CHAPTER

4

Enzymes in Fruit Juice Processing

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4.1 INTRODUCTION

International markets exist for traditional and non-traditional fruits, while recently, the processing of fruits was developed in several countries. Fruit juices (FJ), beverages, and nectars are the most popular fruit products. The consumption of FJ has significantly increased, and this increase has resulted in an upswing in FJ processing in fruit-growing countries, which endeavor to increase FJ production in order to be competitive for export markets. Enzymes are important factors in food processing because they simplify intermediate bio-processes during food production. The commercial utilization of enzymes was first reported in 1930 for FJ preparation (Nisha, 2016; Oslen, 2000). Industrial enzymes fall into various groups, of which, the most important are pectinases, cellulases, and tannases which are used in fruit processing (Nisha, 2016; Sharma et al., 2016; Yao et al., 2014).

Enzymatic treatment of FJ has many advantages over traditional processing. These advantages include an increase in FJ yield, enhanced clarification, increased total soluble solids in FJ, improved pulp liquefaction, and decreased turbidity and viscosity (Kaur and Sharma, 2013; Nadeem et al., 2009; Pal and Khanum, 2011; Sharma et al., 2016). Macerating enzymes are utilized in these FJ processing steps: (1) after fruit crushing, the pulp is macerated, which results in an increase in FJ yield, decreases the processing time, and extracts more bioactive components from the fruits, and (2) after FJ extraction, clarification is carried out, which increases the product stability (Rui et al., 2012). The cloudiness of FJ is due to the presence of pectin, cellulose, starch, proteins, tannins, and lignin (Vaillant et al., 2001). The commercial application of enzyme preparations containing pectinases, cellulases, and tannases benefits the FJ industry (Grassin and Fauquembergue, 1996). These enzymes are known as macerating enzymes, which are used in FJ extraction and clarification (Gailing et al., 2000; Sharma et al., 2014). Pectinases have globally attracted great interest as a biological catalyst in many industrial applications (Rashmi et al., 2008). Pectinase catalyzes degradation of pectic substances through de-esterification (esterases) reactions and depolymerization (hydrolases and lyases) (Kohli and Gupta, 2015; Tariq and Latif, 2012). Cellulases have gained worldwide interest, as they have valuable potential to process cellulosic biomasses and transform them to

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useful products. The synergistic effect of cellulases (i.e., exoglucanases, endoglucanases, and β-glucosidases) is needed for cellulose de-polymerization for transformation to useful products using suitable microorganisms (Sharma et al., 2016). Tannases (tannin acylhydrolases) are important groups of enzymes that are utilized in several industrial applications, including the manufacture of FJ, and tea. Tannases act in a wide range of temperatures and pH, and produce microorganisms, including *Aspergillus*, *Paecilomyces*, *Lactobacillus*, and *Bacillus* (Yao et al., 2014). Enzymes are essential tools in FJ processing in terms of cost savings and quality improvement. Development of the FJ industry became strongly connected with the enzyme industry. Over the last years, many steps in FJ processing were improved, optimized, modified, and rationalized using specific enzymes to improve FJ quality (Ramadan and Moersel, 2007). This chapter discusses the application of pectinases, cellulases, and tanninases in FJ processing.

4.2 PECTINASES AND THEIR APPLICATIONS IN FJ PROCESSING

Pectic materials are complex of colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked with α-(1–4) linkage. Carboxyl groups of galacturonic acid are partially or completely neutralized by potassium, sodium, or ammonium ions and partially esterified by methyl groups (Caffall and Mohnen, 2009; Mohnen, 2008; Sieiro et al., 2012). Side chains of pectin consist of arabinose, galactose, L-rhamnose, and xylose. Pectic substances could be classified into four molecules: protopectin (pectic substance in intact tissue), pectic acids (polygalacturonan contains negligible amounts of methoxyl groups), pectinic acids (polygalacturonan that contains >0%–75% methylated galacturonate units), and pectins (pectinic acid with ca. 75% methylated galacturonate units). Protopectines are water insoluble, while other types of pectic substances are partially or wholly soluble in water (Alkorta et al., 1998; Be Miller, 1986; Kertesz, 1951; Tapre and Jain, 2014). Pectin is a mixture of different molecules with pectinic acid as the main constituent. Pectin (Fig. 4.1) is found in the cell wall and pectin might be linked with polysaccharides or proteins to form protopectin. In the cell wall of fruits, pectin is the main constituent (Anuradha et al., 2010; Kohli and Gupta, 2015). Pectin content (%) of some fresh fruits is given in Table 4.1. Among fresh fruits, currants contain the highest percentage of pectin (0.9%–1.5%).

Pectins have many valuable applications in nutraceuticals and food industries. In the food industry, pectin is used as a gelling agent, as nutritional fiber, as well as a replacement for

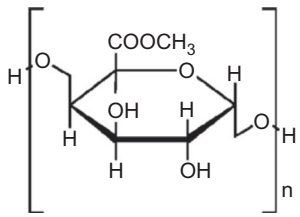

FIG. 4.1 Chemical structure of pectin.

TABLE 4.1 Pectin Content (%) in Some Fresh Fruits (Fogarty and Ward, 1972)

Fruit	Pectin Content (%)
Banana	0.7–1.3
Apricot	0.7–1.3
Apple	0.5–1.6
Currant	0.9–1.5
Grape	0.2–1.0
Guava	0.7–1.5
Tomato	0.2–0.5
Strawberry	0.6–0.7
Peach	0.3–1.2
Pea	0.5–0.8
Pineapple	0.3–0.6

fats and sugars in low-calorie foods (Sakai et al., 1993; Sieiro et al., 2012; Thakur et al., 1997). In addition, pectins contribute to FJ viscosity and turbidity. Mechanical crushing of fruit with high levels of pectin yields a FJ with high viscosity that remains bound to the fruit pulp. It is difficult to extract this FJ using other mechanical methods or by pressing. Pectin is involved in crosslinking cellulose and hemicellulose fibers; thus pectinases helps to enhance the access of cellulases to their substrates (Giacobbe et al., 2014). By treating with pectinases, the viscosity of FJ is decreased, the jelly structure disintegrated, the pressability of the pulp improved, and higher FJ yields are obtained.

4.2.1 Pectolytic Enzymes

Enzymes that hydrolyze pectic materials are known as pectinases, pectic enzymes, or pectinolytic enzymes (Blanco et al., 1999). The first commercial use of pectinolytic enzymes was in 1930 for the preparation of FJ and wine (Oslen, 2000; Tapre and Jain, 2014). In the 1960s, the structure of plant tissues was elucidated, and enzymes began to be used efficiently. Pectinolytic enzymes are very important enzymes in the food industry especially in FJ processing as a prerequisite for obtaining stability and clarification (Girard and Fukumoto, 1999; Lee et al., 2006; Mohnen, 2008; Nisha, 2016; Ribeiro et al., 2010; Tapre and Jain, 2014; Viquez et al., 1981). Pectinases are formed during the fruits' ripening process wherein pectinases split polygalacturonic acid to monogalacturonic acid by breaking glycosidic linkage. Softening of cell walls and increasing the FJ yield from the fruits takes place during this process. Acidic pectinases are mainly utilized in FJ processing (i.e., extraction and clarification), while alkaline pectinases have economic and environmentally friendly industrial applications (Kohli and Gupta, 2015). The detailed

TABLE 4.2 Classification of Pectinases According to Its Mode of Action (Sieiro et al., 2012)

Enzyme	Mode of Action	Main Substrate	Product(s)
Depolymerases			
Lyases			
Endopectate lyase (4.2.2.2)	Transelimination	Pectic acid	Unsaturated oligogalacturonates
Exopectate lyase (4.2.2.9)	Transelimination	Pectic acid	Unsaturated oligogalacturonates
Endopectinlyase (4.2.2.10)	Transelimination	Pectin	Unsaturated methyl-oligogalacturonates
Hydrolases			
Protopectinases	Hydrolysis	Protopectin	Pectine
Endopolygalacturonase (3.2.1.1.5)	Hydrolysis	Pectic acid	Oligogalacturonates
Exopolygalacturonase (3.2.1.6.7)	Hydrolysis	Pectic acid	Monogalacturonates
Esterases			
Pectin methyl esterase (3.1.1.11)	Hydrolysis	Pectin	Pectic acid and methanol
Pectin acyle esterase (3.1.1.6)	Hydrolysis	Pectin	Pectic acid and methanol

classification of pectic enzymes is shown in Table 4.2. Based on its substrate preference and mode of action, pectinases could be classified as:

I. Esterases, that eliminate acetyl and methoxyl residues from pectin giving rise to polygalacturonic acid;
II. Protopectinases, that solubilize protopectin to form soluble pectin;
III. Depolymerases that break the glycosidic linkages between galacturonic residues via either transelimination (pectate lyases and pectin lyases) or hydrolysis (polygalacturonases).

The latter enzymes are further divided into *exo-* if its action pattern is at the terminal end, and *endo-* if its action pattern is random (Fogarty and Kelly, 1983; Sieiro et al., 2012; Whitaker, 1990). Pectinases mainly include pectin esterases, polygalacturonases, pectin lyases (PNL), and pectate lyases with different substrate specificities (Ahlawat et al., 2009; Kohli and Gupta, 2015). Pectin lyases can hydrolyze pectin to oligosaccharides having 4-deoxy-6-*O*-methyl-α-D-galact-D-enuronosyl groups at their ends (Fig. 4.2). Pectinolytic enzymes act on plant cell walls decreasing the intracellular adhesively and tissue rigidity (Pires and Filho, 2005). Pectinases are produced by microbes and plants (Saranraj and Naidu, 2014). There are numerous reports of different types of pectinolytic enzyme production from different pathogenic fungi and bacteria, including pectin methyl esterase, whose isoforms are detected in all higher plants tested so far (Mareck et al., 2012). Pectinolytic microbes were industrially exploited for pectinases that are environmentally friendly enzymes (Kohli and Gupta, 2015). Microorganisms are naturally endowed with the potential

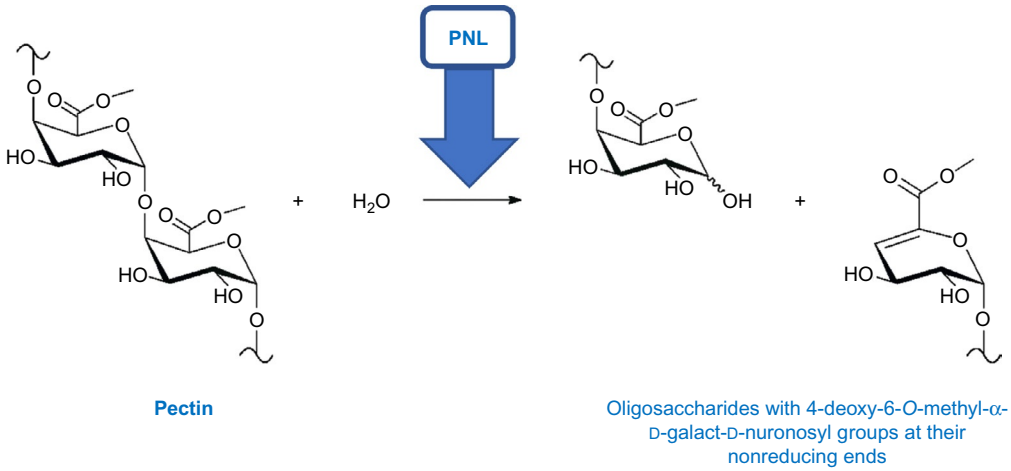

FIG. 4.2 Hydrolysis of pectin using pectin lyase.

to produce enzymes extracellularly, such as Streptomyces GHBA10, which is an efficient producer of pectinases that might be utilized in FJ clarification and extraction processes (Das et al., 2013). Samples for isolation of pectinolytic isolates were from fruit waste processing areas, agro-industry residues, soils rich in pectic waste, sewage of FJ centers of different locations, and waste from the pectin industry (Hoondal et al., 2002; Kohli and Gupta, 2015). Fungal-pectinases are extracellular enzymes, wherein polygalacturonase is the prominent type among them. Pectinases are produced by different fungi, including *Botrytis cinerea*, *Aspergillus* sp., *Fusarium moniliforme*, *Penicillium*, *Rhizopus stolonifer*, *Trichoderma* sp., *Rhizoctonia solani*, *Neurospora crassa*, and *Fusarium* (Joshi et al., 2011; Nisha, 2016).

4.2.1.1 Pectinases in the FJ Industry

Pectinases are important item in the FJ industry and have different food technological applications. Microbial pectinases accounts for about 10% of total global enzyme production. Pectinases act to degrade the complex and long pectin in the fruit pulp, which are found as polysaccharides responsible for pulp turbidity. Applicability of pectinases is pH-dependent, wherein acidic enzymes are mainly used in the beverage industry for clarification and extraction (Alkorta et al., 1998; Blanco et al., 1998) to remove pectic materials responsible for FJ turbidity and consistency (Bonnin et al., 2003). Pectinases are a prerequisite in FJ clarification because they bring down bitterness and cloudiness of FJ, reduce viscosity of fruits, as well as improve pressability of pulp and disintegrate the jelly-like pectin (Kohli and Gupta, 2015). Pectinase treatment was applied with different fruits such as raspberry, strawberry, orange, blackberry, and grape juice and apple pomace, which resulted in improved FJ chromaticity and stability (Kohli and Gupta, 2015; Pasha et al., 2013).

4.2.1.2 Pectinases in the Extraction of FJ

Pectinases are utilized to facilitate FJ extraction and to help in the separation of flocculants precipitated by filtration, sedimentation, and/or centrifugation. If a cloudy FJ is required, it is

pasteurized to inactivate enzymes. Centrifugation removes large-size remains while leaving small particles in the FJ suspension. If a clear FJ is required, those suspended particles should be withdrawn. To perform this, a treatment with commercial enzymes mixtures (cellulases, pectinases, and hemicellulases) is carried out and later the fluid is centrifuged to clarify the FJ (Grassin and Fauquembergue, 1996; Kashyap et al., 2001; Sieiro et al., 2012). Degradation of pectic constituents in the mashed fruit purees is achieved via pectinase treatments resulting in an enhancement in the FJ recovery and its clarification as well as a reduction in the FJ viscosity. In addition, applications with pectinases mixtures could provide filtering (de Gregorio et al., 2002; Fernández-González et al., 2004; Ribeiro et al., 2010; Souza et al., 2003). Tropical pectin-rich fruits are too pulpy to obtain FJ by pressing or centrifugation. Those techniques usually require high amounts of energy and result in meager FJ yield. In fruits like banana, guava, mangoes, and papaya, the expression of FJ is difficult by the traditional techniques. In other fruits like grapes and apples, FJ extraction is usually incomplete because some quantity of FJ is retained in the fruit pomace. In the traditional process of FJ production from soft fruits, the pulp is boiled and the extract is processed (Tapre and Jain, 2014; Waldt and Mahoney, 1967). An enzymatic application enables expression of FJ without drastic processing, and also helps in the FJ clarification (Sreekantiah et al., 1971). The process consists of pulping the fruits and warming them to 65°C for 15 min to inactivate inner enzymes. The pulp is cooled, pectinases are added and left for incubation time, and the FJ is separated by pressing it through cheesecloth or using a centrifuge. The FJ is racked at 3°C to 5°C for 24 to 48 h, and during that time all the suspended particles settle down. The clear supernatant could be clarified and stored after pasteurization.

Pectinases are used in the apple FJ industry to help FJ pressing or FJ extraction and to facilitate the separation of a flocculent precipitate by centrifugation. Schols et al. (1990) reported on rhamnogalacturonase (from *A. aculeatus)* and its effect on the maceration of the apple tissue. Treatment with pectinases takes about 15 to 120 min depending on the enzyme nature and how much is utilized, the reaction temperature, and the apple variety (Kilara, 1982). With pectinase application, there was an increase (up to 30%) in the yield of grape FJ. The FJ yield increased as levels of pectinases increased from 0.05% to 1.50% (Tapre and Jain, 2014; Villettaz, 1993). The FJ recovery of pectinases-treated pulps increased greatly from 38% to 63% in peach, 60% to 72% in pear, 52% to 72% in plums, and 50% to 80% in apricot (Joshi et al., 2011). Ramadan and Moersel (2007) studied the physical and chemical parameters of the *Physalis peruviana* FJ as influenced by enzymatic treatments with enzyme preparations (Rohapect VR-C, Pectinase L 40, and Ultrazym AFP-L). Rohapect VR-C contained a pectinase, protease, and hemicellulose mixture. Pectinase L 40 contains pectinase activity, and a minor polygalacturonase activity. Ultrazym AFP-L contains pectolytic and cellulolytic activities (hemicellulose, pectinase and cellulase). With enzyme treatments, the yield of *P. peruviana* FJ was increased, along with the macro- and micro-constituents. Enzymatic treatments resulted in *P. peruviana* FJ with high pulp content, high total soluble solids, and high acidity. Enzyme-treated *P. peruviana* FJ was characterized by low alcohol-soluble solids and pH. The antioxidant activities of different processed *P. peruviana* FJ were assessed by bleaching of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radicals and the values were correlated with antioxidants found in the FJ. In another study, Sharoba and Ramadan (2011) prepared *P. peruviana* FJ enzymatically-treated with Pectinex Ultra SP-L (300 and 600 ppm), and the FJ was concentrated to 30°Brix and 40°Brix. Rheological characteristics of *P. peruviana* juices were studied

at a shear rate range from 0.3/s to 100/s and at a wide range of temperatures (5°C to 100°C). *Physalis peruviana* FJ concentrates had a definite yield stress and behaved as non-Newtonian fluids. The Bingham and Casson, yield stress, plastic viscosity, consistency index, and flow index were decreased when the temperature and Pectinex Ultra SP-L dose were increased. Arrhenius-type equations described the effect of temperature on the FJ viscosity. The activation energy for viscous flow depended on the TSS. The impact of pectinase obtained from *Paecilomyces variotii* on the extraction and clarification of grapes and pomegranate juices was studied (Nisha, 2016). Different enzyme concentrations (0.5 to 3.5) and incubation times (30 to 160 min) at 50°C to optimize the enzymatic treatment for the yield and clarity of pomegranate juice were examined. Optimum conditions recommended for enzyme treatment for clarification and yield of pomegranate juice were 3.5 mg/20 g pulp for the enzyme concentration, and 180 min for incubation time. There was an increase in the yield of 31.6% and 42.3% of the grape and pomegranate juices when treated with purified enzymes than the untreated juices (Nisha, 2016).

4.2.1.3 Pectinases in the Clarification of FJ

The application of enzymes in FJ clarification was first introduced in Germany and the US in the early 1930s (Neubeck, 1959). The enzymatic-assist clarification is affected by many factors, including the enzyme concentration, incubation time, and temperature (Lanzarini and Pifferi, 1989; Tapre and Jain, 2014). Pectinases hydrolyze pectin and flocculate protein-pectin complexes (Baumann, 1981). The resulting FJ has a much lower level of pectin and a low viscosity, which facilitates the subsequent filtration. Before the technique of enzymatic clarification, heat coagulation or clarification by freezing were techniques adopted to obtain clear FJ. Commercial pectinases were utilized as processing aids for degradation of pectin that settled particles in the suspension. The application of pectinases resulted in higher FJ clarity and yield as well as preserved the nutrients, color, and flavor of the FJ. Traditional clarification methods depend on pectin hydrolysis with pectinases and starch hydrolysis with amylases. Clarifying agents including gelatin, bentonite, or silicasol induce the physical and chemical precipitation of sediments or haze-active components (Cerreti et al., 2016; Mirsaeedghazi et al., 2010; Pinelo et al., 2010; Rinaldi et al., 2013). De-pectinizing actions have two impacts: to form the aggregation of cloud particles and to degrade the viscous-soluble pectin. Pectin carries a negative charge in acidic environments and forms a coat around suspended-proteins which causes them to repel each other. Pectinases degrade the chain of pectin, therefore exposing positively charged proteins. Electrostatic repulsion between the cloud particles is reduced so that they aggregate together (Sorrivas et al., 2006). In the preparation of clarified FJ, cellulase, hemicellulose, and pectinase are effective in the viscosity reduction and filterability enhancement (Jaleel et al., 1978; Koff et al., 1991; Shahadan and Abdullah 1995; Tapre and Jain, 2014). Pectinases have improved the apple FJ clarification with a 35% drop in viscosity (Girard and Fukumoto, 1999; Mondor et al., 2000), pineapple FJ (Carneiro et al., 2002), tangerine FJ (Chamchong and Noomhorm, 1991), as well as peach, plum, pear, and apricot FJ prior to ultrafiltration. Brasil et al. (1995) reported a significant reduction (ca. 63%) in the viscosity of guava FJ when Clarex-L concentrate was used. In addition, Sharma et al. (2005) mentioned that enzyme concentration, temperature, and incubation time affected carrot FJ viscosity (41% reduction) when Pectinex Smash XXL was applied. The viscosity and turbidity of banana FJ are caused mainly by the polysaccharides (pectins) in the banana (Alvarez et al., 1998;

Brasil et al., 1995; Koff et al., 1991; Yusof and Ibrahim, 1994). Pectin makes the clarification step harder due to its fiber-like molecular structure. It was reported that de-pectinization using pectinases might clarify banana pulp (Ceci and Lozano, 1998; Lee et al., 2006; Tapre and Jain, 2014; Vaillant et al., 1999). The commonly utilized enzymes in the apple juice industry are those enzymes that could depolymerize the highly-esterified pectin. Developed techniques for producing apple juice were reported (Jaleel et al., 1978; Tapre and Jain, 2014) whereas by judicious application of pectic enzymes, a sparkling FJ was obtained and 20% of pectin was recovered. Apple juice could be extracted from crushed apple mush with the help of pectic enzymes, followed by pomace liquefaction with a mixture of cellulases and pectinases to complete the extraction and obtain premium FJ (Sieiro et al., 2012; Will et al., 2000).

Kumar and Sharma (2012) studied the impact of enzymatic treatment on pineapple (*Ananas comosus*) juice clarity, yield, and viscosity. The optimized enzymatic treatment conditions were incubation time (446 min), incubation temperature (47°C), and enzyme concentration (0.14 mL/50 g of pulp). The conditions for the enzyme treatment of the same variety of pineapple to improve the FJ recovery and quality were also optimized. The crude enzymes were competitive to the commercial enzymes for the enhancement of pineapple FJ recovery and quality. The comparison was done under optimized conditions using principal component analysis. Saxena et al. (2014) exposed watermelon juice to masazyme enzymes at varying levels (0.01% to 0.1%, *w*/w), different temperatures (30°C to 50°C) and periods (20 to 120 min). Enzymes degraded polysaccharides, resulting in reductions in turbidity, viscosity, and absorbance values, while FJ yield, total dissolved solids (TSS), and lightness were increased. Deshmukh et al. (2015) studied the rheological properties of enzymatic-clarified *Achras sapota* (sapota) FJ at different temperatures (10°C to 85°C) and TSS content (10°brix to 55°brix) corresponding to a water activity (0.865 to 0.986). The effect of TSS content on the viscosity of enzymatic-clarified sapota FJ followed the second order exponential type relationship ($r > 0.99$, rmse % <3.53). The enzymatic-clarified *Achras sapota* FJ behaved like a Newtonian liquid wherein the viscosity (η) values were from 4.34 to 56.41 mPa s depending on the temperature and concentration. The effect of TSS content on flow activation energy was described by using an exponential relationship ($r > 0.95$) and that of water activity, described by using power law relation ($r > 0.99$). An equation representing combined impact of TSS content/water activity and temperature on the viscosity of enzymatic-clarified *A. sapota* FJ was established. Conventional processing of pomegranate into FJ is time-consuming and needs several steps including washing, pressing, clarification, pasteurization, and filtration (Cerreti et al., 2016). Clarification is a basic step in the pomegranate FJ processing to inhibit the substances responsible for FJ turbidity, and to inhibit the development of turbidity during FJ storage, known as haze formation (Cassano et al., 2011; Mirsaeedghazi et al., 2010; Vardin and Fenercioglu, 2003). Removing those particles is an industrial problem, which improves the clarity as well as the color stability. The FJ industry has developed several techniques to solve these problems because consumer interest is driven by FJ quality and appearance (Costell et al., 2010). Cerreti et al. (2016) tested the impacts of pectinolytic and/or proteolytic clarification on the turbidity and the haze from active substances in pomegranate FJ. A synergic impact of the application of protease and pectinase was reported wherein very good results in terms of FJ turbidity and potential haze formation were reached. Although enzymatic treatments with pectic enzymes and proteolytic enzymes did not modify the amounts of protein, pectin, and phenolics, they influenced the haze-forming activity of turbidity-forming molecules. In addition, this kind of enzymatic treatment did not

affect FJ color and levels of anthocyanin. In the orange FJ, where pectin esterases are found, pectins are partially methylated. Polygalacturonases are commonly used for this kind of FJ. During orange FJ extraction, pectinases could be added at the end of the pulp wash to reduce FJ viscosity. This leads to high FJ yield, a better TSS extraction, and a lower viscosity. Pectinases reduce FJ viscosity without attacking the insoluble pectin, which maintains the cloud stability. Enzymes should have the least content of pectin methyl-esterases to avoid clarification of FJ (Kashyap et al., 2001; Sieiro et al., 2012). Manjunatha et al. (2014) evaluated thermos-physical traits of enzymatic-clarified lime (*Citrus aurantifolia*) FJ at moisture levels (30.3% to 89.3%, wet basis). The viscosity of enzymatic-clarified lime FJ and Newtonian viscosity decreased with the increase in the water activity; whereas thermal conductivity and specific heat increased with the increase in the moisture and water activity wherein the thermal diffusivity increased marginally. A correlation between thermos-physical traits and moisture content of enzyme-treated lime FJ was observed. In addition, a significant negative correlation between physical and thermal characteristics was noted.

4.2.2 Cellulases and Their Applications in the FJ Industry

Cellulose is synthesized by microorganisms including some algal species, plants, and also animals (Mohite et al., 2012; Zenga et al., 2011). Cellulose has a multi-level architecture consisting of microfibrils bundles. Each microfibril contains about 36 to 1200 cellulose chains that are linked together by van der Waal forces and hydrogen bonds to form a crystalline structure. A cellulose chain is a non-branched chain of D-glucose monomers that might range from 100 to 20,000 glucose units linked by β-glycosidic bonds (Ioelovich, 2008; Sharma et al., 2016; Zhang and Lynd, 2004). Hydrogen bond interactions in these amorphous regions are sub-optimal, therefore accessible for enzyme attack and water (Lenting and Warmoeskerken, 2001). Generally, cellulose degradation to glucose is achieved by synergistic action of exoglucanases, endoglucanases, and β-glucosidases. Conversion of cellulose polymers using cellulases is a foreseeable approach for the judicial use of abundant agricultural lingo-cellulosic wastes to produce useful products. Cellulases account for an 8% share of the global industrial enzyme demands (Elba and Maria, 2007; Ioelovich, 2008) and the annual globule cellulase market is expected to expand up to $400 million USD (Sharma et al., 2016; Zhang et al., 2006). Cellulases are valuable factors in food technology due to their different applications in several processes. Cellulases are used worldwide due to their useful and promising potential to be exploited in several processes and techniques involved in food technology like FJ clarification and the reduction of nectar viscosity (Bhat, 2000; Efrati et al., 2013; Karmakar and Ray, 2011; Kuhad et al., 2010; Sharma et al., 2014; Singh and Sharma, 2013).

Production of FJ (i.e., apple and pear) includes crushing fruit to pulp mash which is then separated to become clear FJ and pomace (solid phase) by mechanical processing (Galante et al., 1998). The yield and the process performance were increased by using macerating enzymes to clarify the FJ (Sharma et al., 2016; Vieira et al., 2009). There was about 50% decrease in viscosity of passion FJ when a combination of cellulases, pectinases, and amylases were applied (Sandri et al., 2011). The application of *exo*-enzymes in black carrot FJ processing enhanced the antioxidant potential due to the increase in the levels of phenolic compounds and flavonoids (Khandare et al., 2011). Fruit nectars are processed by blending pulpy FJ with sugar syrup and citric acid to form ready-to-drink beverages. The attractive feature of those

beverages to be maintained is cloud stability. Cloud stability of nectars was improved by applying exogenous enzymes. Enzyme preparations like Pectinex Ultra, Rohapect, or preparations containing a combination of pectinases and cellulases, have been found to decrease the nectars viscosity (Kashyap et al., 2001; Sharma et al., 2016). Enzyme preparations were tested in improving the rheological characteristics of mango puree, wherein Rapidase Pomaliq and Rapidase recorded the best results. Those enzyme preparations have high amount of cellulases, pectinases, and xylanases that could reduce puree viscosity in a short time to modify the rheological characteristics to be suitable for commercial use (Brito and Vaillant, 2012; Sharma et al., 2016). Ramadan and Moersel (2007) reported the characteristics of the *P. peruviana* FJ as affected by enzymatic treatments with Ultrazym AFP-L, which contains cellulolytic and pectolytic activities (hemicellulose, cellulase and pectinase). With enzyme treatments, the yield of *P. peruviana* FJ is increased, along with the macro- and micro-components. Fruit juice sensory characteristics include aroma, texture and flavor properties, all of which play an important role in food technology and biotechnology. The fruit sensory traits could be altered with enzyme infusions like cellulases (Baker and Wicker, 1996; Sharma et al., 2016). Enzymatic treatments were found to enhance the nutritional value as well as the aroma of fruits (Shoseyov and Bravdo, 2001). β-Glucosidase, when added to tea beverages, caused aroma enhancement due to the increase in the essential oils content (Contesini et al., 2013; Su et al., 2010). Tea browning could be avoided because immobilized β-glucosidase is able to work at low temperatures when compared to the free enzyme. Enzyme-assisted treatment of FJ was reported to enhance the color, FJ yield, and health-promoting effects. An increase in acidity, total soluble sugar, and β-carotene levels of carrot juice with a decrease in juice viscosity was exhibited due to enzymatic treatment, thus improving the sensory traits of color, flavor, and general acceptability (Kaur and Sharma, 2013; Sharma et al., 2016).

4.2.3 Tannases and Their Applications in the FJ Industry

High levels of tannins in food or feed have negative impacts on nutrition by reducing protein digestibility, inhibiting digestive enzymes, or by systemic toxicity (Curiel et al., 2010). Anti-nutritional effects of tannins might be decreased by treatment with tannase-producing microorganisms, or tannase (Rodríguez-Durán et al., 2011; Yao et al., 2014). Tannase (EC 3.1.1.20, tannin acyl hydrolase) catalyzes the hydrolysis of ester bonds found in gallotannins, ellagitannins, gallic acid esters, and complex tannins (Beniwal et al., 2013). The practical use of tannases is limited due to few reports and knowledge about their traits and purification processes (Yao et al., 2014). They are widely used as clarifying agents in the production of tea, FJ, treating tannin-polluting industrial effluents, and agricultural by-products. Moreover, tannases play an important role in the manufacturing of gallic acid, which is a substrate for the enzymatic or chemical synthesis of propyl gallate (antioxidant). Tannases are produced on tannic carbons such as tannic acid, tea, wheat bran, and coffee husk extract. Microbial tannases are induced extracellular enzymes, produced by solid-state fermentation, and submerged fermentation. Tannase is purified by hydrophobic interaction chromatography and reverse micelle. Most of tannases act in a wide range of temperature and pH, although tannases with acidic pH optima are common. A sequence-based classification spreads tannases in several families, therefore reflecting the molecules' variety (Beniwal et al., 2013; Yao et al., 2014).

Tannases have interesting and useful applications in food and feed industries. The main applications of tannases are instant tea, as well as gallic-acid manufacturing from plant raw materials rich in tannins. Tannases help to reduce the adverse impacts of tannins in FJ and beverages (Yao et al., 2014). Enzymatic treatment of FJ in order to reduce FJ bitterness has advantages like the high quality of FJ due to non-deterioration and the low haze (Beniwal et al., 2013). New FJ (i.e., cranberry, raspberry, pomegranate, and iced tea) were acclaimed for their health-promoting effects and disease-fighting antioxidant traits. High tannin levels in these fruits cause sediment formation, as well as tannin levels responsible for color, bitterness, and astringency of FJ during storage. Because of the inability of traditional FJ debittering processes to effectively eliminate bitterness, enzyme treatments are needed. Oded et al. (1990) reported that enzymes from mutant species CMI CC 324, 626 of *Aspergillus niger* B1 showed activity that endo tannase, β-glucosidase, and anthocyanase can enhance taste and flavor of FJ and the fermented products (Beniwal et al., 2013). Tannase-treated black tea and FJ in high concentrations can be preserved without causing precipitation and clouding, thereby exhibiting excellent quality (Beniwal et al., 2013; Kaoru et al., 2000). Motoichi et al. (2001) reported that FJ could be stored for long periods without showing any precipitation or turbidity when they are treated with tannase and/or chlorogenase (Beniwal et al., 2013). Tannase treatments resulted in 25% degradation of tannin in pomegranate juice, while the combination of gelatin and tannase (1:1, *w*/w) resulted in ca. 49% degradation of tannin (Rout and Banerjee, 2006). Srivastava and Kar (2009) reported the enzymatic treatment of aonla/myrobalan (*Phyllanthus emblica*) juice with 68.8% removal of tannin content resulted in considerable loss of astringency.

4.3 CONCLUSION

Increased health awareness has led to an increase in FJ consumption as an alternative to caffeine-containing beverages. Fruits are usually pectinaceous and pulpy to yield FJ by simple processing techniques. The pectinolytic enzymes cellulases and tanninases are effectively used in FJ biotechnology and FJ processing. Enzymatic processing makes clear FJ by breaking down pectins and allowing the suspended molecules to settle down, as well eliminating undesirable changes in FJ color and stability. Compared with other established processing methods, the costs of producing enzymatically-clarified FJ could be competitive and could have a high production yield.

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