ACTIVITY OF α--AMYLASE FROM *P. OSTREATUS* GROWN ON WASTE SUBSTRATES

MATEJA PRIMOŽIČ,[1] ŽELJKO KNEZ,[1, 2] MAJA LEITGEB[1, 2]

[1] University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory for Separation Processes and Product Design, Maribor, Slovenia
mateja.primozic@um.si, zeljko.knez@um.si, mateja.leitger@um.si
[2] University of Maribor, Faculty of Medicine, Maribor, Slovenia
zeljko.knez@um.si, mateja.leitger@um.si

Abstract Forest and agricultural waste can be a major development and ecological opportunity. Therefore, it is reasonable to use biological waste further to produce energy and for the manufacture of certain products with high added value, such as, for example, the cultivation of fungi and, consequently, the production of biocatalysts with high market value. In addition, the use of agriculture waste for Oyster mushroom (*Pleurotus ostreatus*) growth can be integrated to waste management and the development of the bioeconomy. The cultivation of *P. ostreatus* using waste plant biomass from agriculture (straw, grass, courgettes, cucumbers, peaches, apricots, pears, and peppers) was performed in order to obtain the highest increase in biomass production of the cultivated mushroom and as a potential source of α-amylase, with high catalytic activity. The highest α-amylase activities were achieved when pears or apricots were used as a substrate for *P. ostreatus* cultivation.

Keywords:
P. ostratus,
α-amylase,
activity,
agriculture
waste,
cultivation

University of Maribor Press

DOI https://doi.org/10.18690/um.fkkt.2.2022.7
ISBN 978-961-286-598-6

1 Introduction

One of the economically interesting medical mushrooms is *P. ostreatus*, which is easily cultivated by beginners, as it has an extremely strong undergrowth. The genus *Pleurotus* includes groups of edible ligninolytic fungi with medicinal properties and important biotechnological and environmental applications. One of the most important aspects of *Pleurotus* fungi is related to the use of their ligninolytic system for various applications, such as the biological conversion of agricultural waste into useful animal feed and other food products, and the use of their ligninolytic enzymes for biodegradation of organic pollutants, xenobiotics and industrial contaminants. The cultivation of *P. ostreatus* is important for the production of lignocellulosic and other enzymes. Mushrooms are grown on different nutrient bases, i.e. substrates in which the undergrowth or mycelium develops. These substances, however, contain polymeric organic compounds that are difficult to decompose. In order to consume organic matter, mushrooms must first eliminate certain enzymes that decompose this substrate. The prevalence of mushroom enzymes has long been known and exploited for use in the food industry and in many other industries. Mushrooms respond to their habitat through controlled gene expression and secretion of specific enzymes. The enzymes split the molecules into smaller units, and mushrooms mycelium absorbs these reduced organic molecules as nutrients directly through the cell walls. The conversion of substrates into mushroom biomass is mediated by the secretion of a variety of hydrolytic and oxidative enzymes. In nature, *P. ostreatus* grows on waste wood, but it is also possible to grow it on agrarian waste to produce enzymes to stimulate oxidation reactions (laccase, manganese peroxidase, lignin peroxidase) and hydrolysis (amylase, cellulase, xylanase, tanase)(da Luz et al., 2012). The cultivation of *P. ostreatus*, *Pleurotus eryngii*, *Pleurotus pulmonarius*, *Agrocybe Aegerita* and *Volvariella volvacea* was performed on three types of agricultural waste (wheat straw, cotton waste, and peanut shells) (Philippoussis et al., 2001). High growth rates of all studied mushroom cultures on the agricultural waste were detected. Waste of coffee leaves, eucalyptus bark, with or without the addition of rice bran, was used as the substrate for *P. ostreatus* cultivation. The lignocellulolytic enzyme activity increased with time after inoculation and the substrate composition affected the enzyme activity (da Luz et al., 2012). The production of lignocellulolytic enzymes such as laccase, manganese peroxidase, endoglucanase, exoglucanase and 3-glucosidase was successfully performed by degradation of Chrysanthemum and Rosa residues using *P. ostreatus* (Quevedo-Hidalgo et al., 2015). Rashad et al.

(Rashad et al., 2009) studied the enzymatic activity of amylase, cellulase, invertase, polygalacturonase and pectinase from *P. ostreatus* cultivated on citrus wastes and papaya. A higher activity of amylase was observed in the unsupplemented lemon pulp and papaya waste. Further, the ability of *P. ostreatus* and *Pleurotus sajor-caju* fungi to produce laccase and carboxymethylcellulase enzymes on a variety of agricultural wastes in the solid-state fermentation process (SSF) was studied. Enzymatic activity was highest on substrates that contained bran, had high nitrogen content and low C/N ratio (Kurt & Buyukalaca, 2010). Belšak-Šel et al. (Belšak-Šel et al., 2015) studied the increase in laccase production from *P. ostreatus* by optimizing the culture medium. The level of laccase activity produced by *P. Ostreatus* in the SSF process in wheat bran was significantly influenced by lignocellulose biomass and glucose content. Production of enzymes with SSF offers the advantage of reducing production costs and increasing the rate of mycelium growth. Additional, *P. ostreatus*, as well as other mushrooms (*Lentinula edodes*, *G. frondosa*, *Cantharellus cibarius* and *Agaricus bisporus*) are rich in B vitamins (Solomko & Eliseeva, 1988), organic acids, β - lipids, proteins and micronutrients (e.g. selenium or chromium)(Dikeman et al., 2005; Valentão et al., 2005). Amylases are important in many industrial processes and are one of the most widely used enzymes required for the preparation of fermented foods and now in the light of biotechnology they are considered useful for biopharmaceutical applications. They are useful tools in medicinal and clinical applications (Afzaljavan & Mobini-Dehkordi, 2013). α-Amylases can be obtained from plants, animals and microorganisms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (de Souza & de Oliveira Magalhães, 2010).

Therefore, *P. ostreatus* was cultivated on waste vegetable biomass from agriculture (straw, grass, courgettes, cucumbers, peaches, apricots, pears, and peppers) in combination with wheat bran to maximize mushroom growth as a potential source of α-amylase.

2 Methods

2.1 *P. ostreatus* cultivation

P. ostreatus was cultivated on substrate containing waste vegetable biomass from agriculture and wheat bran in volumetric ratio 1:1 in the presence of growth media from yeast extract and peptone. The *P. ostreatus* was cultivated for 8 days at 27 °C.

2.2 Extraction of enzymes from *P. ostreatus*

The extraction of *P. ostreatus* mycelium was performed using 0.05 M sodium citrate buffer as a solvent and for 90 min by shaking at 200 rpm. The obtained extract was centrifuged 20 min at 5000 rpm. Supernatant was used for the determination of α-amylase activity.

2.3 Determination of total protein concentration and α-amylase activity

The total protein concentration was assayed by the Bradford method (Bradford, 1976) and activity of α-amylase was defined spectrophotometrically at 595 nm. The activity was expressed as the change in absorbance at 595 nm per minute per solid of α-amylase (Čolnik et al., 2016).

3 Results

3.1 Mycelium growth of *P. ostreatus*

P. ostreatus was cultivated on waste vegetable biomass from agriculture (straw, grass, courgettes, cucumbers, peaches, apricots, pears, and peppers) in combination with wheat bran to maximize mushroom growth. The increase in mycelium growth of *P. ostreatus* after 8 days of cultivation on different substrates was pursued (Figure 1).

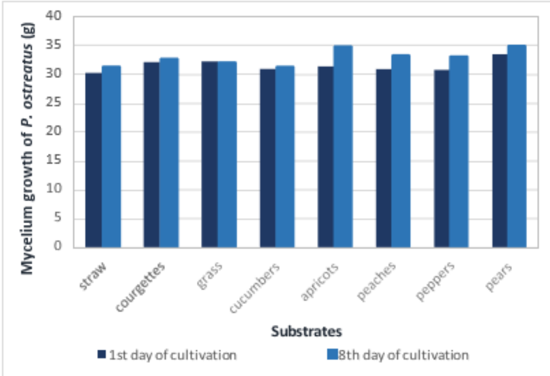

Figure 1: Influence of substrates on mycelium growth of *P. ostreatus*
Source: own.

As can be seen from Figure 1, the highest increase in mycelium growth of *P. ostreatus* after 8 days of cultivation on different substrates was detected when apricot as a substrate was used. Significant increase in mycelium growth of *P. ostreatus* was also detected when straw, peaches, pears, and peppers were used as a substrate.

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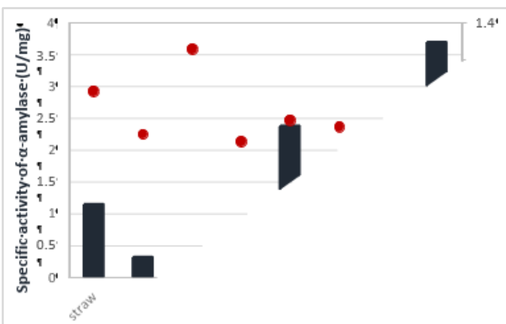

Figure 2: Influence of substrates on total protein concentration and specific activity of α-amylase
Source: own.

4 Conclusion

The *P. ostreatus* fungus was successfully grown on waste vegetable biomass from agriculture (straw, grass, courgettes, cucumbers, peaches, apricots, pears, and peppers) in combination with wheat bran. With increase in the time of cultivation of *P. ostreatus* on selected media, an increase in biomass growth was detected. The most suitable substrate, among those tested, for α-amylase production was composed from pear and wheat bran. Thus, waste vegetable biomass are suitable substrates for the cultivation of *P. ostreatus* for the purpose of α-amylase production. Due to bioconversion of nutrient-rich waste and by-product to value-added products, the environmental burdens imposed by these wastes are alleviated, and consequently, the regional and international economy can be boosted.

Acknowledgement

The authors acknowledge the financial support from the Slovenian Research Agency (research core funding No. P2-0046 - "Separation Processes and Product Design").

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