Performance Of Protease From Paddy Oats Seed Peel (Gnetum Gnemon) And Its Potential As Detergent Additive

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Abstract: Increasing demand of proteases with specific properties has lead biotechnologists to explore newer sources of proteases. Protease, enzyme that cleavage protein can be produced from microorganism, animal and plant. Several protease derived from plants, have been widely used in many industries. Although many proteases have been isolated from plants, there is no previous study of protease from paddy oats. Paddy oats seed is valuable agricultural commodity in some tropical countries, particularly in Indonesia and paddy oats seed peel is only agricultural waste. The industrial demand of protease enzymes, with appropriate specificity and stability to pH, temperature, metal ions, continues to stimulate the search for new enzyme sources. The aim of this research was to detect and to characterize protease in paddy oats seed peel as well as to evaluate its compatibility as detergent additive. To assess the effect of temperature and pH on protease activity, protease enzyme extract were examined at temperature 10, 20, 30, 40, 50, 60 and 70^{o}C, and pH range from 3 to 9. To evaluate the compatability as detergent additive, used detergent available in the local market. To simulate washing conditions, the detergent was diluted in distilled water up to final concentration of 2 mg.mL^{-1}. The solution was boiled for 10 minutes for the inactivation of their protease enzyme contents already present and cooled. Crude extract of protease was added to the detergent solution. The result showed optimum temperature of protease enzyme extract was 40-55^{o}C; optimum pH 6.5-9.0; and blood stain on the fabric could be removed perfectly by detergent-protease enzyme solution in 95 minutes. This study showed that protease enzyme extracted from paddy oats seed peel hold potential as a new source of protease enzyme for biotechnological applications, in particular as detergent additive.

Index Terms: Paddy oats, protease enzyme, plant protease, detergent additive

1 Introduction

Proteases, widely distributed in all living organism, are essential enzymes to the metabolic and regulatory functions such as digestion, development, defense, and apoptosis, and are also involved in pathological disorders. Protease is one of the most important group of industrial enzymes and account for nearly 60% of total enzyme sale. Among the world sale of industrial enzyme of about US$ 300-600 million per annum, 75% of these are hydrolitytic enzyme, of which two-third are protease enzymes [1]. Enzyme market reach US $ 4.4 billion by 2015 [2]. The major uses of protease enzyme occur in dry cleaning, detergents, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation, and virulent wounds. Protease has some interesting characteristics for biotechnology because it has become the most important industrial enzymes. Properties characteristic of plant protease enzyme is also advantageous for applications in the industrial field. Virtually, all plant native proteases remain stable at high temperatures, and stable in a wide pH range. Plants have enzymes with a variety of active sites; however the active sites a specialized to carry out specific activities. The active sites have the best function under its optimum pH, optimum temperature, and substrate specificity.

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On the basis of their acid-base behavior, proteases are classified into three categories mainly acid, neutral, and alkaline proteases [3]. Papain, bromelin, and ficin are the most popular protease enzymes extracted from plants, however, there are many constraints and difficulties for producing them. Many researchers have explored new sources of plant protease enzyme. Protease was isolated from Chaya leaves [4]. Some researcher isolated oryzasin from rice seeds [5]. In dairy field, paper reported that protease extracted from Cyanara L for cheese making coagulant [6]. Protease was also isolated and characterized from sprouts of Pleiobastus hindsii Nakai and roots of sweet potato respectively [7]. The application of protease from Cucumis trigonus Roxb for meat tenderization could increase tenderness of meat [8]. Although many proteases have been isolated from plant latex, fruit, leaves, and seed, there is no information yet that protease enzyme has been from paddy oats (Gnetum gnemon) seed peel. Observation in paddy oats processing workers has led to speculation that paddy oats may contain protease enzyme. It was observed that the fingers of paddy oats processing workers suffered from exfoliation. This evidence suggests the existence of a high activity of protease enzyme in paddy oats seed peel. To the best of our knowledge, this is the first study that investigates the presence of protease in paddy oats seed peel. Thus, further studies on purification and characterization of proteases found in paddy oats seed peel are required in order to elucidate functions and define possible biotechnological applications. In this paper, extract protease of paddy oats seed peel was created as detergent additive.

2 Materials and Method

2.1 Optimum Temperature

To determine optimum temperature of protease extracts of paddy oats seed peel as described in the following steps: The protease extracts were tested at different temperature ranging from 10 to 70^{o}C for 30 minutes at pH 7. After incubation and TCA precipitation, then filtered using Whatman no 1. The

supernatant was measured its optical density (OD) with spectrometer UV at 280 nm [9].

2.2 Optimum pH

To determine optimum pH of protease extracts of paddy oats seed peel, the protease was incubated in different pH. Protease extracts were mixed in substrate solution in the following buffers: 100 mmol.L^{-1} sodium citrate, pH 2-4; 100 mmol.L^{-1} sodium acetate, pH 4.5-5.5; 100 mmol.L^{-1} sodium phosphate, pH 6-8; and 100 mmol.L^{-1} sodium carbonate, pH 8.5-11. The experiment to determine optimum pH of protease activity was repeat three times and average values were taken [10].

2.3 Evaluation of Washing Performance

Clean cotton cloth pieces (5x5cm^2) were stained with blood 50 micro Liter. The stained cloth pieces were subjected to wash treatments with commercial solid detergent dilute in tap water, supplemented with and without protease enzyme. Each flask was incubated at room temperature. Data is time needed to remove blood stain completely. Visual examination of various pieces showed the effect of protease enzyme of paddy oats seed peel in the removal of stains [1] and [11].

2.4 Detergent Compatibility

The compatibility of the protease enzyme with commercially available solid laundry detergents was studied using detergents those are available in Taiwan. Commercial detergents were diluted in tap water to a final concentration of 7 mg/mL to simulate wash solution. The endogenous proteases contained in these detergents were inactivated by heating the diluted detergents for 1 h at 90^{0}C prior to the addition of the enzyme preparation. The protease enzyme was incubated with different detergents for 1 hour at 40^{0}C and then the remaining activities were determined under standard assay conditions. The enzyme activity of a control, without detergent, incubated under similar conditions, was taken as 100% activity [12].

3 Results and Discussion

3.1 Optimum Temperature

Protease enzyme extracted from paddy oats seed peel was evaluated between temperature experiment range 10 and 70^{o}C. Protease enzyme activity increased rapidly by increasing of temperature, reaching a maximum at 40^{o}C. The protease enzyme activity at this point was 63.4x10^{-2}µmol tir.ml^{-1}.min^{-1}, and this activity is not significant different from those activities at temperature 35 and 45^{o}C, indicating that protease enzyme extracted from paddy oats seed peel has a wide temperature range activity. At the optimum temperature, activity of enzyme reaches the highest performance, because the optimum temperature is the best temperature for the active side of enzyme to breakdown substrate. Mostly, protease enzyme from plants resistance to high temperature, and remain active in a broad temperature range, as well as optimum temperature of plant protease enzyme is relatively high. Optimum temperature of serine protease extracted from Cucumis trigonus Rxburghi was reported of 70^{o}C, and the protease activity rapidly decreased as the reaction temperature increase higher than 80^{o}C due to thermal denaturation of the protein of enzyme [13].

3.2 Optimum pH

The result shows that the protease enzyme activity at pH 6.5 was the highest (65,8x10^{-2}µmol tir.ml^{-1}.min^{-1}), however it was not different (p=0.05) from the activity of the enzyme at pH 8 64.7x10^{-2}µmol tir.ml^{-1}.min^{-1}. Protease enzyme extracted from paddy oats seed peel has high activity in a broad range of pH 6.5-8 and the activity decrease sharply at pH under 6.5 and above pH 8. The optimum pH of each enzyme varies depending on the constituent amino acids of the enzyme protein. Protease enzyme activity is affected by the conformation of the active site of the enzyme, whereas the conformation of the active site of the enzyme is affected by the concentration of H+ ions. At the optimal pH conditions, enzyme has highest enzyme activity, because the correspondence between the conformation of the active site of the enzyme with its substrate. Several previous studies reported that protease enzymes extracted from the plant in general is kind of like neutral protease: Protease from chaya leaves has optimum pH around neutral [4]; However, another paper reported the type of enzymes such as acid enzymes extracted from rice seeds [5]. Our study demonstrates that protease enzyme extracted from paddy oats seed peel was stable over a wide range of pH (6.5–8) and the stability profile highlight the suitability of this protease for possible application in industrial process particularly in neutral condition.

3.3 Evaluation of Washing Performance

Stain removal ability of protease of paddy oats seed peel was assayed using cotton cloths stained with blood. Clean cotton cloth pieces (5x5 cm) were soiled with blood 50 micro Liter. The stained cloth pieces were subjected to wash treatments i.e. water, 0.7 % detergent solution, protease solution, detergent + protease solution. Data in this research is the time is needed to remove blood stain completely. The shorter time is needed, the better washing performance of the treatment. Figure 2 shows that the visual examination of cloths stained with blood, after washing process in deferent solution accordance to the treatment, in room temperature. Figure 1 shows the washing performance of protease enzyme extracted from paddy oats seed peel. Stain removal ability is significantly different among treatments. The stain cloths pieces were subjected treatment detergent+enzyme need just only 95 minutes for removing all of blood stain from the cloths perfectly. While the cloths stained blood subjected at water and enzyme solution could not remove all blood stain perfectly up to the observation was stopped at 480th minute. Further, as shown in Figure 1, limited washing performance was observed with detergent only, that need time 115 minutes, however the treatment of detergent supplemented with protease enzyme that extracted from paddy oats seed peel gave a better stain removal. This finding is different from previous works reported by Hmidet, who showed that protease enzyme from Bacillus sp, could remove blood stain from cotton cloth both in the presence or absence of detergent [11]. However, the protease enzyme from paddy oat seed peel has potential as additive in detergent, despite of it has failed to show its ability in stain removal, when that protease enzyme work alone without detergent. Blood contain not only protein but also fat, sugar etc. and protease enzyme works only on protein, like owing this is the main reason why the treatment protease enzyme without detergent could not show the exquisite washing performance.

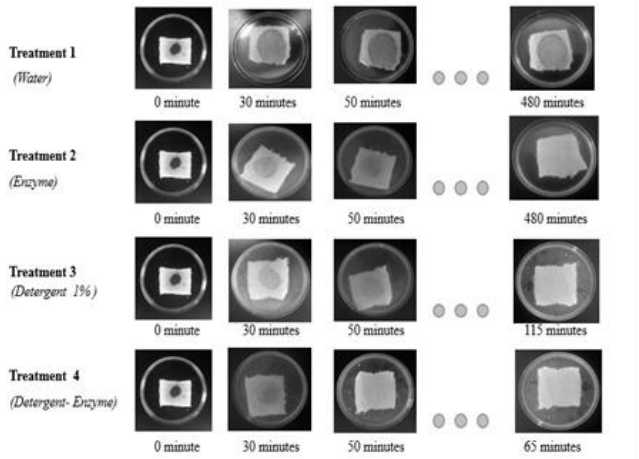

Fig. 1. *Protease enzyme from paddy oats seed peel washing performance.*

3.4 Detergent Compatibility

Many parameters are involved in selecting a protease for detergents, such as compatibility with detergent components, e.g. surfactant, perfumes and bleaches. Protease as detergent additive should be stable and active in detergent. Stability of protease extracted from paddy oats seed peel is around 40% shown in figure 2. Protease stability in the presence of detergents varies according to the source of enzyme and the type of detergent (detergent 1, detergent 2 and detergent 3 are different "famous detergent brand" in Taiwan market). A paper reported that, enzyme from the fungi Conidiobolus coronatus and Nacardiopsis sp, retained 64% and 90% of their activities, respectively [14] whereas protease from the bacteria Bacillus cereus retained more than 60% activity [15].

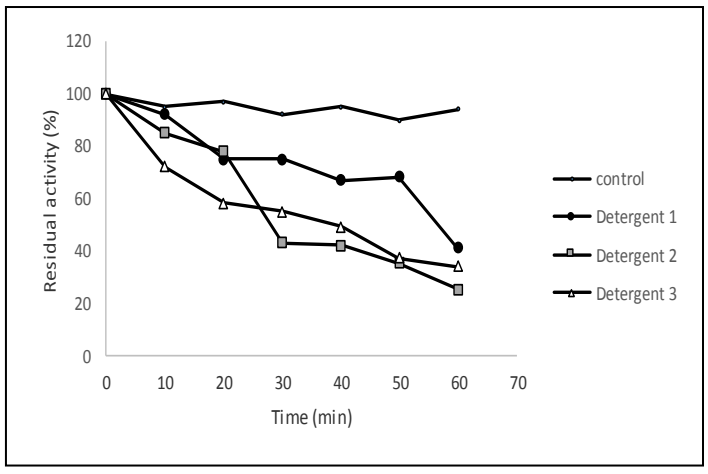

Fig. 2. *The stability of protease from paddy oats seed peel in different detergent solution.*

4 Conclusion

The results obtained in this experiment clearly indicate the paddy oats seed peel contains protease enzyme, that can be used as detergent additive. Protease enzyme solution and detergent 1% with ratio 1:1 removed perfectly blood stain on the fabric in 95 minutes. The optimum temperature of protease enzyme is 35-45^{o}C, pH 6.5-8, and has different stability to different detergent mixture; perhaps it is obstacle the use of this protease enzymes for detergent additives. Its high activity in around temperature 45^{o}C, it may have a potential application in food industry as well as leather industry.

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