shock are used.

2.6.2 pH

Its importance is primarily related to the metabolism of nutrients. Most mushrooms have a good development with pH levels between 6.5 and 7, but there are variations according to the species and strains (Miles and Chang, 1997). The microbiota present in the substrate, according to Zadrazil and Grabbe, (1983), is distinctly influenced by the initial pH level: values below 7.0 usually are good for the development of the mushroom mycelium, but most fungi can develop at pH levels above 7.0. Urben et al. (2003), reporting about the cultivation of *Lentinula edodes* by Jun-Cao technique, stated that the mycelium can grow in pH levels between 3.0 to 6.5, while the ideal range is between 4.0 and 5.5. However, the pH

value between 3.5 and 5.0 is the best for the formation of primordia and development of the fruiting body. For this reason, the pH value should always be monitored when choosing the materials that compose the substrate, the cultivation and the source of water supply (Urben et al., 2003).

The pH is directly linked to the enzymatic reaction of fungus and wood. Each enzyme has its optimum pH value. The pH affects the solubility of the compost which in turn determines its availability to the fungus (Przybylowicz and Donoghue, 1990). The optimum pH value for the wood-rotting fungi *Lentinus* and *Pleurotus* is between 4.5 and 5.5. The pH of the wood is usually 4.5 to 5.0, increasing the acidity with its decomposition. The optimum pH value for fruiting lies between 3.5 to 4.5 (for laboratory culture or artificial medium) and 5.0 for compost with sawdust for *Lentinula edodes* (Przybylowicz and Donoghue, 1990)

2.7 Steps to be followed in the cultivation of mushrooms

For the cultivation of edible fungi the following steps are generally adopted: obtaining primary matrix, the production of seed or Spawn (matrix that will serve as inoculum for the substrate), preparation of substrate or compost, sterilization or pasteurization (when cultivation is done in natural conditions), inoculation and colonization of substrate, inducing primordia (with thermal or water shock when necessary), fruiting and harvest. The production aspects of primary decomposition fungi like *Pleurotus*, *Lentinula edodes*) will be covered here.

2.7.1 Obtaining primary matrix and spawn production

For most mushrooms, the production matrix or mycelium follows the same techniques and recommendations for the cultivation of champignon (*Agaricus*), oyster mushroom (*Pleurotus*), shiitake (*Lentinula edodes*) and jewish ear (Auricularia), with some exceptions (Urben et al., 2003). Two distinct steps are fundamental for the preparation of the matrix: obtaining pure inoculum of the fungus and the preparation of the "spawn" or matrix itself.

Obtaining the primary matrix of mushroom can be performed both by sexual or by asexual process. In this work it will be related as an asexual process. It is relative to the mycelial or vegetative phase of the fungus colonizing a previously sterilized nutritional substrate (growth medium). Its production starts by the isolation of a fungus using tiny fragments of a mushroom, placed in sterile culture medium under aseptic conditions. After mycelial growth in the dark, with a temperature of 24 ± 1^0C (depending on the strain), fragments of this culture (primary matrix) are transferred to the cereal grain or bran or sawdust enriched with bran and incubated for 30 days in the dark at 24 ± 1 ^{0}C. This step corresponds to the production of the "seed" or spawn (Molena, 1986; Eira and Minhone, 1997; Eira and Montini, 1997). The main function of the grain is to serve as means of dispersion of mycelium, since it is impossible to handle the mycelium without damaging the fragile structure of the hyphae walls (Maziero, 1990).

Although the most used media to obtain the primary matrix are potato-dextrose-agar and malt extract (Bononi et al., 1999), the sawdust-dextrose-agar (SDA) medium is the most indicated by avoiding the physiological adaptation that can occur when the used culture medium has very different characteristics of production substrate (Eira and Minhoni, 1997, Eira and Montini, 1997).

The current trend is to produce inoculum from the cultivation substrate. When working with sawdust it is possible to produce the inoculum ("seed") with grain mixed with sawdust

or with sawdust only. In this case "spawn" or "seed" is the substrate colonized by mushroom mycelium, with the goal of facilitating the distribution of the inoculum in different points of cultivation, thereby contributing to a more uniform and rapid colonization of the substrate, reducing the possibility of contamination.

2.7.2 Substrate cultivation

Currently there is a growing tendency to use agro-industrial residues for the cultivation of edible and medicinal fungi. However, traditional methods are still being used like the cultivation of *Lentinula edodes* (shiitake), by some Japanese and Chinese farmers, using oak and hazel logs, although the cultivation in cylindrical tubes (in polypropylene or high density polyethylene-HDPE bags) with enriched sawdust is the most widely used technique.

The technique for the production in sawdust was developed mainly in Japan. Other countries like the Netherlands and the United States are also using this method for the production of *Lentinula edodes* (shiitake), on a large-scale (Bononi at al., 1999). In Brazil the traditional cultivation is done normally on eucalyptus logs and it may also be grown on logs of avocado, mango, walnut, hazel and oak (the last two being widely used for cultivation in Japan) (Eira and Minhoni, 1997). Eucalyptus sawdust is already used in Brazil for the production of this mushroom.

The material used for production of mushroom has to be preferably a residue, easily available, and produced not far away from the cultivation place to lower the production costs. Care should be taken to observe that the waste should be free of chemicals that could affect the growth of the mycelium and not offering toxicity. If a low productivity residue is used, supplementation has to be made with cereal grains or cereal bran (Eira and Minhoni, 1997; Bononi et al., 1999; Przybylowicz and Donoghue, 1990; Stames and Chilton, 1983; Stames, 2000).

The supplements contain a mixture of protein, carbohydrate and fat, where the protein is the main source of nitrogen. They contain minerals and vitamins that also influence the growth of the fungus. The addition of these supplements aims mainly to increase the levels of nitrogen and carbohydrates available. Sugars and starch which are readily available carbohydrates, speed up colonization and the consequent degradation of the substrate, reducing the time of fruiting since the mycelium easily converts these carbohydrates in reserve for the fructification, increasing productivity (Przybylowicz and Donoghue, 1990). Other supplements like limestone ($CaCO_3$) must be added to the cultivation medium, to get the correct pH favorable for the growth of the fungus during the last stages of decomposition since there is an increase in acidity caused by the fungus metabolism. Gypsum is widely used in the mushroom industry to improve the physical structure of the compost and to change the pH value, also acting as a source of calcium (Przybylowicz and Donoghue, 1990). The concentration of 5% (in relation to the dry weight of the substrate) is ideal for the cultivation of shiitake in sawdust, improving structure and porosity of the substrate (Stames and Chilton, 1993)

When working with primary-decomposer fungi as *Pleurotus* and *Lentinus*, i.e. fungi that degrade the structural elements of the residue, it is important to ensure that the material to be used in cultivation has not undergone decomposition by microorganisms during storage. If it is already degraded, colonization by these fungi will be hampered and the attack of other organisms will be facilitated, causing a reduction in productivity (Maziero, 1990).

The sawdust used to prepare the substrate is usually from hardwoods. Sawdusts of conifers are used for *Lentinula* cultivation (shiitake) in areas where there is shortage of hardwood

sawdust and it is therefore necessary to make a mixture of the two kinds of sawdust (Przybylowicz and Donoghue, 1990). Many conifers contain resin and phenolic compounds which inhibit the growth of the fungus. These compounds must be degraded or removed before using this kind of sawdust, or it can be changed with the addition of sodium carbonate to remove these compounds (Przybylowicz and Donoghue, 1990).

Various types of substrates have been used for the production of edible fungi (Guzmán and Martinez; 1986; Guzmán et al., 1993; Maziero, 1990; Bononi et al., 1999; Eira and minhoni, 1997; Miles and Chang, 1997; Stames and Chilton, 1983; Stames, 1993; Urben, 2001; Urben et al., 2003; Zhanhua and Zhanxi, 2001). The most used are: sawdust, wheat straw, corn, rice; corn cobs, sugar cane bagasse, various grasses, supplemented with cereal grain or bran. The choice of one or more residues as supplement to sawdust will depend, among other factors, on cost and availability of these materials (Maziero, 1990; Eira and Minhoni, 1997; Eira and Montini, 1997; Guzmán et al., 1993; Urben et al., 2003; Stames and Chilton, 1983; Stames, 1993).

When using the bagasse of sugar cane it is important to make sure that this residue is not very old, which can reduce productivity. However, the fresh ground bagasse is rich in carbohydrates, allowing other competitors or pathogens to colonize the substrate more quickly. To avoid this problem, the residue should be pre-treated through a process of fermentation or washing (Kurtzman and Zadrazil (1989)

Japanese producers of *Flammulina Velutipes*, *Auricularia* and *Pleurotus ostreatus* use a standard formula with a ratio 4:1 of sawdust and bran respectively, where the sawdust is aged for one year, with the purpose to improve the water retention capacity. An immersion of sawdust in water before mixing with the bran is an effective way used by these producers to achieve an optimum of 60% humidity (Samets and Chilton, 1983). This method, according to Lizuka and Takeuchi (1978) cited by Przybylowicz and Donoghue (1990) is widely used in Asia. In the United States, 80% of sawdust, 10% bran and 10% grain (usually wheat or millet). In Taiwan, the substrate for the cultivation of shiitake is done with 84% of sawdust, 5% of rice bran, 5% wheat straw, 3% soy bran and 3% calcium oxide (Przybylowicz and Donoghue, 1990). The substrate formulations have become unlimited in terms of raw material and agro-industrial residues (Stames, 1993).

Currently, studies are trying to develop a technology that allows the cultivation of edible mushrooms in substrates of low cost and easily available. Perhaps this explains why in Brazil the largest edible fungi producing region is located in São Paulo, where there is a big sugar and alcohol production. This bagasse comes from sugar cane mills, it is homogeneous, it has a fibrous characteristic, and when it is pressed allows aeration for mycelial growth (Rossi, 1999). The sawdust is also a material in abundance in the Amazon region, because of its timber industry.

2.7.3 Pasteurization/sterilization

The pasteurization process is a heat treatment given to the compost for the removal of possible organisms that could compete with the fungus to be cultivated (Maziero, 1990). It can be done in a natural way, in a pasteurizing tunnel or room without heated steam, using only the thermogenesis, with the control of the air that gets in and out in order to control the temperature inside the room, or it can be made with heated steam produced by boilers heated with firewood, diesel or gas.

Pasteurization for the cultivation of lignicol fungi as *Pleurotus*, occurs when after the revolving process of the compost, the temperature of thermogensis, produced by the action

of microorganisms, falls below 45 to 50^0 C (Eira and Minhoni, 1997). The compost is then introduced in the pasteurization chamber. The pasteurization temperature for *Pleurotus* is more severe than for *Agaricus*, being raised to 75^0C during the first 6 hours. After cutting the steam, the temperature falls to 40 to 45^0 C, maintaining a constant ventilation to cool the compost and then proceeding to the inoculation process (Eira and Minhoni, 1997). Depending on the type of cultivation, the substrate to be inoculated can be packed in plastic bags, put in wooden or plastic boxes, shelves or "bed", pressed blocks covered with plastic sheets or in special containers (Maziero, 1990).

2.7.4 Inoculation of the substrate

The sterilization process (used in axenic cultivation) or pasteurization (when working with composted natural substrate) is followed by the inoculation of the substrate, which is made immediately after the cooling of the substrate in aseptic conditions (laminar flow chamber) in case of cultivation in totally axenic conditions. Under these conditions, the substrate to be inoculated is autoclaved at 121^0 C for 2 to 4 hours (Eira and Minhoni, 1997). For cultivation under natural conditions (not axenic), the substrate is inoculated after pasteurization and cooling of substrate that is around 30^0 C, in aseptic place.

There are several types of inoculum ("seed"): with grains, grains with sawdust, and less used liquid inoculum. There are still those that are made of small wooden dowels (wooden rods inoculated with fungus, used for the cultivation of shiitake in logs). Inoculum of sawdust/grain is used for *Pleurotus* and *Lentinus* cultivation, when using sawdust for cultivation. It is important that the inoculum is the same sawdust from the cultivation substrate. The quantity used for the cultivation substrate is also variable. It is usually 0.5 to 5% (v/v) (Chang and Miles, 1997). (Zadrazil and Grabbe, 1983), recommend 0.5 to 5% of wet substrate. Urben et al. (2003), recommend 0.5 to 5% of the wet weight of the substrate for the *Pleurotus* cultivation when using the Jun-Cao technique. Gonçalves (2002) studying the effect of mycelial fragmentation in order to obtain inoculants in suspension (liquid fermentation) for cultivation of shiitake in axenic cultivation, found that inoculants fragmented up to 10 seconds provided greater biological productivity and efficiency in comparison with usual solid inoculants. The author reports that cultivation using liquid inoculants has the advantage of reducing the time for fruiting. However, it has the disadvantage of having predisposition for degeneration and mutation after successive crops (Itaavara, 1993), cited by Gonçalves (2002).

2.7.5 Substrate incubation

Incubation period, also known as the "mycelial race", is the development of the vegetative mycelium on the substrate (Przybylowicz and Donoghue, 1990). It is the mechanism in which the mycelium of the fungus, through an enzymatic process, digests the substrate and stores reserves for fruiting. During this process the mycelium develops and colonizes the whole compost, forming a compact white mass. It is a complex process, characterized by intense biological activity in which molecules of cellulose, hemicellulose and lignin of the compost are attacked by fungal enzymes such as cellulase and lacase that reduce these molecules to phenols and simple sugars which are more easily assimilated. This enzymatic activity lasts from the beginning of colonization until the production of mushrooms, but during the period of growth of the mycelium production it is greater (Bononi et al., 1999). Incubation usually occurs in a room that can be dark or not, depending on the light requirement of the fungus, at a temperature between 22 to 25^0 C for *Pleurotus* (Maziero, 1990).

Guzmán et al. (1993) uses the range 25 to 30^0 C for the cultivation of several *Pleurotus* species in Mexico. Bononi et al. (1999) report that the ideal temperature for the incubation of these fungi varies between species, but in general it should be kept between 25 and 28^0 C (the temperature of the compost). The range 25-30^0 C is also used for the *Pleurotus* cultivation, and 22-25 ^{0} C for *L. edodes* cultivation in Jun-Cao (Urben et al., 2003). Przybylowicz and Donoghue (1990), reported temperatures of 25^0 C that are ideal for *L. edodes*.

It is important to monitor the temperature during the mycelial race to maintain the optimum temperature for the growth of the mycelium. If there is an excessive rise in temperature (a phenomenon that occurs during metabolic activity of *Pleurotus* and micro-organisms present in the substrate) mycelial growth retardation or even its death may occur. Containers with large amounts of substrate mass are avoided since of heat loss is hampered, and generates an increase in temperature. (Maziero, 1990).

The incubation period is approximately three weeks for *Pleurotus* (Maziero, 1990; Bononi et al., 1999). At low temperatures, such as 4-5^0 C, the mycelium of most species ceases its activity, entering "latency", and at temperatures over 35-40^0 C can be lethal to certain species (Bononi et al., 1999). To avoid excessive internal temperature of the substrate during the incubation period, Bononi et al. (1999) recommend keeping the room temperature between 20-22^0 C, and avoid to clutter the bags of the substrates.

The incubation period is variable, because the development of mycelium occurs within variable time, according to the type of the inoculum, the quality of the compost and conditions of the cultivation chamber, but it generally oscillates between 20 and 30 days for *Pleurotus* (Eira and Minhoni, 1997). Urben et al. (2003) report 20-45 days for the total development of the mycelium with Jun-Cao technique. For the cultivation of shiitake in logs, Eira and Minhoni (1997) report that, after two to three months from log incubation, there is already a significant mycelial growth, which can be indicated by a yellow color in the region of the inoculated holes and the region around those holes become soft. In natural conditions of cultivation on logs, this period of maturity of the mycelium for mycelia production, ranges from six months to a year (Przybylowicz and Donoghue, 1990).

During the colonization of the substrate in the cultivation of *Lentinula edodes* using sawdust enriched with rice bran, packed in plastic bags, Bononi et al. (1999) recommend cycles of alternating light and dark, with at least 8 hours of light per day during a period of four to six weeks. The wavelengths between 370 to 420nm and light intensity between 180 to 500 lux are more efficient during the process of colonization, and it can be achieved with cold fluorescent lamps (Przybylowicz and Donoghue, 1990). According to Bononi et al. (1999), after the total colonization of the substrate, the plastic bags are cut and the surface of mycelium begins to turn into a brown skin. The air humidity must be maintained around 80 to 90% and between 40 to 50 days after the opening of the bags the production starts, after the induction of primordia through thermal shock for 24 to 48 hours at 10^0 C.

At the end of the mycelial race for shitake there is a period of mycelial stability or mycelium maturation, which lasts until the hardening and darkening of the mycelial skin that becomes brownish grey (Chang and Miles, 1989). The formation of mycelial cover is very important because it acts as a barrier to moisture loss, being also a defense against contaminants, resulting from the oxidation of poliphenol oxidase, a reaction to light and oxygen (Przybylowicz and Donoghue, 1990).

2.7.6 Induction of primordia, fruiting and harvesting

The induction of the primordia occurs naturally in nature. The sudden change of external physical conditions stimulates primordia formation, which will develop, forming the

fruiting body (Bononi et al., 1999). On the cultivation of mushrooms, it is used to stimulate or speed their formation. During the induction phase and the production of mushrooms, physical factors such as temperature, lighting, gas exchange, water availability in the compost, relative humidity and the methods of induction are aspects that influence the production and the quality of mushrooms (Zadrazil and Grabbe, 1983).

Sudden changes in temperature usually cause induction of primordia. However, there are differences according to the strains (Przybylowicz and Donoghue, 1990). Low temperatures may indirectly induce fruiting in strains of shiitake, because of the reduction of metabolic activity, reducing therefore the available nutrients, leading to a condition of "stress". On the other hand, in other fungi, temperature can have a direct effect, favoring specific metabolic processes that trigger the induction (Przybylowicz and Donoghue, 1990).

Hawker (1966), cited by (Przybylowicz and Donoghue, 1990) reports that studies with various fungi showed that reducing sugars readily available on the substrate (end of vegetative growth) favors the fruiting. During the entire cycle of fruiting, the primordia phase is the most sensitive to environmental changes. The moisture content of the substrate, temperature and relative humidity are important in this process. On the cultivation of shiitake in logs, moisture for primordia induction should be around 55-65% and the temperature depends on the strain (Przybylowicz and Donoghue, 1990).

There are several artificial induction mechanisms of primordia. It can be done by changing the temperature of incubation (± 25^0 C) to lower temperatures (±16^0 C) in *Pleurotus* cultivation "shimeji" (Eira and Minhoni, 1997). Some strains respond well to this temperature variation, others produce more when subjected to thermal shock.

Marino (2002) in a study about genetic improvement with *Pleurotus ostreatus* aiming the axenic cultivation of strains resistant to heat obtained strains that stood out by their early fruiting and productivity, with two production cycles and without the need for thermal shock, using water immersion only.

For the cultivation of shiitake in sawdust, according to Leatham (1985), cited by Przybylowicz and Donoghue (1990), the thermal shock can be done by cooling the cultivation blocks (packed in bags of polypropylene) at a temperature of 5^0-8^0 C for five to twelve days or by putting them into cold water (5-16^0 C) for 12 to 24 hours, packing them later in the fruiting room (16^0C). After some time primordia will appear at the top of the bags. After the development (3-4 days), according to Eira and Minhoni (1997), the mushrooms are ready to be collected.

Additional flushes of fruiting will emerge without the need for new inductions, provided they are kept in conditions of fruiting. Producers can control the flush making synchronized induction by heating the blocks, followed by reduction of temperature or thermal shock. Sprinkling or immersion can also induce the flush (Przybylowicz and Donoghue, 1990).

Treatment for production of mushrooms (Eira and Minhoni, 1997) is done by reducing temperature and/or water logging (covering with clean cold water for 2 to 4 hours) and removing the bag after water drainage.

The thermal shock for *Lentinula edodes* in modified Jun-Cao technology is made by dipping the miceliated substrates in cold or icy water during 7-8 hours. Then, the bags are packed in a shed or green house. When the buttons (primordia) begin to emerge, the plastic bags (high-density polyethylene) are removed, and the substrates are watered twice a day. After spraying, the bags are covered with a plastic for two hours or until the environment is agreeable (Urben et al., 2003). Regional climatic variations need to be considered. The relative humidity varies with the location of cultivation.

In Brazil, the necessary time for the complete development of the shiitake mushroom is not well defined due to climatic variations. The fruiting occurs over a period between three and twelve months after the inoculation, depending on the temperature of the region and the maintenance of moisture in the log (Eira and Minhoni, 1997; Eira and Montini, 1997). To accelerate this process in the cultivation on eucalyptus logs, the authors recommend soaking the miceliated logs for induction, after the incubation period when the first signs of primordia emission (callus or popcorns) which usually appear after 2 to 3 months. Mineral supplementation in water immersion increased the productivity of this mushroom. However, the increase of productivity and the efficiency of energy conversion were only possible in logs well colonized by the fungus (Queiroz, 2002; Eira and Minhoni, 1997; Eira and Montini, 1997).

In relation to water temperature for immersion, there is a controversy, probably because of environmental differences, and observations often without experimental parameters (Eira and Minhoni, 1997; Eira and Montini, 1997). Some Brazilian producers who own cooling bath system report positive results since this system causes a steady temperature differential of 5-10^0 C. However, experiments performed in the Module of Mushrooms of the Faculty of Agricultural Sciences "Universidade Estadual Paulista" (FCA/UNESP), in Botucatu, São Paulo State, these same authors report that, in regions with mild climate and thermal amplitude greater than 10^0 C, the use of ice for cooling did not show significant difference in relation to normal bath immersion.

Induction time depends on environmental conditions and age of the logs and the fruiting temperature varies from 5 to 30^0 C depending on the strain and the spawn used for cultivation. The relative humidity of the location of the logs should be between 80 and 90%. The emergence of primordia will be within two to three days and harvesting can be made after seven to ten days, and in cool seasons the metabolism of the fungus is reduced, increasing the time before harvest (Eira and Minhoni, 1997). The induction bath can be done in stages, depending on the needs of the producer, thus inducing bath of logs can be programmed as a function of demand (Eira and Minhoni, 1997; Eira and Montini, 1997).

3. Material and methods

The study was carried out at the Edible Mushroom Cultivation Laboratory from the Department of Forest Products in the "Instituto Nacional de Pesquisas da Amazônia" - INPA, in the following steps:

3.1 Collection, drying and preparation of material

Wood residue (sawdust) was chosen based on the generation of the wood waste produced by local lumber industry. Collection, drying and preparation of materials were done at CPPF/INPA, using sawdust of *Anacardium giganteum Hanck* ex Engl (cajuí). After the collection of the residues, they were dried (12% of humididy) in a solar dryer at CPPF/INPA, and packaged into plastic bags of 100 L until the preparation of the substrates.

3.2 Production of a primary and secondary matrix and the "spawn"

The strain of *Lentinus strigosus (Schwinitz) Fries* was taken from the collection of fungi at INPA Institute. Mycelial fragments of fungus (stored in test tubes) were transferred to a Petri dish containing malt medium and incubated at 27 ^{o}C until colonization by the fungus

(primary matrix) that was used as a source of inoculum for the secondary matrix . Mycelial disks, 9 mm in diameter, were removed from the primary matrix and transferred to Petri dishes containing SDA medium (sawdust-dextrose-agar), prepared according to Sales-Campos (2008), named secondary matrix. "Spawn" is the source for inoculation of the cultivation substrate, considered here as a tertiary matrix. This matrix was produced from cajuí sawdust, with humidification of 75%. The pH was corrected to approximately 6.5, by adding $CaCO_3$. Then that substrate was deposited on glass bottles of 500 mL, in 200 g portions, which were autoclaved at 121 ˚C for 45 minutes. After cooling, the substrate was inoculated with the secondary matrix. The bottles were partially closed, and kept in special chamber with biochemical oxygen demand (BOD) at 25 ± 2 °C until the complete colonization of the substrate by fungus. This matrix served as a source of inoculation for the cultivation substrates for the production of *L. strigosus* mushrooms

3.3 Preparation of cultivation substrate and processing

The cultivation substrate was prepared from the same residue (cajuí sawdust) as the spawn inoculums. It consisted of 88% of sawdust + 10% of the soy bran as a protein source + 2% of $CaCO_3$, for pH adjustment (6.5). The material was homogenised and humidified to 75%, and packed into bags of high density polyethylene-HDPE (1 kg capacity). Only 500 g of the substrate (wet basis) were put into each bag, with ten repetitions. The substrates were autoclaved at 121 °C for one hour. After that, they were cooled and inoculated with a tertiary matrix under axenical conditions. Each experimental unit (the bag containing the substrate) received 3% of the inoculum in relation to the wet weight of the substrate. They were taken to an incubating chamber until the colonization of the substrate by the fungus. Afterwards, they were transferred to a production chamber. The control samples were also prepared as above, but without inoculation by the fungus. The bags were taken to an oven with air circulation at 55 ± 5 ^{o}C and dried to a constant weight, in order to obtain the dry mass of the initial substrate (DMIS) so that they were used to calculate the productivity, based on the biological efficiency index of substrate (BE) and the loss of organic matter (LOM).

3.4 Experimental conditions

The experiment was conducted indoors. The bags contained the substrates were incubated in a climatic chamber at the temperature of 25 ± 3 °C, in the absence of light and at around 80-85% humidity, in order to allow substrate colonization until the production of primordia. Then, they were transferred to the production chamber. The temperature was reduced from 25 ^{o}C to 22 ^{o}C to induce primordial emission and to allow the production of basidioma (fruit body of the mushrooms) in a way that it would be as uniform as possible. Light intensity was maintained at 2000 Lux, with a photoperiod of 12 hours per day. The relative humidity was scheduled to 95% during the "fructification. The total period of cultivation was 100 days. After "fructification", the mature mushrooms were collected and weighed, and then oven dried for the determination of moisture, dry mass and chemical analyzes. During cultivation, the variables analyzed were: biological efficiency (BE), and loss of organic matter (LOM). Biological efficiency (used to express the productivity of fungus), was calculated according to Tisdale et al. (2006) and Das and Mukherjee (2007):

$$BE = \frac{FMM}{DMS} * 100$$

Where:
BE= Biological efficiency, %
FMM= Fresh mass of mushrooms, g
DMIS= Dry mass of the initial substrate, g

The loss of organic matter (LOM) is the index that evaluates the substrate decomposition by the fungus. It was evaluated according to Sturion (1994), expressed by the following formula:

$$LOM = \frac{DMIS - DMSS}{DMSS} * 100$$

Where:
LOM = loss of organic matter,%
DMSS = Dry mass of the spent substrate, g
DMIS = Dry mass of the initial substrate, g

4. Results and discussion

Table 2 presents the development of the *L. strigosus*. The fructification happened three to five days after the primordia initiation (Table 2), with the development of vigorous mushrooms.

Period (days)						
Mycelial growth	Primordium emission	Fruiting	Cultivation time	Number of flushes	Pileus cm	Stipe cm
11 to 15	33 to 34	35 to 38	100	6	2 to 7	1

Table 2. Profile of the *Lentinus strigosus* mushroom cultivated in cajuí sawdust with soy bran as protein source during 100 days.

The high biological efficiency (BE) of the substrate formulated with cajuí sawdust, supplemented with soy bran demonstrates good productivity of the substrate (Table 3). The result (80%) is superior to other substrates used in cultivation of *P. ostreatus* mushroom formulated with sawdust of *Fagus orientalis* (Yildz et al., 2002), and with *Eucalyptus* sp. according to Marino et al. (2002) which presented BE equal to 8.6 to 64.3% and 11.4 to 43% in their respective studies. The good productivity of this substrate is the result of the quantity of material readily available and absorbed by fungus during the mycelial development process and the soy bran was a good source of protein.
Philippoussis *et al*. (2003) showed that the mycelial growth rate is related to the bio-availability of nitrogen and that the formulation of the substrate influences nutritional levels and porosity (availability of O_2) and Gbolagade *et al.* (2006) stated that each fungus utilizes a specific C/N ratio. The soy bran provided a good source of protein for the fungus as we can see at the table 3 (20%). The results are very important for this edible mushroom, since they present low lipid content (2.5%) and a high fiber level (18%).
The use of alternative substrates, easily obtained at low cost for the cultivation of edible mushrooms have been investigated in many publications (Özçelik and Pekşen, 2007; Philippoussis *et al*., 2007; Royse and Sanchez, 2007). The supplementation of the substrates with a nitrogen source, mainly with cereal bran, has been adopted to achieve a C/N ratio good for the production of mushrooms.

Özçelik and Pekşen (2007), analyzing the application of hazelnut shells in the formulation of substrate for mushroom cultivation *Lentinula edodes*, reported that the biological efficiency of the substrate made with hazelnut shells only, was considered to be low (43.73%). However, when the proportion of hazelnut shells was reduced and combined with wheat straw (25:75) the biological efficiency was considered good (62.24%). The result however, is less than that reported in this study (Table 3).

Philippoussis et al. (2007) tested the productivity of agricultural residues (sawdust of oak, wheat straw and corncobs) in the cultivation of *Lentinula edodes* and found that corncobs and wheat straw presented higher rates of biological efficiency: 80.64% and 75.23% respectively, which were similar to those presented in this research. Alberto and Lechner (2007) however, obtained lower BE (61.93%), cultivating *Lentinus tigrinus* with Salix sawdust.

Royse and Sanchez (2007) tested three formulations for the cultivation of *L. edodes*, combining wheat straw and oak residues. They found that the substrate with higher proportions of wheat straw (in relation to oak residue), provided the best biological efficiency (98.9%) at the end of 4 harvests. These results are superior to the ones obtained in this work. However, 80% of Biological Efficiency presented by *L. strigosus* cultivated in cajuí sawdust supplemented with soy bran in the present study is considered high.

Productivity Results			Nutritional Composition			
Substrate Cajui Sawdust	Biological Efficiency (BE) (%)	Loss of Organic Matter (LOM) (%)	Total Protein (%)	Total Fiber (%)	Lipid (%)	Ash (%)
Standard deviation	6.94	4.85	1.00	2,00	0.30	1.00
Average	**80.0**	**47.51**	**20**	**18**	**2.5**	**5**

Table 3. Results of Productivity and Nutritional Composition of the edible mushroom *Lentinus strigosus* cultivated on cajuí wood waste, supplemented with soy bran.

5. Conclusions

The high biological efficiency of the mushroom in this substrate, formulated with the cajuí sawdust supplemented with soy bran, makes its use feasible for the cultivation of *Lentinus strigosus* mushroom from the Amazon Region. The soy bran provided a good source of protein for the fungus.

The findings presented herein point out the utilization of the Amazon wood waste as substrate for the mushroom cultivation, which will certainly promote the improvement of the social and economical conditions of its people and the sustainability of the biodiversity resources, enabling the establishment of a new economical niche in the region.

L. strigosus can be considered an important food in terms of their characteristics: rich in protein and low in fat, important for nutrition and human health.

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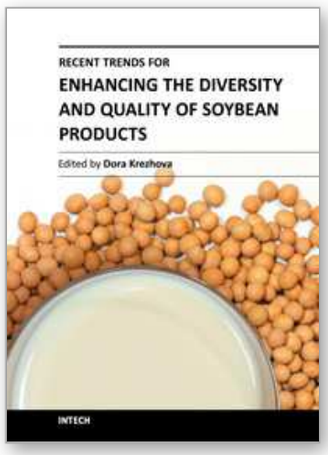

Recent Trends for Enhancing the Diversity and Quality of Soybean Products

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