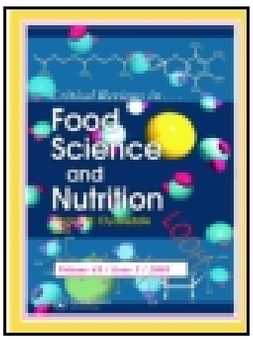

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Enzymatic Extraction and Clarification of Juice from Various Fruits – A Review

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ABSTRACT: Enzymatic treatment for juice extraction is most commonly used now a days. The enzymatic process is claimed to offer a number of advantages over mechanical-thermal comminution of several fruit pulps. Enzymes are an integral component of modern fruit juice manufacturing and are highly suitable for optimizing processes. Their main purposes are: increase extraction of juice from raw material, increase processing efficiency (pressing, solid settling or removal), and generate a final product that is clear and visually attractive. Juice extraction can be done by using various mechanical processes, which may be achieved through diffusion extraction, decanter centrifuge, screw type juice extractor, fruit pulper and by different types of presses. Enzymatic treatment prior to mechanical extraction significantly improves juice

recovery compared to any other extraction process. Enzymatic hydrolysis of the cell walls increases the extraction yield, reducing sugars, soluble dry matter content and galacturonic acid content and titrable acidity of the products. Enzymatic degradation of the biomaterial depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations. We can conclude from the technical literature that use of the enzymes i.e. cellulases, pectinases, amylases and combination of these enzymes can give better juice yield with superior quality of the fruit juice. Pectinase enzyme can give maximum juice yield i.e. 92.4% at 360 minutes incubation time, 37^{o}C incubation temperature and 5 mg/100gm of enzyme concentration. Whereas the combination of two enzymes i.e. pectin methyl esterase (PME) and polygalacturonase (PG) at 120 minutes of incubation time, 50^{o}C of incubation temperature and 0.05mg/100 gm of enzymatic concentration can give the maximum yield of 96.8% for plum fruits. This paper discusses the use of enzymes in fruit juice production focusing on the juice recovery, clarity and effect of the particular enzyme on the biochemical properties of the fruit juices.

KEYWORDS enzymatic treatment, juice extraction, pectinase, juice yield, clarity, enzymatic concentration

1. INTRODUCTION

Fruits and vegetables are important sources of essential dietary nutrients such as vitamins, minerals and fiber. Since the moisture content of fresh fruits and vegetables is more than 80%, they are classified as highly perishable commodities. The world fruit production is about 609,213,509 metric ton in 2010 (FAO STAT, 2010-11). In India, out of the total production of fruits and vegetables, nearly 76 percent is consumed in fresh form, while wastage and losses account for 20-22 percent. Only 4 percent of fruit production are being processed (Indian Horticulture Database, 2013).

Food preservation has an important role in the conservation and better utilization of fruits and vegetables in order to avoid the glut and utilize the surplus during the off-season. It is necessary to employ modern methods to extend storage life for better distribution and also processing techniques to preserve them for utilization in the off-season (Vidhya and Narain, 2011). The fruit can be preserved by converted it in to products like jam, jelly, fruit bar, juice, pickle and murabba to prolong their utilizable lifespan. Fruit juicing is one of the easiest way to preserved fruit.

The production of fruit and vegetable juices is important both from the human health and commercial standpoints. The availability of nutritious components from fruits and vegetables to a wide range of consumers is thus facilitated throughout the year by the marketing of their juices. The production of fruit and vegetable juices requires methods for extraction, clarification and stabilization (Bhat, 2000).

The most traditional method of juice extraction is through the use of presses viz., traditional rack and cloth press, screw presses, Bucher-Guyer horizontal press, and the belt press. Juice extraction can also be done by using diffusion extraction, decanter centrifuge (Beveridge and Rao, 1997), screw type juice extractor, fruit pulper (Lotha and Khurdiya, 1994). The yield of juice using such juice extraction methods can be increased by combining them with various pre-treatments viz., cold, hot and enzymatic extraction (Chadha *et al*., 2003). Enzymatic treatment is one of them, which gives significant increase in juice recovery compare to cold and hot extraction (Joshi *et al*., 1991).

The enzymatic process is claimed to offer a number of advantages over mechanical-thermal comminution of several fruit pulps. In particular, the use of cellulases and pectinases has been an integral part of modern fruit processing technology involving treatment of fruit masses they not only facilitate easy pressing and increase in juice recovery but also ensure the highest possible quality of end products (Kilara, 1982; Roumbouts and Pilnik, 1978). These enzymes not only help in softening the plant tissue but also lead to the release of cell contents that may be recovered with high yield (Sreenath *et al*., 1984).

Clarification is a process by which the semistable emulsion of colloidal plant carbohydrates that support the insoluble cloud material of a freshly pressed juice is “broken” such that the viscosity is dropped and the opacity of the cloudy juice is changed to an open splotchy look. This can be accomplished in one of two general ways: enzymatically and non-enzymatically (Kilara and Van Buren, 1989).

Nonenzymatic clarification involves breaking the emulsion by other means, the most common of which is heat. Other techniques include addition of gelatin, casein, and tannic acid–

protein combinations (Kilara and Van Buren, 1989). Additionally, the uses of honey and combined honey-pectinase treatments have been found to be effective clarification agents. It is believed that the proteinaceous component of honey is responsible for a synergistic effect when honey and pectinase are used in combination (McLellan *et al*., 1985).

Fruit contains pectin and other polysaccharides so it may lead to fouling during filtration through membrane. Enzymatic treatment leads to degradation of pectin. Enzymatically clarified juice resulted in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration. As a result, the juice presents higher clarity, as well as more concentrated flavor and colour (Abdullah *et al*., 2007).

During the early 1930s, when fruit industries began to produce juice, the yields were low, and many difficulties were encountered in filtering the juice to an acceptable clarity (Uhlig, 1998). Subsequently, research on industrially suitable pectinases, cellulases and hemicellulases from food-grade micro-organisms (*Aspergillus niger* and *Trichoderma* sp.), together with increased knowledge on fruit components, helped to overcome these difficulties (Grassin and Fauquembergue, 1996a).

Enzymatic treatment for juice extraction and clarification is most commonly used now a days. Enzymatic hydrolysis of the cell walls increases the extraction yield, reducing sugars, soluble dry matter content and galacturonic acid content and titrable acidity of the products (Joshi *et al.,* 1991). The resultant pulp has a lower viscosity and the quantity of waste pomace is reduced (Dorreich, 1996). Enzymatic degradation of the biomaterial depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations (Baumann, 1981).

Currently, pectinases, cellulases and hemicellulases collectively called macerating enzymes are used for improvement in pressing, extraction and clarification of fruit and vegetable juices (Galante *et al.*, 1998b). In addition, α-amylase and amyloglucosidase, active at acidic pH, were used to process starch containing fruits, especially apples harvested during the early stages in order to prevent haze formation (Grassin and Fauquembergue, 1996a; Uhlig, 1998).

2. ENZYMES

Enzymes are effective protein catalysts for biochemical reactions. The structural components of proteins are L-α-amino acids with the exception of glycine, which is not chiral. The four levels of protein structure are primary, secondary, tertiary, and quaternary structures (Bayindirli, 2010). Primary structure is related to the amino acid sequence. The amino group of one amino acid is joined to the carboxyl group of the next amino acid by covalent bonding, known as a peptide bond. The amino acid side-chain groups vary in terms of their properties such as polarity, charge, and size. The polar amino acid side groups tend to be on the outside of the protein where they interact with water, whereas the hydrophobic groups tend to be in the interior part of the protein. Secondary structure (α-helix, β-pleated sheet, and turns) is important for protein conformation. Right-handed α-helix is a regular arrangement of the polypeptide backbone by hydrogen bonding between the carbonyl oxygen of one residue (i) and the nitrogenous proton of the other residue (i+4). β-pleated sheet is a pleated structure composed of polypeptide chains linked together through interamide hydrogen bonding between adjacent strands of the sheet. Tertiary structure refers to the three dimensional structure of folded protein. Presence of disulfide bridges, hydrogen bonding, ionic bonding, and hydrophobic and van der Waals interactions maintain the protein conformation. Folding the protein brings together amino

acid side groups from different parts of the amino acid sequence of the polypeptide chain to form the enzyme active site that consists of a few amino acid residues and occupies a relatively small portion of the total enzyme volume. The rest of the enzyme is important for the three-dimensional integrity. The quaternary structure of a protein results from the association of two or more polypeptide chains (subunits) (Bayindirli, 2010).

Enzymes are classified into six groups according to the reaction catalyzed and denoted by an EC (Enzyme Commission) number viz., EC1: Oxidoreductases, EC2: Transferases, EC3: Hydrolases, EC4: Lyases, EC5: Isomerase and EC6: Ligases. The first, second, and third–fourth digits of these numbers show class of the enzyme, type of the bond involved in the reaction, and specificity of the bond, respectively. Systematic nomenclature is the addition of the suffix *-ase* to the enzyme-catalyzed reaction with the name of the substrate (Bayindirli, 2010).

2.1 PECTIC SUBSTANCES AND PECTIC ENZYMES

2.1.1 Pectin

Pectins depending on their chemical form are categorized as either soluble or insoluble fibre, which cannot be absorbed by the human digestive tract. However, enzymes are able to modify them to short polysaccharide fragments that may be absorbed. Pectin degradation by enzyme action leads to decrease of raw juice viscosity and, in consequence, increasing of juice yield (Plocharski *et al*., 1998; Voragen, 1992) improving production efficiency.

The pectic substances are classified as galacturonans (polymers of galacturonic acid), rhamnogalacturonans (mixed polymers of rhamnose and galacturonic acid), arabinans (polymers of arabinose), galactans (polymers of galactose) and arabinogalactans (mixed polymers of

arabinose and galactose) (Whitaker, 1984). Pectolytic enzymes can hydrolyze pectic substances present in fruit, so resulting juice has a much lower amount of pectin (Lee *et al.,* 2006). These enzymes, not only help in softening the tissue but also lead to the release of cell contents that recovered with high yield (Sreenath *et al.,*1994).

2.1.2 Pectic enzymes

Pectolytic enzymes are used for the fruit-processing industry to increase yields, improve liquefaction, clarification and filterability of juices, maceration, and extraction of plant tissues, releasing flavour, enzymes, proteins, polysaccharides, starch and agar (Dorrich, 1996; van den Broek *et al*., 1997). Aspergillus niger or Aspergillus aculeatus is used for industrial production of pectolytic enzymes (Naidu and Panda, 1999). The pectic enzymes include pectin lyase, pectin methylesterase, endo and exo-polygalacturonases, pectin acetylesterase, rhamnogalacturonase, endo- and exo-arabinases are used in extraction and clarification of fruits and vegetable juices (Galante *et al.*, 1998b). The fruit and vegetable juice industry uses mainly acidic pectinases of fungal origin, principally from *Aspergillus* spp. Commercial preparations are mixtures of polygalacturonases, pectate lyases and pectin esterases. Pectate lyases can act on the esterified pectin while the polygalacturonases act on the desesterified pectin thus it might require previous action of the pectin esterases. Pectic enzymes treatments vary depending on the type of juice (Sieiro *et al.,* 2012). Biochemical properties of some pectic enzymes shown in table 2.1.2.

2.1.2.1 Pectin methylesterase

Pectin methylesterase (pectin pectylhydrolase, EC 3.1.1.11) is often referred to as pectinesterase, pectase, pectin methoxylase, pectin demethoxylase and pectolipase. The action of pectin methylesterase is to remove the methoxyl groups from methylated pectin substances

(pectin). It is a carboxylic acid esterase and belongs to the hydrolase groups of enzymes. PME de-esterifies the methyl groups on the galacturonic acid backbone of pectin, creating charged regions which complex with Ca^{2+}, forming Ca^{2+} pectate gels which precipitate and clarify the juice (Baker and Bruemmer, 1972). Activity of pectin methylesterase is most readily followed in a pH stat at pH 4-7.5, because of ionization of the carboxyl group of the product to give a proton. Pectin methylesterase from the albedo of oranges has been the best studied (MacDonnell *et al.*, 1950). Action of pectin methylesterase has little effect on viscosity of the pectin-containing solution unless divalent cations are present. In the presence of Ca^{2+}, the viscosity increases due to Ca^{2+} crosslinking of the pectic acid chains (Whitaker, 1984).

2.1.2.2 Polygalacturonases

The polygalacturonases [poly (1, 4-α-D-galacturonide) glycanohydrolase, EC3.2.1.15] hydrolyse the α(l-4) linkages between D-galacturonic acid units. There are four types of polygalacturonases, depending on whether they have a preference for poly[α(1-4)-D-methylgalacturonic acid] (pectin-like substrates) or poly[α(1-4)-D-galacturonic acid] (pectic acid-like substrates) and whether they attack the polymer chain from the end (exo-splitting) or in the interior (endo-splitting). The four types can be distinguished on the basis of substrate requirements, the rate of decrease in viscosity relative to rate of formation of reducing groups and by the nature of the products formed early in the reaction. Polygalacturonases activity is determined on the basis of measuring, during the course of the reaction: (a) the rate of increase in

number of reducing groups; and (b) the decrease in viscosity of the substrate solution (Rexova-Benkova and Markovic, 1976).

2.1.2.3 Pectate lyases

Lyases perform non-hydrolytic breakdown of pectates or pectinates, characterized by a trans-eliminative split of the pectic polymer (Sakai e*t al.*, 1993). The lyases break the glycosidic linkages at C-4 and simultaneously eliminate H from C-5, producing a D 4:5 unsaturated products (Codner 2001; Albersheim *et al.*, 1960). Lyases can be classified into following types on the basis of the pattern of action and the substrate acted upon by them (I) endopolygalacturonate lyase (EndoPGL, E.C. 4.2.2.2); (II) exopolygalacturonate lyase (ExoPGL, E.C. 4.2.2.9); (III) endopolymethylgalacturonate lyase (EndoPMGL, E.C. 4.2.2.10); (IV) exopolymethylgalacturonate lyase (ExoPMGL) (Jayani *et al.*, 2005). Activity of the pectate lyases can be determined by measuring the rate of increase in absorbance at 235 nm due to formation of the double bond. All of the pectate lyases require Ca^{2+}, while the polygalacturonases do not all have this requirement. Ethylenediaminetetraacetic acid (EDTA) is generally an inhibitor of pectate lyase activity, because of chelation of the Ca^{2+} (Whitaker, 1984).

2.2 CELLULOSE AND CELLULASES ENZYME

2.2.1 Cellulose

Cellulose is a crystalline polymer, an unusual feature among biopolymers. Cellulose chains in the crystals are stiffened by inter and intra chain hydrogen bonds and the adjacent sheet which overlie one another are held together by weak Van-der Waals forces. In nature, cellulose is present in a nearly pure state in a few instances whereas in most cases, the cellulose fibers are

embedded in a matrix of other structural biopolymers, primarily hemicelluloses and lignin (Marchessault and Sundararajan, 1993; Lynd *et al*., 1999).

2.2.2 Cellulases Enzyme

Cellulases are defined as a family of enzymes which perform the process of degradation of cellulose into glucose. They are widespread in nature and are particularly common in the world of bacteria and fungi. They are manufactured, among others, by symbiotic bacteria found in multi-compartmental stomachs of ruminants (primarily in the rumen). Most animals, including humans, do not synthesise cellulases and, therefore, are incapable of utilising the entire energy contained in plant material (Kuhls and Lieckfeldt, 1996).

Cellulases enzyme hydrolyze cell wall polysaccharides and substituted celluloses. Cellulases are defined as a family of enzymes which perform the process of degradation of cellulose into glucose. Cellulases is used in extraction and clarification of fruits and vegetable juices for production of nectars and purees, oil extraction from oil seeds, animal feed preparation, Improvement in soaking efficiency, homogeneous water absorption by cereals, the nutritive quality of fermented foods, the rehydrability of dried vegetables and soups, the production of oligosaccharides as functional food ingredients and low-calorie food substituent's and biomass conversion (Beguin and Aubert, 1994; Bhat, 1997). Cellulases are also used in carotenoid extraction in the production of food coloring agents. Fungi including Aspergillus niger, Aspergillus nidulans, Aspergillus oryzae are used for production of microbial cellulases (Sukumaran *et al.*, 2005).

The term cellulase actually includes three enzymes that produce glucose from hydrolyzing cellulose (Clarke, 1996) such as endo-β 1,4-glucanases (EG; EC. 3.2.1.4), exo-β-

1,4-cellobiohydrolases (CBH; EC. 3.2.1.91), and β-glucosidases (BG; EC. 3.2.1.21) (Schulein, 1988). The complete cellulase set including CBH, EG, and BG components synergistically functions to convert crystalline cellulose to glucose. EG and CBH act together to hydrolyze cellulose to small cello-oligosaccharides. The oligosaccharides (mostly cellobiose) are next hydrolyzed to glucose by a core β-glucosidase (Sukumaran *et al.*, 2005).

2.3 OTHER ENZYMES

2.3.1 Hemicellulases

Hemicellulases including endo- and exo-xylanases, galactanases, xyloglucanases and mannanases. Hemicellulases are a diverse group of enzymes that hydrolyze hemicelluloses, one of the most abundant groups of polysaccharide in nature. Xylanases (EC 3.2.1.8) hydrolyze the ß-1,4 bond in the xylan backbone, yielding short xylooligomers. ß-Mannanases (EC 3.2.1.78) hydrolyze mannan-based hemicelluloses and liberate short ß-1, 4-manno-oligomers, which can be further hydrolyzed to mannose by ß-mannosidases (EC 3.2.1.25) (Shallom and Shoham, 2003).

2.3.2 Amylase

Amylase is an enzyme that catalyses the breakdown of starch into sugars. Amylase is present in human saliva, where it begins the chemical process of digestion. Amylase can be derived from bacteria and fungi. All amylases are glycoside hydrolyses and act on α-1,4-glycosidic bonds. Amylases are extensively employed in processed-food industry such as baking, brewing, preparation of digestive aids, production of cakes, fruit juices and starch syrups. Amylase used clarification of fruit juice (Couto and Sanroman, 2006)

Macerating enzymes are generally used in two steps: (1) after crushing, to macerate the fruit pulp either to partial or complete liquifaction, which not only increases the juice yield and reduces the processing time, but also improves the extraction of valuable fruit components, and (2) after the juice extraction, whereby pectinases are used for its clarification, thereby lowering the viscosity of fruit juice prior to concentration and increasing the filtration rate and stability of the final product(Bhat, 2000).

3 ENZYMATIC EXTRACTIONS AND JUICE RECOVERY

3.1 Fruit preparation prior to enzymatic extraction of juice

Fruit is first washed, cut into small pieces and then pretreatments like steaming, cooling or heating prior to enzymatic extraction increased juice recovery (Trappey *et al.,* 2008). Water is added to pulp in difference ratios. The greater degree of tissue breakdown from freezing and thawing of whole fruit coupled with a pectinase enzyme treatment of fruit macerate yield higher solids which agrees with other research reports (Pilnik *et al.,* 1975; McLellan *et al.,* 1985). Hot water extraction with addition of enzyme in apple pomace with a combination of pectinases and cellulases results in higher yield. Al-Hooti *et al.,* 2002, blended date fruit pulp with three times the water before the addition of enzyme for extraction of juice.

3.2 Juice Recovery

Extraction of juice using macerating enzymes claimed to increased juice recovery from various fruits. However, the enzymatic process should be optimized with respect to incubation temperature, time and enzymatic concentration to maximized yield and quantity of various fruit

juices. Table 3.2.1 shows the optimized condition to maximized juice yield from various fruits. In case of bael fruit enzymatic extraction results in 17.5% increased in juice yield from untreated sample at enzymatic concentration 20mg/100g pulp, incubation time 425min. and temperature 47^0C (Singh *et al.,* 2012). Similar Yusof and Ibrahim, 1994, found that the larger the amount of enzyme used and the longer the time of incubation, the greater the yield of juice obtained. They found 41% increase in juice recovery with enzymatic treatment then untreated sample of soursop. The enzyme treatment of plum, peach, pear and apricot have shown clearly that the juice yield increased from 52% (plum), 38% (peach), 60% (pear) and 50% (apricot) to 78% (plum), 63% (peach), 72% (pear) and 80% (apricot), respectively (Joshi *et al.,* 2011). A concentration of 0.5% purified enzyme (pectinol) was found optimum to increase juice yield of plum, peach and apricot (Joshi *et al.,* 1991). Enzymatic concentration of 2% for 2 h at 50^0C resulted in a serum yield of 65% in mango pulp (Gupta and Girish, 1988). Upon enzyme treatment, degradation of pectin lead to reduction in water holding capacity of pectin so free water is release in system hence juice yield increases (kashyap *et al.,* 2001; Lee *et al.,* 2006). The increase in juice yield is attributed to the hydrolysis of pectin thus, releasing the sap inside the cells of the pulp (Broeck *et al.,* 1999). However, the increase varied in different fruits owing to amount of pectin present and the activity of enzymes. The yield of mixed juice and puree from pomace obtained in the enzymatic processing of apples ranged from 92.3% to 95.3%, and increased significantly when compared to the control without the enzymatic pomace treatment (81.8%). These yields are much higher than the values determined in our previous research (Oszmian´ ski *et al.,* 2009). Apple juice can be obtained through a two-step process consisting of a first treatment of the crushed apple mush with pectinases to obtain the premium juice followed

by pomace liquefaction treatment made with a mixture of different pectinases and cellulases for the complete extraction of the juice (Will *et al.*, 2000).

Juice recovery % = Weight of juice / Weight of fruit

Different enzymes in combination claim to increase juice recovery, TSS, clarity, and decreases viscosity and turbidity. Many modern processes of fruit and vegetable juice production frequently employ pectinases, but mixtures of cellulytic and pectolytic enzymes are finding wide application to enhance pulp liquefaction and provide a higher yield of juice with high soluble solids content. Pectinolytic and cellulolytic enzymes are used for the fruit processing industry to increase the extraction yield, reducing sugars, soluble dry matter and titrable acidity of the products from some fruits such as peaches, plums and apricots (Joshi *et al.*, 1991). From table 3.2.2 it is observed by using pectinase, cellulase and amylase in various combination for juice extraction from kiwi fruit that the enzymatic treatments in combinations significantly increases juice yield. The best results were found in combination of Pectinase (0.05 g/kg), amylase (0.025 g/kg) and cellulase (0.025 g/kg) with juice yield of 78.46% compared to 58.44% of control sample. Pectinase and cellulase treatment in combination at 1:1 ratio at 0.025% concentration resulted in juice recovery of 74.75% from pineapple (Sreenath *et al.*, 1994).

4. ENZYMATIC CLARIFICATION AND CLARITY

Fruit juices are naturally cloudy, yet in different degrees, especially due to presence of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (Vaillant *et al*., 2001). As the juice clear appearance is a determinant factor for consumers, the fruit juice industry has been investing in methods that optimize this feature (Tribess and Tadini, 2006). The high concentration of pectin leads to colloid formation, which constitutes one of the main problems during the processing of clear fruit juices. However, although the suspended pulp particles can be removed through filtration, the presence of pectin may make this method difficult (Sulaiman *et al*., 1998). The depectinisation of fruit juices through the use of pectinases has been presented as an efficient alternative to reduce turbidity, in many studies (Kashyap *et al*., 2001; Landbo and Meyer, 2007). Pectinases degrade pectin hence resulting in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration. As a result, the juice presents higher clarity, as well as more concentrated flavour and colour (Abdullah *et al*., 2007; Kaur *et al*., 2004). Pectinase enzymes used in grape juice macerate increased the juice clarity and filterability by 100% according to Brown and Ough (1981). For clarified fruit juices, a juice that has an unstable cloud or whose turbidity is considered ‘‘muddy’’ is unacceptable to be marketed as clear juices (Floribeth *et al*., 1981).

4.1 Clarity

Enzymatic treatment leads to increase the clarity of juice. Juice clarity can be determined in terms of absorbance and transmittance at 660 nm using UV visible spectrophotometer.

Increase in enzymatic concentration increase the rate of clarification by exposing part of the positively charged protein beneath thus reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and eventually settled out (Sin et al., 2006). Clarity showed the lowest absorbance values at highest enzyme concentration, where lower absorbance indicates a clearer juice is being produced. It was also observed that the absorbance values decreased with increasing incubation time at fixed temperature. In general, the time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara, 1982). At the lowest level of temperature, the clarity of banana juice was found to increase rapidly at the beginning but with a slower rate towards the end, with an increase in enzyme concentration. The temperature increases the rate of enzymatic reactions, hence the rate of clarification, as long as the temperature is below denaturation temperature for the enzyme. A similar behaviour for the clarity was observed for the changes in incubation time in case of banana (Lee *et al.,* 2006). The clarity of centrifuged litchi juice increased with an increase in enzyme concentration. Among the different concentrations used for the optimization of pectinase, the litchi pulp added with 500 ppm of pectinase resulted in maximum transmittance of 80% at 660 nm. The clarity of mosambi juice decreases with time up to 90 min and increases thereafter. Similarly at constant time and temperature, the clarity decreases with enzyme concentration and remains constant and increases thereafter. From both the observations, it is evident that there exists an optimum enzyme concentration and time for the juice clarity (Rai *et al.,* 2003).

5. EFFECT OF ENZYMATIC TREATMENT ON PHYSICO-CHEMICAL PROPERTIES OF JUICE

5.1 Effect of Enzymatic treatment on Total Soluble Solids (TSS) of juice

Enzymatic extraction also increases TSS of juice from various fruits. The °Brix value can be determined by measuring the refractive index with a hand held refractometer. TSS of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in table 5.1.1 Yusof and Ibrahim, 1994 found that the use of enzyme for soursop at various enzyme levels significantly increased the soluble solids content from 6.8°Brix to 7.3°Brix within the first hour of incubation. Increasing the incubation time to 2 and 3 h did not cause any significant increase in the total soluble solids content. Brix/acid ratio is decrease from 16.6 to 14.9 (Yusof and Ibrahim, 1994). Pectinase treated apricot, pear, mayhaw, banana had a larger brix levels as compared to untreated juices (Joshi *et al.,* 2011; Trappey *et al.*, 2008; Shahadan and Abdullah, 1995). The use of various enzymes in different combination increases TSS content of juice. Sreenath used pectinase and cellulases enzymes for extraction of pineapple juice at enzymatic concentration of 0.025%. The TSS of the final pooled juice was around 12°Brix. Similarly for carrot, pectinase and cellulases at concentration 2% in (3:2) ratio increase yield of final juice TSS. The increase in TSS is related to greater degree of tissue breakdown, releasing more compounds such as sugars (Chang Tung *et al.,* 1995), which contribute to soluble solids.

5.2 Effect of Enzymatic treatment on Viscosity of juice

The use of enzymes leads to the drop of fruit juice viscosity and disintegrating the jelly structure and making it easier to obtain the fruit juices (Singh *et al.,* 2012). Viscosity can be measured by using a Brookfield viscometer. Viscosity of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in table 5.2.1. The viscosity of the

juice after enzyme treatment had generally decreased. This was also noted in many of the studies reported earlier and is due to the hydrolytic action of enzymes on the cellulosic and pectic materials present in the juice. Therefore to enhance filtration process performance, fruit juices are usually pretreated with enzyme, before filtration, for the purpose of hydrolysing soluble polysaccharides responsible for high viscosity (Cheryan & Alvarez, 1995). Viscosity was significantly reduced with higher enzyme concentration. Incubation time also affected the viscosity at linear terms with a negative effect but to a lesser extent. Incubation time showed a maximum viscosity at 90 min but reduced as the incubation time increased in case of sapodilla juice (Sin *et al.,* 2006). The higher viscosity was observed to affect the rheological properties of the products. Drinkability was reduced, and the samples had more characteristics of a puree than of a beverage. The viscosity of typical cloudy juices has been reported to range between 95 and 134 mPas (Will *et al.,* 2008). The viscosity of the control apple juice was 397 mPas; whereas the viscosity of the samples treated with enzymes ranged from 122.4 (Pectinex Smash XXL) to 291.5 mPas (Pectinex Yield Mash). Abdullah *et al.,* 2007 also reported reduction in viscosity of carambola juice with 0.1% enzyme concentration for 20min at 30^0C incubation temperature. The use of various enzymes in combination also tends to reduce the viscosity of juice. Anastasakis *et al.,* 1987 given enzymatic treatment of pectinase and cellulase in combination at 2% in 3:2 ratios to carrot, he found that the enzymatic treatment of carrot in combination has no significant difference in viscosity compare to only pectinase treatment and has much higher viscosity compare to cellulase treatment.

5.3 Effect of Enzymatic treatment on pH of juice

The pH value of juice decreases with increase in enzyme concentration (Joshi *et al.*, 2011). Results of pectinase treatments on pH shown in table 5.3.1. pH can be measured by digital pH meter. Yusof and Ibrahim, 1994 found that for each level of enzyme used, pH decrease, as a result of incubation time, was not significant for the first hour of incubation. But after 2 h and 3 h of treatment pH values decrease significantly from the pH of the original juice. Nevertheless, the values for 2 and 3 h incubation are almost the same. According to Woodroof and Phillips (1981) a decrease in pH from 4.5 to 3.0 could increase the shelf life about 3 times. Similarly significant decrease in pH was observed in case of date (variety *Deglet Nour, Allig* and *Kentichi*) syrup (Abbes *et al.*, 2011) and carrot (Anastasakis, 1987). Results of pH using enzymatic treatment in combination at optimized condition shown in table 5.3.1.

5.4 Effect of Enzymatic treatment on Ascorbic Acid content of juice.

The ascorbic acid content of clarified juice decreased to 11.8 mg/100 g as compared to that of litchi pulp (17.6 mg/100 g), which could be due to the oxidation of ascorbic acid during the clarification. Ascorbic acid was determined using the 2,6-dichlorophenol indophenol dye titration method (Ranggana, 1977). The effects of enzyme concentration and time of incubation on the ascorbic acid is shown in Table 5.4.1. The enzyme treatment did not seem to increase the ascorbic acid content significantly for soursop juice. Joshi *et al.,* 2011 found that the ascorbic acid remain unaffected with increase in enzyme concentration. The ascorbic acid was found to decrease about 21% after an enzyme treatment. The reduction of 16.9-20.7% ascorbic acid occurs during enzymatic clarification of various juices (Singh *et al.,* 1993).

5.5 Effect of Enzymatic treatment on turbidity of juice

In case of elderberry it was observed very clearly from the turbidity data that ranged from 120–161 FNU with enzyme addition, and thus on average turbidity was 30% lower than those of samples produced without enzyme addition that had turbidity levels ranging between 191–212 FNU (Landbo *et al.,* 2007). Since the turbidity in the juices may be due to pectin and other plant cell wall substances released during the enzymatic prepress maceration, it seems logic that elevated turbidities may transiently result during enzyme catalyzed cell wall degradation, which can partly explain the positive effect coefficient of the enzyme dosage on the turbidity. Turbidity in fruit juices can be a positive or a negative attribute depending on the expectation of the consumers (Hutchings, 1999). In the case of orange and tomato juices, the juices are usually cloudy and have colloidal suspensions. However, this cloud is desirable and acceptable by the consumers. Turbidity of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in table 5.5.1. Increase in enzyme concentration and incubation time might decrease turbidity. Pectin was the main cause of turbidity (Grassin and Fauquembergue, 1996a). As the clarification process took place, the amount of pectin in the juices decreased, therefore reducing the turbidity of the juices (Alvarez *et al*., 1998).

5.6 Effect of Enzymatic treatment on Titrable Acidity of Juice.

Titrable acidity was measured by titration of juice with 0.1N NaOH. Yusof and Ibrahim, 1994 found that the total titratable acidity for enzymatically extracted juice increased significantly from 0.41% to 0.49% for the 1, 2 and 3 h of incubation at the 0.025% enzyme concentration but not at 0.05%, 0.075% and 0.1% concentrations. The acidity values at the latter three concentration levels were almost the same for the three incubation times. Titrable acidity of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in

table 5.6.1. While increase in acidity (as citric acid) of date syrup was observed after the extraction using enzyme. This was explained by the addition of citric acid during enzymatic extraction and liberation of galacturonic acid inducted by pectinase adjunction.

5.7 Effect of Enzymatic treatment on Anthocyanin content of juice.

Total anthocyanins were determined by the pH differential method and anthocyanin concentrations in black currant juice were calculated as cyanidin-3-rutinoside equivalents (Wrolstad, 1976). Anthocyanins are located mainly in the skin of the fruit and during juice pressing it is important to transfer into the juice (Mieszczakowska-Frac *et al.*, 2012). The obtained extraction yields of anthocyanins in the 250 different samples ranged from 900 to 2200 mg/kg wet weight black currant mash equivalent to a span of concentrations of anthocyanins in the juices of 1340–3220 mg/l juice. The anthocyanins yields for blackcurrant juice tended to increase with increased enzyme dosage and increased maceration temperature, but the effects of these parameters as well as the influence of the maceration time varied depending on the enzyme preparation used for the maceration (Landbo and Meyer, 2004). Anthocyanin content of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in table 5.7.1. Pectinase treatment increased release of anthocyanins than the other enzyme treatments in white grape juice. Treatment of raspberry juices with pectolytic enzymes modified the level of individual pigment and the total anthocyanins content varied accordingly. The pectolytic enzymes showed a stationary high level of total anthocyanins over the time (range: 289-306 mg l^{-1}). On the other hand, it was clear a decrease of total anthocyanins, after 6 h. The pectolytic

enzymes showed higher anthocyanins hydrolytic activity in raspberry then in strawberry juices. Anthocyanins yields increased with increased maceration temperature and increased enzyme dose in elderberry juice, while no effect of increased maceration time on anthocyanins was found.

5.8 Effect of Enzymatic treatment on Total Phenols of juice.

Increased enzyme dosage and maceration time together with increased maceration temperature in general increased the total phenols yields, while Landbo *et al.*, 2007 found that the total phenols yields increased with increased maceration temperature, but increased enzyme dose and increased maceration time has unaffected total phenols yield. Total phenols in the juices were determined by the Folin-Ciocalteu procedure with total phenols expressed as mg/l gallic acid equivalents (GAE) (Landbo and Meyer, 2004). Total Phenols at optimized condition for enzymatic treatment of various fruits and vegetable shown in table 5.8.1.

6. CONCLUSION

The use of cellulases and pectinases has been an integral part of the modern fruit processing technology involving treatment of fruit masses. Enzymes in combination claim to increase juice recovery, TSS, clarity and decrease viscosity and turbidity. Cellulytic and pectolytic enzymes mixtures are having wide application to enhance pulp liquefaction and provide a higher yield of juice with high soluble solids content.

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Table 2.1.2: Biochemical properties of some Pectic Enzymes

Microorganism	Enzyme	Optimal pH	Optimal temperature(^{0}C)	References
Bacteria				
Bacillus sp NT-33	Polygalacturonase	10.5	75	Cao *et al.,* 1992
Bacillus sp DT7	Pectin lyase	8	60	Kashyap *et al.*, 2000
Fungi				
Aspergillus niger	Pectinesterase	3.5	45-55	Landbo *et al.*, 2007
Aspergillus ficuum	Pectin lyase	5	50	Yadav *et al.*, 2008
Penicillium frequentans	Endopolygalacturonase	3.5-5	50	Borin *et al.*, 1996
Sclerotium rolfsii	Endopolygalacturonase	3.5	55	Chane & Shewal, 1995
Penicillium paxilli	Pectin lyase	5	35	Szajer & Szajer, 1982
Yeasts				
Saccharomyce cerevisiae	Endopolygalacturonase	5.5	45	Blanco *et al.*, 1994
Kluyveromyces Marxianus	Endopolygalacturonase	4.5	55	Serrat *et al.*, 2002

Table 3.2.1: Optimized conditions for extraction of maximum juice using Pectinase enzyme

Fruit/ Vegetable	Incubation time[a]	Incubation temperatur[b]	Enzyme concentration[c]	Juice Recovery[d]	Referance
Bael (Aegle marmelos correa)	425	47	20 mg/100gm	86.6	Singh *et al.*, 2012
Guava (*Psidium guajava* L.)	436.2	43.3	0.70 mg/100gm	62.2	Kaur *et al.*, 2009
Elderberry *(Sambucus nigra L)*	50	60	0.34 mg/100gm	77.0	Landbo *et al.*, 2007
Tamarind (Variety *Ajanta*)	360	37	5 mg/100gm	92.4	Joshi *et al.*, 2012
Mayhaw (*Crataegus opaca* Hook.)	60	32	0.20%	75.7	Trappey *et al.*, 2008
Plum (variety *Titrone*)	300	45	0.5%	82	Chauhan *et al.*, 2001
Mango (variety *Amrapali*)	360	45	0.9%	59	Chauhan *et al.*, 2001
Mango	120	50	2%	65	Gupta and Girish, 1988
Apricot (variety *Charmagz*)	300	45	0.5%	78	Chauhan *et al.*, 2001

Pear	240	40	2.5%	72	Joshi *et al.*,2011
Black currant (*Ribes nigrum*)	30	60	0.18%	66-78	Landbo and Meyer, 2004
Banana *(Musa sapientum* cv Berangan)	240	44	0.4%	69.4	Shahadan and Abdullah, 1995
Soursop *(Annona muricata* L.)	180	35-40	0.05%	67.2	Yusof and Ibrahim, 1994
Apricot	240	40	2.5%	80	Joshi *et al.*,2011
Pineapple	30	40	0.02%	63-64	Dzogbefia et al., 2001
Date (*Phoenix dactylifera L.*)	300	50	50U	72.25	Abbes *et al.*, 2011

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in mg/100g : Milligram per 100 gram of pulp, % : Percentage on pulp basis, U : Enzyme Unit, [d] Juice recovery in Percentage (%)

Table 3.2.2: Optimized conditions for extraction of maximum juice using enzymes in combination

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation temperatur[c]	Enzyme concentration[d]	Juice Recovery[e]	Referance
Date (*Phoenix dactylifera* L.) variety Deglet Nour	Pectinase and Cellulase	120	50	50U pectinase / 5U cellulase	72.37	Abbes *et al.*, 2011
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and Cellulase	120	50	0.05, 0.025 and 0.025 g/kg, respectively	78.46	Vaidya *et al.*, 2009
Blackcurrant	PME and PG	120	50	0.2 g/kg (2:1)	59	Mieszczakowska-Frac *et al*., 2012
Pineapple	Pectinase and cellulase	30	27-30	0.025% (1:1)	74.75	Sreenath *et al*., 1994
Carrots *(Daucus carrota)*	Pectinase and cellulase	30	50	2% (3:2)	73.5	Anastasakis *et al*., 1987
Date (Variety *Birhi* and *safri*)	Pectinase and cellulase	60-300	40	1% (1:1)	67.5 and 68.22, respectively	Al-Hooti *et al.*,2002

Plum	PME and PG	120	50	0.05g/kg (2:1)	96.8	Mieszczakowska-Frac, 2012

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in g/kg :Gram per kilogram of pulp, % : Percentage on pulp basis, U : Enzyme Unit, [e]Juice recovery in Percentage (%)

Table 4: Optimized conditions for clarification of various fruit juices using Pectinase

Fruit/ Vegetable	Incubation time[a]	Incubation temperature[b]	Enzyme concentration[c]	Clarity[d]	Referance
Banana (*Musa sapientum* cv Berangan)	80	43.2	0.084%	0.009 Abs	Lee *et al.,* 2006
Carambola (*Carambola Averrhoa* L.)	20	30	0.10%	0.019 Abs	Abdullah *et al.*,2007
White Grape *(Vitis vinifera)*	30	27-30	0.048%	0.031 Abs	Sreenath and Santhanam, 1992
Sapodilla (*Achras sapota*)	120	40	0.1%	0.023 Abs	Sin *et al.,* 2006
Mosambi (*Citrus sinensis* (L.) Osbeck)	99.27	41.89	0.0004 w/v%	83.97% T	Rai *et al.,* 2003
Lichi (*Litchi chinensis* L)	120	40	500ppm	80% T	Vijayanand *et al.,* 2010

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in [a] w/v% : Weight per volume, ppm: parts per million, % : Percentage on pulp basis, [d]Clarity in Abs: Absorbance, T: Transmittance.

Table 5.1.1: Effect of Incubation time, Temperature and Enzymatic concentration on TSS at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	TSS[e]	Referances
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.05%	7.30	Yusof and Ibrahim, 1994
Apricot	Pectinase	240	40	2.5%	10.07	Joshi *et al.*,2011
Pear	Pectinase	240	40	2.5%	11.16	Joshi *et al.*,2011
Mayhaw (*Crataegus opaca* Hook.)	Pectinase	60	32	0.20%	8.13	Trappey *et al.*, 2008
Banana *(Musa sapientum* cv Berangan)	Pectinase	240	44	0.4%	26.1	Shahadan and Abdullah, 1995
White Grape *(Vitis vinifera)*	Pectinase	30	27-30	0.048%	13	Sreenath and Santhanam, 1992
Lichi (*Litchi*	Pectinase	120	40	500ppm	16.4	Vijayanand *et*

chinensis L)						*al.*, 2010
Blackcurrant	PME and PG	120	50	0.2g/kg (2:1)	18-19	Mieszczakowska-Frac, *et al.*, 2012
Plum	PME and PG	120	50	0.05g/kg (2:1)	16.55	Mieszczakowska-Frac, *et al.*, 2012
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg, respectively	14.75	Vaidya *et al.*, 2009
Carrots *(Daucus carrota)*	Pectinase and cellulase	30	50	2% (3:2)	12.0	Anastasakis, *et al.*, 1987
Pineapple	Pectinase and cellulase	30	27-30	0.025% (1:1)	15.0	Sreenath, *et al.*, 1994

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b]Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in % : Percentage on pulp basis, ppm: parts per million, g/kg: gram per kilogram of fruit/pulp [e]TSS: Total Soluble Solids in ^{0}Bx: Degree Brix.

Table 5.2.1: Effect of Incubation time, Temperature and Enzymatic concentration on Viscosity at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes	Incubation time[a]	Incubation Temperature[b]	Enzyme Concentration[c]	Viscosity[d]	Referances
Bael (Aegle marmelos correa)	Pectinase	210	35	24 mg/100gm	1.35	Singh *et al.*, 2012
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.05%	4.68	Yusof and Ibrahim, 1994
Apricot	Pectinase	240	40	2.5%	1.11	Joshi *et al.*,2011
Pear	Pectinase	240	40	2.5%	1.17	Joshi *et al.*,2011
Banana *(Musa sapientum* cv Berangan)	Pectinase	240	44	0.4%	14.2	Shahadan and Abdullah, 1995
White Grape *(Vitis vinifera)*	Pectinase	30	27-30	0.048%	1.05	Sreenath and Santhanam, 1992

Banana (*Musa sapientum* cv Berangan)	Pectinase	80	43.2	0.084%	1.89	Lee *et al.*, 2006
Sapodilla (*Achras sapota*)	Pectinase	120	40	0.1%	1.37	Sin *et al.*, 2006
Carambola (*Carambola Averrhoa* L.)	Pectinase	20	30	0.1%	1.33	Abdullah *et al.*,2007
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg, respectively	5.43	Vaidya *et al.*, 2009
Date (Variety *Birhi* and *safri*)	Pectinase and cellulase	60-300	40	1% (1:1)	17.6 (*Birhi*) 14.8 (*safri*)	Al-Hooti *et al.*,2002
Carrots *(Daucus carrota)*	Pectinase and cellulase	30	50	2% (3:2)	2.75	Anastasakis *et al.*, 1987

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in % : Percentage on pulp basis, mg/100gm: milligram per 100 gram of fruit/pulp. [d] Viscosity in cps: centipoises.

Table 5.3.1: Effect of Incubation time, Temperature and Enzymatic concentration on pH at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes	Incubation time[a]	Incubation Temperature[b]	Enzyme Concentration[c]	pH	Referances
Apricot	Pectinase	240	40	2.5%	3.50	Joshi *et al.*,2011
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.05%	3.54-3.7	Yusof and Ibrahim, 1994
Pear	Pectinase	240	40	2.5%	3.46	Joshi *et al.*,2011
Mayhaw (*Crataegus opaca* Hook.)	Pectinase	60	32	0.20%	3.03	Trappey *et al.*, 2008
Banana *(Musa sapientum* cv Berangan)	Pectinase	240	44	0.4%	3.41	Shahadan and Abdullah, 1995
Sapodilla (*Achras sapota*)	Pectinase	120	40	0.1%	4.6	Sin *et al.*, 2006
Mosambi (*Citrus sinensis* (L.)	Pectinase	99.27	41.89	0.0004 w/v%	3.6	Rai *et al.*, 2003

Osbeck)						
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg, respectively	3.50	Vaidya *et al.*, 2009
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour, Allig & Kentichi*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase	3.2, 3.12 & 3.07	Abbes *et al.*, 2011
Date (Variety *Birhi* and *safri*)	Pectinase and cellulase	60-300	40	1% (1:1)	4.09 & 4.11	Al-Hooti *et al.*,2002
Carrots *(Daucus carrota)*	Pectinase and cellulase	30	50	2% (3:2)	5.44	Anastasakis, 1987

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in [a] w/v% : Weight per volume, g/kg : gram per kilogram of fruit/pulp, % : Percentage on pulp basis, U : Enzyme Unit.

Table 5.4.1: Effect of Incubation time, Temperature and Enzymatic concentration on Ascorbic acid at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	Ascorbic acid[e]	Referances
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.05%	1.14	Yusof and Ibrahim, 1994
Apricot	Pectinase	240	40	2.5%	5.55	Joshi *et al.*,2011
Pear	Pectinase	240	40	2.5%	1.60	Joshi *et al.*,2011
Black currant (*Ribes nigrum*)	Pectinase	30	60	0.18%	118.8	Landbo and Meyer, 2004
Lichi (*Litchi chinensis* L)	Pectinase	120	40	500ppm	11.8	Vijayanand *et al.*, 2010
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg, respectively	154.59	Vaidya *et al.*, 2009
Blackcurrant	PME and PG	120	50	0.2g/kg (2:1)	279.4	Mieszczakowska-Frac, *et al.*, 2012

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in % : Percentage on pulp basis, ppm: parts per million. [e] Ascorbic acid in mg/100g: milligram per 100 gram of fruit/pulp, mg/100g: milligram per 100 milliliter of juice.

Table 5.5.1: Effect of Incubation time, Temperature and Enzymatic concentration on Turbidity at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	Turbidity[e]	Referances
Elderberry *(Sambucus nigra L)*	Pectinase	50	60	0.34 mg/100gm	154 FNU	Landbo *et al.*, 2007
Banana (*Musa sapientum* cv Berangan)	Pectinase	80	43.2	0.084%	3.62 NTU	Lee *et al.*, 2006
Sapodilla (*Achras sapota*)	Pectinase	120	40	0.1%	16.44 NTU	Sin *et al.*, 2006
Carambola (*Carambola Averrhoa* L.)	Pectinase	20	30	0.10%	20.30 NTU	Abdullah *et al.*, 2007
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase	186.45 NTU	Abbes *et al.*, 2011
Plum	PME and PG	120	50	0.05g/kg (2:1)	590 NTU	Mieszczakowska-Frac, 2012

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in % : Percentage on pulp basis,

mg/100g: milligram per 100 gram of fruit/pulp. [e] Turbidity in FNU: Formazin Nephelometric Units NTU: Nephelometric Turbidity Units.

Table 5.6.1: Effect of Incubation time, Temperature and Enzymatic concentration on Titrable acidity at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	Titrable Acidity[e]	Referances
Mayhaw (*Crataegus opaca* Hook.)	Pectinase	60	32	0.20%	1.24	Trappey *et al.*, 2008
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.025%	0.48	Yusof and Ibrahim, 1994
Pineapple	Pectinase and cellulase	30	27-30	0.025% (1:1)	1.152	Sreenath, *et al.*, 1994
Blackcurrant	PME and PG	120	50	0.2g/kg (2:1)	4.06	Mieszczakowska-Frac, *et al.*, 2012
Plum	PME and PG	120	50	0.05g/kg (2:1)	1.06	Mieszczakowska-Frac *et al.*, 2012
Kiwi (*Actinidia*	Pectinase,	120	50	0.06, 0.025 and	1.20	Vaidya *et al.*,

deliciosa)	Amylase and cellulase			0.025 g/kg, respectively		2009
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour, Allig & Kentichi*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase	1.25, 1.22 & 1.29	Abbes *et al.*, 2011

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in g/kg : gram per kilogram of fruit/pulp, % : Percentage on pulp basis, g/kg: gram per kilogram of fruit/pulp, U : Enzyme Unit. [e] Titrable acidity in %.

Table 5.7.1: Effect of Incubation time, Temperature and Enzymatic concentration on Anthocynin at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	Anthocynin[e]	Referances
Elderberry *(Sambucus nigra L)*	Pectinase	50	60	0.34 mg/100g	2.4 mg/g	Landbo *et al.,* 2007
Strawberry	Pectinase	120	45	30 g/100kg	323 mg/l	Versari *et al.,* 1997
Raspberry	Pectinase	120	45	30 g/100kg	457 mg/l	Versari *et al.,* 1997
White Grape *(Vitis vinifera)*	Pectinase	30	27-30	0.048%	2.8 mg/l	Sreenath and Santhanam, 1992
Black currant (*Ribes nigrum*)	Pectinase	30	60	0.18%	1.5–2.2 mg/g	Landbo and Meyer, 2004
Plum	PME and PG	120	50	0.05g/kg (2:1)	13.64 mg/100 ml	Mieszczakowska-Frac, 2012
Blackcurrant	PME and PG	120	50	0.2g/kg (2:1)	239.6 mg/100 ml	Mieszczakowska-Frac, *et al.,* 2012

[a] PME: Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in mg/100kg : milligram per 100 kilogram of fruit/pulp, g/100kg: gram per 100kilogram of fruit/pulp, % : Percentage on pulp basis, [e] Anthocynin in mg/g: milligram per gram of fruit/pulp, mg/l: milligram per liter of pulp, mg/100ml: milligram per 100 gram of juice.

Table 5.8.1: Effect of Incubation time, Temperature and Enzymatic concentration on Total phenols at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes	Incubation time[a]	Incubation Temperature[b]	Enzyme Concentration[c]	Total phenols[d]	Referances
Black currant (*Ribes nigrum*)	Pectinase	30	60	0.18%	3.1–4.4 mg/g	Landbo and Meyer, 2004
Elderberry *(Sambucus nigra L)*	Pectinase	50	60	0.34 mg/100g	6.0 mg/g	Landbo *et al.*, 2007
White Grape *(Vitis vinifera)*	Pectinase	30	27-30	0.048%	440 mg/l	Sreenath and Santhanam, 1992
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour, Allig & Kentichi*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase	326.84, 292.34 & 304.28 mg/100 gm respectively	Abbes *et al.*, 2011
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg	240 mg/l	Vaidya *et al.*, 2009

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in mg/100g : milligram per 100 gram of fruit/pulp, % : Percentage on pulp basis, [d] Total phenols in mg/g: milligram per gram of fruit/pulp, mg/l: milligram per liter of pulp, mg/100 gm: milligram per 100 gram of fruit/pulp.

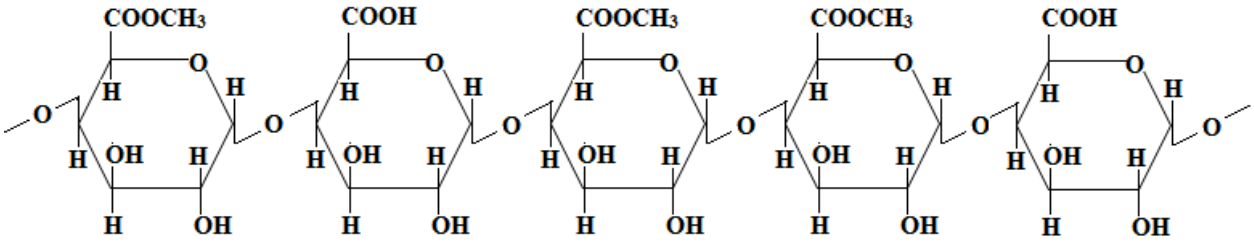

Fig. 2.1.1: Structure of pectin

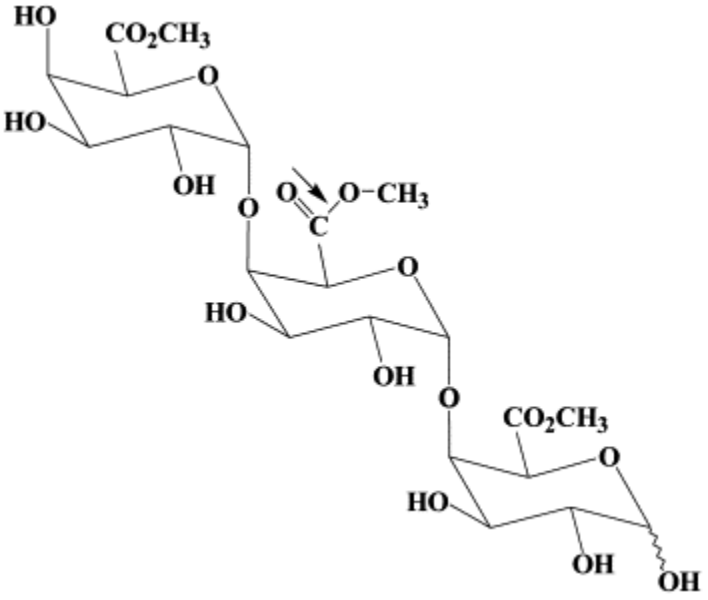

Fig. 2.1.2.1: Structure of Pectin methylesterase

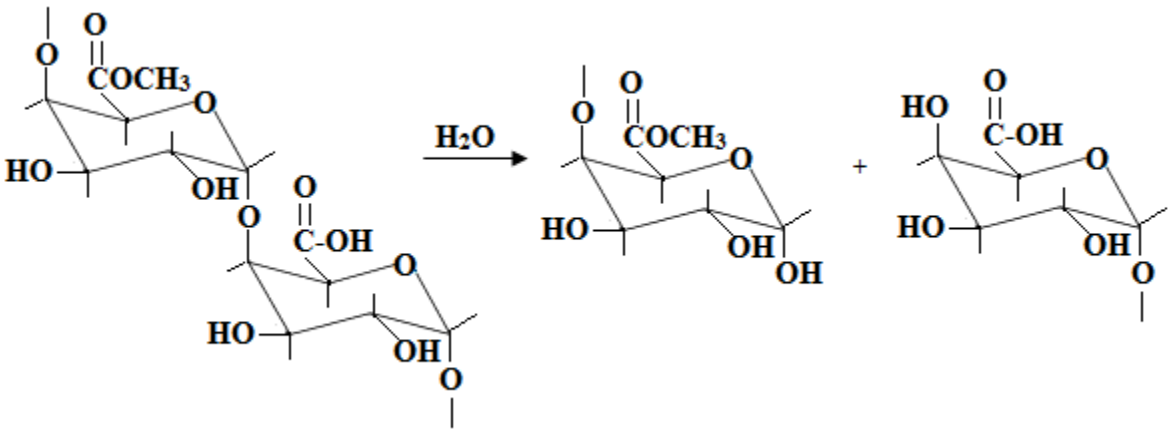

Fig. 2.1.2.2: Structure of Polygalacturonases

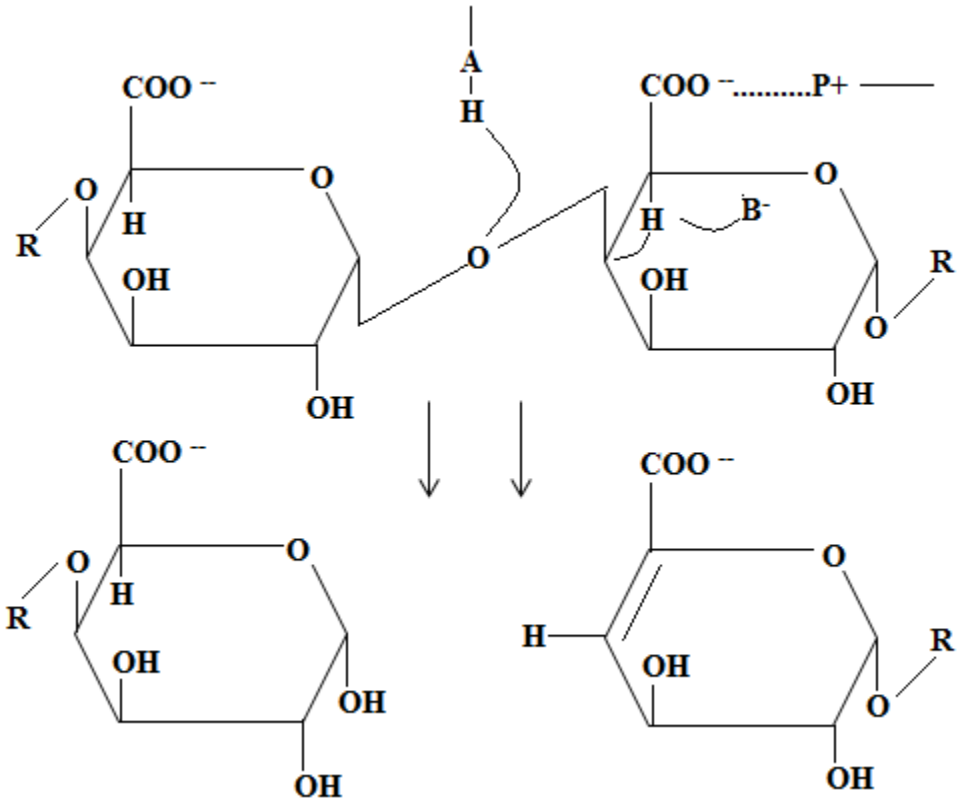

Fig. 2.1.2.3: Pectate lyases

