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Cellulases, hemicellulases and ligninolytic enzymes: mechanism of action, optimal processing conditions and obtaining value-added compounds in plant matrices

Abstract

This review article has focused on the enzymatic hydrolysis (HE) of plant matrices (MV), the mechanisms of action of cellulases, hemicellulases and ligninases; the optimal process conditions most used to obtain products of interest; as well as some factors that reduce catalytic activity. Based on the enzymatic mechanism, a compendium of the applications on different substrates is elaborated, with the idea of reorienting the current application of the hydrolysis of MV (biofuels) and providing an alternative for its use and obtaining compounds of added value. For example, the extraction of phenolic compounds with antioxidant capacity, of xylooligosaccharides and aromatic phenolic compounds from the hydrolysis of cellulose, hemicellulose and lignin, respectively.

Keywords: cellulases, xylanases, ligninolytic enzymes, lignocellulose, phenolic compounds

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Introduction

In the present work, "vegetable matrix" (MV) is understood as the by-products derived from the industrial processing of fruits and vegetables, as well as the lignocellulosic biomass from the agricultural field. The fruits are the main processed materials in the world, resulting in the generation of large amounts of material without an established purpose, these elements are natural and safe sources for the recovery of bioactive compounds.[1,2] For its part, the Food and Agriculture Organization of the United Nations (FAO) reported a production of more than 31 million tons of lignocellulosic biomass for the year 2019. The previously described MV has as its main purpose the production of reducing sugars, and subsequent transformation into biofuels. Less appreciated is its potential to obtain products with high phenolic content. Therefore, some researchers have recently studied the enzymatic activity to obtain pigments and different bioactive compounds from MV. So the present review has focused on the latter. In such a way that it is an approach of extracting compounds synthesized in the plant. Besides, the use of enzymes is an environmentally friendly method.

The cell wall of MVs is mainly composed of complex polysaccharides, peptide compounds, and glycoproteins. The cell wall has a lignocellulosic structure, meaning that it is composed of cellulose, hemicellulose and lignin.[3] Cellulose is a linear polymer made up of D-glucose molecules, linked by β-1,4 glycosidic bonds; hydrogen bonding and van der Waals forces between the parallel-extending strands provide the three-dimensional structure of microfibrils. The properties of such a molecule are related to the degree of polymerization of the chain. Hemicellulose is a branched chain of sugars where the main chain is made up of D-xylose, D-mannose and D-galactose; the side chains are made up of L-arabinose, D-galactose and 4-O-methyl-D-glucuronic acid, its structure differs by the location and source from which it comes. The presence of the side chains provides various three-dimensional structures that incur in the different physical properties of the compound. Hemicellulose binds to cellulose providing rigidity to the cell wall.[4,5] Lignin, for its part, is a copolymer formed by monolignols, mainly p-coumaryl, coniferyl and sinapyl alcohol, arranged temporally and spatially in various structures related to their origin, for which they have diverse chemical properties.[6] Encapsulated by the plant cell wall are many phenolic compounds (PC).[7]

Enzymatic hydrolysis of plant matrices (HEMV) can be considered as a potential alternative for the release of CF or fibers with nutraceutical importance, since it promotes the degradation or breakdown of cell wall structures. As a consequence, the use of MV (biofuels) can be redirected and the search for alternatives can be promoted to obtain value-added products, as well as more natural and safe ingredients.[8–12]

Enzymatic hydrolysis of plant matrices

HEMV consists of a series of processes that, through the addition of specific enzymes, promotes the breaking of certain bonds and, therefore, the specific degradation of the cell wall, releasing targets according to the enzyme used from a lignocellulosic matrix. The pH and the temperature, among other factors, are considered key issues in the correct functioning of the catalytic activity. This process is qualified as ecological and friendly to the environment, since chemical compounds are not required.[5,8,13,14] The enzymes most commonly used to achieve HEMV are trypsin, papain, pectinase, cellulase, protease, amylase, xylanases, and glucanases; the combination of these enzymes can improve the yield of the released products.[8,14,15] HEMV is considered the typical technique for obtaining fermentable sugars and their subsequent transformation into biofuels.[10] In recent years, some studies have focused on enzymatic hydrolysis for other purposes, including the transformation of insoluble to soluble dietary fiber, promoting gastrointestinal benefits; obtaining oligosaccharides and xylooligosaccharides with antioxidant activity, as well as the release and extraction of phenolic compounds.[16–19] Generating a potential alternative for obtaining compounds that can benefit health.

Hemv assisted by glycosil hydrolases

Glycosyl hydrolases (GH) (EC 3.2.1-) are a group of enzymes with a specific biochemical function, they hydrolyze the glycosidic

bond between carbohydrates or between a carbohydrate and oxygen, nitrogen or sulfur. Its nomenclature falls on the specificity to the substrate, thus defined by Carbohydrate-Active Enzymes.[20] Most GH are produced by *Trichoderma reesei*, which is naturally responsible for the degradation of lignocellulosic stock, generating significant amounts of cellulases and hemicellulases at an industrial level. For the production of GH, different types of fungal strains such as Aspergillus and Fusarium have also been used; in addition to fungi, bacteria such as Clostridium, Streptomyces, Cellulomonas, among others, have been of interest for the generation of lignocellulolytic enzymes. It has been estimated that a synergy of enzymes of diverse origin can optimize the results in enzymatic hydrolysis.[20] CAZy[21] classifies GH families according to their amino acid structure, the similarities in the binding fold, as well as the spatial orientation mechanism of the catalytic residues, the latter focused on hydrolyzing α or β glycosidic bonds. From the classification provided, there are 17 families that have an action on the β-1,4-D-glucosidic bonds of cellulose and on β-D-glucans, presenting what is called cellulolytic activity.[22]

Structure and mechanism of action of GH

GHs in their structure have two globular modules, the catalytic site responsible for the hydrolysis reaction and a substrate binding site (SU). The SU of GH is the amino acid sequence within an enzyme that generates the fold to promote carbohydrate binding, which identifies the cellulose matrix, maintains the proximity between the glycosidic bonds and the enzyme, and finally the site where they are attached carries out the disruptive reaction.[23] The reactions of GH on its substrate are catalyzed by SU, two catalytic residues are required to start the hydrolysis, a proton donor element, such as aspartic and glutamic acid, or a nucleophile such as glutamate and aspartame, generating the bimolecular nucleophilic substitution mechanism (SN2).[20,22] Within the SN2 mechanism generated by the enzymes is the conservation and inversion mechanisms. The conservation is a double displacement mechanism, it occurs when the catalytic residues are located at 5.5 Å and glycosylation is promoted, where the nucleophile acts in the anomeric center, which generates a glycosidic enzyme with an acid character, later the carboxylate acid acts as a base and binds to the nucleophile, to hydrolyze the previous enzyme and obtain the hydrolysis product; for its part in the inversion mechanism, the catalytic residues are at a distance of 10 Å and the nucleophile substitutes the molecule in an electrophilic position. In general, both procedures involve a transition state of the oxocarbenium ion that generates SP2 hybridization and the formation of a positive charge on the anomeric carbon.[22,23] Cellulases and hemicellulases are located within the GH families, and are governed by the previously described catalytic mechanism.

Cellulases are the enzymes that catalyze the hydrolysis of cellulose and they were named according to their action on the target matrix. Endoglucanases (EC.3.2.1.4), exoglucanases including cellodextrins (EC.3.2.1.74) and cellobiohydrolases (EC.3.2.1.91), as well as β-glucosidases (EC.3.2.1.21) are the three types of enzymes that act together for the degradation of native cellulose.[23] Cellulose micelles that are highly ordered and linked by hydrogen bonds are called crystalline cellulose and are distinguished from amorphous ones by their well-defined structure. Therefore, the irregular arrangement allows endo-(1,4)-β-D-glucanases to act more easily on the internal β-1,4 glycosidic bonds of amorphous cellulose, and subsequently hydrolyzes crystalline cellulose, opening the reducing and non-reducing ends of the polymer and generating glucan chains of smaller proportions (4-6 units). The action of these enzymes is continued by that of the exo-(1,4)-β-D-glucanases that release β-cellobioses. This process is generated by the hydrolysis of the terminal regions of the cellulose chain. Finally, β-glucosidases act on the reducing ends of β-cellobiose, hydrolyzing them to glucose molecules.[22,24,25]

Inhibition of cellulase activity

In the present work, the factors that generate enzyme inhibition will be classified into two large groups, the first one is the intrinsic factors, specific to the enzyme and the lignocellulosic matrix, and the second is the extrinsic factors generated by external factors (temperature, pH, agitation, etc.). For the first case, which will be addressed in this work, it is possible to classify it into two large groups: the transport or accessibility of the enzyme to the cellulose surface (through different polymers such as hemicellulose and lignin) and the availability of binding sites.[26] In the case of accessibility, they mainly comprise the structural elements present in the substrate. The first of them relies on the degree of cellulose polymerization, since some studies show that it affects the relative activity of GH, by increasing or reducing the SU directly related to the length of the chain.[26,27] As the glucan chain increases, the number of bonds between the microfibrils and the resistance to hydrolysis as well is increased. Cellulose crystallinity also negatively influences enzymatic hydrolysis. Therefore, the shape and size of the pore allows or restricts access to the enzymes, promoting a rapid or decreased reaction rate.[26,27] The elements that surround the cellulose also exert a resistance to enzymatic action. Lignin is considered an impediment to the entry of cellulases into its substrate, mainly due to its proportion in the system that generates a physical barrier. Other factors that influences its chemical structure are the hydrogen bonds, methoxy group, and its aromatic cyclic structure, which can irreversibly trap the enzyme due to its hydrophobic properties.[26,28] However, the number of aliphatic hydroxyl groups in the structure and the hydroxyl groups of the phenolic compounds of the lignin are the main inhibitors. This is because the OH group could form hydrogen bonds with the amino acid residue of cellulases, preventing enzymatic action on cellulose and reducing the hydrolysis yield attributed to the availability of the binding site.[29]

Productive binding of enzymes occurs when it reacts only with its specific substrate. A common reaction that occurs with hemicellulose is acetylation, the acetyl group can interfere with the cellulase active site.[30] Arabinoxylooligomers, specifically the less substituted ones, show a greater affinity to occupy the active site compared to cellulose itself, for which the hydrolysis of arabinoxylan to xylose and arabinose is recommended.[31] There are different soluble toxins in the hydrolytic medium that can cause enzymatic deactivation or cause enzyme precipitation, one of them corresponds to the generation of compounds during the process, such as phenolic compounds derived from lignin or the generation of acetyl groups previously described, the same glucose production can generate the inhibition of β-glucosidase. Other inhibitors are the residues of the pretreatments, mainly of the chemical type to achieve the amorphogenesis of lignin, these can be reduced by carrying out abundant washing of the raw material after treatment.[21] Ahmed et al.[32] conclude that drying after the pretreatment and oxidative metal ions, generated by microorganisms that can easily develop due to optimal hydrolysis temperatures, also modify enzymatic action.[21]

Lignin, as previously described, is a physical barrier and also generates non-productive unions with the enzyme, so lignin degradation promotes hydrolysis, since it is considered one of the main adverse effects for enzymatic degradation.[28] Various treatments have been described to achieve delignification and reduce the barrier due to the structure of the substrate. Among the chemicals, acid and basic hydrolysis are described, with organic solvents and ionic liquids. For the physical, grinding, extruding,

microwaves, electric fields or ultrasound are defined, which also have an effect on the depolymerization of the cellulose chain, as well as decrease the particle size and increase the accessible surface area. Pretreatment with microorganisms is also a good option by having a considerable biodegradability of the matter in question, and for being friendly to the environment.[28,33,34] Different authors agree that the use of pretreatments is essential to improve the enzymatic action on the lignocellulosic matrix, in addition that they can improve the antioxidant activity provided by the phenolic compounds released during the process.[18,19,35] Finally, it is established that a synergistic action of the pretreatments, to achieve access to the enzyme with a productive union, is a key factor that favors enzymatic hydrolysis.

Application of cellulases in MV and obtaining phenolic compounds (antioxidants)

As previously described, the use of GH was primarily focused on the production of fermentable sugars and subsequent transformation into biofuels. An example of this is the enzymatic hydrolysis of the empty bunches of oil palm fruit, in which the combination of cellulases and β-glucosidases presented better results in the production of reducing sugars. The use of β-1,4 glucosidases, in addition to generating hydrolysis to glucose, is necessary to avoid inhibition of the enzyme by excess cellobiose, which inhibits the action of cellobiohydrolases and endoglucanases.[36] For the production of ethanol from Jatropha curcas husks by enzymatic hydrolysis, the association of two enzymes was considered, *Trichoderma resei* cellulase and α-glucosidases (15 FPU/g DM and 15 IU/mL, 50 °C, 150 RPM, 48 h), by increasing the H_2SO_4 concentration (up to 1%) in the experiment, the xylan content decreased, attributed to this characteristic and the synergy with the enzymes, a higher yield was observed for obtaining reducing sugars.[37] Pocan et al.[38] evaluated corn cob, a material with a high lignin content, noting that biomass with this characteristic requires chemical treatments to complete enzymatic hydrolysis, in this case NaOH (2%) was considered; alkali and the association of cellulases and cellobiohydrolases enzymes (26 FPU/g DM and 13 FPU/g DM, 50°C, 150rpm, pH 4.8, 24h, 3% solid / liq) promoted a higher yield in glucose production.

It was previously commented that the peels obtained from the processing of plant foods, as well as the lignocellulosic biomass from the field, can not only be used to obtain biofuels, since they have significant potential as a source of bioactive compounds. It is known that the residual matrices are recognized for having a higher CF concentration than even the edible part. Therefore, its transformation into value-added products allows contributing to the protection of the environment and recovering valuable nutrients.[18,39] Now we proceed to describe some examples of HEMV with the potential to obtain bioactive compounds, applying the use of GH for this purpose and dividing it into two aspects; the first one to obtain products with antioxidant activity as a CF extraction technique and the other to obtain products with antioxidant activity.

To achieve a product with a high phenolic content, which is the example described by Wang et al.[14], who determined the in vitro antioxidant activity of apple peel extracts, this through peroxyl radical scavenging capacity tests (mmol aq. ascorbic equivalent /100g peel), observing values 1.6 times higher in treatments with pectinases and cellulases compared to chemical extraction and boiling water, it should be noted that no significant difference was observed between the enzymatic treatments at 37 °C for 4 hours. A recent work investigated the extraction of CF from *Silphium perfoliatum L* (cup plant), using three types of enzymes, pectinases, cellulases and papain at 1.59% of the complex in the same proportion (50 °C, 22% liq/sol, 66 min and pH 5.1); the presence of galacturonic acid was identified by HPLC. After HE an increment was observed on the percentage of inhibition of free radicals, measured by ABTS and DPPH. The inhibitory properties of α-amylase and α−glucosidase suggest that hydrolyzates can be used as antioxidants and hypoglycemic agents.[40] Pocan et al.[38] carried out the HE on the orange and pomegranate peels, for this they used pectinase as it is a pectin-rich compound, and Biogazyme, cellulase from *Trichoderma* spp. (112 FPU/g and 268 IU/g, 50°C, 3% sol/liq, pH 4.8); the conversion to reducing sugars was improved by 70% under the described conditions. In an alternate publication with citrus residues and where cellobiose was also added, it was observed that the total phenol content was higher for the hydrolyzed residues (21874 mg/g) compared to the control (non-hydrolyzed sample); the hydrolysis residues were effective in inhibiting melanoma (A375) and colon cancer (HCT116) cell proliferations.[41]

To obtain phenolic extracts from the pomegranate peel, an enzymatic treatment was used for the release of compounds and subsequent extraction with supercritical fluids, the enzymes used were acid cellulase, pectinase, alcalase, neutral, acid and alkaline protease, as well as an enzymatic cocktail (pectinase 25%, protease 25 and cellulase 50), observing that the CF concentration doubled in the previously hydrolyzed extracts, giving the best results to the enzyme cocktail, in turn the antioxidant capacity by free radicals increased (TEAC) and free radical scavenging capacity by DPPH; the enzymes worked better at low temperatures (50 °C) except for alcalase, the most suitable pH was close to 5.[42] There are other investigations that frame the benefits of the simultaneous use of enzymes, for example in Southeast Asia there is an increase in the amount of by-products obtained from longan (*Dimocarpus longan*), this product can contain up to 19% lignin in its structure and a high lipid content. The husk was pulverized and defatted to remove lipids that could interfere with the enzymatic process. An increase of more than 40% of the total phenolic content was observed, hydrolyzing the substrate with the synergistic action of cellulases (EC 3.2.1.4), α-amylase (EC 3.2.1.1), protease (EC 3.4.21.62) and α−glucosidase (EC 3.2.1.21) (210/ 200/ 0.48/ 30 units, 50 °C, pH 6.5) the same yield was obtained only hydrolyzate with cellulases (200 units). Using the HPLC technique, the increase in phenolic compounds in the hydrolyzates was observed, such as gallic acid, chlorogenic acid, corilagin, ferulic acid, ellagic acid, o-coumaric acid, quercetin and kaempferol.[43] For its part, Limonia acidissima, also called wood apple, was worked fresh without prior treatment, using a combination of endoglucanases and pectinases. Subsequently, the total phenolic content, DPPH and ABTS were measured, observing an increase of 56, 63 and 85%, respectively, compared to the control samples.[44] Currently, few are the applications of the exclusive use of cellulases, to achieve the hydrolysis of different constituents of the cell wall, the use of enzymatic cocktails is recommended, as well as some physical, chemical or mechanical pretreatments that allow the permeability of the substrate to the enzyme. The previously described results frame the possibility of continuing to work with enzymes, mainly of the GH type, to achieve hydrolysis of the lignocellulosic matrix and obtain value-added products. The hydrolysis conditions mark a tendency to choose, temperatures close to 50 °C, mainly due to cellulolytic enzymes, pH from 4.0 to 6.0 without diminishing the action of cellulases. The enzymatic concentration and reaction time is focused on the type of MV in question. In the works described above, the GH correspond to commercial brands originating mostly from *Trichoderma reesei*. It should be noted that there is a wide possibility of working with plant matrices with a high content of phenolic compounds through HE and in this way finding compounds that can be used and can even generate health benefits.

Hemicellulase assisted hemv

According to the literature, it is known that hemicellulases are obtained from a wide spectrum of microorganisms present in nature, being the most commercial those from fungi and thermophilic bacteria. Their main function is to hydrolyze the polysaccharides present in hemicellulose. As part of the group of hemicellulases are xylanases, endoxylanases, xylosidases, arabinofuranosidases, to mention a few.[45,46] In previous paragraphs, the use of hemicellulases and pectinases as auxiliaries in the disintegration of MV was described, in order to allow permeability to cellulose.

Structure of hemilcellulose and mechanisms of action of xylanases

Hemicelluloses constitute a variety of diverse polysaccharides, including glucuronoarabinoxylans (GAX), xyloglucan (XG), glucuronoxylans (GX), galactomannan (GalM), galactoglucomannans (GGM), glucanans (GM), arabinogalactan (AG), and hemicelluloses mixed link glucan (MLG). These units are produced in the Golgi apparatus, then they are transported to the cell wall generating polymers of 60 to 150 residues, they have a high affinity for cellulose, mainly GM and XG, unlike AG and GGM, which do not bind to her. The cell wall of dicotyledons can have up to 25% of XG. While the main hemicelluloses among monocotyledons are GAX and GalM. However, this ratio can vary depending on the type of plant cell and the stage of tissue development.[47]

The main structure of xyloglucans is formed by β-(1,4)-D-glucan substituted with α-D-xylosyl residues attached to glucose at carbon 6, it has a regular pattern where three glucose units are found substituted and later one unsubstituted, the xylosyl can also be linked in β-(1,2) with a D-galactosyl residue, and this in turn to α−(1,2) of the L-fucosyl residue, however, the patterns can be diverse depending on the matrix in question. Endoxylanases cut the XG skeleton in the unsubstituted position, generating polymers with four glucose residues, these in turn have three xyloses for the substituted glucoses and to a lesser extent galactose and fucose.[48] The main chain of GAX is composed of β-D-xylopyranose, substituting the α−L-arabinofuranosyl residues in O3, or the α-D-glucopyranosyl in O2, ferulic acid has been located randomly in arabinose groups, acetylation occurs to a lesser extent compared to XG. Glucuronoxylans have the same central structure as GAX, but acetylation is more frequent and is located in O2 and O3. GGMs have a β-D-mannopyranose backbone and β-D-glucopyranose is randomly substituted at the O of mannose by β-D-galactopyranose residues, this also generates acetylations at C2 and C3.[49] In general, the different hemicelluloses are made up of a branched chain of sugars, where the main chain is made up of units of D-xylose, D-mannose and D-galactose; and the lateral ones are made up of L-arabinose, D-galactose and 4-O-methyl-D-glucuronic acid in different proportions.[5] Of the hemicellulases, the xylanases (XIL) were selected due to the interest in obtaining oligosaccharides (XOS), which are widely known for their techno-functional and bio-functional activity.

Endo-β-(1,4)-D-xylanases (EC 3.2.1.8) or commonly called xylanases (XIL), are the most important enzymes for the degradation of arabinoxylan, they act on internal β- bonds (1,4) of xylose residues, affecting their functionality given changes in the molecular mass and solubility of arabinoxylan.[50] These enzymes are produced mainly by fungi, and around 300 varieties have been identified, classified into ten of the GH families, family 10 being one of those with proven activity. Being a GH, it follows a SN2 reaction mechanism, that is, double displacement by nucleophilic substitution like cellulases.[20] GH family 10 XILs have a cylindrical catalytic domain, which is made up of a pair of glutamates located in an open cleft at the narrower end, near the carboxy terminus, it also has non-catalytic modules associated with carbohydrate binding.[50]

Application of xylanases in MV and obtaining xylooligosaccharides

XOS are oligosaccharides containing 2 to 10 xylose units linked by β-1,4 glycosidic bonds, obtained from xylan-rich compounds.[51] Table 1 describes some works found in the literature to obtain these compounds, it should be noted that most of them required the prior extraction of xylan by means of a chemical treatment, using acids (H_2SO_4 [1%]), bases (NaOH [1.25 M] or KOH) and NaClO [1%], once the delignification was achieved, ethanol was used to precipitate the xylan.[52] Another technique for separating lignin from xylan was used in Moso bamboo, this material was passed through a polystyrene divinylbenzene resin column, achieving the separation of the parts.[51] After the pretreatments, hydrolysis was performed with the described enzymes, mainly endo-β-(1,4)-D-xylanases from *Aspergillus oryzae*. To carry out the hydrolysis reaction, the pH was adjusted with a sodium citrate buffer solution, observing that the different authors handled pH values between 4 and 5, the reaction temperature was 50 °C except for the treatment with xylanases of B. aerophilus used for the degradation of the xylan of the corn cob, the temperature for this case was 70 °C.[53] Some of the techniques used to measure the progress of hydrolysis were DNS and chromatography (thin layer and ion exchange), for antioxidant capacity it was measured by ABTS and DPPH, and finally FI-TR was used for the structural part.

Table 1 Studies to obtain XOS by endo-β-(1,4)-xylanases

Matrix	Type of enzyme used	Procesiing conditions	XOS activity	Measurement	Author
Corn	Endo-β-(1,4)-D-xylanases *B. aerophilus*	Substrate 5% Enz: 25 IU tem: 70 °C T: 12 noon pH: 4.0	XOS reached IC50 values of up to 1mg/mL, suggesting significant health benefits	DNS High Performance Thin Layer Chromatography (HPTLC) DPPPH	53
Sugar cane husk and coffee	Endo-xylanases α−L-arabinofuranosidase Feruloil esterase	Substrate 2% Enz: 25 IU tem: 50 °C T: 48 hours pH: 5.0	High proportion of XOS (xylobiose, xylotriose and xylooligotetraose) with excellent antioxidant activity, resistant to digestion in vitro	DNS Spectrophotometric evaluation of total antioxidant capacity (TAC), DPPH	52

Table Continued...

Matrix	Type of enzyme used	Procesiing conditions	XOS activity	Measurement	Author
Moso Bamboo	Endo β−1-4-xylanase	Substrate 10% pH 4.8 Temp: 50°C T: 12 h Agitation: 150 rpm	The purified XOS contained 57% xylobiose and xylotriose. They promoted the growth of bifidobacteria adolescentis, stimulating the production of short-chain fatty acids by Lactobacillus acidophilus	Anion exchange chromatography. DPPH and culture of bifidobacteria adolescentis bacteria	51
Wheat Straw	Endo β-1,4 Xylanase	Substrate 5% ENZ:0.30 U/g pH: 5 Temp: 50°C T :24 h	The highest concentration of XOS was obtained under the conditions previously described	DNS abts FT-IR	54

From Table 1 it can be concluded that to obtain xylooligosaccharides the separation of the lignin fraction from the hemicellulose is required, for which chemical hydrolysis is performed with NaOH, H_2SO_4 and sodium hypochlorite, in some cases the combination of these is used. Pretreatment with NaOH generates higher yields of XOS. The polystyrene divinylbenzene resin can separate the XOS by anchoring to the column, while the soluble fraction of hemicellulose passes through it for subsequent enzymatic hydrolysis. The ultrasonic method is another promising pretreatment to modify the fiber and perform the extraction of XOS with β-1,4 xylanases. The XOS obtained showed to be resistant to enzymatic digestion in some cases and to produce lactobacilli that benefit intestinal health, excellent antioxidant values are also attributed to them.[51–54]

Hemv assisted by lignin degrading enzymes

Lignin is mainly composed of three monolignols, these are synthesized in the cell's cytosol from phenylalanine, later the alcohol binds to glucose to provide stability and solubility in the aqueous medium, and thus achieve its transport. Monolignols are linked to each other mainly by the aryl ether bond (β.O-4). There are other links such as β-5-phenylcoumaran, β−β−resinol, 5-5-biphenyl and 4-O-5 diaryl ether. Lignin is a highly heterogeneous compound in the various plant matrices, mainly due to the arrangement of the monolignols, it has highly hydrophobic properties, and together with the diversity of bonds, it generates barriers to biological degradation and promotes recalcitrance.[55,56] Although the chemical degradation of lignin in the lignocellulosic matrix has been extensively studied, these pretreatments can generate unfavorable consequences for the environment and generate enzymatic inhibition by obtaining secondary products.[57] The depolymerization into low molecular weight products by biological conversion is barely studied, however, it is a promising method given the enzymatic selectivity and friendly process conditions, to obtain value-added products such as phenolic and aromatic compounds.[58] There are different species of fungi capable of breaking down lignin; mainly and the most studied are those of white rot (*Phanerodontia chrysosporium*), the only one capable of achieving complete hydrolysis.[59] The development of genetic tools seeks to identify, express and characterize these enzymes in different organisms, it has already been studied in *E.coli*, *S. cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica*. For their part, filamentous fungi such as *Aspergillus oryzae* are known as alternative hosts for production, given their high level of enzymatic secretion.[56]

Structure and mechanisms of action of ligninolytic enzymes

For the degradation of lignin, 2 groups of enzymes are mainly involved: lignin-modifying enzymes (EML) and auxiliary lignin-degrading enzymes (ADL), the latter cannot degrade lignin on their own, but are necessary to complete the process. This type of enzymes can include the oxidative generation of H_2O_2 by sequential action of several proteins. EMLs are phenol oxidases (laccases) and peroxidases belonging to class II of the peroxidases-laccases superfamily, capable of achieving lignin decomposition.[59,60] Within the EML are the lignin peroxidases (LiP / EC 1.11.1.14) that oxidize different phenolic aromatic compounds, as well as their non-phenolic fraction, have a globular structure and are organized into two domains that form a central active cavity composed of a ferric ion, have two glycosylation sites, two Ca2+ binding sites, and four disulfide bridges. Its catalytic mechanism is similar to that of all peroxidases. The enzyme is oxidized by H_2O_2, it generates a radical cation transporter oxoporphyrin [Fe (IV) = O +], subsequently the enzyme is reduced [Fe (IV) = O] by veratryl alcohol (AV), considered the electron donor substrate and generating a radical cation (VA+), again [Fe (IV) = O] continues with the oxidation of VA+ generating a reaction cycle.[60,61]

Another EML is the magnesium-dependent peroxidase (MnP / EC 1.11.1.13) that oxidize Mn2+ to Mn3+, their chelates are propagated to oxidize the minor phenolic fragments of lignin, the catalytic mechanism is similar to LiP except that they use Mn2+ as reducing substrate. The molecular structure of these enzymes also has two domains, α−helix and intercalated heme group, it has 5 disulfide bridges and two Ca2+ ions that ensure enzymatic activity, the Mn2+ binding site is formed by glutamate and aspartame.[59,60] Laccases (LAC / EC 1.10.3.2) act mainly on the phenolic residues of lignin using O_2 as an electron acceptor, however non-phenolic aromatic compounds with high redox potential can also be degraded. LAC are proteins that have four copper atoms as a cofactor, as well as three binding domains. On the surface of the structure is the active site T1 located in domain 3, type II and III trinuclear copper (T2 and T3) are located in domains 1 and 3. T1 interacts directly with lignin, initiating to the oxidation reaction, and transports the electrons from the lignin to the Cu of T1, and then from T1 to T2 and T3, and finally to the molecular oxygen located between the copper ions of T3.[62,63,56] There are few studies of commercial lignolytic enzymes focused on the degradation of lignin, in which it has been observed that the efficacy promoted by native ecosystems has not been achieved and its cost is too high.[56]

The most studied enzymes are laccases, they have activity towards phenolic compounds at pH from acid to neutral, studies carried out at alkaline pH have not yet demonstrated their effectiveness. What has been shown is that hydrolysis at high pH can represent an inhibition factor, since the hydroxide ion can generate a competitive binding to the coppers of the catalytic site.[62] There are alternatives for the use of laccases at alkaline pH (pH 8) where greater delignification and glucose and xylose production were observed under these conditions in wheat straw.[64] Novoa et al.[65] point out that the structural rearrangement surrounding the T1 site is crucial to allow alkaline pH tolerance by laccases. A suitable temperature range for these enzymes

is very wide, from 5 to 55 °C.[66] There are other non-fungal LACs that can reach their maximum activity at 75 °C and have a half-life of 4 hours at 80 °C.[56] Zhang et al.[67] worked with LAC, LiP and MnP at a temperature of 30 °C and a pH of 5, obtaining favorable results for hydrolysis. There are several technologies to identify the progress of ligninolytic enzymes on the substrate, including optical analyzes such as scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), gas chromatography-mass spectrometry (GC-MS), among others.[68]

There are laccase mediator systems, which allow expanding the substrate for LACs and better oxidation, since it can only degrade 10% of the lignocellulosic polymer by itself; the best known and the first described mediator was ethylbenzotizoline-6-sulfonic acid. With the development of these mediators, their application has been expanded to obtain different compounds, including aromatic phenolic compounds.[57] Others can be obtained through these enzymes butane, toluene and xylene, as well as teraphthalic acid, for this a depolymerization of the substrate in oxygenated aromatic monomers is required, later a dehydrogenation and demethoxylation are incorporated using metal catalysts, the formation of hydrocarbons is promoted cyclic aromatics by the hydrogenation of the precursor monomers, these results are still under study. Phenol and vanillin are other aromatic compounds obtained by the catalytic oxidative pathway of lignin.[69] LACs have been used as delignifying enzymes, they have also been used as a tool for the removal of phenolic inhibitors for other enzymes (cellulases and hemicellulases), it decreases the hydrophobicity of lingnin by increasing productive unions of cellulase and its substrate, its main focus as in the case of cellulose, it is not to obtain biofuels but as an auxiliary in HEMV.[57]

Application of ligninolytic enzymes in MV

As has been mentioned, ligninolytic enzymes are capable of degrading hydroxyl ions, which is why it has many applications for different industries, for example, in the management of water from the production of alcoholic beverages and sugar cane, used as bioremediators to degrade OH compound contaminants from wastewater from fertilizers, painkillers, antibiotics and hormones that can cause problems in the endocrine system. They are used as a base for making paper, in the textile industry, as well as in the pharmaceutical industry, in the food industry to promote the oxidation of the dough and generate volume and softness in the bread.[56] Nino et al.[70] identified that the use of laccases in bread also modified the content of proteins and phenolic compounds. In fact, there are proposals to use enzymes, in addition to laccases, to replace chemical additives in bakery products.[71,72]

Zhang et al.[67] studied the degradation of alkaline lignin mediated by three enzymes (LAC, LiP and MnP) individually and in synergy. The LACs reached a hydrolysis of 22.5 %, higher values on the action of peroxidases, the combination of (MiP/LiP)-LAC provided better results. However, the synergy of the three enzymes reaches the highest percentage of degradation close to 28 % (measured by electron microscopy / SEM), this due to the increase in enzymes and oxidative intermediates that generate LAC, initiating the reactions catalyzed by peroxidases. Mass-coupled gas chromatography analysis identified 40 enzymatic degradation products for each enzyme, classified into 8 groups (acids, alcohols, alkanes, ketones, esters, phenols, aldehydes, and aromatics). The LACs present in their composition a higher proportion of alcohols and aromatics, 26% for each one of the total; LiPs have 34.77% aromatics and 22.23% ketones; the MnPs presented the same groups except for esters, being 55.70% aromatic and 21.54% ketones. Therefore, the author points out that the LAC-LiP-MnP complex had an action on benzene rings and lignin bonds, obtaining products of lower molecular weight. Masran et al.[73] carried out a simultaneous laccase-cellulase (Pycnoporus sanguineus-Acremonium) treatment, in order to make it more efficient to obtain fermentable sugars from empty oil palm clusters, the treatment was carried out at 50°C for After 72 hours, the concentration was 45 U/g and 25 FPU/g, obtaining a percentage of hydrolysis 10% higher than that using exclusively cellulase or the enzymes separately. In general, it can be highlighted that the synergistic action of different ligninolytic enzymes can act on the bonds and phenolic compounds of lignin, promoting its degradation, and improving the phenolic content and obtaining aromatic compounds, in turn, it is possible to improve the action of other enzymes such as GH by hydrolyzing lignin into polymers of lower molecular weight, and as a consequence reducing the barrier that it provides to the action of cellulases.

Conclusion

The amount of by-products from fruits and vegetables that are generated both from the field and from the processing industries have called attention to take advantage of the amount and types of bioactive compounds they have. It is well known that bioactive compounds are embedded in the lignocellulosic matrix, which makes their extraction difficult, so the use of enzymes is recommended to destabilize its three-dimensional structure and achieve the release of the bioactive compounds and obtain dietary fiber. Therefore, by treating waste lignocellulosic matrices with enzymes and adjuvants, it is possible to reduce environmental contamination using an ecological method. The literature reports the use of a great versatility of enzymes, which act on cellulose, hemicellulose and lignin. Recent studies indicate that it is more effective to use enzyme mixtures for the release of bioactive compounds and in the production of dietary fiber. As time passes, more is known about its mechanism of action. It has been seen that in order to make the enzymatic activity more efficient, it is important to establish certain processing conditions. In addition, it is crucial to avoid certain factors that will act as inhibitors precisely of the enzymatic activity.

On the other hand, to improve the effectiveness of the enzymes, it is recommended to carry out some type of physical pre-treatment such as particle size reduction, extrusion, microwaves, ultrasound, etc.; or chemicals such as the use of acids, bases, organic solvents or ionic liquids, etc.; recently microorganisms have also been used. The potential use that can be given to the released bioactive compounds and obtained dietary fibers would be in line with what consumers expect today. That is, having products made with more natural ingredients (pigments, phenolic compounds, antioxidants, soluble and insoluble fiber, oligosaccharides, etc.). Some of the natural ingredients have double action, bio-functional and techno-functional. The focus could be on the food industry to achieve a more nutraceuticals products or to target certain diseases like diabetes, cholesterol, and certain types of cancer; in cosmetology can be used natural pigments, and bioactive compounds in anti-aging creams; and including the production of food supplements that no only contains micronutrients but also antioxidants, etc.

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Conflicts of interest

There is no specific conflict of interests.

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