



## Various Extraction Methods of Different Enzymes and their Potential Applications in Various Industrial Sector (a review)

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### Abstract

Enzyme's existence has been reported since the mid-nineteenth century, and they have been widely employed in a variety of industrial operations. Because of their unique properties, they have a wide range of uses in a variety of industries. They have been widely used in textiles because of their biodegradability, non-toxicity, and environmental friendliness. The major areas in the textile sector for enzyme-induced processes are lignin degradation, hydrogen peroxide degradation, textile bleaching, desizing and so on. Amylases, peroxidase, protease, cellulase, laccase and catalase are the most common and widely utilised biocatalysts in the textile business. The current review focuses on the role of different enzymes, their extraction from natural sources and their applications in the textile industry.

Keywords: Amylases, peroxidase, protease, cellulase, laccase; extraction; application

### Introduction

Recently, there is a higher awareness regarding environmental problems. The growing expectation that scientists should improve processes to make them greener and more sustainable had also resulted in the development of green chemistry, which aims to develop new products and processes that are less hazardous to human health and the environment by eliminating or reducing the use and production of harmful substances, as well as reducing harmful or toxic intermediates.[1-6]

Experts define biotechnology as "Harnessing the power of living organisms to make industrial processes cleaner, cheaper, and more sustainable." [7] Biotechnology is expected to revolutionise the textile industry during the next decade. [8] It is expanding into new and unexpected areas of the textile industry, with the potential to lower prices, preserve the environment, solve health and safety concerns, and enhance quality and functionality. [9]

Textile production has frequently been known as one of the most polluting sectors. Processors are increasingly attempting to substitute enzymes that operate as biocatalysts for as many harmful chemicals as feasible.[10] Enzymatic pre-treatments are the most well-established use of biotechnology in textiles.[9]

Enzymes were discovered in the second half of the nineteenth century and have since been widely employed in a variety of industrial operations.[11] Enzymes are proteins that are active and can catalyse biological processes,[11, 12] that comprise metabolism in living systems. [10, 13, 14] This is protein structure has a very high molecular weight that contains over 250 amino acids and has separate active sites at the intramolecular level. These active sites are in charge of carrying out catalytic reactions.[11]

Enzymes are increasingly being employed in the textile industry due to their following nature: non-toxic, environment-friendly, produce cleaner

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processes and minimise the usage of raw materials and waste production.[11, 12]

Enzymes can also conduct a wide range of chemical reactions and are widely employed in the detergent, food, pharmaceutical, diagnostic, and fine chemical industries.[15, 16] Now enzymes are an essential element of the textile manufacturing process.[7, 11, 17-20] They speed up the rate of a chemical process without incurring any permanent chemical change, making them real catalysts. In comparison to the commonly employed chemical catalysts, enzymes proved to be more effective. [11, 14, 21] After the reaction is finished, the enzyme is released, ready to begin another reaction. Most enzymes are typically utilised once and then eliminated following their catalytic function.

All known enzymes are proteins.[18] As a result, they are composed of one or more polypeptide chains and exhibit protein-like characteristics. Many chemical and physical conditions (such as salt content, temperature, and pH) affect the rate of enzyme catalysis,[17] which may be explained by their effect on protein structure. Some enzymes require tiny non-protein molecules known as cofactors to operate as catalysts. [9, 17, 21]

Enzyme use is advantageous for practical applications because of the ease of separation of enzymes from a reaction mixture, higher stability and recyclability, prevention of interactions with interfaces, improved activity, selectivity, specificity, resistance to inhibitions, reused easily, reduced environmental impact, limited usage of water and even improved purity.[1, 7, 11, 12, 14] However, there are several disadvantages to enzymes, such as reduced enzyme activity during the reaction compared to native enzymes and poorer reaction speeds compared to native enzymes.[1, 7, 12]

Commercial enzymes are derived from three basic sources: animal tissue, plants, and microbes.[7] Plants are the primary nutritional supplies of all living species and have been for a long time. Furthermore, plant compounds are employed as enzymes in the food industry and other life-sustaining supplies.[22]

There is a separate enzyme for each sort of reaction in a cell, and they are categorised into six major categories: hydrolytic, oxidising and reducing, synthesising, transferring, lytic, and isomerising. The catalytic function is a key property of enzymes.[12, 17-19]

## Extraction of enzymes from natural sources

### 2.1. Polyphenol oxidase enzyme

Polyphenol oxidase (PPO) is a copper-containing enzyme available in a variety of fruits and vegetables.[23] that can catalyse two distinct processes, including the oxidation of diphenols

molecules to quinones and molecular oxygen.[12, 24] And the hydroxylation of monophenol compounds to diphenols. It should be noted that PPO is found in living species such as birds, fungi, insects, mammals, and numerous types of plants and maybe isolated for diverse purposes.

PPO is a component of the plant's defensive system. When a plant bruises or is cut, phenolic chemicals oxidise in the presence of oxygen, generating a polymeric structure that protects the plant against microbial infection.[23-25]

#### 2.1.1. Extraction of Polyphenol oxidase enzyme from banana peel

The banana is a major food source in poor nations, as well as one of the most important global crops. It has nutritional and health benefits. The goal of this study was to find the best conditions for extracting polyphenol oxidase from banana peel.[23]

Bananas at the yellow-green stage of maturation were obtained at a local market. All experimental techniques were carried out at a temperature of 4 °C. The crude extract was prepared as follows: banana peel was cut into small pieces and then homogenised in phosphate buffer containing ascorbic acid and polyethylene glycol, followed by extraction using a blender. The crude extract was filtered, and the filtered extract was centrifuged. And the suspension was utilised as a crude enzyme.[23]

#### 2.1.2. Extraction of Polyphenol oxidase enzyme from apricot, apple, eggplant and potato tubers

Plants, fruits and vegetables are major sources of enzymes and other biological substances.[11] Our study's goal was to extract and characterise polyphenol oxidase from apricot, apple, eggplant, and potato.[24]

Apricot, apple, eggplant and potato tubers were purchased from the local market. The enzyme was isolated by homogenizing sliced fruits with cold potassium phosphate buffer, then filtering through a cheese cloth and centrifuging the homogenate. By adding cold acetone (-5°C) and gently stirring for 60 minutes, the enzyme was precipitated from the supernatant. The precipitate was dissolved with potassium phosphate buffer after the mixture was centrifuged. This crude enzyme extract was utilised to characterise the enzyme.[24]

#### 2.1.3. Application of Polyphenol oxidase enzyme

Polyphenol oxidase enzyme is used as an effective reagent for cleaning wastewater containing polyphenol and also applied as a reducing agent in the eliminating of dyes from the textile substrate. [26]

### 2.1.3.1. Application of Polyphenol oxidase enzyme in the textile industry

In this study Purified oxidase (PPO) from banana fruit pulp, which is easily available all year, was used for the decolourization of several textile colours and does not require any redox mediators, lowering the process cost. PPO enzyme performs oxidative breakdown of the dyes, resulting in no synthesis of amines, which are poisonous and carcinogenic. The application of polyphenol oxidase may be applied to textile industry pollutants.[26]

### 2.1.3.2. Application of Polyphenol oxidase enzyme in decolourization of wastewater

Textile manufacturers produce millions of gallons of treated effluents into public waterways each day, which are eventually released into rivers. These dangerous compounds not only colour the water but are also extremely poisonous to marine and other types of life.

Enzymatic decolourization and degradation are more environmentally friendly and cost-effective and it is an alternative way to the traditional chemicals. This strategy is growing rapidly. Polyphenol oxidase enzymes are capable of acting efficiently on a wide variety of substrates and catalysing the elimination of organic contaminants found in extremely low concentrations at polluted areas in the waste water.[26]

## 2.2. Protease enzyme

Proteases are a broad and diversified collection of hydrolytic enzymes that catalyses the hydrolysis of peptide bonds generated by certain amino acids as well as the cleavage of C-N, C-O, and C-C bonds, characterised by their place of action, active site structure, and unique reaction mechanisms.[11, 27]

Proteases are enzymes found in all organisms that hydrolyse peptide bonds to maintain systemic homeostasis. Proteases are generated from plants, animals, and microorganisms. Among the numerous proteases, bacterial proteases are more important than animal and fungus proteases.[15]

Proteases have a wide range of commercial uses, including the leather, food, brewing and pharmaceutical industries. Protease can hydrolyse protein peptide bonds, a process known as proteolysis, [12, 28] accounting for nearly 60% of total global enzyme sales. Alkaline protease is the most popular protease, accounting for 89 percent of all sales.[29] And the optimum temperature of the enzyme is 37°C and the optimum pH is 8. [30, 31]

### 2.2.1. Extraction of protease enzyme from Moringa Oleifera leaves

Plants are an important source of protease enzymes. [15] Moringa Oleifera is a well-known medicinal plant that is extensively spread in tropical areas. It includes a combination of various hydrolytic enzymes, the most important of which are proteases, which have been shown to have a pharmacological effect. [30]

Moringa Oleifera mature leaves were taken from a plant, samples were crushed homogeneous with a mortar and pestle in a phosphate buffer solution pH 7.0, and centrifuged using a centrifuge machine. The supernatant was collected and treated with more filtration. The crude enzyme protein was precipitated with ammonium sulphate and then purified and we get the crude enzyme [15]

### 2.2.2. Application of protease enzyme

Proteases have a bright future in a variety of industrial applications.[29]

#### 2.2.2.1. Application of protease enzyme in Silk degumming

Sericin or silk gum must be routinely removed from raw silk using a degumming procedure in an alkaline soap solution. Alkaline protease enzymes outperform soap-soda degumming techniques. Because of their managed system and enhanced strength of the final silk thread, The cloth is given excellent handling, pill resistance, and shrink resistance after bio washing and enzymatic finishing with protease.[11]

The protease enzyme is the best choice for removing Sericin without damaging the fibre. It has been demonstrated that fibre break is not accessible, and silk fibres have been discovered to be stronger than earlier conventional treatments.[32]

#### 2.2.2.2. Application of protease enzyme in protein hydrolysis

Proteases are used in the food industry to improve the storage life of all available protein sources. [33] The use of alkaline protease results in high nutritional value preparations of protein hydrolysates. Alkaline proteases are extremely important in meat tenderization. [27, 32]

#### 2.2.2.3. Application of protease enzyme in Food industry

Proteases are added to milk during cheese manufacture. The fundamental role of these enzymes in cheese production is to hydrolyse the particular peptide bond that results in parak-casein and macro

peptides.[12] In the baking industry, protease is used to partially hydrolyse gluten to speed up the production of dough.[7] protease enzyme is also used to hydrolysis of proteins into amino acids and the bioactive peptides created by the hydrolysis of certain food proteins play a vital function in cell antioxidants.[27, 32]

#### 2.2.2.4. Application of protease enzyme in Leather industry

Proteases are essential in the treatment of raw leather in tanneries.[29] Because of the issues with effluent disposal, the use of chemicals in the leather industry is not considered environmentally friendly. The use of enzymes as a substitute has resulted in enhanced leather quality and reduced pollution.[33]

The utilisation of these enzymes is linked to the structure of animal skin as raw material and employed to eliminate undesirable components. In the soaking step, alkaline proteases are applied. This enhances water uptake by dry skins, removes and degrades protein, dust, and lipids, and lowers processing time.[12]

The elastolytic and keratin lytic activity of protease has led to increased utilisation in developing leather industries. Protease is proven to be useful in the Soaking, Bating, and Dehairing phases of skin preparation led to a cleaner and stronger surface, softer leather, and fewer defects. [27, 32]

#### 2.2.2.5. Application of protease enzyme in Detergent industry

Proteases have been widely employed on a commercial basis in the detergent industry.[7] Several detergent industry products containing proteases as an essential component or ingredient have been utilised for cleaning home laundry, dentures, or contact lenses. [27] These proteases facilitate the elimination of any stain, such as blood, egg, or gravy, even under high pH conditions.[29, 33]

And the use of protease in the detergent industry accounts for around 20% of the total. Traditionally, detergents act at high temperatures, but there has been a growing interest in searching for and developing alkaline proteases that work at a wide range of temperatures.[32]

### 2.3. Peroxidase enzyme

Peroxidase is a biotechnologically significant and common enzyme belonging to the oxidoreductase class.[7, 34-37] Peroxidase is the enzyme that catalyses a variety of oxidative processes by using different peroxides (ROOH) as electron acceptors.[13, 38-40]

This enzyme normally catalyses both oxidation and reduction reactions in a wide range of

substances,[7, 41] a reaction between hydrogen peroxide as an electron acceptor and a variety of substrates, [41, 42] through oxygen liberation of a wide spectrum of phenolic and non-phenolic substrates.[7, 8, 11, 14, 35, 37, 43, 44]

The enzyme is naturally found in plants such as potato tuber, horseradish, beet, soybean, tomato, banana, papaya, carrot, turnip, wheat, dates, beets, and strawberries.[34, 38, 45, 46] Peroxidase genes may be found in practically all kingdoms of life [8, 36, 42, 47, 48] And classified into two primary superfamilies: one found mostly in bacteria, fungi, and the other found primarily in animals, and plants. [1, 40, 41, 48-51]

They are Versatile peroxidases that have catalytic properties such as Lignin peroxidase, Manganese peroxidase, and other microbial peroxidases that breakdown down aromatics. [7, 48]

Some innovative uses of peroxidases have been proposed, such as the treatment of wastewater containing phenolic chemicals. [41] Some researchers have recently documented decolourization and removal of textile natural and synthetic dyes from contaminated water and dyeing effluents utilising soluble and immobilised peroxidase. [7, 13, 39, 51]

#### 2.3.1. Extraction of Peroxidase from Horseradish

Few plant enzymes are as well represented in the scientific and patent literature as horseradish peroxidase (see **Figure 1**). currently, horseradish is the primary source of commercially available peroxidase.[11, 34, 45]



**Figure 1:** An early representation of the horseradish plant [52]

Horseradish peroxidase (HRP) isoenzyme is a single polypeptide of 308 amino acid residues.[41, 47, 52] They are widely used in research fields such as enzymology, biochemistry, medicine, genetics,

physiology, history and cyto chemistry. Because of their ease of availability, low cost, and high catalytic activity.[40]

Horseradish Peroxidase has several unique uses, the most recent of which include the treatment of wastewater containing phenolic compounds, waste decolourization,[37] and the removal of peroxide from foodstuffs and industrial effluents.[50] Although the word horseradish peroxidase is used a lot fairly, the plant's root includes a variety of different peroxidase isoenzymes. [52]

Horseradish roots were crushed into small pieces and floated in phosphate buffer solution (pH 7.5), then vacuum filtered through filter paper. The volume was increased with a working buffer, and the extract was sonicated continuously in ice. Then the solution was centrifuged after three sonication times. The residue with low peroxidase activity was removed, and the clear supernatant fluid with high HRP activity was recovered. The crude extract is the name given to this preparation.[34, 50]

### 2.3.2. Extraction of Peroxidase from turnip roots

The current research aims to propose a methodology for the easy, quick, and low-cost synthesis of a peroxidase-rich enzymatic extract suitable for use. not only in biotransformation processes but also for industrial applications. The procedure is targeted to produce peroxidase-rich enzyme extracted from turnip roots, [7] collected from a marketplace and ready to be used as a catalyst in biotransformation activities. The turnip root was chosen because of its availability. [1]

The turnip roots should be washed and peeled, And then the peeled turnip roots, diced. Add a cold phosphate buffer with a pH of 6.5 to the chopped root. Using a professional blender, homogenise the mixture while immersed in ice until there are no lumps. To remove suspended fibrous solid particles, filter the homogenised mixture using cotton fabric. Centrifuge the filtrate, then collect and freeze the supernatant. The result showed that it produced an extract with high levels of protein per mL and high total peroxidase activity in the shortest amount of time and with the fewest intermediary steps.[1]

### 2.3.3. Extraction of Peroxidase from soybean seed

Plant peroxidases are gaining popularity due to their diverse bioactivation capabilities and prospective uses in clinical, biochemical, biotechnological, and other fields. Recent improvements have been achieved in employing them to synthesise organic molecules, which are very valuable chemicals, under moderate and regulated

conditions. [51] According to the literature, soybean has 35 - 50% protein, 18 - 22% fat, and 23 - 25% carbs. Soybean also contains peroxidase ferment, which is a bioindicator for plant resistance.[36]

This study analysed the purifying strategies of PO from soybean meal and reviewed the extraction parameters. [36, 39] Soybean peroxidase (SBP), is one of the cheap byproducts of soybean seed hulls.[7] It has been found in the root, leaf, and seed hulls of soybeans. When compared to PO derived from other components of the soybean plant, PO obtained from seed hulls has the highest activity. [51]

An enzyme derived from soybean hulls is used as a formaldehyde substitute in adhesives, abrasives, protective coatings, and other applications. Because soybean peroxidase is more practical and less costly than horseradish peroxidase (HRP), which has been the focus of most wastewater research, its use to catalyse the polymerization and precipitation of aqueous phenols by hydrogen peroxide is potentially promising. [51]

In the local market, soybean hulls were obtained. All materials were kept at 4°C until testing. [39] Soybean hulls were immersed in phosphate buffer, followed by stirring at room temperature to allow for cell structure destruction and protein solubilization, Extracts were centrifuged. And then the extract was filtered through four layers of cheesecloth, and the filtrate was centrifuged again to eliminate cell debris. The supernatant was collected and kept at 4°C as a source of crude SBP enzyme. The enzyme solution was quickly warmed to room temperature before use.[34, 36, 39, 51]

### 2.3.4. Extraction of peroxidase enzyme from sweet potato

Potato and sweet potato peroxidase might be proposed as a replacement option for horseradish peroxidase, particularly in Ethiopia, where both plants can be procured cheaply and relatively fresh. [34] Sweet potato peels are reported to be rich in peroxidase enzyme, so we are going to determine the enzyme activity and yield of the enzyme peroxidase isolated from sweet potato. [45]

Sweet potatoes were peeled and the peelings were utilised as the sample. A phosphate buffer solution with a pH of 7.0 was added to the sample and well homogenised using a mortar and pestle. The enzyme was filtered via cloth. This extract was centrifuged, and the supernatant was kept. The supernatant was first boiled and then being placed in an ice bucket. This crude extract was kept in a refrigerator and was brought to room temperature for future use (see **Figure 2**).[45, 46]



**Figure 2:** The blank and crude extract enzyme [45]

### 2.3.5. Extraction of peroxidase enzyme from Garlic

Chemical treatment using traditional methods is typically limited by significant waste creation, high prices, the need for an external reagent, and is time-consuming. As a result, alternative treatment methods based on biotechnological principles have grown in favour in recent years. One of these biotechnological processes is the extraction of peroxidase from garlic plants.[47]

Peeled garlic was cut into tiny pieces and then homogenised in a pH 6.5 phosphate buffer solution and maintained at 29 °C with continual shaking on a mechanical shaker at low speed. The homogenate was filtered through two layers of cheesecloth. Centrifugation was performed on the filtrate. The supernatant was collected and kept as crude enzyme extract at 10°C till used.[47]

### 2.3.6. Extraction of peroxidase enzyme from waste cabbage leaves

It has been discovered that various fruit and vegetable wastes include a variety of enzymes. Many reusable goods of high value with various industrial uses like agro-waste residual matter may be transformed into cost-effective commercial products using appropriate technology.

Due to the lack of storage facilities, cabbage leaves and other vegetables typically rot and go to waste at the market. Therefore the waste of cabbage leaves can be used as a source of peroxidase and may be more effective as it does not compete with food consumption and is a method of recycling agro-waste pollution, which causes environmental problems. As a result, the purpose of this study was to isolate waste cabbage peroxidase to determine its potential capacity to biodegrade azo dyes and phenol from an aqueous solution. [44]

The cabbage waste was collected from the vegetable market. Peroxidase was isolated from waste cabbage leaves using a slightly modified technique. Waste cabbage leaves were cut into small

pieces and then homogenised with HCl buffer. The homogenate was filtered using a clean cloth stacked in two layers, and the filtrate was centrifuged. The supernatant was carefully collected and filtered using filter paper into a clean tube. The crude waste cabbage peroxidase extract was incubated in a water bath and chilled on ice.[44]

### 2.3.7. Application of peroxidase enzyme

Peroxidases are essential in terms of industrial applications because of their capacity to catalyse the oxidation-reduction process of a wide range of phenolic and non-phenolic substrates in the presence of hydrogen peroxide. [1, 38, 40] Peroxidases have been used in a variety of applications, including agriculture, bioremediation, textile, synthetic dye decolourization, polymer synthesis, the paper and pulp industry, biosensor development, and medical supplies.[8, 14, 40, 41]

#### 2.3.7.1. Application of peroxidase enzyme in Textile Industry

The textile industry, as one of the main industrial sectors, consumes significant amounts of water, chemical products, and synthetic colours, and produces large volumes of wastewater with a high organic load, which is responsible for acute or chronic toxicity on ecosystems, so it is necessitating the use of proper treatment technology.[1, 8]

Peroxidases are used for dye bleaching in textile industries because they are excellent in removing a wide range of synthetic dyes [7, 48] Peroxidases have been proven to have a high potential for reducing pollutant residues such as phenolic compounds and most synthetic dyes with complex aromatic structures in the textile sector during the decolourization process, [7] and a wide range of chemical groups may be degraded efficiently and without the need of redox mediators. [35, 41, 42, 48] And it is considered an eco-friendly solution for removing hydrogen peroxide from the wastewater and also from the fabric during bleaching and before dyeing without causing fibre damage.[8, 11, 53] Short-time exposure or enzymatic pre-treatment were shown to be enough for improving fabric whiteness. [11]

The substitution of hydrogen peroxide with an enzymatic bleaching method would not only result in higher product quality due to less fibre damage but would also result in significant savings in washing water, energy, the time required for hydrogen peroxide removal (see **Figure 3**). [17]



**Figure 3** bleaching process using enzymes [11]

### 2.3.7.2. Application of peroxidase enzyme in Food Industry

Elimination of remaining hydrogen peroxide whenever possible, such as in, milk production, egg product processing, cheese manufacturing, also used as a leavening agent in baking and other applications where hydrogen peroxide is utilized as an oxidant or a disinfectant. [48]

### 2.3.7.3. Application of peroxidase enzyme in pulp and paper industry

Lignin, is a phenolic polymer, has a negative influence on the pulp and paper industry. As a result, lignin should be eliminated before the creation of high-quality paper. Lignin breakdown using enzyme is proposed as a preferable option. Peroxidase is effectively utilized in the paper industry for selective delignification, which facilitates the creation of high-quality paper. [8, 12]

### 2.3.7.4. Application of peroxidase enzyme in Hair dyeing

Traditionally, hair colouring dyes are created using the oxidative polymerization of dye components. This approach employs the utilisation of hydrogen peroxide (3%) to activate the polymerization reaction. When used regularly, it might cause hair damage. Enzymes like peroxidases have been recommended as preferable possibilities for achieving a softer colouring with a milder oxidation process. [8]

## 2.4. Lipase enzyme

Lipase enzymes play a significant role in the changeover of these water-insoluble molecules, which make up a major portion of the earth's biomass. [16]

Lipases are becoming more important among all enzymes. They are employed in the majority of enzyme applications. Many researchers have investigated enzymatic hydrolysis of fats and oils as an appealing alternative to the currently utilised high pressure and high-temperature techniques for

commercial fatty acid synthesis. Lipases from various origins and optimum conditions for high lipolytic activity were studied. [20]

Lipases are a kind of enzyme that catalyses the hydrolysis of a wide variety of carboxylic esters synthesised from glycerol and long-chain fatty acids. [12, 20, 54] Lipases have been isolated from a wide range of plant, animal, bacterial, fungal, and yeast species. [20] Lipases derived from microbes are commercially important and employed in a variety of sectors, including dairy, food, detergents, textile, pharmaceutical, cosmetic, and biodiesel. [16, 54, 55] Many lipases are active in organic solvents, catalysing a variety of beneficial processes such as esterification. [54]

### 2.4.1. Extraction of lipase enzyme from Sunflower Seed

Plant lipases, which have high activity above room temperature, are cheap, and simple to produce, are particularly appealing for some industrial uses. [55]

Sunflower seeds are significant food and industrial oil seeds for the majority of the world's inhabitants. The seed proteins remain in the meal after the oil from the seeds is extracted, and the meal is commonly used to feed animals in many nations. We believe that using sunflower seed enzymes for industrial, food, and research purposes is a good idea. [55]

For preliminary studies, sunflower seeds were utilised. The dried, crushed seed was agitated in three batches with three cold solvents of varying polarity for defatting. The solvent phase was decanted after each stirring interval, and the fresh solvent was added to the crushed seed. The same method ratio was then applied for the chosen seed and solvent. Unless otherwise specified, this protocol was followed in each experiment. The extractions were all done at 4 degrees Celsius. The defatted seed powder was left to air dry, which effectively eliminated all solvents. The residue was suspended in phosphate buffer (pH 7) with  $\text{CaCl}_2$  overnight (as a lipase activator). In the preliminary trials, the buffer concentration, as well as the calcium chloride concentration, were changed. The extract's activity was shown to be highest when the phosphate buffer concentration was combined with calcium chloride. To eliminate seed cell remnants, the buffer suspension was gently centrifuged. Fractional salt precipitation was performed on the entire supernatant. And then the lipase enzyme was recovered after the filtration of the solution. [55]

## 2.4.2. Application of lipase enzyme

Lipases have interesting applications in organic chemical processing, detergent formulations, biosurfactant synthesis, the oleochemical industry, dairy industry, agrochemical industry, paper manufacturing, nutrition, cosmetics, and pharmaceutical processing.[20, 54]

### 2.4.2.1. Application of Lipases in the detergent industry

Lipases are widely used as additives in commercial laundry and home detergents due to their capacity to hydrolyze lipids. When exposed to water, they catalyse the breakdown of chemical bonds, allowing the fats to be removed.[12]

Detergent enzymes make up nearly 32% of the total lipase sales. [12, 20] Lipase for detergent applications must be thermostable and active in the alkaline environment of a normal machine wash.[10] Detergent lipases are specifically chosen to meet the following criteria: (1) a low substrate specificity, the ability to hydrolyze fats of various compositions, (2) the ability to resist relatively harsh washing conditions, (3) the ability to resist damaging surfactants and enzymes, which are important ingredients in many detergent formulations. [54]

### 2.4.2.2. Application of Lipases in the food industry

Lipases have the potential to be used as emulsifiers in foods, medicines, and cosmetics.[12]

Fats and oils are major food components. Lipases allow us to change the characteristics of lipids by rearranging the fatty acid chains in the glyceride and substituting one or more fatty acids with new ones. In this technique, a low-cost, less desired lipid can be converted into a higher-value fat. Lipases have also been required to develop flavours in cheese ripening, bread products, and drinks. Lipases are also utilised to help in the removal of fat from meat and fish products. [20, 54]

Cocoa butter is a fat with a significant commercial value in the confectionery industry, particularly in the chocolate sector, due to its beneficial characteristics. The high price of cocoa butter is caused by its unavailability. As a result, interesterification in common and less costly fats such as illipe fat, shea butter, sal fat, and kokum butter by using lipase enzyme provides a viable option for the creation of less expensive cocoa butter alternatives.[20]

### 2.4.2.3. Application of Lipases in Ester Synthesis

Lipases have been employed effectively as a catalyst in the production of esters. Short-chain fatty acid esters can be used as flavouring agents in the food sector. Long-chain acid methyl and ethyl esters have been utilised to enhance diesel fuels.[54]

## 2.5. Catalase enzyme

Catalase is found in a broad variety of species, including mammals, plants, and nearly all aerobic microbes.[56-58] The catalase enzyme is an oxidoreductase enzyme because it is important in reducing reactive oxygen species (H<sub>2</sub>O<sub>2</sub>) and produces water and oxygen.[58] As a result, it functions as an antioxidant and protects the cell from oxidative damage. Catalase has one of the greatest turnover rates, with one molecule of enzyme hydrolyzing nearly a million molecules of the substrate (hydrogen peroxide) each second. [59] Additionally, it eliminates the electrons that might contribute to the formation of superoxide free radicals. Catalase has the potential to be used in the textile, paper, food, and dairy sectors, not only as an environmentally friendly alternative to carcinogenic chlorine but also to recycle enormous amounts of water. [56]

### 2.5.1. Extraction of Catalase from Sweet Potato Tubers

The current study addresses the extraction of catalase from a crude sweet potato extract utilising a quick and inexpensive approach known as three-phase partition by optimising parameters such as t-butanol concentration, salt concentration, and pH.

Three-phase partition TPP has been used to purify proteins due to its comparatively easy and low-cost purification process. TPP is made up of protein precipitation in the intermediate phase when crude protein extract is combined with t-butanol as an organic solvent and ammonium sulphate as a salt. The higher organic solvent phase has more enzyme inhibitors, whereas the lower aqueous phase contains more polar components. [56]

Catalase was extracted from fresh potato tubers. In a nutshell, fresh potato roots were properly cleansed with distilled water before the skins were removed. A sample was then broken into small pieces and homogenised in PVP, of phosphate buffer, pH 7, with EDTA, and KCl (potassium chloride) before being homogenised and mixed in a +4 °C refrigerator for a minute. The homogenate was centrifuged after being filtered through five layers of cheesecloth. The clear supernatant was used to create a crude enzyme extract for future investigation. TPP precipitation of proteins resulted in greater catalytic efficiency in

some circumstances. The protein exhibits great flexibility as a result of structural alterations caused by TPP enzyme molecule treatment, and increased conformational flexibility demonstrates better catalytic activity compared to untreated protein. [56]

### 2.5.2. Application of catalase enzyme

Catalase is thought to play a significant part in the breakdown of hydrogen peroxide which leads to the formation of oxygen and water.[11] The use of a commercial catalase to remove hydrogen peroxide residues from cotton fibres significantly enhanced dyeing behaviour and colour output using a reactive bifunctional mono-florotriazinyl dye.[12, 53, 60] Also shown the use of catalase in the treatment of H1N1 pneumonia. Aside from its usage in biomedical and clinical diagnosis, catalase has been patented for the removal of hydrogen peroxide from contact lenses. [61]

#### 2.5.2.1. Application of catalase enzyme in the textile industry

The bleaching of textile fabric or yarn is now the most prevalent procedure in the textile industry. The bleaching reaction is catalyzed by hydrogen peroxide and the enzyme. Once the bleaching step is finished, the residual hydrogen peroxide in the container is left out, and total elimination of hydrogen peroxide is required for the dyeing process. Unfinished or partial hydrogen peroxide removal is inefficient and results in poor dyeing, which results in a decline in colour, shade, strength, and uneven dye circulation.[11]

#### 2.5.2.2. Application of catalase enzyme in the food industry

Catalase is related to food processing and helps to improve the nutritional content of food.[58] Catalase also promotes health and protects against a variety of age-related diseases.[61] Vegetables and fruits contain a high concentration of catalase. The most often employed catalase enzyme in the food business is glucose oxidase, which is utilised for food preservation and egg processing which prevents food from oxidizing, and is useful for removing sulphhydryl groups formed by heat induction and which create bad flavour in ultra-pasteurized milk. Catalase enzyme is also used in the processing of milk to eliminate hydrogen peroxide before the creation of cheese. [58]

## 2.6. Cellulase enzyme

Cellulases are one of the most often used industrial enzymes, Because of their capacity to

change cellulosic fibres in a controlled and preferred fashion to increase fabric quality.[11, 12] and they have been commercially accessible for more than 30 years. These are inducible enzymes produced by a variety of microorganisms, including bacteria and fungi, during their growth on cellulose materials. Bacterial and fungal cellulases are typically composed of two or more functional and structural components linked by a peptide linker. [10]

Cellulases, are more stable at high temperatures than other plant cell wall dissolving enzymes, making them a better choice for industrial applications. As a result, significant attempts have been made to isolate cellulases microbes. Bacterial cellulose has several benefits when compared to fungal cellulase, which includes enhanced specific activity, and greater stability. [10]

### 2.6.1. Extraction of Cellulase enzyme from fungi

Fungi are a varied collection of microorganisms that play a variety of roles in human life. Therefore, the isolation of viable fungal species is important to their use in several areas, including the enzyme sector. We wanted to isolate local fungus species and use them to make cellulase enzymes. [62]

To extract cellulase enzyme, commercial crude cellulase powder generated by fungi by solid-state fermentation was combined with water, stirred gently at 30°C for 1 hour, filtered, and centrifuged. The crude enzyme was the cleared supernatant.[63] Approximately the crude enzyme was saturated with ammonium sulphate and left overnight at 4°C. For further purification, the material was centrifuged again and dissolved in sodium acetate buffer.[64]

### 2.6.2. Application of Cellulase enzyme

Even though cellulases have been used in commercial sectors for more than three decades, this enzyme remains a topic of interest for both academic research and industry. Their wide uses in the textile, animal food, medical, detergent, and paper processing industries placed them second in the worldwide industrial enzyme market in terms of sales volumes. [10, 62]

#### 2.6.2.1. Application of Cellulase enzyme in the textile industry

The use of enzymes, particularly in textile manufacturers, has a long history. Enzymes are employed in various pre-treatment and finishing procedures for cotton. They are also used to treat other natural fibres. Enzymatic degumming of silk with sericinases, felt-free finishing of wool with proteases, and so on are examples. [17]

### 2.6.2.1.1. Application of Cellulase enzyme in Bio polishing

Bio polishing is a finishing technique that enhances fabric quality by eliminating fuzziness and pilling in cellulosic fibre and making it flexible and soft.[11] The process's goal is to eliminate cotton microfibrils by the action of the cellulase enzyme. [10] In the traditional method Once the fibres' chemicals are removed, the surface and texture of the fabric are destroyed, whereas biopolishing using enzymes is a form of permanent treatment that keeps the fabric in excellent condition after multiple repeated washing cycles and also improves the product's quality.[11]

The following are the primary qualities given to the fabric during the biopolishing treatment using enzyme: (a) cleaner surface with a cooler feel. (b) As a result, lustre is obtained. (c) The fabric becomes softer. (d) The fabric's tendency to pull at the ends. [17]

### 2.6.2.1.2. Application of Cellulase enzyme in treatment of denim

Denim is a high-quality cotton fabric, in which the dye is mainly adsorbed over the surface of the fibre.[11]

In the conventional washing procedure of denim fabric, sodium hypochlorite or potassium permanganate were employed as Pumice stones and this process has many disadvantages such as back-staining which is common with the use of pumice stones, A considerable quantity of pumice stones is required, and they cause a lot of wear and strain on the equipment.[11, 17]

These limitations give the way to the procedure of using enzymes. In denim washing, the enzyme cellulase is utilised. In a technique known as "Bio-Stonewashing," cellulase works by releasing the indigo dye on the denim. [10, 11] It eliminates the disadvantages caused due to use of the stones, such as damage to the washers and garments, handling and environmental problems.[12] A little amount of enzyme can substitute for several 1kg of pumice stones. Using enzymes leads to less damage to garments and machines, as well as less dust in the laundry environment.[17]

### 2.6.2.1.3. Application of Cellulase enzyme in Bioscouring

The scouring process includes the removal of synthetic or natural material existing on the cotton surface. The mixture of Cellulase and pectinase enzymes are utilised for bio-scouring. Pectinase helps in the destruction of the cotton cuticle by flouting pectin and eliminating the connectivity

between the cuticle and the cotton fibre, while cellulase dissolves the main wall cellulose. The process is soft during enzymatic scouring and aggressive during alkali scouring. Enzymatic scouring reduces exposure to health problems because no harsh chemicals are used.[11]

### 2.6.2.2. Application of Cellulase enzyme in Animal Feed Industry

Another application for cellulase enzyme is in the animal feed sector. It may be used to increase the nutritional value of animal feed by pre-treatment of grain feed and agricultural straw.[12] Furthermore, cellulases destroy anti-nutritional components such as oligosaccharides and lignin, which have an impact on animal health. [10]

### 2.6.2.3. Application of Cellulase enzyme in olive oil extraction

Olive oil has several health advantages and is often used at home. Cellulases are used in the extraction of olive oil. Their use leads to reduced waste, an increase in antioxidant components, and improved quality and extraction yield.[10]

## 2.7. Amylase enzyme

Amylases are hydrolytic enzymes that break down glucosidic linkages in starch to low molecular weight sugars, dextrin and maltose. [11, 17, 65] There have been reports of amylase sources in plants, animals, and microorganisms. [66] Amylases are among the most important enzymes and have a significant impact on biotechnology, representing a class of industrial enzymes that accounts for roughly 25% of the global enzyme market. Today, a huge variety of amylases are commercially available, and they have nearly replaced chemical starch hydrolysis in the starch processing application. [65]

### 2.7.1. Extraction of amylase enzyme from malted rice

The tests in grain malting have resulted from the need for an alternate source to replace the expensive imported microbial -amylase enzymes. Malting is a process that involves soaking, germination, and drying of cereal seeds with the main aim of encouraging the formation of hydrolytic enzymes that are not present in raw seeds. According to the results of research on various grains, rice has the highest -amylase production during malting.[66]

Paddy rice was purchased locally and kept at an average room temperature. The rice seeds were washed in water, the cleaned grains were then immersed in new water in a plastic container at 28°C

for 24 hours. The soaked grains were allowed to grow for 12 days at a temperature of 26°C while being irrigated twice a day. Using a blender machine, the dried samples for each day were processed to finer particles, the powder was kept in a refrigerator. Distinct extraction mediums were employed. Initially, sodium phosphate buffer at pH 8 was utilised as the extraction medium for the quantitative evaluation of amylase enzymes produced throughout the 12-day malting phase, because of the high extractive ability of amylase from malted rice. The milled malt sample was first placed in the centrifuge, followed by the phosphate buffer. After 30 minutes, the enzymes were allowed to extract in the extraction media and then filtered and kept in a cold place. [66]

### 2.7.2. Application of amylase enzyme in the textile industry

Amylases are used in the textile industry to help in the desizing process. Sizing agents such as starch which added to yarn before fabric manufacture to facilitate a quick and secure weaving process.[11] Starch is a highly appealing size because it is inexpensive, widely available in most parts of the world, and removed easily. [65] Amylases are used to eliminate starch-based sizing for better and more uniform wet processing. The benefit of these enzymes is that they are starch specific, eliminating it without destroying the support fabric. [11, 12] An amylase enzyme can be employed for desizing procedures at low temperatures (30 to 60°C), with an ideal pH of 5.5 to 6.5.[11, 17]

### 2.7.3. Application of amylase enzyme in Detergent industry

Enzymes are mostly used in the detergent industry. The use of enzymes in detergent formation improves the detergent's capacity to remove hard stains while also making the detergent environmentally friendly. Amylases are the second kind of enzyme utilised in the formation of enzymatic detergent, and they are found in 90% of all liquid detergents. These enzymes are employed in detergents for laundry and automatic dishwashing machines to break down starchy food residues such as potatoes, chocolate, and others to dextrins and other smaller oligosaccharides. Amylases have activity at lower temperatures and alkaline pH, allowing them to maintain essential stability under detergent conditions. Amylases' oxidative stability is one of the most essential reasons for their usage in detergents when the washing environment is highly oxidising. [65]

### 2.7.4. Application of amylase enzyme in the food industry

Amylases are widely used in the processed-food industry, including baking, brewing, the production of cakes and fruit juices. These enzymes may be added to bread dough to decompose starch in flour into smaller dextrins, which are then fermented by yeast. The addition of -amylase to the bread increases the speed of fermentation and decreases the viscosity of the bread, resulting in increased volume and texture of the product. Furthermore, it produces extra sugar in the bread, which improves the bread's flavour, crust colour, and baking properties. [65]

## 2.8. Lacasse enzyme

In recent years, enzymes have gained increasing importance in the industry; laccases are one type of enzyme that is available in nature.[11] Laccases are the most ancient and well-studied enzymatic systems. Laccases are multicopper enzymes that belong to the blue oxidase family, [67] and oxidise diphenol using molecular oxygen as an electron acceptor. [68] This enzyme is highly specific, environmentally friendly, and an effective catalyst.[69]

This enzyme's biotechnological importance derives from its capacity to oxidise both phenolic and non-phenolic lignin-related chemicals.[68]

Laccases are found in a broad variety of higher plants, bacteria, fungi, and insects. they can be found in plants such as cabbages, turnips, potatoes, pears, apples, and other vegetables.[67-69]

Laccase catalysis occurs as a result of the reduction of one oxygen molecule to water. [68] Followed by the oxidation of one electron with a wide spectrum of aromatic compounds, including polyphenol.[69]

### 2.8.1. Extraction of Lacasse enzyme from potato peels

Potatoes, along with wheat, rice, and corn, are among the most essential staple crops for human nutrition. It is a major source of starch in a vegetarian diet. [70]

Potato peels include a diverse range of chemicals that might be employed in both food and non-food applications.

Potatoes are often peeled during processing, resulting in large volumes of peels that provide a major disposal problem for the industry. The purpose of this study was to determine the presence of laccase in potato peels. This was achieved by extracting, purifying, and characterising laccase from potato peels.[68]

Potato peels were collected from a nearby potato chips company. The obtained material was combined with distilled water. This suspension was diluted and

then spread over the surface of Potato dextrose agar with Chloramphenicol for 7 days at 30°C.

In a potato dextrose agar plate, the fungal strain was inoculated. Following incubation, the plates were examined for the development of reddish-brown zones surrounding the colony. Potent strains were grown in a liquid medium and incubated on a rotating shaker for 15 days at 37°C. Following the incubation period, the flask contents were filtered through filter paper and the solution was centrifuged. The resulting supernatant was used as the enzyme extract in the research. The protein solution was then put into a beaker with a magnetic bar and stirred at 4°C and a little amount of ammonium sulphate were added, allowing it to dissolve before adding the next quantity. Finally, the beaker was left to stand overnight and the enzyme was obtained.[68, 70]

### 2.8.2. Application of Lacasse enzyme

Laccase is useful as it oxidises both harmful and nontoxic substrates. Laccase is now attracting a lot of interest because of its numerous applications, such as dye decolorization, and bioremediation applications in the textile, food, and wood processing industries, as well as the pharmaceutical and chemical industries.[67, 68, 70]

#### 2.8.2.1. Application of Lacasse enzyme in Dye Decolorization

Wet processing in the textile industry requires a considerable amount of water and chemicals. These chemicals include both inorganic and organic substances. When dyes are exposed to light, water, and other substances, their chemical structure prevents them from fading. Laccase destroys dye, that's why laccase-based techniques that contain synthetic dyes have been created and are currently employed in the industry.[69]

#### 2.8.2.2. Application of Lacasse enzyme in Paper and Pulp Industry

At the industrial level, chlorine and oxygen-based chemical oxidants are employed for the isolation and degradation of lignin, which is necessary for the manufacturing of paper. However, other issues remain, such as recycling, cost, and toxicity. As a result, we decided to use Lacasse enzyme rather than a chemical substance.[69]

#### 2.8.2.3. Application of Lacasse enzyme in Food Processing Industry

Laccase is used in the food sector to remove unwanted phenolic compounds in baking, juice

preparation, and wastewater biological treatment.[69]

### Summary

Enzymes are presented in the perspective of sustainable technologies as biodegradable, biological agents capable of catalysing a wide range of reactions in water and organic media, and this study includes the extraction of different enzymes from various natural sources and their application in the industry which are used in a wide range as they are ecologically friendly and have become an alternative solution for all chemical-based procedures in nearly all textile processes.

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