Functional Applications of Lignocellulolytic Enzymes in the Fruit and Vegetable Processing Industries

Sazzad Hossen Toushik, Kyung-Tai Lee, Jin-Sung Lee, and Keun-Sung Kim

Abstract: Cellulose, hemicellulose, pectin (carbohydrate), and lignin (noncarbohydrate) polymers are the main substrates of lignocellulose-degrading enzymes. They are present in large amounts in the primary cell wall and dietary fibers of major fruits and vegetables. During processing of fruits and vegetables to the corresponding final food products, lignocellulosic substrates are hydrolyzed by different lignocellulolytic enzymes. Currently, lignocellulolytic enzymes such as cellulases, xylanases, pectinases, and laccases are extensively used during the processing of fruits and vegetables, in applications like texturizing and flavoring of products in the food industries. The present article provides an updated overview of functional applications of lignocellulolytic enzymes in the juice processing, oil extraction, and alcoholic beverage processing industries. Extensive use of lignocellulolytic enzymes in different food processing industries not only accelerates the production rates but also improves product quality. It is also possible to ensure the efficient use of fruits and vegetables globally by employing lignocellulolytic enzymes in the corresponding processing industries to convert them into food commodities, which will not only raise their economic value in the global market but also increase food availability, which will help mitigate nutritional problems worldwide.

Keywords: cellulases, functional applications, laccases, pectinases, xylanases

Practical Application: Lignocellulosic substrates (cellulose, hemicellulose, pectin, and lignin) are abundantly present in fruits and vegetables. Various lignocellulolytic enzymes have been used to hydrolyze these substrates during fruit and vegetable processing. Lignocellulolytic enzymes are widely used in the conversion of fruits and vegetables into various food commodities, from texturizing to flavoring, and their immense potential for such applications makes them very attractive to food manufacturers. Lignocellulolytic enzymes have been extensively used in fruit and vegetable juice processing, vegetable oil extractions, and wine processing.

Introduction

Lignocellulose is a generic word used to describe key structural components of woody and nonwoody plants and represents plant biomass, the most promising, naturally abundant, renewable organic resource (Iqbal and others 2013). The building blocks of lignocellulose are the noncarbohydrate polymer lignin and the carbohydrate polymers cellulose, hemicellulose, and pectin, with ash and extractive components as minor constituents. All these components have an important chemical effect on the tertiary structure of lignocellulose. The intricate physical and chemical properties of the lignocellulosic components make them biotechnologically valuable (Pothiraj and others 2006; Ravindran and Jaiswal 2016). Due to their enormous availability in the environment, nearly 50% of the world biomass is produced from natural lignocellulosic materials. It has been reported that the yearly utilization of lignocellulosic biomass is in the range of 1×10^{10} to 5×10^{10} tons (Detroy 1981; Sánchez and Cardona 2008), whereas approximately 1.5×10^{13} tons of cellulosic biomass are individually produced from nature every year via photosynthesis (Gupta and others 2013). Lignocellulosic substrates like cellulose, hemicellulose, and lignin are used as a food source by extensively distributed biomass-utilizing organisms that include bacteria, archaea, fungi, protists, plants, and invertebrate animals (such as insects and crustaceans) with their symbiotic gastrointestinal microbes. These organisms contain cellulase, hemicellulase, and other ligninolytic enzymes, which can potentially transform lignocellulose into glucose and other soluble sugars, the cheapest and most feasible alternative energy source on earth (Sweeney and Xu 2012; Iqbal and others 2013). Lignocellulolytic enzymes actively degrade lignocellulosic substrates, and are used in various applications in several food industries, such as fruit and vegetable juice, vegetable oil processing, winemaking, brewing, and baking industries (Bhat 2000).

Consumption of fruits and vegetables is vital to meet the recommended daily intake of vitamins, minerals, and fibers. The dietary fibers in these fruits and vegetables consist of lignocellulosic substrates (Poli and others 2011) such as cellulose, hemicellulose, pectin, and lignins (Table 1). Fruits and vegetables are also sources of phytochemicals with protective or disease-preventive properties. To avoid chronic diseases like cancer, diabetes, obesity, and heart disorders, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) have suggested consuming at least 400 g of fruits and vegetables daily, which can also mitigate

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Table 1–Lignocellulosic substrate composition in major fruits and vegetables.

Major fruits and vegetables	TDF	Cellulose[a] (%)	Hemicellulose[a] (%)	Pectin[a] (%)	Lignin[a] (%)	Reference
Apple pomace	78.20-89.8	40.0-43.6	19.0-24.4	9.0-11.7	15.0-20.4	(Nawirska and Kwaśniewska 2005; Chen and others 1988; Figuerola and others 2005)
Orange pulp	35.40-36.9	25.32	5.35	15.7-16.3	2.2-3.0	(Grigelmo-Miguel and Martín-Belloso 1998; Larrea and others 2005)
Pineapple shell	70.60-81.8	40.55-41.57	28.69-29.0	2.49-2.70	10.1-10.39	(Larrauri and others 1997; Pardo and others 2014)
Grape pomace	66.50-77.89	6-17.75	18.0-31.0	0.25-4.0	59.0-64.0	(Saura-Calixto and others 1991; Botella and others 2005; Valiente and others 1995aa)
Pear pomace	43.90-94.0	34.5	18.6	13.4	33.5	(Martin-Cabrejas and others 1995; Nawirska and Kwaśniewska 2005)
Peach pomace	31.0-36.0	28.7-30.0	18.6-20.0	20.5-23.8	5.35-6.0	(Grigelmo-Miguel and others 1999; Pagan and Ibarz 1999; Augusto and others 2011)
Cherry pomace	58.0-71.44	16-20	10.7	1.51	69.4	(Kosmala and others 2009; Nawirska and Kwaśniewska 2005; Basanta and others 2014)
Black currant	66.80	12.0	25.3	2.73	59.3	(Wawer and others 2006; Nawirska and Kwaśniewska 2005)
Mango pulp	30.70-74.0	11.5	7.0	3.9	4.2	(Gourgue and others 1992; Holloway and others 1985)
Olive	80.0	31.9-36.4	21.9-26.8	16.2-17.44	26.0-39.2	(Valiente and others 1995bb; Fernández-Bolaños and others 1999; Rodríguez and others 2008)
Chokeberry pomace	90.83	34.6	33.5	7.85	24.1	(Nawirska and Kwaśniewska 2005)
Carrot pomace	37.0-65.1	51.6	12.3	3.88	32.2	(Nawirska and Kwaśniewska 2005; Bao and Chang 1994; Chau and others 2004)
Tomato pomace	44.90-59.03	19.02	12.0	7.55	36.0	(Andres and others 2016; Del Valle and others 2006; Carvalheiro and others 1994)
Sweet potato	75.10-75.28	31.9	11.38	15.65	16.86	(Mei and others 2010)
Sugar beet pulp	67.0-67.30	22.0-30.0	24.0-32.0	24.0-32.0	3.0-4.0	(Spagnuolo and others 1997; Vural and others 2004)
Potato pulp	84.3-91.3	17.0-21.7	14.0	2.2	2.6	(Theander and others 1995; Meyer and others 2009; Steinbuchel and Mayer 1998)
Cauliflower	40.0-65.0	35.0-66.97	13.6-21.0	5.51	14.44	(Femenia and others 1997; Rani and Kawatra 1994)
Cabbage	27.3-28.1	63.02	15.20	6.79	14.88	(Kahlon and others 2008; Wennberg and others 2003; Rani and Kawatra 1994)

TDF, total dietary fiber.
[a]Values correspond to percentage of dry weight.

micronutrient deficiency. The 2011 global production of fruits and vegetables was 6.4×10^8 and over 1.0×10^9 metric tons, respectively, and the production is increasing significantly every year (FAO 2013). Because fresh fruits and vegetables contain more than 80% moisture, they are categorized as extremely perishable food. Modern techniques must be developed to preserve surplus food and food products during the off-season and extend their storage life for better distribution. Converting fruits and vegetables into various food commodities such as juice, jam, jelly, pickles, and fruit bars will not only extend their lifetime as food products but will also generate economic opportunities for poor farmers in developing countries (Vidhya and Narain 2011; Sharma and others 2016).

Enzymes have been widely used as biological catalysts intentionally or unintentionally for food processing since time immemorial. The commercial use of enzymes for starch hydrolysis by the food industry began during the 1960s. Nowadays, active research continues to significantly improve various enzymes to make them more convenient for use in many applications in the food industries. It has been reported that commercial enzymes are obtained from a wide range of organisms: almost half (< 50%) are from yeasts and other fungi, over one-third (< 35%) are from bacteria, and the rest (> 15%) are either from plant or animal sources (Anisa and Girish 2014). According to previous research data, the market value of commercially available enzymes has already reached US$4.2 billion in 2014 (Rohan 2016) and is expected to exceed US$6.0 billion in 2016 (Jaramillo and others 2015) and US$6.2 billion worldwide by 2020 (Rohan 2016). In industrial enzyme production, hydrolase enzymes dominate the global market and have a total market share of approximately 77% to 85% (Sharada and others 2014). Presently, lignocellulolytic enzymes such as cellulase, hemicellulase, and pectinase together represent more than 20% of worldwide sales of commercially available enzymes (Polizeli and others 2005; Jaramillo and others 2015). The functional use of cellulases and hemicellulases began as a means to produce animal feeds and later to produce food, textiles, and paper in the 1980s (Bhat 2000; Polizeli and others 2005). In the 21st century, extensive research makes it possible to produce tailor-made enzymes, which are more convenient and popular to use in specific reactions in various functional applications (Bhat 2000). The aim of this article is to review various functional applications of lignocellulolytic enzymes in the fruit and vegetable processing industries.

Lignocellulosic Substrates and Degrading Enzymes

Lignocellulosic substrates are made of 3 carbohydrate polymers, cellulose, hemicellulose, and pectin, and a noncarbohydrate polymer, lignin, in vascular plants. The substrates are mostly present in

dietary fibers (Poli and others 2011) and the primary cell wall of fruits and vegetables (Nagar and others 2012; Szymanska-Chargot and Zdunek 2013). Two major carbohydrates, cellulose and hemicellulose, are covalently cross-linked with noncarbohydrate lignin, which imparts strength to the cell wall of fruits and vegetables, and removal of lignin can accelerate the hydrolysis rates of cellulose and hemicellulose (Pérez and others 2002; Yuan and others 2013). The cell wall of vascular plants contains relatively more cellulose and hemicellulose than pectin, whereas the cell wall of fruits and vegetables contains more pectin than cellulose and hemicellulose (Chylińska and others 2014). A schematic illustration of lignocellulosic substrates is shown in Figure 1.

Cellulose and the cellulase enzyme

Cellulose is a linear homopolysaccharide composed of anhydrous repeating (1,4)-D-glucopyranose units linked together by regioselectively and enantioselectively β-1,4-glycosidic bonds. The repeating smallest unit of cellulose is called cellobiose and $(C_6H_{10}O_5)_n$ symbolizes the chemical formula of cellulose, where "n" indicates the number of glucose groups, which is called the degree of polymerization. Cellulose chains bound with hydrogen bonds constitute the microfibrils of the plant cell wall, which contain approximately 36 glucan chains and each chain contains an average of 500 to 15000 glucose molecules (Buchanan and others 2015; Ravindran and Jaiswal 2016) with an approximate molecular weight of 10 to 150 kDa (Gupta and others 2013). The cell wall polysaccharides of fruits and vegetables contain approximately 20% to 35% of cellulose in dry weight. The properties and structure of the cell walls of fruits and vegetables change gradually during the maturation and ripening period called "softening." Due to the lack of information about molecular size and crystalline and noncrystalline regional structures, the microfibril structure of celluloses in fruits and vegetables remains unknown (McFeeters 1985; Houben and others 2011; Nyman and Haskå 2013).

The hydrolysis of cell wall polysaccharides and the breaking of the β-1,4-glycosidic bonds in cellulose are catalyzed by a complex mixture of enzymes called the cellulase complex, which results in the formation of glucose (Glc) or other simple sugars and short cellodextrins. The cellulase complex comprises 3 different enzymes: endoglucanase or endo-1,4-β- D -glucanase (EG; EC 3.2.1.4), exoglucanase or exo-1,4-β-cellobiohydrolase (CBH; EC 3.2.1.91), and β-glucosidase (BG; EC 3.2.1.21). Because the cellulase complex has a broad spectrum of catalytic mechanisms, it was classified as glycoside hydrolases (EC 3.2.1.-) with family numbers 1, 3, 5, 6, 7, 8, 9, 12, 26, 44, 45, 48, and 51. Family numbers 61 and 74 were also proposed owing to a cellulase like activity (Zhang and Zhang 2013). In cellulose hydrolysis, endoglucanase (EG) and exoglucanase (CBG) enzymes 1st work synergistically to transform cellulose into small cellooligosaccharides, and then β-glucosidase (BG) hydrolyzes the cello-oligosaccharides into simple sugars or glucoses (Sathya and Khan 2014; Sindhu and others 2016).

Hemicellulose and the hemicellulase enzyme

Hemicellulose is a short chain and a highly branched heterogeneous polymer containing different pentose (β-D-xylose and α-L-arabinose) and hexose (β-D-glucose, α-D-galactose, and β-D-mannose) monomers in the form of *O*-acetyl-4-*O*-methylglucuronoxylans and *O* -acetyl-galactoglucomannans. α-D-glucuronic, α-D-4-*O*-methylgalacturonic, and α-D-galacturonic acids (GalA) may also be present in small amounts in the composition of hemicellulose, and thus give rise to an uronic acid form of the polymer, but the presence of these compounds varies from plant to plant and from location to location within a plant. Xylan is the most abundant hemicellulose polymer in monocotyledonous species, but xyloglucan is the most abundant in dicotyledonous species (Vicente and others 2009). Hemicelluloses are tightly bound to celluloses through non-covalent interactions of hydrogen bonds (van der Waals), and bound to lignin through covalent bonds. As a copolymer, hemicelluloses are composed of different quantities of various saccharide molecules, which makes it distinct from cellulose (Chen 2014). Hemicellulose comprise approximately 15% to 19% in dry weight of cell wall polysaccharides of fruits and vegetables (Luis and others 2010; Nyman and Haskå 2013), and the hemicellulose backbone contains approximately 500 to 30000 sugar units per chain with an approximate molecular weight of 30 kDa (Sun and others 2014; Shahzadi and others 2014).

Hydrolysis of hemicellulose by the hemicellulase enzyme is crucial not only for the degradation of the cell wall structure but also for enhancing the hydrolysis of tightly linked cellulose by the cellulase enzyme. Formation of valuable polysaccharides from hemicellulose is initiated after the breakdown of hemicellulose by 2 hemicellulase enzymes, endoxylanases and exoxylanases, because xylan is the most abundant hemicellulose in the cell wall of fruits and vegetables. Hemicellulases are classified based on their catalytic activity into glycoside hydrolases (GHs), carbohydrate

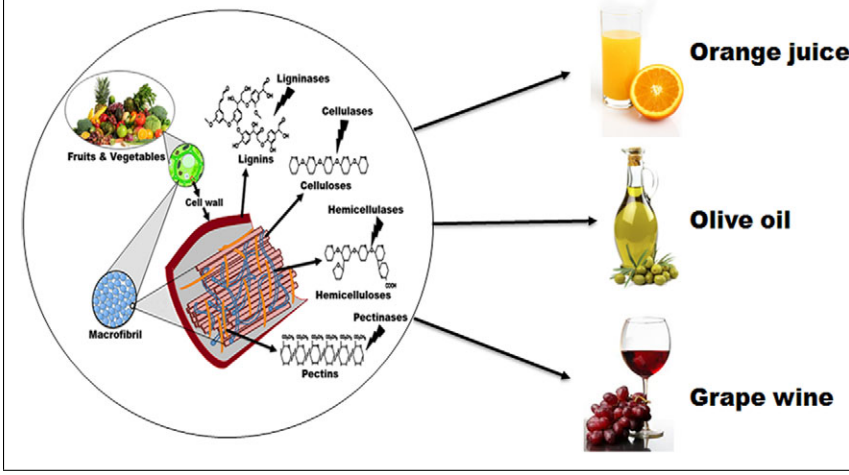


Figure 1–Schematic illustration of lignocellulosic substrates (cellulose, hemicellulose, pectin, and lignin) in fruits and vegetables and their applications in related food industries.

esterases (CEs), and polysaccharide lyases (PLs), which catalyze hydrolysis of glycosidic bonds, hydrolysis of ester linkages of acetate or ferulic acid side groups, and cleavage of glycosidic bonds, respectively. In addition to xylanase, hemicellulases include glucuronidase, mannanase, PGase, β-glucanase, arabinase, and acetyl xylan esterase (Howard and others 2004; Sweeney and Xu 2012; Sindhu and others 2016).

Pectin and the pectinase enzyme

Pectin is a naturally complex, hydrocolloid acidic polysaccharide. Its backbone is composed of D-GalA residues linked by α-1,4-glycosidic bonds and its side chains are mostly made of L-arabinose, D-xylose, D-galactose, and L-rhamnose molecules. Pectin is located between cellulose microfilaments in the cell wall, and the glycosidic macromolecules of pectin form the main component of the middle lamella in higher plants. Chemically, GalA residues of pectin are partly esterified by methyl ester in their carboxylic group and neutralized partly or entirely by sodium, potassium, or ammonium ions. Pectin is divided into 2 major groups: high-ester or high-methoxyl (HM) pectin and low-ester or low-methoxyl (LM) pectin (Figuerola and others 2005). The behavior of pectin in food applications depends on the ratio of esterified to nonesterified GalA and on the degree of esterification. Based on physicochemical characteristics, the American Chemical Society (ACS) categorized pectin into 4 different classes: protopectin, pectic acid, pectinic acid, and pectin (Jayani and others 2005). Pectic acid, pectinic acid and pectin are either partly or entirely soluble in water, but protopectin is completely insoluble in water (Alkorta and others 1998). Fruits and vegetables contain the highest amount of pectin at under-ripe or just ripe conditions, approximately 35% to 40% in dry weight (Luis and others 2010). Fruits and vegetables contain approximately 200 GalA residues per chain, with an approximate molecular weight of 25 to 50 kDa, as the pectin backbone (Chen 2014; Kumar and others 2015).

The cleavage of the different bonds of pectin or pectic substances in the cell wall is catalyzed by pectinases, a complex group of enzymes. Based on catalytic activity, most of the pectic polymer-degrading enzymes belong to family number 28 in the group of glycoside hydrolases, and these enzymes have been classified into 3 major groups: (1) PGases, also known as pectin depolymerases, that hydrolyze the α-1,4-glycosidic linkages in GalA residues; (2) pectin lyases (PL), that cleave the α-1,4-glycosidic linkages by transelimination of GalA residues and form galacturonide with an unsaturated bond at the non-reducing end of GalA; and (3) pectinesterases (PE), also known as pectin methyl esterases (PME), that catalyze de-esterification of the methyl group in GalA residues. PGases have been subdivided into endo-PGase and exo-PGase (exo-PGase-I and exo-PGase-II) based on the location of the cleaved bond in GalA residues (Suberkropp 2005; Khan and others 2013).

Lignin and the ligninolytic enzyme

Lignin is a complex, heterogeneous, and amorphous macromolecule consisting of phenylpropane units (ppu) linked by carbon–carbon (C–C) and carbon–oxygen (C–O) ether bonds. Monolignols or lignin monomers differ structurally only in the substitution number of the methoxy groups on the aromatic ring. Lignin polymers are composed of 3 main lignin monomers or monolignols: nonmethoxylated *p*-coumaryl alcohol, monomethoxylated coniferyl alcohol, and dimethoxylated sinapyl alcohol, which form the hydroxyphenyl alcohol (H), guaicyl alcohol (G), and syringyl alcohol (S), respectively (Barros and others 2015). Lignin polymers are mainly located between and around cellulose and hemicellulose complexes, filling the gaps and acting as glue. Because in vascular plants lignins are mostly abundant in the secondary cell wall of fruits and vegetables, these molecules can have unfavorable effects on texture and palatability. Lignin polymers are present in small amounts (less than 2% in dry weight), and their molecular weight may vary from plant to plant (Duckworth 1966; Mongeau and Brooks 2016).

Lignin contains aromatic polymers with phenolic and nonphenolic structures, and imparts strength to the cell wall of fruits and vegetables. Therefore, removal of lignin facilitates the hydrolysis rates of cellulose and hemicellulose. Ligninolytic enzymes capable of degrading lignin polymers in cell walls have been classified into 2 major families, peroxidases and phenol oxidases. Peroxidases are heme glycoproteins belonging to the fungal oxidative lignin enzyme (FOLy) family LO2 that requires hydrogen peroxide as an oxidant, and can be subdivided into 3 major classes: lignin peroxidase (LiP; EC 1.11.1.14), manganese peroxidase (MnP; EC 1.11.1.13), and versatile peroxidase (VP; EC 1.11.1.16). LiP, MnP, and VP have the capacity to degrade both phenolic and nonphenolic aromatic portions of lignin polymers through lipid peroxidation reactions. Phenol oxidase corresponds to the multicopper protein enzyme, laccase (E.C. 1.10.3.2), that belongs to the FOLy family LO1 and that oxidizes lignin phenolic compounds directly and non-phenolic compounds indirectly using active redox mediators. Glyoxal oxidase (EC 1.1.3.13), aryl alcohol oxidase (EC 1.1.3.7), and glucose oxidase (EC 1.1.3.4) also have the ability to degrade lignin polymers in cell walls (Marco-Urrea and Reddy 2012; Sweeney and Xu 2012; Pollegioni and others 2015).

Applications of Lignocellulolytic Enzymes in the Fruit and Vegetable Processing Industry

Lignocellulolytic enzymes have a wide range of important potential applications in the food processing industries ranging from the fruit and vegetable juice industry to the baking industry as described previously. However, this review will focus on applications of lignocellulolytic enzymes in fruit and vegetable processing industries, particularly on the fruit and vegetable juice industry including the production of nectars and purees, vegetable oil processing industry, and winemaking industry. A schematic illustration of lignocellulosic substrates and their applications in related food industries is shown in Figure 1.

Juice processing industry

The richness and availability of nutrients in fruits and vegetables encourage food processors to convert them into various food commodities. Juice products are typical food commodities most commonly produced from fruits and vegetables that are important not only for human health but also from a commercial point of view. During the early 1930s, the fruit and vegetable juice industry faced numerous difficulties related to low yields and filtering to obtain acceptable clarity. Presently, different enzymatic treatments are widely used as an integral constituent of modern juice processing from fruits and vegetables, and cellulases, hemicellulases, and pectinases are highly suitable for optimizing the process. The use of these enzymes is considered superior to mechanical and thermal comminution processing of several fruit and vegetable pulps. The main purpose of using cellulases, hemicellulases, and pectinases or a cocktail of these 3 enzymes in juice processing is to accelerate juice extraction from raw materials, to increase processing efficiency in pressing and in solid settling or removal, and to generate a clear and visually attractive final product. The important steps

involved in the production of juices from fruits and vegetables are illustrated in Figure 2.

Peel removal and juice extraction. Prior to the extraction of juices from fruits and vegetables, raw materials are processed through washing, sorting, chopping, or crushing. After washing, peeling is the 1st step for juice extraction depending on the variety of the fruit or vegetable. Most citrus and tropical fruits and vegetables such as bananas, avocados, oranges, beets, and pumpkins require removal of the skin and need to be chopped to pieces before juice extraction. Nowadays, enzymatic methods for peeling are replacing chemical, mechanical, steam, or freeze methods due to reduced damage to the flesh of fruits and vegetables as well as low peeling losses, which increase the quality and quantity of juice production. Concentrated mixture of pectinases, hemicellulases, and cellulases are used as enzymatic treatment for peeling of apricots, nectarines, and stone fruits, for example, peaches. For removal of orange skins, only pectinases or a mixture of hemicellulases with PG and arabinase enzymes provide good results at mild temperatures (Sharma and others 2013). Different commercial peeling enzymes such as Peelzyms I, II, III, and IV (NOVO Nordisk Ferment) have been widely used in the processing of citrus and other fruits. Among these peeling enzymes, Peelzym II (mixture of cellulases, hemicellulases, arabinases, and PGase) showed better peeling activity on lemon, orange, and grape fruits (Pagán and others 2010; Pagán and others 2011; Sanchez-Bel and others 2012). If the fruits and vegetables do not require a peeling method, juice extraction might be treated directly with pectic enzymes (for example, pectinases) to facilitate pressing and reduce the separation time of pulps by sedimentation, filtration, or centrifugation. A blend of pectinases with cellulase, arabinase, and xylanase are being used to enhance pressing efficiency for juice extraction from fruits and vegetables (Gailing and others 2000). The pectinase-like enzyme rhamnogalacturonase, obtained from a food-grade microorganism (*Aspergillus aculeatus*), has been used prior to juice extraction for better separation of pulps in some varieties of apple, like Golden Delicious, which are otherwise difficult to process effectively (Kashyap and others 2001). Thus, for better juice extraction, an enzymatic treatment is most effective for fruits and vegetables, which are mostly mature and come directly from cold storage (Lozano 2006).

Clear juice production from fruits and vegetables. Juices from fruits and vegetables typically contain highly concentrated polysaccharides like pectin, cellulose, hemicellulose, starch, and bound lignin (Vaillant and others 2001). These components make juices highly opaque and viscous after extraction, and enzyme treatment is necessary in the clarification step to produce haze-free, clear juices. Enzymes like pectinases have been used for decades in fruit and vegetable processing during pressing and straining of juices to reduce opaqueness and viscosity and to remove suspended pectinious substances. The use of these enzymes also enhances the extraction process by making juices more transparent. Using chitosan as a clarifying agent, a commercial pectinolytic enzyme Citrozym-Ultra L (from *A. aculeatus* and *Aspergillus niger*; Novozymes) showed better results in the production of clear açaí palm juices by reducing the lipids, insoluble solids, and other substances that can produce haze in açaí palm juices (Cesar and others 2014). In addition, infusion of pectinases and β-glucosidase activity have also shown better results in altering texture, flavor, and other sensory properties such as aroma and volatile characteristics of fruit and vegetable juices during processing (Kuhad and others 2011). A cocktail of pectinase (acid and thermostable), PGase, pectin esterase, and pectin transeliminase may increase the

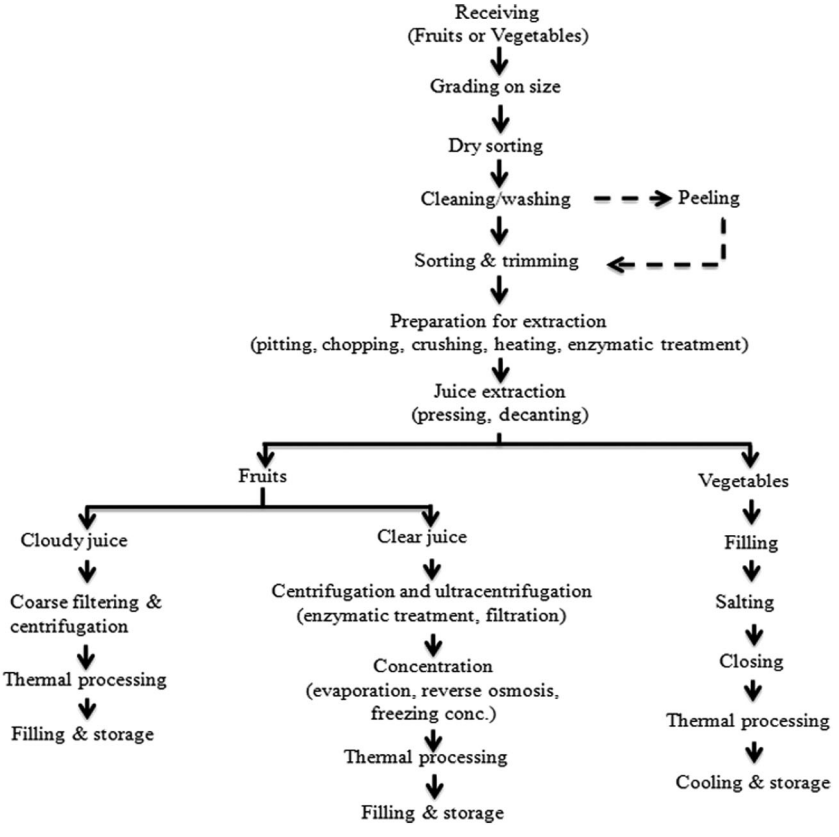


Figure 2–Steps involved in juice processing from fruits and vegetables. The peeling step is optional depending on the variety of fruit or vegetable.

juice drop rates of viscous stone fruits (for example, mangoes, peaches, cherries, and so on) by degrading the fruit tissues and may improve the juice color and reduce the pressing times of fruit mashes (Uhlig 1998). Although most fruits and vegetables are pectin-rich, certain fruits and vegetables like pineapples, tomatoes, and apples contain considerable amounts of xylans because the corresponding hemicellulose is xylan-rich. Therefore, treatment of pineapples, tomatoes, and apples with xylanases (from *Bacillus pumilus* SV-85S) enhances the corresponding juice transparency by 22.20%, 19.80%, and 14.30%, respectively, and increase juice yields by 23.53%, 10.78%, and 20.78%, respectively (Nagar and others 2012). A cocktail of xylanases with pectinase, carboxymethyl cellulase, and amylase has also been used for clarification of juices from apples, pineapples, and tomatoes. Enzymatic treatments of apple, grape, and orange juices with xylanase (from *Streptomyces* sp. AOA40) significantly increased juice clarity by 17.85%, 19.19%, and 18.36%, respectively, and improved the reducing sugar concentrations by 17.21%, 16.79%, and 19.57%, respectively (Adigüzel and Tunçer 2016). In addition, a lignin-degrading enzyme, laccase, in conjunction with cross-flow filtration (ultrafiltration), has shown better results in producing stable and clear juice concentrates with color and flavor stability in apple and sour cherry juice production (Minussi and others 2002; Osma and others 2010).

Mixtures of enzymes such as cellulases (endoglucanase, exoglucanase, and β-glucosidase), hemicellulases (endo- and exoxylanase, mannanase, glucuronidase, and xyloglucanase), and pectinases (PL, pectin acetylesterase, pectin methylesterase, endo- and exo-PGase, and endo- and exo-arabinases) have been widely used as macerating enzymes in the fruit and vegetable juice industries (Bhat 2000). During juice processing, macerating enzymes are used in 2 steps: after crushing the fruits and vegetables and after juice extraction. Macerating enzymes can hydrolyze the cell walls and soluble pectins of fruits and vegetables, helping to reduce juice viscosity and release more flavor, proteins, polysaccharides, and enzymes during processing while maintaining the textures of fruit and vegetable juices (Sharada and others 2014). These steps increase juice yield and transparency, improve stability, texture, flavor, and aroma, and reduce viscosity of nectars and purees from tropical fruits such as plums, papayas, peaches, mangos, apricots, citrus fruits, kiwifruits, apples, bananas, and pineapples. During the production of bilberry juices, different commercial lignocellulolytic enzyme preparations such as Pectinex BE XXL and Vinozym Ultra FCE (Novozymes), Rohapect 10 L, Rohapect PTE 100, and Rohament CL (AB Enzymes) have been used to increase the yields and total anthocyanin contents of juices. It has been reported that increasing doses of the above enzyme preparations from 0.5 nkat/g (manufacturer's recommended dosage) to 10 nkat/g significantly increased the yields (5% to 17%) and total anthocyanin contents (10.1% to 41.9%) of bilberry juices (Heffels and others 2016). In a sense, macerating enzymes are key to improve juice production from a wide range of fruits and vegetables in different juice processing industries, which resulted in a worldwide increase in demand for this enzyme (Bhat 2000).

Production of nectars and purees from fruits

Fruit and vegetable juices, nectars, and purees are internationally popular with consumers because these products contain most of the important components of the original fruits and vegetables, including flavor and taste. Fruit nectars are homogeneous products, which contain diluted purees or a certain amount of suspended particles to make it pulpy, and they can be blended with sweeteners, citric acid, and other stabilizers. Tropical fruits like mangos, peaches, guavas, bananas, and avocados have mostly been used to produce nectars and purees from fruits, and those products have been diluted or blended with other fruit products to make even more delicious fruit cocktails. PGase with high propectinase and low cellulase activities can partially hydrolyze the propectin of fruits to produce highly viscous purees from fruits (Bhat 2000). After mixing water, sugar syrup, and citric acid with nectars, the cloud particles tend to settle down and sometimes form gels, resulting in a clear supernatant layer, which may reduce the desirability of these products. Therefore, a very important quality factor of nectars is that cloud particles should be stable. In the production of fruit nectars and purees, use of exogenous enzymes prior to the pulping stage not only results in cloud stability but also generates higher yields. Several commercial enzyme preparations such as Rapidase TF (DSM), Pectinex UltraSP (Novozymes), and Rohapect PTE (AB Enzymes) are commonly used to reduce the viscosity of pulpy tropical fruit juices and enhance the flavor of fruit and vegetable juices (Grassin and Coutel 2010). Because tropical fruits contain more cellulose and hemicellulose components compared to other fruits, use of pectinases combined with hemicellulase and/or cellulases is recommended not only for fruit maceration but also to develop cloud stability, texture, and proper concentration of nectars and purees in the final product by reducing viscosity (Kashyap and others 2001).

Vegetable oil processing industry

Vegetable oils (cooking oils) from coconut, canola, sunflower seed, kernel, palm, and olive are commonly extracted with the organic solvent hexane, which is a potential carcinogen. The addition of cell-wall-degrading pectinolytic enzymes in the extraction of vegetable oils allows an aqueous process by softening the structural components and degrading the cell walls in oil-containing crops, and improves oil quality by increasing the extraction of phenolic compounds with high antioxidant activities (Kashyap and others 2001; Sharma and others 2013).

In recent years, olive oil gathered attention worldwide as a healthier alternative to other types of cooking oil. Olive oil contains 71% of monounsaturated fats, which are considered healthier than trans-fatty acids found in other oils. In addition, it may be possible to refine polyunsaturated fats during food processing. Olive oil corresponds to 3% of global use because of its premium market value, although usage of olive oil is increasing daily (Barjol 2013). Extraction of olive oil includes (1) crushing and grinding of olives using stone or hammer mills; (2) after that, the minced olive paste will pass throughout a series of malaxeurs and horizontal decanters; and (3) lastly, centrifugation at high speeds to recover the oil. Higher oil yields have been obtained from fully ripened olives when processed above room temperature, but the resulting oil showed high acidity, rancidity, and poor aroma. To overcome this problem, plant cell wall-degrading enzymes have been widely used in the olive oil extraction process. The 1st commercially available enzyme preparation, Olivex (from *A. aculeatus*, Novozymes), contained pectinases with low levels of cellulase and hemicellulase, and its use led to better stability of olive oils during the storage period after the extraction process (Galante and others 1998; Chiacchierini and others 2007). Furthermore, 2 commercial enzyme preparations such as Cytolase 0 (Gist-Brocades), a mixture of pectinase (from *Aspergillus* sp.), hemicellulase, and cellulase (from *Trichoderma* sp.), as well as some minor enzymes and Rapidase adex D (Gist-Brocades), a mixture of pectolytic with

cellulolytic and hemicellulolytic enzymes, can be used in olive oil preparations to enhance the aroma, increase polyphenols and vitamin E contents, and reduce the rancidity of extra-virgin olive oils (Chiacchierini and others 2007). Additionally, Rapidase adex D enzyme complex shows endo-PGase activity that can emulsify minute oil droplets by degrading the vegetable colloids such as hemicelluloses, pectin substrates, and proteins (Ranalli and others 2003).

Wine improvements in the alcoholic beverage industry

Use of enzyme technology in the production of alcoholic beverages, for example, wine processing, has a successful history. Lignocellulolytic enzymes such as glucanases and related polysaccharidases play a critical role in wine processing, not only by increasing the quality of products but also by improving overall yield (Galante and others 1998).

Wine-producing industry. In wine production, yeast strains and exogenous enzymes have both been used to enhance the process of grape juice fermentation and to increase overall wine production. Three main exogenous enzymes, pectinases, β-glucanases, and hemicellulases, have been widely used for hydrolyzing the polysaccharides in cell wall to enhance the skin maceration process, to improve the extraction of natural color from grape skin, to make must clarification and filtration processes easy, and finally to improve wine quality and stability. β-glucosidases are also employed to modify glycosylated precursors, naturally present in grapes, to improve the aroma during wine preparation. The 1st commercial use of pectic enzymes such as pectin esterase (PE), PGase, and PL (with small amounts of hemicellulase) was introduced in wine production around the 1960s (Galante and others 1998; Leisola and others 2001). Nowadays, pectinases (from *A. niger, Penicillium notatum*, or *Botrytis cinerea*) are being used in 3 stages during wine preparation to improve haze condition and pressability of grapes to reduce filtration time, and to increase must volume. Consequently, addition of pectinases increases the amount of extractable phenolic components such as polymeric anthocyanins and tannins, which are responsible for enhancing flavor and color intensity in wines (Kashyap and others 2001; Sharma and others 2013; Garg and others 2016). It has been reported that Cytolase 219 (Danisco-Genencor), a commercially available macerating enzyme (a mix of cellulase, pectinase, and xylanase), has been widely used in wine preparation from 3 varieties of white grapes: Soave, Chardonnay, and Sauvignon from Northern Italy. The use of this maceration enzyme resulted in increased extraction and filtration rates of 10% to 35% and 70% to 80%, respectively. The observed decrease in must pressing time and viscosity was 50 to 120 min and 30% to 70%, respectively. The overall improvement corresponded to a reduction of 20% to 40% in energy use of the fermenter cooling system and resulted in enhanced wine stability (Galante and others 1998). A mixture of commercial pectinase enzyme Vinozym FCEG (Novozymes) with yeast VR5 and commercial tannins enhances the extraction of anthocyanins during wine preparation and wine color stabilization (Li and others 2015). Furthermore, the lignin-degrading enzyme laccase has been used occasionally during preparation of must from grapes to avoid the undesirable alteration in organoleptic characteristics of wine by removing unwanted polyphenols. Laccase has also been used commercially in the production of cork stoppers for wine bottles, where the enzyme reduces by oxidation the cork taint and astringency problems in aged wine bottles (Minussi and others 2002; Osma and others 2010).

Future Trends and Perspectives

During fruit and vegetable processing, color and appearance, flavor (taste and aroma), texture or cloudiness, and bioactive compound (phytochemicals or phenolics) availability are important quality parameters depending on the product. Enzymatic degradation of fruit and vegetable lignocellulose can improve the qualities and yields of fruit and vegetable products, prompting the search for various microbial lignocellulolytic enzymes that can be applied in the fruit and vegetable processing industries. Microbial lignocellulolytic enzymes have been used as processing aids to improve product quality parameters or increase the efficiency of processing operations of value-added fruit and vegetable products.

The present review describes the main studies involving the application of lignocellulose-degrading enzymes in the fruit and vegetable processing industries. However, further improvement and novel applications are needed. In addition, the number and variety of value-added fruit and vegetable products are expected to increase significantly in the future. Therefore, understanding of the lignocellulose-degrading enzymes from alternative microbial sources, structure and kinetic mechanism of the enzymes, and improved production processes should be increased. This information can be used to improve production yields and decrease overall production costs. Multidisciplinary studies related to lignocellulose-degrading enzymes should be performed to (1) design a new process or improve the available process, (2) develop novel enzyme preparations with functionally synergistic effects, and (3) develop new methods for easier and cheaper production of these enzymes to fulfill the demands of the fruit and vegetable processing industries. Extensive knowledge of innovative process operations technologies, biochemical and kinetic behavior of lignocellulose-degrading enzymes, and recombinant DNA and downstream processing technologies will be important for further investigations.

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