

Title : Extraction of castor plant peroxidase by aqueous two-phase system

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## ABSTRACT

Peroxidase is an enzyme in the oxido-reductase group that catalyzes the oxidation of various substrates, both organic and inorganic, with hydrogen peroxide as the electron acceptor. Peroxidase can be found commonly in plants. This research aimed to extract peroxidase from castor plants using the aqueous two-phase systems (ATPS). Peroxidase activity was determined by using guaiacol as a substrate in the presence of hydrogen peroxide. Protein content was determined by Bradford method. In this aqueous two-phase extraction experiment, different amounts (%w/w) of polyethylene glycol (PEG) and sodium sulfate were used under five different combinations in crude extract giving the final volume of 10 mL as follows: the first 14% PEG 1500 and 12% sodium sulfate, the second 18% PEG 1500 with 8% sodium sulfate; the third 14% PEG 4000 and 8% sodium sulfate; the fourth 18% PEG 4000 with 8% sodium sulfate; and the last 18% PEG 6000) with 8% sodium sulfate. Phase separation was allowed to proceed for 3 hours. Results showed the total activity of peroxidase in the bottom sodium sulfate phase to be higher than that of the top polyethylene glycol phase and thus become the phase of interest. Specific activity of peroxidase in each salt phase was 1.744, 3.158, 2.820, 2.654 and 2.647 units/mg protein, respectively. Thus, the corresponding purification fold was calculated as 1.61, 2.92, 2.60, 2.45 and 2.44, respectively, when compared with the crude extract. Results showed that the use of 18% PEG 1500 with 8% sodium sulfate gave the highest peroxidase purity. In the protein profile examination of the salt phase samples by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and semi-native polyacrylamide gel electrophoresis (Semi-native-PAGE), the salt phase extracts in all conditions showed colored bands of tetra-guaiacol after substrate staining, confirming that the extracts contained peroxidase among other proteins that can be further separated.

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