3 Role of Enzymes in Fruit and Vegetable Processing Industries: Effect on Quality, Processing Method, and Application

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Abstract

The significance of enzymes and their application in food processing industry is increasing rapidly. Different kinds of enzymes are extensively used based on their effective application. In fruits and vegetables processing, several endogenous enzymes and newly developed enzymes are used. Enzymes present in fruits and vegetables play a huge role in determining the texture, colour, flavour, and taste attributes of the processed products. The continued enzymatic activity in fruits and vegetables affects the storage quality, shelf life, and palatability of the product. Therefore, several processing methods such as grinding, crushing, slicing, juices, or preservation are used to prolong the shelf life and reduce wastage of fruits and vegetables. Hence, it is very important to control the stability and activity of endogenous enzymes present in fruits and vegetables during food processing. Apart from the conventional techniques of thermal processing such as blanching, heating, ohmic, and microwave, new and highly advanced processing techniques like high hydrostatic pressure (HHP), high-pressure homogenization processing (HPHP), pulsed electric field (PEF), and ultrasound processing have been introduced successfully. The major advantage of these new techniques is the use of non-thermal technology, which helps in retaining the sensory attributes and nutritional content of the product. These non-thermal processing techniques are effective at ambient or sub-lethal temperatures and minimize the adverse thermal effects on the nutritional content and quality of fruits and vegetables. Nonetheless, multiple advantages are rendered by these techniques in food processing industry over conventional thermal techniques which affect not only the enzymes, but also the texture, taste, and colour of the product compelled for further investigation and improvement.

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Abbreviations

CAGR	Compound annual growth rate
HG	Homogalacturonan
HHP	High hydrostatic pressure
HPHP	High-pressure homogenization processing
HTLT	High temperature-long time
HTST	High temperature-short time
OH	Ohmic heating
PEF	Pulsed electric field
PME	Pectin methylesterase
POD	Peroxidase
PPO	Polyphenol oxidase
RG-II	Rhamnogalacturonan-II

3.1 Introduction

Enzymes play a very critical role in the growth and maturation of fruits and vegetables. They also make significant contributions during the post-harvest phase in maintaining stability of raw food materials and improving their quality attributes like aroma, colour, texture, flavour, and nutritional quality. Apart from these, enzymes also have the ability to act as catalysts in transforming raw materials to food products during processing. They are also found to have extensive applications in food processing and production by enhancing the nutritional, safety, functional qualities, and overall acceptability of ingredients and processed products. Moreover, enzymes are also known for their substrate specificity, effectiveness in catalytic reactions, and enhancement rate under controlled conditions of moisture, pH, and temperature (Berg et al., 2010).

Due to rapid growth of population and fast changing lifestyle, the production and demand of convenience and processed food products have also been gradually increasing. As per the study of Markets and Markets (2020), the fruit and vegetable processing enzyme global market is expected to touch around \$41.39 billion by 2022 at 6.7% CAGR from 2016 to 2020. During this corresponding period, the Asia-Pacific region enzyme market is projected to grow at the highest CAGR due to high growth of the food and beverage industry in big emerging economies like India and China. Therefore, productive usage of the existing enzymes and the development of new enzymes have become very significant in meeting the ever-increasing demands in food processing industries. Hence, rapid advancement in enzymology is given prime focus in the food processing sector.

Currently, new enzymes based on their sources (fungi and bacteria) and types (amylase, pectinase, xylanase, and cellulase) have been isolated, characterized, and developed commercially for productive usage either in enhancing the desirable or delaying the unfavourable characteristics of foods during processing. Nonetheless, the importance and significance of endogenous enzymes in the present scenario of food processing industries cannot be overlooked for their productive usage in meeting the desired colour, texture, flavour, appearance, and stability of foods during industrial processing.

In light of the importance of enzymes in food processing, the uses of enzymes and their application in fruit and vegetable processing industries are indispensable in order to increase the productivity and shelf life, reduce cost of production, and also develop new products.

3.2 Fruit and Vegetable Composition

Knowing the composition of fruit and vegetable is the first step in determining the benefits and drawbacks of them. Fruits and vegetables are mainly composed of cellulose (7.20–43.60%), hemicelluloses (4.26–33.50%), pectin (1.50–13.40%), lignin (15.30–69.40%), and starch (3–21%) (Dhillon et al., 2013). The primary cell walls in the young plants comprise largely of cellulose and tend to thicken and become higher in hemicelluloses and lignin as they grow (Albersheim et al., 1996). Now, let's discuss in detail the different types of composition in fruits and vegetables.

3.2.1 Cellulose

Cellulose is one of the main components in fruits and vegetables ranging from 7.20% to 43.60%. It is a linear polymer of D-glucose present in the primary cell wall. It forms microfibrils, which give a stiff shape, structure, and tensile strength to the cell wall. It also helps to form a resistance against the degradation of this polymer (Szymańska-Chargot et al., 2017). These microfibrils are coated with hemicelluloses and help in binding firmly to their surface (Carpita & Gibeaut, 1993). Cellulose is known for its diverse applications in food processing industry. It is used as an emulsifier, bulking agent, texturizer, and as a fat substitute (Grassino et al., 2016). However, the application of celluloses during the extraction process needs extra precaution in order to avoid breaking down the cellulose network in maceration processes as it decreases the quantity of juice production.

3.2.2 Hemicellulose

Hemicellulose is another major component in fruits and vegetables with a variable range of 4.26–33.50%. It is a matrix polysaccharide often present along with cellulose in the cell walls and comprises of xyloglucan, arabinoxylan, glucomannan, and galactoglucomannan. Xyloglucan is the most abundant hemicellulose with similar structure as D-glucose backbone in cellulose. Hemicelluloses are bound via hydrogen bonds, forming a network of cross-linked microfibrils between pectin and cellulose. Such interconnections play a major role in the integrity of the pectin cross-linking network. Vincken et al. (1994) demonstrated that the key structure in the apple cell wall is xyloglucan and it helps in breaking down cell-wall embedded in cellulose if it gets hydrolysed. In apple cell wall matrix, xyloglucan accounts for 24% of the total amount of sugar, whereas the cellulose–xyloglucan complex accounts for 57% approximately. In food processing industries, hemicelluloses are primarily used as enhancing agents in viscosity, stabilizing, and gelling. Moreover, it is also widely used in technical and pharmaceutical fields as coatings, films, gel materials, and adhesives (Wang et al., 2010).

3.2.3 Pectin

Pectin is one of the least present components in fruits and vegetable ranging from 1.50% to 13.40%. It forms a class of complex polysaccharides commonly found in the cell walls of higher plants. It provides structure and firmness to the plant tissue in the primary cell wall and middle lamella component involved in intercellular adhesion (Thakur et al., 1997). There are three pectic polysaccharides, namely, homogalacturonan, rhamnogalacturonans, and substituted galacturonans isolated from primary cell walls. Homogalacturonan (HG) is a homopolymer, a linear chain of 1,4-linked α-D-galactosyluronic residues, known for its gel forming ability. In HG, some of the carboxyl groups are methylesterified at the C-2 and C-3 positions. Similarly, the backbone of Rhamnogalacturonan-II (RG-II) is composed of 1,4-linked D-galactosyluronic residues like HG, with a non-saccharide and an octa-saccharide side chains attached to C-2 and two disaccharides attached to C-3 of the backbone residues. RG-II is significantly used in winery and fruit juice industries because of its exceptional quality of binding heavy metals, thereby reducing the toxicity level in the final product. It also has immunomodulating activities (Grassin & Coutel, 2009). Galarturonan fractions are generally separated from other high molecular weight pectin fractions by degrading purified pectins specifically present in the galacturonan backbone either enzymatically or chemically. In fact, as explained above, the different types of pectins perform certain specific functions, but pectins in general are largely used as gelling and thickening agent in dairy and baking. Also, pectins are being widely used as a carrier of drug delivery system in cosmetic and pharmaceutical industries (Kollarigowda, 2015).

3.2.4 Starch

Starch is another main component in fruits and vegetables which is known very commonly. It is a polysaccharide composed of amylose and amylopectin. Amylose has linear chains of about 840–22,000 units of α-D-glucopyranosyl residues linked by 1→4-α-D-glucan, whereas amylopectin is usually comprised of highly branched α-1→6 and α-1→4 glucosidic linkages. Starch is a form of carbohydrate or energy reserve mostly found in storage organs, seeds, unripe fruits, and vegetables. The most important quality of starch is its water holding capacity which depends on the specific shape and size of each granule. For example, a potato granule can hold approx. 5–100 μm water/glucose unit (Jobling, 2004). Moreover, each granule contains 'blockets' of amylopectin which are crystalline and amorphous in nature. They are responsible for absorption of water, swelling, lose crystallinity, and seep amylose. However, the swelling capacity tends to be lower with higher amylose content and thus results in reduction in gel strength (Li & Yeh, 2001). Therefore, because of its inconsistent natural properties due to the vagaries of weather and agricultural conditions, most of the suppliers constantly try to make uniform starches as functional ingredients. In food processing industry, the usage of starch is manifold. It acts as an innate natural ingredient with different added functionalities like an emulsifier, stabilizer, water binder, thickener, and gelling agent.

3.2.5 Lignin

Lignin is one of the major components present in fruits and vegetables ranging from 15.30% to 69.40%. It represents a class of natural aromatic polymers of 4-hydroxyphenylpropanoids units connected by ether and carbon-carbon linkages. Lignins are generally considered a part of dietary fiber. They are mainly present in cereals, fruit, and vegetables, in which wheat bran has the richest source of lignin. Lignins have efficient antioxidant properties, mainly scavenging superoxide and hydroxyl radicals. It also inhibits the activity of xanthine oxidase, glucose-6-phosphate dehydrogenase, and non-enzymatic and enzymatic lipid peroxidation (Lu et al., 1998). In addition to this, its concentration and composition influence the adsorption capacity of cell walls. Therefore, due to these properties, lignins are considered as efficient adsorbers of hydrophobic heterocyclic aromatic amines (Funk et al., 2006). Although lignin has many applications in other different industries, in food industry it is mainly used as an additive, provides roughage to foods, and as sequestering agents.

3.3 Enzymes Used in Industrial Processing of Fruits and Vegetables

As discussed, the role of enzymes in the overall natural growth, maturation, and ripening of fruits and vegetables is very critical. This very importance of enzymes remains the same or even more in the industrial processing of fruit and vegetables. Another important positive aspect for enzymes is their ability in keeping the quality of fresh fruits and vegetables post-harvest and during storage conditions, which ultimately plays a very pivotal role in the industrial food processing.

In industrial processing, the ripening process with the application of enzymes is a major step, wherein several changes like alterations in the cell wall, middle lamellae, and membrane occur resulting in softening of tissues. Naturally, most of the enzymes in fruits and vegetable tissues are important for the maintenance of metabolism; however, they are also associated with undesirable effects on colour, flavour, odour, texture, and nutritional value. For example, in some vegetables, flavour and odour development is affected by lipoxygenase, lipase, and peroxidase (Fleuri et al., 2016). Furthermore, phenol oxidases result in discolouration and unfavourable effects on the taste and nutritional quality of fruits and vegetables. Also, fruits and vegetables containing pectic substances and α-amylases have major effects on their textural integrity (Berg et al., 2010; Fleuri et al., 2016).

With the advancement in enzymology, different types of new enzymes have been developed and are being used in improving the quality of products, development of new products, and in processing aids such as peeling, extraction of juice, and clarification, thereby increasing the efficiency in processing operation. For example, amylases, cellulases, and pectinases facilitate maceration, liquefaction, and clarification in processing of fruit juice, and hence they are cost-effective and increase the yield. Moreover, different enzyme extracts from plant tissues, bacteria, yeast, and fungi are applied in fruit and vegetable processing industries for the same purpose (Leadlay, 1993). In Table 3.1, some of the important microorganism origin enzymes used in industrial processing and its application are summarized. In Table 3.2, different types of enzymes, product type, and its application in fruit and vegetable processing are highlighted.

Enzymes are classified broadly into four types: Pectinases, Cellulase, Xylanase, and Amylase. In the proceeding paragraphs, we will be discussing in brief the different types of enzymes in fruit and vegetable processing industry.

3.3.1 Pectinases

Those enzymes whose primary role is to break down pectin, a structural heteropolysaccharide found in primary cell walls of fruits and vegetables, cereals, and fibers, are known as pectinases (Singh et al., 2003). The pectin substances are of high molecular weight acidic heteropolysaccharide primarily composed of α-(1–4)-linked D-galacturonic acid residues (Kavuthodi & Sebastian, 2018). Pectic acids are water-soluble substances having variable degrees of methyl ester groups and for

Table 3.1 Enzymes derived from microorganisms and their application in fruits and vegetable processing

Enzyme	Microorganism	Action	Application
Pectinase	*Aspergillus* spp., *Penicillium funiculosum*	Pectin hydrolysis	Degradation of pectins, increases the overall juice production, fruit juice clarification
Pectinesterase	*Aspergillus* spp.	Remove methyl groups from galactose units of pectin	Increase firmness of vegetables and also used with pectinase depectinisation technology
Protopectinase	*Kluyveromyces fragilis*, *Galactomyces reesei*, *Trichosporon fragilis*, *Bacillus subtilis*	Catalyse pectin solubilization	Clarification and reduction of viscosity in fruit juices
Hemicellulase	*Aspergillus* spp., *Bacillus subtilis*, *Trichoderma reesei*	Hemicellulose hydrolysis	Helps extraction of fruit juices, vegetable oils, and aromatic compounds, acts on hydrolysis of soluble pectin and cell wall components with pectinases, lowers viscosity and texture
α-Amylase	*Aspergillus* spp., *Bacillus* spp., *Microbacterium imperiale*	Random hydrolyses α-1,4 bounds to rupture starch and produce maltose	Hydrolysing starch to reduced viscosity, liquefying adjunct, helps in sugar production, for softness and increases volume of fruit juices and vegetables
Glucoamylase	*Aspergillus niger*, *Rhizopus* spp.	Hydrolyse dextrin from starch in glucose	Fruit juice extraction and also used for corn syrup and glucose production
Glucose isomerise	*Streptomyces rubiginosus*, *Streptomyces lividans*, *Actinplanes missouriensis*, *Bacillus coagulans*	Conversion of glucose to fructose	Helps in high-fructose corn syrup production (beverage sweetener)
Cellulase	*Trichoderma* spp., *Aspergillus niger*	Hydrolyses cellulose	Liquefaction of fruit in juice production

Note: Adapted from "Enzymes in food and beverage processing", Fleuri, L. F., Delgado, C. H. O., Novelli, P. K., Pivetta, M. R., Do Prado, D. Z., & Simon, J. W., 2015, p. 257, London, New York: CRC Press, Taylor & Francis Group

neutralization, which form gels with the addition of sugars and acids under favourable conditions (Guo et al., 2014). Pectic substances are classified into protopectins, pectic acids, and pectin which are partially soluble in water (Uneojo & Pastore, 2007). These substances are generally degraded by the enzyme pectinases. They are further classified into different sub-types, namely,

Table 3.2 Enzymes used in fruit and vegetable processing

Name of fruit	Product type	Enzyme	Application
Apple (*Pyrus malus* L.)	Juice	Amylase	Decrease starch concentration to improve durability
Banana (*Musa sapientum* L.)	Juice	Pectinase	Decrease turbidity and viscosity
Blueberry (*Vaccinium myrtillus* L.)	Juice	Pectinase	Improve juice yield and anthocyanin level
Citrus (*Citrus sinensis* L. Osbeck)	Juice	Pectinase and cellulase	Decrease the turbidity of juice
Cherry (*Prunus avium* L.)	Juice	Pectinase and protease	Decrease turbidity and increase stability
Cloudy ginkgo (*Ginkgo biloba* L.)	Juice	Amylase and protease	Reduce hydrolysis time to improve stability
Grape (*Vitis vinifera* L.)	Juice	Pectinase	Decrease turbidity and soluble solids
Lemon (*Citrus sinensis* L. Osbeck)	Soft drink punch	Pectinase	Clarification of peel extract to produce soft drinks
Pineapple (*Ananas comosus* L.)	Juice	Pectinase and cellulase	Improve soluble solids and aromas
Pomegranate (*Punica granatum* L.)	Juice	Pectinase	Improve concentration of antioxidants and decrease turbidity
Carrot (*Daucus carota* L.)	Juice	Pectinase	Improved nutritional properties as the content of polyphenols and flavonoids
Date syrup (*Phoenix dactylifera* L.)	Syrup	Cellulase and pectinases	Reduce turbidity and increase the extraction of soluble solids
High-fructose corn syrup (HFCS—*Zea mays* L.)	Syrup	Amylase	Conversion of glucose to fructose
Olive oil (*Olea europaea* L.)	Oil	Pectinase, cellulase, and hemicellulase	Oil extraction from olive residue and improve soluble solids on oil extraction
Sunflower oil (*Helianthus annuus* L.)	Oil	Pectinase	Improve oil yield

Note: Adapted from "Enzymes in food and beverage processing", Fleuri, L. F., Delgado, C. H. O., Novelli, P. K., Pivetta, M. R., Do Prado, D. Z., & Simon, J. W., 2015, p. 258, London, New York: CRC Press, Taylor & Francis Group

methylesterases (based on mechanical action), polygalacturonases, and lyases (based on their mode of action).

Then, polygalacturonases are further sub-classified into endo-polygalacturonases (E.C. 3.2.1.15) and exo-polygalacturonases (E.C. 3.2.1.67). Similarly, lyases are also sub-classified into three types, namely, pectin methylesterases (E.C. 3.1.1.11), pectatelyases (E.C. 4.2.2.9 and E.C. 4.2.2.2), or pectin lyases (E.C. 4.2.2.10) (Kc et al., 2020).

As per a recent study, it is reported that pectinases from microbial origin account for about 25% of global industrial enzymes market which is projected to reach USD 6.3 billion by 2021 (Oumer, 2017; Oumer & Abate, 2018). This clearly highlights the significance of microorganism origin enzyme as a reliable source of industrial enzyme production. Pectinases or pectinolytic enzymes are also naturally produced by many other organisms like insects, bacteria, nematodes, and protozoans (Khairnar et al., 2009). Some of the other commonly used microorganisms for extensive production of pectinases are *Aspergillus* spp., *Bacillus* spp., *Erwinia* spp., and *Penicillium* spp. (Oumer, 2017).

In food processing industries, especially in fruits and vegetables processing, pectinases have multiple usage. They are mainly used during processing of citrus juice. Moreover, pectinases are also generally recommended for use with the combination of other enzymes such as cellulases and hemicellulases. Such combinations of enzymes are generally used in facilitating the process of maceration, liquefaction, and clarification. It ultimately helps in increasing the extraction yield and enhancing the concentration of acids, colourants, and flavourings (Oumer & Abate, 2018). Apart from these, pectinases are also widely used in wine clarification, concentration, and fermentation of tea, cocoa, and coffee. Further, these enzymes are also used regularly in pickling, preparation of jam and jellies, syrups, starches, and vegetable oil extraction (Kubra et al., 2018).

3.3.2 Cellulases

Cellulases are enzymes that promote the process of hydrolysis of "cellulose", a fibrous, tough and water-insoluble polysaccharide and a homopolymer comprising of several glucose units joined by β-1,4 linkages. They act synergistically as a biocatalyst to release the sugar molecules, which helps in converting into ethanol and organic acids (Fleuri et al., 2016). Cellulases are generally produced as a multi-enzyme system comprising of glucosidase (β-D-glucoside gluco-hydrolase, E.C. 3.2.1.21), β-1,4-endoglucanase (1,4-β-D-glucan 4-glucan hydrolase, E.C. 3.2.1.4), and cellobiohydrolase or exoglucanase (1,4-β-D-glucan cellobiohydrolase, E.C. 3.2.1.91) (Jecu, 2000). Cellulases are produced by bacteria and fungi (Sharma et al., 2017). However, for commercial preparation of cellulases, different filamentous fungi such as *Trichoderma reesei* (Megazyme) and *Aspergillus niger* (Cellulocast from Novozyme) are used. Some other common cellulases producing fungi are *Aspergillus*, *Chaetomium*, *Fusarium*, *Humicola*, *Neocallimastix*, *Penicillium*, *Piromonas*, *Thermoascus*, *Trichoderma,* etc. (Singhania et al., 2010; Bansal et al., 2011).

In food processing industry, majorly in beverage industry, cellulase enzymes are extensively used in the production of fruit juices and wine processing. It facilitates the extraction of juice and maceration process by breaking the cellulose chains present in the plant cells. It also helps in extracting pigments and flavouring substances present in the grape skin. Besides releasing the flavouring substances and improving the aroma and flavour of beverages, it also breaks the unpleasant-tasting compounds present in the fruits and vegetables (Juturu & Wu, 2013).

3.3.3 Hemicellulase and Xylanase

Hemicellulase is an enzyme complex that breaks down the backbone of xylan and arabinose side chains and releases pentoses (xylose and arabinose) (Yang et al., 2017). Similarly, xylanases (endo-1,4-β-D-xylanohydrolase; E.C. 3.2.1.8) are hydrolytic enzymes involved in depolymerization of xylan. They are usually present in superior plants, such as cereals, grasses, and trees that present noncellulosic polysaccharides, such as D-glucose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucuronic, and D-galacturonic acid (Cunha & Gandini, 2010). These enzymes also degrade hemicellulose polymers, including acetyl xylan esterase (E.C. 3.1.1.72), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C. 3.2.1.55), arabinase (endo-α-L-arabinase, E.C. 3.2.1.99), endo-xylanase (endo-1,4–βxylanase, E.C. 3.2.1.8), feruloyl esterase (E.C. 3.1.1.73), α-glucuronidase (α-glucosiduronase, E.C. 3.2.1.139), and β-xylosidase (xylan-β-1,4-xylosidase, E.C. 3.2.1.37) (Juturu & Wu, 2013). Polymers such as xylan and arabinoxylan are completely hydrolysed by the synergistic action of several xylanolytic enzymes: endo-1,4-β-D-xylanases. The hydrolysis of these polymers degrades the β-D-xylan linkages; β-D-xylosidases release a xylose monomer from the cleavage of the non-reducing end of xylo-oligosaccharides and xylobiose (Terrasan et al., 2010).

Currently, commercial xylanases are produced on a large scale in many countries, such as United States, Japan, Finland, Germany, Ireland, Canada, and Denmark, by fermentation processes using bacteria, yeast, and fungi (Polizeli et al., 2005). Substantial increase in the production of xylanases has been observed after the development of improved microbial strains and efficient fermentation techniques and recovery systems. It has several applications in food industries, agriculture as well as in human health.

Furthermore, during processing of beer, the cellular wall is generally hydrolysed releasing long chains of arabinoxylans which in turn increase the viscosity of beer rendering it “muddy” in appearance. Then, xylanases are added to hydrolyse arabinoxylans in order to lower oligosaccharides, thereby reducing the viscosity and consequently improving its appearance (Dervilly et al., 2002). Xylanases in combination with amylases, cellulases, and pectinases provide multiple advantages such as increase yield of juice, stabilization of fruit pulp, and increased recovery of flavours and aromas, essential oils, mineral salts, vitamins, etc.

3.3.4 Amylases

Amylases are enzymes that act as catalysts in the hydrolysis of starch into sugars such as glucose and maltose (Sundarram & Murthy, 2014). These enzymes are extensively found in plant, animal, and microbial kingdoms. Amylases are classified into exo-amylases and endo-amylases based on their action. Exo-amylases are involved in hydrolysis of α-glucan into maltose and glucose, whereas endo-amylase hydrolyses α-glucan-forming oligosaccharides. Starch which is a polysaccharide that is an essential factor of structure, consistency, and texture in foods is hydrolysed by β-amylase. Moreover, the non-reducing terminals in starch polysaccharides and malto-oligosaccharides are also hydrolysed by β-amylase (Fleuri et al., 2016). Such enzymes are responsible for the degradation of starch and its related polymers to yield products characteristic of individual amylolytic enzymes.

Amylase enzymes have some common types, namely, amylolytic, α-amylase, β-amylase, and glucoamylase. These enzymes are largely present in a wide range of organisms, including plants, animals, and microorganisms. *Aspergillus* spp. is the most extensively used fungi for the production of amylolytic enzymes. The major sources of α-amylase enzymes are *A. niger*, *Aspergillus oryzae*, *B. circulans*, *Bacillus amyloliquefaciens*, *B. subtilis, B. licheniformis,* and *B. stearothermophilus*. β-Amylase enzymes are obtained from species like *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. However, out of these enzymes, the production of α-amylases accounts for about 30% of enzymes in world market for their extensive use in food industrial processes, such as in baking, brewing, fruit juices, syrups, starch, etc. It is also widely used in the production of drugs and other pharmaceutical products (van der Maarel et al., 2002).

3.4 Significance of Enzymes in Food Quality

In recent years, the significance of enzyme application in food processing industries has been increasing. It is due to the diversified role played by them in increasing the rate of biochemical processes. But, sometimes enzymes are also liable to unfavourable biochemical and physiological changes in fruits and vegetables. These changes alter the colour, texture, aroma, and flavour in them. Therefore, it is important to understand and characterize the interrelationships between the quality components and the associated enzymes. It also helps in determining the optimal post-harvest handling procedures and processing techniques to provide high-quality products to consumers.

3.4.1 Enzymatic Reaction on Colour of Fruits and Vegetables

The colour of a product is the primary assessment factor of quality more than any other factor. So, maintaining the natural colour of food is very critical. The colour of food products is affected during pre-harvest and post-harvest factors which are coupled by enzymatic reactions. The main enzymes responsible for changes of colour in fruits and vegetables are polyphenol oxidase (PPO), peroxidase (POD), and β-glucosidase (Zabetakis et al., 2000). Among the various processing changes, enzymatic browning causes major problem in food processing (Terefe et al., 2014).

It is due to the oxidation of phenolic compounds to quinones via polymerization reactions resulting in the production of dark colour compound called melanin (Marshall et al., 2000). Either to inhibit or delay this particular phenomenon, the fruits and vegetables are treated with anti-browning agents prior to further processing. The most commonly used anti-browning agents are citric acid, ascorbic acid, and calcium chloride. They can be used either singly or in combination with other chemicals. Ascorbic acid and citric acid are extensively used as anti-browning agents since they act as a reducing agent on the enzymatic reaction of PPO. Similarly, calcium chloride ($CaCl_2$), also known for PPO inhibitors, reduces enzymatic browning by acting on pH and as a chelating agent. However, combined treatments were found to be more effective to prevent the browning effect.

Polyphenol oxidases (PPOs) are a group of copper containing enzymes, capable of catalysing the oxidation of several phenols to *o*-quinones (Oliveira et al., 2011). These *o*-quinones in turn react with molecules which undergo non-enzymatic secondary reactions, resulting in the formation of melanin, brown complex polymers, and cross-linked polymers with protein functional groups (Rolff et al., 2011). PPOs are abundantly present in various fruits and vegetables, such as, apple, peach, banana, potato, mushroom, coffee bean, microorganisms, etc. (Eisenmenger & Reyes-De-Corcuera, 2009). Conversion of phenolics substrates into *o*-quinones occurs in two oxidation steps. In the first step, hydroxylation of *ortho*-position adjacent to an existing hydroxyl group, "monophenolase" or "monophenol oxidase", is generally referred to as cresolase or hydroxylase activity. The second step is the oxidation of *o*-dihydroxybenzenes to *o*-benzoquinones, "diphenolase activity" or "diphenol oxidase", referred to as oxidase or catecholase activity (Yoruk & Marshall, 2003).

The oxidation of phenolics compounds by polyphenol oxidase is the main reason for most of the enzymatic browning in foods occurred during harvesting, handling, storage, and processing (Eisenmenger & Reyes-De-Corcuera, 2009). It was reported that the PPO activity in apple, tomato, and tobacco is encoded by multiple genes and regulates tissue damage (Kim et al., 2001). In another study, expression of PPO response was observed in both damaged and non-damaged leaves (Constabel & Ryan, 1998). The expression of PPO in peaches was observed for 48 h of storage and it was found that there was a slight or gradual decrease in the PPO activity in intact tissues. On the other hand, in damaged tissues, there was a rapid increase in PPO activity (Tourino et al., 1993). In peaches, enzyme inhibitors such as ascorbic acid, sodium metabisulfite, β-mercaptoethanol, and cysteine were found to be effective against PPO activity (Belluzzo et al., 2009).

Furthermore, enzymatic browning can also be controlled by several processing techniques, such as freezing and heating coupled with natural and synthetic inhibitors (Marshall et al., 2000). However, many PPO inhibitors are associated with off-flavours, food security, and sustainability. For example, sulphites as PPO inhibitors are reported to cause allergies and thus seek natural alternatives (Loizzo et al., 2012). 4-Hexylresorcinol is a natural compound; when used along with ascorbic acid, cysteine, or kojic acid, it resulted in the reduction in PPO activity and changes colour in mango puree and apple juice (İyidoğan & Bayındırlı, 2004).

Kojic acid is another natural compound; it chelates metal ions such as Fe^{3+} and Cu^{2+} in addition to impounding of free radicals (Kim & Uyama, 2005).

Proteases are another group of enzymes that catalyse the hydrolysis of proteins which are degraded to peptides and amino acids (Omaña-Molina et al., 2013). It inhibits PPO activity through proteolysis or binding to specific sites. According to a study report, it was found that the browning reaction was decreased in apple when papain was used (Labuza et al. 1992). Also, when pineapple juice and high-pressure technique were applied, the browning reaction was reduced in apple slices. Such inhibitory effect is due to the presence of bromelin, sulfhydryl groups, citric, or malic acid (Perera et al., 2010). Similarly, the prevention of browning effect was also observed in peeled banana when pineapple juice was used (Chaisakdanugull et al., 2007). However, in some studies, bromelin was found to be ineffective in preventing browning of apple juice (Tochi et al., 2009).

Peroxidases (POD) are generally isolated from plants, animals, and microorganisms. These are oxidase enzymes which use hydrogen peroxide as a catalyst in the oxidation reaction of polyphenols, aminophenols, monophenols, and diphenols (Fatibello-Filho & Vieira, 2002). They are heat-stable and hence used as a parameter to increase the efficiency in bleaching (Aguero et al., 2008). POD lead to undesirable changes in colour, flavour, texture, and nutritional values in foods (Gonçalves et al., 2007). The browning of sugar cane juice is due to the presence of enzymes, POD and PPO, which oxidize phenolics compounds (Qudsieh et al., 2002). The enzymatic activities of POD and PPD enzymes were studied in different cultivars of grapes. In these studies, the POD extracts showed similar activity in both the soluble and bound fractions, and highest PPD activity was observed in cultivar Ruby. PPD and POD activities in cultivars Benitaka and Ruby decrease when juice extracts were treated with higher temperature and longer duration. It was observed that enzyme inactivation was achieved at 85 °C with 10 min exposure time, but the thermal treatments were not sufficient to inactivate the enzymes completely. For example, the thermal treatment in jam, jellies, and juices causes reduction in PPO and POD activities, but not sufficient to inactivate the enzymes (Freitas et al., 2008). Similar activities were also reported in processing of guavas (Zanatta et al., 2006).

In other studies, it was shown that apple peel from Fuji and Gala cultivars when compared to its pulp had elevated enzymatic activity both for PPO and POD. When heat treatment was done in the concentrated extracts of pulp and peel, there was a decline in PPO enzyme activity and total inactivation was also achieved after 10 min of heat treatment at 75 °C. However, such case of total inactivation was not observed for POD enzyme activity (Valderrama et al., 2001). Studies have shown that the enzymatic activity of PPO in fresh broccoli was higher compared to bleached broccoli. In case of POD, the activity was found to be lower in bleached broccoli, indicating that bleaching was partially effective in denaturation of these enzymes (Lopes & Clemente, 2002).

3.4.2 Enzymatic Reaction on Texture of Fruits and Vegetables

Texture of fruits and vegetables is an important indicator in determining the quality of the product. It is dependent on the cellular structure of cell walls. Any changes in the composition and structure of cell wall polysaccharides result into changes in the firmness (Alkorta et al., 1998). It influences the keeping quality such as storage time, handling damages, and consumer acceptance of fruits and vegetables after harvesting. Degradation in the textural quality may be due to several factors such as growth of microorganisms, infestation by insects, rodents, and other environmental conditions.

The texture quality of fruits and vegetables is also affected by a number of enzymes. Pectin which is an important cell wall polysaccharide helps in maintaining the texture by avoiding the breakdown of cellular structure. The enzymatic effect on the texture of fruits and vegetables happens when enzymes such as pectinases, cellulases, and hemicellulases break the cell walls. So, the inactivation of these enzymes during processing would help in preserving the texture of the food. Generally, pectin is used as a gelling and texturizing agent. It also improves the taste and appearance of processed foods like jams, jellies, and marmalades (Alkorta et al., 1998). However, pectins also have some major disadvantage in food processing, especially in juice processing. Soluble pectins in fruits are responsible for cloudiness or haziness in different fruit juices. Hence, the texture of such final juice product may or may not have positive feedback from consumers. But, these days, cloudy juices from tropical fruits are generally accepted and their market is growing. Nonetheless, clear fruit juices from apples, grapes, oranges, etc. are still the preferred choice of the consumers as they have appealing texture. Therefore, the enzymatic application on such hazy or cloudy juices becomes important for improving their texture and ultimately increasing consumer acceptability in the market. In order to overcome this issue, clarification process is conducted for the degradation of pectins by the application of enzyme pectinases (Alkorta et al., 1998). Different types of pectinases are used in this process and some names are pectin lyase, pectin esterase, and polygalacturonase (Kant et al., 2013). But, the pectinolytic enzymes from A. niger are commonly used in fruit and vegetable processing to improve the texture of the final products (Dinu et al., 2007).

Transglutaminases is another important enzyme which has been used extensively for food texture and new product development in food processing industries. The enzyme helps in the catalytic cross-linking reactions between proteins which transfer the glutamine residue, namely, γ-carboxyamide of one protein to the ε-amino group of lysine residue of the same protein or another protein (Nandakumar & Wakayama, 2015). This results in the improvement in food properties, such as firmness, elasticity, viscosity, and water-binding capacity. The main microbial sources of enzymes for improving the texture in food are *Bacillus* sp., *Streptomyces* sp., and *Corynebacterium* sp. (Zhang et al., 2012; Placido et al., 2008). Glutaminase is another enzyme that helps in texture by improving the protein properties, thereby increasing its solubility, gelation, emulsification, etc. (Nandakumar & Wakayama, 2015).

3.4.3 Enzymes in Flavour and Aroma Production

Taste and flavour are the two major attributes in determining the overall quality of various fruits and vegetables. Taste is determined by the contents of sugar, tannins, phenols, organic acids, and other compounds. Analysis of flavour compounds has given us an inclusive knowledge on the chemical compounds responsible for flavour sensations of fruits and vegetables. However, different enzymes have multiple effects on these attributes of fruits and vegetables. Enzymes such as peroxidases and lipoxygenases are responsible for off-flavour formation in fruits and vegetables (Bhowmik & Dris, 2004). The enzyme peroxidases are responsible for deterioration in colour, flavour, texture, and loss in nutritional qualities in raw or processed fruits and vegetables. The off-flavour is often linked with the oxidation of phenolic compounds and indigenous lipids. It was observed that enzyme inactivation was achieved at 70 °C with 15 min exposure time (Sessa & Anderson, 1981). Therefore, inhibition of such enzymes during processing is a must in order to retain or improve the aroma and flavour.

Another enzyme that has a major effect in flavour and aroma is lipogenase. This enzyme produces free radicals and conjugated unsaturated hydroperoxy acids by catalytic oxidation of polyunsaturated fatty acids. Then, these free radicals interact with other constituents like proteins, vitamins, phenolics, etc. present in fruits and vegetables which helps in enhancing the flavour and aroma of the product. However, these aromatic compounds may produce off-flavours in *Brassicaceae* family. According to the reports of Sheu and Chen (1991), increase in colour losses and development of off-flavour were observed in broccoli and asparagus during storage of non-blanched and under-blanched products.

Furthermore, the volatile compounds such as aldehydes, alcohols, ketones, esters, lactones, etc. are related to flavour and aromatic characteristics in foods (Beaulieu & Baldwin, 2002). For example, the compounds present in alcohols and aldehydes are extensively used as food additives due to the aroma referred to as 'green touch', a characteristic observed in freshly harvested fruits (Schwab et al., 2008). These volatile compounds are synthesized using various substrates like amino acids, fatty acids, and carotenoids (Goff & Klee, 2006). The main enzymes involved in the synthesis of volatile compounds from fatty acids are lipoxygenase, alcohol dehydrogenase, hydro peroxide lyase, and (3*Z*): (2*E*)-enal isomerise (Schwab et al., 2008). These enzymes are used in the extraction from fruits and vegetables such as banana, soy, tomato, and olive where they subsequently react with fatty acids to produce volatile compounds (Akacha & Gargouri, 2009).

In order to improve the taste and flavour in citrus fruits, enzymes limoninase and naringinase can be enzymatically tailored by degrading the bitter taste compounds such as limonin and naringin (Ribeiro et al., 2010). The formation of limonin can also be prevented by using limonoate dehydrogenase, as it catalyses the oxidation of its precursor lactone-A-ring to 17-dehydrolimonoate, a non-bitter derivative which cannot be changed into limonin (Merino et al., 1997). Bitter compounds in citrus fruits can be reduced by using adsorbing polymers, such as Amberlite XAD-16HP and Dowex Optipore L285 resins. Besides acting as a debittering agent, Dowex

Optipore L285 can also induce other modifications in juice processing like reduction of total titratable acidity (TTA), increasing total soluble solids (TSS), the ratio of TSS to TTA, pH, etc. (Kola et al., 2010).

3.5 Application of Enzymes in Fruit and Vegetable Processing

The significance of enzymes and its application in food processing industry is increasing rapidly. Different kinds of enzymes are used extensively based on their effective application. In fruits and vegetables processing as well, several endogenous enzymes and newly developed enzymes are used because of the following advantages:

- Low temperature requirements
- Low energy requirements during processing
- Increased product yield especially in juice processing. They make the juice stable without the addition of additives
- Less by-product formation during processing
- Improved product quality (Grassin & Coutel, 2009)

In the following paragraphs, detailed analyses are discussed on the use of enzymes in different fruit and vegetable processing techniques.

3.5.1 Fruit Firming

Texture is one of the important quality attributes of fruits and vegetables (Pan et al., 2014; Grassin & Coutel, 2009; Guillemin et al., 2008; Jensen et al., 2004; Degraeve et al., 2003). However, the texture of processed fruits and vegetables, especially the softer ones, such as strawberry, raspberry, and tomatoes, is adversely affected by thermal processing treatments like blanching, sterilization, freezing or pasteurization, and other mechanical method which may result in softening (Grassin & Coutel, 2009; Guillemin et al., 2008; Degraeve et al., 2003).

The texture and structural integrity of fruits and vegetables depend on the composition of cell walls present in them. These cell walls are composed of an interlinked fibrous structure of cellulose embedded in a matrix of pectin, hemicelluloses, and celluloses (Guillemin et al., 2008; Sila et al., 2008). The enzyme pectin methylesterase (PME) bound to the cell wall is a pectin degrading enzyme which results in demethoxylation of pectin (de-esterification of the methylated carboxy groups of polygalacturonic pectin), releasing methanol and forming carboxylated pectin. This carboxylic acid is said to interact with calcium, resulting in firmness of fruits by strengthening the cell wall (strong pectate network with added calcium) (Pan et al., 2014; Sila et al., 2008; Guillemin et al., 2008; Degraeve et al., 2003). This process is known as chelation, and it helps to overcome the adverse effects of thermal and mechanical processing treatments resulting in reduced fruit damage and disintegration (Grassin & Coutel, 2009; Degraeve et al., 2003).

Studies were conducted as early as 1965 and 1972 to assess the role of PME in increasing the firmness of canned tomatoes and potatoes, respectively. In both the studies, heat treatments such as blanching in case of tomatoes and preheating of potato slices in water were used to assess the role of PME with firmness (Bartolome & Hoff, 1972; Hsu et al., 1965).

As per a study conducted on peaches, vacuum infiltration of blanched peaches with PME of citrus fruit for 2 h along with calcium chloride increased the firmness of peaches (Javeri et al., 1991). Another study also demonstrated that vacuum impregnated PME (*Aspergillus niger*) along with calcium chloride increased the firmness in pasteurized fruits, namely, apples, strawberries, and raspberries (Degraeve et al., 2003). Similarly, a study demonstrated enzymatic firming of red pepper by using exogenous pectinesterase (Rheozyme from Novozyme A/S) and retention of texture even after freezing or heating (Jensen et al., 2004). Thus, many fruits and vegetables may be processed by using PME and calcium, thereby retaining the texture of the products (Grassin & Coutel, 2009).

3.5.2 De-Skinning (Enzymatic Peeling)

De-skinning means removal of peel/skin of fruits and vegetables. It was initially done mostly by different methods such as manually, chemical, steam, mechanical, or freeze method. But, these methods involved high labour cost, high water consumption, and peeling losses (Toushik et al., 2017; Pretel et al., 2007a, 2010). Now, considering their disadvantages, these methods have been replaced almost by enzymatic peeling in processing industry. In this method, the product is infused in enzyme preparation of pectic substances such as pectinases, hemicellulases, and cellulases (Berry et al., 1988; Bruemmer et al., 1978). Once infused, the vacuum is applied which helps the enzymatic solution to enter the intercellular spaces (Pretel et al., 2008). In the process, cellulase releases pectin from albedo, while pectinases break down the cell wall by hydrolysis of polysaccharides (Ben-Shalom et al., 1986).

Nowadays, many commercial enzymatic preparations are available and pectolytic preparation (mixture of pectinases, cellulase, and hemicellulase) is one of them. It is obtained from *Aspergillus species* fungi. It has been observed that the enzymatic solution easily diffuses when there are large intercellular spaces in albedo, resulting into easy peeling (Baker & Bruemmer, 1989). However, many studies have found that the efficiency of enzymatic peeling effect depends on the treatment methods such as heat (scalding), vacuum infusion, temperature, and pH (Pagán et al., 2005; Suutarinen et al., 2003; Toker & Bayındırlı, 2003; Rouhana & Mannheim, 1994). Details of some recent studies are given in Table 3.3.

Furthermore, the role of enzymes in the peeling process of vegetables such as potato, carrot, onion, and Swedish turnips was examined by Suutarinen et al. (2003). It was observed that enzymes did help in peeling of carrot and onions; however, potato and Swedish turnips could not be peeled properly due to the presence of cutin

Table 3.3 Studies assessing role of enzymes in enzymatic peeling

Reference	Fruit/ vegetable	Pre-processing condition	Enzymatic solution	Result
Prakash et al. (2001)	Grapefruit	Scalding, 1–4 min	Polygalacturonase, cellulase and pectin methylesterase, incubated for 12 min	Peeling improved
Suutarinen et al. (2003)	Potato, carrot, Swedish turnip, onion		Cellulase, polygalacturonse	Difficulty in removing peel of potato and Swedish turnips
Liu et al. (2005)	Mandarins		Pectinase, polygalacturonse, and cellulase, 20 min	Peeling efficiency improved
Pretel et al. (2007b)	Orange, Thomson, and Mollar	Hot water bath (45 °C)	Pectinase, polygalacturonse, and cellulase, 40 min	Peeling improved
Pretel et al. (2007a)	Orange, Sangrina	Hot water bath (45 °C)	Pectinase, polygalacturonse, and cellulase, 30 min	Peeling improved

or suberin in vegetable surface (Suutarinen et al., 2003). As per a study by Toker and Bayindirli, it was observed that the enzymatic peeling was easier at relatively moderate temperatures such as 44–47 °C for nectarines, 41–46 °C for peaches, and 45 °C for apricots with a pH ranging from 3 to 4.1 (Toker & Bayındırlı, 2003). The steps involved during enzymatic peeling are illustrated in Fig. 3.1.

The first step is selection of fruits or vegetables and washing them with water. At times, hot water treatment or scalding is done prior to enzymatic peeling to improve the peeling process and have a high quality end product (Pretel et al., 2008, 2010). A study on grapefruit as early as 1974 demonstrated dipping the fruit in a water bath of 60 °C for 30–35 min resulted in a good quality product (Bruemmer et al., 1978). The advantage of scalding is that it reduces the viscosity of pectin as well as enhanced the ability of the peel to absorb the enzyme solution, thereby helping in enzymatic peeling process (Pretel et al., 2008) (Fig. 3.1).

Therefore, enzymatic peeling has wide range of applications in fruits and vegetables such as grapefruit, orange, mandarin, apricot, peaches, potato, carrot, and Swedish turnips (Suutarinen et al., 2003; Toker & Bayındırlı, 2003). This method is a significant alternative in food processing industry because of its advantages in improving the quality of products, reducing wastage, minimal water use, reduction in contamination, and being cost-effective (Toker & Bayındırlı, 2003).

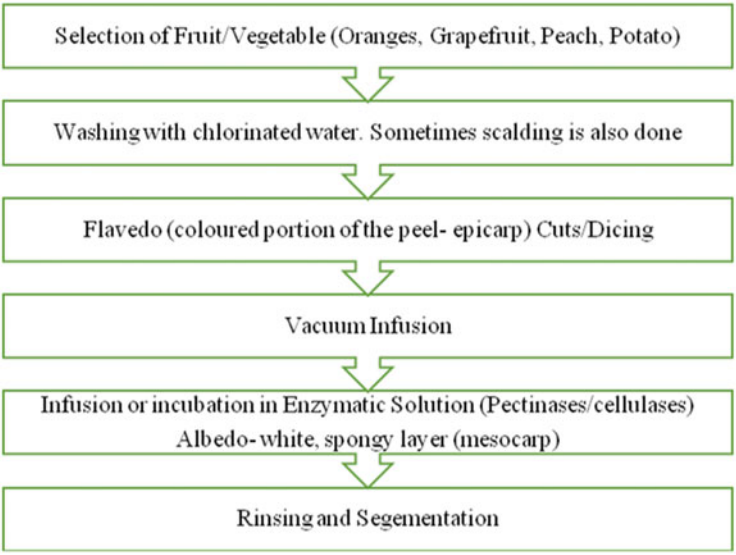

Fig. 3.1 Steps in enzymatic peeling. (Adapted from Pretel MT, Sánchez-Bel P, Egea I & Romojaro F. (2010). Enzymatic peeling of citrus fruits. In Bayindirli (Ed.), Enzymatic processing of fruits and vegetables: Chemistry and engineering applications (pp. 145–174). CRC Press, Taylor and Francis Group)

3.5.3 Application of Enzymes in Fruit and Vegetable Juice Processing

The natural liquid substance contained in fruits and vegetables is commonly known as juice. It can be obtained or prepared directly by extraction, pressing, or diffusion from fruits or vegetables and such juices are meant for direct consumption. They can be broadly categorized as juices without pulp (clarified/cloudy) or juices with pulp such as purees, nectars, and pulps (Ceci & Lozano, 2010; Cautela et al., 2010). In juice processing, different enzymes are extensively used in fruit and vegetable for higher yield, clarification, and improvement in filtration, resulting in higher quality of juices (Fleuri et al., 2016). Some of the commonly used enzymes in fruit and vegetable juice processing are pectinases, hemicellulases, and cellulases. In juice processing of fruits and vegetables, multiple steps are involved and below are the standard procedures. The steps involved in fruit juice processing are also illustrated in Fig. 3.2.

3.5.3.1 Selection of Fruit, Followed by Washing and Peeling

Enzymatic preparations can be used for peeling of fruits such as oranges, lemon, or vegetables such as pumpkin and beet (Toushik et al., 2017) as discussed in Sect. 3.4.2. This is followed by juice extraction.

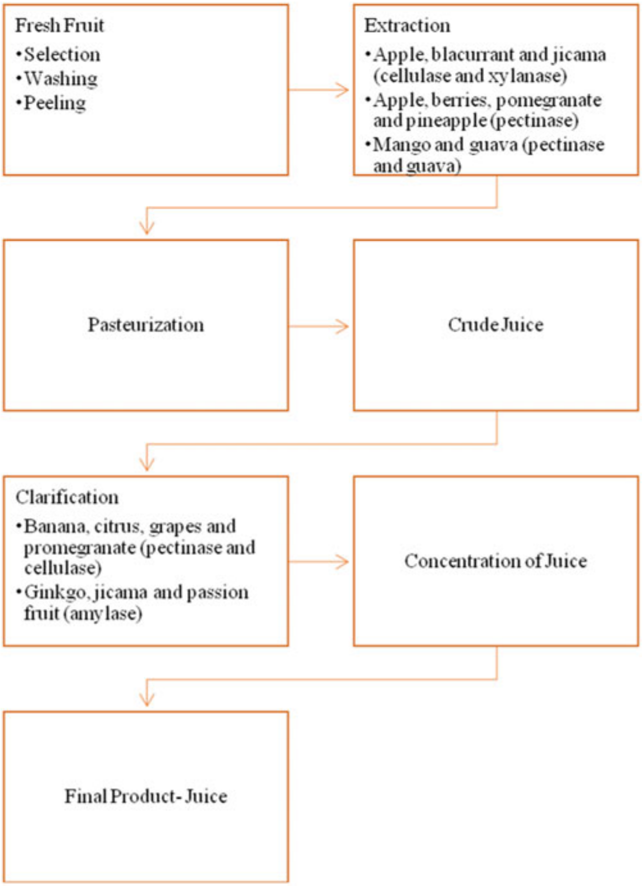

Fig. 3.2 Steps in juice processing. (Adapted from Fleuri, L. F., Delgado., C. H. O., Novelli, P. K., Pivetta, M. R., do Prado, D. Z., & Simon, J. W. (2016). *Enzymes in food and beverage processing* (pp. 255–280). CRC Press, Taylor and Francis Group)

3.5.3.2 Juice Extraction

Juice can be extracted by a number of methods—centrifugation, diffusion, extraction, and ultrafiltration. Enzyme preparations help in breakdown of cell wall and release the juice contained within the structure in fruits such as apples, grapes, berries, pears, and citrus fruits (Ribeiro et al., 2010). Among apples, the activity of endogenous enzymes is low; therefore, commercial pectinases from *Aspergillus* species are added so they can be macerated before pressing (Aehle, 2007). The extracted juice is pasteurised for microbial stability (Grassin & Coutel, 2009). A study on processing of blackberry juice observed that application of pectinolytic enzymes during pressing resulted in an increase in juice extraction as high as 81.73% in three cultivars as compared to 53.79% in the control arm (Granada et al., 2001).

Another important aspect in juice processing is the discolouration of juices during pasteurization due to presence of anthocyanins. This issue can be prevented by the use of β-glucosidase which hydrolyses anthocyanins into anthocyanidins, thereby minimizing discolouration due to its low colour intensity and solubility (Villena et al., 2007; Palma-Fernandez et al., 2002).

3.5.3.3 Clarification and Fining

Turbidity in juices is due to scattering of light caused by insoluble substances such as cell fragments that come from pulpy tissue (pectin from cell walls) in suspension. It is usually undesirable except when present in citrus fruits (Ribeiro et al., 2010). Pectin is the cause of turbidity in juices especially apple juice (Aehle, 2007). Another reason for the haziness in extracted juices is due to presence of starch in unripe fruits (Ceci & Lozano, 2010). At this stage, the role of enzymes becomes critical as enzymes like pectinase help in reducing turbidity by attracting pectin and forming clusters (aggregation of cloud particles). Then, these particles usually settle down and can be easily removed by filtration or centrifugation (Uneojo & Pastore, 2007). Apart from enzymes, flocculating or fining agents such as gelatin and bentonite may be added to aid in clarification process (Ceci & Lozano, 2010). Similarly, citrus juices, especially lemon juice, are also clarified by pectinolytic enzyme preparations (Fleuri et al., 2016).

Apple juice contains about 15% of starch which results in turbidity, slow filtration rate, and gelling after concentration. The addition of amylase along with pectinase hydrolyses this starch and removes the haze or cloudiness (Ceci & Lozano, 2010; Carrín, 2004). Sometimes, haziness occurs in juice, so adding of fining agents can prevent post-bottling haziness, thereby optimizing fining and ultrafiltration process. Similarly, araban present in certain fruits may be responsible for post-concentration haze in juice, and to prevent such haze formation, arabanase is added to the juice (Ceci & Lozano, 2010).

3.5.4 Role of Enzymes in Wine Processing

The role of enzymes in the production process of alcoholic beverages such as brewing of beer and winemaking is very important (Toushik et al., 2017; Gómez-Plaza et al., 2010; Aehle, 2007). Different enzymes, namely pectinases, amylases, glucanases cellulases, glycosidases, lyzozymes, and ureases, are used. They help in improving the quality and yield of the product (Toushik et al., 2017; Gómez-Plaza et al., 2010; Aehle, 2007).

3.5.4.1 Enzymes Used in Processing of Wine

Wine is a type of alcoholic beverage made from fermentation of fresh grapes (Bruchmann & Fauveau, 2009). Pulp and skin of the grapes account for about 75–85% and 6–7%, respectively. The cell wall of grapes is composed of hemicelluloses, pectin, and structural proteins like chitinase. Also, the cellulose and pectin present in cell walls provide rigidity to the grapes, thus hindering juice

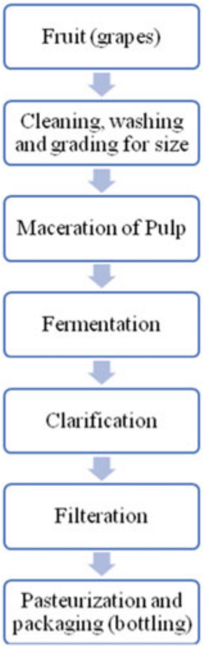

Fig. 3.3 Steps in wine-making. (Adapted from Fleuri, L. F., Delgado., C. H. O., Novelli, P. K., Pivetta, M. R., do Prado, D. Z., & Simon, J. W. (2016). *Enzymes in food and beverage processing* (pp. 255–280). CRC Press, Taylor and Francis Group)

extraction, clarification, and filtration (Claus & Mojsov, 2018). Moreover, the skin and cell walls of grapes contain several essential compounds such as anthocyanins and tannins. These compounds are responsible for colour and structure of wine (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009).

The natural enzymes present in grapes and those obtained from yeast, fungi, and bacteria collectively play an important role in wine making process (Gómez-Plaza et al., 2010). Usually, the manufacturers extend the action of endogenous enzymes by adding exogenous enzymes during production (Gómez-Plaza et al., 2010). The commonly used commercial enzymes include pectinases, glucanases, glycosidases, lysozymes, and ureases (Gómez-Plaza et al., 2010). The application of these exogenous enzymes and yeast strains during processing increases the rate of production, yield, and quality of the wine (Toushik et al., 2017).

The standard steps in wine processing are illustrated in Fig. 3.3.

These steps can be broadly categorized in three phases, namely pre-fermentation, fermentation, and post-fermentation.

Pre-fermentation

This phase involves crushing the fruit and extraction of juice. In red wine preparation, the skin is not separated, while it is separated in white wine. During the processing of red wine, pulp, skin, and seeds of grapes are kept together after crushing and during fermentation in order to extract colour and flavour (Byarugaba-Bazirake, 2008). The colour of red wine is due to the presence of anthocyanins and tannins in grape skin and seed (Gómez-Plaza et al., 2010). In this phase, exogenous enzymes derived from *Aspergillus* species such as pectinases, cellulases, and hemicellulases (xylanases and galactanases) are employed, which help in expediting the extraction of juice and colour control (Espejo, 2020; Toushik et al., 2017; El Darra et al., 2016; Kelebek et al., 2007; Bautista-Ortín et al., 2005). These macerating enzymes also modify the stability, taste, structure, and mouth feel of red wine (Gao et al., 2019).

Furthermore, maceration of the cell walls by pectinases results in the liberation of liquid and other phenolic compounds (Espejo, 2020; Gao et al., 2019). It has been observed in different studies that wines prepared by enzyme treatment tend to have phenolic and tannin content, as well as colour intensity (Romero-Cascales et al., 2008; Revilla & Gonzalez-San Jose, 2003). Sometimes, maceration is accompanied by pressing (wine press extract must or wine from crushed grapes), resulting in better yield (Gómez-Plaza et al., 2010). Another important attribute in wine making is its aroma; reductases such as glycosidases and polyphenol reductase help in aroma extraction and polyphenol reduction, respectively (Espejo, 2020). Glycosidases improve the aroma of wines by releasing aromatic compounds from their non-aromatic precursors or non-odouriferous sugar compounds (Zhu et al., 2014). As per a study, treatment of Albarino wine with pectinolytic preparations and glycosidase resulted in an improved aroma (Armada et al., 2010). However, in industrial processing, usually exoglycosidases and β-glucosidase from *Aspergillus niger* are used (Gómez-Plaza et al., 2010).

Fermentation

In this phase, yeast is added to the must in red wine to initiate fermentation (Byarugaba-Bazirake, 2008). During the process, the sugars present in the juice are converted into alcohol and release carbon dioxide. In this period, pectin plays an important role by preventing the diffusion of intercellular components such as phenolic and aroma compounds into the must (Claus & Mojsov, 2018; Bruchmann & Fauveau, 2009).

Post-fermentation

This phase involves different steps such as clarification, filtration, and microbial stabilization with the help of different enzymes. Proteases are used at the time of clarification, while pectinase and β glucanase are used during filtration and lysozyme for microbial stabilization (Espejo, 2020). After pressing, the grape must is quite turbid and is rich in solid particles (Armada & Falqué, 2007). Thus, clarification is an important step to improve the quality of wine, especially in white wine (Gómez-Plaza et al., 2010). Moreover, activated carbon, bentonite, and pectinase may be added to aid in clarification or physical removal of suspended matter in the must (Gómez-Plaza et al., 2010). Commercial enzymes that help in clarification are:

(a) Pectin lyase which results in destabilization of the cloud, thereby reducing viscosity.
(b) Pectin methylesterase (PME) results in demethylation of pectin, aiding polygalacturonase in hydrolysis of pectin and leading to cloud flocculation

Table 3.4 Enzymes and their roles in wine processing

Step	Enzyme	Action
Clarification	Pectinase	Removal of suspended particles
Maceration	Pectinases, hemicellulase, cellulase	Degradation of pectin
Maturation	Pectinases and glucanases	Ageing results in mouth feel and is done at the end of fermentation
Filtration	Pectinases and glucanases	Glucanases are applied to the must to shorten the glucan chains preventing problems during filtration
Addition to wine/ must	Urease	Elimination of urea and preventing the formation of ethyl carbamate
Addition to wine/ must	Lysozyme	Elimination of bacterial
Late phase of fermentation (white wine)	Beta glucosidase	Increased aroma by splitting glucose residue from odourless precursors
Stabilization/haze formation	Proteases	Stabilization of wine, reducing betonite demand

Note: Adapted from Gómez-Plaza et al., 2010. Use of enzymes in wine production. In Bayindirli (Ed.), Enzymatic processing in fruits and vegetables: Chemistry and engineering applications (pp. 215–243). CRC Press, Taylor and Francis Group

and clarification of must (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009).

Furthermore, during ageing or maturation, cellulases and β-glucanases are used to improve the mouthfeel (Espejo, 2020; Gómez-Plaza et al., 2010). Other than the above mentioned advantages of the macerating enzymes, studies have also observed that pectinases are found to improve haze condition, increase pressability of grapes to reduce filtration time, and increase must volume (Sharma et al., 2017; Garg et al., 2016). Table 3.4 illustrates different enzymes and their actions during wine processing.

Other important enzymes used in commercial wine production are cinnamyl esterase, cinnamyl decarboxylase, glucanase, urease, and lysozyme. The yeast-derived enzymes, cinnamyl esterase and cinnamyl decarboxylase, may be present in commercial pectin preparations as a side activity and can cause off-flavours due to volatile vinyl phenols (Bruchmann & Fauveau, 2009). At times, grapes infected with *Botrytis cinerea* result in formation of glucans. These glucans prevent the natural sedimentation of cloud particles and cause filtration problems as well. Thus, glucanase enzyme is added to prevent the development of such glucans during wine making process (Gómez-Plaza et al., 2010). Urease is another enzyme that is added occasionally during wine preparation. This enzyme hydrolyses urea into ammonia and carbon dioxide and prevents the formation of ethyl carbamate in wine which is carcinogenic at high concentration (Gómez-Plaza et al., 2010).

Lysozyme is used in winemaking to prevent or delay malolic fermentation and sulphur dioxide is added to the must or wine (Gómez-Plaza et al., 2010).

3.6 Effect of Processing Methods on Enzymes and Vegetables

Enzymes present in fruits and vegetables play a huge role in determining the texture, colour, flavour, and taste attributes of the processed products (Oey, 2010). The continued enzymatic activity in fruits and vegetables affects the storage quality, shelf life, and palatability of the product. Therefore, several processing methods such as grinding, crushing, slicing, juices, or preservation (pickling) are used to prolong the shelf life and reduce wastage of fruits and vegetables (Oey, 2010). Hence, it is very important to control the stability and activity of endogenous enzymes present in fruits and vegetables during food processing (Oey, 2010).

Apart from the conventional techniques of processing such as blanching, heating, or freezing, new and highly advanced processing techniques like high hydrostatic pressure (HHP), pulse electric field (PEF), and ultrasound have been introduced successfully. The major advantage of these new techniques is the use of non-thermal technology, which helps in retaining the sensory attributes and nutritional content of the product (Oey, 2010; Jaiswal & Sharma, 2016). In HHP technique, it employs high pressure range of 100–600 MPa, resulting in enzyme and microbial inactivity which may affect the shelf life of the products (Briones-Labarca et al., 2015). In PEF technique, being a non-thermal processing, it uses a series of short and high voltage pulses to inactivate microbes or enzymes (such as peroxidases and polyphenol oxidases) in food (Segovia et al., 2015). However, in ultrasound processing technique, high frequency short pulses are used for inactivation (Jaiswal & Sharma, 2016). These non-thermal processing techniques are effective at ambient or sub-lethal temperatures and minimize the adverse thermal effects on the nutritional content and quality of fruits and vegetables (Tiwari et al., 2009).

However, these non-thermal processing techniques still have several drawbacks, such as high equipment and processing cost, tedious to operate, hazardous, requires stringent process control operations, etc. (Jaiswal & Sharma, 2016). Nonetheless, multiple advantages are rendered by these techniques in food processing industry over conventional thermal techniques, which affect not only the enzymes, but also the texture, taste, and colour of the product compelled for further investigation and improvement (Oey, 2010).

3.6.1 High-Hydrostatic Pressure (HHP) Processing

HHP, also known as High-Pressure Processing (HPP), is based on the Le Chatlier principle, i.e. pressure is equally applied in all directions (Augusto et al., 2018; Terefe et al., 2016; Keenan et al., 2012). As the name implied, this technique uses very high pressure for application of uniform pressure to a product or food, leading to the inactivation of certain microbes and enzymes in the food (Augusto et al., 2018). This technique can be applied in solid, semi solid liquid, or particulate food products (Augusto et al., 2018).

The high pressure (HP) may increase the shelf life of fruits and vegetables, especially when combined with temperature by resulting in enzyme activity or inactivity and pressure-induced gel formation (Guerrero-Beltrán et al., 2005). In HHP technique, enzyme inactivation may be affected by the type of food, pH, temperature, and duration of treatment (de Castro Leite Júnior et al., 2017; Guerrero-Beltrán et al., 2005).

The HHP unit consists of a pressure vessel, pressure generator, temperature control, and pressure handling system. The HHP processing can be of two types—batch (closed vessel system) or semi-continuous (number of closed vessels). In this process, the hydrostatic pressure at a given point is transformed rapidly and uniformly in all directions. The intensity of HHP is determined by the process parameters like pressure, treatment duration, and temperature. The entire HHP processing cycle takes a few minutes and it can be performed at pressures as high as 1400 MPa and temperatures between less than 0–150 °C (Oey, 2010) (Augusto et al., 2018; Oey, 2010). The maintenance of a product inside a vessel at a high-pressure results in molecular changes affecting the enzymes (de Castro Leite Júnior et al., 2017).

Several studies have been conducted to determine the effect of HHP (at times combined with thermal processing) on enzyme activity in various fruits and vegetables as shown in Table 3.5. Enzymes like PME, POD, and PPO are considered baroresistant with POD and PME being the most and least resistant, respectively (Augusto et al., 2018). Hence, some studies have found that PPO and POD are not affected substantially by HHP (Augusto et al., 2018).

3.6.2 High-Pressure Homogenization Processing (HPHP)

HPHP, also called Ultra High-Pressure Homogenization, is a non-thermal processing technique, especially used in fruit juice processing. In this method, the fluid is pressurized with high or ultra-high pressure to flow through a narrow valve and the shear stress distribution across the product helps to inactivate microbes and enzymes present in the fluid (Augusto et al., 2018; de Castro Leite Júnior et al., 2017). It is a technique which can be used only in fluids. HPH is a continuous process and is often called dynamic high-pressure processing (Augusto et al., 2018; de Castro Leite Júnior et al., 2017). The high shear and sudden drop in pressure, turbulence, and caviation result in changes in processed foods (de Castro Leite Júnior et al., 2017).

In HPH, factors such as enzyme structure, fluid, equipment, media, and process parameters determine its effect on enzyme activity (Augusto et al., 2018). To ascertain its efficacy, HPH was used to treat oranges and PME activity was measured at five pressures (0–250 MPa) and three inlet temperatures (22, 35, 45 °C). A reduced activity of 70% can be achieved at 45 °C and 250 MPa (Welti-Chanes et al., 2009). Similarly, HPH has also been found to result in complete inactivity of

Table 3.5 Effect of HHP on enzyme activity

Reference	Fruits/ vegetables	Enzyme	Processing	Enzyme stability after application of HHP processing
Andreou et al. (2016)	Tomato juice	PME	800 MPa, 15 min, 65 °C	70% reduction in enzyme activity
		PG	500 MPa, 10 min, 55 °C	Complete inactivation of enzyme activity
Terefe et al. (2016)	Pear	POD	600 MPa, 3 min, 40–100 °C	Enzyme activation by 23% at 40 °C, while reduction by 92% at 100 °C
		PPO	600 MPa, 3 min, 100 °C	Reduction in activity by 90%
		PME	600 MPa, 3 min, 100 °C	Reduction in activity by 83%
Marszałek et al. (2015)	Strawberry puree	POD	500 MPa, 15 min, 50 °C	Reduction of about half of enzyme activity
		PPO	500 MPa, 15 min, 50 °C	Reduction of 72% of enzyme activity
Castro et al. (2008)	Green bell pepper	POD	100–200 MPa, 10 and 20 min, 18–20 °C	70% of reduction in enzyme activity
	Red bell pepper	POD	100–200 MPa, 10 and 20 min, 18–20 °C	40% of reduction in enzyme activity
	Green bell pepper	PPO	100–200 MPa, 10 and 20 min, 18–20 °C	50% reduction in enzyme activity
	Red bell pepper	PPO	100–200 MPa, 10 and 20 min, 18–20 °C	No effect

the enzyme pectate lyase at pressures more than 150 MPa (Calligaris et al., 2012). Therefore, it is not recommended to use HPH independently and preferably; it needs to be coupled with other processing methods for microbial or enzyme inactivation (Augusto et al., 2018; de Castro Leite Júnior et al., 2017; Calligaris et al., 2012; Welti-Chanes et al., 2009).

3.6.3 Ultrasound Processing (Ultrasonication)

In ultrasound processing technique, ultrasonic waves are employed with frequencies above the hearing range of humans. The ultrasonic waves are of two types: high frequency ultrasound (2–20 MHz) corresponding with low sound intensity (0.1–1 W/cm^2) and power ultrasound (20–100 kHz) with high sound intensity (10–1000 W/cm^2) (Feng et al., 2008). These techniques are used in processing industry for improving the shelf life of fruits and vegetables by inactivating the enzymes like PME, PPO, and POD present in the food (Bourke et al., 2010). But, when the technique, high power ultrasound with low frequency, is combined with temperature/heat, the process is known as thermosonification or pressure (manosonification) (Bourke et al., 2010). The combination of heat and ultrasound helps to ensure product stability and inactivation of enzymes and microbes, thereby resulting in retaining the quality and extending the shelf life of fruits and vegetables, especially juices (Rojas et al., 2017; Saeeduddin et al., 2015). The inactivation of microorganisms or enzymes in this technique may be due to physical factors such as caviation or mechanical effects (Bourke et al., 2010; O'Donnell et al., 2010).

Ultrasonic processing in fruits and vegetables focuses on the inactivation of endogenous enzymes which are more resistant to heat treatments (Feng et al., 2008). Studies have It has been observed that ultrasonic processing either in combination with heat or pressure has minimal effect on quality of fruit juices such as orange juice, guava, and strawberry juice (Bourke et al., 2010). Further, it has been observed that high ultrasound and longer processing times may be required for enzyme inactivation as the pulp complicates the inactivation process (Rojas et al., 2017). Hence, ultrasound technique is usually combined with a thermal process to increase the rate of inactivation of enzymes in juices (Chen et al., 2019; Rojas et al., 2017; Anaya-Esparza et al., 2017; Abid et al., 2014). PME was also found to be inactivated in lemon juice (Knorr et al., 2004) and mousambi (Siwach & Kumar, 2012) by using ultrasonic processing along with heat. Likewise, peroxidase enzyme present in watercress was also found to be inactivated by the use of thermosonication (Cruz et al., 2006).

3.6.4 Pulsed Electric Field (PEF)

In PEF processing technique, electrochemical effects and ohmic heating are responsible for changing the structure and function of enzymes present in the food. At times, large specific energy inputs are required to inactivate enzymes (Poojary et al., 2017). Enzyme activity can be affected by a number of factors such as properties of the enzyme, treatment parameters, processing conditions as well as the condition of the medium (Poojary et al., 2017).

The PEF unit consists of a treatment chamber, pulse generator, a pulse monitoring system, and a temperature monitor. It can be conducted in two ways—continuous or batch processing. The unit also contains a fluid handling system for liquid foods in continuous PEF processing (Min et al., 2007). The design of the treatment chamber plays a key role in distribution of uniform temperature inside the PEF chamber (Oey, 2010). In PEF, the duration of processing takes substantially lesser time (micro to milli seconds) as compared to other methods (Oey, 2010).

However, in PEF processing, the activity on enzyme may either limit its activation/inactivation or may not be affected at all (Poojary et al., 2017). In this process, multiple factors such as food matrix, pH, enzyme dissolving medium, and certain treatment conditions affect the enzyme treatment in fruits and vegetables (Poojary et al., 2017; Oey, 2010). Two important parameters that affect the intensity of PEF processing are electric field intensity and the total duration of treatment per energy

(Oey, 2010). A higher electric field and treatment duration may result in enzyme inactivation, while a lower electric field and duration may result in enzyme activation (Poojary et al., 2017). Also, increasing pulse width lowers enzyme activity (Poojary et al., 2017).

Furthermore, several studies have been conducted to assess the effect of PEF processing on various food enzymes such as polyphenol oxidase, lipoxygenase, peroxidase, and pectin methyl esterase (Poojary et al., 2017; Aguiló-Aguayo et al., 2009; Noci et al., 2008; Espachs-Barroso et al., 2006) and are given in Table 3.6. The findings from these studies indicate that a longer treatment duration, higher electric field intensity, and pulse width result in enzyme inactivation.

3.6.5 Thermal Processing

In spite of advancement in alternative non-thermal treatments in food processing industry, thermal treatments are still widely considered the most cost-effective and simple method to ensure enzymatic and microbial inactivity in foods (Rawson et al., 2011). However, these treatments have many drawbacks as well, such as slow in conduction and convection of heat transfer, loses in nutritional and functional properties, reduction in sensory attributes, etc. (Baysal & Icier, 2010; Gonzalez & Barrett, 2010). In addition, food matrix, complexity of products, and the microorganism also affect the efficacy of thermal processing (Chen et al., 2013). As a result, optimization of thermal treatment becomes important to preserve the sensory attributes, nutritional quality, and safety of the product.

The common thermal treatments (conventional and non-conventional) employed during processing of fruits and vegetables are High temperature-long time (HTLT), High temperature-short time (HTST), and Ohmic and Microwave heating (Lee et al., 2015). Brief explanations of these treatments are discussed below.

High temperature-long time (HTLT) is a conventional thermal treatment. In this, the processing of juice and beverages is done at temperature 80 °C with >30 s holding time (Miller & Silva, 2012). It inactivates or reduces the enzymatic activities of polyphenoloxidase (PPO), pectin esterase (PE), peroxidase (POD), etc. (Marszałek et al., 2017). During the treatment, it was observed that antioxidant activity and PME enzymatic activity were reduced to 75% and 83% in mombin juice and litchi beverage, respectively (Swami Hulle & Rao, 2016; de Carvalho et al., 2015).

High temperature-short time (HTST) is another conventional thermal treatment used in food processing. It is carried out at temperature ≥80 °C with ≤30 s holding time. Here, priority is given for destruction of microorganism than nutrient degradation (Achir et al., 2016). For example, HTST treatment reduces PME and PPO activity by 95.3% and 90.9% in apple juice, respectively (Aguilar-Rosas et al., 2013). In another study based on apricot nectar, complete inactivation of POD, PPO, and PME was observed (Huang et al., 2013).

Ohmic heating (OH) is a common non-conventional thermal treatment. This treatment is primarily used in fruit juices processing for its effectiveness. Fruit juices

Table 3.6 PEF and effect on enzyme activity

Reference	Fruits/ vegetables	Enzyme	Processing	Enzyme stability after application of HP processing
Aguiló-Aguayo et al. (2009)	Tomato juice	Lipoxygenase	Electric field: 35 kV/cm for 1000, pulse width: 1–7 μs, frequency: 50–250 Hz	An increase in frequency or pulse width reduced enzyme activity
	Strawberry juice	Polygalacturonase	Electric field: 35 kV/cm for 1000 μs, treatment time: 1700 μs, frequency: 100 Hz, pulse width: 4 μs	Enzyme activity inactivated by 26%
	Strawberry juice	PME	Electric field: 35 kV/cm for 1000 μs, treatment time: 1700 μs, frequency: 100 Hz	Enzyme inactivation
Noci et al. (2008)	Apple juice	PPO	Electric field: 40 kV/cm, pulse width: 1 μs, treatment time: 6000 μs, frequency: 15 Hz, no. of pulses: 100	About 50% reduction in enzyme activity
Espachs-Barroso et al. (2006)	Carrot Banana Tomato Orange	PME	Pulses of 13.2–19.1 kV/cm for 40 μs, time: 1.6 ms, frequency: 0.5–5 Hz	PME inactivation—45% banana, 83% carrot, 87% orange and tomato. Enzyme inactivity increased with increased treatment time and electric field and pulses

that contain water and ionic salts in higher amount are found to be more effective with this treatment (Miller & Silva, 2012). In other foods, this process has many advantages like uniform and rapid heating which ultimately helps in nutritional and sensory attributes of the processed products. As per the study by Somavat et al. (2013), *B. coagulans* (ATCC 8038) in tomato juice when treated with OH at 60 Hz and 10 kHz expedites the inactivation process as compared to conventional treatment (Somavat et al., 2013). However, microwave heating is another common non-conventional treatment which is mainly used in urban areas. Even though it is a thermal treatment, minimal thermal exposure is required for enzymatic inactivation (Arjmandi et al., 2016).

3.7 Regulatory Aspects of Food Enzymes Used in Fruit and Vegetable Processing

The regulation of enzymes for its application in food industry is very complex and differs from county to country. The European Union (EU) classifies food enzymes as processing aids of food additives. Majority of the enzyme preparations used in food processing are categorized as processing aids by European Union since they have a role in the technological aspect of food processing stage and not in the final product (Aehle, 2007). The processing aid may not be labelled (Bruchmann & Fauveau, 2009). The enzyme lysozyme used in wine processing is considered as a food additive (Bruchmann & Fauveau, 2009) since it has a role to play in the final product and is regulated under Food Additives Directive (95/2). A vertical legislation of EU on fruit juices (Council Directive 93/77/EEC) allows pectinolytic, proteolytic, and amylolytic enzymes, while another regulation (Council Regulations 82/87/EEC) allows only pectinolytic enzymes on wines (Aehle, 2007). The Organization Internationale de la Vigne et du Vin (OIV) regulates the enzymes used in wine processing in EU (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009; Aehle, 2007) and has recognized the important role of pectinases, hemicellulases, cellulose, β-glucanase, and glycosidase. The European Commission Regulation 1493/1999 authorises the use of pectinases from *Aspergillus niger*, β-glucanase from *Trichoderma harzanium* and urease from *Lactobacillus fermentum*. Pectinases are assigned a GRAS status by United States Food and Drug Administration (Bruchmann & Fauveau, 2009). In India, Food Safety and Standards Authority of India (FSSAI) regulates the use and safety of food enzymes. As per the regulation Food Safety and Standards (Food Products Standards and Food Additives) (Amendment) Regulations, 2015, the food regulator had permitted the use of processing aids in bread. The enzymes permitted for usage are glucose oxidase, lipase, and xylanase. They are obtained from various microbial sources.

In some EU countries, namely Denmark, France, and United Kingdom, approval is required for the use of any food enzymes (Aehle, 2007). However, in countries like Poland, China, Japan, Australia, New Zealand, Canada, Brazil, and Mexico, approval is needed for the use of enzymes produced traditionally, especially if they are new enzymes (Aehle, 2007). On the other hand, countries such as Thailand, Korea, and Taiwan require registration prior to their enzyme use, while USA requires a GRAS (generally regarded as safe) assessment, GRAS notice, or Food additive (Aehle, 2007). Even though countries have different set regulations, enzyme preparations must comply with specifications recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by Food Chemical Codex (FCC) for food enzymes (Bruchmann & Fauveau, 2009; Grassin & Coutel, 2009).

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