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Effect of Temperature and Fermentation Duration on Protease Enzyme by *Aspergilus niger*

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Abstract
The effects of Temperature and duration of fermentation on protease enzyme by *Aspergilus niger* were investigated in this work. Different Temperature were used as to test the best one to produce the protease enzyme from this isolate. Also different duration of fermentation were test. Temperature increase from 25 to 30 °C and also won an award in both enzymatic activity and specific activity at temperature rises above 30°C. The best duration of fermentation was at 21 days.

Keywords: proteolytic activity; Wheat bran; rice hulls, enzyme activity

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1. Introduction

Microorganisms are an ideal source for enzymes production that are reproductive very quickly and in a short time [1]. Protease is one of the most Worthwhile enzymes for biotechnology and its global market has been growing significantly. Proteases are the efficient executioners of a common chemical reaction: the hydrolysis of peptide bonds[2]. Protease is obtained mainly from *Aspergillus* and *Penicillium*. The different hydration rates were used in material fermentation Solid, the humidity effect on protease production of *A. flavus* was ranged between 35-80% while the effect on *A. oryzae* was at 50%[3]. The wheat bran medium is better than soybean medium in production protease enzyme by *A. flavus*. It gives a productivity higher than that of soybeans by approximately a half times[4]. Also It was found that wheat bran medium is in development protease production by *A. oryzae* was the best among solid fermentation media[5]. This study evaluate the effect of agro-waste for potential protease product by *Aspergilus niger*.

2. Experimental Part

Aspergillus niger was used to produce protease. Two waste materials were used: bran and rice hulls as basic materials for the production of protease enzyme. Available solids of 10 gm/flask were used. The above-mentioned solids are moistened using them Including phosphate at a concentration of 0.2 molar and no pH 7, with a hydration ratio of 1:5) by volume /weight (by adding 50 ml of buffer Phosphate to 10 grams of solid and binder Wet the oil with solids and sterilize the flasks It is sealed with a temperature resistance of 121 °C and a pressure of 15psi for 15 minutes. The flasks were inoculated with the suspension Spores: 610 spores/10 gm and incubated for 72°C. Murachi method [6] was applied to evaluate Protease using casein %0.0 with different types of basic materials and defines the unit Enzymatic: It is the amount of enzyme that is amplified in Optical wavelength at wavelength 220 nanometers per minute under standard conditions. Protein concentration can also be measured by strength loss According to the method described by Bradford[7].

3. Optimal conditions of protease production

Determine the optimal conditions for protease production in the medium Solid fermentation. A study was conducted that affects some factors as a percentage Hydration and nutritional value of the medium vary Temperature and duration of fermentation affect the production of protease by local isolation of *A. niger* at medium bran, 10 grams per flask And the use of phosphate buffer in humidification .All the circumstances referred to above were recorded in the judgment. The required factor has a message and its effect. For determine the optimal temperature for enzyme production, inoculate wheat bran medium with spore extraction 1×610 spore/10 gm is a very hot substance and was incubated at high temperatures various (25, 28, 30, 32 and 35) 72 hours, then the enzyme was extracted and estimated enzymatic activity.

4. Results and Discussion

For the purpose of study the efficiency of selected local isolate *A. niger* To produce the protease enzyme

using fermentation technology. When planting hardwood, waste of plants were used as a culture media to test the best solid fermentation medium to produce the protease enzyme from this isolate. Wheat bran and rice hulls A (solid media, separately). It turns out that the center of wheat bran is better fermentation medium for enzyme production compared to another medium. With wheat bran medium the enzymatic activity was at 1600 units/ml and effectiveness was at 1300 units/mg Protein. While the enzymatic activity with rice hulls medium was at 350 units/ml and effectiveness 290 units/mg protein. The reason for the superiority of wheat bran over the medium (rice hulls) was may be to Class A (for the high content of planets, as its protein content 14.62% compared while rice hulls contains 9.93 of protein. Also, the physical properties of materials that used in the fermentation of solid culture effects the production of various enzymes such as the size of particles. The surface area exposed to the action of living organisms Microstructure and porosity of the medium[8,9]. Previous study found that the wheat bran medium is better than soybean medium in production protease enzyme by *A. flavus*. It gives a productivity higher than that of soybeans by approximately a half times[4]. Also It was found that wheat bran medium is in development protease production by *A. oryzae* was the best among solid fermentation media[5].

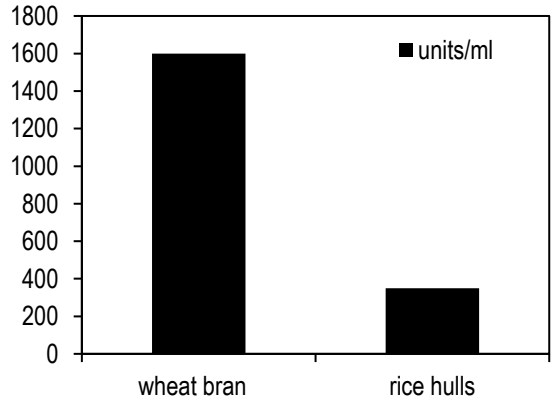

Fig. (1) Effect of formation media on protease enzyme activity

Protease enzyme productivity from *A. niger* elevated when temperature increase from 25 to 30 °C and also won an award in both enzymatic activity and specific activity at temperature rises above 30°C. For a long time The optimum temperature for enzyme production was 30°C, at enzymatic activity is 1610 units/ml and is effective 1500 units/mg protein and varies, changes in Both the enzymatic activity and specificity of the enzyme at Temperature rises above 30°C. The drop in temperature below the optimum level. It leads to slow growth and delayed enzyme synthesis. This temperature is suitable for mushroom growth. On the other hand Enzyme stability on the other hand, the optimum temperature for the production of protease enzyme. This study is 30 m in length, along with the results of other studies. It dealt with the production of protease enzymes by species where *Aspergillus* temperatures rise 30 °C. The optimum for the production of these enzymes between 28°C. These studies indicated a decrease in the effectiveness of the enzyme.

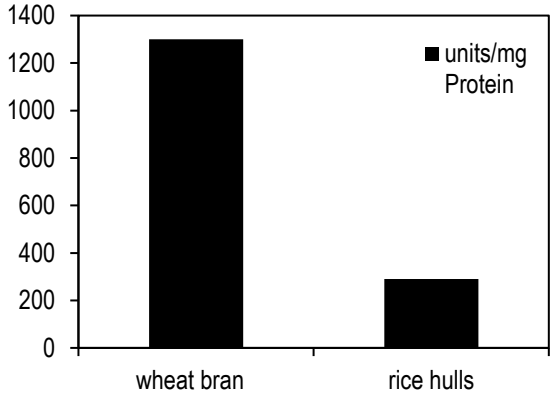

Fig. (2) Effect of formation media on protease enzyme activity

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