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Purification and Characterization of Peroxidase from Broccoli (Brassica oleracea l. Var. Italica) Stems

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ABSTRACT

Enzyme activity was increased during purification which could exert beneficial effect on plants. Methods and Findings: Enzyme purification included extraction, (NH4)2SO4 precipitation, dialysis followed by sequential chromatographies with Sephadex G-75 and Sephadex DEAE A-25. The purified enzyme was characterized with time, pH stability, metal ions, thermostability, and substrate kinetics was determined using guaiacol as substrate. The purification fold for purified broccoli stem peroxidase was 72.83 with 1.5% yield. The optimum time for enzyme activity was 6 min. and it remained stable between pH 4 to 8 having optimum pH of 6 using guaiacol as substrate. The enzyme showed maximum activity at 30°C and remained stable upto 50°C. The Km value of Broccoli Peroxidase was 0.35 m.mol/ml and 33 U/ml for guaiacol substrate by using Lineweaver-Burk graph and similarly using Michaelis Menten graph values was 0.34 m.mol/ml. Metals such as Na+, Ca2+, K+, Mg2+ and Zn2+ exhibited no effect on enzyme activity. Conclusion: These properties recommend that peroxidase could be auspicious tool for various applications in different analytical determination as well as in treatment of industrial waste.

Peroxidases are large group of enzyme family called oxidoreductase that catalyze the reduction of peroxides, such as hydrogen peroxide (H2O2) and are responsible for the oxidation of various organic and inorganic compounds. Peroxidase binds with other substrates such as ascorbate and ferricyanides and break them into harmless substances by donating electrons. Peroxidase catalyzes the oxidation of phenolic compounds and aromatic rings which occur naturally in plant tissues.

Peroxidases represent group of specific enzymes, such as iodine peroxidase, glutathione peroxidase and NADH peroxidase as well as a wide range of non-specific enzymes simply called as peroxidases. The molecular weight of peroxidases ranges from 30 to 150 kDa. Peroxidases are associated with cell wall biosynthesis of plants. Their activity increases with plant age, with mechanical grazes and after vaccination with viruses, so they provide host resistance mechanism to plants.

In research areas such as biochemistry, medicine, genetics, enzymology and physiology, Peroxidases as a major enzyme group have occupied specific position in heat processing of vegetables. The analysis of this enzyme have not only showed its negative effect on flavor, color and degradation of pigments of vegetables but also have positive energetic effect on vegetarian food.

The wide range of plant peroxidases have been purified and studied from different sources, such as Oil palm oil, Orange peel, Turnip root, Olives, Wheat, Sweet potato tuber, Green asparagus, Melon , Strawberry, Apple, Papaya fruit, Vanilla bean, Spinach, Red cabbage and cabbage leaves, Red

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beet, Spanish broom, Lettuce stem, Pearl millet, Sprouted green gram roots, Horseradish roots, Cauliflower, Drumstick tree leaves, Banana, Avocado, Rosemary leaves and Sweet gourd. Multiple isoenzymes have been purified from all these sources which differ in their thermal stability, molecular mass, substrate specificity, pH optimization and their physiological role.

Peroxidases are also produced from microbial sources such as bacteria (Pseudomonas spp., Bacillus subtilis, Bacillus sphaericus, Citrobacter spp.), fungi (Chrysosporium, Candida krusei, Coprinopsis cinerea, actinomycetes, phanerochaete), Cyanobacteria (Anabaena spp.) and yeast (Pichia pastoris) have various industrial applications. Despite the purification of peroxidase from these sources, one of the most important source rich in peroxidase is broccoli (Brassica oleracea l. Var. Italica). Broccoli is dicotyledonous plant vegetable belong to the family Brassicaceae or Cruciferae. Peroxidase purified from the broccoli (Brassica oleracea. L. var. italica) processing waste (mostly stem) have many clinical and industrial applications.

Plants have been used for purification of enzymes to meet present day industrial demand. In present study a peroxidase enzyme was purified from broccoli stems. The purified enzyme was characterized with time, pH stability, metal ions, thermostability, and substrate kinetics was also determined using guaiacol as substrate. The properties of this enzyme recommend that peroxidase could be auspicious tool for various applications in different analytical determination as well as in treatment of industrial waste.

Keywords: Peroxidase; Brassica oleracea L. var. italic; Chromatography; Dialysis; Purification.