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Use of Rosemary (*Rosmarinus officinalis*) Processing Waste for *Pleurotus ostreatus* Cultivation

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Abstract: Rosemary processing waste (RPW) generated after extraction of antioxidant components for the food industry, in combination with crushed corn seeds, represented a suitable growth substrate for the cultivation of *Pleurotus ostreatus*. On average, fruit body yields were 49% lower on substrate mixtures containing RPW-1, containing residual acetone from the antioxidant extraction process, compared to substrates incorporating RPW-2 which had been allowed to stand in well-aerated conditions in order to remove residual solvent. The highest Biological Efficiency value (67%) were recorded with a substrate mixture containing 30% (w/w) crushed corn seeds (CCS) mixed with RPW-2 and 2% $CaCO_3$. Increasing proportions of wheat bran (WB) in the mixtures decreased the C/N ratio, had a positive effect on mycelial growth rates, but generally resulted in lower fruit body yields. The highest vegetative growth rate (9.9 cm in 23 days) was recorded on substrate mixtures with a C/N ratio of 24 and containing RPW-2 supplemented with 30% (w/w) CCS and 30% (w/w) WB.

Key words: agricultural by-products; mushroom cultivation; *Pleurotus ostreatus*; rosemary processing waste

In 1997, *Pleurotus* species accounted for 14.2% of the total edible mushroom production worldwide[1]. The popularity of these species has increased due to ease of cultivation, high yield potential, and high nutritional value[2]. Although commonly grown on pasteurized wheat or rice straw, these mushrooms can be cultivated on a wide variety of lignocellulosic substrates and can play an important role in the management of organic wastes in cases where disposal is problematic[3]. In addition to rice straw[4], many different substrate types have been used for cultivation including cotton stalks and sorghum stover[5], cottonseed hulls[6], banana straw[7], cardboard[8], different types of grass[9], spent brewery grains[10] and weed plants[11].

Rosemary biomass is primarily a feed-stoc for extracting antioxidants for use as foo additives through a patented procedure[12] After extract processing, disposal of the wast rosemary biomass represents a financial burde to the antioxidant producers. For example Vitiva Corporation, located in the Markov municipality of Slovenia, generates up to 70 tonnes of waste rosemary biomass annually Since producers have to pay for disposal of th waste at designated dumping sites, they ar constantly seeking for other methods t upgrade the material into value - adde products. To this end, we have explored th suitability of rosemary processing waste as substrate for cultivating *P. ostreatu* mushrooms.

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1 Materials and Methods

1.1 Preparation of fungal inoculum

P. ostreatus, strain Pl. o4, was obtained from the fungal culture collection at the Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana, Slovenia and maintained on Potato Dextrose Agar (PDA).

1.2 Substrate preparation

Rosemary Processing waste (RPW) was obtained from Vitiva Corporation, Markovci, Slovenia and stored in plastic bags until use. Two kinds of RPW were used in cultivation experiments: RPW-1 was used directly and had a high content of residual acetone from the antioxidant extraction procedure; RPW-2 was placed on plastic sheets in a well - aerated location and mixed daily until no acetone smell was detectable. RPW-1 or RPW-2 was mixed with different proportions of wheat bran and/or crushed corn seeds (Table 1) and 2% $CaCO_3$, and 400 grams of the mixture were transferred to 720 mL glass jars at a packing density of 62 g substrate per 100 mL volume. The jars were covered with punctured lids (9 mm hole closed with a cotton plug), and sterilized for three hours at 121 ℃. Four replicates of each substrate mixture were prepared.

Table 1 Substrate mixtures used for *P. ostreatus* cultivation

RPW (%)	Wheat bran (%)	Crushed corn seeds (%)	RPW (%)	Wheat bran (%)	Crushed corn seeds (%)
38	30	30	68	20	10
48	20	30	68	30	0
48	30	20	78	0	20
58	10	30	78	10	10
58	20	20	78	20	0
58	30	10	88	0	10
68	0	30	88	10	0
68	10	20	98	0	0

1.3 Substrate inoculation

After cooling, each glass jar was inoculated with a 9 mm diameter disc of mycelium cut from PDA cultures grown in the dark at 24 ℃, and incubated at 24 ± 1 ℃.

1.4 Mycelial growth measurements

The average mycelium growth rate on substrate mixtures containing RPW - 2 was calculated by first taking the mean of the fastest and slowest mycelium growth front point in each jar, and then averaging the values obtained for all four replicates.

1.5 Mushroom cultivation

When the fungal mycelium reached the bottom of the glass jars (28 to 105 days), the cultures were transferred to the mushroom cultivation room maintained at 16 ± 1 ℃, 90 ± 5% relative humidity, approximately 1300 ppm CO_2 and subjected to a 10 : 14 h light : dark cycle. Mushrooms were harvested before the caps started to invert and until cessation of fruiting (up to 90 d cultivation). Fruit bodies were cleaned of excess substrate and weighed. Biological efficiency (BE), defined as the weight of fresh fruit bodies divided by the initial weight of dry substrate × 100, was calculated for each substrate mix. The entire experiment was repeated and the average of the two BE determinations was recorded. Experiments were performed at the Mycological Laboratory, Institute of Natural Sciences, Podkoren, Slovenia.

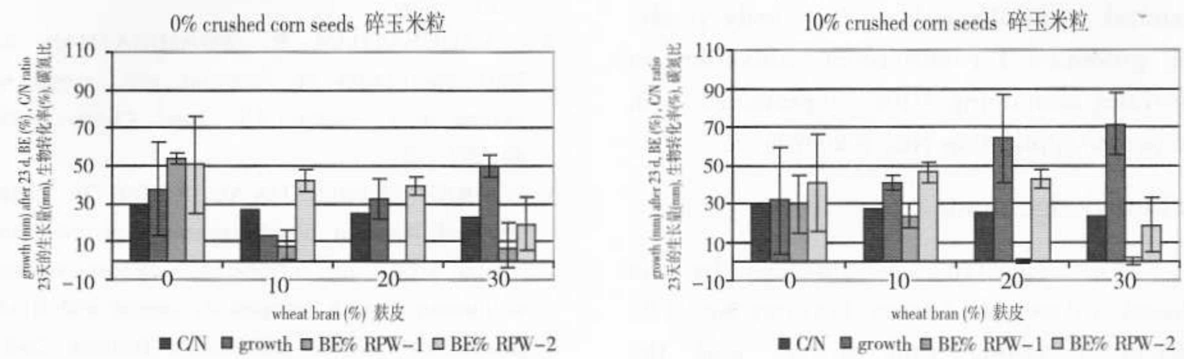
1.6 C/N determination

Total and organic carbon (C) and total nitrogen (N) contents of the substrate mixtures were determined after dry combustion and incineration at 900 ℃ in a CNS analyzer (Vario MAX CNS, Elementar Corp., Germany), and the C/N ratio was calculated.

2 Results

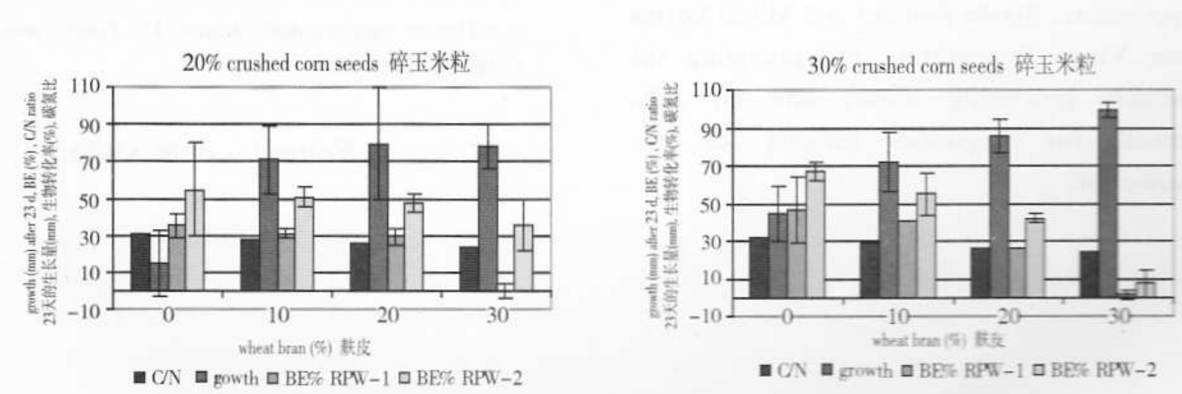
With four exceptions (those mixtures containing 30% wheat bran), all the substrate combinations containing RPW-2 resulted in BE values of 40% or higher (Figs. 1-4). The highest BE value (67%) were recorded with the substrate mixture containing 30% (w/w) crushed corn seeds (CCS) mixed with RPW-2 and 2% $CaCO_3$ (Fig. 4). Increasing proportions of wheat bran (WB) in the mixtures generally resulted in a decrease in fruit body yields (Figs. 1-4). However, increasing supplementation with WB decreased the C/N ratio in the substrate and had a positive effect on mycelial growth rates (Figs. 1-4). The highest vegetative growth rate (9.9 cm in 23 days) was recorded on substrate mixtures with a C/N ratio of 24 and containing RPW supplemented with 30% (w/w) CCS and 30% (w/w) WB (Fig. 4).

In all tested substract formula, fruit body yields were lower when RPW-1, containing residual acetone from the antioxidant extraction process, was incorporated into the mushroom substrate mixture. On average, BE values on mixtures containing RPW-1 were 49% lower compared to substrates incorporating RPW-2.



Figures 1 and 2 Influence of RPW supplements on BE values, mycelium growth rates and C/N ratios associated with substrate mixtures containing 0% and 10% CCS.

图 1 & 图 2 CCS 添加量 0%&10%水平培养料中 RPW 对生物转化率、菌丝生长率和 C/N 的影响



Figures 3 and 4 Influence of RPW supplements on BE values, mycelium growth rates and C/N ratios associated with substrate mixtures containing 20% and 30% CCS.

图 3 & 图 4 CCS 添加量 20%&30%水平培养料中 RPW 对生物转化率、菌丝生长率和 C/N 的影响

3 Conclusions

Processing waste remaining after the extraction of antioxidants from rosemary plants is an appropriate substrate component for cultivating *P. ostreatus* mushrooms and BE values of up to 67% were attainable. Data from this study contradict our previously unpublished results obtained from experiments using substrates containing spent brewery grains showing that *P. ostreatus* mycelium growth rates and BE values declined with decreasing C/N ratios. This indicates that substrate characteristics linked to the added supplements other than those affecting the C/N ratio influence vegetative growth and fruiting in *P. ostreatus*. For more efficient *P. ostreatus* cultivation, the acetone content in the RPW should be reduced to a minimum since this chemical drastically reduces fruit body yields. The procedure for mushroom cultivation on substrates containing RPW is protected with the patent application No. P-200700296.

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