



## Extraction and Purification from Local Horseradish Peroxidase

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### ABSTRACT

Horseradish peroxidase is a comprising enzyme which can be appropriate to oxidoreductases and is intricate in degradation of definite intractable organic complexes like phenol and substituted phenol via free radical polymerization. Peroxidase was extracted and purified from horseradish using ammonium sulphate for dialysis, gel filtration and precipitation chromatography. Spectrophotometer was used for assay of horseradish peroxidase in this present research. Horseradish peroxidase increased activity from 5.211 to 9.246 U/ml by purification. It gained 29.67 folds purification at this final step. In addition to its beneficial in characterization of glucose in blood; moreover it can destroy microbial strains.

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### Introduction

Peroxidases are widely distributed in nature and can be easily extracted from most plant cells, some animal organs and tissues [1-2]. Horseradish peroxidase HRP (E.C. 1.11.1.7) as a single chain glyco-hemoprotein containing 308 residues is the most abundant member of the peroxidase family [3].

Peroxidases are ubiquitous oxidoreductases that use hydrogen peroxide or alkyl peroxides as oxidants. Peroxidases catalyze the one- or two-electron oxidation of various organic and inorganic substrates in the presence of hydrogen peroxide [4]. Horseradish has medicinal applications; it is widely used as antibiotic and anti-inflammatory ability. It is also helpful in the determination of glucose in blood [5].

Horseradish peroxidase is most commonly used in enzyme labeled antibody in rapid, sensitive and specific enzyme immunoassays e.g. ELISA test used for labeling antibody and also, the produced antibody has many application in research, clinic and education. This polyclonal antibody can be used for diagnosis and monitoring of free light chain producing disease [6-7]. The obvious values of peroxidases, their present commercial uses are limited, primarily by the low stability of peroxidases in the presence of their natural substrate, hydrogen peroxide. Presence of high concentrations of hydrogen peroxide led to inactivation of hemoproteins, including peroxidases [8].

Therefore, development of purification method associated to diagnosis of diverse diseases is of abundant status. The goal of this study is to discover the original natural resources for medication of different diseases.

### Materials and Methods

All the chemicals and reagents were of analytical grade. Horseradish of about 40 g was obtained from local market.

#### Extraction of peroxidase

Horseradish peroxidase extraction comprises the following steps:

#### Enzyme extraction

The enzyme was extracted at a extraction ratio 1:5(w/v) by cutting down about 40 gm of horseradish into small pieces after washed with water and homogenized by using cooled blender for 5 min, 200 ml of distilled water were added, the mixture was centrifuged at 6,000 rpm for 15 min at 4°C, then filtration

through Whatman paper (No.1). Enzyme assay was performed for this extract.

#### Enzyme Assay and Protein Estimation

Enzyme assay was measured by Bernhard and Whitaker [9] method according to the below steps:

Buffer substrate solution was prepared as follow:

1. 0.1M Sodium acetate buffer(PH=5.5)
2. 0.02M H<sub>2</sub>O<sub>2</sub>
3. 0.05M Guaiacol

The reagents were mixed with distilled water in ratio (1:1:1:7), the peroxidase assay was carried out by placing 6 ml of the mixture in the cell of the spectrophotometer and absorbance was measured after 3 min., the activity was calculated using Sadasivam & Manickam equation [10]. Unit definition

One unit of enzyme activity is defined as the amount of enzyme which catalysis the conversion of one micromole of Guaiacol per under assay conditions.

#### Protein estimation

Protein contents of the enzyme extract at all steps were measured by Moss and Bond [11].

#### Purification of peroxidase

##### Ammonium Sulphate precipitation

The enzyme was precipitated by Ammonium Sulphate for partial purification at the concentration of 80% (w/v), as described by Bentely (1962) [12]

#### Dialysis

Before dialysis procedure, the dialysis bag was boiled with 0.1 M sodium carbonate solution for about 1 h and then retained overnight. By this procedure, the dialysis bag was opened. The precipitates obtained by ammonium sulphate, then dialyzed in dialysis bag with 0.2 M phosphate buffer (pH 6.5) with constant stirring on magnetic stirrer for 2 h. The precipitates were subjected to enzyme assay and protein estimation. Normally, dialysis was completed after ammonium sulphate precipitation.

#### Gel filtration chromatography using Sephadex G-75

The reagents used were 0.2 M phosphate buffer (pH 6.5), Sephadex G-75 and 0.5% dextrin blue. Sephadex G-75 was soaked in 500 ml phosphate buffer (pH 6.5) containing 0.1 g of sodium azide and was incubated at room temperature for 24 h.

After soaking, the gel was degassed by direct drive rotary vacuum pump and then was poured into the column. The packed column was washed with 0.2 M phosphate buffer (pH 6.5). Dextrin blue (0.5% w/v) was washed for the determination of its void volume. Dialysed extract was applied on the column and fractions each of 3 ml were collected. Each fraction was then assayed for enzyme activity and total protein. Sephadex G-75 column for gel filtration chromatography out of a total of 20 fractions, 5 have the maximum activity of 9.246 U/ml.

**Table 1: Purification of Horseradish Peroxidase**

Sample	Activity (U/mL)	Protein Contents (mg/mL)	Specific Activity (U/mg)	Purification Fold
Crude	5.211	6.255	0.8331	1.00
Ammon. Sulph. Precip.	5.844	1.778	3.2868	3.94
Dialysis	6.774	0.588	11.5204	13.82
After Gel Filtration	9.246	0.374	24.7219	29.67

### Results and Discussion

Horseradish peroxidase was extracted by blending it, the activity and specific activity of crude extract obtained were 5.211U/mL and 0.8331U/mg respectively. To purify the enzyme, the crude extract was subjected to 80% saturation with  $(\text{NH}_4)_2\text{SO}_4$  to remove unwanted proteins. It was shown that the activity was increased to 5.844 U/ml and 3.2868 U/mg specific activities by  $(\text{NH}_4)_2\text{SO}_4$  precipitation. The protein contents were decreased from 6.255 mg/ml of crude extract to 1.778 mg/ml which indicate that unwanted proteins have been removed. Most of the investigators recorded that peroxidase is fractionally precipitated by salting out with ammonium sulphate. Favzana Alyas [13] recorded that peroxidase extracted from soybean seeds was precipitated by using ammonium sulphate at a concentration between 50%-85% saturations with fold of purification about 3.58 and specific activity 5.68(U/mg). Horseradish peroxidase in Pakistan [14] was fractionally precipitated by using ammonium sulphate at a concentration between 20%-80% saturation with fold of purification about 14.8 and specific activity 12.77(U/mg). The peroxidase from tomato [15] was partially purified by using ammonium sulphate at a concentration between 50%-90% saturation with specific activity 0.546(U/mg). After the precipitation step of peroxidase by ammonium sulphate, the enzyme was concentrated using dialysis tube. This purification step increases the specific activity from 3.2868 (U/mg) to 11.5204(U/mg) and fold of purification increased up to 13.82 fold.

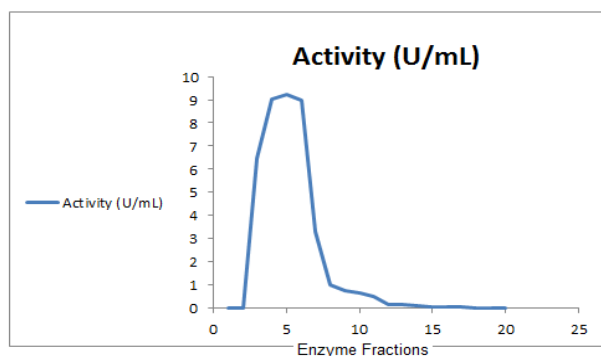


Figure 1: Activity versus enzyme fractions

The enzyme fraction having highest activity after dialysis was passed through Sephadex G-75 column for gel filtration chromatography. The maximum activity of 9.246 U/ml was obtained in the 5th fraction during the experiment, with 0.374

mg/ml of protein contents and 24.7219 U/mg of specific activity.

Table 1 shows the summary of peroxidase purification from local horseradish, while Figures 1, 2 and 3 shows the results curves which indicate the horseradish peroxidase enzyme was purified up to 29.67 fold.

It is concluded that horseradish is good source of peroxidase enzyme whose activity and production in commercial scale can be enhanced by improving the purification techniques.

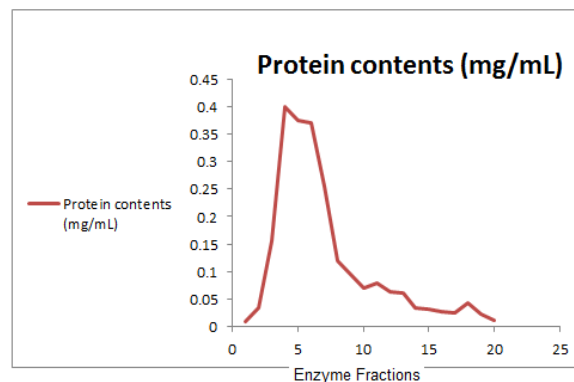


Figure 2: Protein contents versus enzyme fractions

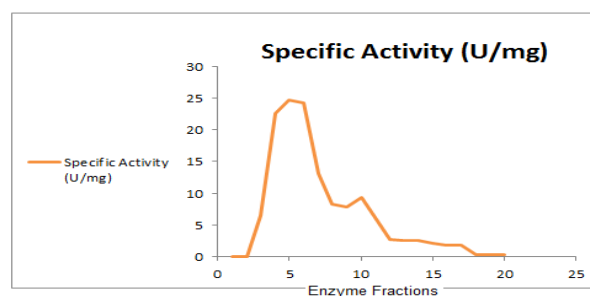


Figure 3: Specific activity versus enzyme fractions

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