IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2022 Vol.:23, Issue:2

Production of Protease Enzyme by Using *Neolamarckia cadamba* in Solid-State Fermentation

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Submitted: 21 December 2021
Accepted: 26 December 2021
Published: 30 January 2022

Keywords: *Neolamarckia cadamba*, *Aspergillus awamori*, Solid-state fermentation

ABSTRACT

The present research work deals with the production of Protease enzyme using *Neolamarckia cadamba* leaves as substrate in solid-state fermentation by microorganism *Aspergillus awamori.* Proteases are a complex and large group of enzymes that plays an important role in nutritional and regulatory activities. Proteases are very important for the physiological functions of living organisms, they help in the breakdown of proteins and food materials into amino acids that can be used for energy, and also plays an active role in essential processes like blood clotting, cell division. Solid-state fermentation is explained as an activity that occurs on a non-soluble material that may act both as support and a source of nutrients, with a minimal amount of water under the process of fermentation. For the production of protease enzyme various parameters like incubation time, incubation temperature, pH, inoculum level, and moisture content were determined. The incubation time of 72hrs, the temperature of 30^{0}C, pH 5, inoculum level of 60%v/w, and moisture content of 70%v/w were observed optimum for the production of protease. Different carbon components were screened as a carbon source for their effect on enzyme production; they are glucose, fructose, sucrose, and lactose used as carbon supplements. For nitrogen source potassium nitrate was used and 0.3% w/w observed optimum for the enzyme production.

INTRODUCTION

Proteases are a complex and large group of enzymes that plays an important role in nutritional and regulatory activities. Proteases are very important for the physiological functions of living organisms, they help in the breakdown of proteins and food materials into amino acids that can be used for energy, and also plays an active role in essential processes like blood clotting, cell division. Microbial proteases are degenerating enzymes, which catalyze the total hydrolysis of proteins[1,2]. Protease enzymes are observed in a wide diversity of sources such as plants, animals, and microorganisms and they are mainly produced by various bacterial and fungal species. Protease enzymes are the most essential industrial enzymes that produce about 60% of the total enzymes market in the world[3,4]. Proteases enzyme exhibits various medical and pharmaceutical applications. Microbial proteases are produced extracellular and can be secreted in the fermentation medium[5,6]. Protease enzymes are predominantly used in food industries, meat processing, cheese making, detergents, leather, silver recovery from photographic film, production of digestive and also used in the treatments of inflammation and virulent wounds. Solid-state fermentation is determined as an activity that occurs with a minimal amount of water under the process of fermenting agent on a non-soluble material that may act both as support and a source of nutrients. Solid-state fermentation has more advantages than submerged fermentation because of superior volumetric productivity, use of simpler machinery, use of inexpensive substrates, simpler downstream processing, and lower energy requirements[7,8]. Microorganisms like bacteria and fungi produce a wide variety of proteolytic enzymes. They can potentially grow under various environmental conditions such as time, temperature, pH moisture content, etc, utilizing a wide variety of substrates as nutrients[9,10]. The environmental conditions of the fermentation medium play an avital role in the growth and development of microorganisms. Nutrients can influence the growth and development of the microorganisms either indirectly by affecting the availability of nutrients present in the medium or directly by acting on the cell surfaces[11]. The present research work deals with the optimization of process parameters for the production of protease from *Neolamarckia cadamba* using *Aspergillus awamori* under Solid-state fermentation.

MATERIAL AND METHODS:

Substrate: *Neolamarckia cadamba* leaves were collected from college ground and dried naturally and powdered, packed, and stored until further use.

Microorganism: *Aspergillus awamori* was used for the production of protease enzyme using *Neolamarckia cadamba* leaves as substrate. Potato dextrose agar medium was used for the maintenance and sub-culturing of the microorganism.

Preparation of Inoculum: Streaking is done from the old cultures of *Aspergillus awamori* pure potato dextrose agar slants and incubated at 30^0C for 3 days.

Development of Inoculum:10ml of sterile distilled water was added to the cells from 3 days old slant, from that 1ml of suspension containing approximately 10^5-10^6spores/ml was used as the inoculum to each flask.

Fermentation condition: Solid-state fermentation was carried out in 250ml conical flask containing 10g of substrate with 10ml of production medium (g/l) containing glucose 10g/l; peptone 5g/l; yeast 5g/l; K_2HPO_4 1g/l; $MgSO_4$ 2g/l; Na_2CO_3 10g/l; NaCl 5g/l; $FeSO_4$ 1g/l. The inoculum was placed in the production medium and incubated with continuous shaking. Shaker fermentations were carried out at 36^0c with controlled agitation at 150-200 rpm. At the end of the fermentation period, the whole culture broth was centrifuged at 1000rpm for 1 hour to remove debris; the supernatant was collected and used for further experiments.

Determination of Enzyme Activity:

Enzyme assay: Protease activity of culture filtrate was determined by a colorimetric method using casein as the substrate. 0.5mL enzyme solution was added to 3.0mL casein solution (0.6% w/v casein solution prepared in 20mM Borax-NaOH buffer, (pH 10) and allowed to react for 10 min at 36°C. The reaction was terminated by the addition of a 3.2mL stopper solution trichloroacetic acid mixture. The reaction mixture was then kept for 10 min the absorbance was measured at 280nm and related to the protease activity. The effect of various factors like incubation time, temperature, inoculum size, pH, moisture content, carbon source, and nitrogen sources on the production of protease was studied.

RESULTS AND DISCUSSION:

Proteases enzyme exhibits various medical and pharmaceutical applications. Protease enzymes are predominantly used in food industries, meat processing, cheese making, detergents, leather, silver recovery from photographic film, production of digestive and also used in the treatments of inflammation and virulent wounds. Microbial proteases are degenerating enzymes, which catalyze the total hydrolysis of proteins. Microbial proteases

are produced extracellularly and can be secreted in the fermentation medium. To optimize the effect of time on protease enzyme production, the medium was incubated at different time intervals and the maximum protease activity was noted at 72hrs. After 72hrs, production was decreased due to the depletion of nutrient materials. Protease production at different time intervals is shown in fig.1.

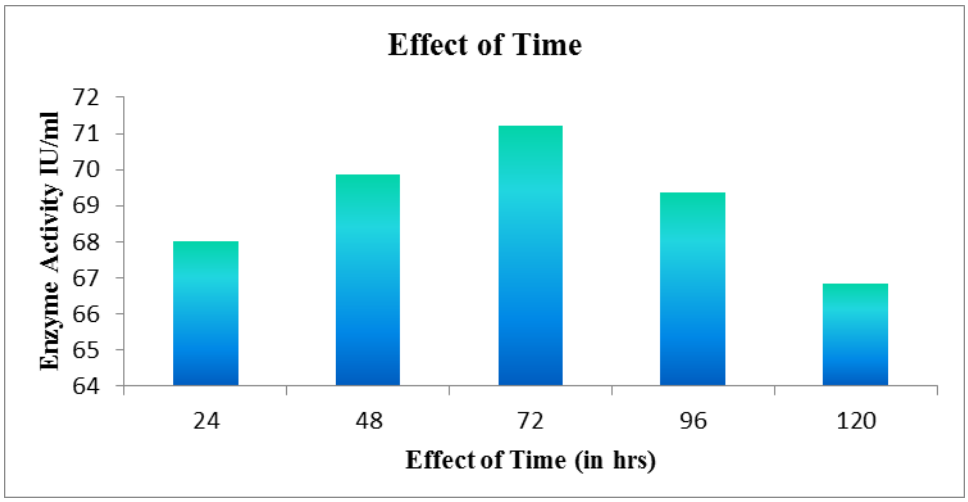

Figure no.1 Effect of time on enzyme production

The temperature plays a critical role in solid-state fermentation as it ultimately influences the growth of the microorganism. The maximum yield of protease was observed at 30^0c temperature Fig.2.

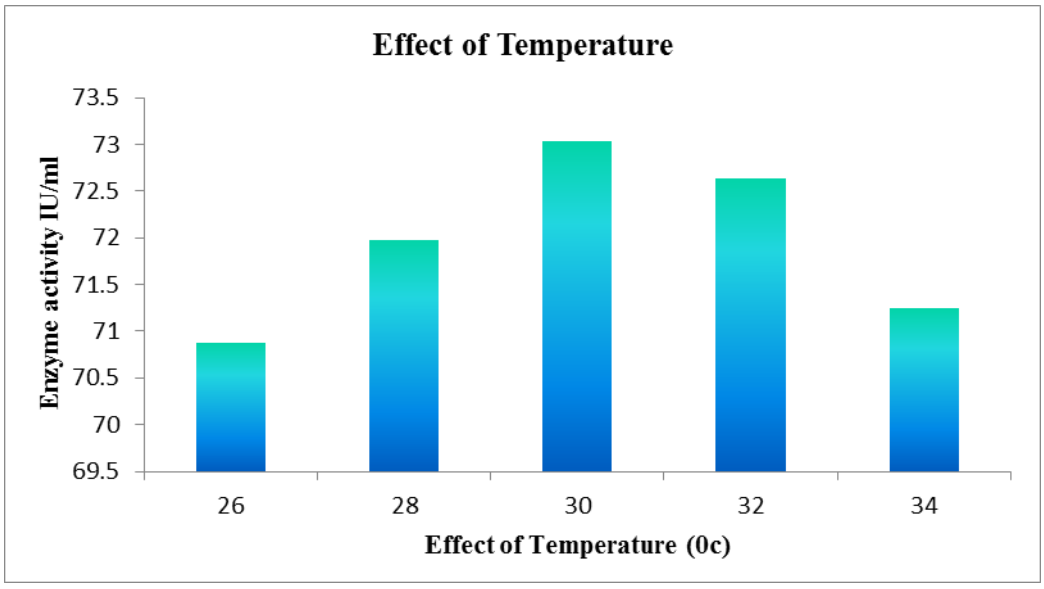

Figure no.2. Effect of temperature on enzyme production

To determine the effect of pH, the bacterial nutrient medium was made with different pH ranges 3, 4, 5, 6, and 7. The maximum yield of protease production was observed at pH 5 fig.3.

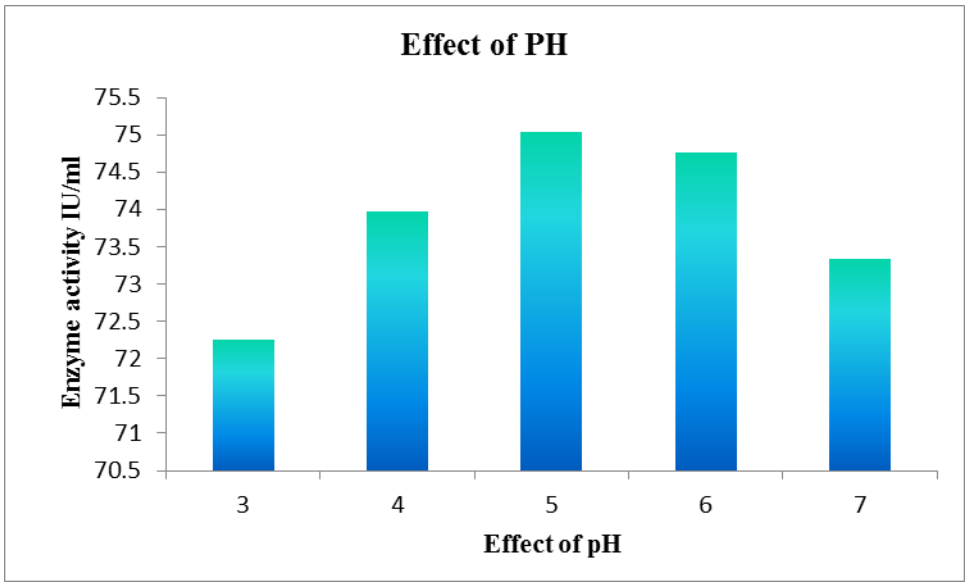

Figure no.3. Effect of pH on enzyme production

Different inoculum levels were made for the production of protease enzyme 40%, 50%, 60%, 70%, 80% v/w. The maximum enzyme yield was observed at 60% v/w of inoculum fig.4.

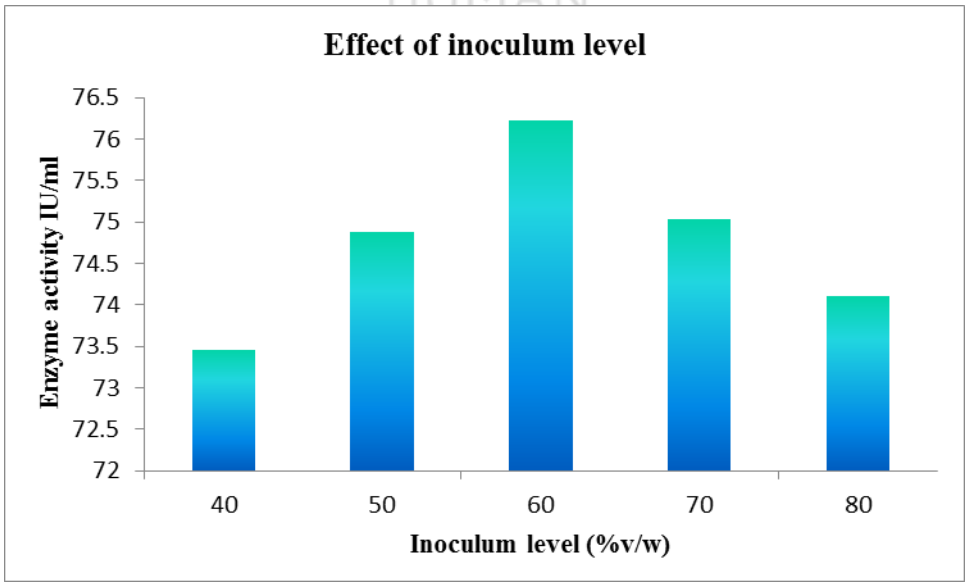

Figure no.4. Effect of inoculum level on enzyme production

Moisture content plays an important role in the production of enzymes in solid-state fermentation. High moisture content shows decreased substrate porosity, which prevents

oxygen penetration that causes bacterial contamination. Different moisture content 40%, 50%, 60%, 70%, 80%, 90%, and 100% v/w were optimized. The maximum enzyme activity was observed at 70% v/w of the moisture content fig.5.

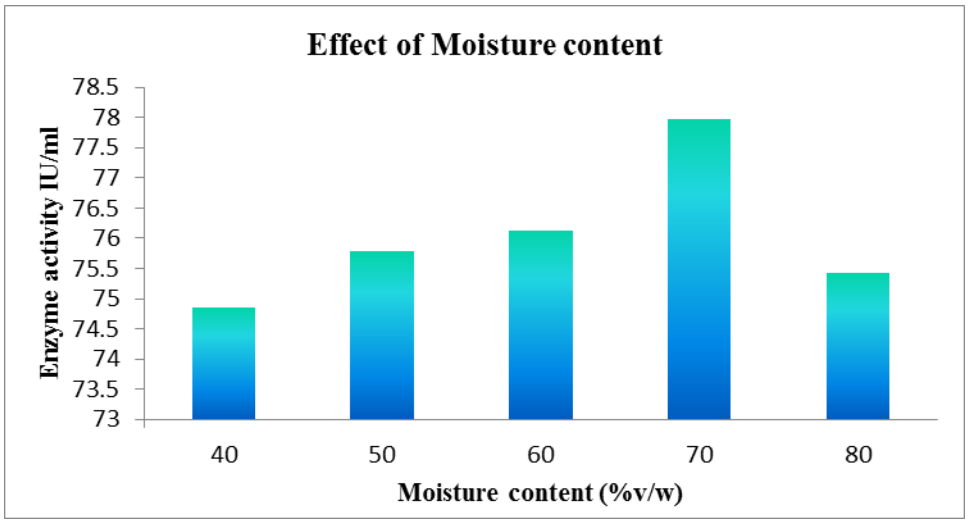

Figure no.5. Effect of moisture content on enzyme production

Different carbon sources were screened for the production of protease enzyme which includes sucrose, glucose, fructose, and lactose. The results showed that maltose supplementation gave marginally improved protease enzyme production than other supplementations. Production medium was made with different concentrations of sucrose like 1, 2, 3, 4, 5, and 6 %w/w. The result shows that maximum enzyme production was recorded at 4% w/w of maltose concentration fig.6.

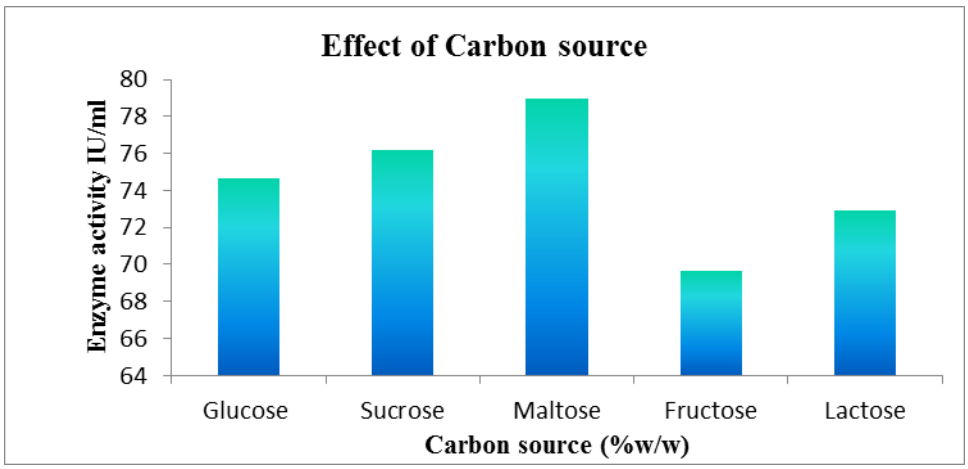

Figure no.6. Effect of carbon source on enzyme production

To determine the influence of nitrogen source on protease enzyme production, the production medium was prepared with different concentrations of potassium nitrate like 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%w/w were optimized. The result indicates that maximum enzyme production was recorded at 0.3% w/w concentration fig.7.

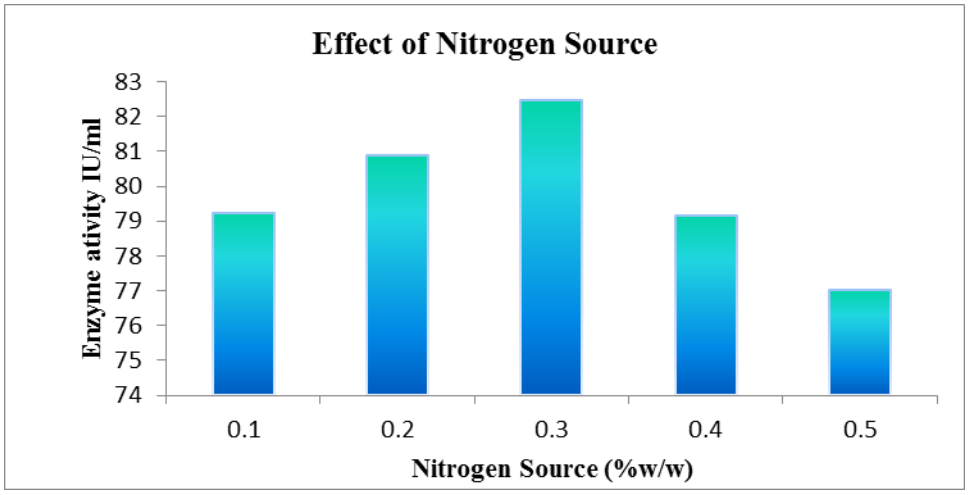

Figure no.7. Effect of nitrogen source on enzyme production

CONCLUSION:

Finally, we concluded that *Aspergillus awamori* is a promising microorganism, giving a significant yield when the carbon and nitrogen sources are added to the basal medium(82.46IU/ml) using *Neolamarckia cadamba* leaves under solid-state fermentation. As *Neolamarckia cadamba* is a low-cost substrate, easily available raw material and showing suitability for solid-state cultivation of microorganisms, the lab-scale study on protease production from *Neolamarckia cadamba* leaves as a major substrate might give the basic information of further development for large scale protease production.

ACKNOWLEDGMENT

The authors express their sincere thanks to the management, Mother Teresa Pharmacy College, Sathupally, Telangana for providing the necessary facilities to carry out the research work.

REFERENCES:

1. Haq I, Mukhtar ZA and Riaz N. Protease biosynthesis by a mutant strain of Penicillium griseoroseum and cheese formation.Pakistan Journal Biological Science. 2004; 7: 1473–1476.
2. Raju K, Jaya R. and Ayyanna C. Hydrolysis of casein by Bajara protease importance. Biotechnology Coming Decade. 1994; 181: 55–70.

3. Chouyyok W, Wongmongkol N, Siwarungson N. and Prichnont S.Extraction of alkaline protease using an aqueous two-phase system from cell-free Bacillus subtilis TISTR 25 fermentation broth. Process Biochemistry. 2005; 40: 3514–3518.
4. Godfrey T. and West S. Industrial Enzymology, 1996; 2nd ed., Macmillan Publishers Inc., New York.
5. Al-Shehri MA. Production and some properties of protease produced by Bacillus licheniformis isolated from TihametAseer, Saudi Arabic. Pakistan Journal Biological Science.2004; 7: 1631-1635.
6. Barindra S, Debashish G, Malay S. and Joydeep M. Purification and characterization of a salt, solvent, detergent, and bleach tolerant protease from a new gamma Proteobacterium isolated from the marine environment of the Sundarbans. Process Biochemistry.2006; 41: 208–215.
7. Paranthaman R, Alagusundaram K. and Indhumathi J.Production of protease from rice mill wastes by Aspergillus niger in solid-state fermentation. World Journal of Agricultural Science. 2009; 5(3): 308-312.
8. Verma OP, Kumari P, Shukla S, Singh A. Production of Alkaline Protease by Bacillus subtilis (MTCC7312) using Submerged Fermentation and Optimization of Process Parameters. European Journal of Experimental Biology. 2011; 1:124-129.
9. Lakshmi BKM, Ratnasri PV, AmbikaDevi K, Hemalatha KPJ. Screening, optimization of production, and partial characterization of alkaline protease from haloalkaliphilic Bacillus sp. Journal of Research Engineering Technology. 2014; 3:435- 443.
10. Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P. and Srinivasulu B. Optimization of process parameters for glucoamylase production under solid-state fermentation by a newly isolated Aspergillus species. Process Biochemistry.2002; 38: 615- 620.
11. Haq, IU, Mukhtar H. and Umber H. Production of protease by Penicillium chrysogenum through optimization of environmental conditions. Journal of Agricultural Social Science. 2006; 2(1): 23–25.

Conflict of Interest	No Conflict of Interest
Funding Information	No funding from external source
Authors' Contribution	All authors contributed in the research work.
Acknowledgement	The authors express their sincere thanks to the management, Mother Teresa Pharmacy College, Sathupally, Telangana for providing the necessary facilities to carry out the research work.