PURIFICATION OF PEROXIDASE FROM SOYBEAN THROUGH AFFINITY CHROMATOGRAPHY ON IMMOBILIZED CRATYLIA MOLLIS LECTIN (CRAMOLL 1,4-SEPHAROSE)

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Peroxidase (EC 1.11.1.7) catalyzes oxidation reactions using peroxide and oxygen as hydrogen acceptors. This enzyme (POD) has been found in species of plants, with a wide cellular distribution. It has been used in histochemistry, immunological assays and in enzymatic determination of uric acid, glucose and cholesterol. The aim of this work was the isolation of peroxidase from soybean seeds through affinity chromatography on *Cratylia mollis* lectin (Cramoll 1,4) immobilized on Sepharose CL-4B (Cramoll 1,4-Sepharose). Extracts from soybean in phosphate buffer were precipitated with ammonium sulphate and, after exhaustive dialysis, fraction F0-60 was obtained. Extract and fraction were submitted to peroxidase activity by qualitative assay using diaminobenzidine (DAB) and quantitative assay using guaiacol. F0-60 was chromatographed on Cramoll 1,4-Sepharose; desorbed proteins were analyzed by SDS-PAGE and peroxidase activity. Cramoll 1,4 was successfully immobilized (yield of 100%) and efficiently retained commercial peroxidase. Extract and fraction presented high peroxidase activity to DAB and guaiacol assays. Affinity chromatographies revealed bioselectively adsorbed fractions (all with enzymatic activity) and specific binding of peroxidase (glucose eluted). In conclusion, Cramoll 1,4-Sepharose was efficient in the purification of active soybean peroxidase; the affinity matrix is promising to obtain the enzyme from different sources.

Key Words: Soybean; Enzyme purification; Peroxidase; *Cratylia mollis* lectin; Immobilization.

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