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Systematic Review on Characterization of Tannase from Agricultural By-Products

Tinjauan Sistematis Karakterisasi Enzim Tannase dari Produk Sampingan Limbah Pertanian

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Abstract

Tannin-rich compounds are widely produced as by-products of many agro-industrial processes. Tannase is an attractive hydrolase for the bioconversion of tannin-rich materials into value-added products by accelerating the hydrolysis of ester and depside linkages. It has opened new opportunities in several industrial sectors, such as food, drinks, and medicines. Primary sources of tannase are microorganisms, particularly bacteria. Tannases or tannin acyl hydrolases, are an important group of biotechnologically relevant enzymes in several industrial applications. Microbial tannases are mostly induced extracellular enzymes produced by submerged and solid-state fermentation. Tannins containing low-value agro-industrial wastes are being extensively used in industries. This review provides a more in-depth knowledge of the research related to the biochemical characteristics of the tannase enzyme activity (in terms of molecular weight, the effect of pH, the effect of temperature, the effect of metal ions, inhibitor, and chelator), extraction, and purification methods. Additionally, the potential use of agricultural waste as a substrate for tannase production has also been reviewed, including the utilization of pomegranate peel waste (PmPW), banana peel waste (BPW), or potato peel waste (PtPW).
Keywords: agricultural waste, tannase, tannin

Abstrak

Senyawa kaya tanin diproduksi secara luas sebagai produk sampingan dari banyak proses agroindustri. Tannase *adalah hidrolase yang menarik untuk biokonversi bahan kaya tanin menjadi produk bernilai tambah dengan mempercepat hidrolisis ikatan ester dan depside. Hal tersebut telah membuka peluang baru di beberapa sektor industri, seperti makanan, minuman, obat-obatan, dan sebagainya. Sumber utama* tannase *adalah mikroorganisme, terutama bakteri.* Tannase *atau tanin asil hidrolase, adalah kelompok penting dari enzim yang relevan secara bioteknologi yang digunakan dalam beberapa aplikasi industri.* Tannase *dari mikroba sebagian besar diinduksi enzim ekstraseluler dan diproduksi oleh fermentasi terendam dan fermentasi keadaan padat. Tanin yang terkandung dalam limbah agroindustri bernilai rendah banyak digunakan dalam industri. Ulasan ini memberikan pengetahuan yang lebih mendalam tentang penelitian terkait karakteristik biokimia aktivitas enzim tannase (dari segi berat molekul, pengaruh pH, pengaruh suhu, pengaruh ion logam, inhibitor, dan chelator), metode ekstraksi dan pemurnian. Selain itu juga telah dikaji potensi pemanfaatan limbah pertanian sebagai prekursor ekstrak tannase, antara lain pemanfaatan limbah kulit buah delima (PmPW), limbah kulit pisang (BPW), atau limbah kulit kentang (PtPW).*
Kata Kunci: *limbah pertanian,* tannase, *tanin*

INTRODUCTION

Most commercial enzymes utilized today are of microbial origin since they typically demonstrate considerably increased activity compared to enzymes of plant and animal origin, exemplifying an alternate source of enzymes as reported previously by Dhiman et al. (2018). A significant number of industrial enzymes (about 65%) are "hydrolases". Tannase (E.C.3.1.1.20) is the popular name for the

tannin acyl hydrolase, which may catalyze the hydrolysis of bonds in hydrolysable tannin molecules (Aharwar & Parihar, 2018; Mahmoud et al., 2018) into simple phenolic compounds such as gallic acid. Tannase is widely present in the industry with applications in food, animal feed, cosmetics, pharmaceutical, chemical, and leather industries, among others (Al-Mraai et al., 2019; Mahmoud et al., 2018). The bacteria that generate tannase are a significant source for industries. *Aspergillus* sp. and *Penicillium* sp. are two of the most frequent tannase-producing fungi, and they may utilize both hydrolyzable and condensed tannin. On the other hand, the most prevalent tannase-producing bacteria is *Bacillus* sp. (Aharwar & Parihar, 2019).

Although of great commercial importance, this enzyme has not been fully explored due to a lack of information on the production medium or solid substrates, as well as the catalytic activity and characteristics (Aharwar & Parihar, 2018). A few studies have been reported on the production of tannase from agricultural wastes (Mahmoud et al., 2018). Due to their genetic complexity, fungi degrade substrate rather slowly and are difficult to manipulate genetically, which is a major issue in using fungal strains for industrial purposes (Jana et al., 2014). While several studies have focused on the screening, generation, characterization, and purification of tannase from various sources, these reviews have yet to investigate biomass applications derived from natural sources thoroughly. As a result, this review attempted to examine the natural tannins involved in tannase production (Lekshmi et al., 2021). These by-products (peels, seeds, shells, pomace, and leaves) contain bioactive compounds (phenols, peptides, carotenoids, anthocyanins, and fatty acids) as well as fibers and enzymes that can be used to make functional foods, drugs against acute and chronic diseases, and antioxidants for the cosmetic industry. The Food and Agriculture Organization (FAO) defines these by-products as food losses and waste (FLW) that occur throughout food production (Osorio et al., 2021). Economic feasibility of the pomegranate peel waste (PmPW), banana peel waste (BPW), or potato peel waste (PtPW) valorization route is dependent on a wide range of variables. Over 1,000 academic papers have been published in the past decade, nearly 90% on the different PmPW, BPW, or PtPW valorization routes globally. In animal feed, soil amendment, or as bio-adsorbents, PmPW, BPW, or PtPW waste may be utilized directly or after treatment, and the outputs may be obtained via the biorefinery process (biogas, bioethanol, and biohydrogen). PmPW, BPW, or PtPW have been extensively studied for their potential to be converted into high-value goods (such as essential oils, enzymes, food, and pharmaceuticals) via various methods. Consumers' acceptance of reused or waste-based goods must be encouraged, as must their understanding of valorization and marketing prospects in other industries. Innovative upgrading technologies must be coupled with new business models and marketing strategies to use of AgW and by-products efficiently.

Food waste has recently become a significant problem in Malaysia, a country recognized for its amazing food (Md Sani et al., 2022). The Star (2021) reported that Malaysians create around 38,699 tonnes of solid trash daily, at least 1.17 kg per person. Although Malaysia has many landfills, the number is insufficient to handle the massive waste generated. As the population grows, solid waste is expected to continue stacking up. The world's waste production has skyrocketed in the last several decades and shows no signs of slowing down. The amount of municipal solid waste generated worldwide in 2050 is predicted to rise by 70%, reaching 3.4 billion metric tonnes. Many food businesses are researching methods to turn waste into profit in response to this concern. Growing concern about the effects of excessive material consumption has given rise to attempts in many places to reduce the quantity of garbage transported to the landfill. By-products from one business may be useful commodities in another, resulting in less waste (El-Maghraby et al., 2014). However, compared to fungal tannase, prior assessments have focused less on bacterial tannase.

The present communication summarizes the current knowledge and accomplishments in bacterial tannase. Significant papers on bacterial tannase have been published, including discoveries (Dhiman et al., 2022; Jana et al., 2014). Since the 1990s, there has been a considerable emphasis on bacterial strains producing tannase. As a result, it is crucial to compile all accessible information on bacterial tannase for future research. Previously, the sources of bacterial tannase, culture techniques, downstream processing, key biochemical and structural properties, immobilization, recombinant synthesis, and future possibilities have been explored (Jana et al., 2014). The main purposes of this review are to provide more in-depth knowledge of the research related to the biochemical characteristics of the tannase enzyme activity (in terms of molecular weight, the effect of pH, the effect of temperature, the effect of metal ions, inhibitor, and chelator), extraction and purification methods. Additionally, the potential use of agricultural waste

as a precursor to extract tannase has also been reviewed, including the utilization of pomegranate peel waste (PmPW), banana peel waste (BPW), or potato peel waste (PtPW).

METHODS

Selection of Articles

Papers published from 2009 to 2021 were reviewed from significant scientific databases to enrich the most current literature (Science Direct, Research Gate, Scopus, and Google Scholar) to gain information on agricultural wastes (AgW) and to determine the biochemical characterization of tannase enzyme activity. The search term used to retrieve the published articles, were 'tannase' and 'agricultural waste' under the (Article title, Abstracts, and Keywords). In addition, the term 'tannase' was used under the [Search within results] to be specific. Papers that lacked relevant information on the tannase, agricultural waste, and potential application were eliminated. The literature review was advanced by reviewing the abstracts, significant findings, and relevant text of the publications to narrow the field of proper research. The chosen papers were uploaded to a desktop referencing system for full-text analysis and data extraction. Relevant details and metadata were collected, tabulated, and counted for qualitative and quantitative analysis. About 3,658 articles were found through a database search, and six extra articles, including government reports and newspaper articles. Figure 1 illustrates the PRISMA diagram, which explains the methods for identifying suitable research projects.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagram

Researchers reviewed each article after generating the list of references to see whether it should be included along with data extraction and analysis. The screening and quality evaluation were carried out in two stages; (1) selection of primary studies based on titles and abstracts, and (2) assessment based on the authors of the study's full text. Figure 1 shows the procedure in the PRISMA diagram, which includes the number of studies discovered throughout the search, screening, and quality evaluation phases.

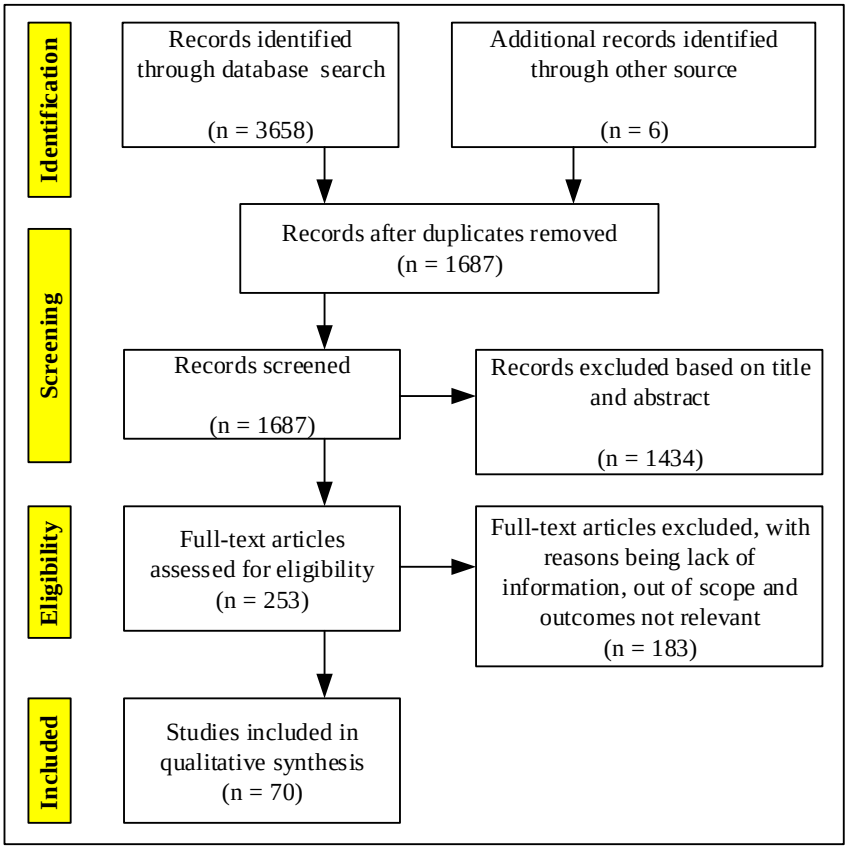

Figure 1. PRISMA Flow Chart Demonstrating the Selection Process and Criteria

DISCUSSION

Agricultural Waste

Agricultural waste (AgW) is residual material from numerous agricultural products, such as coffee pulp from the coffee industry, cereal husks from cereal production, and starch-based peels from the starch-based industry. Every year, a substantial number of these residues are created as agricultural operations result, which will ultimately degrade and negatively impact agricultural resources, human and animal health, and the environment if suitable waste management measures are not implemented (Yusree et al., 2021). As mentioned by Donner et al. (2021), agricultural waste (AgW) has three lignocellulosic components consisting of hemicellulose (10.5-40.4 wt.%), cellulose (25.0-44.2 wt.%) and lignin (21.7-44.0 wt.%) with an unconsidered quantity of ash and extractives. Depending on the biomass amount, AgW and by-products can be converted into valuable resources through enhanced conversion processes, resulting in new value-added products such as bioenergy, bio-fertilizers, biomaterials, and biomolecules. The conversion of residues is critical for assisting in decoupling economic growth and human well-being from the use of primary resources, as well as preventing land pressure, detrimental impacts on biodiversity, and risking global food security (United Nations Environment Programme & International Resource Panel, 2011). Previously fruit waste was also valuable in decreasing tannic-acid absorption in tannery effluent (Irfan et al., 2020).

The potato, pomegranate, and banana industries create large amounts of waste yearly. Landfills can contaminate surface water, groundwater, air, and soil. Degradation of AgW by landfill microorganism results in changes in their chemical composition, forming several pollutants consisting of CO_2, CH_4, CO, H_2S, and others. Landfill leachates interact with soil, surface water, and groundwater, deteriorating their quality and impacting populated biodiversity. It contains inorganic substances (Cd^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}), chloride (Cl), sulfates (SO_4^{2-}), organic matter (volatile fatty acids), xenobiotics (pesticides and herbicides), microbes, aromatic hydrocarbons, phenols, oil, hydraulic fluids, and other chemicals.

These wastes also pose pollution hazards during their treatment and valorization, such as the impact of organic additions on soil organisms (El Barnossi et al., 2021). The most common problems with landfill disposal include fires and explosions, vegetation damage, landfill odors, air pollution, climate change, and groundwater pollution. Landfills can harm human health by contaminating soil, air, surface water, or groundwater. Direct health risks include the absorption of harmful compounds produced by landfills and direct or indirect exposure to contaminated soil and water from consuming contaminated food from the environment. Many other publications have linked public exposure to odor control systems, especially landfills, to respiratory and other non-specific diseases.

Agricultural Waste (AgW) and By-Products Conversion

Donner et al. (2021) highlighted that depending on the biomass amount, AgW and by-products could be converted into valuable resources through enhanced conversion processes, resulting in new value-added products. The conversion of residues is critical for assisting in decoupling economic growth and human well-being from the use of primary resources, as well as preventing land pressure, detrimental impacts on biodiversity, and risking global food security (United Nations Environment Programme & International Resource Panel, 2011).

According to Torres-León et al. (2018), food waste is a natural resource with enormous potential and diverse uses in food formulations, notably in underdeveloped nations where it is generated. These biomolecules may be isolated from the biological matrix and utilized as food to maximize the nutritional and functional components found in food waste and byproducts. New government measures are required to develop infrastructure and technology that permits waste and byproducts to be used in production and storage (Figure 2). In general, several established traditional methods exist for disposing of fruit peels, PmPW, BPW, and PtPW, including landfilling and incineration. Traditional systems cannot handle the daily volume of peels. These practices harm the environment and human health, and their use costs the global economy billions of dollars every year. Thus, their physicochemical and microbiological features must be accurately determined to valorize wastes properly. This part explores the utilization of PmPW, BPW, and PtPW without regard to their composition and then the valorization of these peels regarding their chemical, microbiological, and product contents.

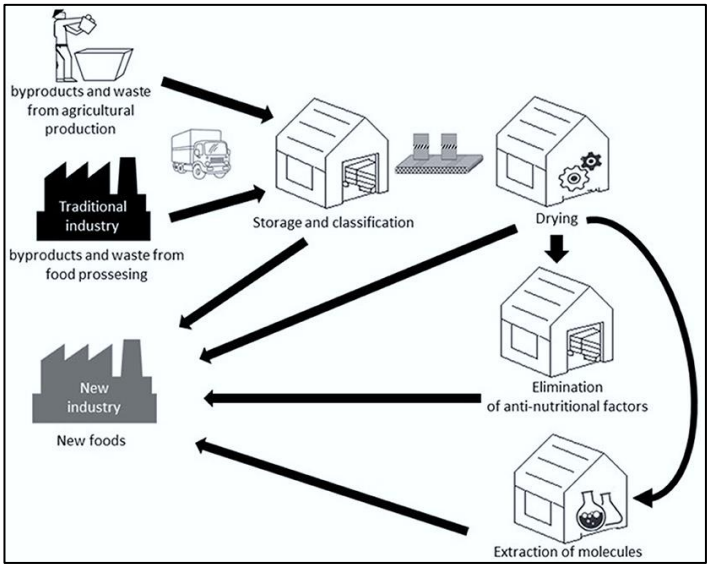

Figure 2. Technical Ideas for Recycling Food Waste and By-Products in Food Development (Torres-León et al., 2018)

The Tannase Enzyme Production from Agricultural Waste (Agw)

Pomegranate (*Punica granatum* L.*)* peel is a significant by-product of fruit processing. It is an attractive source of natural antioxidants and bioactive ingredients, yet accounts for about 40% of the fruit's total weight; however, it is often discarded. It is worth considering how to extract the natural antioxidants from pomegranate peel to boost the exploitation of this by-product (Silva et al., 2021). A "superfruit" known as pomegranate, rich in ellagitannins, has been utilized in functional beverage production due to its intense human antioxidant activity. A subclass of hydrolyzable tannins known as ellagitannins contains one or more hexahydroxydiphenoyl (HHDP) molecules in a glucopyranose core, which the tannase enzyme may destroy during lactic acid fermentation. Several powerful antioxidant phenolic acids are made from ellagitannins, including gallic, ferulic, quinic, caffeic, and ellagic acids (Ruiz Rodríguez et al., 2021).

The most important agricultural products after wheat, corn, and rice, the staple food for 1.3 billion people, are potatoes (*Solanum tuberosum* L.*),* with more than 368 million tonnes produced worldwide in 2018. About 5,000 types of potato are known, making it the most genetically diverse crop among cultivated species. In addition to being one of the most consumed crops, potato production is also linked to one of the largest food processing industries across the world (Sampaio et al., 2020). According to previous research, potato peel is a significant source of antioxidants and antibacterial chemicals, such as bioactive phenolics and glycoalkaloids (Thakur et al., 2021). Even though glycoalkaloids are poisonous, they have therapeutic value (Ostreikova et al., 2022). Potato peel is a lignocellulosic waste that may be used to make bioethanol and biobutanol. The carbohydrate content of potato peel is high (approximately 25%), but the number of fermentable sugars is low. Enzymatic hydrolysis of peel starch is necessary to recover reducing sugars for fermentation-related applications. Alternatively, polysaccharides may be converted into valuable biomolecules. It has been found that the enzymatic degradation of water-soluble polysaccharides in potato peel generated functional oligosaccharides (Ben Amara et al., 2022).

Bananas are tropical fruits available year-round that belong to the Musaceae family and are native to Southeast Asian nations. The particular species is *Musa sapientum* (El Barnossi et al., 2021). Banana is the fourth most significant food crop in developing nations, after rice, maize, wheat, and the world's second most important fruit crop. It is also the most widely consumed fruit in the world (Bhushan et al., 2019). Edible components of the fruit are just 12% of the plant's total weight, resulting in a massive quantity of agro-industrial waste such as peel, which is usually employed in industrial processes to create new goods (chips, dried pulps, jams, sauces, beer, and wine). Banana peels account for 30-40% of the fruit's weight. After bananas are processed, a substantial peel is collected and discarded as waste. As they are spread throughout the planting area or burnt, these leftovers pose a severe pollution hazard. The increased number of banana processing companies has resulted in a tremendous increase in the

accumulation of banana trash, necessitating the development of an effective technique to utilize these leftovers (Torres-León et al., 2018).

Extraction of the Tannase Enzyme

Enzyme extraction from AgW has long been known due to its potential industrial uses. The capacity to lower production costs and increase enzyme performance for industrial uses is considerably boosted by the valorization of AgW. Actinobacterial enzymes derived economically from AgW have been investigated as a means of reusing waste biomass. It is a tannic acid hydrolysis enzyme that breaks the ester and side bonds with the glucose and gallic acid in its primary components (Figure 3 and Figure 4) in its central units (Aharwar & Parihar, 2018; Al-Mraai et al., 2019) tannic acid, methyl gallate, ethyl gallate, propyl gallate, and isoamyl gallate are some of hydrolysable.

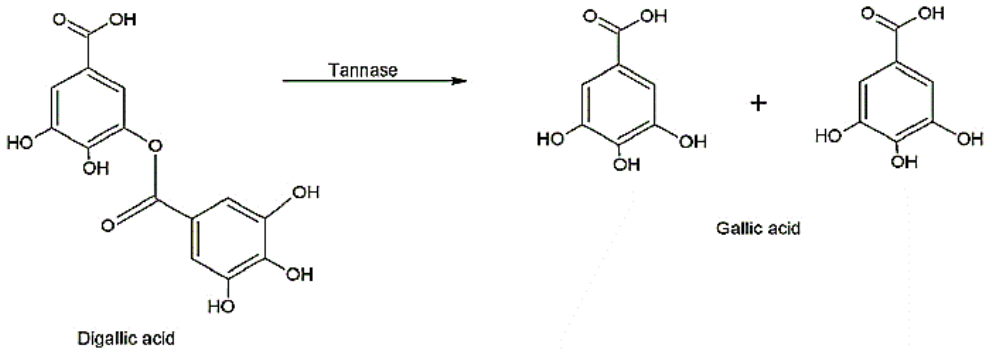

Figure 3. Depsidase Activity of Tannase – the Breakdown of Digallic Acid

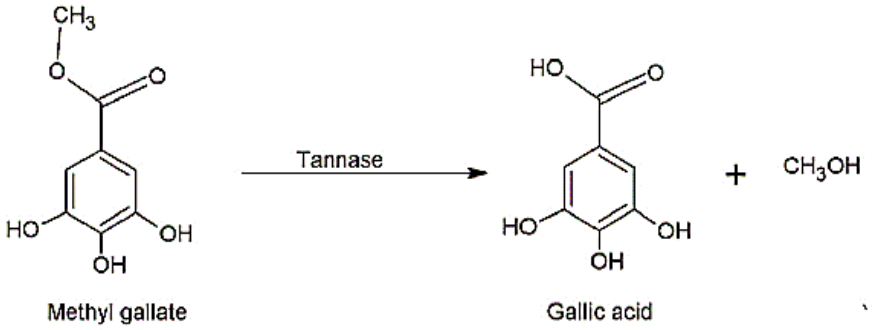

Figure 4. Esterase Activity of Tannase – the Breakdown of Methyl Gallate

Tannase Production

Submerged fermentation (SmF) and solid-state fermentation (SSF) were the two kinds of fermentation systems that were most often used in the production of bacterial tannase until recently. SmF is based on cultivating microorganisms in a liquid medium containing nutrients. SSF is based on the microbial growth and product formation of solid particles in water's absence (or near absence). However, the substrate contains sufficient moisture for the microorganism's growth and metabolism (Martins et al., 2011). Determining a competent production method depends on various factors, such as the strain used throughout the production process, nutrient accessibility, variability, and substrate quality (Dhiman et al., 2018).

Submerged Fermentation (SmF)

Jana et al. (2014), state that a high oxygen-concentration liquid nutrient medium is used to grow microorganisms in submerged fermentation. The SmF approach is widely employed in commercially manufactured tannase using bacteria and yeast (Aharwar & Parihar, 2018). Producing tannase via SmF

has several benefits, including greater process control, more efficient substrate usage, and a shorter incubation period.

Tannase synthesis by SmF might vary widely depending on many nutritional and physicochemical variables. Tannic acid, a carbon source, has been used as an inducer at a 1% concentration. However, some researchers reported improved tannase synthesis above this concentration. As a carbon source, gallic acid has an inductive effect in certain circumstances (Aharwar & Parihar, 2018). For the tannase enzyme glucose formation, fructose and arabinose are among the additional carbon sources. Generally, above 1% glucose content inhibits tannase synthesis and the development of the microorganism. Nitrogen sources such as $NaNO_3$, NH_4Cl, and NH_4NO_3 are used to boost the synthesis of inorganic forms of nitrogen. Yeast extract, for example, is an organic nitrogen source, but its complexity and low concentration reduce output (Aharwar & Parihar, 2018). Traces of metal ions are needed in the manufacture of tannase, such as Mg^{2+} ($MgSO_4$), Fe^{2+} ($FeSO_4$), and Ca^{2+} ($CaCl_2$) ions, while Mo^{6+} ($NaMoO_4$), Mn^{2+} ($MnCl_2$), and Zn^{2+} ($ZnSO_4$) ions were employed in a very few occasions in the production medium. Temperature, pH 5.0 – 7.5, 100-300 rpm agitation, and 24 – 91 h incubation duration may to determine the amount of tannase produced in SmF (Aharwar & Parihar, 2018).

Solid-State Fermentation (SSF)

SSF has piqued the interest of researchers in recent years, as several studies have shown that SSF may result in higher yields and productivity, as well as better product characteristics, compared with the SmF. Furthermore, because low-cost agricultural and agro-industrial residues are used as substrates, capital, and operating costs are lower than SmF. The low water volume in SSF significantly impacts the process's economy owing to smaller fermenter sizes, reduced downstream processing, reduced stirring, and lower sterilization costs (Martins et al., 2011). The main disadvantage of this type of cultivation is the difficulty in scaling up the process, primarily due to heat transfer and culture homogeneity issues (Martins et al., 2011). However, research efforts have focused on developing bioreactors to overcome these challenges.

As reported by Meini et al. (2021), SSF, which uses agro-industrial waste as a support and carbon source, has shown to be a viable approach for synthesizing industrial enzymes. The microbial growth happened on a wet solid substance or inert support in a continuous gas phase and the lack of free-flowing water in SSF (Jana et al., 2014). However, the advantage of SSF in the generation of tannase was extensively documented. The use of microorganisms in the study for enhanced tannase gives a way to increase the tannin-rich biomass conversion, eventually leading to "greener" technology. SSF is appropriate for manufacturing tannase from natural tannin-rich agricultural leftovers because it mimics the circumstances in which fungi thrive naturally. Compared to SmF, SSF-mediated bacterial tannase synthesis has been used to a lesser degree. According to recent research on SSF tannase synthesis, it has higher productivity, higher activity titers, and greater resilience to pH and temperature fluctuations than SmF (Aharwar & Parihar, 2018). However, unlike submerged tannase synthesis, a literature review revealed that bacterial tannase production by SSF has yet to receive much attention. SSF may also increase solid substrates' nutritional content and detoxify harmful chemicals. Tannic acid is employed as a supplementary carbon source alongside the solid substrate to improve tannase synthesis, with a 1-12% concentration optimal for maximal output. Furthermore, gallic acid, glucose, glycerol, lactose, and sucrose were used as carbon sources to manufacture tannase (Wang et al., 2013).

Gallic acid, on the other hand, had a repressive impact on tannase synthesis, while glucose concentrations below 1% considerably stimulated production (Mandal & Ghosh, 2013). However, beyond this concentration, it acts as an inhibitor. Additional nitrogen sources in simplex forms, such as ammonium chloride, ammonium nitrate, sodium nitrate, and potassium nitrate, were shown to promote tannase synthesis. However, yeast extract inhibited tannase production (Aharwar & Parihar, 2018). Mg^{2+} ($MgSO_4$) is a common metal ion that boosts tannase synthesis in SSF.

Biochemical Characterization of Tannase Enzyme

The characteristics of tannase vary depending on the culture and source; for example, post-translational changes are limited in bacterial tannases, whereas in fungi and yeast, the recognized tannases are glycoproteins. The characteristics of tannase generated by microorganisms vary due to changes in culture conditions and glycosylation (Lekshmi et al., 2021). Several microorganisms have been

researched for their tannases and the many features of tannase, including their molecular weight, pH, temperature, and metal ions.

The Molecular Weight (MW) of Tannase Enzyme

Estimated the MW of tannase using SDS-PAGE in a polyacrylamide gel using standard proteins, as shown in Figure 5, which explains the relationship between the logarithm of the MW of standard protein and relative movement of the protein itself, revealing that the MW of tannase was 95.49 kDa was produced from *Aspergillus niger* (A_{13}) by SDS-PAGE (Al-Mraai et al., 2019).

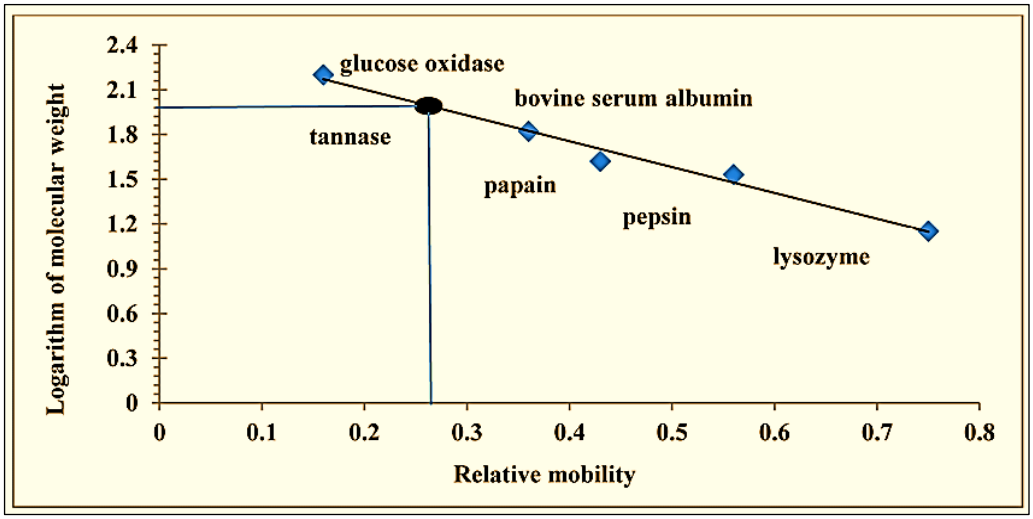

Figure 5. Standard Curve to Determine the Molecular Weight of Tannase (Al-Mraai et al., 2019)

Depending on the producer microorganisms, the qualities of bacterial tannase might vary. The physicochemical characteristics of bacterial tannase are one of the least known aspects of the enzyme (Jana et al., 2014). According to Yao et al. (2014), the molecular weight of the tannases enzyme was discovered to be between 50-320 kDa and is naturally monomeric. It has also been observed that tannases comprise two or more subunits. In contrast, Aharwar & Parihar (2018) indicated that bacterial tannases have low MW ranging from 31 to 90 kDa, but fungal tannases have large MW ranging from 45 to 310 kDa. Until now, all known tannases from yeast and fungi have been glycoproteins in nature, whereas bacterial tannase has shown no such post-translational changes.

Effect of pH on Tannase Activity and Stability

Microorganism growth in the SSF, as well as fermentation performance, are greatly influenced by this crucial physical element. According to Jana et al. (2014), the ionization state of basic and acidic amino acids is highly impacted by pH, which impacts the enzyme reactions. The improper ionization state of amino acids might affect the ionization bonds that determine the enzyme's three-dimensional structure, resulting in substrate binding failure at the active site, enzyme inactivation, or enzyme instability. As a rule of thumb, the pH range of 4.5-7.0 is ideal for tannase activity. Certain enzymes are optimally active even at severe alkalophilic pH values (8.0 and 8.9). In contrast with Al-Mraai et al. (2019), the optimal pH for enzyme activity (Figure 6) is 5, with an enzymatic activity of 525.2 (unit/mL). The optimum pH for tannase activity was 5.5, while the results of the optimum pH for tannase stability produced from the local isolation *A. niger* (A13) ranged between (3-6), as shown in Figure 7, where the enzyme kept most of its activity for a 2h incubation, while observed decreasing in pH at the values of 8, 8.5, and 9 where the remaining activity was 15, 20, and 25% respectively, this decline is attributed to the influence of the pH in change the secondary and tertiary structure of the enzyme molecule and change the form of the active site (Al-Mraai et al., 2019).

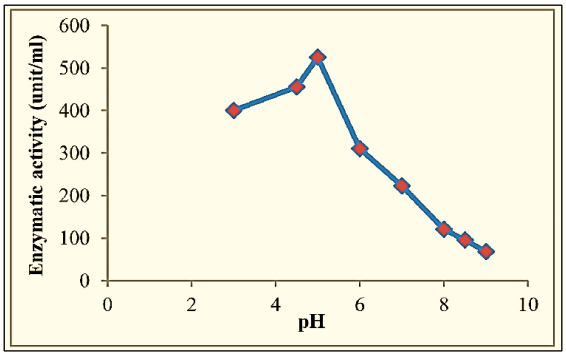

Figure 6. Optimum pH for Tannase Activity (Al-Mraai et al., 2019)

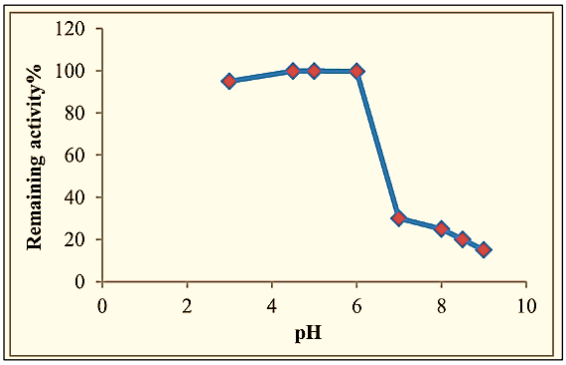

Figure 7. Optimum pH for Tannase Stability (Al-Mraai et al., 2019)

As the pH of the basal medium increases, the amount of gallic acid produced decreases since tannase is acidic, and its maximal synthesis occurs at a pH of 5.5. The tannase enzyme biosynthesis decreases when the pH increase over 5.5 (Saeed et al., 2022). Because of its function in the solubility of medium substrates, its effect on the ionization of the substrate and its availability for bacterial growth, pH impacts on enzyme production. Furthermore, pH impacts productivity and enzyme stability (Muslim et al., 2015).

Effect of Temperature on Tannase Activity and Stability

The optimum temperature of tannase may differ across the species of fungus being used. The temperature optimum for microbial tannase is typically between 20-60 °C (Jana et al., 2014; Yao et al., 2014). Bacterial tannase has been shown to be most active in the mesophilic range, about 20-45 °C. Stability across a broad range of pH 3.0-8.0 and temperature 25-70 °C has been reported in most situations. It has been shown that the higher the aliphatic index of tannase protein sequences, the better its thermostability. As Saeed et al. (2022) highlighted, tannase and gallic acid production decreased over time due to the breakdown of enzyme globular structures at higher temperatures.

Figure 8 shows that increasing the temperature causes the enzymatic activity to increase until it reaches a maximum of 520.5 (unit/mL) at 35 °C then progressively decreases to 250 (unit/mL) at 50 °C, and continues to decline until it reaches 25.7 (unit/mL) at 80 °C. Tannase was discovered to be thermally stable in a temperature range of 30-50°C, as it maintained its total activity after 60 min of incubation at these temperatures, then lowered the activity rapidly with rising temperature, even losing its total activity at 90 °C, while an enzyme maintained about 50% and 38.4% at temperatures of 60 °C and 70 °C, respectively, as shown in Figure 9. The findings were comparable to those of previous researchers who determined the optimal temperature for enzyme stability, which varied between 25-45 °C, and discovered that tannase was thermally stable at temperatures ranging from 40-60 °C (Al-Mraai et al., 2019). The enzyme's instability might be due to microbial strain denaturation at higher temperatures (Aharwar & Parihar, 2018).

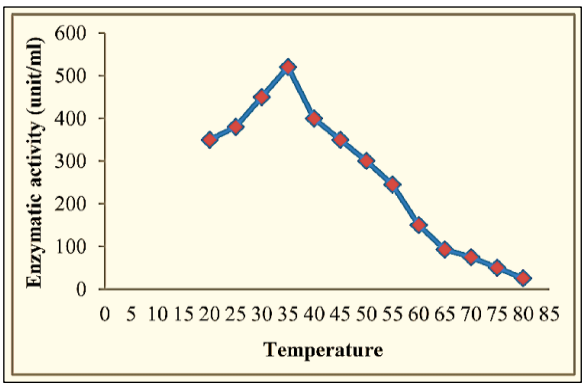

Figure 8. Optimum Temperature for Tannase Activity (Al-Mraai et al., 2019)

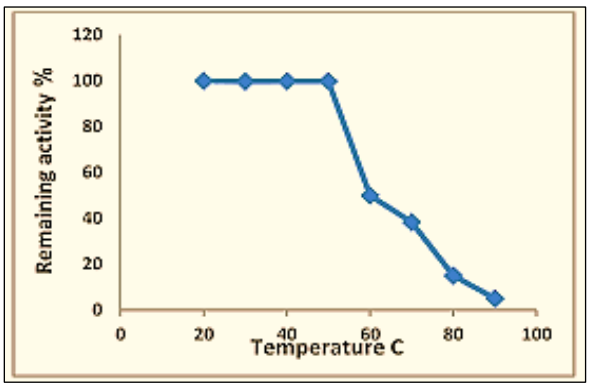

Figure 9. Optimum Temperature for Tannase Stability (Al-Mraai et al., 2019)

Lower and higher temperatures reduce activities due to the thermal impacts of these temperatures on microbe growth and the enzymatic reaction rate inside the cells, which reflects on the crucial enzyme synthesis. When the temperature increases, the kinetic energy of the substrate and enzyme molecules also increases, influencing the reaction rate. The contacts frequency between tannase activity and its substrate, tannic acid, increases with increasing temperature, resulting in increased activity. Once the optimum temperature has kicked in, the chemical potential energy increases, weakening the three-dimensional shape of the proteins and eventually breaking the bond leading to the denaturation of tannase protein and inactivation of its activity. Thus, exceeding the optimal temperature decreased the tannase catalytic rate as either the enzyme or substrate became denatured and inactive (Muslim et al., 2015).

Tannase Purification and Recovery

Previously Lekshmi et al. (2021) reported that enzyme purification could help boost their catalytic activity and prevent other undesirable enzymatic reactions or stability. Considerable research on partial and complete purification of tannase has been documented. The procedure involves a series of solvent extraction or salt precipitation phases, ultrafiltration, pH, gel permeation, or ion exchange chromatography**.** Other options include affinity chromatography, which is less common and unsuitable for commercial processes. Most documented purification processes include several steps, such as protein concentration followed by ion exchange or gel filtration chromatography. The researchers stated that the purified tannase from *Enterobacter* sp. using DEAE-cellulose chromatography followed by Sephadex G-100 gel filtration column, with 7.1% recovery and purified tannase from *B. subtilis* using a two-step purification protocol consisting of $(NH_4)_2SO_4$ precipitation (80%) followed by Sephadex G-75 gel filtration chromatography, with 5.04% recovery (Jana et al., 2014). Conventional multi-step techniques are expensive, complex, time-consuming, and challenging to replicate and may result in significant product loss. *L. plantarum* recombinant tannase was isolated in four steps: ammonium sulphate precipitation at 60%, Q-Sepharose, hydroxylapatite (HAP), and MonoQ column chromatography with low recovery (4.8%). Rather than $(NH_4)_2SO_4$ precipitation, the researchers employed ultrafiltration to extract tannic acid from the crude enzyme (100 kDa cut-off membrane) to concentrate the enzyme further (30 kDa cut-off membrane) (Jana et al., 2014).

Enzyme extraction is a critical step in downstream processing since it accounts for a significant proportion of industrial enzyme synthesis costs. Meanwhile, the tannase recovery from culture media is essential since downstream processing affects the product yield and cost of enzyme synthesis. Tannase is an extracellular enzyme made by bacteria and fungi. As a result, extraction is simpler than intracellular enzymes, requiring the addition of three or more volumes of buffer or twice distilled water (Lekshmi et al., 2021). The fermentation technique influences the tannase recovery from fermented broth/mass. Enzyme secretion is often attached to microbial cells via non-covalent interactions, including hydrogen bonds, hydrophobic forces, and Van-der-walls forces. An appropriate solvent solution must be chosen to extract an enzyme from ferments based on its unique hydrophilic and hydrophobic properties (Jana et al., 2014). Researchers used centrifugation at 10,000 rpm to extract extracellular tannase from the fermented broth. *S. ficaria* cell-associated tannase was recovered by centrifugation followed by cell washing with (0.1 M, pH 3.5) citrate buffer. Because tannase is extracellular, it is easy to recover in SSF and can be readily removed with water or buffer. The tannase was recovered from the fermented substrate using citrate buffer, shaking, and filtering using Whatman No. 1 filter paper.

Tannase Applications in Industry and the Environment

Lekshmi et al. (2021) mentioned that several plants contain tannins, which are polyphenolic chemicals that harm animal nutrition. Animals fed on tannin-rich forages have slower development and lower productivity because of the tannins in the feed (Verma et al., 2021). These tannins are found in several sections of the plant, including the seed, the root, the fruit, the leaf, and the wood. Their MW ranges from 500 to 3000 kDa. In contrast to other plant phenols, tannin is one of the most common secondary chemicals. Condensed tannins and proanthocyanidins are difficult to hydrolyze. The hydrolyzable tannins in tea and other food drinks may have biological and pharmacological characteristics. Organoleptic characteristics, appearance, and flavor are all influenced by tannins in fruit juices. When polyphenol oxidase is activated, it produces a brown color using tannins as a substrate (García Méndez et al., 2021). Also, pomegranate juice is dark in color because of the mixture of

anthocyanins and tannins. Tea and wine tannins reacted with salivary protein to produce astringency in the mouth. According to discoveries, tannins also interact with membrane-bound proteins, oral tissues, epithelial cells, and receptors to produce astringency (Rossetti et al., 2009).

Leather, tannery, pharmaceutical, and food industries use tannase in various ways. It is also used as a clarifying agent in coffee-flavored drinks. Gallic acid, a chemical intermediary, has long been employed in the pharmaceutical business. Antioxidant chemicals can be made through enzymatic biosynthesis, which uses enzymes. Gallic acid is used in ink production and has various medicinal benefits. The use of tannase in food and drink has helped to lessen any adverse effects that may have occurred. Adding tannase to the drinks decreased the formation of insoluble precipitates at lower temperatures. Caffeine and phenolic chemicals combine in drinks to generate precipitates. Precipitate production in fruit juices was decreased because of the addition of tannase. Enzyme treatment results in a brighter hue and a more fragrant flavor. Enzymatic approaches for bitterness reduction in fruit juices increased the beverage's quality. The production of sediment and the development of a bitter taste were caused by increased concentration of tannins in raspberry juice and other liquids at lower temperatures. Fruit juices with high tannin concentration might benefit from enzymatic treatment to restore their quality. Gelatin and tannase, for example, decreased tannin levels in pomegranate juice by more than 40 percent despite the usage of tannase. A high tannin concentration was found in several sorghum varieties, making them unsuitable for animal feed or bioethanol production. In order to use sorghum as a media component for animal feed formulations and bioethanol production, the media must be treated with beneficial bacteria or tannase (Chuck-Hernández et al., 2011). To minimize production costs, ethanol generation from agricultural and industrial waste has recently gotten much attention. Lignin and phenols are present in these agro-industrial wastes. The hydrolytic activity of cellulose was previously found to be reduced by phenolic compounds. This enzyme is essential for eliminating phenolic compounds and increasing the activity of other enzymes (Tejirian & Xu, 2011). It has been utilized in laundry detergent manufacture and tannin degradation in the leather processing industry. Black tea infusion's sensory properties were enhanced, coloring was improved, and cream production in tea was altered by *E. cloacae* 41 tannases, which were created. Bacterial sources of tannase lowered tannins in fruit juices and reduced the fruit liquids' bitterness (Govindarajan et al., 2021). The bitterness of the fruit juice was decreased by immobilizing tannase in various mediums or directly treating the juice. In the alginate bead, the tannase immobilization may preserve around 90% of its activity. For removing tannins in fruit juices, the immobilized enzyme retains more than 90% effectiveness until the third cycle and around 80% at the end of the sixth cycle, significantly improving over conventional enzymes. Tannase, which reduces tannin levels, may help with juice clarity issues.

CONCLUSIONS

Correct re-utilization of wastes and by products with green technologies helps limit the harmful and destructive consequences of waste disposal while also producing value-added compounds, contributing to the implementation of the circular economy. The cascading usage of agro-industrial wastes poses a challenge to their industrial valorization. SSF may be a good technique for this. A low-cost biotechnological method, SSF has gained attention in recent years because of its potential to manufacture lignocellulolytic enzymes from AgW and agro-industrial wastes.

However, microbial sources play a crucial role in tannase synthesis. Hence, considerable study has been done on tannases' bacterial and fungal sources to optimize their productivity and applications. Molecular structures and the active site of tannase from bacteria are now accessible. For gallic acid and tannase production, it is determined that AgW with a high quantity of tannin can be used as an alternative substrate for tannin extraction. Increasing in industrial demand for gallic acid necessitates using efficient fungal degraders to bio-transform locally accessible and inexpensive substrates. Furthermore, much emphasis is required on enzyme regulation, the design of novel bioprocess systems, the development of new expression systems, the development of new strategies for large-scale cultivation processes using tannin-rich agro-industrial wastes, novel downstream processing for industrial application, and the simple design of new tannase applications. More study is needed to build a new bioprocess, a novel expression system, a low-cost culture system, and a new protocol for producing low-cost enzymes from tannin-rich agro-residues.

References

Aharwar, A., & Parihar, D. K. (2018). Tannases: Production, properties, applications. *Biocatalysis and Agricultural Biotechnology*, *15*(June), 322–334. https://doi.org/10.1016/j.bcab.2018.07.005

Aharwar, A., & Parihar, D. K. (2019). Talaromyces verruculosus tannase production, characterization and application in fruit juices detannification. *Biocatalysis and Agricultural Biotechnology*, *18*(January), 101014. https://doi.org/10.1016/j.bcab.2019.01.052

Al-Mraai, S. T. Y., Al-Fekaiki, D. F., & Al-Manhel, A. J. A. (2019). Purification and characterization of tannase from the local isolate of Aspergillus niger. *Journal of Applied Biology and Biotechnology*, *7*(1), 29–34. https://doi.org/10.7324/JABB.2019.70106

Ben Amara, F., Bouzid, M., Sahnoun, M., Ben Nasr, Y., Jaouadi, B., Bejar, S., & Jemli, S. (2022). Valorization of potato peels starch for efficient β-cyclodextrin production and purification through an eco-friendly process. *Starch - Stärke*, *74*(9–10), 2200037. https://doi.org/10.1002/star.202200037

Bhushan, S., Rana, M. S., Mamta, Nandan, N., & Prajapati, S. K. (2019). Energy harnessing from banana plant wastes: A review. *Bioresource Technology Reports*, *7*, 100212. https://doi.org/10.1016/j.biteb.2019.100212

Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E., & Serna-Saldívar, S. O. (2011). Sorghum as a multifunctional crop for bioethanol production in Mexico: Technologies, advances and improvement opportunities. *Revista Mexicana de Ingeniera Quimica*, *10*(3), 529–549.

Dhiman, S., Mukherjee, G., Kumar, A., & Majumdar, R. S. (2022). Purification, characterization and application study of bacterial tannase for optimization of gallic acid synthesis from fruit waste. *Journal of Scientific & Industrial Research*, *81*(10), 1029–1036. https://doi.org/10.56042/jsir.v81i10.55236

Dhiman, S., Mukherjee, G., & Singh, A. K. (2018). Recent trends and advancements in microbial tannase-catalyzed biotransformation of tannins: a review. *International Microbiology*, *21*(4), 175–195. https://doi.org/10.1007/s10123-018-0027-9

Donner, M., Verniquet, A., Broeze, J., Kayser, K., & De Vries, H. (2021). Critical success and risk factors for circular business models valorising agricultural waste and by-products. *Resources, Conservation and Recycling*, *165*, 105236. https://doi.org/10.1016/j.resconrec.2020.105236

El-Maghraby, A., El-Kady, M. F. M., Taha, N. A., El-Hamied, M. A. A., & Hung, Y.-T. (2014). Beneficial Reuse of Waste Products. In *Handbook of Environment and Waste Management* (pp. 425–489). WORLD SCIENTIFIC. https://doi.org/10.1142/9789814449175_0006

El Barnossi, A., Moussaid, F., & Iraqi Housseini, A. (2021). Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review. *Biotechnology Reports*, *29*, e00574. https://doi.org/10.1016/j.btre.2020.e00574

García Méndez, M. G., Morales Martínez, T. K., Ascacio Valdés, J. A., Chávez González, M. L., Flores Gallegos, A. C., & Sepúlveda, L. (2021). Application of lactic acid bacteria in fermentation processes to obtain tannases using agro-industrial wastes. *Fermentation*, *7*(2), 48. https://doi.org/10.3390/fermentation7020048

Govindarajan, R. K., Mathivanan, K., Khanongnuch, C., Srinivasan, R., Unban, K., Charli Deepak, A., Al Farraj, D. A., Mohammed Alarjani, K., & Al Qahtany, F. S. (2021). Tannin acyl-hydrolase production by Bacillus subtilis KMS2-2: Purification, characterization, and cytotoxicity studies. *Journal of King Saud University - Science*, *33*(3), 101359. https://doi.org/10.1016/j.jksus.2021.101359

Irfan, M., Bakhtawar, J., Sadia, S., Shakir, H. A., Khan, M., & Ali, S. (2020). Utilization of fruit wastes for enzyme production in submerged fermentation. *International Journal of Biology and Chemistry*, *13*(2). https://doi.org/10.26577/ijbch.2020.v13.i2.11

Jana, A., Halder, S. K., Banerjee, A., Paul, T., Pati, B. R., Mondal, K. C., & Das Mohapatra, P. K. (2014). Biosynthesis, structural architecture and biotechnological potential of bacterial tannase: A molecular advancement. *Bioresource Technology*, *157*, 327–340. https://doi.org/10.1016/j.biortech.2014.02.017

Lekshmi, R., Arif Nisha, S., Thirumalai Vasan, P., & Kaleeswaran, B. (2021). A comprehensive review on tannase: Microbes associated production of tannase exploiting tannin rich agro-industrial wastes with special reference to its potential environmental and industrial applications. *Environmental Research*, *201*(July), 111625. https://doi.org/10.1016/j.envres.2021.111625

Mahmoud, A. E., Fathy, S. A., Rashad, M. M., Ezz, M. K., & Mohammed, A. T. (2018). Purification and characterization of a novel tannase produced by Kluyveromyces marxianus using olive pomace as solid support, and its promising role in gallic acid production. *International Journal of Biological Macromolecules*, *107*, 2342–2350. https://doi.org/10.1016/j.ijbiomac.2017.10.117

Mandal, S., & Ghosh, K. (2013). Optimization of tannase production and improvement of nutritional quality of two potential low-priced plant feedstuffs under solid state fermentation by Pichia kudriavzevii isolated from fish gut. *Food Biotechnology*, *27*(1), 86–103. https://doi.org/10.1080/08905436.2012.755929

Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., & Teixeira, J. A. (2011). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnology Advances*, *29*(3), 365–373. https://doi.org/10.1016/j.biotechadv.2011.01.008

Md Sani, A. N., Mohd Adzahan, N., & Ismail-Fitry, M. R. (2022). Valorization of malaysian tropical fruit seeds: A review of their nutrition, bioactivity, processing and food application. *Food Bioscience*, *50*, 102156. https://doi.org/10.1016/j.fbio.2022.102156

Meini, M.-R., Cabezudo, I., Galetto, C. S., & Romanini, D. (2021). Production of grape pomace extracts with enhanced antioxidant and prebiotic activities through solid-state fermentation by Aspergillus niger and Aspergillus oryzae. *Food Bioscience*, *42*, 101168. https://doi.org/10.1016/j.fbio.2021.101168

Muslim, S. N., Mahammed, A. N., Musafer, H. K., AL_Kadmy, I. M. S., Shafiq, S. A., & Muslim, S. N. (2015). Detection of the optimal conditions for tannase productivity and activity by Erwinia carotovora. *Journal of Medical and Bioengineering*, *4*(3), 198–205. https://doi.org/10.12720/jomb.4.3.198-205

Osorio, L. L. D. R., Flórez-López, E., & Grande-Tovar, C. D. (2021). The potential of selected agri-food loss and waste to contribute to a circular economy: Applications in the food, cosmetic and pharmaceutical industries. *Molecules*, *26*(2), 515. https://doi.org/10.3390/molecules26020515

Ostreikova, T. O., Kalinkina, O. V., Bogomolov, N. G., & Chernykh, I. V. (2022). Glycoalkaloids of plants in the family solanaceae (nightshade) as potential drugs. *Pharmaceutical Chemistry Journal*, *56*(7), 948–957. https://doi.org/10.1007/s11094-022-02731-x

Rossetti, D., Bongaerts, J. H. H., Wantling, E., Stokes, J. R., & Williamson, A.-M. (2009). Astringency of tea catechins: More than an oral lubrication tactile percept. *Food Hydrocolloids*, *23*(7), 1984–1992. https://doi.org/10.1016/j.foodhyd.2009.03.001

Ruiz Rodríguez, L. G., Zamora Gasga, V. M., Pescuma, M., Van Nieuwenhove, C., Mozzi, F., & Sánchez Burgos, J. A. (2021). Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages. *Food Research International*, *140*(July 2020), 109854. https://doi.org/10.1016/j.foodres.2020.109854

Saeed, S., Bibi, I., Mehmood, T., Naseer, R., & Bilal, M. (2022). Valorization of locally available waste plant leaves for production of tannase and gallic acid by solid-state fermentation. *Biomass Conversion and Biorefinery*, *12*(9), 3809–3816. https://doi.org/10.1007/s13399-020-00989-3

Sampaio, S. L., Petropoulos, S. A., Alexopoulos, A., Heleno, S. A., Santos-Buelga, C., Barros, L., & Ferreira, I. C. F. R. (2020). Potato peels as sources of functional compounds for the food industry: A review. *Trends in Food Science & Technology*, *103*, 118–129. https://doi.org/10.1016/j.tifs.2020.07.015

Silva, L. de O., Garrett, R., Monteiro, M. L. G., Conte-Junior, C. A., & Torres, A. G. (2021). Pomegranate (Punica granatum) peel fractions obtained by supercritical CO2 increase oxidative and colour stability of bluefish (Pomatomus saltatrix) patties treated by UV-C irradiation. *Food Chemistry*, *362*, 130159. https://doi.org/10.1016/j.foodchem.2021.130159

Tejirian, A., & Xu, F. (2011). Inhibition of enzymatic cellulolysis by phenolic compounds. *Enzyme and Microbial Technology*, *48*(3), 239–247. https://doi.org/10.1016/j.enzmictec.2010.11.004

Thakur, M., Rai, A. K., Mishra, B. B., & Singh, S. P. (2021). Novel insight into valorization of potato peel biomass into type III resistant starch and maltooligosaccharide molecules. *Environmental Technology & Innovation*, *24*, 101827. https://doi.org/10.1016/j.eti.2021.101827

The Star. (2021, October). Tackling the Rising Amount of Trash. *TheStar*.

Torres-León, C., Ramírez-Guzman, N., Londoño-Hernandez, L., Martinez-Medina, G. A., Díaz-Herrera, R., Navarro-Macias, V., Alvarez-Pérez, O. B., Picazo, B., Villarreal-Vázquez, M., Ascacio-Valdes, J., & Aguilar, C. N. (2018). Food waste and byproducts: An opportunity to minimize malnutrition and hunger in developing countries. *Frontiers in Sustainable Food Systems*, *2*, 1–17. https://doi.org/10.3389/fsufs.2018.00052

United Nations Environment Programme & International Resource Panel. (2011). *Decoupling Natural Resource Use and Environmental Impacts from Economic Growth*.

Verma, S., Taube, F., & Malisch, C. S. (2021). Examining the variables leading to apparent incongruity between antimethanogenic potential of tannins and their observed effects in ruminants—A review. *Sustainability*, *13*(5), 2743. https://doi.org/10.3390/su13052743

Wang, F., Ni, H., Cai, H.-N., & Xiao, A.-F. (2013). Tea stalks – a novel agro-residue for the production of tannase under solid state fermentation by Aspergillus niger JMU-TS528. *Annals of Microbiology*, *63*(3), 897–904. https://doi.org/10.1007/s13213-012-0541-5

Yao, J., Guo, G. S., Ren, G. H., & Liu, Y. H. (2014). Production, characterization and applications of tannase. *Journal of Molecular Catalysis B: Enzymatic*, *101*, 137–147. https://doi.org/10.1016/j.molcatb.2013.11.018

Yusree, F. I. F. M., Peter, A. P., Mohd Nor, M. Z., Show, P. L., & Mokhtar, M. N. (2021). Latest advances in protein-recovery technologies from agricultural waste. *Foods*, *10*(11), 2748. https://doi.org/10.3390/foods10112748