Evaluation of Different Colombian Agroindustrial Wastes as Substrates for the Growth and Production of *Lentinula edodes*. Berk. Pegler (Shiitake)

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Abstract: The objective of this study was to determine the best substrate or substrates for the development and production of *Lentinula edodes*. Berk. Pegler, well known as "Shiitake". This is a white rot mushroom, very appreciated as a nutritional food. The substrates used were Colombian agroindustrial wastes (cotton gin waste, sugarcane bagasse) and sawdust of different vegetal species (*Eucalyptus* sp., *Pinus* sp. and *Quercus* sp.). The substrates were packed into plastic bags and sterilized before inoculation with *L. edodes* spawn cultured on wheat grains. The extent and speed of the mycelium growth were evaluated as well as basidiocarp diameter, weight, biological efficiency and organoleptic properties. The best substrates for the production of high quality shiitake were sugarcane bagasse and *Eucalyptus* sp. sawdust with B.E of 93.8% and 76%, respectively.

Key words: Biological efficiency, *Lentinula edodes*, quality, shiitake, substrates, agroindustrial wastes

1 Introduction

Shiitake is a traditional mushroom, native to Japan, Korea and China; it is valued both for its flavor as well as for its healthful properties.[1] It has been cultured in the mountainous regions of Asia for over a thousand years using traditional techniques, and only in the last thirty years has attention been paid to the development of superior culture techniques in order to reach adequate crop yields for its commercialization to the western world.[2]

The substrates used in the western world for shiitake production are commonly limited to logs of different deciduous hardwood trees such as oak, walnut and hornbeam, or their residues in the form of sawdust. However, these methods involve long incubation times and often have associated contamination, and generally present economic losses in the production stage. It has been shown that various lignocellulosic residues from other industrial crops, such as cotton gin waste and sugarcane bagasse among others, can provide the mushroom with the nutrients required for the spawn run and fruiting stage which, under controlled conditions and procedures result in an optimum product yield.[3-5]

In Colombia, the national demand for *L. edodes* has been on the rise over the last few years, and modern research centers have not produced satisfactory results with regard to its production. It is for this reason that this study is expected to adapt oriental culture techniques to the conditions of our country, to start its production, using native agroindustrial residues.

2 Materials and Methods

The agroindustrial residues used were cotton gin waste, sugarcane bagasse, coffee pulp and coconut fiber and sawdust from cedar, eucalyptus, pine and oak trees (oak sawdust served as a "control"). All the substrates were

prepared following the same formulation: 78% of agroindustrial residue, 20% wheat bran, 1% sugar and 1% $CaSO_4$. The mixture was packed in 10 bags each weighing 1 kg and 5 1-litre bottles, for the fruiting and spawn run assays respectively. All the substrate mixtures were sterilized at 121°C for 1 hour and were inoculated with 2-3% *Lentinula edodes* spawn in a wheat grain base. The incubation was carried out at for 70 days at a temperature of 23-25°C and a relative humidity of 65-70%. The fruiting stage was carried out at a temperature of 14-22°C, a relative humidity of 80-95% and light incidence of 100 lux units. All the harvested mushrooms were measured and weighed to determine the yield of each of the substrates through the percentage of biological efficiency, while the quality and flavor of the basidiocarps produced under each condition were determined by a sensory test with a panel of tasters.
Statistical analysis was carried out by a frequency distribution for central tendency measurements, where the analysis of variance compared the means of the data collected on mushroom development, and the non-parametric Kruskal-Wallis test was used to analyze the data of the sensory test, in order to determine the optimum values of Shiitake production and quality in the different substrates used.

3 Results and Discussion

3.1 Analysis of Shiitake growth on different substrates

An adequate substrate for Shiitake production is that which can provide a good biological efficiency in the production of high quality mushrooms in a minimum amount of time. To determine these characteristics it became necessary to study the growth of the mushroom, in its vegetative phase as well as its reproductive phase to analyze the rate of development and productivity.
In this study the vegetative phase or spawn run of Shiitake mushrooms was characterized by showing the highest growth rates in pine and eucalyptus sawdust (Fig. 1). Values were even higher than those observed for the control oak sawdust. However, statistical analysis showed that the growth rates observed in eucalyptus and oak sawdust are similar. These results suggest that the resins present in pine residues (sigiril and guaiasil) and eucalyptus residues (eucalyptol) do not play a role in the vegetative development of the mushroom, and that the metabolism of this organism is different in the spawn run with respect to the fruiting stage.
For the induction of the mushroom's reproductive phase all substrates underwent the same thermal shock after 70 days of incubation, resulting in 2-3 harvests per substrate. In this study, coffee pulp, coconut fiber and cedar sawdust cultures did not reach the fruiting stage after the 70 days, and they are excluded from the following discussion.
At the end of the harvests, the productivity of the substrates was calculated and expressed as percentage of biological efficiency, where the highest yield was obtained with sugar cane bagasse at 93.8% which was statistically similar to eucalyptus sawdust (76%) but superior to that of the control (66%) (Fig. 2). The biological efficiency obtained with sugar cane bagasse is superior to that reported in the study carried out by Gaitan,[3] where Shiitake production reached only 65.42%. The yield observed with sugar cane bagasse can be explained by it's resemblance to hard woods in terms of chemical composition, where amounts of cellulose and lignin are very similar. Additionally the availability of simple sugars within the sugar cane bagasse turns produces higher yields than with oak sawdust.

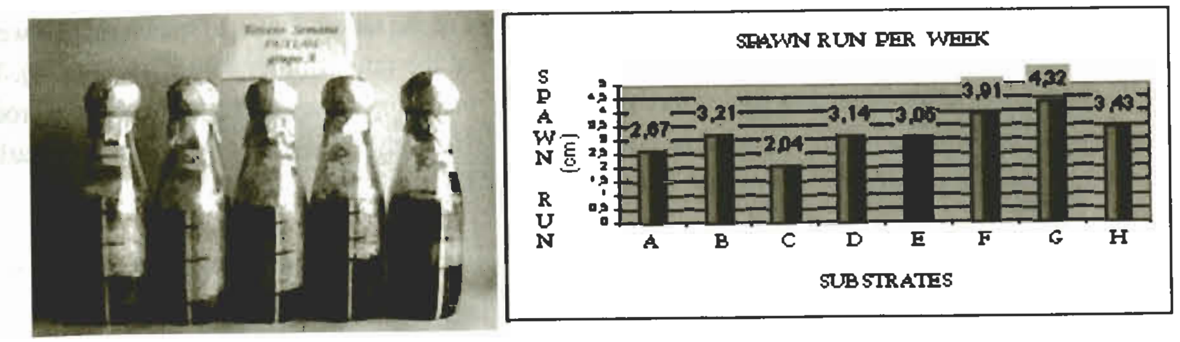

Figure 1. Spawn run on the different substrates
A: cotton; B: bagasse; C:coffee; D: cedar: E: coconut; F: eucalyptus.

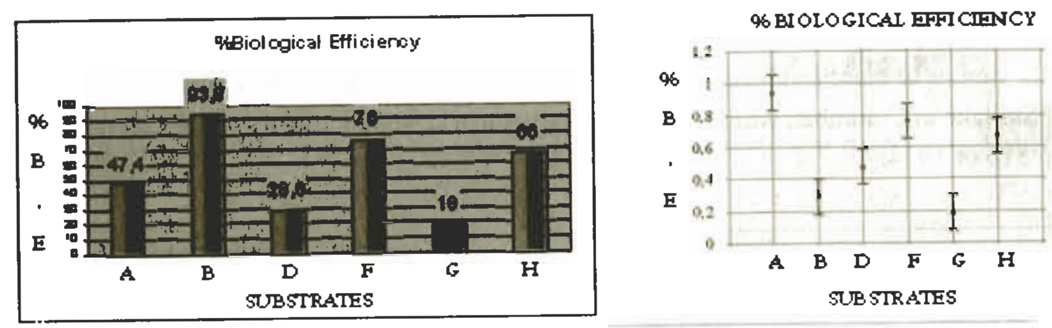

Figure 2. Percent biological efficiency of the different substrates
A: cotton; B: bagasse; D: cedar; F: eucalyptus; G: pine; H: oak.

In the case of the biological efficiency for cotton residues, eucalyptus and oak sawdust, the yields obtained were higher than those reported by Royse[6] in natural cultures on trees, where 33% efficiency was reached after 6 years of culture. This study also showed higher values than those reported by Rinker,[7] where Shiitake production with artificial, mixed wood, trees reached a biological efficiency of 56%, a lower percentage than that observed with sugar cane residue (93.8%) and eucalyptus sawdust (76%) used in this study.
The amount of harvested mushrooms and the total fresh weight of production (Fig. 3) show an inverse relation between these two variables. This is due to the fact that the nutrient concentration in the bag determines the productivity and quality of the crop, showing that the substrate is the limiting factor in this study. The clearest example of this relationship was observed with the cotton gin waste substrate: with a mean of 17 mushrooms, statistically superior to the 6.4 mushrooms of the control, shows a mean weight of 142.2 g, one of the lowest values in the total fresh weight of the harvest (Fig. 3). That is, the mushrooms produced by these substrates showed relatively low sizes and weights, while a smaller amount of mushrooms in the substrates statistically similar to the control developed better size and commercial weight.

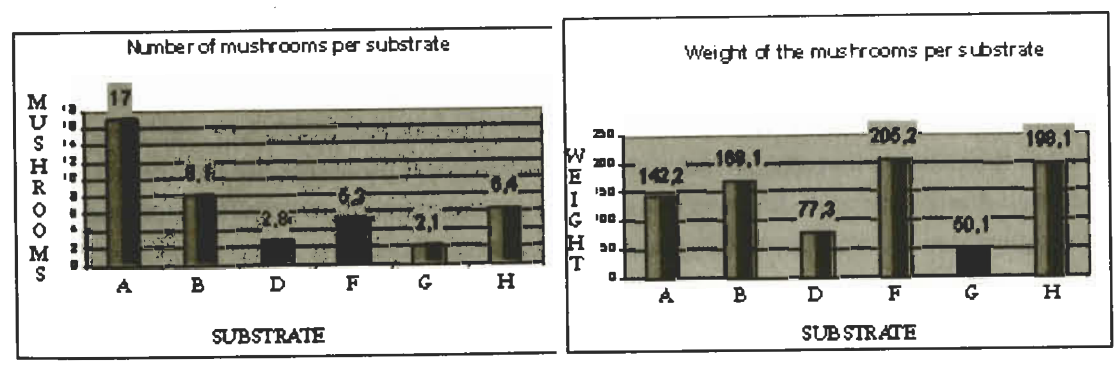

Figure 3. Number and weight of the mushroom harvested per substrates
A: cotton; B: bagasse; D: cedar; F: eucalyptus; G: pine; H: oak.

It is worth noting that the cotton gin waste showed, on average, one of the slowest rates in spawn run. However, in terms of fruiting, development not only equalled but surpassed the rate of other substrates (Fig. 4). This suggests that cotton gin waste possesses some kind of compound that induces the development of the reproductive phase. One of the keys could be in the initial composition of the cotton residue, which presents a carbon-nitrogen ratio of 11/1,[8] delaying the spawn run but accelerating the fruiting.

3.2 Evaluation of the commercial quality parameters during the production of Shiitake mushrooms

The quality of Shiitake mushrooms in reference to size and weight, standardized by the Asian states[9] allowed an objective analysis of the mushrooms harvested in this study. We observed that the substrate based on eucalyptus sawdust produces, on average, the highest yields in biological efficiency in the production of export-quality mushrooms. However this value was not statistically different to those observed for sugar cane bagasse and the control; that is, the mushrooms produced by this substrate are of excellent size and commercial weight, and statistically similar to those produced with oak sawdust. These substrates provide new alternatives for the production of Shiitake in Colombia, which had been brought to a standstill, mainly by the ban on oak lumbering by Resolution 316 of 1974.

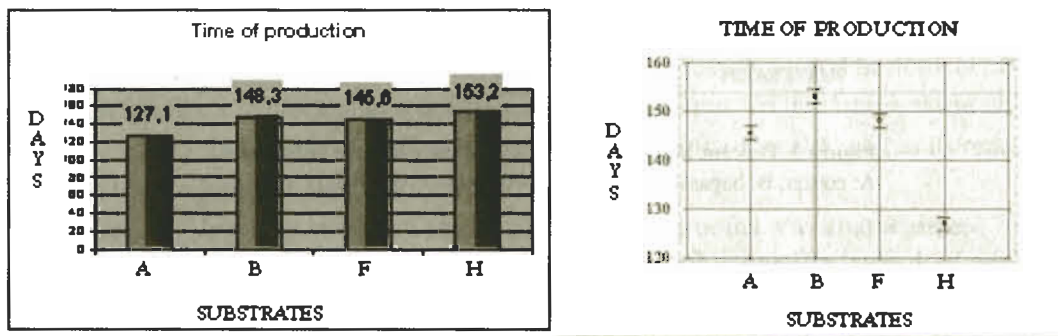

Figure 4. Total days of production of Shiitake on different substrates
A: cotton;B: bagasse; F: eucalyptus; H: oak.

3.3 Sensory analysis of the carpophores produced on each substrate

In the sensory test carried out by untrained tasters, we found that the mushrooms produced in pine sawdust showed significant differences in the absence of defects and shape of the carpophore in comparison to the mushrooms produced in other substrates, which are statistically similar to the control. The production of mushrooms with an irregular morphology in pine sawdust could be consequence of the resins present in the chemical composition of the wood, which could be interfering in the differentiation and development of the mushroom in the reproductive phase, similar to the basidiocarps reported by Ting[9] (Figs 5 and 6).
In the organoleptic test carried out on cooked Shiitake, no significative differences were found between the mushrooms harvested on the different substrates, that is; the flavor and texture characteristics do not vary with the substrate composition. These results suggest that although the mushroom develops on different substrates it does not absorb the extracts that may be present in the media, or it possesses the metabolic mechanisms to degrade them and thereby prevents any intervention in its organoleptic properties. Therefore, none of our substrates affected mushroom development physiologically or metabolically.

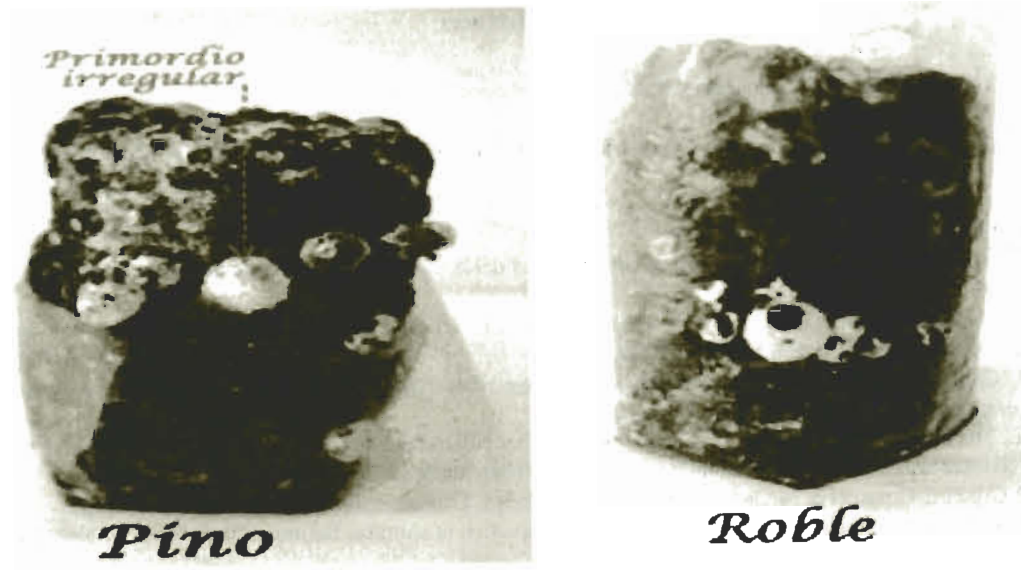

Figure 5. Irregular shape of pine sawdust substrate primordium vs normal substratecontrol primordium

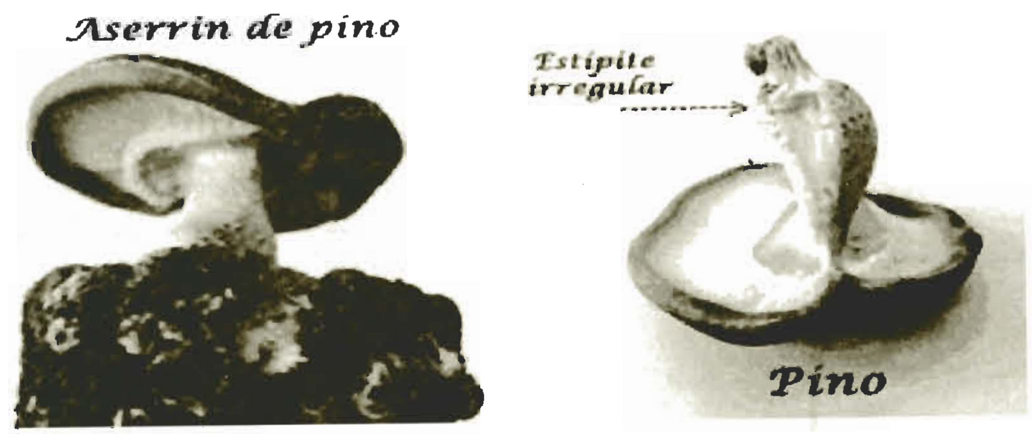

Figure 6. Irregular shape of pine sawdust substrate mushroom

4 Conclusions

The best substrate for the development and production of Shiitake is sugar cane bagasse, which showed a biological efficiency of 93.8% over a 70-day incubation period and a total production time of 5 months. It demonstrated excellent spawn run development, and a fruiting stage with excellent organoleptic characteristics suitable both for export as well as for national distribution. Eucalyptus sawdust is an excellent substrate for shiitake cultivation, with a biological efficiency of 76% over 5 months, with a mushroom production that meets international standards. Cotton gin waste produces a large volume of mushrooms suitable for national distribution, with a biological efficiency of 47.2% and a culture time of 4.5 months. The different substrates used for production did not interfere with the organoleptic properties of the shiitake mushroom.
The recommendations for further investigation are to compare mixes of different substrates in order to increase biological efficiency in the production of shiitake mushrooms using the artificial block system. It would also be interesting to carry out chemical analyses on the composition of each basidiocarps, and to evaluate the secondary metabolites of the shiitake mushrooms harvests from different materials for possible therapeutic and nutritional uses.

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