Self-pasteurised substrate for growing oyster mushrooms (*Pleurotus* spp.)

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As part of participatory research with farmers from the municipality of Cacahoatán, Chiapas, Mexico, an experiment was conducted to determine the technical feasibility of using substrate self-pasteurisation to grow oyster mushrooms (*Pleurotus* spp). In the first step, the substrate treatment (alkaline immersion disinfection versus self-heating pasteurisation) was evaluated, and after determining the better treatment, the productivity of 10 strains of four *Pleurotus* species, *Pleurotus ostreatus* (ECS-0152 and ECS-1123), *P. eryngii* (ECS-1290 and ECS-1138), *P. citrinopileatus* (ECS-1338 and ECS-01337) and *P. djamor* (ECS-0143, ECS-0142, ECS ECS-0144 and ECS-0149) was evaluated. Comparison of the substrate preparation treatments using *P. ostreatus* strain ECS-0152 showed significant differences in Biological Efficiency (BE) between alkaline immersion and self-heating pasteurisation, which had BE values of 57.6 and 78.6%, respectively (p = 0.001). The yield was compared in three levels of the substrate inside the crate used for self-heating pasteurisation, and no significant differences between the levels were found (p = 0.103). In the evaluated strains, five strains (ECS-0152, ECS-01123, ECS-1338, ECS-0142 and ECS-0149) produced mushrooms with BE values between 53.5 to 111.9%, whereas the other five strains did not produce mushrooms. The highest rate of production was observed in the strain ECS-1123. It can be concluded that the self-heating pasteurisation method is feasible at the field level, and furthermore, five strains of *Pleurotus* spp. that are able to be cultivated on substrate pasteurised in this way were identified.

Key words: Alkaline immersion, self-heating substrate pasteurisation, edible mushrooms, mushroom cultivation.

INTRODUCTION

To grow oyster mushrooms (*Pleurotus* spp) in the state of Chiapas, Mexico, two substrate preparation methods are used: alkaline immersion and heat pasteurisation (Sántiz de la Cruz, 2007). Although, heat pasteurisation allows for better substrate protection and is generally used for commercial purposes, it is more expensive and requires greater investment. In Chiapas, almost 98% of oyster mushroom growers use alkaline immersion. The alkaline disinfection method is simple: no thermal treatment is applied, and the substrate is soaked for 12 to 48 h in limed water. This method works well in rural areas of the state; however, there are some deficiencies, as bacterial contamination is possible and fly eggs survive the treatment (De León-Monzón et al., 2004).

Hernández et al. (2003) developed an alternative method of pasteurisation involving stacking the substrate for 2 to 3 days to allow for self-heating without an external energy source. These authors demonstrated that the application of this method was feasible, but when used under non-experimental conditions, they found that pasteurisation was achieved only at the central and upper
layers of the substrate; the lower layer was not pasteurised. Barrios-Espinoza et al. (2009) and Sánchez et al. (2011) increased the substrate mass and volume and used a wooden 1 m³ crate containing approximately 230 kg substrate at 65% moisture, which resulted in the pasteurisation of the whole substrate under experimental conditions.

The next step was then to test the self-pasteurisation procedure under rural conditions. Thus, farmers interested in running an oyster mushroom farm were invited to participate in this participatory research study. To test this procedure, mushroom production was compared using the self-heating pasteurisation and alkaline immersion disinfection methods and after determining the better treatment, the productivity of 10 strains of *Pleurotus* spp was evaluated.

**MATERIALS AND METHODS**

This study was conducted in the municipality of Cacahotán, Chiapas, México (N 15°04’. W 92°11’), at an altitude of 830 m, a mean annual temperature of 21.7°C and 80% mean relative humidity. Farmers who were already engaged in coffee production as members of the “Productores de Café La Central” (Procacen) organisation participated in this study. They built a 7 m x 5 m incubating/fruiting room with cement block walls, a cement floor and a laminar ceiling for this purpose.

**Strains, culture media and substrate**

Ten strains from four species of *Pleurotus* were used: *Pleurotus ostreatus* (ECS-0152 and ECS-1123), *P. eryngii* (ECS-1290 and ECS-1138), *P. citrinopileatus* (ECS-1338 and ECS-01337) and *P. djamor* (ECS-0143, ECS-0142, ECS-0144 and ECS-0149). These strains were obtained from the fungal culture collection of El Colegio de la Frontera Sur (ECOSUR). The strains were grown and preserved on malt extract agar (Bioxon). Spawn preparation was performed following the guidelines of Chang (1982) using sorghum grains (500 g per bag, autoclaved at 121°C for 30 min).

The Pangola grass *Digitaria decumbens* was used for mushroom production. The grass was ground or cut as required: 2 to 2.5 cm long for self-heating and 15 to 25 cm long for alkaline immersion. Before spawning, the substrate was treated in one of two ways: self-heating pasteurisation (Sánchez et al., 2011) or alkaline immersion disinfection (Contreras et al., 2004). Alkaline immersion disinfection was used as a control, as it is the most widely used method in the region.

**Experimental trials**

Four experimental trials were performed: A, B, C and D. Two of them (A and C) were designed to evaluate substrate treatment by the following methods: 1) stacking (self-heating) or 2) alkaline immersion. For trials A and C, only the strain *P. ostreatus* ECS-0152 was used. The other two trials (B and D) were conducted to evaluate the mushroom production of 10 *Pleurotus* spp. strains cultivated using the previously selected self-heating method. During the first two trials (A and B), problems in attaining pasteurisation temperature (50 to 55°C) were encountered. To solve these problems, 2 cm layer of polyurethane was applied to the sides and upper cover of the wooden box to improve the insulation and retain the self-generated heat. Trials A and B were pasteurised in a non-insulated wooden crate, and trials C and D were pasteurised in an insulated crate.

**Substrate preparation and spawning**

The substrate was prepared by mixing 80 kg dry grass with 2% calcium hydroxide (Ca(OH)_2, Grijalva, México), and the moisture was adjusted to 65%. To self-heat the substrate, a crate made from Temepixtle (*Ocotea sp.*) wood that was similar to the one described by Sánchez et al. (2011) was used (1 m³ with forty-eight 0.5 cm diameter holes on its base, and a 20 cm diameter, 15 cm long central chimney at the top to allow for aeration). For trials A and B (non-insulated crate), the mixture was incubated for 50 h. For trials C and D, the substrate remained inside the crate for 48 h. Temperature measurements and substrate sampling were performed at three different positions (levels) of the substrate as previously described (Hernández et al., 2003). Level 3 consisted of approximately 1/3 of the total mass and were located at the bottom of the crate; the temperature was measured 15 cm above the bottom. Level 2 consisted of the middle 1/3 of the substrate, and the temperature was measured at the centre of the crate. Finally, level 1 consisted of the top 1/3 of the substrate, and the temperature was measured 15 cm below the upper substrate surface. Twenty-four hours after substrate stacking, the whole mass was rotated once by removing the substrate and then returning it to the crate. During this process, the substrate levels were arranged such that the portion of substrate that started at the bottom of the crate was now located in the middle level, the substrate at the middle was moved to the top, and the substrate that started at the top was moved to the bottom.

After the self-heating treatment, each level of substrate was removed from the crate and cooled to the environmental temperature by manual aeration. Each substrate level (1, 2, 3) was spawned, bagged and incubated separately. Alkaline immersion was performed as in Contreras et al. (2004). Seventy kilograms of substrate was immersed in 350 L water with 2% calcium hydroxide for 12 h. After immersion, the substrate was drained over perforated trays and exposed to the sun to eliminate excess water and adjust the moisture to 65%.

The spawning rate was 3% on 3.4 kg of substrate. The spawned substrate was placed in tightly closed 40x60 cm polyethylene bags and then incubated at 22 ± 2°C for 15 days. One day after spawning, approximately 80 holes were cut in the plastic bags to allow for aeration. Once the mycelium had colonised the substrate, the plastic bags were discarded. The substrate was watered regularly, and fresh, moist air was introduced to induce fruiting. After the first flush, the substrate was soaked in water to recover lost moisture. Two flushes of mushrooms were harvested when the mushroom pilei were horizontally extended (Ziombra, 2000).

**Sampling**

Fifteen grams of substrate were harvested for pH and moisture measurements. Three types of samples were taken: a) wet substrate, before processing; b) self-heated substrate taken from each substrate level at the time of substrate removal (24 h process) and at the end of pasteurisation before spawning; and c) alkaline immersion substrate, once after treatment.

**Evaluated parameters**

Moisture content of the substrate: A five-gram sample was placed on a Moisture Analyzer thermo balance (A&D M.F. 50, Tokyo, Japan). Relative humidity: This parameter was measured using a digital thermo-hydrometer Oakton model 35612-10, Vernon Hills, IL.
Hydrogen Potential (pH): A ten Gram sample was placed in 90 ml of distilled water and mixed for 10 mins before being measured using an Orion pH/ISE meter model 710A. C/N ratio: A 200 g sample was dried in an oven at 65°C for 5 days. The dry sample was ground to a fine powder and sent to the Bromatology laboratory (Ecosur). The carbon and nitrogen contents were determined using an auto analyser Leco (Leco Corporation, 1988).

Substrate temperature: This parameter was measured using 30 cm bimetallic thermometers. During self-heating, temperature measurements were made every three hours throughout the two day process. The temperature was recorded in three places at the front side of the crate: a) 15 cm below the upper surface of the substrate, b) at the centre of the crate and c) 15 above the bottom of the crate. The environmental temperature and relative humidity were also recorded.

Biological efficiency (BE) was determined using the following relationship: fresh mushroom weight divided by dry substrate weight and multiplied by 100. The production rate (PR) was calculated by dividing BE by the number of days needed to obtain two flushes (Royse, 1989).

The incidence of contamination was estimated visually: at the end of the spawn run, the percentage of contaminated area per each substrate bag was estimated. The result was expressed as an average.

Statistical analysis

For the comparison of BE in the three substrate levels and the comparison of the treatments (self-heating versus alkaline immersion), a randomised design with 20 replicates was used. For the productivity evaluation of 10 Pleurotus strains, a randomised design with five replicates per strain was used. For each case, analyses of variance were made, and a mean separation by Tukey’s test was performed (p< 0.05).

RESULTS

Temperature profile during self-heating

The temperature profiles of the three substrate levels during self-heating pasteurisation were monitored throughout the experiment. Four experimental trials were performed. For trials A and B, the wooden crate was not insulated, while trials C and D were carried out using the same wooden crate covered with a 2 cm layer of polyurethane as thermal insulation. For trials A and B (data not shown), the temperature profiles were similar between trials. In the upper layer (L₁), there was an increase of up to 50°C after a 30 h process, which was followed by a slow decrease in the substrate temperature. The middle layer (L₂) reached a temperature of 40°C at approximately 36 h, which was maintained until the end of process. The lower layer (L₃) experienced a slower and practically linear increase, reaching a maximum of 36°C at 50 h.

The experimental results presented in Figure 1 (trials C and D) reveal very different temperature profiles compared to those of the previous trials A and B but also show similarities among them. Trials C and D exhibited fewer differences between levels than trials A and B. The three levels reached almost 50°C in 24 h, with maximum temperatures between 60 to 69°C at 36 to 40 h. The treatment was stopped at 48 h. Statistical analysis showed a significant difference in temperature over time in all levels (p=0.0001).

pH and C/N ratio

The evolution of pH, moisture and C/N ratio in the substrate during self-heating and alkaline soaking is shown in Table 1. In the self-heating samples, the pH decreased from 8.7 to 8.0 at the end of the process. The moisture was steady at approximately 65%, and the C/N ratio decreased from 59.1/1 to 52.1/1 at the end of stacking. In the alkaline soaking samples, the pH rose
Table 1. pH, moisture and C/N ratio during the preparation of the substrate (Pangola grass) for Pleurotus spp mushroom cultivation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>0</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-heating</td>
<td>pH</td>
<td>8.7±0.5</td>
<td>8.1±0.5</td>
<td>8.0±0.5</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>65.7±5.0</td>
<td>64±2.1</td>
<td>64±1.4</td>
</tr>
<tr>
<td></td>
<td>C/N ratio</td>
<td>59:1</td>
<td>54.6:1</td>
<td>52:1</td>
</tr>
<tr>
<td>Alkaline immersion</td>
<td>pH</td>
<td>-</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td></td>
<td>65±1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/N ratio</td>
<td>59.1</td>
<td>58.6</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as the mean of four replicates ± Std Dev.

Table 2. Yield, Biological efficiency and Production Rate (PR) of Pleurotus ostreatus ECS-0152 grown on Pangola grass prepared using two different methods (self-heating or alkaline immersion). Self-heating was performed in a wooden crate covered by a 2 cm polyurethane layer. Data are presented as the mean of 20 repetitions ± Std. Dev.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (g/3.4kg)</th>
<th>BE (%)</th>
<th>PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Immersion</td>
<td>684.7±196.6*</td>
<td>57.6±15*</td>
<td>1.8±0.46</td>
</tr>
<tr>
<td>Self-heating</td>
<td>935.6±178*</td>
<td>78.6±16.5*</td>
<td>2.5±0.5</td>
</tr>
</tbody>
</table>

*Statistical difference among treatments α=0.05.

from 7.5 to 8.5 at the end of treatment (24 h), and the moisture was 65%, as expected. The C/N ratio presented virtually zero variation, changing only from 59.1/1 to 58.6/1.

Alkaline immersion versus substrate self-heating

The mushroom production among levels 1, 2 and 3 was compared. BE values varied between 66 to 77%, and statistical analysis did not reveal significant differences among the levels (p=0.103). The alkaline immersion and self-heating treatments were compared in two trials. The first batch A was prepared in a regular wooden crate, and the third batch C was prepared in the same crate covered with polyurethane. For the first trial (A), the biological efficiency varied between 62.8 and 68.2% for alkaline immersion and self-heating, respectively. Statistical analysis did not show a significant difference between the treatments (p =0.591) (data not shown). For trial C, the mushroom production was 684.7 and 935.6 g/bag, the Biological Efficiency (BE) values were 57.6% and 78.6%, and the Production Rate (PR) was 1.8 and 2.5% for alkaline immersion and self-heating, respectively. Statistical analysis identified a significant difference between these two methods (p=0.001) and revealed that treatment by self-heating was superior to that by alkaline soaking (Table 2).

Strain evaluation

The production data (yield, BE and PR) collected while cultivating the studied strains on Pangola grass pasteurised by self-heating in an insulated wooden crate are presented in Table 3. Of the 10 strains evaluated, only five produced mushrooms: *P. ostreatus* (ECS-0152 and ECS-1123), *P. citrinopileatus* (ECS-01338) and *P. djamor* (ECS-0142 and ECS-0149). The strains that failed to produce mushrooms were as follows: *P. eryngii* (ECS-01290, ECS-1138), *P. citrinopileatus* (ECS-1337) and *P. djamor* (ECS-0143 and ECS-0144). These strains did not colonise the substrate after a spawn run of 15 days. The grass used in this study may not have been adequate for the growth of these strains.

The yield varied between 896 and 1331 g fresh mushrooms/bag, and the BE and PR values varied between a minimum of 75.3 and 2.51% and a maximum of 111.9 and 3.1%, respectively. Statistical analysis showed significant differences for yield and BE (p=0.019), and accordingly, the strains were separated into two groups: group “a”, with higher values, including strains *P. ostreatus* (ECS-1123 and ECS-0152) and *P. djamor* ECS-0149; and group “b”, which included strains with
lower values (ECS-0149, ECS-01338 and ECS-0142). For PR the statistical analysis did not find significant difference among strains.

Contamination

*P. ostreatus* growth during the spawn run was homogeneous in the case of strain ECS-0152 on substrates prepared by self-heating or alkaline immersion. The contaminant fungi encountered were *Trichoderma* sp. (during colonisation) and *Coprinus* sp. (at the beginning of fruiting). Both fungi have already been identified as contaminants of oyster mushroom cultivation processes in the area (López et al., 1996). A contaminant fungus encountered in batch A after the first flush was *Fusarium* sp. It appeared after the first flush, most likely because the lower portion of the crate did not reach a sufficiently high temperature for pasteurisation. *Fusarium* contamination occurred later because the substrate units were immersed in water to recover the moisture lost during incubation after harvesting. In subsequent experiments, colonised substrate was soaked in water + 0.5% Ca (OH)₂ to avoid contamination.

The surface area of substrates treated with alkaline immersion were colonised by the green mould *Trichoderma* sp. on 14% of its surface area, while substrates pasteurised by self-heating showed green mould on 4.4% of their surface areas. *Coprinus* sp. affected 5% of the substrate treated by alkaline immersion and 2% of the self-heated substrate. *Coprinus* is generally considered to be an indicator of insufficient pasteurisation temperatures, and this role is confirmed by the temperatures observed in the lower layer during the first run (A). During this run, the most important contaminant fungus observed was *Fusarium* sp. It was observed in 36% of the self-heated synthetic logs. During the second trial (B), *Fusarium* sp. was observed on 10% of the surface area of cultivated synthetic logs.

Trial C and D did not show any contaminated spots during the evaluated two-harvest period. It is noteworthy that similar and homogenous temperatures between different layers of substrate were observed during self-heating pasteurisation (Figure 1). In contrast, in trials A and B, the lower layer temperature rose too slowly and was insufficient to prevent contaminant survival.

DISCUSSION

In this work, we tested the self-heating treatment reported to pasteurise the substrate used to cultivate oyster mushrooms at the field level. The temperatures observed in trials A and B were lower than those reported by Sanchez et al. (2011), although a similar crate and the same substrate were used. They reported that the upper layer reached 50°C after an 18 h process. The environmental conditions most likely affected the microbial activity (as the conditions differed at the experimental sites) and, consequently, the temperature profile. Additionally, Sanchez et al. reported 26°C and 80 to 90% relative humidity, whereas the air temperature was 21 to 22°C and the relative humidity was 76 to 96% in this experiment. Thus, the environmental temperature was 5°C lower in our case, and this difference certainly affected the temperatures inside the non-insulated crate. Trials C and D showed higher temperatures (up to 69°C) and also exhibited minimal variation between levels. These results were certainly due to the thermal insulation caused by the polyurethane layer insulating the crate. This layer kept the heat produced by microbial metabolism inside the crate and enabled not only higher temperatures but also less temperature variation between levels. In fact, polyurethane has a thermal conductivity coefficient of 0.027 W/m.K, which is lower than those of glass and fiberglass and much lower than that of wood (IRAM, 2002).

Under the environmental conditions of this experiment (air temperature 21 to 22°C), the substrate at the bottom level of the crate did not reach 40°C, resulting in insufficient pasteurisation. Because of this insufficient temperature, contamination occurred in trials A and B.

Table 3. Yield, Biological Efficiency (BE) and Production Rate (PR) of different strains of *Pleurotus* spp. cultivated on Pangola grass + 2% Ca(OH)₂ and 65% moisture. Pasteurisation by self-heating was performed in a wooden crate covered by a 2 cm polyurethane layer. Data are presented as the mean of 5 repetitions ± Std. Dev.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Yield (g/3.4kg)</th>
<th>BE (%)</th>
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<tr>
<td><em>P. ostreatus</em> ECS-1123</td>
<td>1331.6±161.6ᵃ</td>
<td>111.9±18.2ᵃ</td>
<td>3.1±0.50</td>
</tr>
<tr>
<td><em>P. ostreatus</em> ECS-0152</td>
<td>1224.5±186.8ᵃ</td>
<td>102.9±15.7ᵃ</td>
<td>2.85±0.43</td>
</tr>
<tr>
<td><em>P. djamor</em> ECS-0149</td>
<td>993.6±254.6ᵇ</td>
<td>83.5±21.4ᵇ</td>
<td>2.78±0.71</td>
</tr>
<tr>
<td><em>P. citrinopileatus</em> ECS-1338</td>
<td>929.4±90.4ᵇ</td>
<td>78.1±7.6ᵇ</td>
<td>2.44±0.23</td>
</tr>
<tr>
<td><em>P. djamor</em> ECS-0142</td>
<td>896.0±117.8ᵇ</td>
<td>75.3±9.9ᵇ</td>
<td>2.51±0.33</td>
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*Different letters in the same column indicate significant differences between strains (α=0.05).*

P. ostreatus growth during the spawn run was homogeneous in the case of strain ECS-0152 on substrates prepared by self-heating or alkaline immersion. The contaminant fungi encountered were *Trichoderma* sp. (during colonisation) and *Coprinus* sp. (at the beginning of fruiting). Both fungi have already been identified as contaminants of oyster mushroom cultivation processes in the area (López et al., 1996). A contaminant fungus encountered in batch A after the first flush was *Fusarium* sp. It appeared after the first flush, most likely because the lower portion of the crate did not reach a sufficiently high temperature for pasteurisation. *Fusarium* contamination occurred later because the substrate units were immersed in water to recover the moisture lost during incubation after harvesting. In subsequent experiments, colonised substrate was soaked in water + 0.5% Ca (OH)₂ to avoid contamination.

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This result demonstrates that environmental temperature strongly affects the substrate temperature profile inside the crate because of 1) the heat exchange at the interphase between the crate and the surrounding air temperature and 2) the air entering the crate to aerate the substrate and prevent anaerobic conditions. Additionally, a lower environmental relative humidity around the crate will induce more substrate moisture evaporation, thus slowing the pasteurisation temperature increase. Thus, the method is environmentally dependent, and to reduce heat loses, it is advantageous to use a good insulating material, such as polyurethane.

The temperatures observed (under the environmental conditions mentioned) in level 3 of A and B do not guarantee safe substrate pasteurisation because they only just exceeded 40°C, while in cases C and D (crate insulated), the substrate temperature was greater than or equal to 60°C for approximately 15 h, which resulted in effective pasteurisation by impeding microorganisms that are noxious for mushroom growth. According to Overtijns (1981), 6 h at 60°C is sufficient to destroy green mould originating in raw materials, and milder time/temperature treatments are sufficient to kill other types of organisms, such as flies and nematodes.

Barrios-Espinoza et al. (2009) reported higher BE in both treatments (BE values of 112 and 62% for self-heating and alkaline immersion, respectively). They also found a significant difference between treatments. Additionally, in this study, the contamination observed on substrates from the non-insulated crate was higher than in their report (13 and 6% for alkaline immersion and self-heating, respectively). It is worth noting that, in their study, the environmental conditions were experimentally controlled, whereas in our study, cooperative farmers obtained these results under natural conditions. Finally, the difference in the BE values between trials A and C could be due to the polyurethane layer, which helped maintain an improved temperature profile inside the crate and thus resulted in a better and more uniform substrate pasteurisation.

The fact that five strains did not produce mushrooms indicates that the self-heating pasteurisation method is not feasible for all Pleurotus spp strains, and furthermore, a strain must be tested before being used commercially. In fact, Estrada et al. (2009) successfully cultivated P. eryngii on autoclaved sawdust. de Siqueira et al. (2012) also confirmed that P. eryngii was not able to grow on composted and steam-pasteurised substrate but did grow on an autoclaved substrate.

The self-heating pasteurisation treatment occurs aerobically, similar to composting; however, this treatment cannot be called composting because the objectives of these two processes are quite different: Composting is a long process seeking to produce humic substances and high-quality organic fertiliser (Martin 1991). Self-heating pasteurisation is also strongly aerobic; however, it occurs over a short time frame (two days), and bioconversion of materials is only desirable because it produces heat. Rather than bioconversion, the objective of the treatment is to promote a temperature increase to kill most contaminating organisms. Once the temperature profile has reached desirable standards (60°C for more than 6 h or alternative time/temperature treatments), the treatment is stopped. Of course, some compositional changes are observed, such as a decrease in sugar content and a change in C/N. However, the main objective is producing heat and retaining it for pasteurisation purposes. From this point of view, the method may still be improved by defining conditions that induce a faster temperature increase so that the thermal treatment is supplied earlier.

The self-heating pasteurisation method has several advantages over the alkaline immersion disinfection method and other alternative treatments, such as hot water pasteurisation, chemical disinfection, etc. (Ali et al., 2007; Oseni et al., 2012), as it provides substrate protection based on pH and a thermal treatment to the substrate. As observed here, the temperature increase depends on several factors, including substrate mass and volume, environmental conditions and stacking time. Additionally, the selection of a well-chopped substrate is important to the efficacy of this method: Shahid et al. (2006) reported a yield of 295 g/1.5 kg substrate while growing a strain of P. sajor-caju on wetted (overnight) straw with 2% lime.

Self-pasteurisation saves water and allows for precise control over the substrate moisture. It is also somewhat inconvenient as it lasts longer (two days) and requires substrate removal and because the substrate needs to be cooled down quickly at the end of pasteurisation. However, this method is a good alternative as it saves energy costs. In fact, the self-heating treatment described here may be considered an ecological method: it saves energy by using the natural "spontaneous" heat produced by stacked organic matter. No external energy source is used. If stacking conditions are controlled (mass, volume, pH, moisture, particle size, aeration and insulation), the grower has a good alternative method to prepare a selective substrate for cultivating several oyster mushroom strains from different species.

Finally, this experiment found that the self-heating pasteurisation method is suitable at the farm level. Two strains that were previously reported in laboratory experiments were used (P. ostreatus ECS-0152 and ECS-1123), and three alternative strains were also tested (P. djamor ECS-0149 and ECS-0142 and P. citrinopileatus ECS-1338).

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