

**TITLE:** PRODUCTION AND PARTIAL PURIFICATION OF POLYPHENOL OXIDASE OF THE *PLEOROTUS PULMONARIUS* (PP21) BY SOLID STATE FERMENTATION USING AGROINDUSTRIAL RESIDUES AS SUBSTRATE

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**ABSTRACT:**

Polyphenol oxidase (PPO), EC 1.14.18.1, is an enzyme widely distributed in nature that oxidizes phenolic compounds to o-quinones acting on the enzymatic darkening of plants and influencing the organoleptic and nutritional properties of the processed derivatives. The use of PPO for biotechnology and industrial purposes depends upon its being obtained and sometimes of its purification. The using of agroindustrial residues by edible mushrooms by SSF is a sustainable alternative because these fungi are easily cultivable and showed as a good producers of PPO. Thus, the objective of this work was verify the better moisture to PPO producing by Solid State Fermentation (SSF) using agroindustrial residues as substrates and partially purify the enzyme using ammonium sulphate precipitation. The SSF were carried out by inoculating four 8mm plugs of the mycelium fungus into peach-palm residue and cocoa seed skin for 3, 5, 8, 12 and 15 days at 28°C. Subsequently, the SSF was performed with five moisture variations (70, 75, 80, 85 and 90%) for 5 days at the same temperature. All the experiments were carried out in 3 replicates. The extracts were obtained from the addition of 20 mL of distilled water to the cultures and further extraction by vacuum filtration and centrifugation at 12,000 rpm for 15 minutes at 4°C. The enzymatic assay was performed using 50 mM of catechol as substrate. The readings were obtained in kinetic mode by spectrophotometer at the absorbance of 420 nm. One unit of PPO activity was defined as the increase in absorbance of 0.001/min/mL. For partial purification of the enzyme was held precipitation with 60% ammonium sulphate. Total protein content was determined by the Bradford method to perform the calculation of specific activity and purification index. The extract obtained from SSF for 5 days showed activity of 107.6 U/mL. After the moisture variation, the highest enzymatic activity was observed in the crude extract obtained from SSF with 90% moisture content, which presented 307.95 U/mL, with 0.095 mg protein/mL and a specific activity of 3241.58 U/mg protein. The ammonium sulphate showed as an efficient purification method to PPO with the purification index of 2.1 and the specific activity of 6752.62 U/mg protein.

**Keywords:** Enzymes, Mushroom, Peach-palm residue, Cocoa seed skin.

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