**How to Cite:** Noreen S, Siddiqa A, Fatima R, Anwar F, Adnan M, Raza A. 2017. Protease Production and Purification from Agro Industrial Waste by Utilizing *Penicillium digitatum*. International Journal of Applied Biology and Forensics 1(4): 119-129

**REVIEW ARTICLE**

# Protease Production and Purification from Agro Industrial Waste by Utilizing *Penicillium digitatum*

Saima Noreen[1], Ayesha Siddiqa[2], Ridda Fatima[3], Fakhra Anwar[4], Muhammad Adnan[5] and *Akash Raza[6]

[1, 2, 5, 6] Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat, Pakistan
[4] Department of Zoology, University of Gujrat, Gujrat, Pakistan
[3] Department of Biochemistry and Molecular Biology, COMSAT Institute of Information and Technology, Islamabad, Pakistan
***Correspondence:** akash.raza@gmail.com*

## Abstract

Proteolytic enzymes are important enzymes for food and leather industry. Commercial proteases are almost 60% of the total industrial enzymes. Proteases are one of the three largest groups of industrial enzymes and are used in detergents as well as in leather industry and pharmaceutical industries. Proteases are widely distributed in nature, microbes are valuable sources of proteolytic enzymes because of their rapid growth, limited space required for the culture and they can be easily genetically manipulated to produce new enzymes with modified properties that are desirable for various applications. This review highlights the investigation of the culture influence of fungal strain *Penicillium digitatum* using different agro-industrial residues (Apple pomace, lemon peel) and their by-products as growth supported substrates. As microbes are cheaper source for enzymes production due to their rapid growth. In this review a fungal strain *Penicillium digitatum* was screened for protease production.

**Keywords:** Protease; Protein; *Penicillium digitatum*; Enzyme; Leather industry.

## Introduction

Proteolytic enzymes are also known as proteases. These enzymes have the capability of hydrolyzing peptide bonds in protein molecules. Proteolytic enzymes are everywhere and found almost in all organisms, and these enzymes are very important for cell differentiation and also for cell growth (Sharma et al, 2004). Growth and enzyme production of the body are strongly influenced by the components of the environment as sources of carbon and nitrogen. Further nutritional factor cultural parameters such as temperature, pH and incubation time also play an important role in the production of the enzyme (Krishnaveni et al, 2012) and optimization of components and media settings culture is the main task in a biological process.

These proteolytic enzymes find numerous applications in pharmaceutical industry, food and also detergent industries (Boominadhan et al, 2009). Proteases or proteolytic enzymes are usually found in all living organisms, like viruses, animals and also in humans. Proteolytic enzymes are very useful as they have the great importance in the field of medical especially in pharmaceutical industries. Their importance in these fields is due to their key role in biological processes. Proteolytic enzymes play an important role in life cycle of many pathogens. In biotechnology and industrial sectors the proteases or proteolytic enzymes are extensively applied. Proteolytic enzymes play an important role in many research applications. These research applications include nucleic acid purification, digestion of unwanted proteins, cell culturing, diagnostic of many diseases and in proteomics proteolytic digestion of proteins. In nucleic acid purification proteases play their important role by digestion of unwanted proteins.

Proteases are also useful and important elements in biopharmaceuticals such as enzymatic cleaners and enzymatic contact lens deriders (Saleemuddin and Anwar, 1997). Protease enzymes comprise of the very important classes of industrial enzymes which are widely used in waste management, pharmaceutical, food industry like in cheese making and brewing, baking, in hydrolysis of proteins and also in leather industries (Synowiecki 2010; Seifzadeh et al, 2008; Dias et al, 2008). Almost 75% of enzyme applications in industries comprise of hydrolytic enzymes and proteases make up 60% of it. Proteases are important class of industrial enzymes, which are approximately 60% of the total world-wide enzyme production. With the growing demand for industrial biocatalysts in difficult circumstances in all industrial processes, isolation and characterization of new strains is performed. Although to date over 3,000 different enzymes approval and found biotechnological and industrial applications, current tool box is not sufficient to meet all demands. A major reason for this is that many available enzymes not resist industrial reaction conditions. Consequently, characterization of microorganisms that are reliable thrive in extreme environments received much attention.

## Scope of the Review

Protease enzymes are also known as proteolytic enzymes because they can be hydrolyzed peptide bonds present in protein molecules, they are also known as peptidases. The proteases are vital for human, animals and microbes including viruses due to their physiological function. The classification of proteases is mainly based on their origin or source of production, they may be microbial produced from microbes like bacteria, fungi and viruses or they may be known as plant and animal enzymes. Proteases are also classified as endo and exopeptidase due to their sit of action. Endopeptidase hydrolyzes peptide bond from the end of polypeptide chain while exopeptidase hydrolyzes the peptide bond from N- or C- terminal of substrate.

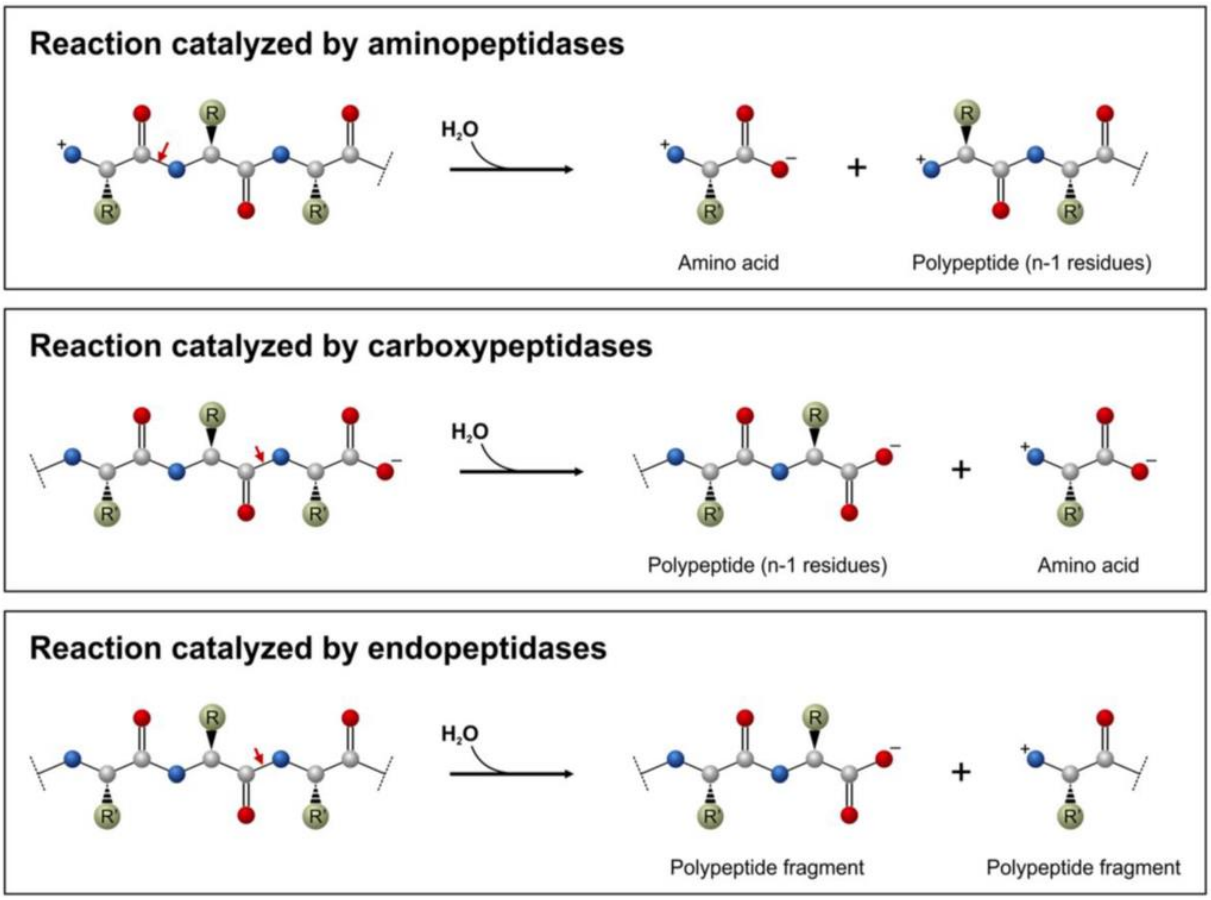


**Figure 1: Mechanism of action of Endopeptidase and Exopeptidase**
(http://www.mdpi.com/2218-273X/3/4/923/htm)

## Sources of Protease Production

Plants, animals and microbes are important source for protease production (Geethanjali and Subash, 2013). Microbial proteases are produced from many bacterial and fungal species *i.e. Bacillus* sp., *Alterneria alterneta, Penicillium digitatum, Aspergillus niger* grown under submerged culture. Microbial sources are preferred for the production of enzymes owing primarily to several advantages such as purity, economy, consistency, ease for process modification, availability of cheaper substrates and easier setting up production. With increasing industrial demands of biocatalysts, the extractions and purification of essential enzymes from microbial sources is becoming very popular (Smita et al, 2012).

**Table 1: Types of Protease Enzyme and Source**

| Enzyme | Source | References |
|---|---|---|
| Alkaline Protease | *Aspergillus oryzae* | Ortiz et al, 2016 |
| Alkaline Protease | *Penicillium sp. LCJ228* | Benluvankar et al, 2015 |
| Metallo Protease | *Bacillus sp. KG5* | Ahmetoglu et al, 2015 |
| Invertase | *Aspergillus niger* | Ahmed et al, 2014 |
| Serine Protease | *Bacillus licheniformis* | Nadeem et al, 2013 |

## SSF Strategy for Protease Production

SSF is also known for many centuries and it was mainly used for the production of many useful enzymes as well as for vital foods. Now a day's SSF is widely used for the production of many microbial enzymes like proteases. SSF has many advantages as compared to SmF. Several researches on SSF have been reported like in production of many chemicals (Vandenberghe et al, 2000; Roukas, 1999; Kar et al, 1998; Soccol et al, 1994), production of useful enzymes (Pandey et al, 2000; Suresh and Chandrasekaran, 1999), production of antibiotics (Kota and Sridhar, 1999; Yang & Swei, 1996; Ohno et al, 1996) and as immune suppressants (Murthy et al, 1999; Sekhar and Balaraman, 1998; Sekhar et al, 1997). SSF is widely used in those countries which have agro industrial wastes in abundance because these agro industrial wastes are used as raw material for enzyme production.

Many organisms have been used in SSF in order to produce useful enzymes but it is very necessary that optimum environmental and growth conditions should be provided to these organisms to enhance enzyme production. The conditions that facilitate protease production are very different from those conditions that facilitate cell growth (Amrita et al, 2012). SSF is very important because it reduces cost by using of cheap raw materials. SSF is a very simple process because it requires less energy than SmF. Proteases can also be produced by using SmF due to its apparent advantages in consistent enzyme production characteristics with defined medium and process conditions and advantages in downstream in spite of the cost intensiveness for medium components. SSF has gained renewed interest and fresh attention from researchers owing to its importance in recent developments in biomass energy conservation, in solid waste treatment and in its application to produce secondary metabolites (Rathakrishnan et al, 2012).

**Figure 2: *Penicillium digitatum* Cultured in Department of Biochemistry & Molecular Biology, University of Gujrat, Pakistan**

Protease enzymes not only enhance yield but decrease the environmental pollution and also improve leather quality. The use of proteases has become vast in leather industries as they help in environmental friendly processes. Like proteases, lipases are specifically used to remove grease. As

leather industries are main cause of air pollution and cause many hazards to environment and human health. Microbial enzymes like proteases are very useful to reduce these hazards in leather processing like dehairing, soaking and degreasing. In leather processing dehairing by protease enzymes can be helpful in obtaining desirable characteristics to leather (Sinha and Satyanarayana, 1991). Some microbial enzymes produced from microbial sources are very beneficial in food industry as well as in textile industries (Aleksieva and Peeva, 2000; Benslimane et al, 1995). The free proteases are mainly used in industries like as detergents, cheese making and in dry cleaning as well as in medical treatments of virulent wounds (Nout and Rombouts, 1990) stated that protease are very useful for improving water uptake by dry skins and reduce processing time of protein digestion. Proteases assist chemical process in leather dehairing. Proteases are most important group of industrial enzymes (Ahmed et al, 2007). Proteases are used in a variety of industrial application like textile industry, laundry and in health care centers. Due to ecofriendly nature of neutral proteases have vast applications in food processing like baking and brewing. Proteases are also used in therapy of many diseases. They can be helpful in immune regulation and in inflammatory conditions (Coelho et al, 1978). Mohan et al, 2005 stated that proteases find major applications in detergents, medical treatments and in virulent wounds. Extracellular proteases can digest insoluble nutrients like cellulose and proteins and these digested products then transported in cells where they can be used as essential source of growth (Gibb and Strohl, 1987; Oh et al, 2000).

Proteases can be purified by using different methods. After separation from fermentation broth culture supernatant concentrated by ultrafiltration (Kang et al., 1999; Smacchi et al., 1999), salting out by ammonium sulphate precipitation (Kumar et al, 2002), or by acetone in solvent extraction method (Kumar et al, 1999; Thangam and Rajkumar, 2002) and ethanol (EI-Shanshoury et al, 1995). Enzymes can also be beneficial as therapeutic agents for many fatal diseases like cancer and AIDS (Rao et al, 1998). Microbial proteases are of great interest for treatment of cancer, cardiovascular disorders and inflammation (Chanalia et al, 2011; Hellgren and Vincent, 1986).

Proteases can also be used as immune stimulatory agents (Biziulenvicius, 2006). Proteases are widely used in pharmaceutical industries for preparation of many medicines and drugs.

Proteases are also used in cleaners and in detergent industry (Gupta et al, 2002). Patil and Shastri (1985) carried out a work on a fungal strain *Alternaria alternate.* The main purpose of this study was the purification of alkaline proteases. Wheat bran was used as substrate for *Alternaria alternate. Alternaria alternate* secreted neutral as well as alkaline proteases. Neutral protease was found to be cysteine protease and alkaline protease was serine protease. Production of alkaline proteases was enhanced by fructose. This study also demonstrated that fructose can enhance characteristics of alkaline protease with respect of *V*max.

In 1994 Venkata & Goli studied the effect of different parameters such as substrate concentration, moisture content, inoculum size, incubation temperature, nitrogen sources, pH, incubation period and carbon sources in SSF for maximum yield of proteases by *Bacillus Cereus.* This protease was fibrinolytic protease. The substrate used in this experiment was apple pomace. The optimal culture conditions were investigated. Optimum pH 8, moisture content 70%, temperature 37°C and incubation period was 3 days. Fructose was found to be best carbon source for the production of fibrinolytic protease. El-Safey and Raouf (2004) studied the production and purification of protease enzyme by *Bacillus subtilis.* This organism was grown in broth culture for the purpose of protease production as well as purification. The optimum conditions were provided to *Bacillus subtilis* for enzyme production like an optimum substrate concentrations 1 %; optimum incubation period of 32 h; optimum incubation temperature was 35 ºC; the optimum pH was 6.5; and the buffer for the production of enzyme was phosphate buffer of pH 7.0 and optimum inoculum size used for protease production was 2 mL. The carbon source was glactose. The purification of protease enzymes was carried out by ammonium sulfate precipitation and sephadex G 100 by gel filtration. The activity of purified protease increased while increasing the enzyme concentration and substrate concentration. They emphasized the production and purification of proteases for industrial scale application.

In 2006 Gullon et al, Studied that the apple pomace can be used as potential raw material for

the production of many useful industrial enzymes. They measured the potency of apple pomace for the production of enzymes and other food products. Samples of this raw material were assayed for enzymatic digestibility. Oven dry apple pomace contained 30-40% of monosaccharide and oligo-saccharides. The main components of pomace were glactose and fructose. 60-70% mass of pomace was comprised of alcohol-insoluble compounds. Apple pomace showed the high potency for microbial enzyme production.

Sumantha et al, (2006) studied the use of rice bran as a substrate in SSF for the production of proteases. *Rhizopus microsporus* was used for enzyme production as well as for optimizing the culture conditions. The moisture content for fermentation process was almost 45%, temperature was 35 °C for 75 h. These conditions were found optimum for protease production in SSF. Casien addition resulted into a significant increase in protease production. Then SSF was carried out under control conditions for obtaining crude enzyme extract and then this crude enzyme was purified by filtration and precipitation. The protease obtained from this method was found as metalloprotease which has maximum activity at temperature 65 °C and pH 7.0.

In view of the large industrial applications protease, this review highlights the influence of culture conditions on the potential of locally isolated new strain of *Penicillium digitatum* taking technique SSF generation protease present its potential application in an industrial environment. In this study, a new approach has been applied fully focused on the use of different residues (lemon peel and apple pomace) for the production of useful microbial enzyme, protease. This study also focuses on providing a potential solution for managing large-scale solid waste.

Chutmanop et al, (2008) carried out a work to demonstrate that cheaper agro industrial wastes can be used to produce commercially valuable enzymes such as protease. *Aspergillus oryzae* can produce valuable commercial enzymes in SSF by utilizing cheaper agro industrial wastes. Rice bran was found to be best substrate for *Aspergillus oryzae* to produce protease. Morphology of rice bran can be improved by using certain amount of wheat bran. The result of this study demonstrated that *Aspergillus oryzae* can produce valuable amount of protease at pH 8 and moisture content of 55%. Dias et al, (2008) studied that among the various proteases, microbial proteases play a fundamental role in various biotechnological processes. Alkaline proteases production has much importance because alkaline proteases can be used in many industries like detergents, food industry, pharmaceutical industry and as well as leather industry. In recent years a number of reports have been published to characterize alkaline protease from different microorganisms. Many alkaline proteases which are applied for industrial purposes have to face some limitations such as low stability towards surfactants and production cost of the enzymes arisen from growth medium.

Paranthaman et al, (2009) studied that the proteases production by bioprocesses has a good value in respect to agro industry residues. A comparative study was carried out by them on the production of protease enzymes. They studied that by using different varieties of Rice from waste of rice mills as a substrate in SSF by *Aspergillus niger* proteases can be produced in valuable yield. The proteases produced from this waste can be used in various industries like food and leather industry. Jayasree et al, (2009) studied the production of protease enzyme from *Streptomyces pulvereceus* in submerged fermentation by using malt extract and soybean as substrates. The enzyme production was investigated during different growth phases and it was demonstrated that the maximum yield of protease was during stationary phase. Optimum culture conditions were provided and different parameters were investigated during enzyme production such as pH was 9.0, inoculums size 3 and the optimum temperature was 33°C. The carbon sources were xylose, fructose, maltose and starch. Among all these carbon sources starch found to be best carbon source for optimum enzyme production. The protease produced can be used as good source for a number of various industrial applications.

Mussatto et al, (2010) demonstrated by their research that a large amount of agro industrial wastes is produced from many industrial processes. Some of these wastes can be burned for alternation of elimination and some wastes can be utilized as animal feed. However, these wastes are usually comprised of large amount of sugars and proteins therefore; these wastes cannot be considered as "wastes" but as raw materials for other commercial industrial processes. Microorganisms use these raw materials as residue in SSF because solid support and nutrient source is provided by these agro

industrial wastes. They demonstrated that solid state fermentation can give high yield of enzymes and other valuable industrial products. In addition, SSF is cost effective because reuse of industrial wastes.

Saxena and Singh (2010) carried out a work on protease enzyme produced from isolated *Bacillus* strain. High stability by this protease was exhibited even in the presence of ions such as many divalent as well as monovalent ions. Agro-industrial wastes used in this work were Gram Husk, Mustard Oil Cake and Wheat Bran in SSF. Among all these substrates rice bran was found to be best for optimum enzyme production. The optimum pH was 8 and temperature was 60°C. Some metal ions, K+, Zn++, Ca++, Mg++ Cu++, Na+ Mn++, and Hg++ can enhance the protease activity produced by this work.

Ire et al, (2011) carried out a work for the evaluation of *Aspergillus carbonarius* for enzyme production such as protease by using different standard methods. This fungus was cultured and it produced a valuable amount of protease. Protease production by *Aspergillus carbonarius* can be influenced by using different carbon as well as nitrogen sources. Glucose was found to be best carbon source for protease production. *Aspergillus carbonarius* produced maximum yield of protease by applying optimized culture conditions i.e. pH 6.0, incubation period 9 days and 5% glucose. The results demonstrated that *Aspergillus carbonarius* can produce appreciable value of protease by using cheaper raw materials from industries.

Sevinc and Demirkan (2011) carried out a work on different strains of *Bacillus* for protease production. Almost fifty-four strains of *Bacillus* were isolated from soil and these strains were tested for valuable enzymes production such as proteases. One strain which gave the maximum protease yield was selected. Different medium ingredients such as nitrogen and carbon source as well as metal ions were investigated in order to enhance the protease production. Many carbon sources were used and among all these carbon sources fructose showed greatest potential for protease production. Skim milk found to be best nitrogen source. Organic nitrogen sources are very affective as compared to inorganic nitrogen sources. Combination of metal ions enhanced the enzyme production such as combination of Mg2+ and Ca2+ found to be best because these two ions could not be effective alone. Optimum pH for enzyme activity was 7.0 and optimum temperature was 55°C. This study demonstrated that enzyme is very stable in alkaline condition. This purified enzyme was also thermostable. Muthulakshmi et al, (2011) carried out a work to investigate the protease production using *Aspergillus flavus* by SSF. This study evaluated different agro industrial wastes for protease production by *Aspergillus flavus*. Wheat bran was found to be best substrate for *Aspergillus flavus* to produce protease. The results of this study demonstrated that *Aspergillus flavus* can give maximum yield of protease at different optimal conditions e.g. pH 5.0, substrate concentration 4% and temperature *35 °C.* Purified protease had molecular weight of 46kDa. Results demonstrated that enzyme activity can be enhanced by some metal ions such as $Cu^{2+}$ and $Zn_2$ and it can be inhibited by some metal ions such as $Ca^{2+}$ and $Mg^{2+}$.

Sharmila et al, (2012) stated that proteases are fundamental components for all living organisms. Proteases are found almost in all living bodies. Microorganisms like bacteria, yeast as well as fungus are major sources for proteases. Proteases account for 60 % of total worldwide enzymes as they are very important in various industries. This work carried out for isolation of protease enzyme from leaves of various plants. Then activity and pH level for this enzyme was checked. Leaves of different plants were tested but results demonstrated that leaves of *Nicotiana tobaccum Protea* was found to be best for optimum protease production. The optimum pH was 7.2 for this purified enzyme. Josephine et al, (2012) carried out a work on *Bacillus sp.* for production of various extracellular enzymes mainly proteases. Optimized conditions were applied e.g. pH 7.0, incubation period 24h and optimum temperature was 37 °C. Crude protease was purified by ammonium sulphate. This study revealed that *Bacillus sp.* can produced substantial amount of protease by using cheaper and easily available agro industrial wastes. Alnahdi (2012) studied the protease production from a bacterial strain *Bacillus* sp. Bacteria are known to have potency of enzyme excretion in environment. *Bacillus* sp. is known to be very important organism for production of a variety of extracellular enzymes such as proteases. First of all *Bacillus* sp. was isolated from marine samples for protease production. The main purpose of this work was to investigate the protease production from various bacterial strains isolated

from marine. Six bacterial strains were isolated and screening of these strains was performed for protease production. Among all these strains *Bacillus* sp. was found to be best strain for maximum protease production.

Nadeem et al, (2013) carried out a work for purification of protease produced from *B. licheniformis* for various industrial applications. Produced protease was purified by ammonium Sulphate precipitation and by gel filtration chromatography using sephadex G-100. Specific activity of purified protease was increased up to 36.83 fold. Molecular weight of purified protease was 36.12 kDa which was determined by SDS-PAGE. The *V*max value was found to be 61.58ìM/mL/min. The optimal conditions for purified proteases were pH 11, incubation time 24 hrs and temperature to 50°C. The protease purified in this study was found to be serine protease. Metal ions such as Mg2+ and Ca2+ enhanced the activity of purified protease. This purified protease had many applications as laundry detergent.

Ali and Vidhale (2013) studied that agro industrial wastes are very valuable for microbial enzyme production. Protease production was carried out in SSF by *Fusarium oxysporum.* Agro industrial Rice bran was used as substrate. This substrate was found to be best for maximum protease production by *Fusarium oxysporum* in SSF. Maximum protease production was achieved by optimum conditions such as moisture content of 50%, temperature 30°C, incubation period of 70h and pH 7.0. This study revealed that maximum protease production can be achieved by optimized conditions in SSF by *Fusarium oxysporum.* Yadav (2013) studied different aspects of SSF for protease production by *Bacillus sp.* and its advantages over other types of fermentation. SSF is very useful in industries for production of various microbial enzymes. SSF is cost effective, low energy consumption, higher yield and low bacterial contamination. *Bacillus sp.* produced valuable amount of protease by low energy consumption and optimum pH level.

Ahmed et al, (2014) studied the optimum conditions applied in SmF for the production of various industrial and microbial enzymes. Three industrial enzymes, Invertase, α-Amylase and Glucose isomerase were produced from *Aspergillus niger by* using agricultural wastes like cotton stalk, rice husk and sunflower waste. These wastes were the source of carbon for organism and the sources of nitrogen were Corn steep, Nitrate Albumin and Sulphate Urea Yeast. Various factors were also investigated for the optimum production of enzyme like the time period which was 24-240 hours, pH was 4-9 and temperature was 40-60°C. It was investigated that *Aspergillus niger* gave the highest yield of invertase in that culture medium whose nitrogen source was yeast extract. Thermo stable strains of *Aspergillus niger* can also be used for enzyme production in industries. But the activity of various enzymes was very low for industrial applications although their activity can be enhanced by using nitrogen sources.

Vanitha et al, (2014) carried out a work to isolate suitable soil bacteria for production of alkaline protease. Identification of optimized conditions i.e. pH, incubation period, inoculum size and carbon source for this bacterium for protease production is another important point. Agro industrial wastes from different industries were used as substrates. *Bacillus subtilis* was used for protease production by maintaining optimal conditions. Crude enzyme was purified by ammonium sulphate. The purified enzyme had molecular weight of 55 kDa. Maltose and yeast extract were found to be best carbon and nitrogen source respectively for protease production. Purified protease has many industrial applications.

Sawant and Nagendran (2014) focused on the important industrial applications of proteases. They play a vital role in a number of industries like food, detergent and pharmaceutical industries. Microbial Proteases are of great interest in this regard because they are ecofriendly and have many commercial applications.

Thakur et al, (2015) studied that the use of agro industrial waste in SSF is very useful for microbial enzyme production as well as for solving pollution problems. They emphasized on the biochemical conversion of agro-industrial waste of soybean seed after extraction of oil. While extraction of oil from these seeds excessive heat is supplied so the quality of fodder is reduced and cannot be used for animal feed. *Aspergillus oryzae* shows excellent potential for this soybean meal as a substrate in SSF for the production of protease enzyme. Protease activity was measured by using different parameters like pH, moisture content and incubation time.

Benluvankar et al, (2015) studied the SSF for the production of protease by *Penicillium sp.* using agro industrial wastes. Agro industrial wastes

like cotton seed, peanut shells were used as substrates in SSF. Among all these substrates peanut shell was found to be best substrate for protease production at industrial scale. The culture conditions, for maximum yield of protease were investigated and various factors were measured. The best carbon source found for maximum protease production was glucose and the best nitrogen source for maximum yield of proteases was yeast extract. The suitable protease production inducer was casein. Optimum pH was 9.5. A moisture content of 90% was found to be optimumĪ for protease production and purification. From this study it was proved that *Penicillium sp*. is best for high yield protease under SSF by using cheap agro industrial wastes. Racheal et al, (2015) carried out a work for protease purification and characterization from *Aspergillus niger* by using yam peels. Purification of protease was carried out by ion exchange column and gel filtration. Effect of different parameters like pH, temperature and incubation time on the production of protease was investigated. Physicochemical characteristics of purified protease were also investigated. Optimized conditions for production and purification of protease were temperature 37$^{o}$C, pH 7.0 and incubation period of 42 hrs. The results of this study showed that enzymatic activity of purified protease is more than crude enzyme. The specific activity of purified protease was 8.51 (U/mg) as compared to specific activity of protease which was 0.51 (U/mg). The enzyme showed its maximum activity by using casein as substrate. Metal ions such as Cu2+, Fe2+ and Mg 2+ inhibited protease activity while Na+ enhanced protease activity. The molecular weight of purified protease was found to be 46.90 kDa.

Malik and Shinde (2016) studied the comparison of SSF and SmF. Those enzymes which are produced from microorganisms are very useful for industrial applications and can be survive under extreme alkaline conditions. This worked carried out for comparative of SmF and SSF to find which type of fermentation is better for optimum microbial enzyme production. In this work four fungal strains were isolated and screened. These fungal isolates were screened by clear zones formed due to protein hydrolysis by protease production. The strain which gave maximum clear zone was selected for protease production in comparative study of SSF and SmF. Standard culture conditions were applied for enzyme production. The protease produced by this work was very active at pH 9.5 and 10. At pH 11, the enzyme activity was started to decline. By this work, it was proved that between two types of fermentation the SSF showed double activity as compared to SmF. So SSF can be used at commercial level due to its high activity and cost effective.

## Future Scope

Protease enzymes are known as one of the most important class of enzymes because these enzymes have large commercial applications in industries as well as in therapeutics. Protease enzymes have synthetic as well as degradative properties. Proteases are found in animals, microbes as well as in plants. Among all these sources microbes are considered to be best source for protease due to their several properties including rapid growth and limited planetary for cultivation. Microbial proteases are widely used in many industries including food, leather and detergent industries since earliest times. Many researches had proved that protease can be used as targets for the development of therapeutic agents for treatment of many deadly diseases such as AIDS and cancer. Microbial proteases can be considered as ideal for many biotechnological researches. Biotechnologists mainly focus on development and improvement of microbial strains used in industries for the production of microbial enzymes.

## Conclusion

In conclusion, the present study describes the protease production, purification and characterization from agro residues (lemon peel and apple pomace) in term of SSF. Pakistan is an agricultural country and a lot of agro industrial waste is produced every year so, it is a good step to produce useful products including microbial enzymes from this waste. It will be very beneficial for economy of country. This study focused on production of protease by using a fungal strain *Penicillium digitatum*. This work will also be beneficial for many industrial applications because protease produced from *Penicillium digitatum* can be used in detergent industry as detergent additive and also in cosmetic industries in hair removing creams.

## References

Ahmetoglu N, Bekler FM, Acer O, Guven RG, Guven K. 2015. Production, purification and characterisation of thermostable metallo-protease from newly isolated *Bacillus* sp. KG5. *Eurasia. J. Biosci*. 9: 1-11.

Ahmed K, Valeem EE, Ul-Haq Q, Mahmood I, Dahot MU. 2014. Optimal conditions for the production of industrial enzymes by Aspergillus Niger using agricultural wastes as source of carbon. *Fuuast. J. Biol*. 4: 205-211.

Ahmed SA, Saleh S, Fattah AF. 2007. Stabilization of *Bacillus licheniformis* ATCC 21415 alkaline protease by immobilization and modification. Aust. J. Basic Appl. Sci. 1: 313-322.

Alnahdi H. 2012. Isolation and screening of extracellular proteases produced by new isolated *Bacillus* sp. J. Appl. Pharm Sci. 2: 71-74.

Ali SS, Vidhale NN. 2013. Protease production by *Fusarium Oxysporum* in solid-state fermentation using rice bran. Amr. J. Microb. Res. 3: 45-47.

Aleksieva P, Peeva L. 2000. Investigation of acid protinase biosynthesis by the fungus *Humicola Lutea* 120-5 in an airlift bioreactor. Enzymes. Microb. Technol. 26: 402-405.

Amrita R, Nancy K, Pujari N, Bhattacharya S, Das A, Rajan S. 2012. Enhancement of protease production by *Pseudomonas aeruginosa* isolated from dairy effluent sludge and determination of its fibrinolytic potential. Asia-Pac J. Trop. Biomed 19: 1845-51

Benluvankar V, Jebapriya GR, Gnanadoss JJ. 2015. Protease production by *Penicillium* Sp. LCJ228 under solid state fermentation using groundnut oilcake as substrate. Life Sci. Bio. 5: 2250-2480.

Benslimane C, Lebrihi A, Lounes A, Lefebvre G, Germain P. 1995. Influnce of dextrins on the assimilation of yeast extract amino acids in culture of *Streptomyces ambofaciens* producer of spiramycin. Enzymes. Microb. Technol. 17: 1003-1013.

Biziulenvicius GA. 2006. Where do the immune stimulatory effects of oral proteolytic enzymes (systemic enzyme therapy) come from? Microbial proteolysis as a possible starting point. Rev. Med. Microb. 67: 1386-1388.

Boominadhan U, Rajakumar R, Karpaga P, Sivakumaar V, Melvin MJ. 2009. Optimization of Protease Enzyme Production Using *Bacillus Sp.* Isolated from Different Wastes**.** Bot. Res. Intl. 2: 83-87.

Chutmanop J, Chuichulcherm S, Chisti Y, Srinophakun P. 2008. Protease production by *Aspergillus oryzae* in solid-state fermentation using agroindustrial substrates. J. Chem. Technol. Biotechnol. 83: 1012–1018.

Chanalia P, Gandhi D, Jodha D. 2011. Applications of microbial proteases in pharmaceutical Industry: an over view. Rev. Med. Microb. 22: 96-101.

Coelho R, Drozdowicz A. 1978. The occurrence of actinomycetes in a cerrado soil in Braz. Tox. 15: 459–73.

Dias DR, Vilela,DM, Silvestre MC, Schwan RF. 2008. Alkaline protease from *Bacillus* sp. isolated from coffee bean grown on cheese whey. World J. Microb. Biotechnol. 24: 2027-2034.

El-Safey EM, Abdul-Raouf UM. 2004. Production, purification and characterization of protease enzyme from *Bacillus Subtilis*. J. Innov. Res. Sci. Eng. Techn. 5: 12-17.

El-Shanshoury R, El-Raheem AR, El-Sououd S, Awadalla O, El-Bandy N. 1995. Effects of *Streptomyces corchorusii*, *Streptomyces mutabilis*, *pendimethalin*, and *metribuzin* on the control of bacterial and Fusarium wilt of tomato. Cand. J. bot. 74: 1016-1022.

Geethanjali S, Subash A. 2013. Optimization and Immobilization of Purified *Labeo rohita* Visceral Protease by Entrapment Method. Appl. Microbiol. Biotechnol 32: 276-290

Gibb GD, Strohl WR. 1987. Physiological regulation of protease activity in *Streptomyces peucetius.* Can. J. Microbiol. 34: 187-190.

Gupta R, Beg QK, Lorenz P. 2002. Bacterial Alkaline Protease: Molecular approaches and industrial application. Appl. Microbiol. Biotechnol. 59: 15-32.

Gullon B, Falque E, Alonso JL, Parajo JC. 2006. Evaluation of apple pomace as a raw material for alternative applications in food industries. Food Technol. Biotechnol. 45: 426-433.

Hellgren V, Vincent J. 1986. Proteases of Antarctic Krill- a new system for effective enzymatic debriment of necrotic ulcerations. Cel. Mol. Life Sci. 42: 403-404.

Ire FS, Okolo BN, Moneke AN, Odibo FJ. 2011. Influence of cultivation conditions on the production of a protease from *Aspergillus*

*Carbonarius* using submerged fermentation. Afr. J. Food Sci. 5: 353-365.

Jayasree D, Kumari DS, Kishor K, Lakshmi V, Narasu L. 2009. Optimization of production protocol of alkaline protease by *Streptomyces pulvereceus*. Fuuast. J. Biol. 4: 507-522.

Josephine FS, Ramya V, Devi N, Ganapa SB, Siddalingeshwara K, Venugopal G, Vishwanatha T. 2012. Isolation, production and characterization of protease from *Bacillus Sp* isolated from soil sample. J. Microb. Biotech. Res. 2: 163-168.

Kar B, Benerjee R, Bhattacharya BC. 1998. Biotransformation of tannic acid to gallic acid by solid state fermentation. Adv. Biotechnol. 2: 67-74.

Kang KI, Meng X, Bouhouche I, Chadli A, Catelli MG. 1999. The molecular chaperone Hsp90 can negatively regulate the activity of a glucocorticosteroid-dependent promoter. J. Basic Appl. Sci. 3: 144-156.

Kota KP, Sridhar P. 1999. Solid state cultivation of *Streptomyces clavuligerus* for Cephamycin C production. Process Biochem. 34: 325–28.

Krishnaveni KJ, Mukesh K, Balakumaran MD, Rameshand SP, Kalaichelvan T. 2012. Production and optimization of extracellular Alkaline Protease from*Bacillus subtilis* isolated from dairy effluent. J. Appl. Pharm Sci. 4: 10-98.

Kumar CG, Malik RK, Tiwari MP. 1999. Novel enzyme-based detergents: An Indian perspective. Curr. Sci. 75: 1312-1318.

Kumar A, Harrison PM, Cheung KH, Lan N, Echols N, Bertone P, Miller P, Gerstein MB, Snyder M. 2002. An integrated approach for finding overlooked genes in yeast. Nat. Biotechnol. 20: 58-63.

Malik N, Shinde V. 2016. Comparative analysis of alkaline protease production by solid state and submerged fermentation. Int. J. Res. 6: 6972-697.

Mohan FN, Dileep D, Deepthi D. 2005. Potential application of protease isolated from *Pseudomonas auriginosa* PD100. Biotechnol. Ind. 8: 197-203

Murthy MV, Mohan EV, Sadhukhan AK. 1999. Cyclosporine A production by *Tolypocladium-inflatum* using solid state fermentation. Process. Biochem. 34: 269-80.

Muthulakshmi C, Gomathi D, Kumar DG, Ravikumar G, Kalaiselvi M, Uma C. 2011. Production, purification and characterization of protease by *Aspergillus flavus* under solid state fermentation. J. Bio.Sci. 4: 137-148.

Mussatto I, Solange E, Ballesteros F, Martins S, Teixeira JA. 2010. Use of Agro-Industrial Wastes in Solid-State Fermentation Processes. Enzymes. Microb. Technol. 7: 346-349.

Nadeem M, Iqbal J, Qazi, Syed Q, Gulsher M. 2013. Purification and characterization of an alkaline protease from *Bacillus licheniformis* UV-9 for detergent formulations. J. Sci. Technol. 35: 187-195.

Nout MJR, Rombouts FM. 1990. Recent developments in tempe research. J. App. Bacterial 69: 609-633.

Ohno A, Ano T, Shoda M. 1996. Production of Lipopeptide antibiotic, surfactin by recombinant *Bacillus subtilis* in solid state fermentation. Biotechnol. Bioeng. 47: 209-14.

Oh YS, Shih IL, Tzeng YM, Wang SL. 2000. Protease produced by *Pesudomonas aeroginosa* K-187 and its application in the deproteinization of shrimp and crab shell wastes. Enzymes. Microb. Technol. 27: 3-10.

Ortiz GE, Noseda DG, PonceMora MC, Recupero MN, Blasco M, Alberto E. 2016. A comparative study of new *Aspergillus* strains for proteolytic enzyme production by solid state fermentation. Biochem. Eng. J. 80: 76–82.

Pandey A, Soccol CR, Mitchell D. 2000. New developments in solid-state fermentation bioprocesses and products. Process Biochem. 35: 1153-69.

Paranthaman R, Alagusundaram K, Indhumathi J. 2009. Production of Protease from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation. World J. Agr. Sci. 5: 308-312.

Patil M, Shastri NV. 1985. Purification and properties of proteases produced by *Alternaria alternata* (Fr.) Keissl, regulation of production by fructose. J. Biosci. 9: 1-11

Rathakrishnan P, Nagarajan P, Rajesh RK. 2012. Optimization of Process Parameter using a Statistical Approach for Protease Production by *Bacillus Subtilis* using Cassava Waste. Int. J. Chem. Tech Res. 4: 749-760

Racheal OO, Ahmed TF, Ndigwe EV, Morakinyo SD. 2015. Extraction, purification and characterization of protease from *Aspergillus Niger* isolated from yam peels. Int. J. Nut. Food Sci. 4: 125-131.

Rao MB, Tanskale AM, Ghatger MS, Deshpande V. 1998. Molecular and Biotechnological

aspects of Microbial proteases. J. Sci. Technol. 63: 596-63.

Roukas T. 1999. Citric acid production from carob pod by solid-state fermentation. Enzyme. Microb. Tech. 24: 54-59.

Saxena R, Singh R. 2010. Metal ion and pH stable protease production using agro-industrial waste. J. Eco. Biotechnol. 2: 1-5.

Sawant R, Nagendran S. 2014. Protease: An enzyme with multiple industrial applications. World J. Pharm. Sci. 3: 568-579.

Saleemuddin M, Anwar A. 1997. Chemical stability and prop of proteases. Biotech. App. Biochem. 25: 43-46.

Sekhar C, Balaraman K. 1998. Optimization studies on the production of cyclosporine A by solid state fermentation. Bioprocess Eng. 18: 293-96.

Sekhar C, Rajasekar VW, Balaraman, K. 1997. Production of cyclosporine A by solid state fermentation. Bioprocess Eng. 17: 257-59

Sevinc N, Demirkan E. 2011. Production of protease by *Bacillus* sp. N-40 isolated from soil and its enzymatic properties. J. Biol. Environ. Sci. 5: 95-103.

Seifzadeh S, Sajedi RH, Sariri R. 2008. Isolation and characterization of thermophilic alkaline proteases resistant to sodium dodecyl sulfate and ethylene diamine tetraacetic acid from *Bacillus* sp. GUS1. Iran. J. Biotechnol. 6: 214-221.

Sharma S, Aneja MK, Mayer J, Scholter M, Munch JC. 2004. RNA fingerprinting of microbial community in the rhizosphere soil of grain legumes, Fems. Microbiol. Lett. 240: 181-86.

Sharmila S, Jeyanthi LR, Das MP, Saduzzaman M. 2012. Isolation and partial purification of protease from plant leaves. J. Chem. Pharm. Res. 4: 3808-3812

Sinha N, Satyanarayana T. 1991. Alkaline protease production by thermophilic *Bacillus licheniformis*. Ind. J. Microb. 31: 425–430.

Smacchi M, Gobbetti E, Semeraro M, Fox PF, Lanciotti R, Cogan T. 1999. Purification and characterization of an extracellular proline iminopeptidase from *Corynebacterium variabilis* NCDO 2101. J. Appl. Microb. 5: 134-136.

Smita BCD, Brahmbhatt MN. 2010. Selective culture media for the recovery of Aeromonas species from poultry meat. Ind. J. Vet. Res. 20: 66-68.

Soccol CR, Marin B, Raimbault M, Lebeault JM. 1994. Potential of solid state fermentation for production of L (+) - lactic acid by *Rhizopusoryzae*. Appl. Microbiol. Biotechnol. 41: 286-90.

Suresh PV, Chandrasekaran M. 1999. Impact of process parameters on chitinase production by an alkalophilic marine *Beauveriabassiana* in solid state fermentation. Process Biochem. 34: 257-67.

Sumantha A, Deepa P, Sandhya C, Szakacs G, Soccol CR, Pandey A. 2006. Rice bran as a substrate for proteolytic enzyme production. Braz. Arch. Biol. Technol. 49: 843-851.

Synowiecki J. 2010. Some applications of thermophiles and their enzymes for protein processing. Afr. J. Biotechnol. 9: 7020-7025.

Thakur AS, Nemade, S. N., & Sharanappa, A. (2015). Solid State Fermentation of Overheated Soybean Meal (Waste) For Production of Protease using *Aspergillus Oryzae.* Int. J. Innov. Res. Sci. Eng. Technol. 4: 11-14.

Thangam EB, Rajkumar GS. 2002. Purification and characterization of alkaline protease from *Alcaligenes faecalis*. Biotechnol. Appl. Biochem. 35: 149-54.

Vandenberghe LPS, Soccol CR, Pandey A, Lebeault JM. 2000. Solid-state fermentation for the synthesis of citric acid by *Aspergillusniger*. Biores. Technol. 74: 175-78.

Vanitha N, Rajan S, Murugesan AG. 2014. Optimization and Production of Alkaline Protease enzyme from *Bacillus subtilis* 168 isolated from food industry waste. Int. J. Curr. Microb. App. Sci. 3: 36-44.

Venkata EN, Goli D. 1994. Effect of physiochemical parameters on fibrinolytic protease production by solid state fermentation. World J. Pharm. Sci. 3: 1937-1954.

Yang SS, Swei WJ. 1996. Cultural condition and Oxy-tetracycline production by *Streptomyces rimosus* in solid state fermentation of corn cob. World J. Microb. Biotechnol. 12: 43-46.

Yadav MM. 2013. Alkaline protease production by isolated *Bacillus* SP. in submerged fermentation & solid state fermentation. J. Bio. Innov. 2: 161-167.