International Journal of Food Engineering and Technology
2021; 5(2): 68-73
http://www.sciencepublishinggroup.com/j/ijfet
doi: 10.11648/j.ijfet.20210502.17
ISSN: 2640-1576 (Print); ISSN: 2640-1584 (Online)

Production and Industrial Application of Microbial Aspartic Protease: A Review

Fikadu Hailemichael

Food Science and Nutrition Research, National Fishery and Aquatic Life Research Center, Ethiopian Institute of Agricultural Research, Sebeta, Ethiopia

Email address:
fikaduh.michael11@gmail.com

To cite this article:
Fikadu Hailemichael. Production and Industrial Application of Microbial Aspartic Protease: A Review. *International Journal of Food Engineering and Technology*. Vol. 5, No. 2, 2021, pp. 68-73. doi: 10.11648/j.ijfet.20210502.17

Received: October 19, 2021; **Accepted**: November 22, 2021; **Published**: December 11, 2021

Abstract: Proteases are one of the predominant groups of industrial enzymes and it represents for about 65% of the total global enzyme market. Proteases of microbial origin have great importance over plant sources because they minimize industrial production costs, increase characteristics of the desired products and widely used in biotechnological process. Among the protease enzymes, aspartic proteases are the most important groups of proteolytic enzymes which are mainly produced by plants, animals and many microorganisms to degrade large polypeptides into peptides and amino acids. Microorganisms are also mainly preferred in the production of aspartic protease since they have most of the characteristics desired for biotechnological application rather than plant protease. Aspartic proteases produced from microbial sources are widely used in pharmaceutical, protein hydrolysis, detergent, cheese-making, photographic, baking, meat, leather, food and beverage industries. Although acid protease is vital to enhance the demands of many food and other industries, there are factors affecting the production of aspartic protease. Hence, aspartic protease production using microorganisms is highly affected by various carbon and nitrogen substrates, divalent metal ions, pH, incubation temperature, time, agitation speeds, age of inoculum and density. This review highlights on the production and applications of microbial aspartic proteases.

Keywords: Production, Aspartic Protease, Microbial Enzymes, Submerged Fermentation, Solid State Fermentation

1. Introduction

Enzymes are large macromolecules composed of polymers of amino acids combined by amide bonds, ranging from insulin to ribosome in molecular mass. They are produced by living organisms that catalyze the biochemical reaction in highly efficient ways and environment friendly. They have considerable benefits over chemical catalysts, in its specificity, high catalytic activity, its capability to work at moderate temperatures, and the ability to be produced in large amounts [25]. Microorganisms are the most important sources for the production of intracellular and extracellular enzymes. Each single strain of organism produces a large number of enzymes, hydrolyzing, oxidizing or reducing, and metabolic in nature [2]. Selection of the right organism for enzyme production plays a key role in enzyme productivity [19].

Based on the nature of the microorganisms and activity of enzymes, microbial enzymes can be produced by using submerged and solid state fermentation techniques. Solid-state fermentation is mainly used for production of fungal enzyme (pectinases, amylases, acid-proteases, amyloglucosidases, etc) at industrial scale [20]. Solid state fermentation (SSF) has the potential for the higher protease yield. Economically this type of fermentation possesses many advantages, including superior volumetric productivity, use of simpler machinery, use of an inexpensive substrate, simpler downstream processing, lower energy requirements and low waste water output [29, 41].

Among various industrial enzymes, microbial proteases are one of the three largest groups of industrial enzymes which dominate the world enzyme market due to their potential application in many industries like food, pharmaceutical, detergent, leather and textile industries. In food processing, proteases are widely used for the modification and improvement of protein functionality, production of protein hydrolyzates, meat tenderization, utilization of different by-products, as well as for catalysis of the plastein reactions [36, 32].

Proteases are highly diverse enzymes having different active sites. They are classified into exopeptidases, that attack the ends of protein molecules, and endopeptidases which cleave peptide bonds within polypeptide chains [10]. Protease account for about 65% of the total worldwide enzymes sales and they 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 optima and stability profile [32]. At present, proteases are classified depending on three major criteria: (i) type of reaction catalyzed, chemical nature of the catalytic site, and (iii) evolutionary relationship with reference to structure [11]. Aspartic proteases (EC3.4.23), also known as acidic proteases, are a subfamily of endopeptidases that have been isolated from diverse sources, including viruses, bacteria, fungi, plants, and animals [9].

Acid protease has a wide range of application in various industries to make a change in product taste, texture, and appearance and in waste recovery. Besides this, they have extensive applications in food industry, beverage industry, and pharmaceutical industry [11]. The increasing importance of these enzymes and their numerous applications in different industries made us to investigate acid protease production from a new source. The isolation and characterization of new promising strains are possible ways to increase the yield of such enzymes. Therefore, the aim of this paper was to review on production, optimization and application of acid protease.

2. The Discovery of Enzyme

The use of enzymes was established when human civilization has been increased for centuries. For instance, Egyptians used enzymes to preserve food and beverages. Moreover, the process of cheese making included the use of enzymes from as early as 400 BC and hence the term 'enzyme' was first introduced in 1877 by Wilhelm Friedrich Kuhne who was a professor of physiology at the University of Heidelberg. He was the first person to observe the scientific terminology of the protein molecule [4]. On the other hand, their catalytic action was first studied by the Swedish chemist Jon Jakob Berzelius in 1835. James B. Sumner of Cornell University, on the other hand, first isolated pure form of enzyme in 1926, which earned him a Nobel Prize in 1947 [23].

The aspartic proteases have a long history. Chymosin, in the form of rennet, has been used for millennia in cheese making. During the periods of 5500-2000 B.C., cave paintings in the Libyan Sahara have shown different types of appearances in milk processing steps. In Egypt, the tombs of Horus-aha, the second king of the first dynasty (3000-2800 B.C.), pots were found with remains of cheese. Cheese production is also mentioned in hieroglyphic texts. Hence, microbial aspartic proteases have been used in China for making soy sauce, first mentioned during the Zhou dynasty (1028 220 B.C.) the creators of the Great Wall [37].

2.1. Proteases

Proteases are a group of enzymes that catalyze the hydrolysis of peptide bonds [16]. They are one of the three largest groups of industrial enzymes and account for about 60% of the worldwide enzyme sale [25]. In 2014, the global market for industrial enzymes was estimated about $4.2 billion and expected to develop from 2015 to 2020 to reach nearly $6.2 billion [31].

Among the world sale of industrial enzymes, 75% of these are hydrolytic enzymes, of which two-thirds are proteolytic enzymes [26]. Proteases are highly specific during hydrolysis process and hence they have widely used in different industries, such as food, laundry detergent, leather and pharmaceutical industries [1].

2.2. Classification of Proteases

Proteases contain a very large and complex group of enzymes, which can be classified on the basis of their substrate specificity, active site and catalytic mechanism, pH and temperature optima and stability profile. Proteases can be classified in different ways [3]. For example, depending on their catalytic mechanism proteases can be categorized as serine proteases, aspartic proteases, cysteine proteases and metallo-proteases [28]. Proteases are also classified into different clans and families depending on their amino acid sequences and evolutionary relationships [12].

Proteases can be categorized as endopeptidases and exopeptidases based on their site of action on polypeptide. They are also classified into acid, alkaline and neutral proteases based on their optimal pH for their activity [33]. Acid proteases, commonly known as acid proteases, are rennin-like proteases from microbes which are mainly used for cheese production [38]. Aspartic proteases have an optimum pH of 2-4 [40]. Neutral proteases are secreted by both fungi and bacteria. They are relatively unstable and require ions such as Ca^{2+}, Na^{+} and Cl^{-} for stability. They have narrow pH range for their activity and are not very stable at higher temperatures [3]. Alkaline proteases are more stable at high temperatures and in the alkaline range 9 – 11 [21].

2.3. Sources of Protease

Proteases are essential for the physiological process of living organisms and hence they are ubiquitous and widely distributed in plants, animals and microbes. The major plant proteases studied and currently in use, with an important role in food and pharmaceutical industries are papain, bromelain and ficin [6]. Pancreatic trypsin, chymotrypsin, pepsin, and rennin are the well-known examples of animal proteases and usually released from their zymogens by autolysis or due to the proteolytic action of other enzyme [12].

2.3.1. Animal and Plant Protease

Different types of proteins such as pancreatic trypsin, chymotrypsin, pepsin and rennin are the most familiar proteases of animal origin. Those of the aforementioned proteins are commonly prepared in pure form in bulk quantities. But their production mostly depends on the availability of livestock for slaughter, which in turn is governed by political and agricultural policies. In addition, plants can be used as a source of proteases. However, they are

governed by several factors such as the availability of land for cultivation and the suitability of climatic conditions for growth [13]. However, the number of industrially important proteases from plant origin is limited [12].

2.3.2. Microbial Proteases

Due to the discouragement of protease production from plant and animal sources, it has directed to an increased interest in microbial proteases to meet the present world demands of the enzyme [12]. The presence of desired characteristics for biotechnological applications in microbial protease enzymes helps it to be preferred over plant and animal proteases [22]. Microorganisms represent an excellent source of enzymes owing to their wide biochemical diversity and their susceptibility to easy genetic manipulation, due to their fast growth and simplicity of life for the generation of new recombinant enzymes with desired properties. Furthermore, most of the enzymes in microorganisms are extracellular, with no need for cellular disruption. Microorganisms have over a two-third share for commercial protease production in the enzyme market across the world. About 40% of the total global enzyme sales are from microbial sources [11].

2.4. Factors Affecting Aspartic Protease Production

The production of extracellular protease from microorganisms is greatly influenced by media components and physicochemical factors. Thus, various carbon and nitrogen substrates, divalent metal ions, environmental and fermentation parameters such as pH, incubation temperature, time, agitation speeds, inoculums ages and density have significant effect on protease production [24].

2.4.1. Carbon and Nitrogen Source

Enzyme production is highly influenced by carbon and nitrogen sources. A number of substrates such as glucose, sucrose, fructose, glycerol, and wheat bran, rice bran, soya bean cake, lupine sunflower meal can be used as carbon sources for protease production. Different studies indicated that a high carbohydrate concentration can suppress enzyme production and hence optimum amount of carbohydrates can be added either continuously or in aliquots (fed-batch) throughout the fermentation to complement the exhausted component [22].

Organic nitrogen sources like soya bean meal, corn step liquor, soya oil, corn glutan as well as inorganic nitrogen such as nitrates or ammonium salts and amino acids are the most important nutrients used for protease production. Therefore, complex nitrogen source is preferred as it is slowly degraded in medium resulting in the availability of low levels of amino acids/peptides in the medium which act as inducers of protease production [21].

2.4.2. Temperature and Incubation Time

Temperature and incubation time are the most important factors on acid protease production. Most of aspartic protease producing microorganisms requires an optimum temperature ranging from 30-55°C [11]. Microorganisms also require an incubation period ranging from 24 to 144 h for the maximum yield of protease [32].

2.4.3. Initial Media pH and Metal Ions

Most microorganisms require optimum pH for their maximum growth and enzyme production. The culture pH also strongly affects many enzymatic processes and transport of various components across the cell membrane [22]. Most of the aspartic protease producing microorganisms shows the best activity at low pH (pH 3 to 6) [5, 11]. In addition, most of the aspartic proteases (Aps) have isoelectric points in the pH range of 3 to 4.5 [11].

2.4.4. Fermentation

Based on the type of fermentation, solid or submerged strongly impacts the growth of the microorganism and their enzyme production [35]. Solid state fermentation is a fermentation process in which microorganisms grow on solid state material without presence of free liquid. In submerged fermentation, microorganisms grow in suspended form in liquid media supplemented with protein-rich sources like soya bean meal, wheat bran, casein, corn step liquor and soya oil [30].

Solid-state fermentations contain, undissolved substrate, in which microbial cultures are grown on a moist solid with little or no free water, although capillary water may be present. Based on their cost of economics, solid state fermentation has more efficient than liquid state fermentation [35].

2.5. Application of Microbial Acid Protease

Proteases are one of the most essential groups of enzymes and have various applications in different industries, such as detergents, foods, pharmaceuticals, and leather [17]. Certainly, proteases which are used in food and detergent industries are prepared in bulk quantities and used as crude preparations; whereas those that are used in medicine are produced in small amounts but require extensive purification before they can be used [28].

Among the protease enzyme, microbial acid proteases have extensively used in three industries: food, beverage, and pharmaceuticals [38]. They have potential in application of various food and feed industry such as dairy industry (milk protein; casein and whey protein hydrolysis for use in cheese flavor development), baking industry (treatment of flour in the manufacture of baked goods and improvement of dough texture, flavor, and color in cookies.), brewing industry, wine industry, soy protein hydrolysis, soy sauce production, gelatin hydrolysis, meat protein recovery, fish protein hydrolysis and meat tenderization and improves digestibility of animal feeds [35, 11].

2.5.1. Dairy Industry

In dairy industry, acid proteases are mainly used for the manufacture of cheese. In cheese making, the primary function of aspartic proteases is to hydrolyze the specific peptide bond to generate *p*-k-casein and macro peptides [17]. The most important property of acidic proteases is the ability to coagulate milk proteins (casein) to form curds from which cheese is prepared after the removal of whey. Hence, based on

such properties, microbial acidic proteases have primarily replaced the calf enzyme (rennet) and enabling the development of the cheese manufacture industry. The proteases produced by GRAS (generally regarded as safe) microbes such as *Bacillus subtilis*, *Mucor michei*, and *Endothia parasitica* have been used for cheese production. Furthermore, proteases are involved in lactose reduction and flavor modification in dairy industry [8, 40].

During normal pasteurization process, the proteolytic activity of aspartic protease produced from fungal origin is highly reduced. Aspartic proteases are primarily produced by some fungal genera such as *Aspergillus, Mucor, Entothia, Rhizopus, Penicillium* and *Fusarium*. In most cases, these fungi are characterized based on their adaptation in solid-state fermentation (SSF) using cheap substrates. Hence, solid state fermentation improves the recovery of extracellular enzymes with high yields, solve pollution problems and reduce the capital costs of process [41, 27].

2.5.2. Beer Industry

In brewing industry, acid proteases are used for extracting sufficient proteins from malt barley and in the production of brewing wort. Proteases are used to solubilize protein from barley adjuncts, thereby releasing peptides and amino acids which can fulfill the requirement of the nitrogen supply. Moreover, proteolytic enzymes are used in chill proofing, a treatment designed to prevent the formation of precipitates during cold storage [3]. Due to the presence of proteinanceous substances, hazes are becoming the most challenging in brewery industries. In beer, these substances also have the ability to precipitate polyphenols and oligosaccharides. Therefore, to prevent the accumulation of insoluble complex, there is a need to hydrolyzing protein components using aspartic protease enzyme. The degradation of turbidity complex from various protein-rich foods improves the quality of alcoholic liquors [35].

Various features of beer such as clarity, color, and foam are vital for consumers. For example, foam affects the consumers' views; flavor and mouth feel about the beer. Brewers desire the presence of sufficient, stable, white, and finely textured foam to satisfy consumers' concerns [14]. In beer production, haze formation is a major problem since it affects the qualities of the end product [34]. In such process, proteins and polyphenols extracted from the plant tissue may interact and form haze [15].

Beer haze has contains various components: the most common organic parts are proteins (40–75%), polyphenols (in combination with proteins), and, to a smaller percentage carbohydrate (2–15%). The group of proteins that contributes to haze formation is called "cystine-rich proteoses" [11]. During beer fermentation, the addition of acidic protease efficiently inhibits chill haze formation in bottled beer without affecting the desirable beer foam [15]. In addition, aspartic protease has a significant role to increase fuel ethanol production by improving the capability of the yeast to metabolize soluble proteins [7].

2.5.3. Wine Industry

The production of clear wine, particularly for white wines, is one of the most important parameters from the consumer's perspective. Therefore, maintaining the stability of wine before bottling is a challenging and crucial step in the winemaking process [39]. The microbial stability of wine can be maintained with the addition of sulfur dioxide and filtration, whereas tartrate stability is kept by three techniques, such as cold stabilization, ion exchange resins, and/or electrodialysis [42]. However, heat unstable grape protein could remain and cause a hazy appearance in final wine products. Under certain conditions, grape proteins exist as unstable, aggregate into light-dispersing particles and cause wines to appear turbid. Specifically, grape pathogenesis-related (PR) proteins, such as thaumatin-like proteins (TLPs) and chitinases, contribute for wine haze formation [18].

3. Conclusion

In conclusion, aspartic proteases are amongst the most important groups of protease enzymes that can speed up thousands of biochemical reactions under acidic conditions. Aspartic protease is commonly produced by plants, animals and microorganisms. Due to several reasons, microorganisms are best candidates for aspartic protease production. Even though aspartic proteases are used in different industries, it is commonly applied in food, beverage, and pharmaceutical industries. Moreover, different environmental and physiological factors have an influence for the maximum production of aspartic protease. Therefore, having further investigation, aspartic proteases have promising future in different industrial applications.

Conflicts of Interest

The authors declare that they have no competing interests.

Data Availability

Data will available on request.

References

[1] Abidi, F., Chober T, J. -M., Haertlé, T. & marzouki, M. N. (2011). Purification and biochemical characterization of stable alkaline protease Prot-2 from Botrytis cinerea. *Process Biochemistry,* 46, 2301-2310.

[2] Alariya, S. S., Sethi, S., Gupta, S. & Lal, G. (2013). Amylase activity of a starch degrading bacteria isolated from soil. *Archives of applied science Research,* 5, 15-24.

[3] Daniel Yimer & Ameha Kebede. (2014). *Production and Characterization of Bacterial Protease from Isolates of Soil and Agro-Industrial Wastes.* Haramaya University.

[4] Ghani, M., Ansari, A., Aman, A., Zohra, R. R., Siddiqui, N. N. & Qader, S. A. U. (2013). Isolation and characterization of different strains of Bacillus licheniformis for the production of commercially significant enzymes. *Pak. J. Pharm. Sci,* 26, 691-697.

[5] Gomri, M. A., Rico-Díaz, A., Escuder-Rodríguez, J.-J., EL Moulouk Khaldi, T., González-Siso, M.-I. & Kharroub, K. 2018. Production and characterization of an extracellular acid protease from thermophilic Brevibacillus sp. OA30 isolated from an algerian hot spring. Microorganisms, 6, 31.

[6] González-rábade, N., Badillo-Corona, J. A., Aranda-Barradas, J. S. & Del Carmen Oliver-Salvador, M. (2011). Production of plant proteases in vivo and in vitro—a review. *Biotechnology advances,* 29, 983-996.

[7] Guo, Z. P., Zhang, L., Ding, Z. Y., Wang, Z. X. & Shi, G. Y. (2010). Improving the performance of industrial ethanol-producing yeast by expressing the aspartyl protease on the cell surface. *Yeast,* 27, 1017-1027.

[8] Gupta, R., Beg, Q., Khan, S. & Chauhan, B. (2002). An overview on fermentation, downstream processing and properties of microbial alkaline proteases. *Applied microbiology and biotechnology,* 60, 381-395.

[9] Hassan, N., Rafiq, M., Rehman, M., Sajjad, W., Hasan, F. & Abdullah, S. (2018). Fungi in acidic fire: A potential source of industrially important enzymes. *Fungal Biology Reviews*.

[10] Hsiao, N.-W., Chen, Y., Kuan, Y.-C., Lee, Y.-C., Lee, S.-K., Chan, H.-H. & Kao, C.-H. (2014). Purification and characterization of an aspartic protease from the Rhizopus oryzae protease extract, Peptidase R. *Electronic Journal of Biotechnology,* 17, 89-94.

[11] Jermen Mamo & Fassil Assefa (2018). The role of microbial aspartic protease enzyme in food and beverage industries. *Journal of Food Quality*.

[12] Khandelwal, H. B. (2013). Production, purification and characterization of fungal alkaline protease and its applications.

[13] Kumar, D. M., Premavathi, V., Govindarajan, N., Nalakumaran, M. & Kalaichelvan, K. (2012). Production and purification of alkaline protease from Bacillus sp. MPTK 712 isolated from dairy sludge. *Global Veterinaria,* 8, 433-439.

[14] Lewis, M. J. & Lewis, A. S. (2003). Correlation of beer foam with other beer propeties. *Technical quarterly & the MBAA communicator*.

[15] Lopez, M. & Edens, L. (2005). Effective prevention of chill-haze in beer using an acid proline-specific endoprotease from Aspergillus niger. *Journal of agricultural and food chemistry,* 53, 7944-7949.

[16] Maitig, A. M. A., Alhoot, M. A. & Tiwari, K. (2018). Isolation and Screening of Extracellular Protease Enzyme from Fungal Isolates of Soil. *Journal of Pure and Applied Microbiology,* 12, 2059-2068.

[17] Mandujano-Gonzalez, V., Villa-Tanaca, L., Anducho-Reyes, M. A. & Mercado-Flores, Y.(2016). Secreted fungal aspartic proteases: a review. *Revista iberoamericana de micología,* 33, 76-82.

[18] Marangon, M., Van Sluyter, S. C., Robinson, E. M. C., Muhlack, R. A., Holt, H. E., Haynes, P. A., Godden, P. W., Smith, P. A. & Waters, E. J. (2012). Degradation of white wine haze proteins by Aspergillopepsin I and II during juice flash pasteurization. *Food Chemistry,* 135, 1157-1165.

[19] Mishra, S. & Behera, N. (2008). Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African Journal of Biotechnology,* 7.

[20] Mukhtar, H. (2009). Production of acid protease by Aspergillus niger using solid state fermentation. *Pakistan Journal of Zoology,* 41.

[21] Nadeem, M. (2009). *Biotechnological production of alkaline protease for industrial use.* University of the punjab, Lahore, Pakistan.

[22] Nirmal, N., Shankar, S. & Laxman, R. (2011). Fungal proteases: an overview. *International Journal of Biotechnology & Biosciences,* 1, 1-40.

[23] Patel, G. (2015). Isolation and characterization of starch degrading bacteria from garden soil, Ganpat University, Gujarat, India. *Indian Journal of Microbiology Research,* 2, 111-114.

[24] Qureshi, A. S., Bhutto, M. A., Khushk, I. & Dahot, M. U. (2011). Optimization of cultural conditions for protease production by Bacillus subtilis EFRL 01. *African Journal of Biotechnology,* 10, 5173-5181.

[25] Sandhya, C., Nampoothiri, M. K. & Pandey (2005a). Microbial protease, in Microbial Enzyme and Biotransformation. *J. L. Barredo*, 165–180.

[26] Sandhya, C., Sumantha, A., Szakacs, G. & Pandey, A. (2005b). Comparative evaluation of neutral protease production by Aspergillus oryzae in submerged and solid-state fermentation. *Process biochemistry,* 40, 2689-2694.

[27] Sathya, R., Pradeep, B., Angayarkanni, J. & Palaniswamy, M. (2009). Production of milk clotting protease by a local isolate of Mucor circinelloides under SSF using agro-industrial wastes. *Biotechnology and Bioprocess Engineering,* 14, 788-794.

[28] Sawant, R. & Nagendran, S. (2014). Protease: an enzyme with multiple industrial applications. *World Journal of Pharmacy and Pharmaceutical Sciences,* 3, 568-579.

[29] Shivakumar, S. (2012). Production and characterization of an acid protease from a local Aspergillus sp. by Solid substrate fermentation. *Archives of Applied Science Research,* 4, 188-199.

[30] Siala, R., Frikha, F., Mhamdi, S., Nasri, M. & Sellami Kamoun, A. (2012). Optimization of acid protease production by Aspergillus niger I1 on shrimp peptone using statistical experimental design. *The Scientific World Journal.*

[31] Singh, R., Kumar, M., Mittal, A. & Mehta, P. K. 2016. Microbial enzymes: industrial progress in 21st century. 3 Biotech, 6, 1-15.

[32] Souza, P. M., Werneck, G., Aliakbarian, B., Siqueira, F., Ferreira filho, E. X., Perego, P., Converti, A., Magalhães, P. O. & Junior, A. P. (2017). Production, purification and characterization of an aspartic protease from Aspergillus foetidus. *Food and Chemical Toxicology,* 109, 1103-1110.

[33] Souza, P. M. D., Bittencourt, M. L. D. A., Caprara, C. C., Freitas, M. D., Almeida, R. P. C. D., Silveira, D., Fonseca, Y. M., Ferreira FILHO, E. X., Pessoa Junior, A. & Magalhães, P. O. (2015). A biotechnology perspective of fungal proteases. *Brazilian Journal of Microbiology,* 46, 337-346.

[34] Steiner, E., Becker, T. & GASTL, M. (2010). Turbidity and haze formation in beer—Insights and overview. *Journal of the Institute of Brewing,* 116, 360-368.

[35] Sumantha, A., Larroche, C. & Pandey, A. (2006). Microbiology and industrial biotechnology of food-grade proteases: a perspective. *Food Technology and Biotechnology,* 44, 211.

[36] Synowiecki, J. 2010. Some applications of thermophiles and their enzymes for protein processing. African Journal of Biotechnology, 9, 7020-7025.

[37] Szecsi, P. B. (1992). The aspartic proteases. *Scandinavian Journal of Clinical and Laboratory Investigation,* 52, 5-22.

[38] Theron, L. W. & Divol, B. (2014). Microbial aspartic proteases: current and potential applications in industry. *Applied microbiology and biotechnology,* 98, 8853-8868.

[39] Van sluyter, S. C., Mcrae, J. M., Falconer, R. J., Smith, P. A., Bacic, A., Waters, E. J. & marangon, M. (2015). Wine protein haze: mechanisms of formation and advances in prevention. *Journal of agricultural and food chemistry,* 63, 4020-4030.

[40] Vishwanatha, K. (2009). *Acid protease from Aspergillus oryzae: Structure-stability and enhancement of the activity by physical, chemical and molecular biological approaches.* University of Mysore.

[41] Vishwanatha, K., Rao, A. A. & Singh, S. A. (2010). Acid protease production by solid-state fermentation using Aspergillus oryzae MTCC 5341: optimization of process parameters. *Journal of industrial microbiology & biotechnology,* 37, 129-138.

[42] Waters, E. J., Alexander, G., Muhlack, R., Pocock, K., Colby, C., o'neill, B., Høj, P. & Jones, P. (2005). Preventing protein haze in bottled white wine. *Australian Journal of Grape and Wine Research,* 11, 215-225.

[43] Yegin, S. & Dekker, P. (2013). Progress in the field of aspartic proteinases in cheese manufacturing: structures, functions, catalytic mechanism, inhibition, and engineering. *Dairy science & technology,* 93, 565-594.