CULTIVATION OF OYSTER MUSHROOMS ON CASSAVA WASTE

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ABSTRACT

Cassava is a major food crop for approximately 700 million people, especially in African countries. A large quantity of waste is produced after processing of cassava, mainly consisting of tuber peels. Although previous research has shown that these peels can be an ingredient for substrate to cultivate mushrooms, yields were usually inferior compared to traditional substrates such as saw dust. In a project funded by the European Union (http://www.fp7-gratitude.eu/) trials were done for the production of oyster mushrooms using fermented peels and stems from cassava crop produced in Ghana. Four mushroom strains representing two species (*Pleurotus ostreatus* and *P. pulmonarius*) were grown on fermented substrates made from cassava waste (peels & stems) without further heating/sterilisation. Peels and cassava stems were tested in different ratio's and supplemented with different amounts of rice or wheat bran. All the substrate samples colonized quickly (15-16 days) and time for pinning varied between 18 and 24 days. The *P. pulmonarius* strains produced three flushes within 47 days (starting from inoculation) and the *P. ostreatus* strains needed 57-63 days completing flush 3. Biological efficiencies after 3 flushes varied between 38% and 100%. The effect of bran supplementation on yields depended on the concentration (0, 1, 6 and 12% w/w), type of bran (rice or wheat) and strain used. The trials have shown that cassava waste (stems and peels) can be used well for the production of oyster mushrooms and that substrates containing up to 75% cassava peels have productions well comparable to yields obtained on the traditional saw dust based substrates.

Keywords: *Pleurotus* spp., cassava waste, biological efficiency, fermentation

INTRODUCTION

During the processing of cassava, large quantities of peels and, to a lesser extent, plant stems are produced. At present, only part of this waste is used, mainly as feed for goats. In many places where cassava is cultivated and processed, heaps of cassava peels are discarded along the roads and cause unpleasant odours and unhygienic conditions. Previous reports on the utilisation of cassava waste for the cultivation of grey oyster (*Pleurotus ostreatus*) and lung oyster (*Pleurotus pulmonarius*) mushrooms have shown that yields were reasonably well but still lower than those obtained on traditional substrate prepared from saw dust [1, 2]. It is known that waste from cassava production is low in nitrogen, one of the ingredients known to enhance the productivity of mushrooms. In this paper trials are presented in which substrate was prepared from cassava waste (peels and stems) supplemented with rice or wheat bran. Cultivation of both oyster mushroom species for 3 flushes showed that the addition of these supplements increases yields considerable up to levels comparable or even better than on traditionally used sawdust based substrates. In addition, cassava waste based substrates are colonized in a shorter time than saw dust and thus more crop cycle per year increasing the productivity even more. The cassava peels and stems were fermented for six days and inoculated subsequently with spawn without sterilization of the substrate. No infections were seen indicating that, when done properly, cultivation on cassava waste can be done also at lower costs since sterilization or high temperature pasteurization costs a lot of energy.

MATERIALS AND METHODS

Substrate

Fermentation of substrate and cultivation of mushrooms were done at Unifarm (test facility of Wageningen University and Research Centre). Cassava peels and stems were obtained via the Food Research Institute, Accra, Ghana (Mary Obodai) and originate from a regular cassava production site in Ghana. Dried peels and stems were packed in nylon sacks and submerged in water for 3 days and subsequently drained for one day to remove excessive water. Mixtures of peels and

stems were made and subsequently chalk and bran were added and mixtures were homogenized well. For each type of substrate mixture (Table 1) 3 trays were filled, each containing ca. 27.8 kg of substrate. Trays were put in a room heated up by steam up to 45 °C. This was done to mimic fermentation in large heaps where a preheating to start fermentation is not needed. Trays were fermented for 6 days. Most substrates have been at least for 8 hours above 65 °C. All trays were weighed to measure weight loss (which was 3-6 kg/tray). After cooling to 22 °C, each substrate type was mixed with spawn (60 ml, i.e. 30 grams of spawn/kg) and distributed over bags (2 kg inoculated substrate per bag). Bags were obtained from Microsac (http://www.saco2.com/) and taped off after filling. Spawn run (mycelial growth) was done in a growing room at 25 °C for ca 15 days.

Table 1. List of substrate mixtures made for the cultivation of *Pleurotus* species

Substrate mixtures	Cassave peels % (w/w)	Cassave sticks % (w/w)	CaCO3 % (w/w)	Rice bran % (w/w)	Wheat bran % (w/w)
1	73.5	24.5	2	0	0
2	72.75	24.25	2	1	0
3	69	23	2	6	0
4	64.5	21.5	2	12	0
5	72.75	24.25	2	0	1
6	69	23	2	0	6
7	64.5	21.5	2	0	12
8	48.5	48.5	2	1	0
9	49	49	2	0	0
10	46	46	2	6	0
11	43	43	2	12	0
12	48.5	48.5	2	0	1
13	46	46	2	0	6
14	43	43	2	0	12

Varieties

Varieties tested were obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO): One *P. ostreatus* (MES14030) and one *P. pulmonarius* strain (MES14029), gift from Agnes Asagbra at FIIRO, and 2 strains from the Plant Breeding collection of Wageningen UR: one commercial *P. ostreatus* (MES 03448) and one *P. pulmonarius* strain originating from Brazil (MES01997).

Cultivation

After colonization, 4 slits were made in each bag (one in all four sides) with a sharp knife to allow the formation of mushrooms. The room was subsequently vented with fresh air and cooled to 20 °C with relative humidity of 88%. Light was on for 12 hours/day.

RESULTS AND DISCUSSION

Fermentation of substrates

Fermentation of each type of substrate was done in trays each containing 27.8 kg substrate. Degradation of starch in the peels and other easily degradable compounds by microorganisms will usually heat up the substrate resulting in killing insects and pathogens and making the substrate more selective for growth of white rot fungi. Since only a small amount of substrate is present in each tray, the heat was easily lost to the environment. For that reason the environment (the room) was heated up to 45 °C to mimic fermentation in large heaps (where temperature is easily built up). Especially addition of wheat bran

lead to a quick heating of the substrate up to temperatures above 70 °C, i.e. 25 °C above the room temperature indicating an active growth of thermophilic microorganisms. Not all trays reached this temperature (minimum temperature achieved 61°C) but we expect that this is due to the small volumes in each tray and will be higher in large heaps. A visual inspection of the substrates after fermentation showed that most substrates were well colonized by thermophilic microorganism (whitish appearance, fire fang). Especially trays with wheat bran showed a dense colonization by thermophiles. The addition of wheat bran up to 6 and 12% seems too much since the substrate smells a bit (ammonia). The weight loss of substrates during fermentation was on average 16% (based on wet weight).

Production profile

Daily inspections by eye showed that fifteen days after spawning (inoculation), most substrates were colonized well. Four slits were then made on the bags (on each side one). Three days later the room temperature was decreased to 20 °C to initiate fruiting. Averaged over all types of substrates, pins were formed between 19 days (commercial *P. pulmonarius* strain) to 24 days (*P. pulmonarius* from Nigeria) after inoculation whereas the *P. ostreatus* strains produced pins 23 days after spawning. Twenty three days after inoculation the first mushrooms were picked from the fastest *P. pulmonarius* strains while the slowest *P. ostreatus* strains were ready to be picked 27 days after inoculation. The *P. pulmonarius* strains had produced a third flush by 43 days after inoculation while the *P. ostreatus* strains needed on average 12 days more. An overview of the production profile is given in Table 2. The statistical analysis (see appendix) showed that for time between inoculation (spawning) and pinning there is a significant difference between strains and the amounts of rice bran added. In addition, there is also an interaction between strains and amount of bran added, i.e. not all strains react in the same way. For wheat bran there is a significant difference in time to pinning between strains.

Table 2. Production profiles of varieties of 2 oyster mushroom species cultivated on substrate prepared from different mixtures of cassava waste (see Table 1). Significant differences were seen on time between spawning (inoculation) and pinning and between spawning and first flush between strains. This was significantly influenced by the amount of bran added (see appendix for statistical analysis).

		Rice Bran	Wheat Bran	Rice Bran	Wheat Bran
Strain	Amount of bran added % (w/w)	Time between spawning and pinning (days)		Time between spawning and first flush (days)	
P. ostreatus (Brazil)	0	23	22	27	26
P. ostreatus (Brazil)	1%	22	21	27	25
P. ostreatus (Brazil)	6%	23	23	25	27
P. ostreatus (Brazil)	12%	21	26	27	29
P. ostreatus (Brazil)	*average*	*23*	*23*	*27*	*27*
P. ostreatus (FIIRO)	0	24	21	28	25
P. ostreatus (FIIRO)	1%	21	22	24	25
P. ostreatus (FIIRO)	6%	22	26	25	28
P. ostreatus (FIIRO)	12%	18	27	24	34
P. ostreatus (FIIRO)	*average*	*23*	*23*	*27*	*27*
P. pulmonarius (commercial)	0	19	18	24	24
P. pulmonarius (commercial)	1%	17	17	23	22
P. pulmonarius (commercial)	6%	19	21	24	24
P. pulmonarius (commercial)	12%	19	20	24	24
P. pulmonarius (commercial)	*average*	*19*	*19*	*24*	*25*
P. pulmonarius (FIIRO)	0	23	24	27	25
P. pulmonarius (FIIRO)	1%	16	22	25	24
P. pulmonarius (FIIRO)	6%	20	23	23	26
P. pulmonarius (FIIRO)	12%	35	27	23	29
P. pulmonarius (FIIRO)	*average*	*24*	*24*	*25*	*25*

For time between spawning and first flush there are significant differences between strains and the amount of bran added. This indicates that for each variety there might be different optima of type and amount of supplements on the production profile.

Yields on substrate fermented in trays

Averaged over all substrates, the total yields after 3 flushes varied between the different strains (Table 3). The *P. pulmonarius* variety from Nigeria yielded 319 grams of fresh mushrooms per bag of 2 kg whereas the *P. ostreatus* of Nigeria yielded 583 grams per bag of 2 kg. The yield in fresh weight mushrooms for each substrate type varied considerably. Best yields

were for the *P. ostreatus* strain from FIIRO on cassava waste supplemented with 1% rice bran, i.e. 632 grams per 2 kg of substrate in 3 flushes (fresh weight productions and biological efficiency for all treatments are presented in the appendix). Expressed as biological efficiency (BE; fresh mushrooms per kg dry substrate), there are significant differences in yields due to the amount of either rice or wheat bran added. BE varied from as low as 47% up to 99% with different rice bran amounts added and from 42% up to 98% with different amount of wheat bran added. The general trend is that for rice bran the optimal amount is around 6% and for wheat bran 1% (wet weight/wet weight). This might reflect the amount of protein present in these two types of bran. As expected, fruiting bodies of the *P. ostreatus* had a light grey color whereas those of *P. pulmonarius* were dark brown (Fig.1). It is important to mention that none of the bags were infected, despite the fact that the substrates were only fermented for 6 days and maximum temperatures reached varied between 60 and 72 °C.

Table 3. Yields of mushrooms produced on the different substrate mixtures. Yields were recorded for 3 flushes. Especially the *P. ostreatus* variety of FIIRO showed a high production well comparable to yields on the traditional sawdust substrates. Statistical analysis for biological efficiency (fresh mushrooms per dry weight substrate) showed significant differences in yield between varieties and for the amount of bran added (see appendix).

		Yields (grams/bag of 2 kg substrate)		Biological efficiency	
Strain	Bran added	Rice bran	Wheat bran	Rice bran	Wheat bran
P. ostreatus (Brazil)	0	482	508	77%	79%
P. ostreatus (Brazil)	1%	542	513	85%	80%
P. ostreatus (Brazil)	6%	559	551	87%	86%
P. ostreatus (Brazil)	12%	511	440	78%	74%
P. ostreatus (Brazil)	average	524	503	80%	80%
P. ostreatus (FIIRO)	0	560	590	88%	92%
P. ostreatus (FIIRO)	1%	615	632	97%	98%
P. ostreatus (FIIRO)	6%	632	613	99%	94%
P. ostreatus (FIIRO)	12%	608	483	93%	80%
P. ostreatus (FIIRO)	average	604	579	91%	91%
P. pulmonarius (commercial)	0	471	504	74%	79%
P. pulmonarius (commercial)	1%	516	523	81%	82%
P. pulmonarius (commercial)	6%	526	514	82%	79%
P. pulmonarius (commercial)	12%	539	408	83%	68%
P. pulmonarius (commercial)	average	513	506	77%	77%
P. pulmonarius (FIIRO)	0	298	349	47%	54%
P. pulmonarius (FIIRO)	1%	364	352	57%	55%
P. pulmonarius (FIIRO)	6%	365	319	57%	49%
P. pulmonarius (FIIRO)	12%	400	254	61%	42%
P. pulmonarius (FIIRO)	average	357	319	52%	52%

Figure 1. Representative photographs of fruiting bodies of flush 1 of the 4 varieties grown on substrate prepared from cassava waste

CONCLUSION

The trials done with substrate prepared with cassava crops from Ghana showed clearly the potential of cassava waste as raw and safe (Obodai *et al.*) [3] materials for the production of oyster mushrooms. Although one has to keep in mind that cultivation conditions used here are much more optimal than on average in African countries the following conclusions can be drawn:

- Substrate based on cassava waste only can give yields of oyster mushrooms well comparable to traditional substrates such as saw dust. Yields close to 100% biological efficiency in only 3 flushes make this crop economically sustainable.
- Substrate based on cassava waste only (peels & stems) are more quickly colonized than the traditional saw dust based substrates.
- Fermentation of cassava waste can be done within 6 days. When done under proper conditions and inoculation under hygienic conditions, a sterilisation (or heating up to 100 °C) is not needed.

ACKNOWLEDGEMENTS

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APPENDIX

Statistical analysis.

Statistical analysis of time between spawning and pinning (days) on supplementation with rice bran. There is a significant difference between strains and between the amount of rice bran added. There is also a significant interaction between strains and the amount of rice bran added: not each strain reacts in the same way on the amount of bran added.

Analysis of variance

Variate: spawning_pinning

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Herhaling stratum						
rice_bran	3		3.71	1.24		
Residual	-2	(1)	2.27			
Herhaling.*Units* stratum						
Strains	3		563.31	187.77	13.77	<.001
rice_bran	3		382.31	127.44	9.34	<.001
Strains.rice_bran	9		1164.54	129.39	9.49	<.001
Residual	47	(103)	640.94	13.64		
Total	63	(104)	1331.73			

Statistical analysis of time between spawning and first flush (days) on supplementation with rice bran. There is a significant difference between strains and between the amount of rice bran added but no significant interaction between strains and the amount of rice bran added.

Analysis of variance

Variate: spawning_pinning

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Herhaling stratum	1	(1)	0.08	0.08	0.00	
Herhaling.*Units* stratum						
Strains	3		744.99	248.33	12.77	<.001
wheat_bran	3		345.02	115.01	5.91	0.002
Strains.wheat_bran	9		140.10	15.57	0.80	0.618
Residual	47	(103)	914.18	19.45		
Total	63	(104)	1331.73			

Statistical analysis of time between spawning and pinning (days) on supplementation with wheat bran. There is a significant difference between strains. There is no significant difference between the amount of wheat bran added and no interaction between strains and the amount of bran added.

Analysis of variance

Variate: Spawning_flush_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Herhaling stratum	2	9.083	4.542	0.56	
Herhaling.*Units* stratum					
Strains	3	251.875	83.958	10.44	<.001
wheat_bran	3	362.250	120.750	15.01	<.001
Strains.wheat_bran	9	210.000	23.333	2.90	0.003
Residual	150	1206.500	8.043		
Total	167	2039.708			

Statistical analysis of time between spawning and first flush (days) on supplementation with wheat bran. There is a significant difference between strains and between the amount of wheat bran added but no significant interaction between strains and the amount of rice bran added.*Volgensmij is eenwaarde van 0.003 wel significant.*

Analysis of variance

Variate: Spawning_flush_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Herhaling stratum					
rice_bran	3	4.76	1.59		
Residual	-1	4.32			
Herhaling.*Units* stratum					
Strains	3	251.88	83.96	8.36	<.001
rice_bran	3	169.00	56.33	5.61	0.001
Strains.rice_bran	9	102.60	11.40	1.13	0.342
Residual	150	1507.14	10.05		
Total	167	2039.71			