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Research Article

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Immobilization of plant protease using calcium alginate beads

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ABSTRACT

Proteases are the most important group of enzymes from a biotechnological perspective. They have a wide variety of applications in many fields. In this study, protease was isolated from Nicotiana tabacum, Moringa oleifera, Murraya koenigii and Coriandrum sativum .This extracted proteases were immobilized on calcium alginate beads and the specific activities of the immobilized enzyme were estimated and compared. It was found that immobilized protease activity was more in Nicotiana tabacum (4.40 units/mg of protein) followed by Moringa oleifera (2.46 units/mg of protein), Murraya koenigii (2.02 units/mg of protein) and was less in Coriandrum sativum (1.56 units/mg of protein). The optimum pH was also determined and was found to be 7.2 for all the species. The activity with respect to time of production of these samples were also analyzed. For Murraya koenigii the activity was high at 10th min and in case of Moringa oleifera and Nicotiana tabacum, it was at 15th min. Coriandrum sativum had highest activity at the 20th min.

Keywords: Protease- Immobilization- *Nicotiana tabacum -Moringa oleifera- Murraya koenigii - Coriandrum sativum*- Specific activity.

INTRODUCTION

Enzymes are biocatalyst used to increase the rate of the reaction. Varieties of enzymes are available in enzyme market. Among which proteases are the most essential group. They account for about 65% of world wide enzyme sale [1]. Protease are made up of complex group of enzymes and they differ in some of their properties such as substrate specificity, catalytic mechanism, temperature and pH [2]. Protease have wide applications in industrial process such as Laundry detergents, food, pharma, chemical, leather and silk, protein recovery, degradation of gelatin on X-ray films and organic synthesis[1][3][4]. They are also used in bioremediation processes [5].

Generally proteases are widely distributed in microorganisms especially bacteria and fungi and Algae [6]. Among various proteases, bacterial proteases are the most significant compared with animal and fungal proteases [7]. Plants are the rich source of protease enzyme. Green berg and Winnick [8] listed eleven plant proteases. Agro waste may also be used for the production of protease enzyme [9].

Enzyme immobilization has attracted a wide range of interest from fundamental academic research to many applications [10]. The basic idea of this immobilization is to entrap protein in a semipermeable support material, which prevents the enzyme from leaving while allowing substrates, products and cofactors to pass through [11]. The matrix should be nondegradable and compatible with the enzymes and process should be mild enough so as not to keep the enzyme stable during preparation. When an immobilized enzyme is used in vivo, the support material should also prevent immune recognition especially if the enzyme is of non-human origin [12].

Alginate is the most widely used polymer matrix for immobilization and micro encapsulation technologies[13][14]. Alginate is a seaweed extract composed of chains of alternating α-L guluronic acid and β-D mannuronic acid residues [15]. It is made by cross linking the carboxyl group of α-L guluronic acid with a solution of cationic cross linker such as calcium chloride, barium chloride [16][17].

In this study dried leaves of *Nicotiana tabacum* and green leaves of *Moringa oleifera, Murraya koenigii* and *Coriandrum sativum* were selected to isolate protease enzyme. These proteases were partially purified and were immobilized in sodium alginate beads. Then their specific activity, time taken for maximum activity and optimum pH were studied.

EXPERIMENTAL SECTION

1).Extraction of enzyme
The samples were homogenized using mortar and pestle with phosphate buffer pH 7.0 and was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and was subjected to further purification.

2). Partial purification of enzyme
The protein from the crude enzyme was precipitated using 70% ammonium sulphate. Then it was fed into sephadex column for desalting.

3). Estimation of protein concentration
The protein concentrations of the samples were analyzed by Bradford method [18].

4). Immobilzed Protease activity
The partially purified enzyme solution (0.5 ml) was mixed with 1ml of sodium alginate. The mixture was pipetted out into calcium chloride using sterile syringe. Beads were formed and were counted. These beads containing enzyme were mixed with 0.5ml casein and 10%TCA was added to it. Then it was centrifuged and the supernatant was taken. The supernatant was mixed with protease reagent and folins phenol reagent and OD was taken at 650nm. Activity was checked for every 5 min to find out the time for maximum activity.

5). Estimation of pH
The optimum pH for the protease was checked out using phosphate buffer at pH 5.7, 6.2, 6.7 and 7.2.

RESULTS AND DISCUSSION

Enzyme immobilization is used to prevent enzymes leaving while allowing substrate and products to pass through. In this study, protease was isolated from the leaves of *Nicotiana tobacum, Moringa oleifera, Murraya koenigii* and *Coriandrum sativum* and was partially purified. After purification protein concentration was estimated. The protease was then immobilized on sodium alginate matrix and their specific activity were compared.

Table.1 Specific activity of immobilized protease

S.No	Sample		Specific Activity(units/mg of proteins)	Effectiveness Factor
1.	*Nicotiana tabacum*	Free	5.6	1
		Immobilized	4.40	0.79
2.	*Moringa oleifera*	Free	4.27	1
		Immobilized	2.46	0.58
3.	*Murraya koenigii*	Free	3.32	1
		Immobilized	2.04	0.614
4.	*Coriandrum sativum*	Free	2.47	1
		Immobilized	1.56	0.63

Table.1 shows the maximum specific activity of immobilized protease and free enzyme [19] from the four plants analysed. The maximum activity was shown by *Nicotiana tabacum* (4.40 units/mg of protein) followed by *Moringa oleifera* (2.46 units/mg of protein), *Murraya koenigii* (2.04 units/mg of protein) and finally less in *Coriandrum sativum* (1.56 units/mg of protein). The immobilized protease activity was slightly decresed when compared to the free enzyme in all four plants analyzed. The decreased enzyme activity might be due to the immobilization of

enzyme in calcium alginate beads. The result is in accordance with previous reports that the activity of immobilized enzyme was reduced in the calcium alginate beads due to interaction between protein and calcium ions [20][21].

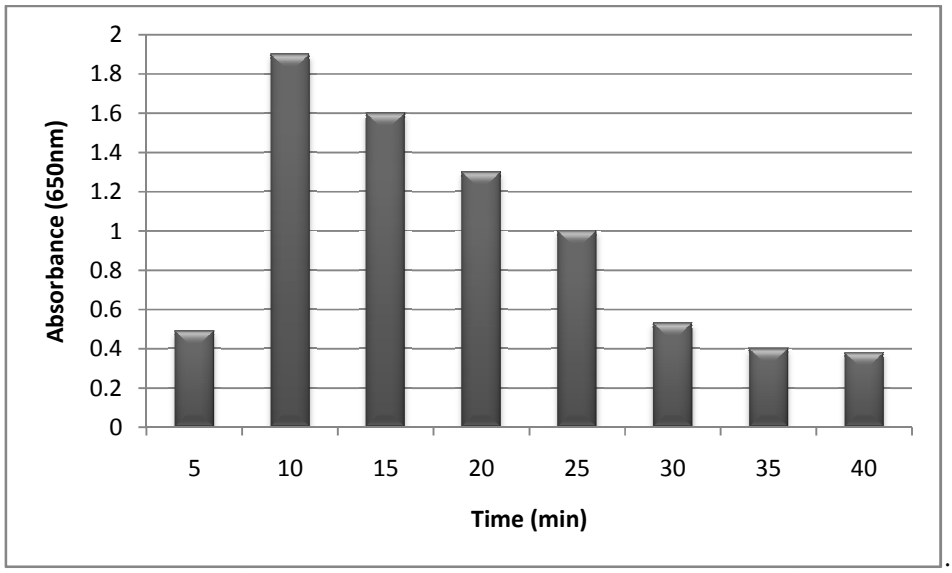

Fig.1 Immobilized protease activity of *Murraya koenigii*

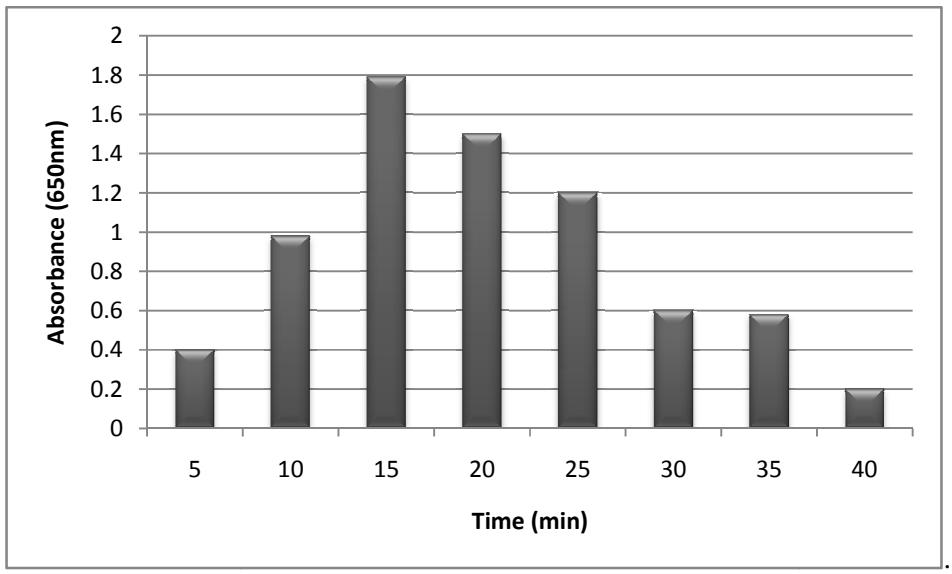

Fig.2 Immobilized protease activity of *Moringa oleifera*

Fig.1 shows that the immobilized protease activity of *Murraya koenigii* was maximum at 10th min where as for *Moringa oleifera* at 15th min (Fig.2). *Coriandrum sativum* showed highest activity at 20th min (Fig.3). Fig.4 showed that the highest activity for *Nicotiana tobacum* at 15th min.

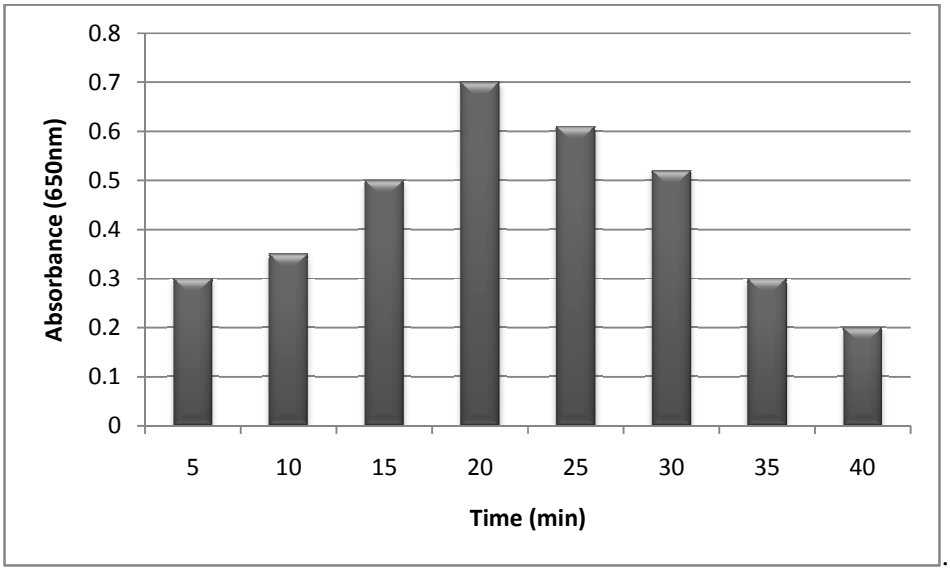

Fig.3 Immobilized protease activity of *Coriandrum sativum*

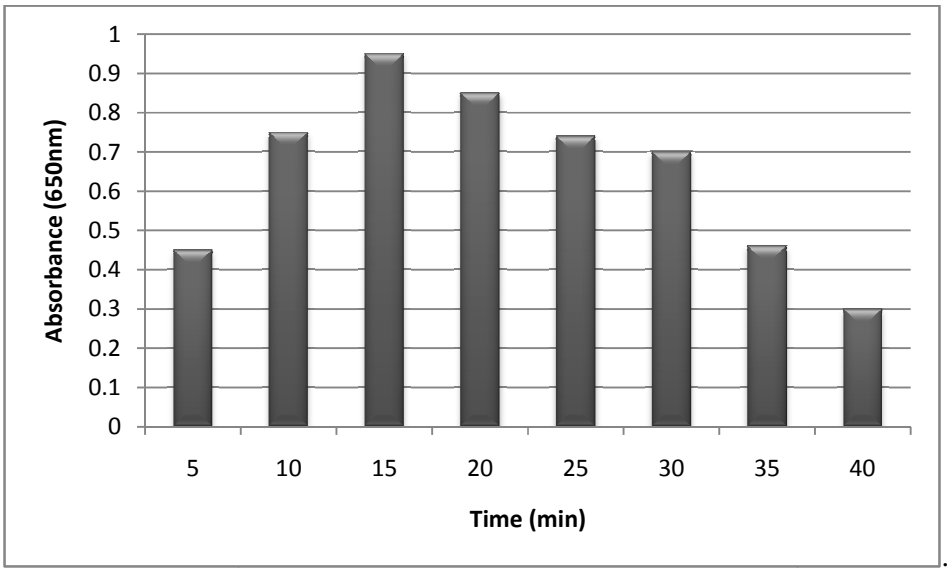

Fig.4 Immobilized protease activity of *Nicotiana tabacum*

Estimation of pH

The optimum pH was checked out for all the four immobilized protease samples at four different pH and it was found that 7.2 is the optimum pH for all the samples.

CONCLUSION

This study illustrates a method for preparing immobilized protease isolated from plant leaves such that the biological agent is protected in the inner biocompatible alginate core and its pH was optimized. In future, other parameters of immobilized protease can also be investigated for maximum production at a cheaper rate.

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