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REVIEW ARTICLE

Enzymatic extraction and clarification of juice from various fruits-A review

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Abstract

Enzymes are an integral component of modern fruit juice manufacturing processes. Their main functions are to: 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. The use of the enzymes like cellulases, pectinases, amylases alone and their combination can give better juice yield with superior quality of the fruit juice. The present article discusses the use of enzymes in fruit juice production focusing on the juice recovery, clarity and effect on the biochemical properties of the fruit juices.

Keywords: Enzymatic treatment, juice extraction, juice yield, clarity, enzymatic concentration

Introduction

Fruits and vegetables are important sources of essential dietary nutrients such as vitamins, minerals and fibers. Since the moisture content of the fresh fruits and vegetables is more than 80% (wb); they are highly perishable commodities. The world fruit production was about 609 million MT in 2010 -11 (FAO, 2010). According to the estimate, nearly 20-40% of the fruits are lost due to spoilage, mishandling during transportation and lack of cold storage and processing techniques (Singh *et al.*, 1994).

Food preservation ensures conservation and better utilization of fruits and vegetables through avoiding the glut and utilizing 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 converting it into products like jam, jelly, fruit bar, juice, pickle, murabba etc. to prolong their utilizable lifespan. Fruit juice preparation is one of the easiest ways to preserve 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 process of fruit and vegetable juices includes steps like extraction, clarification, and stabilization (Bhat, 2000).

The traditional method of juice extraction is through the use of mechanical 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 *et al.*, 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

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(Chadha *et al*., 2003). Enzymatic treatment 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 the modern fruit processing technology involving treatment of fruit masses. The enzyme treatment not only facilitates easy pressing and increase in juice recovery but also ensures the highest possible quality of the 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". During this process the viscosity of the juice is dropped and the opacity of the cloudy juice is changed to an open splotchy look. This can be accomplished in one of the two general ways: enzymatically and non-enzymatically (Kilara and Van Buren, 1989).

Non-enzymatic 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 results 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 early 1930s, when fruit processing industries began to produce juices, 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, 1996).

Enzymatic treatment for juice extraction and clarification is most common now- a-days. Enzymatic hydrolysis of the cell walls increases the extraction yield, reducing sugars, soluble dry matter content, 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 (Bauman, 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*., 1998). 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, 1996; Uhlig, 1998).

Enzymes

Pectic substances and pectic enzymes

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, as a consequence, leads to increase in juice yield (Plocharski *et al*., 1998; Voragen, 1992) and improved 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, resulting in juice that has much lower amount of pectin (Lee *et al*., 2006).

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 (Dorreich, 1996; van den Broek *et al*., 1997). *Aspergillus niger* or *Aspergillus aculeatus* is used for industrial production of pectolytic enzymes (Naidu and

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Panda, 1999). The pectic enzymes including pectinlyase, pectinmethylesterase, endo and exo-polygalacturonases, pectinacetylesterase, rhamnogalacturonase, endo- and exo-arabinases, are used in extraction and clarification of fruits and vegetable juices (Galante *et al.*, 1998). 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 are shown in Table 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 forms complex with Ca^{2+}, forming Ca^{2+} pectate gels which precipitate and clarify the juice (Baker and Bruemmer, 1972).

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).

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).

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), and (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 have this requirement. Ethylenediaminetetraacetic acid (EDTA) is generally an inhibitor of pectate lyase activity because of chelation of the Ca^{2+} (Whitaker, 1984).

Cellulose and cellulases enzyme

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 (Marchesseault and Sundararajan, 1993; Lynd *et al*., 1999).

Cellulases: 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 synthesis cellulases and, therefore, are incapable of utilizing the entire energy contained in plant material (Kuhls and Lieckfeldt, 1996).

Cellulases are 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

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Table 1: Biochemical properties of some pectic enzymes

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

ingredients and low-calorie food substituent's and biomass conversion (Beguin and Aubert, 1994; Bhat and 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).

Other Enzymes

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).

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 as well as in the 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 liquefaction, 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).

Enzymatic extractions and juice recovery

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 increases 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 (Pilnik *et al.*, 1975;

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McLellan *et al.,* 1985). Hot water extraction with addition of enzyme in apple pomace with a combination of pectinases and cellulases results in 37% increase in juice yield (Will *et al.,* 2000). Al-Hooti *et al.* (2002), blended date fruit pulp with three times the water before the addition of enzyme for extraction of juice and the juice recovery was 67-68%.

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 maximize yield and quantity of various fruit juices. Table 2 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 425 min and temperature 47°C (Singh *et al.*, 2012). Similarly, Yusof and Ibrahim (1994) found that the larger the amount of enzyme used and the longer the time of incubation, the greater was the yield of juice. They found 41% increase in juice recovery with enzymatic treatment than the 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°C 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 that free water is release into the 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%) (Oszmianski *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).

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 (Bhat, 2000). 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). Use of pectinase, cellulase and amylase in various combinations for juice extraction from kiwi fruit significantly increased juice yield (Table 3). The best results were found in combination of pectinase (0.05 g/kg), amylase (0.025 g/kg) and cellulases (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).

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 clear juice 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 is one of the main problems during the processing of clear fruit juices. However, 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 maceratation increased the juice clarity and filterability by 100% (Brown and Ough, 1981). Clarified fruit juice, that has an unstable cloud or whose turbidity is considered ‘‘muddy’’ is unacceptable to be marketed as clear juices (Floribeth *et al*., 1981).

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Table 2: Optimized conditions for extraction of maximum juice using pectinase enzyme

Fruit/ Vegetable	Incubation Time[a]	Incubation Temperature[b]	Enzyme Concentration[c]	Juice Recovery[d]	References
Bael (*Aegle marmelos correa*)	425	47	20 mg/100g	86.6	Singh *et al.* (2012).
Guava (*Psidium guajava* L.)	436.2	43.3	0.70 mg/100g	62.2	Kaur *et al.* (2009).
Elderberry *(Sambucus nigra L)*	50	60	0.34 mg/100g	77.0	Landbo *et al.* (2007).
Tamarind (Variety *Ajanta*)	360	37	5 mg/100g	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 min, [b]Incubation temperature in °C, [c]Enzyme concentrations in mg/100g : mg per 100 g of pulp, % : Percentage on pulp basis, U : Enzyme Unit, [d] Juice recovery in Percentage (%)

Table 3: Optimized conditions for extraction of juice using enzyme combinations

Fruit/ Vegetable	Enzymes	Incubation Time[a]	Incubation Temp[b]	Enzyme Concentration[c]	Juice Recovery[d]	References
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	pectin methyl esterase (PME) and polygalacturonase (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 (*Birhi)* 68.22(*safr*)	Al-Hooti *et al.* (2002).
Plum	pectin methyl esterase (PME) and polygalacturonase (PG)	120	50	0.05g/kg (2:1)	96.8	Mieszczakowska-Frac (2012)

[a]Incubation time in min, [b] Incubation temperature in °C, [c] Enzyme concentrations in mg/100g : mg per 100 g of pulp, % : Percentage on pulp basis, U : Enzyme Unit, [d] Juice recovery in Percentage (%)

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Clarity

Enzymatic treatment leads to increase the clarity of juice. Increase in enzymatic concentration increase the rate of clarification by exposing part of the positively charged protein beneath. This causes reduction in electrostatic repulsion between cloud particles causing these particles to aggregate into larger particles and eventually settled out (Sin *et al.,* 2006). Lowest absorbance value of clarity at highest enzyme concentration indicates a clearer juice. Moreover, the absorbance values decreases 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. Data on effect of different enzymes on juice clarity is presented in Table 4.

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 (Rai *et al.,* 2003). From both the observations, it is evident that there exists an optimum enzyme concentration and time for the juice clarity.

Effect of enzymatic treatment on physico-chemical properties of juice

Effect of enzymatic treatment on total soluble solids (TSS) of juice

Enzymatic extraction also increases TSS of juice from various fruits. TSS of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in Table 5. 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 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 TSS content and the brix/acid ratio 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. Pectinase and cellulases enzymes were used for extraction of pineapple juice at enzymatic concentration of 0.025%. The TSS of the final pooled juice was around 12°Brix (Sreenath et al., 1994). 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 (Sreenath *et al.,* 1984), which contribute to soluble solids.

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 of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in Table 5. 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 and Alvarez, 1995). Viscosity was significantly reduced with higher enzyme concentration. Incubation time also affected the viscosity negatively. 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 20 min at 30°C incubation temperature. The use of various enzymes in combination also tends to reduce the viscosity of juice. No significant difference in viscosity was observed for a combined enzyme treatment (pectinase and cellulase 2% in 3:2) given to

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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]	Reference
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 min, [b]Incubation temperature in °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.

carrot juice, compared to only pectinase treatment, which yielded juice with higher viscosity compared to cellulase treatment (Anastasakis *et al.*, 1987).

Effect of enzymatic treatment on pH of juice

The pH value of juice decreased with increase in enzyme concentration (Joshi *et al.,* 2011). Results of pectinase treatments on pH shown in Table 5. Yusof and Ibrahim (1994) found that for each level of enzyme used, decrease in pH was not significant for the first hour of incubation. As the incubation time increased (2-3 h), the decrease in pH values of the fruit juice was significantly different from the initial value. 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 of juice to 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). Effect of enzymatic treatment on pH is shown in Table 5.

Effect of enzymatic treatment on ascorbic acid content of juice

The ascorbic acid content of clarified juice decreased to 11.8 mg/100 g sample 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 (Joshi *et al.,* 2011). The effects of enzyme concentration and time of incubation on the ascorbic acid is shown in Table 5. The enzyme treatment did not seem to increase the ascorbic acid content significantly for soursop juice. Joshi *et al.* (2011) also found in apple pomace that the remaining ascorbic acid content was unaffected by the increase in enzyme concentration. The total ascorbic acid content was found to decrease about to 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). The effect of enzyme treatment on the ascorbic acid content of the fruit juice is presented in Table 5.

Effect of enzymatic treatment on turbidity of juice

The turbidity in the juices may be due to pectin and other plant cell wall substances released during the enzymatic prepress maceration. It seems logical 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, they 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 6. Increase in enzyme concentration and incubation time might decrease turbidity. Pectin was the main cause of turbidity (Grassin and Fauquembergue, 1996). 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).

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Table 5: Effect of incubation time, temperature and enzymatic concentration on TSS, viscosity, pH and ascorbic acid at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes	Incubation time[a]	Incubation Temp.[b]	Enzyme Conc.[c]	TSS[d]	Viscosity	pH	Ascorbic acid[f]	References
Bael (Aegle marmelos correa)	Pectinase	210	35	24 mg/100g	-	1.35	-	-	Singh *et al.* (2012)
Soursop *(Annona muricata* L.)	Pectinase	180	35-40	0.05%	7.30	4.68	3.54-3.7	1.14 mg/100 g	Yusof and Ibrahim (1994)
Apricot	Pectinase	240	40	2.5%	10.07	1.11	3.50	5.55 mg/100 g	Joshi *et al.* (2011)
Pear	Pectinase	240	40	2.5%	11.16	1.17	3.46	1.60 mg/100 g	Joshi *et al.* (2011)
Mayhaw (*Crataegus opaca* Hook.)	Pectinase	60	32	0.20%	8.13	-	3.03	-	Trappey *et al.* (2008)
Banana *(Musa sapientum* cv Berangan)	Pectinase	240	44	0.4%	26.1	14.2	3.41	-	Shahadan and Abdullah (1995)
White Grape *(Vitis vinifera)*	Pectinase	30	27-30	0.048%	13	1.05	-	-	Sreenath and Santhanam (1992)
Lichi (*Litchi chinensis* L)	Pectinase	120	40	500ppm	16.4	-	-	11.8 mg/100 mg	Vijayanand *et al*. (2010)
Blackcurrant	pectin methyl esterase (PME) and polygalacturonase (PG)	120	50	0.2g/kg (2:1)	18-19	-	-	118.8 mg/100 g	Mieszczakowska-Frac, *et al.,* 2012
Plum	pectin methyl esterase (PME) and polygalacturonase (PG)	120	50	0.05g/kg (2:1)	16.55	1.33	-	-	Mieszczakowska-Frac, *et al.* (2012)
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg,	14.75	1.05	3.50	154.59 mg/100ml	Vaidya *et al.* (2009)
Carrots *(Daucus carrota)*	Pectinase and cellulase	30	50	2% (3:2)	12.0	2.75	5.44	-	Anastasakis, *et al*. (1987)
Pineapple	Pectinase and cellulase	30	27-30	0.025% (1:1)	15.0	-	-	-	Sreenath *et al*. (1994)
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	4.6	-	Sin *et al.* (2006)
Carambola (*Carambola Averrhoa* L.)	Pectinase	20	30	0.1%	-	1.33	-	-	Abdullah *et al.* (2007)
Date (Variety *Birhi* and *safri*)	Pectinase and cellulase	60-300	40	1% (1:1)		17.6 (*Bi* 14.8 (*safr*	4.09 & 4.	-	Al-Hooti *et al.* (2002)
Mosambi (*Citrus sinensis* (L.) Osbeck)	Pectinase	99.27	41.89	0.0004 w/v%		-	3.6	-	Rai *et al.*, 2003
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour, Allig and Kentichi*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase		-	3.2, 3.12 3.07	-	Abbes *et al.* (2011)

[a]Incubation time in min, [b] Incubation temperature in °C, [c] Enzyme concentrations in % : Percentage on pulp basis, ppm: parts per million, g/kg: gram per kilogram of fruit/pulp, w/v% : Weight per volume, U : Enzyme Unit, [d]TSS: Total Soluble Solids in °Bx: Degree Brix., [e]Viscosity in cps: centipoises

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Table 6: Effect of incubation time, temperature and enzymatic concentration on titrable acidity, turbidity, Anthocynin and total phenols at optimized condition using enzymatic treatments**.**

Fruit/ Vegetable	Enzymes	Incubation time[a]	Incubation Temp[b]	Enzyme Conc.[c]	Titrable Acidity[d]	Turbidity[e]	Anthocynin[f]	Total phenols[g]	References
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	pectin methyl esterase (PME) and polygalacturonase (PG)	120	50	0.2g/kg (2:1)	4.06	-	239.6 mg/100ml	-	Mieszczakowska-Frac, *et al.* (2012)
Plum	pectin methyl esterase (PME) and polygalacturonase (PG)	120	50	0.05g/kg (2:1)	1.06	590 NTU	13.64 mg/100ml	-	Mieszczakowska-Frac *et al.* (2012)
Kiwi (*Actinidia deliciosa*)	Pectinase, Amylase and cellulase	120	50	0.06, 0.025 and 0.025 g/kg, respectively	1.20	-	-	240 mg/l	Vaidya *et al.* (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	-	-	326.84, 292.34 & 304.28 mg/100 g res.	Abbes *et al.* (2011)
Elderberry *(Sambucus nigra L)*	Pectinase	50	60	0.34 mg/100g	-	154 FNU	2.4 mg/g	6.0 mg/g	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)
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	440 mg/l	Sreenath and Santhanam (1992)
Black currant (*Ribes nigrum*)	Pectinase	30	60	0.18%	-	-	1.5–2.2 mg/g	3.1–4.4 mg/g	Landbo and Meyer (2004)

[a]Incubation time in min, [b] Incubation temperature in °C, [c] Enzyme concentrations in % : Percentage on pulp basis, mg/100g: milligram per 100 g of fruit/pulp, [d] Titrable acidity in %, [e] Turbidity in FNU: Formazin Nephelometric Units NTU: Nephelometric Turbidity Units

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Effect of enzymatic treatment on titrable acidity of juice

Yusof and Ibrahim (1994) found that the total titratable acidity for enzymatically extracted juice from soursop 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 later 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 6. 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.

Effect of enzymatic treatment on anthocyanin content of juice

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 6. Pectinase treatment increased release of anthocyanins than the other enzyme treatments in white grape juice (Joshi *et al.,* 2012). Treatment of raspberry juices with pectolytic enzymes modified the level of individual pigment and the total anthocyanins content varied accordingly (Versari *et al.,* 1997). The pectolytic enzymes showed a stationary high level of total anthocyanins over the time (range: 289-306 mg). On the other hand, it was a clear 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.

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 increase in maceration temperature, but increased enzyme dose and increased maceration time does not affect total phenols yield (Landbo and Meyer, 2004). Total Phenols at optimized condition for enzymatic treatment of various fruits and vegetable shown in Table 6.

Conclusions

Enzymatic treatment in juice extraction is one of the most commonly methods used now-a-days. The enzymatic process is claimed to offer a number of advantages over mechanical-thermal comminution of several fruit pulps. Since, cellulose and pectin present in fruit pulp and skin adversely affect the juice recovery; use of hydrolytic enzymes can prove beneficial in overcoming the shortfalls. 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.

References

Abbes F, Bouaziz M, Blecker C, Masmoudi M, Attia H and Besbes S (2011). Date syrup: Effect of hydrolytic enzymes (pectinase/cellulase) on physicochemical characteristics, sensory and functional properties. *Journal of Food Science and Technology*, 44: 1827-1834.

Abdullah AGL, Sulaiman NM, Aroua MK and Megat MNMJ (2007). Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. *Journal of Food Engineering*, 81: 65-71.

Albersheim P, Neukom H, and Deuel H (1960). Splitting of pectin chain molecules in neutral solution. *Archives of Biochemistry and Biophysics*, 90: 46-51.

Al-Hooti SN, Sidhu JS, Al-Saqer JM and Al-Othman A (2002). Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. *Food Chemistry*, 79: 215-220.

Alvarez S, Alvarez R, Riera FA and Coca J (1998). Influence of depectinization on apple juice ultrafiltration. *Colloids and Surfaces A:*

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Physicochemical and Engineering Aspects, 138: 377-382.

Anastasakis M, Lindamood JB, Chism GW and Hansen PMT (1987). Enzymatic hydrolysis of carrot for extraction of a cloudstable juice. *Food Hydrocolloids*, 3: 247-261.

Baker RA and Bruemmer JHJ (1972). Pectinase stabilization of orange juice cloud. *Journal of Agricultural and Food Chemistry*, 20: 1169-1173.

Bauman JW (1981). Application of enzymes in fruit juice technology. In: Enzymes and Food Processing (Birch et al., Eds). Applied Science Publications, pp 129-147.

Beguin P and Aubert JP (1994). The biological degradation of cellulose. *FEMS Microbiology Revives*, 3: 25-58.

Beveridge T and Rao MA (1997). Juice extraction from apples and other fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 37(5): 449-469.

Bhat MK (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18: 355-383.

Bhat MK and Bhat S (1997). Cellulose degrading enzymes and their potential industrial applications. *Biotechnology Advances*, 5: 583-620.

Blanco P, Sieiro C, Diaz A and Villa TG (1994). Production and partial characterization of an endopolygalacturonase from Saccharomyces cerevisiae. *Canadian Journal of Microbiology*, 40: 974-977.

Borin MDF, Said S and Fonseca MJV (1996). Purification and biochemical characterization of an extracelular endopolygalacturonase fron Penicillium frequentans. *Journal of Agricultural and Food Chemistry*, 44: 1616-1620.

Broeck IVD, Ludikhuyze LR, Weemaes CA, Van Loey AM and Hendrickx ME (1999). Thermal inactivation kinetics of pecin esterase extracted from oranges. *Journal of Food Processing and Preservation*, 23: 391-406.

Brown MR and Ough CS (1981). A comparison of activity and effects of two commercial pectic enzyme preparations on white grape musts and wines. *American Journal of Enology and Viticulture*, 32: 272-276.

Cao J, Zheng L and Chen S (1992). Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of ramie. *Enzyme and Microbial Technology*, 14: 1013-1016.

Chadha R, Kumbhar BK and Sarkar BC (2003). Enzymatic hydrolysis of carrot for increased juice recovery. *Journal of Food Science and Technology*, 40(1): 35-39.

Chane PS and Shewal JG (1995). Pectinase production by Sclerotium rolfsii. Effects of culture conditions. *Folia Microbiologica*, 40: 111-117.

Chauhan SK, Tyagi SM and Singh D (2001). A pectinolytic liquefaction of apricot, plum, and mango pulps for juice extraction. *International Journal of Food Properties*, 4(1): 103-109.

Cheryan M and Alvarez JR (1995). Food and beverage industry application. Membrane separation technology principles and applications, 443-465.

Clarke AJ (1996). Biodegradation of cellulose: enzymology and biotechnology. *CRC Press, Boca Raton, FL.*

Codner RC (2001). Pectinolytic and cellulolytic enzymes in the microbial modification of plant tissues. *Journal of Applied Bacteriology*, 84:147–60.

Couto SR and Sanroman MA (2006). Application of solid-state fermentation to food industry- A review. *Journal of Food Engineering*, 76: 291-302.

Dorreich K (1996). Investigations on production of apple juice without the utilisation of presses. In XII International congress of fruit juice report of congress (pp. 183-197). IFU, Interlaken, 20-24 May.

Dzogbefia VP, Ameko E, Oldham JH and Ellis WO (2001). Production and Use of Pectolytic Enzyme from Saccharomyces cerevisiae for the Extraction of Pineapple Juice. *Food Biotechnology*, 15(1): 25-34.

FAO (2010). Food and Agriculture Organization Statistical Production Yearbook. 2010-2011, *FAO, Rome, Italy.*

Floribeth V, Celsa L and Cooke RD (1981). A study of the production of clarified banana juice using pectinolytic enzymes. *Food Technology*, 16: 115-125.

Galante YM, De Conti A and Monteverdi R (1998). Application of Trichoderma enzymes in food and feed industries. *Trichoderma and Gliocladium—Enzymes, Biological Control and Commercial Applications*, 2: 327–342.

Grassin C and Fauquembergue P (1996). Fruit juices. *In. Industrial Enzymology*, 2: 226.

Gupta TR and Girish K (1988). Studies on mango pulp filtration for concentration by pectolytic enzyme treatment. *Indian Food Packer*, 42(5): 7-14.

Hutchings JB (1999). Food colour and appearance. *An Aspen Publication*, 2: 19-21.

Jayani RS, Saxena S and Gupta R (2005). Microbial pectinolytic enzymes: A review. *Process Biochemistry*, 40: 2931-2944.

Joshi AA, Kshirsagar RB and Sawate AR (2012). Studies on standardization of enzyme concentration and process for extraction of tamarind pulp, variety Ajanta. *Journal Food Processing and Technology*, 3(2): 1-3.

Joshi VK, Chauhan SK and Lal BB (1991). Extraction of juice from peaches, plumes and apricot by pectinolytic treatment. *Journal of Food Science and Technology*, 28(1): 64-65.

Joshi VK, Parmar M and Rana N (2011). Purification and characterization of pectinase produced from apple pomace and evaluation of its efficiency in fruit juice

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extraction and clarification. *Indian Journal of Natural Products and Resources*, 2(2): 189-197.

Kashyap DR, Chandra S, Kaul A and Tewari R (2000). Production, purification and characterization of pectinase from Bacillus sp. DT7. *World journal of Microbiology and Biotechnology*, 16: 277-282.

Kashyap DR, Vohra PK, Chopra S and Tewari R (2001). Applications of pectinases in the commercial sector: a review. *Bioresource Technology*, 77: 215-227.

Kaur G, Kumar S and Satyanarayana T (2004). Production, characterization and application of a thermostable polygalacturonase of a thermophilic moul Sporotrichum Termophile. *Bioresource Technology*, 94: 239-243.

Kaur S, Sarkar, BC and Sharma HK (2009). Optimization of enzymatic hydrolysis pretreatment conditions for enhanced juice recovery from guava fruit using response surface methodology. *Food and Bioprocess Technology*, 2: 96-100.

Kilara A (1982). Enzymes and their uses in the processed apple industry: a review. *Process Biochemistry*, 17: 35-41.

Kilara A and Van Buren J (1989). Clarification of apple juice. In Processed Apple Products. D.L. Downing (Ed.), *Van Nostrand Reinhold, New York.*

Kuhls K and Lieckfeldt E (1996). Molecular evidence that the asexual industrial fungus Trichoderma reesei is a clonal derivative of the ascomycete Hypocrea jecorina. *Proceedings of the National Academy of Sciences*, 7755-7760.

Landbo AK and Meyer AS (2004). Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice. *Innovative Food Science and Emerging Technologies*, 5: 503-513.

Landbo AK, Kaack K and Meyer AS (2007). Statistically designed two step response surface optimization of enzymatic prepress treatment to increase juice yield and lower turbidity of elderberry juice. *Innovative Food Science and Emerging Technologies*, 8: 135-142.

Lee WC, Yusof S, Hamid NSA and Baharin BS (2006). Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, 73: 55-63.

Lotha RE, Khurdiya DS and Maheshwari M (1994). Effect of storage on the quality of kinnow mandarin fruit for processing. *Indian Food Packer*, 48(2): 25-38.

Lynd LR, Wyman CE and Gerngross TU (1999). Biocommodity engineering. *Biotechnology Progress*, 5: 777-793.

Marchesseault RH and Sundararajan PR (1983). Cellulase, The Polysaccharides, 11-95.

McLellan MR, Kime RW and Lmd LR (1985). Apple juice clarification with the use of honey and pectinase. *Journal of Food Science*, 50: 206-208.

Mieszczakowska-Frac M, Markowski J, Zbrzezniak M and Plocharski W (2012). Impact of enzyme on quality of blackcurrant and plum juices. *Journal of Food Science and Technology*,, 49: 251-256.

Naidu NGS and Panda T (1999). Performance of pectolytic enzymes during hydrolysis of pectic substances under assay conditions: a statistical approach. *Enzyme and Microbial Technology*, 25: 116-124.

Oszmianski J, Wojdylo A and Kolniak J (2009). Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity, and turbidity of cloudy apple juices. *Journal of Agricultural and Food Chemistry*, 57(15): 7078-7085.

Pilnik W, Voragen AGJ and Devas L (1975). Enzymatic liquefaction of fruits and vegetables. *Die Januar-Ausgabe von FLÜSSIGES OBST*, 42: 448-451.

Plocharski W, Szymczak J and Markowski J (1998). Auswirkung enzymatish Apflemaischenbehandlung auf Saftsbeute und Pektinstoffmenge. *Die Januar-Ausgabe von FLÜSSIGES OBST*, 6: 325-330.

Rai P, Majumdar GC, DasGupta S and De S (2003). Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology, *Journal of Food Engineering*, 64: 397-403.

Rexova´-Benkova´ L and Markovic´ O (1976). Pectic enzyms. *Advances in Carbohydrate Chemistry*, 33: 323-385.

Roumbouts FM and Pilnik W (1978). Enzymes in fruits and vegetable juice technology. *Process Biochemistry*, 13: 9-13

Sakai T, Sakamoto T, Hallaert J and Vandamme EJ (1993). Pectin, pectinase and protopectinase: production, properties and applications. *Advances in Applied Microbiology*, 39: 231-294.

Serrat M, Bermúdez RC and Villa TG (2002). Production, purification and characterization of a polygalacturonase from a new strain of Kluyveromyces marxianus isolated from coffee wet-processing wastewater. *Applied Biochemistry and Biotechnology*, 97: 193-208.

Shahadan S and Abdullah A (1995). Optimizing enzyme concentration, pH and temperature in banana juice extraction. *ASEAN Food Journal*, 10(3): 107-111.

Shallom D and Shoham Y (2003). Microbial hemicellulase. *Current Opinion in Microbiology*, 6: 219-228.

Sieiro C, Garcia-Fraga B, Lopez-Seijas J, Silva AF and Villa TG (2012). Microbial Pectic Enzymes in the Food and Wine Industry. In: Food Industrial Processes - Methods and Equipment, pp 201-218.

Sin HN, Yusof S, Sheikh Abdul Hamid N and Abdul Rahman R (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *Journal of Food Engineering*, 73: 313-319.

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Singh A, Kumar S and Sharma HK (2012). Effect of enzymatic hydrolysis on the juice yield from bael fruit (Aegle marmelos Correa) pulp. *American Journal of Food Technology*, 7(2): 62-72.

Singh C, Poonia GS and Toor GS (1994).Distribution pattern of fruit processing industry in Punjab. *Indian Food Packer*, 8(1):47.

Singh NGI, Madaiah N and Najundaswamy AM (1993). Prelimary studies on clarification of fruit juices by ultra-filtration. *Indian Food Packer*, 47: 9-15.

Sreenath HK and Santhanam K (1992). The use of commercial enzymes in white grape juice clarification. *Journal of Fermentation and Bioengineering*, 73(3): 241-243.

Sreenath HK, Frey MD, Scherz H and Radola BJ (1984). Degradation of a washed carrot preparation by cellulases and pectinases. *Biotechnology and Bioengineering*, 26: 788-796.

Sreenath HK, Sudarshanakrishna KR and Santhanam K (1994). Improvement of juice recovery from pineapple pulp/residue using cellulases and pectinases. *Journal of Fermentation and Bioengineering*, 78(6): 486-488.

Sukumaran RK, Singhania RR and Pandey A (2005). Microbial cellulases; production, application and challenges. *Journal of Scientific and Industrial Research*, 64: 833-834.

Sulaiman MZ, Sulaiman NM and Liew SY (1998). Limiting permeate flux in the clarification of untreated starfruit juice by membrane ultrafiltration. *Chemical Engineering Journal*, 69(2): 145-148.

Szajer I and Szajer C (1982). Pectin lyase of Penicillium paxilli. *Biotechnology Letters*, 4: 549-552.

Trappey AF, Johnson CE and Wilson PW (2008). Use of a commercial pectolytic enzyme to extract juice from frozen Mayhaw (Crataegus opaca Hook.) fruit. *International Journal of Fruit Science*, 7(1): 77-86.

Tribess TB and Tadini CC (2006). Inactivation kinetics of pectin methyesterase in orange juice as a function of pH and temperature-time process conditions. *Journal of the Science of Food and Agriculture*, 86: 1328-1335.

Uhlig H (1998). Industrial enzymes and their applications. *New York: John Wiley and Sons, Inc*. 435.

Vaidya D, Vaidya M, Sharma S and Ghanshayam V (2009). Enzymatic treatment for juice extraction and preparation and preliminary evaluation of Kiwifruits wine. *Natural Product Radiance*, 8(4): 380-385.

Vaillant F, Millan A, Dornier M, Decloux M and Reynes M (2001). Strategy for economical optimisation of the clarification of pulpy fruit juices using crossflow microfiltration. *Journal of Food Engineering*, 48: 83-90.

Van den Broek LAM, den Aantrekker ED, Voragen AGJ, Beldman G and Vincken JP (1997). Pectin Lyase is a key enzyme in the maceration of potato tuber. *Journal of the Science of Food and Agriculture*, 75: 167-172.

Versari A, Biesenbruch S, Barbanti D, Farnell PJ and Galassi S (1997). Effects of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices. *Food Research International*, 30(10): 811-817.

Vidhya R and Narain A (2011). Formulation and Evaluation of Preserved Products Utilizing under Exploited Fruit, Wood Apple (Limonia acidissima). *American-Eurasian Journal of Agricultural and Environmental Sciences*, 10(1): 112-118.

Vijayanand P, Kulkarni SG and Prathibha GV (2010). Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice. *Journal of Food Science and Technology*, 47(2): 235-239.

Voragen AGJ (1992). Tailor-made enzymes in fruit juice processing. *Fruit Processing*, 7: 98-102.

Whitaker JR (1984). Pectic substances, pectic enzymes and haze formation in fruit juices, *Enzyme and Microbial Technology*, 4: 341-349.

Will F, Bauckhage K and Dietrich H (2000). Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. *European Food Research and Technology*, 211: 291-297.

Will F, Roth M, Olk M, Ludwig M and Dietrich H (2008). Processing and analytical characterisation of pulp-enriched cloudy apple juices. *Food Science and Technology*, 41(10): 2057-2063.

Woodroof JG and Phillips GE (1981). Beverages: Carbonated and Non-carbonated. *The Art Publishing Co. Inc. Westport, Connecticut*, 2: 213-32.

Yadav S, Yadav P, Yadav D and Yadav K (2008). Purification and characterization of an acidic pectin lyase produced by Aspergillus ficuum strain MTCC 7591 suitable for clarification of fruit juices. *Annals of Microbiology*, 58: 61-65.

Yusof S and lbrahim N (1994). Quality of soursop juice after pectinase enzyme treatment. *Food Chemistry*, 51: 83-88.

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