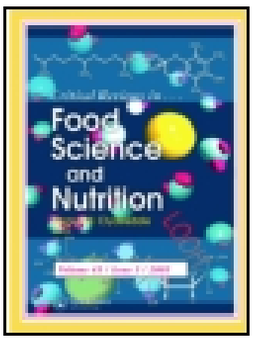

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REVIEW

Enzymatic treatment, unfermented and fermented fruit-based products: current state of knowledge

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ABSTRACT

In recent years, food manufacturers are increasingly utilizing enzymes in the production of fruit-based (unfermented and fermented) products to increase yield and maximize product quality in a cost-effective manner. Depending on the fruits and desired product characteristics, different enzymes (e.g. pectinase, cellulase, hemicellulase, amylase, and protease) are used alone or in combinations to achieve optimized processing conditions and improve nutritional and sensorial quality. In this review, the mechanisms of action and sources of different enzymes, as well as their effects on the physicochemical, nutritional, and organoleptic properties of unfermented and fermented fruit-based products are summarized and discussed, respectively. In general, the application of enzymatic hydrolysis treatment (EHT) in unfermented fruit-based product helps to achieve four main purposes: (i) viscosity reduction (easy to filter), (ii) clarification (improved appearance/clarity), (iii) better nutritional quality (increase in polyphenolics) and (iv) enhanced organoleptic characteristic (brighter color and complex aroma profile). In addition, EHT provides numerous other advantages to fermented fruit-based products such as better fermentation efficiency and enrichment in aroma. To meet the demand for new market trends, researchers and manufacturers are increasingly employing non-*Saccharomyces* yeast (with enzymatic activities) alone or in tandem with *Saccharomyces cerevisiae* to produce complex flavor profile in fermented fruit-based products. Therefore, this review also evaluates the potential of some non-*Saccharomyces* yeasts with enzymatic activities and how their utilization helps to tailor wines with unique aroma profile. Lastly, in view of an increase in lactose-intolerant individuals, the potential of fermented probiotic fruit juice as an alternative to dairy-based probiotic products is discussed.

KEYWORDS

Enzymatic hydrolysis treatment; fermentation; viscosity; organoleptic properties

Introduction

Due to increasing health awareness among consumers, the demand for fresh fruit products is rising exponentially (Grunert 2017; Tóth et al. 2020). In fact, this rapid demand is propelled by the numerous health benefits associated with the consumption of fresh fruit products. Fruits contain nutrients such as dietary fiber, vitamins (e.g. vitamins C and E) and minerals, which are essential for maintaining a healthy and balanced diet (Dreher 2018). As such, incorporating fresh fruits or fresh fruit products into our daily regimen will help to reduce the risk of various degenerative diseases. On the other hand, high sugar consumption is associated with a variety of health issues such as obesity, diabetes and coronary heart disease (Malik et al. 2019; Malik and Hu 2019). In general, the beneficial effects of fruits consumption are far greater than their disadvantages (Bosy-Westphal and Müller 2015).

Most fruits contain a high-water content which makes them highly perishable with a relatively short shelf life. According to Food and Agriculture Organization (FAO), up to 45% of fruits and vegetables are wasted annually due to spoilage during post-harvest processing or rejected due to lower quality standards. Additionally, preserving these fresh fruit products over a longer period of time is not only costly but also energy-consuming. Therefore, modern techniques are employed to transform them into higher-quality products such as fruit bars, pastes, jams, and juices to extend the shelf life of fruits. Among all the strategies, juicing is the most popular and easiest way while retaining the essential nutrients and phytochemicals.

Fruits contain soluble pectin, starch and in the presence of hydrophilic -OH group, water is attracted to produce viscous juices (Kumar 2015). The traditional mechanical method for fruit juice preparation is costly and less efficient, and the product is not esthetically appealing. Presently, the fruit juice market offers a wide range of fruit products with clear and pulp-enriched cloudy types. In general, clarified juices are proven to be much more popular than unclarified cloudy juices since they are visually more attractive.

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Beveridge (2002) reported that sedimentation and turbidity of apple juice were negatively perceived as a product defect by consumers. Therefore, depectinization and destarching are frequently required for juice clarification since pectin and starch not only lead to membrane fouling but filtration retardation as well. In recent years, individuals seem to focus predominantly on nutritional labels apart from attractive packaging (Włodarska, Pawlak-Lemańska, et al. 2019). With this in mind, both the intrinsic (nutritional and taste) and extrinsic (color and clarity) cues of the product have to be fully researched to improve the attractiveness and competitiveness of the product. In addition, citrus juices contain some bitter compounds (e.g. naringin, limonin, and nomilin), which may make the product unacceptable. Traditionally, β-cyclodextrins and resins are utilized to absorb those bitter compounds (Ni et al. 2014). However, these substances may simultaneously absorb many other nutrients (e.g. amino acids, vitamins) and introduce other off-odors (Claus and Mojsov 2018).

Apart from the above-mentioned products, perishable fruits are also converted into fermented fruit-based products (e.g. alcoholic beverages, probiotic juices) with improved health properties. However, the fermentation process is sometimes plagued with problems such as (i) the loss of color and aroma in wine products during filtration before bottling, (ii) long clarification and maturation process, and (iii) low juice yield and cloudiness (Kim and Park 2017; Guo et al. 2018; Jiang, Lu, and Liu 2020; Viegas et al. 1989). In addition, some fruit wines or alcoholic beverages also suffer from haze formation during storage (Bezerra et al. 2015; Narnoliya et al. 2019). The haze formation is normally caused by the oxidation of tannins, leading to the formation of a turbid appearance. Various chemicals such as bentonite and poly(vinylpolypyrrolidone) are used for wine clarification in industry. However, the disadvantage is that bentonite may bind desirable aroma compounds during wine clarification, thereby causing a loss of important aromatic compounds. Furthermore, many fermented fruit wines or alcoholic beverages also suffer from chemical or enzymatic discoloration, which might be caused by the oxidation of abundant phenolic compounds (Verma et al. 2018).

Moreover, proteins can contribute to the turbid appearance and haze of the fermented fruit wines during storage. In general, proteins are derived from fruits or microbial cells such as yeast and lactic acid bacteria. Although most of these proteins can be removed after fermentation, pathogen-related proteins, synthesized by the plants in response against bacterial or fungal infections will remain in the product (Hmid et al. 2016). On the other hand, during fermentation, the production of extracellular polysaccharides caused by several lactic acid bacteria (e.g. *Pediococcus* spp.) can impede grape wine filtration (Claus and Mojsov 2018), and these colloidal polysaccharides cannot be easily removed by using adsorbents, filtration, and flocculants.

Benefits of enzymatic hydrolysis treatments (EHT) in fruit-based products

To date, enzymes have been increasingly used by the fruit processing industry to improve the yield and recovery of fruit products in a cost-effective manner. It is postulated that the enzyme market in fruit and vegetable processing is estimated to reach USD 41.39 billion by 2022 (Markets and Markets 2017). This increase is spurred by an increasing demand for fruit-based products and a high specificity of enzymes in various biochemical reactions. Depending on the fruit type and desired product characteristics, different enzymes are commonly used alone or in combination during fruit juice processing (Sharma and Patel 2016). Some macerating enzymes (e.g. pectinase, cellulase and tannase) are used during fruit crushing to increase juice yield and extract more bioactive compounds or after juice extraction for clarification (Domingues et al. 2012). In addition, a combination of several enzymes is increasingly gaining market traction. Manufacturers such as Novozymes, DuPont, Amway and Roche are producing this kind of enzyme blends that largely comprises of various enzymes (ReportsnReports 2019). It was reported that mixed enzymes could speed up the hydrolysis rate and reduce the enzyme dosage required, and thus, this may help the industry to save time and reduce the overall cost (Adsul et al. 2020). For debittering citrus juices, naringinase neither binds to nutrients nor introduces any undesirable odor, making it a potentially perfect replacement of β-cyclodextrins and resins.

In fact, enzymes are often employed instead of mechanical treatments during juice extraction and clarification and have shown several advantages: (i) improvements in juice yield and clarity, (ii) reduced viscosity, (iii) beneficial physicochemical changes (increased total soluble solids, sugars and organic acids) (Saxena et al. 2014), (iv) increases in nutritional benefits (release of phytochemical and polyphenolic compounds) (Teles Cesar et al. 2014), (v) enhanced sensory quality (e.g. increase in color intensity due to the release of carotenoids) and (vi) increases in flavor intensity (Roy et al. 2018). Therefore, there are unlimited possibilities and approaches to the use of enzymes to fulfill different demands for various fruit products.

Leveraging on the beneficial impacts of EHT, enzymes are now used in various biotransformation reactions such as (i) pre-fermentation, (ii) post-fermentation and (iii) wine-aging for fast and reliable filtration to optimize the color, taste, and aroma of the end-products. Several studies revealed the beneficial impacts of EHT on the alcoholic beverages processing (Phuoc Minh, Thinh Pham, Thi Thao, et al. 2019; Kim and Park 2017), such as (i) accelerated settling time (Claus and Mojsov 2018), (ii) reduction in filtration time, (iii) accelerated clarification, (iv) increased juice yield, and (v) improvement in aroma and color intensity (Piemolini-Barreto, Antônio, and Echeverrigaray 2015).

Generally, *Saccharomyces cerevisiae* is predominantly involved in winemaking worldwide. Despite obtaining wines with uniform quality, the wine or alcoholic beverages produced by *S. cerevisiae* alone lack aromatic complexity. Some non-*Saccharomyces* yeasts, on the other hand, contain certain extracellular and/or cell wall-associated enzymatic activities which are beneficial in fermentation. For instance, non-*Saccharomyces* yeasts belonging to the genera *Debaryomyces*, *Candida* or *Pichia* possess different

β-glucosidase (BG) activities (likely cell wall-associated), which can hydrolyze glycosidic precursors, thereby releasing aroma compounds (Mateo and Maicas 2016). Besides BG activity, yeast strains possessing pectinolytic activity like *Metschnikowia pulcherrima* are also of interest since they can contribute to both clarification and aroma improvement (Belda et al. 2016a). In particular, *Kluyveromyces marxianus* has been widely studied for its pectinolytic activity and its potential applications in various fermented fruit products. It has been reported that the use of this yeast not only helped to produce superior products with pleasant flavor/taste and aroma, but also increased the contents of polyphenolics and anthocyanins (Rollero et al. 2018; Piemolini-Barreto et al. 2015).

Presently, food scientists and food manufacturers are also making great efforts to create highly nutritious foods with added benefits due to the emerging concept of utilizing foods as medicine ("functional foods") (Chugh and Kamal-Eldin 2020). Probiotics including clinically proven lactobacilli and bifidobacterial are beneficial microorganisms that are important for human health to prevent certain diseases (Lu, Putra, et al. 2018). Therefore, incorporating probiotics into fruit juices has major implications (White and Hekmat 2018). Some studies have proven that probiotics utilized fruit sugars (e.g. glucose, fructose) as substrates for energy to produce organic acids (Lu, Tan, et al. 2018; White and Hekmat 2018).

EHT and EHT sources

Fruits contain polysaccharides such as pectin, cellulose, hemicellulose, lignin, and starch. Other than the above-mentioned components, proteins and tannins are also present in certain fruits. Based on the different compositions, different enzymes can be employed in the hydrolysis of these substrates. To this end, it is important to have better insights into the mechanisms of action and sources of different enzymes, which are summarized in Table 1.

Table 1. Categories of different enzymes and their reaction mechanisms during EHT.

Enzyme Name	Subcategories	Functions	EC No.	Sources	References
Pectinase	Pectin methylesterase	De-esterification of methoxyl group of pectin to form pectic acid	3.1.1.11	*Aspergillus* spp.	Garg et al. (2016)
	Hydrolases				
	Polygalacturonase (PG)	Hydrolyze α-1,4-glycosidic in polygalacturonic acid		*Bacillus* sp. NT-33 *Penicillium frequentans*	
	Endo-PG	Random hydrolysis of pectic acid	3.2.1.15	*Sclerotium rolfsii* *Saccharomyce cerevisiae*	
	Exo-PG	Sequential hydrolysis of pectic acid	3.2.1.67	*Kluyveromyces marxianus*	
	Polymethylgalacturonase (PMG)	Hydrolyze α-1,4-glycosidic bonds			
	Endo-PMG	Random cleavage of pectin, preferentially highly esterified pectin			
	Exo-PMG	Sequential cleavage of pectin from the non-reducing end of pectin chain			
	Lyase				
	Polymethylegalacturonate lyase (PMGL)	Breakdown of pectin by trans-eliminative cleavage		*Bacillus sp* DT7 *Aspergillus ficuum*	
	Endo-PMGL	Random cleavage of α-1,4-glycosidic linkages in pectin	4.2.2.10	*Penicillium paxilli*	
	Exo-PMGL	Sequential breakdown of pectin by trans-eliminative cleavage			
	Polygalacturonate lyases (PGL)	Cleave α-1,4-glycosidic linkage in pectic acid by trans-elimination			
	Endo-PGL	Random cleavage of pectic acid	4.2.2.2		
	Exo-PGL	Sequential cleavage of pectic acid	4.2.2.9		
	Protopectinase	Degrade insoluble protopectin to form soluble pectin		*Kluyveromyces fragilis*. *Galactomyces reesei* *Trichosporon fragilis* *Bacillus subtilis*	
Cellulase/Glycosidase	Endo-$\beta-1$,4-glucanases	Break non-covalent interactions in the endocellulose	3.2.1.4	*Aspergillus niger* *Trichoderma reesei*	Singh, Verma, and Kumar (2016)
	Exo-β 1,4-cellobiohydrolases	Hydrolyze the chain ends to break polymers into smaller sugars	3.2.1.91	*Trichoderma reesei*	
	β-glucosidase	Hydrolyze disaccharides and tetrasaccharides into glucose	3.2.1.21	*Trichoderma reesei*	

(*continued*)

Table 1. Continued.

Enzyme Name	Subcategories	Functions	EC No.	Sources	References
Hemicellulase	Xylanase	Hydrolyze β-1,4 bond in the xylan backbone to yield short chain xyglooligomer	3.2.1.8	*Aspergillus spp., Bacillus subtilis, Trichoderma reesei*	Brigham, Adney, and Himmel (2018)
	β-mannanase	Hydrolyze mannan-based hemicellulose and liberate short β-1,4 manno-oligomers	3.2.1.78		
	β-mannosidase	Hydrolyze manno-oligomer to mannose	3.2.1.25		
Amylase	α-amylase	Break α-1,4-glycosidic bond of starch into sugars	3.2.1.1	*Aspergillus spp., Bacillus spp., Microbacterium imperiale*	Mojsov (2016)
	β-amylase	Catalyze hydrolysis of α-1,4-glycosidic bond, cleaving 2 glucose units	3.2.1.2	*Bacillus spp.,*	
	Glucoamylase	Cleave α-1,6-glycosidic bonds and α-1,4-glycosidic bond at the non-reducing end of amylose and amylopectin to produce glucose	3.2.1.3	*Aspergillus niger, Rhizopus* spp.	
Laccase		Oxidize phenolic compounds to yield free radicals, which are transformed into quinones. Quinones and free radicals will then undergo polymerization.	1.10.3.2		Mate and Alcalde (2017)
Naringinase		Hydrolyze naringin to yield rhamnose and naringenin	3.2.1.40	*Aspergillus niger, Aspergillus oryzae, Penicillium decumbens, Phomopsis citri, Aspergillus usamii, Cochliobolus miyabeanus, Rhizoctonia solani*	Naqash, Masoodi, and Rather (2019)
Protease		Hydrolyze peptide bonds in proteins to yield amino acids and polypeptide chains	3.4.21.63	*Escherichia coli*	Raveendran et al. (2018)
Tannase		Cleave ester bonds in gallotannins, complex tannins and gallic acid esters to liberate gallic acid	3.1.1.20	*Aspergillus niger, Aspergillus flavus, Trichoderma* spp.	Yao et al. (2014)

Pectinases

Pectinase is one of the first few enzymes to be applied commercially in fruit and wine processing industries. To date, this enzyme has accounted for about 25% of the global enzyme market, especially in the fruit juice industry to produce clarified juices, with higher yields and better flavor profiles (Oumer 2017).

Pectinase primarily works by degrading pectic polymers into galacturonic acid. This enzyme is commonly categorized into three major groups: (i) pectinmethylesterases (PME), (ii) protopectinase and (iii) depolymerizing enzymes, based on its catalytic mechanism and the pectic substrates it acts on. PME catalyzes the de-esterification of pectin into pectic acid; protopectinase helps to solubilize protopectin into highly polymerized pectin by hydrolyzing the inter-site of protopectin that contains polygalacturonic acid or the outer site of protopectin that connects the cell wall components with polygalacturonate, and depolymerizing enzymes hydrolyze α-1,4-glycosidic linkages between adjacent galacturonic units. In general, a combination of these enzymes is required to degrade pectic substances fully. Conventionally, pectinases such as polygalacturonases (PG), pectin lyases (PL) and PME are obtained from fungal sources like *Aspergillus*.

Pectic substances are complex polysaccharides that are composed of a linear homogalacturonan chain that is bonded via α-1,4-linkages, in which the galacturonosyl residue can be esterified with methanol or being acetylated. This complex structure is composed of rhamnogalacturonan side chains, consisting of L-rhamnose and neutral sugars (arabinose, galactose, and xylose). Therefore, depending on the chemical structure (e.g. degree of esterification), pectic substances are classified into pectic acid, pectinic acid, protopectin (found in the unripe fruit, insoluble in water) and pectin (found in the ripe fruit, soluble in water), each with different physical and functional properties (Lara-Espinoza et al. 2018). The cell walls of unripe fruits are rigid due to the presence of protopectin which binds to cellulose microfibrils (Lara-Espinoza et al. 2018). During fruits ripening, endogenous pectinase can hydrolyze protopectin into water-soluble pectinic acid and pectin, leading to tissue softening (Bhardwaj, Degrassi, and Kumar Bhardwaj 2017). In addition, pectin can be de-esterified with PME to yield pectin acid, a water-soluble galacturonan. Since the cellular structure and cell integrity are composed of these pectin acids, any changes in the polymer's composition will affect the rheological and textural properties of fruit products. In general, the presence of pectin molecules with a higher degree

of esterification and molecular weight are responsible for the viscous texture.

Therefore, based on the desired organoleptic properties of the final product, enzyme manufacturers can customize the specific type of pectinase to preferentially degrade or modify pectic substances. Figure 1A shows the degradation reaction of pectic substrates by different types of pectinase into simpler molecules like galacturonic acid. Depending on the fruit type and the pectinase composition, degradation can occur through various mechanisms and yield different results. This is because the dissolution of protopectin into pectin can lead to an increase in viscosity while the depolymerization of pectin will lead to a decrease in viscosity.

Cellulase and β-glucosidase

The use of cellulases has also been an integral part of modern fruit processing technology. Cellulases such as endoglucanase, exoglucanase and cellobiase are often used in combination for the extraction and clarification of fruits, to increase the nutritional quality of fermented food as well as to produce oligosaccharides as functional food ingredients.

Cellulases refer to a multi-enzyme mixture that aid in the hydrolysis of cellulose into glucose (Raveendran et al. 2018). Commercial cellulases are often manufactured and isolated from bacteria or fungal sources such as *Aspergillus niger*, *A. nidulans* and *A. oryzae* (Table 1). This enzyme can be categorized into three major groups known as (i) endo-β-1,4-glucanases (EG), (ii) exo-β-1,4-cellobiohydrolases (CBH) and (iii) β-glucosidases (BG). In general, these enzymes work synergistically to convert crystalline cellulose to glucose (Figure 1B). The catalysis achieved via endohydrolysis of β-1,4D-glycosidic bonds in cellulose and β-D-glucans by EG, followed by the hydrolysis of β-1,4D-glycosidic bonds from the non-reducing ends in the presence of CBH, thereby releasing cellobiose units (Singh et al. 2019). BG hydrolyzed cellobiose to release glucose from the non-reducing ends of cello-oligosaccharides (Singh et al. 2019). Commonly consumed fruits and vegetables such as apple (8.3–9.3 g/100 g of dry pomace), cucumber (15.2–17.0 g/100 g of dry pomace) and carrot (9.8–10.2 g/100 g of dry pomace) contain a high composition of cellulose (Szymańska-Chargot et al. 2017). Therefore, the utilization of cellulase is highly favored due to the immense benefits this enzyme can provide in terms of viscosity reduction, clarification, and extraction purposes.

Additionally, another hydrolytic enzyme in the fruit processing industry is BG. BG is responsible for the hydrolysis of β-1,4- and β-1,6-glucosidic bonds of β-D-glucopyranoside (Gong et al. 2014). In fact, this enzyme plays a crucial role in catalyzing the hydrolysis of cellulose whereby BG can break down short oligosaccharides into glucose monomers which can be fermented by yeast into ethanol (Singh, Verma, and Kumar 2016). BG can be obtained from different organisms which include eukaryotes, bacteria, and archaea (Singh, Verma, and Kumar 2016). Depending on the substrate specificity, this enzyme can be categorized into three groups: (i) aryl-β-D-glucosidase which cleaves aryl-β-D-glucosides, (ii) cellobiase which hydrolyzes disaccharides and (iii) other glucosidase that hydrolyzes a range of substrates (Srivastava et al. 2019). To date, BG has been utilized in the beverage industry due to its key role in flavor liberation and nutritional enhancement, making it highly desirable in food applications.

Hemicellulase

In general, macerating enzymes such as pectinase and cellulase are often used in conjunction with hemicellulase to process clear fruit pulps, nectars and juices from raw fruit materials. With the knowledge of different fruit compositions, the use of enzymes can help to enhance the extraction process, thereby delivering visually appealing products and improving processing efficiency. Viscozyme L, a commercial enzyme containing cellulase, hemicellulase, arabanase and xylanase were shown to effectively improve extraction yield (15.8%), increase total soluble solids and antioxidative capacity of mulberry fruits as compared to the untreated sample (Nguyen and Nguyen 2018). Presently, the commercial sale of lignocellulolytic enzymes (pectinase, cellulase and hemicellulase) accounts for approximately 20% of all enzymes worldwide. Apart from pectinase and cellulase, the use of hemicellulase in fruit juice processing is becoming essential due to the high hemicellulose content (15–19%) in the cell wall polysaccharides of fruits and vegetables such as apple, grape, black current, mango and pear (Toushik et al. 2017). Therefore, the addition of hemicellulase in fruit juice processing is vital to effectively degrade the cell wall structure (hemicellulose) in these fruits and to enhance the breakdown of tightly linked cellulose by cellulase.

Hemicellulose is classified according to the presence of sugar residues such as D-galactan, D-mannan and D-xylan. This lignocellulosic substrate is tightly bound to cellulose by hydrogen bonds while covalently bonded/bound to lignin. Therefore, apart from using cellulase in the degradation of cellulose, hemicellulase is required for the complete degradation of the polysaccharide cell wall. In general, hemicellulose-degrading enzymes include endo- and exo-xylanases, xyloglucanases, galactanases and mannanases. Xylan is the most abundant hemicellulose in fruits and vegetables, and hence xylanase is required to hydrolyze the β-1,4- bond in the xylan backbone (Figure 1C). D-xylanase is generally produced by fungi, bacteria, insects, crustaceans and plants. In particular, D-xylanases of fungal origin have an optimum pH of 3.5 to 5.5 and a temperature of 50 °C. Alongside xylanases, D-galactanases are hydrolytic enzymes that are able to degrade D-galactans and L-arabino-D-galactans. This enzyme is specific for 1,3- and β-1,4-D-galactopyranosyl linkages. The presence of endo-D-galactanase is able to degrade D-galactan randomly to produce D-galactose and D-galacto-oligosaccharides, respectively. On the other hand, mannase helps to hydrolyze mannan-based hemicelluloses to yield β-1,4-manno-oligomers. This oligomer can be further hydrolyzed into mannose by β-mannosidases. Due to its ability to break down complex polysaccharides in the cell wall, hemicellulase is a promising enzyme with great potential in the processing of fruit-based products.

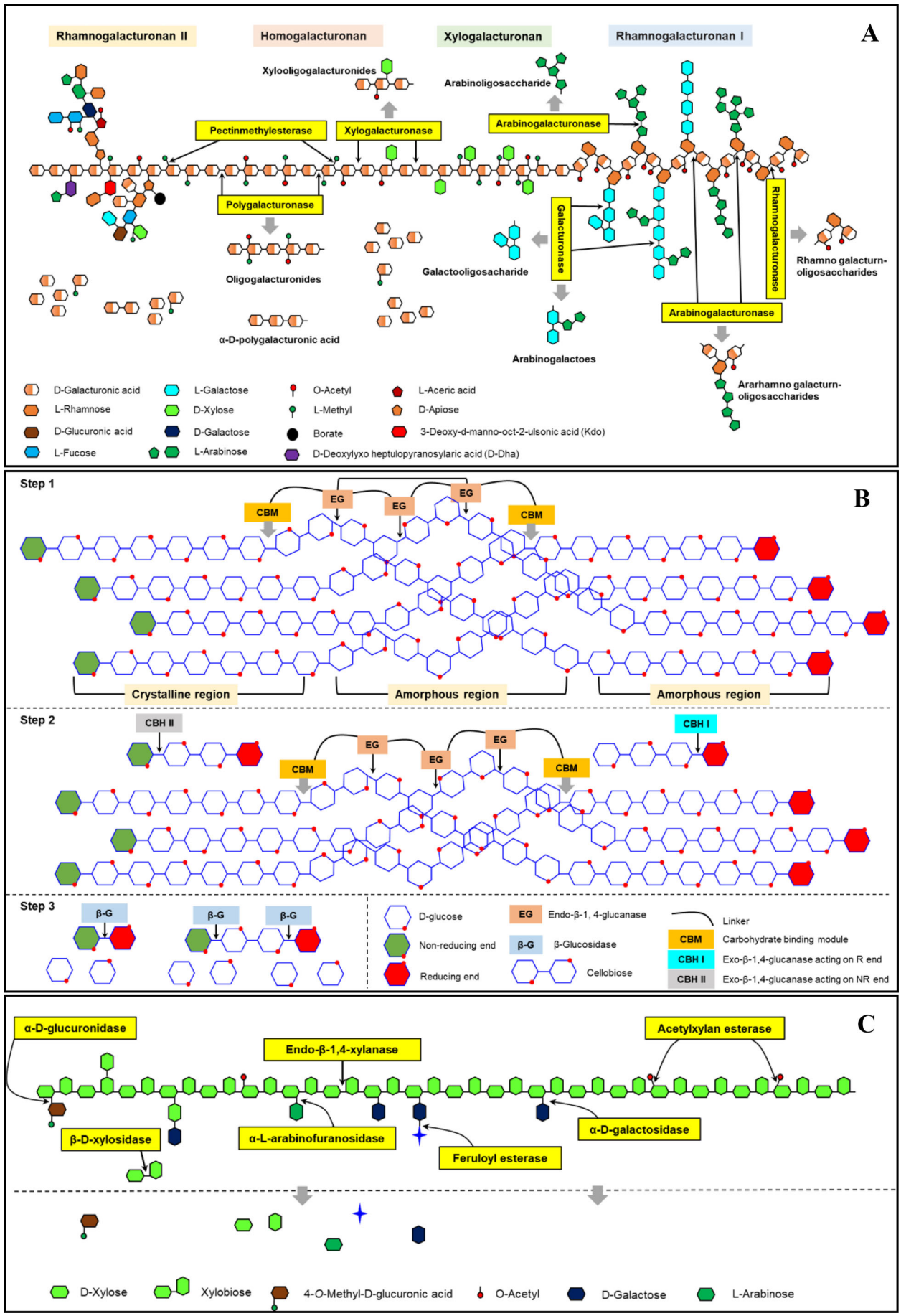

Figure 1. (A) Reaction mechanisms of pectinase on pectin (Tan et al. 2018); (B) cellulase on cellulose (Behera et al. 2017; Dutta and Wu 2014); (C) hemicellulase on hemicellulose (Dutta and Wu 2014).

Amylase

Amylase is one of the first few enzymes being commercially applied in food biotechnology, fermentation, and the pharmaceutical industries. This class of enzyme is naturally produced in plants, animals, fungi, yeast, and bacteria. As shown in Figure 2A, α-amylase leads to the hydrolysis of α-1,4-glycosidic linkages in starch (e.g. amylose and amylopectin) into glucose, maltose, and dextrins. While other amylolytic enzymes (e.g. glucoamylase) are required in the complete breakdown of starch, α-amylase plays the most important role in the hydrolysis of starch.

Depending on the origin of the enzyme, various amylases have significant differences in their pH, temperature and thermostability (Mehta and Satyanarayana 2016). For instance, fungal amylase, *A. oryzae* is the least thermostable while bacterial amylase, *Bacillus subtilis* has a higher thermal stability (Dal Magro et al. 2016). Apart from α-amylase and glucoamylase, exo-acting enzymes can catalyze the

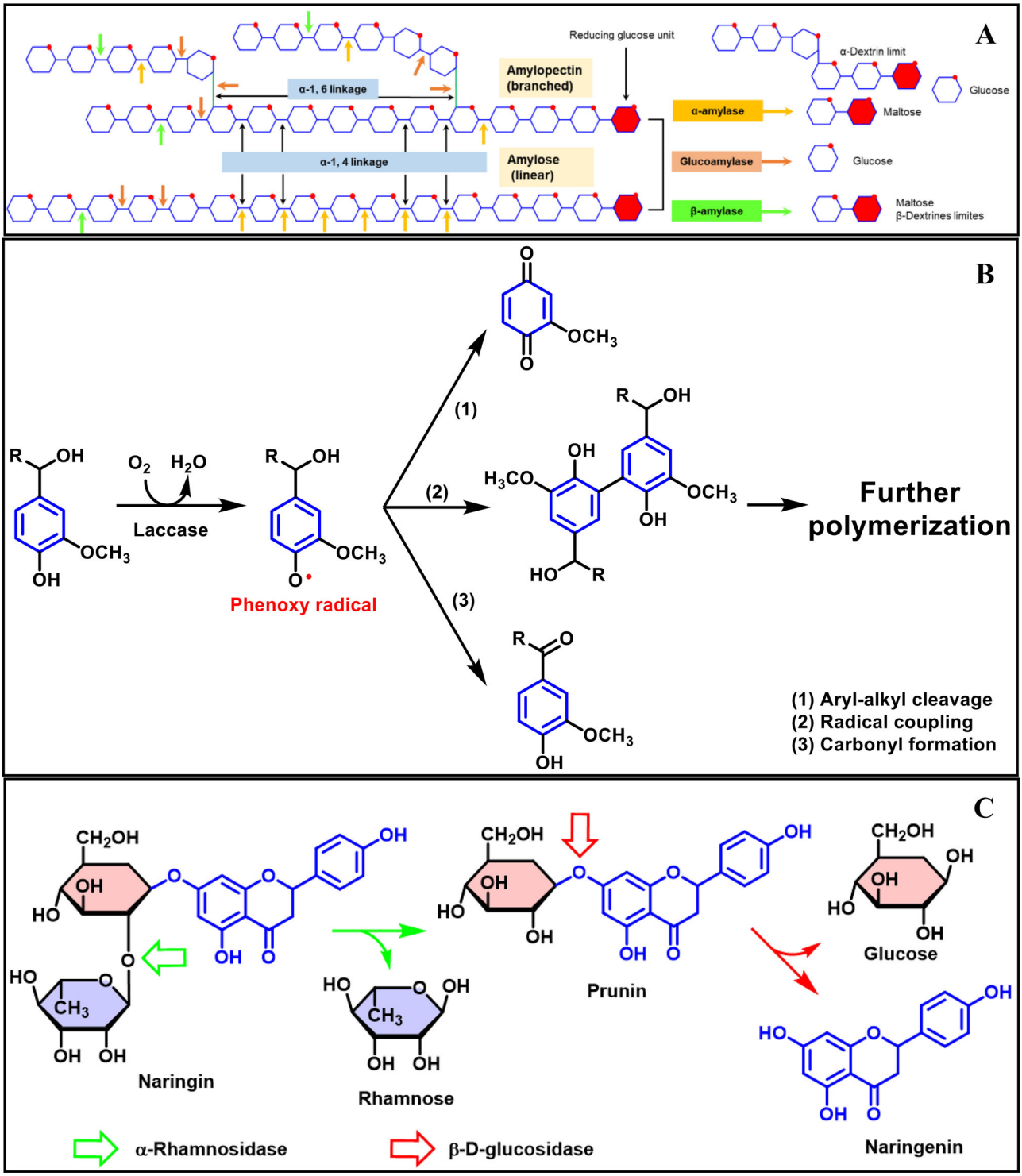

Figure 2. (A) Reaction mechanisms of amylase on starch (Contesini et al. 2013); (B) laccase on phenolic substrates (Minussi, Pastore, and Durán 2002); (C) naringinase on naringin (Singh et al. 2019).

hydrolysis of polysaccharide starch from the non-reducing end, thereby releasing β-glucose. These enzymes are mainly isolated from *A. awamori*, *A. niger* and *Rhizopus oryzae*. Currently, glucoamylases are widely applied in the production of fermented products such as sake and soya sauce (Raveendran et al. 2018). This is because glucoamylase treatment can help in the metabolization of dextrins by converting them into fermentable sugars and thus increase alcoholic level in final products (Raveendran et al. 2018). Due to the immense benefits, amylase can be employed commercially to provide effective clarification and viscosity reduction in highly viscous fruits.

Laccase

Laccase, an enzyme that can oxidize both phenolic and non-phenolic lignins, is of great interest in the food and beverage processing industry. In particular, some main applications of this enzyme include brewing and color enhancement. In general, this enzyme could be produced from fungi, plants, bacteria or insects (Yang et al. 2017) and be further categorized into low or high redox potential. Low redox potential laccase can be found in bacteria, plants and insects while high redox potential laccase is typically found in fungi. In general, phenolic compounds are laccase substrates which can be oxidized to phenoxyl free radicals before undergoing radical rearrangements or coupling-based polymerization (Figure 2B). In recent years, the use of laccase has been on the rise due to its ability in resolving haze formation in conjunction with color and flavor enhancement (Yin et al. 2017; Narnoliya et al. 2019; Bezerra et al. 2015).

Naringinase

In recent years, there has been an increased attention in the use of naringinase for its hydrolytic function on naringin which releases L-rhamnose and naringenin (Table 1). In general, this enzyme complex is composed of α-rhamnosidase and BG which facilitate the breakdown of naringin (Figure 2C). α-Rhamnosidase first hydrolyzes naringin to purnin and rhamnose. Purnin is then hydrolyzed to naringenin and glucose by BG (Singh et al. 2019). These enzymes are often obtained from bacteria (*Aspergillus* species and *Bacillus* species) or fungi. In fact, both naringin and naringenin are associated with similar health promoting benefits which include free-radical scavenging activities and DNA repair (Cavia-Saiz, Busto, Pilar-Izquierdo, Ortega, Perez-Mateos, and Muñiz 2010). Therefore, the conversion of naringin into naringenin is not expected to alter the health-promoting effects of various fruit juice products. Naringinase is mainly responsible for the debittering effect of citrus fruits. Aside from the debittering effect, this enzyme also plays an important role in improving wine aroma when used in conjunction with BG and arabinosidase (Claus and Mojsov 2018). In fact, naringinase has an array of food applications but more studies have to be conducted to achieve a cost-effective enzyme production. All in all, the enzymatic debittering technology seems promising due to its high specificity, nutrient retention and its ability to remove bitter compounds from the juice. In general, this enzyme can be exploited in the near future to produce nutritive fruit based products with reduced bitterness.

Protease

Proteases have been widely used in the food processing industry and account for approximately 60% of the total worldwide sale of enzymes (Souza et al. 2015). The fruit processing industry is interested in using these enzymes since they have broad substrate specificities and are active over a wide range of temperature and pH. Protease is able to hydrolyze the peptide bonds of proteins into peptides and/or free amino acids (Figure 3A). This enzyme can be obtained from different sources, namely, fungi, plants, bacteria, and animals. However, to date, microbial proteases are the most preferred since they are inexpensive and more resistant to heat. In general, proteolytic enzymes can also be categorized into four different groups: (i) acid protease, (ii) serine protease, (iii) sulfhydryl protease and (iv) metal-containing protease. Acid proteases include pepsin, renin, and other microbial proteases with low optimum pH values (pH 2.5–5.0). Serine proteases include chymotrypsin, thrombin, elastase and trypsin, which have an imidazole group at the active sites as well as a seryl residue which is involved in the active site. Sulfhydryl protease has active sites which contain cysteine and a histidine group that is required for enzyme activity. Metal-containing proteases are exopeptidases that require metal for the hydrolysis activity and they include carboxypeptidase A and B, which removes amino acids from the end of the peptide chains that contains a free α-carboxyl group.

Tannase

Another clarifying agent that is also widely utilized in the processing of fruit products and wine is tannase. Tannase participates in the hydrolysis process whereby the ester bonds present in the hydrolyzable tannins and gallic acid esters are cleaved. As a result, glucose and gallic acid are produced (Figure 3B). In general, this enzyme can be obtained by plants, animals, and microorganisms (bacteria, yeast and fungi). Among the various organisms, tannase is often obtained from microorganisms as it is able to produce stable enzymes at a higher yield. Additionally, this enzyme is able to hydrolyze tannin, a naturally occurring substrate that is found in a wide variety of plants (Yao et al. 2014). In fact, the presence of tannin contributes to the bitter taste in food, which is undesirable to consumers (Yao et al. 2014). Moreover, high tannin levels are also responsible for sediment formation and organoleptic changes (astringency, bitterness and color) in the fruit juice (Rinaldi and Moio 2018). Hence, in order to control the quality and taste acceptability of the fruit juice, tannase can be applied in the fruit beverage industries.

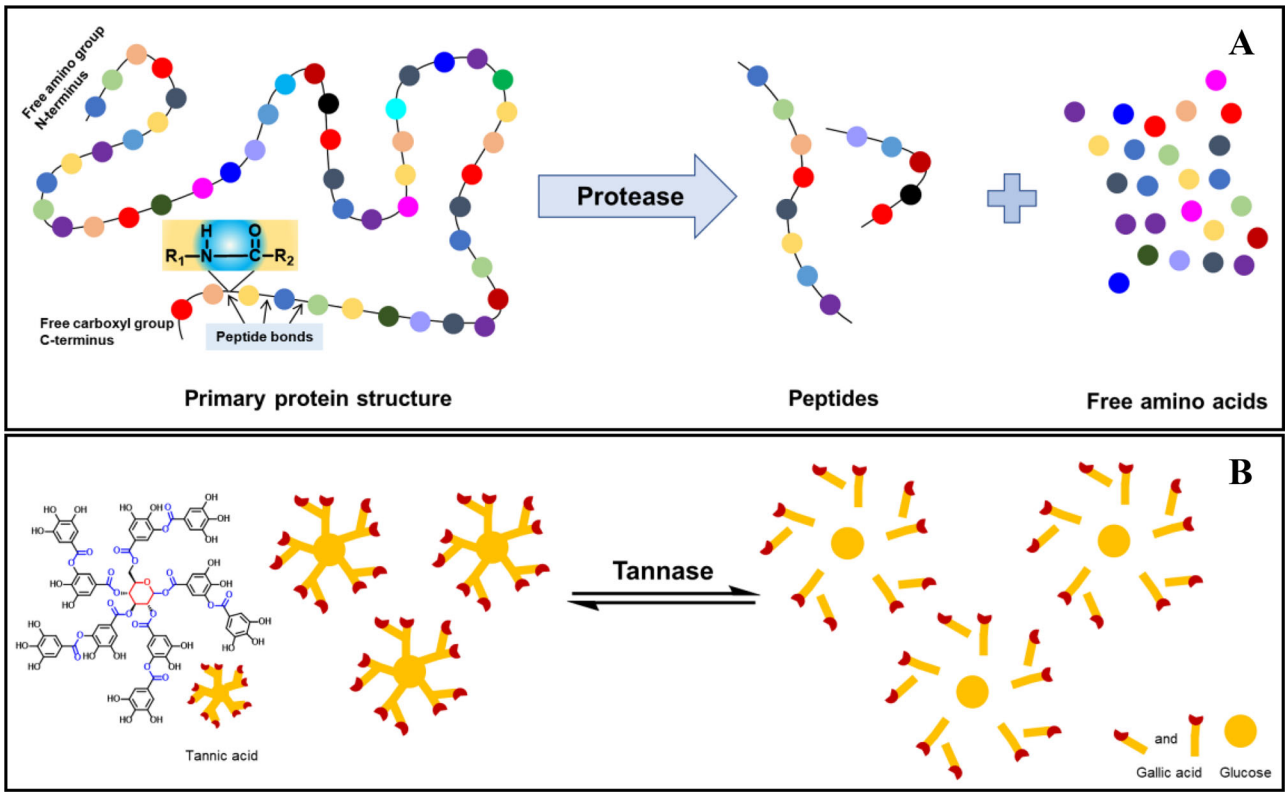

Figure 3. (A) Reaction mechanisms of protease on protein (Kiewiet, Faas, and De Vos 2018); (B) tannase on tannic acid (Zheng, Shi and Chi 2019).

Application of EHT in unfermented fruit products

Physicochemical impacts of EHT on unfermented fruit products

The use of enzymes has been examined extensively over the years. Among the various enzymes, pectinase, cellulase and hemicellulase have been widely used singly or in combination to reduce viscosity and/or to increase juice yield during fruit juice processing (Table 2). Through the enzymatic process, cellulosic and adhesive or sticky substances which form a highly viscous gel-type layer are broken down to facilitate filtration. For instance, pectinase helps to increase juice yield by (i) degrading pectic polysaccharides in the cell wall and the middle lamella, thereby allowing greater release of intracellular liquid; (ii) disintegrating the gel structure to further decrease viscosity and improve pressability. In addition, cellulase helps to increase juice yield and reduce viscosity by breaking down the cellulosic structures. Ongaratto and Viotto (2016) reported that pectinase and cellulase helped to reduce the viscosity of pitanga juice by up to 15% and 23%, respectively. A combination of these enzymes was reported to be more efficient than that of single enzyme treatment both in honey jackfruit and banana juices (Handique, Bora, and Sit 2019; Wong and Tan 2017), which were ascribed to the synergistic effects of different types of enzymes.

Apart from pectin and cellulose, starch is also responsible for the high viscosity of some types of fruit juice products. Therefore, amylase is helpful in viscosity reduction of starchy fruits like banana and apple. In general, α-amylase and polygalacturonase were shown to effectively reduce the viscosity of apple juice by 40% (Dey and Banerjee 2014). In addition, a combination of different enzymes (e.g. pectinase, cellulase, hemicellulase and amylase) was more effective in viscosity reduction by breaking down different substrates of banana juice, and significantly improved the processing efficiency (Handique, Bora, and Sit 2019).

As described above, the reduction in viscosity is ascribed to the degradation of polysaccharide components (e.g. pectin, cellulose and starch), which in turn affects the particle size (decrease or increase the particle size) of the substrates (Table 2). A previous study reported that the decrease of particle size effectively inhibited coalescence and therefore contributed to the stability of the product during storage (Fasawang and Anprung 2014). In addition, the degradation of polysaccharides also affected zeta potential, an important indicator of the surface charge of particles. Additionally, this parameter is related to the degree of electrostatic repulsive forces between the particles. Furthermore, the changes in zeta potential will lead to changes of interaction of interparticles and dispersion stability.

Generally, the use of pectinase leads to the breakdown of negatively charged pectin, and this results in the decrease of zeta potential. However, in some cases, the mean particle size increased after pectinase treatment (Garg et al. 2016). This is because the partial degradation of negatively charged pectin potentially caused the exposure of positively charged protein, leading to an aggregation of cloudy particles via electrostatic attraction (Garg et al. 2016). This indicates that the reduction of viscosity facilitated the flocculation process. In order to gain more insights into the effects of EHT on

Table 2. Beneficial effects of EHT on physicochemical, nutritional and organoleptic properties of unfermented fruits products.

	Target substrate	Potential enzymes	Applications	References
Physiochemical effects				
Viscosity	Cellulose, Pectin, Starch	Cellulase, Pectinase, Hemicellulase, Amylase	Lychee, Jamun, Watermelon, Araca, Apple, Papaya	Dey and Banerjee (2014); Fasawang and Anprung (2014); Ghosh, Pradhan, and Mishra (2016); Sandri et al. (2014); Saxena et al. (2014); Vishal et al. (2015)
pH	Cellulose, Pectin	Cellulase, Pectinase, Hemicellulase	Grape, Blueberry, Apple	Dal Magro et al. (2016); Dey and Banerjee (2014); Siddiq et al. (2018)
°Brix	Cellulose, Pectin, Starch	Cellulase, Pectinase, Hemicellulase, Amylase	Grape, Jamun, Apple, Papaya, Pumelo	Dal Magro et al. (2016); Dey and Banerjee (2014); Ghosh, Pradhan, and Mishra (2016); Ni et al. (2014); Vishal et al. (2015)
Zeta potential	Cellulose, Pectin, Protein	Cellulase, Pectinase, Hemicellulase, Protease	–	–
Particle size	Cellulose, Pectin, Protein	Cellulase, Pectinase, Hemicellulase, Protease	Lychee	Fasawang and Anprung (2014)
Juice yield	Cellulose, Pectin	Cellulase, Pectinase, Hemicellulase	Grape, Pineapple, Mango, Watermelon, Blueberry Pumelo,	Dal Magro et al. (2016); Jori et al. (2015); Ni et al. (2014); Saxena et al. (2014); Siddiq et al. (2018)
Nutritional effects				
Antioxidant capacity	Cellulose, Pectin	Cellulase, Pectinase, β-Glucosidase, Hemicellulase	Grape, Blueberry, Araca, Apple, Papaya, Pumelo,	Dal Magro et al. (2016); Dey and Banerjee (2014); Ni et al. (2014); Sandri et al. (2014); Siddiq et al. (2018); Vishal et al. (2015)
Bioaccessibility	Cellulose, Pectin, Glycosides compounds	Cellulase, Pectinase, Hemicellulase; β-Glucosidase,	Araca, Blueberry;	Ghandahari Yazdi et al. (2019); Schröder et al. (2014); Siddiq et al. (2018)
Bioavailability	Flavonoid rutinosides, Tannins	Naringinase, β-Glucosidase, Tannase	Grape pomace; Pistachio green hull	Ghandahari Yazdi et al. (2019); Martins et al. (2016); Naqash, Masoodi, and Rather (2019)
Organoleptic effects				
Mouthfeel	Cellulose, Pectin, Starch	Cellulase, Pectinase, Hemicellulase, Amylase, Naringinase, Tannase	Lychee, Jamun, Watermelon, Araca, Apple, Papaya	Dey and Banerjee (2014); Fasawang and Anprung (2014); Ghosh, Pradhan, and Mishra (2016); Sandri et al. (2014); Saxena et al. (2014); Vishal et al. (2015)
Clarity	Lignin, Pectin, Cellulose, Hemicellulose, Starch, Lignin, Protein, Tannin	Pectinase, Cellulase, Hemicellulase, Amylase, Laccase, Protease, Tannase	Lychee, Pineapple, Mango, Jamun, Watermelon, Blueberry, Araca, Apple	Dey and Banerjee (2014); Ghosh, Pradhan, and Mishra (2016); Jori et al. (2015); Sandri et al. (2014); Saxena et al. (2014); Siddiq et al. (2018); Yin et al. (2017);
Color	Cellulose, Pectin, Lignin	Cellulase, Pectinase, Laccase, Hemicellulase	Chokeberry	Lachowicz, Oszmiański, and Kolniak-Ostek (2018)
Volatile flavor profile	Pectin, Cellulose, Prunin, Phenolic compounds, Aglycons, Glycosidically bound volatiles	Pectinase, Cellulase, β-Glucosidase, Hemicellulase, Laccase, Naringinase,	Lychee; Grape; Citrus fruit	Fasawang and Anprung (2014); Singh, Verma, and Kumar (2016)
Sweetness	Cellulose, Starch, Pectin, Hemicellulose	Cellulase, amylase, Pectinase, Hemicellulase	Kinnow	Kaur et al. (2018)
Sourness	Cellulose, Pectin, Hemicellulose	Cellulase, Pectinase, Hemicellulase	Kinnow	Kaur et al. (2018)
Bitterness	Naringin, Tannin, Prunin	Naringinase, Tannase, β-Glucosidase	Pumelo, Kinnow, Orange	Kaur et al. (2018); Ni et al. (2014)

particle size and zeta potential, it is important to delve deeper into the electrostatic interactions between the enzymes and fruit substrates.

Apart from the decrease in viscosity after EHT, an increase in total soluble solids and a decrease in pH were also widely observed (Dal Magro et al. 2016; Dey and Banerjee 2014). This is attributed to the breakdown of pectic and cellulosic substances, which produces smaller, soluble molecules such as D-galacturonic acid and neutral sugars like L-rhamnose, D-arabinose, D-galactose, and D-xylose. In addition, the entrapped solutes can also be released during the disintegration process. Furthermore, the breakdown of the cell wall and the middle lamella disrupts the cell integrity and thus, enhancing the release of intracellular liquids.

Nutritional impacts of EHT on unfermented fruit products

EHT is used to increase the bioaccessibility and/or bioavailability of nutrients (e.g. phenolics, carotenoids). In general, the degradation of pectic and cellulosic polysaccharides in the cell wall and middle lamella leads to an increase in wall porosity, and thus enhances the release of cell wall bound phytonutrients, especially polyphenols and carotenoids from the fruit matrix. Considering the human body is neither able to synthesize certain nutrients nor contains relevant enzymes to break down the cell wall, EHT is able to improve the bioaccessibility of these nutrients. Siddiq et al. (2018) reported that pectinase and cellulase significantly improved the total anthocyanin and total phenolic contents by up to 9.8 and 6.4 times respectively as compared to the untreated blueberry juice. In another study, Schröder et al. (2014) reported the effects of BG on the nutritional quality of fruit-based beverages. The study documented that BG is able to cleave the phenolic and phytoestrogen glucosides which can in turn enhance the nutritional quality of the product. However, Krumreich et al. (2018) revealed that nutrients like vitamin C were lost during EHT. This is probably due to thermal oxidation after prolonged periods of EHT at relatively high temperatures.

Naringinase, on the other hand, showed a positive impact on the nutritional quality of unfermented fruit products via converting flavonoid rutinosides into glucosides (Naqash, Masoodi, and Rather 2019). The removal of rhamnose groups from flavonoid rutinosides could help to enhance intestinal absorption, thereby improving the bioavailability of these compounds. Aside from rutin, other glycosides such as hesperidin, quercetin, diosgenin can also be hydrolyzed to develop health-promoting products like supplements.

Other enzymes such as tannase can also enhance the nutritional quality of unfermented fruit products. Tannase can increase total phenolic contents (e.g. proanthocyanidins) via hydrolyzing the depside and ester bonds of tannins and galloyl esters, releasing monomeric and oligomeric polyphenolic compounds from their conjugates, thereby enhancing absorption in the upper gastrointestinal tract. Martins et al. (2016) reported that tannase significantly helped to release phenolic acids (e.g. gallic acid), which are highly correlated with an increase in antioxidative activity in grape pomace. In fact, combining of cellulase, pectinase and tannase is more effective in enhancing the release of phenolic acids, especially gallic acid, which is then used to synthesize other antioxidants such as propyl gallate in fruit products. Similarly, Ghandahari Yazdi et al. (2019) showed that the extraction efficiency of polyphenolic compounds increased when a mixture of enzymes (tannase, pectinase and cellulase) was employed in treating pistachio green hulls.

Organoleptic impacts of EHT on unfermented fruit products. EHT is an efficient way to modify or improve the organoleptic properties (e.g. clarity, color, flavor profiles and tastes) of unfermented fruit-based products. Pectinase has been widely used for clarification and extraction purposes during fruit processing. Amylase prevents the agglomeration of starch molecules with protein and pectin, thereby eliminating haze formation. Besides, by leveraging on the use of other macerating enzymes like cellulase, the turbidity of fruit juice can be further decreased, therefore facilitating the clarification process. Furthermore, the formation of protein-polyphenol haze in various fruit beverages (e.g. lychee, apple, and banana juices) can be easily prevented with the use of laccase (Yin et al. 2017; Narnoliya et al. 2019; Bezerra et al. 2015). For instance, Narnoliya et al. (2019) demonstrated a 41-64% reduction in turbidity after laccase treatment of banana pseudo-stem juice, apple fruit juice and sorghum stem juice. Moreover, laccase treatment aided in the color and flavor stability of apple juice (Bezerra et al. 2015), therefore, contributed to the improvement of sensory characteristics (e.g. appearance) as compared to untreated ones (Verma et al. 2018). This is because phenolic compounds were oxidized to form polymers and then precipitated out after laccase treatment, therefore helped to increase the clarity of fruit juices. It could be inferred that laccase has the potential in clarifying other fruit-based products that are plagued with the problem of phenolic oxidation.

Protease is another enzyme that helps in the clarification process by removing haze-active proteins. Hmid et al. (2016) illustrated that protease treatment had an immediate clarifying result on pomegranate juice. This clarifying effect was attributed to less protein-pectin interactions. In addition, the degradation of cell membrane and cellular organelles (e.g. extensins) aids in the clarification process. In fact, protease helps in reducing turbidity in a short time while pectinase may be useful in reducing turbidity of products with long storage time.

Apart from good appearance (e.g. clarity, color), another key selling point to attract consumers is to produce better-flavored fruit products. For instance, pectinase, cellulase and hemicellulase can be used in tandem to modify the overall aroma profile of fruit products. As demonstrated by Fasawang and Anprung (2014), a marked increase in glycosidically bound aroma compounds was reported after pectinase treatment. As a result, new aromatic compounds were formed/liberated in the lychee fruit pulp, which showed notable differences in the aroma characteristics as compared to the untreated sample. In addition, cellulase can also release flavor volatiles that are entrapped in the cell wall and gel structure. Other than the above-mentioned enzymes, BG is also able to help release aromatic compounds by hydrolyzing flavorless precursors present in fruits (Singh, Verma, and Kumar 2016). For instance, grapes contain flavorless glycoconjugate precursors linked by α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-xylopyranosyl and β-apiofuranosyl bonds. These glycosides can be released as volatile compounds after BG treatment.

Laccase is used to improve the flavor via scavenging oxygen to prevent the formation of undesirable off-flavor precursors that are derived from the reaction of oxygen with fatty acids, amino acids, and alcohols. In addition, naringinase helps to improve aroma profiles via releasing glycosidically bound volatiles. Ni et al. (2015) reported that naringinase treatment on pummelo juice helped to maintain

the aroma profile similar to that of the fresh juice as compared to resin treatment during the debittering process.

Apart from the flavor, the taste of fruit products plays an important role in consumers' choice and acceptance. As described above, the EHT generally causes an increase in total soluble solids (reducing sugars) and a decrease in pH (release of organic acids); however, low pH may result in the conversion of A-ring lactone into limonin, which contributes to the undesirable bitter taste in citrus fruit juices (e.g. orange, grapefruit and lemon). β-Cyclodextrins and resins are traditional debittering materials, but they may simultaneously absorb many other nutrients (e.g. amino acids, vitamins) and introduce other smelly odors. In contrast, naringinase neither binds to any nutrients nor introduces any undesirable odor, making it a potential perfect replacement in debittering. Ni et al. (2014) reported that the combination of pectinase and naringinase significantly improved the juice yield and decreased bitterness in pumelo fruit. Similarly, there was a maximum decrease of 79% of naringin content in kinnow juice after EHT (Kaur et al. 2018). These results indicated the effectiveness of naringinase treatment in bitterness reduction. Additionally, reducing sugars (glucose, fructose, and galactose) in fruit products can undergo a significant change when cellulase, amylase or glucose isomerase are utilized. Since fructose is 2.3 times sweeter than glucose and 1.3 times sweeter than sucrose, an increased composition of fructose in the product may lead to an increase in sweetness.

Color is another important organoleptic parameter which is associated with consumers' acceptance of various fruit products. This is because color is a reflection of various quality attributes such as aroma, taste and nutritive value. Lachowicz, Oszmiański, and Kolniak-Ostek (2018) reported that chokeberry treated with pectinase was 7% redder than that of control (no EHT). This was attributed to the release of anthocyanins.

Application of EHT on fermented fruit products

Physicochemical impacts of EHT on fermented fruit products

It has been well established that the rheological and textural properties of fermented fruit products are largely determined by the structure and chemical composition of the fruit itself and many studies have thus used either endogenous or exogenous enzymes in modulating the consistency of these products (Guo et al. 2018; Nikhanj, Kocher, and Boora 2017). It is a common practice to degrade the fibrous cell wall of fruits (e.g. apple, pear and orange), which are rich in pectin, because incomplete hydrolysis of these structures can cause detrimental effects during fermentation (Patidar et al. 2018).

Commercial enzymes such as glucanases (from *Trichoderma* sp. and *Taleromyces versatilis*) were employed during fruit wine fermentation (Table 3) to reduce viscosity (Claus and Mojsov 2018). In addition, the hydrolysis of fibers from palm dates facilitated better filtration and distillation during the fermentation process. This is because enzymes (a combination of pectinase and cellulase) accelerated the settling process of fibers through liquefying polysaccharide components as well as eliminating colloidal particles. This hydrolyzed fiber is known to settle much faster than unhydrolyzed fiber, which resulted in faster separation of the fiber and the palm date juice. Similarly, an increased extraction (10–35%) and shorter filtration rate (70–80%) was also reported when Cytoclase 219 (a mixture of cellulase, pectinase and xylanase) was used in the preparation of white grape wine (Toushik et al. 2017). Other than the above-mentioned advantages, the EHT also helped to reduce the pressing time while reducing the viscosity of fruit wine products (Toushik et al. 2017).

Nutritional impacts of EHT on fermented fruit products

The EHT (pectinase treatment) enhanced the release of cell wall-bound phytonutrients (e.g. polyphenols and carotenoids) through degrading pectic polysaccharides in the cell wall structure. The greater release of phenolics and carotenoids contributed to a higher antioxidant capacity (Table 3). In general, it was believed that the release of polyphenolic tannins and anthocyanins during wine fermentation was beneficial in achieving good color, nutritional value and aging potential of the wine (Ivanova, Vojnoski, and Stefova 2012). This is because pectinase helps to enhance the release of phenolic compounds such as anthocyanins which contribute to the flavor and color intensity in wine. For instance, the use of Vinozym G (from Novozymes, Denmark) with *S. cerevisiae* VR 5 enhanced anthocyanins release as well as stabilized color during wine production (Li et al. 2015).

Apart from pectinase, protease can also be used to improve the nutritional value (breakdown of protein into amino acids), digestibility and flavor of fermented fruit products (e.g. jujube and banana). Cellulase, on the other hand, can be utilized for the extraction of phenolic compounds from fruits like grape pomace (Kabir, Sultana, and Kurnianta 2015). Piemolini-Barreto et al. (2015) reported that the use of pectinase increased the anthocyanins content in grape wine, which contributed to a higher antioxidant capacity in the final fermented product. Moreover, the release of carotenoids and anthocyanins can impart a bright color to the product which is desirable to consumers. On the other hand, processing fruit products using enzymes (45–60 °C) may deteriorate heat-labile nutrients such as vitamin C, a natural antioxidant which is essential for body functions (e.g. collagen synthesis).

Organoleptic impacts of EHT on fermented fruit products

Organoleptic characteristics (e.g. appearance, clarity, color, and taste) are very important parameters that are used to evaluate the quality of fermented fruit products. Guo et al. (2018) reported that protease treatment prevented the cloudiness of dates alcoholic beverages via removing the proteins prior to alcoholic fermentation. Apart from proteases, β-glucanases can also aid in wine clarification and filtration due to their pivotal roles in removing haze forming glucans. Therefore, EHT can be employed as an effective

Table 3. Enzymes used in the representative fruits fermentation and their effects.

Fruit	Product	Type of microorganism used for fermentation	Enzymes used	Fermentation stage (Addition of enzyme)	Effects of enzymatic treatment	References
Guava	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Increase in juice clarity	Nikhanj, Kocher, and Boora (2017)
Guava	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Decrease in viscosity and ease filtration	Phuoc Minh, Thinh Pham, Thi Tre, et al. (2019)
Black Raspberry	Wine	*Saccharomyces cerevisiae*	Pectinase Hemicellulase Cellulase *β*-glucosidase	Pre-fermentation	• Higher fruity and floral aroma • Increase in terpene, ethyl acetate, ethyl lactate and 2-phenylethyl acetate after enzymatic treatment	Kim and Park (2017)
Jackfruit	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Increase in reducing sugars • Increase in ascorbic acid	Jadhav et al. (2018)
Blueberry	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Increase in polyphenolic content in wine	Zhang, Li, and Gao (2016)
Mango	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Higher yield, better clarity and lower viscosity	(Phuoc Minh, Thinh Pham, Thi Thao, et al. (2019)
Pineapple	Wine	*Saccharomyces cerevisiae*	Pectinase Cellulase Hemicellulase Amylase	Pre-fermentation	• Increased in reducing sugars	Alvarenga et al. (2015)
Kiwi	Wine	*Saccharomyces cerevisiae* Jiuqu	Pectinase	Pre-fermentation	• Increased in alcohols	Chen et al. (2019)
Blueberry	Wine	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Released of glycosidically bound terpenoids	Liu et al. (2019)
Jujube	Brandy	*Saccharomyces cerevisiae* Daqu	Pectinase Cellulase	Pre-fermentation	• Increases in fermentable sugars	Li et al. (2016)
Jujube	Alcoholic beverage	*Torulaspora delbrueckii*	Pectinase Cellulase Protease	Pre-fermentation	• Increases in fermentable sugars • Increases in antioxidant • Improvement in flavor profiles	Guo et al. (2018)
Pineapple	Wine	*Saccharomyces cerevisiae*	Pectinase Cellulase Hemicellulase Invertase	Pre-fermentation	• Increases in ethanolic level • Increase in acetate and ethyl esters which increase the fruity character of the wine	Roda et al. (2017)
Banana	Alcoholic beverage	*Saccharomyces cerevisiae*	Pectinase	Pre-fermentation	• Increases in total soluble solids	Pauline et al. (2017)
Mango peel	Butanol production	*Clostridium acetobutylicum*	Pectinase Cellulase Amylase	Pre-fermentation	• Increases in juice yield and sugars	Avula, Reddy, and Reddy (2015)
Cherry	Wine	*Saccharomyces cerevisiae* Viniflora Oenos Viniflora CH35	*Pectinase* *β*-Glucosidase	Pre-fermentation	• Enhancement in wine aroma • Improvement in sensory quality	Wilkowska and Pogorzelski (2017)
Grape	Wine	*Kluyveromyces marxianus* *Saccharomyces cerevisiae*	Pectinase	During fermentation	• Breakdown of cell wall • Greater volatile release	Rollero et al. (2018)

(*continued*)

Table 3. Continued.

Fruit	Product	Type of microorganism used for fermentation	Enzymes used	Fermentation stage (Addition of enzyme)	Effects of enzymatic treatment	References
Grape	Wine	*Aureobasidium pullulans, Filobasidium capsuligenum Rhodotorula dairenensis Cryptococcus saitoi Saccharomyces cerevisiae*	Pectinase	During fermentation	• Better aroma, color and clarity	Merín and de Ambrosini (2015)
Grape	Wine	*Kluyveromyces marxianus*	Pectinase	During fermentation	• Ethanol production • Higher sugar profile • Higher galacturonic acid content	Williams et al. (2019)
Grape	Wine	*Saccharomyces cerevisiae Kluyveromyces marxianus*	Pectinase	During fermentation	• Enhancement in wine aroma	Sieiro et al. (2014)
Grape	Wine	*Kluyveromyces marxianus*	Pectinase	During fermentation	• Reduction of viscosity • Aid in clarification • Enhancement in color intensity • Increases in phenolic contents and antioxidant	Piemolini-Barreto et al. (2015)

and attractive alternative approach in contrast to the use of fining agents during the clarification process.

Additionally, enzymes could potentially maintain/improve the color and aroma that were otherwise lost during filtration. For instance, it was reported that EHT (pectinase treatment) improved the color intensity of fermented fruit products via releasing cell wall-bound phytochemicals and pigments (Liu et al. 2019). Wilkowska and Pogorzelski (2017) also reported that EHT cherry wines showed a higher release of aroma compounds from their glycosidic counterparts as compared to the control wines (without EHT). In another study, naringinase and limonoate dehydrogenase converted bitter-tasting naringin and limonin into non-bitter derivatives, which significantly improved the taste characteristics of the fermented citrus products (Singh et al. 2019).

To overcome chemical or enzymatic discoloration, color stabilization of fermented fruit-based products is often carried out using laccase, which could selectively catalyze the polymerization of polyphenols to form polyphenolic complexes. These complexes can be easily removed via filtration without altering the organoleptic characteristics of the wine. In addition, the addition of tannase can potentially inhibit haze formation by hydrolyzing tannic acid (Figure 3B). Besides, tannase can also alter wine taste through hydrolyzing chlorogenic acid to quinic acid and phenolic acids such as caffeic acid.

The flavor characteristic of each fruit is contributed by the complex interactions between flavor volatiles and certain taste components (e.g. organic acids and sugars). Other than rheological modifications, enzymes such as pectinase have been shown to cleave glycosidically bound flavor precursors into aroma active aglycones, thus potentially offering a novel or enhanced flavor profile of fermented fruit products (Hjelmeland and Ebeler 2015). Typically, in many fruits, most of the flavor compounds exist as nonvolatile glycoconjugates such as glycosides, which are also known as glycosidic aroma precursors. These odorless glycosides are able to release odor-active compounds upon EHT (e.g. β-glucanases/BG treatments), therefore potentially enhancing and enriching the flavor complexity of the fermented products. For instance, BG can not only improve the odor attributes but also improve wine aroma intensity. Likewise, the liberation of volatile aglycones (e.g. β-damascenone, vitispirane and theaspirane) after EHT can enable greater aroma perception (Molina et al. 2016). Additionally, aromatic volatile terpenes can be released when sugar-conjugated precursors undergo EHT. This process often involves the cleavage of terminal sugars by rhamnosidase, apiosidase or arabinosidase which is accompanied with the release of terpenes by β-D-glucopyranosidase, thereby enhancing the odor characteristics of the final fermented product (Mateo and Maicas 2016). Therefore, by utilizing enzymes, winemakers can produce richer and fruitier fruit wines (Mateo and Maicas 2016).

Additionally, other various volatiles can also be produced after EHT in fermented fruit products. For example, Sun

et al. (2018) examined the effects of commercial enzymes on Cabernet Gernischt dry red wine and concluded that more esters, phenylethyl esters and terpenes were produced. Kim and Park (2017) evaluated the effects of three different glycosidases on Korean black raspberry wine. After EHT, the black raspberry wine yielded higher concentrations of terpenes and esters, particularly ethyl acetate (fruity), 2-phenylethyl acetate (apple, rose) and ethyl lactate (fruity, buttery). These compounds contributed to a positive sensory characteristic (floral and fruity aroma) to the raspberry wine. This is because terpenes are able to interact with one another synergistically, thereby increasing the aroma of wine products.

Effects of EHT on fermentation efficiency

A significant reduction in viscosity due to EHT greatly helps in improving clarification, filtration and increasing in juice yield, which is highly desirable, thereby improving fermentation efficiency. In fact, wineries maximize their efficiency by employing enzymes, which will result in a reduction in energy consumption, henceforth improving profits. According to Alvarenga et al. (2015), when EHT was carried out prior to fermentation, there was a significant increase in reducing sugars in pineapple pulp, which eventually improved the fermentation process. Holistically, an increase in fermentation substrates (e.g. sugars, amino acids) and a decrease in physical attributes (e.g. viscosity, particle size) will lead to increase in productivity and fermentation efficiency. Therefore, the use of EHT is an efficient processing aid in producing fermented fruit products (e.g. alcoholic beverages, vinegars) as it helps to achieve a lower energy consumption, reduce chemical usage and reduce waste generation.

Overall, EHT can yield reproducible improvements in fermented fruit products, particularly in the area of (i) improved color stability (Merín and de Ambrosini 2015), (ii) taste improvement, (iii) improved filtration and processing efficiency, (iv) enhanced aroma (Sieiro et al. 2014), (v) increased yield (Avula, Reddy, and Reddy 2015), (vi) increased clarity (Nikhanj, Kocher, and Boora 2017) and (vii) increased nutritional benefits (Zhang, Li, and Gao 2016).

Non-Saccharomyces yeast strains with enzymatic activities

During the pre-fermentation process, exogenous enzymes are normally used (i) to increase the release of aromatic compounds, (ii) to help release phenolic compounds, (iii) to reduce viscosity, and (iv) to facilitate juice yield/filtration. In recent years, the use of non-*Saccharomyces* yeast species with specific enzyme production (e.g. pectinase) were reported. Although *S. cerevisiae* strains are commonly used in fruit wine fermentation, they have limited pectinolytic activity. Wines produced by pure *S. cerevisiae* yeasts normally lack flavor complexity. Therefore, there is a rising interest in combining non-*Saccharomyces* and *Saccharomyces* yeasts in wine fermentation (Lu et al. 2015). Overall, yeasts with extracellular enzymatic properties are capable of improving the technological and sensorial properties of fruit wine products.

To date, yeast strains such as *Candida spp.*, *Pichia kluyveri*, *Sporodiobulus salmonicolor*, *M. pulcherrima* and *K. marxianus* showed pectinolytic activity (Belda et al. 2016a; Escribano et al. 2017). Due to the enzymatic activity, structural polysaccharides in the fruit substrate were degraded during fermentation, therefore increasing yield and improving filterability of the fermented products (Escribano et al., 2017). Belda et al. (2016a) demonstrated the effects of pectinolytic yeast, *M. pulcherrima* ES-EM-34 in fruit wine clarification. The authors concluded that the use of *M. pulcherrima* NS-EM-34 with *S. cerevisiae* not only improved wine clarity, but also increased anthocyanin content and color intensity in comparison to wines fermented with pure *S. cerevisiae*.

In particular, yeast strains like *K. marxianus* showed promising results in maceration of the pectinolytic cell wall due to its polygalacturonase activity (Williams et al. 2019). This yeast significantly reduced the viscosity of grape pomace and was beneficial on both the color and nutritional content (release of anthocyanin) of fruit wines (Piemolini-Barreto et al. 2014). In addition, the use of *K. marxianus* also resulted in a higher product yield and a more complex volatile profile (Rollero et al. 2018). Furthermore, the total volatile contents in wines produced with *K. marxianus* were significantly higher than wines processed with commercial pectinase in pre-fermentation, likely as a result of yeast metabolism. Similarly, Sieiro et al. (2014) reported that Albariño wine fermented with *K. marixanus* had a stronger floral, citric, balsamic and spicy aroma than wines produced with a commercial pectic enzyme in pre-fermentation.

Apart from the above-mentioned advantages, *K. marixanus* could produce ethanol via fermenting a variety of sugars such as galactose, glucose, fructose and mannose (Williams et al. 2019). In addition, polygalacturonase released from *K. marixanus* showed great stability in high ethanol concentration and low pH, indicating that it would be an ideal candidate to facilitate fermentation. Furthermore, commercial pectinolytic enzymes are not pure and normally contain some lyases and PMEs, which can hydrolyze methoxyl groups and release methanol in the final wine products. In comparison, the products fermented with *K. marixanus* had trace levels of methanol or none, thereby making the products safe for consumption (Sieiro et al. 2014). Above all, the use of *K. marixanus* revealed promising results in physicochemical, nutritional as well as color and aroma profiles of fermented products.

A recent study showed that some non-*Saccharomyces* yeasts with β-lyase activity could help release aromatic compounds (e.g. terpenes and thiols) in wines (Belda et al. 2016b). Some *S. cerevisiae* yeasts also showed β-lyase activity, but lyase could be totally inhibited in a high nitrogen environment (Thibon et al. 2008). Therefore, non-*Saccharomyces* yeasts like *Hanseniaspora osmophila* acting as

an alternative should be studied in depth for aroma enhancement in wine.

BG from non-*Saccharomyces* yeasts (e.g. *Issatchenkia terricola*) was also utilized to enhance the varietal characteristics of wine products. de Ovalle et al. (2018) reported that the use of BG from *I. terricola* helped to liberate norisoprenoids, which significantly contributed to the wine aroma of Cabernet Sauvignon red wine. Despite changes in wine aroma, no effect was observed on wine color. Similar results were obtained when grape juice was fermented by *S. cerevisiae* with the aid of BG from *M. pulcherrima* HX-13, *Pichia kudriavzevii* F2-24 and *I.* terricola SLY-4, respectively (Zhang et al. 2020). In addition, during the co-fermentation of *Saccharomyces* and non-*Saccharomyces* yeasts, various aroma compounds such as esters were elevated, which helped to increase the fruity and floral note, thereby adding more complexity to the flavor profile. Based on these studies, the use of BG from non-*Saccharomyces* yeasts in fermented fruit products seems to be effective in improving flavor complexity without negative effects on the physicochemical properties of the final product.

Other enzymes that affect aroma compound profiles of fermented fruit products include esterase and lipase. In general, lipase contributes to increase in free fatty acids during grape wine fermentation while esterase catalyzes the synthesis of various esters (also hydrolysis of esters). Several yeast strains such as *Williopsis pratensis*, *Sporodiobulus salmonicolour*, *M. puplcherrima*, *P. kluyveri* and *Lachancea thermotolerans* exhibited both esterase and lipase activities (Escribano et al. 2017). However, in certain circumstances, the liberation of medium-chain fatty acids (e.g. octanoic and decanoic acids) will trigger stuck fermentation (Viegas et al. 1989).

As discussed above, protease is mostly used to inhibit haze formation in winemaking. Currently, no extracellular protease activity was observed in *S. cerevisiae*. Non-*Saccharomyces* yeast strains such as *Wickerhamomyces anomalus* (Belda et al. 2016b) and some dairy yeasts such as *Yarrowia lipolytica* and *Debaryomyce hansenii* with extracellular high protease activity should be investigated for their potential in removing haze-forming proteins (Patrignani, Iucci, Vallicelli, Guerzoni, Gardini, and Lanciotti 2007). This may provide novel applications of non-*Saccharomyces* yeasts and dairy yeasts with proteolytic activity.

Potential popular health products derived from fruit fermentation

It was reported that the use of probiotics to ferment fruit juice or pulp (with or without EHT) may serve as a potential avenue to develop novel nondairy fruit-based functional beverages to deliver probiotics (Di Cagno et al. 2020; Lu, Putra, et al. 2018; Mantzourani et al. 2018; Mustafa et al. 2019; Nguyen et al. 2019). Several functional fruit beverages fermented with probiotic lactic acid bacteria were successfully developed. For example, Nguyen et al. (2019) developed a probiotic pineapple juice beverage fermented with *Lactobacillus plantarum* 299 V. The overall microbial population did not significantly decrease even after two months of storage. In addition, Mustafa et al. (2019) investigated the fermentation feasibility of several probiotics including *Lactobacillus plantarum*, *Lactobaccilus casei*, *Lactobacillus bulgaricus* and *Lactobacillus salivarius* in pomegranate juice. The pomegranate juice fermented with *L. casei* showed a higher biomass density and maintained a strong antioxidant activity. Furthermore, Santos Monteiro et al. (2020) developed a probiotic powder from passion fruit pulp fermented with *Lactobacillus reuteri*. Moreover, probiotic orange, grape and apple juices fermented with *Lactobaccillus rhamnosus* GR-1 have also been developed and proven to be acceptable alternatives of dairy based probiotic drink (White and Hekmat 2018). On the other hand, Rosenberg (1999) stated that to produce energy, Lactobacillus has the ability to break down complex carbohydrates in fruit media, so the growth rate is higher than that in milk media. According to Charalampopoulos et al. (2002), *L. plantarum* was more favorable on medium containing maltose, sucrose, glucose, and fructose. Overall, all these studies showed the potential of fruit-based fermented products as an alternative to dairy-based products, which is catered to lactose intolerant consumers.

While these fruit juices serve as an ideal food matrix to carry probiotic bacteria, challenges such as a loss of probiotic viability in stimulated gastrointestinal conditions (e.g. pH and bile, which may destroy the cell membranes of probiotics) (Succi et al. 2005) and during long storage should be addressed to ensure that sufficient probiotic strains ($>10^6$ cfu/mL) are present during consumption (Nguyen et al. 2019). Some strategies have been developed to ensure probiotics viability in fermented fruit-based beverages. For example, selection of robust strains and/or adjustment of pH of the media are potential ways to improve the survival and sustainability of probiotics in acidic fruit juices (Sheehan et al. 2007). In addition, other methods including co-culturing of selected yeasts (Liu and Tsao 2009; Lu, Putra, et al. 2018) and the supplementation of metabolizable sugars (Corcoran et al. 2005) are also effective ways. Lu, Putra, et al. (2018) found that sequential inoculation of *Williopsis saturnus* NCYC22 (yeast) significantly enhanced the survival of *L. casei* L26 (probiotics) in a functional beverage from durian pulp. Corcoran et al. (2005) found that the survival of *L. rhamnosus* GG was enhanced by $\sim 10^6$ to 10^8-fold when supplemented with glucose in simulated juice, this was explained that glucose provided ATP to F0F1-ATPase via glycolysis, which enhanced the survival of *L. rhamnosus* via activating proton exclusion. Furthermore, microencapsulation has also been used to preserve probiotics viability in fruit juices and achieved good results (Krasaekoopt and Watcharapoka 2014; Nualkaekul et al. 2013; Ying et al. 2013).

Examples of application of EHT in fermented fruit-based products

Although EHT has been widely used in various fermented fruit-based products, banana and mango are selected as examples in this review due to their popularity and large

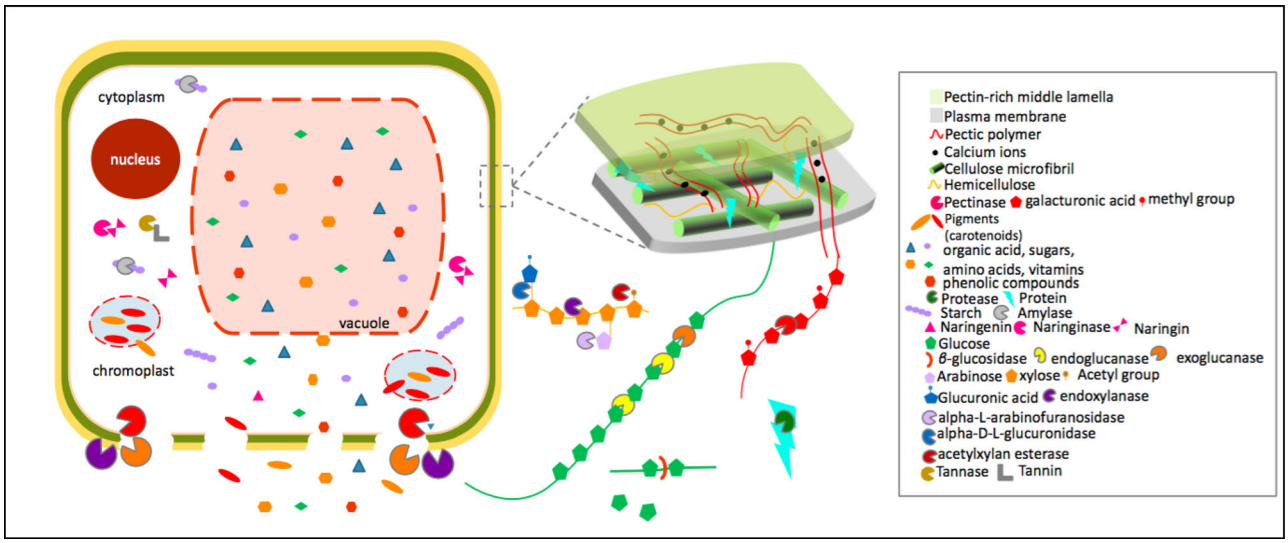

Figure 4. Overall reaction mechanisms of various enzymes on fruit-based substrates.

amount production. Banana and mango are normally consumed fresh after harvest due to their limited shelf life at room temperature (Baez-Sañudo et al. 2009; Alikhani 2014). Fermentation of banana and mango pulp to produce fermented products (e.g. alcoholic beverages and probiotic beverages) offers an alternative way as compared to conventional processing methods like drying or freezing.

Application of EHT in fermented banana products

Bananas (Musa sp., Musaceae) grow whole year-round and are one of the highest yield fruit crops worldwide. However, banana is also one of the most wasted fruit worldwide, accounting for almost 20% of food waste produced in United Kingdom (UK). As such, these fruit waste can be converted into higher value-added products (e.g. banana juice and beverages) so as to eliminate environmental issues and to improve farming economies (Padam et al. 2014). Other than banana juice, bananas have also been fermented into alcoholic beverages, wines, or vinegars (Ogodo et al. 2015; Qamar and Shaikh 2018). However, one major concern is that the end product is often viscous, gray and cloudy due to high pectin and starch content.

During banana processing, various enzymes (e.g. pectinase, amylase and cellulase) can be utilized prior to fermentation. Pectinase is often added to hydrolyze pectin. After the EHT, viscosity will be reduced which can thereby shorten the clarification process. Alongside pectinase, amylase can also be added to degrade starch into smaller oligosaccharides, hence, preventing haze formation. This EHT process is necessary before fermentation in order to improve the clarity, taste, aroma and mouthfeel of the fermented banana product. In addition, the synergistic effects of pectinase and amylase during pretreatment of banana will be able to enhance the hydrolysis of complex carbohydrate, hence increasing reducing sugars which are required during fermentation. Besides, this process accelerated the clarification process by 4-fold. Moreover, the juice yield increased significantly from 38% (no enzymatic treatment) to 63.4% (enzymatic treatment). Apart from juice yield, there was also a significant increase in the reducing sugar content of the banana juice. Other reported physicochemical changes include a decrease in pH and a higher titratable acidity, which may be explained by the degradation of pectin and the release of galacturonic acid (Pauline et al. 2017). The EHT (pectinase treatment) that was applied in banana vinegar fermentation significantly improved the fermentation efficiency in ethanol (alcoholic fermentation) production as well as acetic acid (acetic acid fermentation) production. Furthermore, with the EHT, the fermented banana product achieved good aroma profile with a high consumer acceptability score during sensory evaluation.

Application of EHT in fermented mango products

Mango (*Mangifera indica)* is one of the most popular fruits worldwide due to its delicious taste and nutritional benefits. Additionally, mango is rich in dietary fiber, polyphenolic compounds (mangiferin, rhamnetin, ellagic acid, kaempferol and rhamnentin) and vitamins (vitamins A and C), which have anti-oxidative and anti-inflammatory effects (Siddiq, Brecht, and Sidhu 2017). Despite its popularity and functionality, consumers' purchasing decision and acceptance are highly dependent on several quality attributes such as color, taste, nutritional value, flavor and aroma. As such, enzymes have been used in many mango related products. This EHT helps to overcome the drawbacks of conventional juice extraction process which often result in low juice yield. Besides, the use of enzymes is also a cost-effective method which can enhance the organoleptic and nutritional quality of the final product.

The main carbohydrates of the unripen mango is starch which is converted to monosaccharides and disaccharide such as sucrose, glucose, and fructose during ripening. Due to high concentrations of fermentable sugars, mango puree is a suitable fermentation material. However, the fermented mango samples normally showed a highly viscous and cloudy nature, which was caused by the dietary fiber, lignin, pectin, β-glucans and gums in mango tissues (Li et al. 2013). To date, several commercial enzymes (e.g. pectinase,

cellulase and hemicellulase) were utilized to degrade mango puree before fermentation. The use of pectinase and BG significantly increased the sugar content in mango wine, which was attributed to the hydrolysis and the release of glycosidically bound saccharides (Li et al. 2013). On the other hand, the suitability of mango peel extract as a substrate in biofuel production was investigated (Avula, Reddy, and Reddy 2015). An increase in reducing sugars was observed after EHT which aided in the production of butanol after fermentation. In general, the EHT (pectinase and BG treatment) helps to improve the release of volatile compounds including alcohols (e.g. isoamyl alcohol, isobutyl alcohol, n-propanol, phenethyl alcohol) and esters (ethyl acetate, ethyl hexanoate and ethyl octanoate) in mango wine fermentation (Li et al. 2013).

Apart from the physicochemical and sensorial advantages, EHT has promising effects on the nutritional content of fermented fruit products. For instance, commercial enzymes are known to help release anthocyanidins, vitamin C and polyphenolic compounds, which are responsible for the antioxidative capacity in fruits like mulberry (Nguyen and Nguyen 2018). Therefore, EHT on mango pulp prior to fermentation may help release phytochemicals (e.g. carotenoids, vitamin C) that are present in the polysaccharide cell wall, thereby enhancing the nutritional quality of the fermented product.

Future direction

To date, many studies have examined the effects of pectinase and cellulase on various fruit products. However, research on other enzymes (e.g. laccase, naringinase, protease, amylase, glycosidase, and tannase) remains limited. In order to achieve the optimum yield and efficiency during EHT, it is necessary to investigate and optimize different enzyme combinations and conditions on different fruit products in order to (i) enhance physicochemical properties (e.g. reduce viscosity and increase yield); (ii) improve nutritional properties (e.g. maximize the bioavailability and bioaccessibility of polyphenolic contents in the final product) and (iii) enhance sensorial properties (improve color, odor attributes and taste profile). Currently, few studies have been carried out to look at the effects of EHT on zeta potential and particle size of both unfermented and fermented fruit products. These two parameters are vital because they can indicate the stability of the colloidal suspension after EHT as well as phase separation. Additionally, further understanding of non-*Saccharomyces* yeast strains (with enzymatic activity) are warranted in terms of its other benefits and effectiveness on different fruit materials. It is also important and crucial to source for other organisms that may show potential enzymatic activity as well as their effects on the maceration and clarification of fruit products.

Conclusion

In conclusion, the use of enzymes in unfermented and fermented fruit-based products plays an increasingly vital role in modern food processing technology. EHT helps to modify and improve product quality of the unfermented and fermented fruit products via various enzymatic reactions (Figure 4). There is a cascading flow of possibilities of using enzymes in fruit-based products in juice extraction, clarification, nutritional and flavor improvements to further create differential fermented fruit products (e.g. vinegar, spirits, cider, and wine). Although commercial enzymes have been widely used in fruit juice processing to release flavor compounds, the effects of enzymes in fermentation of fruit-based products are still scant and merit further investigation. Lastly, it will be worthwhile to look at the effects of multi-enzyme combinations on the different fruit materials and to explore alternative organisms (non-*Saccharomyces* yeast strains) to develop new products.

Disclosure statement

The authors declare that there are no conflicts of interest.

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