Vacuum Soaking of Wood Chip Shiitake *Lentinula Edodes* Logs to Reduce Soak Time and Stimulate Mushroom Yield (1)

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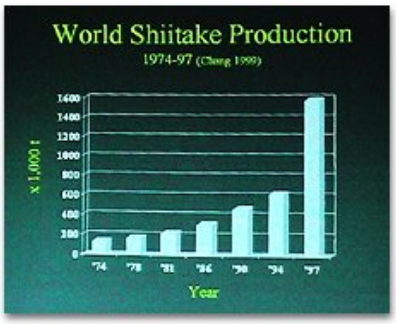
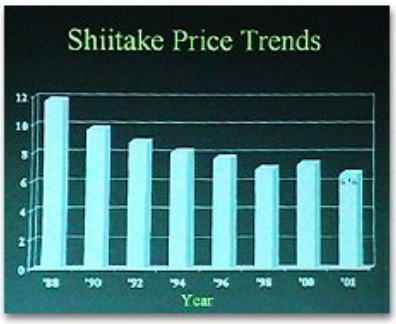
INTRODUCTION

Worldwide commercial production of shiitake [*Lentinula edodes* Berk. (Pegler)] increased to 1,564,000 t in 1997 [last date production statistics were available (Chang 1999)]. This is an increase of 738,000 t from 1994 or nearly double the output in three years. Most of the increase in world production came from China where nearly 10 million part- and full-time farmers cultivate shiitake (Luo 1998, Wang 1998, Zhang and Lai 1993). Shiitake is widely consumed in China, yet nearly one-third is exported (Chiu *et al*. 1999). In 1997, China produced approximately 88% of the total world output (Chang 1999).

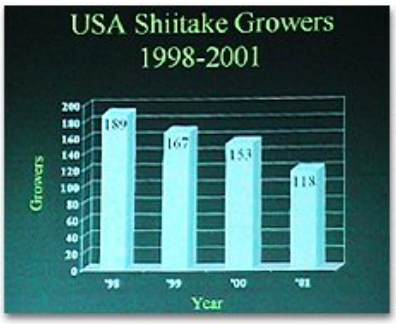


World Shiitake Production

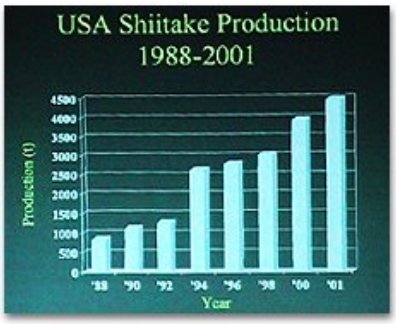


Shiitake Price Trends

In the United States, production of shiitake is a relatively new enterprise, having begun only in the late 1970s (Royse 1997). In 2000, the United States produced 3,714 t, down one percent from the previous season but 30% higher than 1998 output. Farms continue to increase in size as the number of growers has decreased during the last three years (from 189 growers in 1998 to 153 growers in 2000). The average farm in the United States produced their production costs and increased productin while other farmers have abandoned the business due to production inefficiencies.



USA Shiitake Growers



USA Shiitake Production

Sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing shiitake in the United States (Miller and Jong 1987, Royse and Sanchez-Vazquez 2001) but other basal ingredients may include straw, corn cobs, or both. Starch-based supplements

(30~70% dry weight) such as wheat bran, rice bran, millet, rye, and maize may be added to the mix. These supplements serve as nutrients to provide a more optimum growth medium (Royse 1996, Royse 2001).

As part of the mushroom production process, shiitake logs are soaked in water at the end of the browning (or curing) period to stimulate mushroom production. During the curing process (four to five weeks), logs loose approximately 500 to 800 g each of water due to evaporation and mycelial respiration. In order to replenish this lost water, logs are submerged completely in a soak tank for 3 to 24 hours depending on the stage of the crop. Soak times generally increase for each flush of mushrooms due to the increasing hydrophobic nature of the logs as they age. Thus, soak times may average 3 to 4 hours on the first soak, 8 to 16 hours on the 2nd soak, and 14 to 24 hours on the 3rd soak. In addition, logs may not absorb water at the same rate, so some logs may re-hydrate faster than others. Thus, growers are presented with a major challenge in maintaining optimum water contents in their population of thousnads of logs. Some growers have learned to compensate for different moisture contents in logs by placing drier logs on the bottoms of the soak tanks. It was learned several years ago that logs on bottoms of the tanks take on more water than thoses logs near the surface of the water. Thus, the preferential placement of logs has helped to provide more uniform log weights after soaking, but this process does not entirely achieve the goal of obtaining more uniform moisture contents in logs. We sought, therefore, to evaluate the effects of vacuum soaking on log weight uniformity, log soak time and yield and mushroom size.

MATERIALS AND METHODS

Substrates and preparation

Substrates were prepared from mixed hardwood sawdust collected from a commercial sawmill in Centre County, Pennsylvania. The predominant species found in the sawdust was the northern red oak (*Quercus rubra* L.). The sawdust was collected in the spring of 2000 and was stored in an enclosed building until it was used. The moisture content of the fresh sawdust was approximately 38% by weight. The general substrate formulation (all ingredients based on oven dry substrate wt) consisted of 45% sawdust, 30% millet, 15% wheat bran and 10% rye.

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 0.283 m^3 paddle mixer. Injecting live steam into the mixer and allowing the substrate to heat to 111'C pasteurized the substrate. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. Passing cold tap water through a jacket fitted to the mixer rapidly cooled the substrate. Sterility of the mixture was maintained by injecting filtered air into the mixer during cool down and bagging to create a positive airflow. When the substrate (55kg wet wt) had cooled to below 27'C, it was spawned with 210 g rye grain spawn contained in a 500 ml Erlenmeyer flask. When then spawn was thoroughly mixed with the substrate, the resulting mixture was bagged in unused polyethylene bags (20.3 cm * 12.7 cm * 50.8 cm) and closed with a twist-tie. The amount of substrate was weighed at time of filling with a digital scale placed under the bagging port of the mixer. Each bag contained 2.5 kg spawned substrate at 59% moisture (1.03 kg oven dry weight). Dry substrate weight was determined by drying 100 g of the processed substrates in an oven for 18 hour at 105'C.

Substrates

Spawned bags

Experimental design and statistical treatment

The experiments were completely randomized design with 24 to 48 replicates per treatment. Data for yield and size were subjected to Welch's Analysis of Variance [ANOVA (SAS1998)]. For log weight analyses, data were subjected to ANOVA and means were separated using Student's t-test (SAS1996). Standard deviations (SD) were calculated for comparisons of variation in log weights for type of soak treatment (Steel *et al*. 1997). The experiments were repeated three times and designated Crops I, II, and III.

Spawned logs

soaking

Isolate and spawn

Isolate R26 was selected because it is a commercially used cultivar. The isolate was maintained on potato-dextrose yeast-extract agar as outlined previously by Jodon and Royse (Jodon and Royse 1979). Spawn of R26 was prepared as outlined previously by Royse and Bahler (1986).

Spawn run and log browning

After a spawn run of 7 days, 20 slits (5 mm each) were made in the top of each bag with a sharp scalpel to provide gas exchange. At the end of 22 days incubation at 22 ¡¾ 1'C, the plastic bags were removed and the synthetic logs moved to a "browning room". In the browning room (93~98% relative humidity, 18 ¡¾ 1'C air temperature), the synthetic logs were hand-watered lightly with a 600-hole reseface nozzle each day. At the end of 14 days in the browning room all logs were rotated 180' to provide a more uniform browning of the logs' surface. Three hr of light were provided daily by cool-white fluorescent bulbs. Sufficient air changes were maintained to hold CO_2 levels below 1,900 ppm (1,200 ¥il/liter).

Regular soak

At the end of 28 days in the browning room, the logs were soaked in cool water (13 ¡¾ 2'C) until each weighed approximately 2.5 kg. For regular soaking, 12 logs were placed in three layers only (to minimize depth of immersion effect) and secured 15 cm below the lip of 115-L plastic containers (garbage cans). Water then was added to fill the container to run off and containers were periodically topped off with additional water to compensate for water absorbed by the logs. Two monitor logs, submerged in water in 19-L containers, were used to determine when the target weight was achieved. Soaked logs then were placed on shelves in a production room where relative

humidity (90%), CO_2 level (2,000~2,200 ppm) and lighting (500 lux, cool white fluorescent, 4 hr/day) were controlled automatically. After each flush of mushrooms was harvested, logs were resoaked to increase log weight to approximately 2.5 kg. All logs were weighed immediately after removal from the soak containers.

Vacuum soak

Two logs were placed in a metal container [35 * 25 * 25 cm (L * W * H); 25 L capacity] and secured in place with a locking perforated lid. Water was added until the level reached 8 cm above the restrained logs. The container then was placed in a sealed oven and a vacuum pump was used to withdraw air from the oven creating a partial vacuum. For the first soak, the desired partial vacuum (16" Hg) was reached within 60 seconds and this supplied the target log weight after the vacuum was released. For the second soak, 80 sec was required to reach the desired partial vacuum (22" Hg). Approximately 60 and 75 sec was required to release the vacuum for the first and second soaks, respectively. After removal from the soak container, logs were immediately weighed and returned to production shelves for mushroom development.

Determination of moisture contents in three zones of synthetic logs

Logs were cut in half trasnversely with a hacksaw blade before or after regular and vacuum soak. Substrate samples (50 g wet wt) were collected from each of three zones (outside, middle and interior) and dried in an oven (105'C; 18hr) to determine moisture contents of each zone (Figure 1). Sampling consisted of two logs each for each break in Crop I and three logs each for each break in Crop II). The outside, middle and interior zones represented approximately 67%, 28% and 5% of the total log volume, respectively.

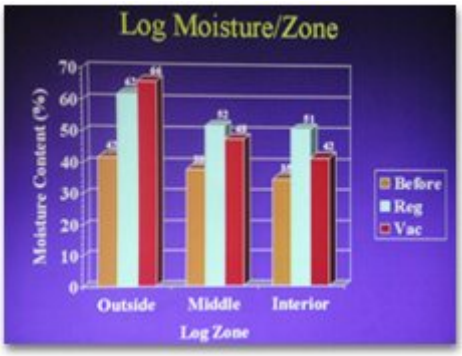


Figure 1. Log Zone

Harvesting

Mushrooms were harvested from the substrates at the same time each day, when the veil had broken and the gills were fully exposed. The mushroms were then counted and weighted. At the end of the harvest period (28 days), the accumulated data were used to calculate the yield and mushroom size.

RESULTS

An average of 3 and 15 hr was required to reach target weights of 2.5 kg for a regular soak of logs for first and second flush (one harvest or break of mushrooms; occurs every 16 to 20 days on synthetic logs), respectively. For vacuum soak, first break soaks required a vacuum cycle (achievement of partial vacuum then release) of 2 min while the second soak required 2.6 min per cycle. The longer time for the second soak was due to the need for a greater vacuum to re-hydrate the logs to the desired moisture level.

Fate of water in vacuum vs. regular soak logs

Prior to soaking, moisture contents(%) were in the low to mid-40s range in the outside zone of logs

while in the middle and interior zones, percentage moisture contents were in the 30 to 40 range (Figure 1). All logs showed a gradient of higher moisture content of the outside to lower moisture content toward the interior. Mean moisture contents for outside, middle and interior zones of non-soaked logs were 42%, 38% and 35%, respectively (Figure 1).

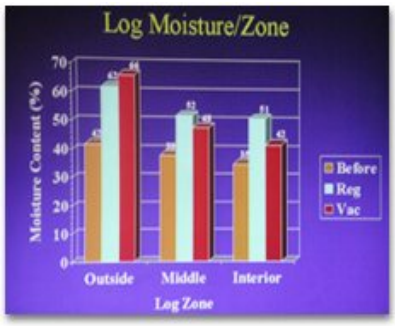


Fig 1. Log Moisture / zone

Figure 1. Mean percentage moisture contents of ten logs (four logs from Crop I and six from Crop II) for three zones of synthetic shiitake *Lentinula edodes* logs before soak and after regular and vacuum soak for two flushes for two crops. Logs were incubated for seven weeks (three weeks spawn run, four weeks browning) before soak.

Water tended to accumulate more in the outside zone in the vacuum-soaked logs than in the regular-soaked logs (Figure 1). Mean moisture contents for Crop I and II for outside, middle and interior zones of vacuum-soaked logs were 66%, 48% and 42%, respectively (Figure 1). Mean moisture contents for outside, middle and interior zones for regular-soaked logs of both crops were 62%, 52% and 51%, respectively (Figure 1).

Yield and mushroom size

The effect of regular and vacuum soaking of synthetic shiitake logs on yield and mushroom size Crop I. for a 28-day production period for three crops is shown in Figure 2. Overall yields were higher for Crops II and III, compared to Crop I. However, trends were similar for all crops. Yields were significantly (P=0.05) higher from vacuum soaked than from regular-soaked logs for all three crops. Mean yiels for all three crops was 456 g/log for regular soak compared to 580 g/log for vacuum soaked logs (27.7% difference).

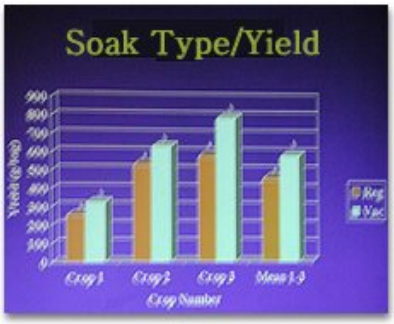


Fig 2. Soak Type / Yield

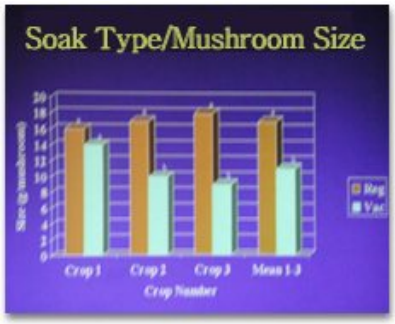


Fig 3. Mushroom Size

Figure 2. Effect of regular and vacuum soak of synthetic shiitake *Lentinula edoeds* logs on yield and mushroom size for a 28-day production period for three crops.

For yield, there were significant differences (P=0.05) between regular soak and vacuum soak for each crop. For mushroom size, there were significant differences (P=0.05) between regular soak and vacuum soak for Crops II and III but not for Crop I.

In contrast to an increase in yield with vacuum soaking, mushroom size showed an opposite trend (Figure 3). Mushroom size (g/mushroom) was significantly less for vacuum soaked logs than for regular soaked logs for crops II and III but not for Crop I. Overall crop means for mushroom size showed approximately a 4.8-g difference for regular (17g) vs. vacuum (11.2g) soak.

DISCUSSION

The trend toward greater use of synthetic media compared to natural logs (USDA 2000) is

attributed to greater yield potential and reduced time required to produce a crop on synthetic media (Royse and Bahler 1986, Royse et al. 1990, Royse 1996, Royse and Sanchez-Vazquez 2001). Most synthetic media used commercially are composed of approximately 50% each of wood chips and nutrient supplements such as millet, wheat bran and rye. Synthetic logs are routinely soaked in water to stimulate mushroom production. In the present study, we examined the effect of vacuum soaking on yield and mushroom size.

The principal merit of our research is the noticeable increase in mushroom yield when synthetic logs are vacuum-soaked. The distribution pattern of water within the log may explain the yield response. Higher moisture levels were consistently observed in the outer most zones of the vacuum-soaked logs when compared to regular-soaked logs. Available water for the developing basidiome preferentially may come from the outside zone leaving the interior zones for the substrate with freer air space. Work by Ohga (1990) on wood chip particle size has demonstrated ghat that oxygen (O2) depletion was the cause of reduced mycelial biomass development in substrates containing smaller particle size. Air spaces saturated with higher moisturee contents may slow gas exchange from deep within the interior of the synthetic log th the surface thereby slowing growth and development of the basidiomes. Since fresh mushrooms may contain 80~85% moisture, higher water availability nearer the site of basidiome formation might result in more efficient growth and development. In addition, lower moisture levels in the interior of the log would allow freer air space and, thus, greater air exchange capacity.

While growers may be able to substantially increase yield with vacuum soaking, basidiome size may decrease appreciably. This may adversely affect the prices growers receive for their product so they carefully should weigh the relative merits of increased yields at the potential expense of reduced mushroom size. In some markets, reduced size may not present a problem and therefore, growers could see an immediate return through increased product yield.

These studies demonstrated that vacuum soaking of substrates was effective in reducing time required for soaking and stimulated mushroom yield. Further study is needed to determine the effect of water availability in various zones of the synthetic log and how this availability may influence yield and mushroom size.

ACKNOWLEDGEMENTS

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