CHAPTER

Plant extracts as enzymes

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10.1 Introduction

Enzymes are the biological catalysts that enhance the speed of biochemical reactions. In nature, enzymes are specific and effective in less amounts as well as they react under the mild conditions of temperature and pH. All enzymes have been obtained from certain natural sources and get inactivated after the desired transformation of any substrate (Dziezak, 1991). Enzymes, unlike any other inorganic catalysts, are more specific and catalyze only one substrate or a group of closely attached compounds by breaking the specific bond. Owing to this specific nature of enzymes, the formation of by-products has been reduced in high-amount reactions. As enzymes react under optimum conditions of temperature and pH, reduced energy cost has been achieved. The low utility amount of enzymes makes them practical and economical candidates for commercial applications (Dziezak, 1991). Enzymes have been acknowledged as non-toxic, natural food components and have been chosen over chemical products in food industry as they have isolated from the plant and other microbial sources (Simpson & Haard, 1987). In food industry, processing of food can be defined as the methods and practices performed in the food and beverages to alter the raw food material and make it adequate for consumption (Heldman & Hartel, 2007; Monteiro & Levy, 2010).

10.2 History of enzyme use in food production

In food manufacturing, enzymes extracted from different sources such as plants, tissues of animals, and microbes have been used for the centuries. Rennet, a natural enzyme, is an enzyme mixture isolated from the stomach of domestic animals (calves) and used in cheese production. Rennet has a protease enzyme which coagulates the milk and separated the solid curd part from the liquid whey. Some other enzymes have also been produced by the yeast that is used for the fermentation of grape juice to make wine (Shinde, Deshmukh, & Bhoyar, 2015).

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10.3 Plant extracts as enzymes

Mainly four different types of plant extracts namely proteases, amylases, lipases, and cellulases have been used as the enzymes in food industry

10.3.1 Protease

Milk coagulation is the main step in cheese production procedure, and some coagulating enzymes like proteases play an important role in cheese manufacturing since thousands of years. This process of cheese manufacturing has seemed to be the oldest known function of protease enzyme. Plant proteases in the crude form or purified form, have been act as a milk coagulant in cheese production process (Harboe, Broe, & Qvist, 2010; Jacob, Jaros, & Rohm, 2011).

10.3.1.1 Source

The crude plant extract can also be further purified to get purified enzyme depending on the degree of purification. Precipitation technique with ammonium sulfate has been an efficient method to yield significant amount of active proteases from *Cynara cardunculus* flowers (Barros et al., 2003). Cardosin A and B, a type of protease enzyme has been extracted from the stigma and stylet of *C. cardunculus* flower (Silva, Allmere, Malcata, & Andrén, 2003). Proteases have been extensively isolated from the stigma of *Cynara scolymus*, dried flowers of *Moringa oleifera* and fresh flowers of *Silybum marianum* (Pontual et al., 2012; Sidrach, García-Cánovas, Tudela, & Rodríguez-López, 2005; Vairo-Cavalli, Claver, Priolo, & Natalucci, 2005). Partially purified protease enzyme extract (onopordosin) has been extracted from the stigma and style of *Onopordum acanthium* flowers (Brutti, Pardo, Caffini, & Natalucci, 2012). Hieronymain, a protein extract was obtained from the fruits of *Bromelia hieronymi* (Bruno et al., 2010). Protease enzyme extract was also obtained from peeled ginger rhizomes (Hashim, Mingsheng, Iqbal, & Xiaohong, 2011). Various plant seeds have also been used to prepare the plant extract as a protease source for cheese making process. Latex of fig tree has also been used for the extraction of protease enzyme and assessed for milk clotting properties (Kumari, Sharma, & Jagannadham, 2012; Sharma, Kumari, & Jagannadham, 2012).

10.3.1.2 Extraction procedure

Proteases have been present in almost all plant tissues and extracted from their natural sources or by in vitro culture techniques to get continuous supply of plant proteases. Protease enzyme have been isolated from a variety of plant parts including seed, latex, roots, leaves as well as flower and extensively studied for its role in milk coagulation (González-Rábade, Badillo-Corona, Aranda-Barradas, & del Carmen Oliver-Salvador, 2011; Shah & Mir, 2014; Shah, Mir, & Paray, 2014). Generally, protease enzyme has been extracted from plant parts using aqueous maceration process. The aqueous extract of these plant parts can be prepared by several ways. For this process, the whole or crushed dried plant parts are soaked in water for variable time period at room temperature. After soaking, filtrate or crude extract is collected and used as an enzyme (milk coagulant) (Roseiro, Barbosa, Ames, & Wilbey, 2003). An alternative process has included grinding of dried plant part with crude salt in presence of warm milk, stain the filtrate and solubilization of enzyme (Sousa & Malcata, 2002). The detailed procedure of enzyme extraction has been demonstrated in Fig. 10.1.

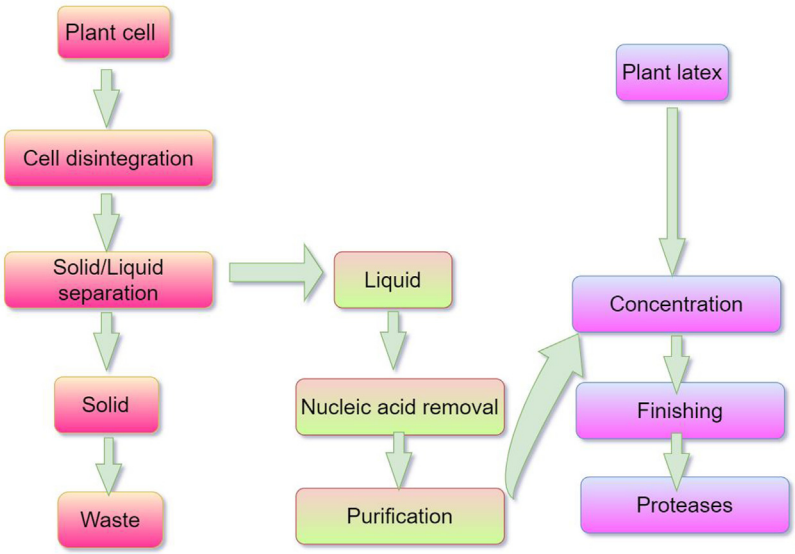

FIGURE 10.1

Extraction procedure of protease enzyme.

10.3.1.3 Types

Mostly, milk coagulant belongs to the aspartic protease group; however, some milk coagulants have also reported from other protease group like serine and cysteine proteases under proper conditions.

10.3.1.3.1 Aspartic proteases

Aspartic proteases possess two aspartic residues at the catalytic site of the enzyme. At the acidic pH level, aspartic proteases have been the most active and demonstrate special cleavage specificity for peptide bonds present between hydrophobic amino acid residues (Domingos et al., 2000). Some aspartic proteases with milk coagulant activity reported from various plant sources including *C. scolymus*, *Onopordum turcicum*, milk thistle, *Centaurea calcitrapa* and rice kernels (Asakura, Watanabe, Abe, & Arai, 1997; Domingos et al., 2000; Llorente, Brulti, & Natalucci, 1997; Tamer, 1993; Vairo-Cavalli et al., 2005). In Mediterranean region, flowers of *C. cardunculus* have been traditionally used for the cheese manufacturing as a source of aspartic proteases (Barros et al., 2003). The *C. cardunculus* have produced cardosins and cyprosins, a type of aspartic proteases that only get accumulated in mature flower parts including petals and pistils however, this enzyme has not accumulated in leaves and seeds (Cordeiro, Pais, & Brodelius, 1998). Cardosin A, an aspartic protease has also been isolated from pistils of *C. cardunculus* abundantly. Three cyprosins (aspartic proteases) that have milk clotting activities were also isolated, purified and characterized from dried flowers of *C. cardunculus* (Heimgartner et al., 1990). The specificity and kinetic parameters of these aspartic proteases are similar to chymosin and pepsin (Veríssimo et al., 1996; Veríssimo, Esteves, Faro, & Pires, 1995).

10.3.1.3.2 Papain

Papain has been classified in the cysteine proteases that processes the protein more broadly than pancreatic protease compounds. Papain mostly comprises a single peptide chain with a sulfhydryl gathering and three sulfide spans. Papain has been isolated from the papaya latex that assembled from the dried unripen papaya. The movement of protein relied upon the unripen papaya natural product. Fundamentally, papain has been settled by disulfide connects and collapsed around these scaffolds. This structure made a functioning site accessible for the cooperation of new particles. 3D structure of papain has principally comprising of two unmistakable complex particles with a cleavage between them that conveyed a functioning site for the catalysis. Papain has a sub-atomic load of 23,406 Da and globular structure with 212 amino acids. It has been steady and dynamic under a wide scope of pH, temperature and fixation. Papain has stayed dynamic at high temperature go, 3−9 pH go and has protection from higher grouping of denaturing substances (Edwin & Jagannadham, 2000). As a crystalline suspension, papain can be steady for 6 years at 50C utilizing settling operators like EDTA, cysteine and dimercaptopropanol. Papain assumed different jobs in food industry be that as it may; enzymatic combination of peptides, amino acids and different atoms has been the most widely recognized employments of papain (Esti, Benucci, Lombardelli, Liburdi, & Garzillo, 2013). Papain has additionally assumed pivotal jobs in food, organic procedures and medication industry. Papain has a major proteolytic action toward amino acid esters, amide joins, short chain peptides, and proteins (Mamboya, 2012).

10.3.1.3.3 Bromelain

Bromelain belongs to the family of sulfhydryl proteolytic enzymes and mainly obtained from the pineapples. Bromelain is a mixture of enzyme that has been used for the digestion of proteins. It has been mainly extracted from the pineapple, some fruits and stem as well. The one extracted from the fruits has known as fruit Bromelain and from the stem has known as stem Bromelain. It has been consisting of 212 amino acids and has molecular weight of 33 kDa (Babu, Rastogi, & Raghavarao, 2008). During protein breakage, it has remained stable at temperature range of 40°C−60°C in 3−7 pH range (Mohapatra, Rao, & Ranjan, 2013; Srujana & Narayana, 2017). This enzyme showed the maximum activity at 50°C and pH 7 at simple extraction and higher proteolytic activity at 60°C and pH 8 (Martins et al., 2014). Similar to papain, Bromelain also played major role in pharmaceutical, food, cosmetics and other industries. Bromelain has also been used for the tenderization of meat, solubilization of grain proteins, clarification of beer and cookies baking. In fresh apple juices, Bromelain has acted as a enzymatic browning inhibitor (Mohan, Sivakumar, Rangasamy, & Muralidharan, 2016).

10.3.1.3.4 Ficain

Ficain, a proteolytic enzyme is also known as Ficin. It has been extracted from the fig tree and belonging to the family of sulfhydryl or proteinases enzymes. It has been isolated from the clarified latex of fig tree. As an specific enzyme, Ficain can hydrolyzed the chemical bonds of natural proteins that helps on the proper digestion of protein. The structure and hydrolysis mechanism of Ficain has been much similar to the papain. It has a good stability and wide applications in the different industries including healthcare and food industry. The separation and purification of raw

Ficain can be processed via various methods like precipitation, chromatography and electrophoresis (Arribére, Caffini, & Priolo, 2000).

10.3.1.4 Role in food industry

In cheese making process, plant extracts have been used since ancient times as milk coagulants. Enzyme extract isolated from vegetables as a coagulant has also been used for the cheese manufacturing in areas like Mediterranean, south European countries and West Africa. In Spain and Portugal, largest variety of cheese has been produced through vegetable coagulant using *Cynara* spp. (Roseiro et al., 2003). The vegetable coagulant of *Cynara* spp. has been used for the production of Portuguese Serra cheese, Serpa cheeses, Spanish Los Pedroches, La Serena, Torta del Casar cheeses (from ewes' milk), Los Ibores cheese (from goats' milk) and Flor de Guía cheese (mixture of ewes' and cows' milk) (Fernández-Salguero & Sanjuán, 1999; Fernández-Salguero, Sanjuán, & Montero, 1991; Macedo, Faro, & Pires, 1993; Roa, López, & Mendiola, 1999; Sanjuán et al., 2002). Traditionally, in Nigeria and Republic of Benin, the extract of *Calotropis procera* has been used in cheese production processes (Roseiro et al., 2003). However, most vegetable coagulants have excessive proteolytic nature that limited the use of coagulant in cheese production as it lowered the texture, flavor and yield of cheese (Lo Piero, Puglisi, & Petrone, 2002). The search of new prospective milk coagulant enzyme from plant sources is in continuous process to make the enzymes more useful in industries and complete the increasing global demand for high quality and diversified cheese production (Hashim et al., 2011).

10.3.2 Lipases

Lipases have been the most extensively used class of enzymes (Hasan, Shah, & Hameed, 2006; Schmid & Verger, 1998). Lipases are found in almost all unicellular and multicellular organisms and are ubiquitous in nature. In industrial applications, mostly lipases have been obtained from yeast and fungi (Sharma & Kanwar, 2014). However, lipases can also obtained from different sources like bacterial, fungal, animal, plant and algal (Patil, Chopda, & Mahajan, 2011). Plant can be used as a novel source of lipase enzyme because of their advantages including easy acceptability, low cost source, specific application of plant enzyme and their direct applications as biocatalyst. Lipases extracted from plant tissues have included different types of non-specific monoacylglycerol, lipid acylhydrolases, triacylglycerol lipases and phospholipases A1, A2, B, C, D. the type TAG1 lipase have been mainly present in the plant seeds as energy reservoir (Beevers, 1969; Hutton & Stumpf, 1969; Lin et al., 1982). Now-a-days many lipases have been isolated and purified from different plant sources including latex of *Carica papaya* and scutella of *Z. mays*. In oilseeds, lipases have been localized in the oil bodies and glyoxysomes (Lin & Huang, 1983; Rosnitschek & Theimer, 1980). The extraction procedure of lipases from seeds is given in Fig. 10.2.

10.3.2.1 Role in food industry

In food industry, plant lipases have focal points over different sources including microbial lipases as a result of their adequacy. Plant lipases exhibit extraordinary soundness in solvent-catalyzed responses like interesterification. Plant lipases likewise have an extra significance as these lipases have minimal effort of handling and creation. Lipase extracted from *C. papaya* (CPL) has been viably utilized for the

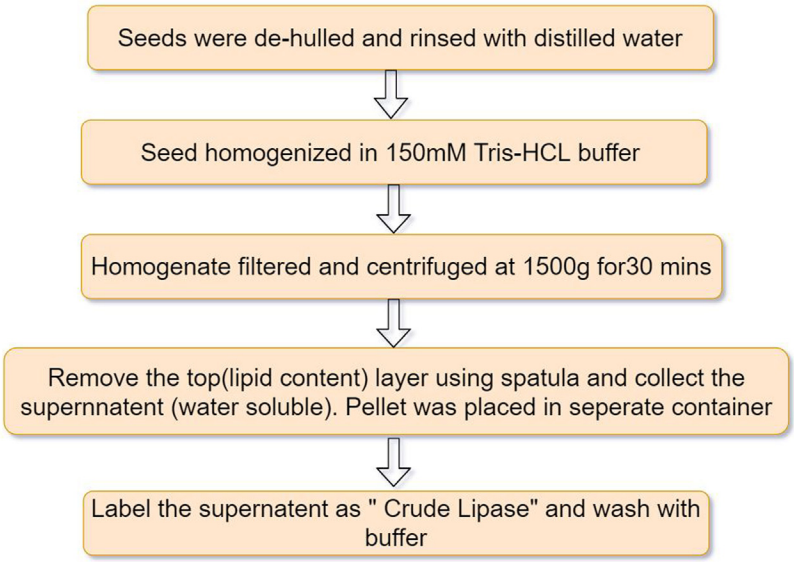

FIGURE 10.2

Flow sheet for extraction of lipase from seeds.

low calorie short and long chain triacylglycerols union for the newborn child formulae. These papaya lipases have likewise been utilized for the creation of triacylglycerols utilizing ethyl esters interesterification with the assistance of tri palmitin (Gandhi & Mukherjee, 2001). The significant expense and unavailability of human milk fat can be overwhelmed by union of human took after milk fat. The human took after milk fat has been done by trans-esterification of unsaturated fat of rapeseed oil with tri palmitin utilizing papaya latex (Mukherjee & Kiewitt, 1998).

Nowadays, CPL has been self-immobilized in papaya latex and servesas a substitute for human milk fat. This CPL has go about as a biocatalyst and utilized for the creation of business lipases as a minimal effort substitute (Tecelão, Rivera, Sandoval, & Ferreira-Dias, 2012). CPL has likewise been utilized for the union of cocoa margarine that can be utilized for the chocolate creation requiring little to no effort. CPL has additionally created some side-effects when contrasted with the synthetic amalgamation (Pinyaphong & Phutrakul, 2009). In TAG interesterification, fat and oil adjustment have additionally been finished utilizing CPL dependent on sound system selectivity (Villeneuve, Pina, Skarbek, Graille, & Foglia, 1997). In this way, CPL has been end up being a savvy plant catalyst arranged from the unrefined papaya latex and had different business applications (Lin & Huang, 1983).

10.3.3 Cellulase

In various industries, cellulase is the most commonly used enzyme after proteases and has a variety of applications in industrial biotechnology. In the processing of lignocellulosic materials, cellulase

has played the important role along with hemicellulase and pectinase for the production of fuel and feedstock's.

10.3.3.1 Role in food industry

In today's context, during the time of urbanization, climate change, and increase in population, food production has become a major concern for the human beings. In agriculture practices, food production has been increased with the technological advances and required vigorous food and beverage industries for the processing, preservation and value addition of raw food materials. Across the globe, these industries categorized as major industries and significantly contribute toward the catering to the people's needs. The food industry has mainly processed the food materials by improving its nutritional quality and reduced the concerns of human health with extending shelf life of the food products. Food industries have also improved the flavor, texture, color, packaging and consumption of the food products (Chandrasekaran, 2012). In food industry, many of these roles have been played by the enzymes to achieve the specific target.

Cellulases have been used in the food industry to increase the nutritional value of the food products. Generally, cellulases have been used in combination with hemicellulase and pectinases in food industries (Bhat, 2000; Kumar, 2015). The macerating enzymes have been made up of the combination of these three enzymes and used for the extraction of olive oil. They have been used to increase the extraction yield, lower rancidity, more antioxidants, better quality and reduced wastage (Galante, DE Conti, & Monteverdi, 1998). Fruits in their natural state have tend to rot fast hence enzymes can be used to convert them to the juice or puree can extend the shelf life of fruits. The addition of maceration enzyme in this process has yield better product and less browning of product (Sims & Bates, 1994).

10.3.4 Amylase

Commercially, amylase has been considered as an interesting enzyme as it hydrolyzes the starch material present in food products. Amylolytic (amylase) enzymes have been widely spread among the plant tissues (storage tissues) and vegetative organs including seeds, tubers and leaves. α-Amylase has been the major starch hydrolyzing enzyme in plant parts but in leaves, sometimes, the activity of amylase has been substantial (Dreier, Schnarrenberger, & Börner, 1995). Previously, amylase has been isolated and purified from different plant sources including soybean, sweet potato, pea, barley and rye apart from other microbial sources (Yamamoto, 1988).

10.3.4.1 Role in food industry

Starch is the main source of carbohydrate that originates from the plant sources. Starch derivatives like glucose syrup, maltodextrin, cyclodextrin, hydrolysates, and other modified starch have significant roles in different food, feed, and beverage industries. Starch modified enzymes have important role in production of starch derivatives and it has been a growing industries. According to updated information, around 11 amylases have been used in food industries, 215 amylases have applied in food processing industries like fruit juice preparation, brewing, baking, starch syrup etc. (Mobini-Dehkordi & Javan, 2012). In dough, starch has been broken down to the limited dextrins, an intermediate product of starch hydrolysis along with fermentable sugars during bread making process. This dextrin has been further fermented to yield the alcohol and carbon dioxide (Prakash &

Jaiswal, 2010). The formation of low molecular weight dextrins has reduced the hardness in bread. In wheat flour, β-amylase has present in abundance however; α-amylase has been absent. This β-amylase has catalyzed the undamaged native starch granules present in wheat flour. In dough, starch hydrolysis has been achieved by heterogeneous action of α-amylase and β-amylase. In the milling process, starch granules present in the flour have been broken down and made them more susceptible to amylase activity. In the baking process, these starch granules get gelatinized and their liquefaction occurred with the help of α-amylase. In flour, β-amylase has also converted the dextrin into maltose and later maltose has been fermented with baker's yeast. This enzymatic hydrolysis has maintained vigorous yeast fermentation so that lively dough has been produced with large loaf volumes (Dekker, 1994). The diversified role of amylase has been demonstrated in Fig. 10.3.

During the storage of baked products, properties of the products may be affected by the staling effect that causes distasteful changes in the products including reduced crust crisp, crumb firmness, loss of flavor, and moisture content. The short amylopectin side chains present in the fresh, soft bread has been crystalized gradually during storage. Owing to starch crystallization, moisture has been migrated within the crumb that increased the crumb firmness and reduced the crumb resilience. To stop this changes during storage, thermostable α-amylase have been used as anti-staling agents. α-amylase has limited the recrystallization, network formation of amylopectin and reduced the water immobilization which helped in the softness retention and shelf life improvement in the baked products (Jana et al., 2013).

10.3.5 Lipoxygenase

Lipoxygenase also known as lipoxidase has been widely distributed among the plants, animals, and fungi. It has been used to catalyze the oxidation reaction of cis, cis-diene units of fatty acids, and converted them to hydroperoxidienoic compounds. Recently, the pH effect of Lipoxygenase has been identified on ionic strength of substrate. It has been abundantly present in the plant sources

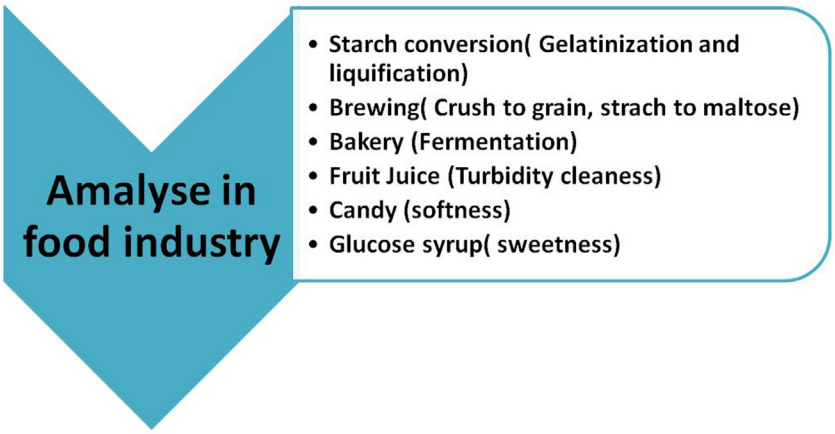

FIGURE 10.3

Role of amylase in food industry.

like legumes and potato tubers. Generally, Lipoxygenase has catalyzed the main polyunsaturated fatty acids (linoleic and linoleic acids) of the plant tissues. This enzyme has found in the vegetative tissues and played crucial role in plant defense system however, its amount in plant vegetative tissues is very less. It has been reported that Lipoxygenase used to eliminate the production of assonate and proteinase inhibitors from potato leaves and reduced the susceptibility of insect attack (Royo et al., 1999).

Lipoxygenase has been widely distributed in plants, and thus different methods have been used to isolate and purify it. The main source of Lipoxygenase enzyme is wheat. The extraction process involved isolation and purification of enzyme through chromatography. The purified enzyme has been characterized on the basis of various enzymatic parameters including thermal sensitivity, pH and amino acid composition (Shiiba, Negishi, Okada, & Nagao, 1991).

10.3.5.1 Role in food industry

Lipoxygenase plays both positive and negative roles in food industry. In a positive manner, it acts as an ingredient in bread production and also as an aroma enhancer. However, it has also affect flavor, color and anti-oxidant properties of food in a negative manner (Barrett, 1975; Baysal & Demirdöven, 2007).

10.3.6 Pectinases

Pectinases have been used for the hydrolysis of glycosidic bonds present in the pectic polymers. These pectic polymers have been generally found in the citrus fruits like pineapple, apple, tomato, orange, lemon pulp and act as the natural substrate for the pectinase. On the basis of enzyme functions, pectinase can be categorized as pectin esterases (remove acetyl and methoxyl groups from pectin), polygalacturonases (hydrolyze glycosidic α-(1−4) bonds), pectin lyase, and pectate lyase (Saadoun, Dawagreh, Jaradat, & Ababneh, 2013). Apart from plant sources, pectinase can also be produced from the natural and recombinant microbial sources with increased yield and thermostability (Rebello et al., 2017). Pectinases can work on both smooth and hairy regions of the pectin. There have been acidic and alkaline pectinases based on their pH and categorized in endo and exopectinases. When this enzyme has cleaved any substrate randomly, it called as endopectinases however; when terminal ends have been targeted, it called as exopectinases (Pedrolli, Monteiro, Gomes, & Carmona, 2009).

10.3.6.1 Role in food industry

Pectinase enzyme has been used in various industries including food industry, paper bleaching, and remediation (Pasha, Anuradha, & Subbarao, 2013). Pectinase-added juices have clearer appearance and filterability than enzyme-less juices (Saadoun et al., 2013). Pectinase have also been used to improve the flavor and color of drinks apart from reducing turbidity and haze generation from natural fruit juices like banana and apple. Haze removal has been the most costly part of juice production. To overcome this problem, pectinase has been added in the fruit juices along with gelation that increased the viscosity and turbidity of the juices and reduced the haze. Biogenic enzymes like pectinases have acted nearly nine times better than mechanical maceration in juice production to get good results (Rebello et al., 2017).

10.3.7 Peroxidase

Plants are the main source of peroxidase enzyme, and peroxidase is mainly located in the roots and sprouts of the higher plants including beetroot, potato tuber, horse radish, soybean, banana, carrot, tomato, papaya, wheat, turnip, beats, dates, and strawberries (Ambreen, Rehman, Zia, & Habib, 2000; Reed, 1975). The plant peroxidase superfamily has comprised of heme containing glycoproteins that vary in their catalytic properties and structure. It has been isolated and characterized from different plant sources such as tubers, fruits, grains and leaves. The availability of peroxidase with high specificity and stability has been used for the improvement of immune enzymatic analytical kit, development of new analytical methods and potential industrial processes (Idesa, 2018; Rosa et al., 2020).

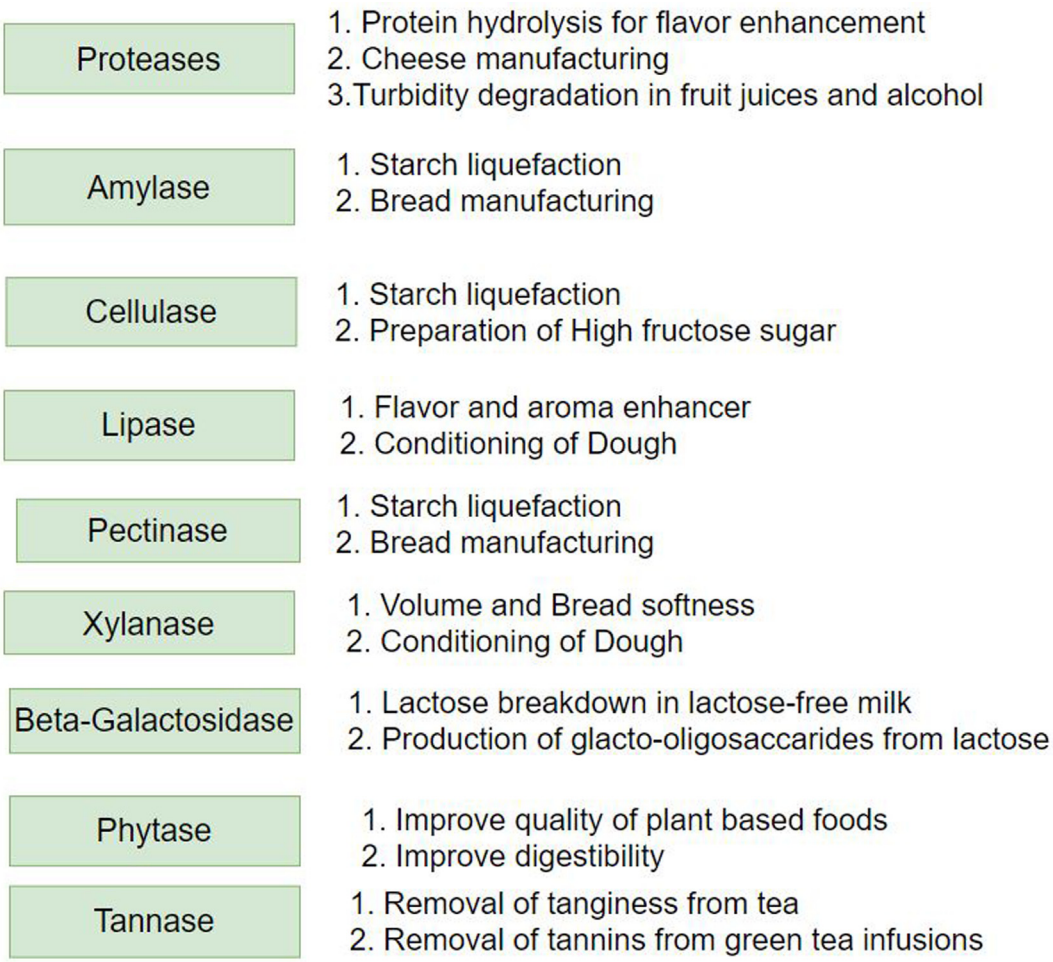

FIGURE 10.4

Major applications of plant enzymes in food industry.

This enzyme has been used to catalyze the wide range of substrate using different peroxide such as hydrogen peroxide (Holm, 1995). In food industry, peroxidase has been used for flavor, texture, and color production as well as for the improvement of food nutritional quality (Bansal & Kanwar, 2013).

10.4 Applications of plant enzymes in food industry

Enzymes have been used for the production and quality enhancement of different types of food in the food industry including yoghurt, cheese, and bread syrup. Prior to our knowledge of enzymes, some food processes were done traditionally like cheese manufacturing, brewing meat tenderization, and condiment preparation using papaya leaves based on proteolysis. The major applications of plant extract as enzyme are mentioned in Fig. 10.4.

10.5 Conclusion

The magnificence and enchantment of plant extricates as enzymes utilized in food ventures have been broadened. In not so distant future, the worldwide interest of enzymes has been anticipated to be ascending at a quick pace. Catalysts are sensitive to obtain under extraordinary ecological conditions in the past decades, and in this way various businesses were held to grasp enzyme innovation. In this field, a few methodologies were utilized to produce novel compounds from the nature for upgrading the synergist properties, catalyst specialization to serve new capacities, again structuring of biocatalysts, and advancement of protein planning. The advances in plant biotechnology have proposed various techniques to manufacture the enzymes that can be utilized in food industry, and their applications will improve the nature of human life. These exercises indicated creative methodologies for the planning of improved biocatalysts with greater steadiness (pH, temperature), less reliance on metal particles, and diminished weakness to inhibitory specialists while keeping up focused action as well as advancing novel exercises. This has been important for the utilizations of enzymes in food and feed ventures that permitted upgraded execution under ideal conditions that has diminished the danger of microbial tainting.

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