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# SOYBEAN SEED COAT CONTAINS HIGHER PEROXIDASE THAN OTHER PARTS OF THE PLANT

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## ABSTRACT

Peroxidase was extracted from seed coat and leaves of one kind of Iranian soybean grown in Northern Iran near the Caspian Sea. The extracted enzyme was purified and identified by HPLC. The quantity and biological activity of peroxidase isolated from different parts of the soybean plant were compared. It was shown that, although the biological activities of peroxidase extracted from leaves and seedcoat were very similar, but seed coat contained higher quantity of peroxidase than the leaves. The biological activities of both peroxidase were comparable with the values reported in literature for peroxidase extracted from soybeans in other countries.

**Key words** : Peroxidase, Extraction, Soybean seed coat, Soybean leaves, Biological activity, Biological diversity

## INTRODUCTION

Soybean seed coat peroxidase (SBP) is a peroxidase with extraordinary stability and catalytic properties. It is a glycoprotein with a molecular weight of 37 kD that is expressed in the seed coat of soybean 20 days after anthesis[1].

Peroxidases (PODs, E.C. 1.11.1.7) are haemoproteins that are very widespread in nature. They have the ability to catalyse the oxidation of a large variety of substrates through a reaction with hydrogen peroxide. They are widely used in clinical biochemistry and in enzyme immunoassays[2,3]. Some novel applications of peroxidases include treatment of wastewater containing phenolic compounds, synthesis of various aromatic chemicals and removal of peroxide from materials such as foodstuffs and industrial wastes[4]. Horse raddish root tubers are commonly employed as a commercial source for peroxidase production[5–7]. However, other cultivated species may provide PODs exhibiting similar or better properties, especially recombinat species[8]. Soybean peroxidase is a special kind of peroxidase with high biological activity and very good resistance to temperature and other changes in physical conditions.

Soybean is a plant grown in many parts of the world and because of its ease of growth, high yield of product and many different uses, it is almost grown in every country. The seads (Figure 1) are high sources of many proteins and this is the reason, why soya is a good replacement for meat, especially for people suffering from high levels of uric acid and cholesterol in their blood.

The aim of this research was to extract and purify peroxidase from two different parts of soybean and to measure the peroxidase activity in the extracted mixture and then compare the total amount of peroxidase found in two different parts of the plant.

## MATERIALS AND METHODS

The leaves (Figure 2) were collected from a soybean plant grown in our laboratory and the seeds were kindly provided from a farm in Astaneh (northern part of Iran).

Ammonium sulphate, sodium chloride and polyethylene glycol were purchased from Sigma chemical company. Comassie brilliant blue G–250, guaiacol and hydrogen peroxide were from Fluka Chemie AG.

In order to prepare a crude extract, the fresh leaves were washed thoroughly with distilled water at room temperature, and cut into pieces. On the other hand, seeds were soaked in distilled water at room temperature for 24 hours and the husks were separated by filtration. Both samples were then homogenized with distilled water for 10 minutes at room temperature using a homogeniser.

The extracts were filtered using four layers of cheesecloth to remove suspended fibrous solid particles. The clear filtrates were used as crude extracts for further purification. Crude extracts were also prepared using 10 mM phosphate buffer by exactly the same procedure. It was shown that the activity and stability of the enzyme was similar in both the extracts. Therefore, the crude extracts prepared from distilled water were used in all further experiments.

Peroxidase activity was determined at room temperature with spectrophotometer following the formation of tetraguaiacol ($A_{max} = 470$ nm $\varepsilon = 26.6$ $mr^{-1}$ $cm^{-1}$) in a 3.0 mL reaction mixture containing 1 mL of 2–methoxyphenol (guaiacol); 1 mL of 3 mM $H_2O_2$; and 50 mL of enzyme extract. The reaction was carried out for 3 minutes. One unit of peroxidase activity (U) represents the amount of enzyme catalyzing the oxidation of 1 mmol of guaiacol in 1 $min^4$. Protein was determined by Comassie brilliant blue G–250 method using bovine serum albumin as standard[9].

## RESULTS AND DISCUSSION

Figure 3 compares the quantity of peroxidase extracted from seed coat and leaves of soybean. It is clear from this graph that seed coats contain about 20% more peroxidase than leaves of soybean plant. However, the specific activities of the enzyme extracted from both parts are very similar. In Figure 4, it is shown that the specific activity of extracted peroxidase does not highly depend on the solvent used for preparation of the crude extract.

From the results obtained, it can be concluded that soybean peroxidase is a very stable enzyme that can be extracted from soybean seed coats using distilled water as the extraction solvent. It is, therefore, a completely soluble enzyme and does not easily loose its activity. As

soybean seed coat peroxidase have many commercial and medical uses and its extraction is possible through a relatively simple procedure from soybean, we can conclude that the procedure described here can be used to produce higher quantities of peroxidase from soybean.

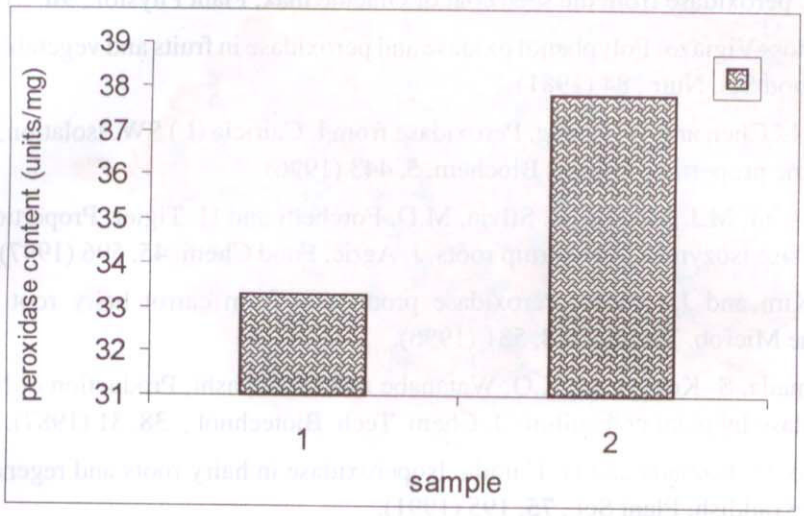


**Figure 3. The quantity (measured in terms of specific activity) of peroxidase extracted from leaves (1) and seed coats (2) of soybean**

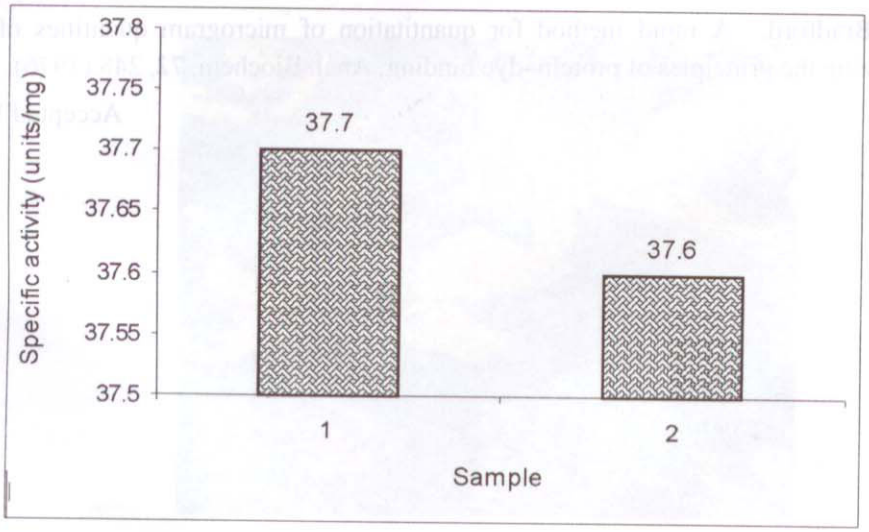


**Figure 4. Comparison between the specific activity of peroxidase extracted from seed coat of soybean by distilled water (pH 7.0, sample 1) and phosphate buffer (pH 6.0, sample 2)**

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Figure 1 Seeds of soybean containing the husks.

Figure 2 The soybean leaves in summer time.