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Mini Review

Pectinases: Enzymes for fruit processing industry

*Tapre, A. R. and Jain, R. K.

Department of Food Processing Technology, A.D. Patel Institute of Technology, New Vallabh Vidya Nagar, Anand, Gujarat 388121, India

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Abstract

The use of enzymes in juice industry has contributed in increasing the yield and production of various types of juices. Pectinolytic enzymes are one of the important groups of enzymes used in fruit processing industry. These are one of the upcoming enzymes of fruit processing industries. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. This review discuses the occurrence and nature of pectic substances in fruits, chemistry of pectic substances, types of pectinases and their applications in the fruit processing industry.

Introduction

Enzymes catalyses various reactions involved in the preparation of different food products. It is one of the important tools in modern food industry because while processing many intermediate processes are simplified due to use of enzymes. Bulk of the industrial enzymes fall into different groups, out of these the most important group of enzymes used in fruit and vegetable processing industry is pectinases. Pectinases were some of the first enzymes to be used in homes. Their commercial application was first observed in 1930 for the preparation of wines and fruit juices (Oslen, 2000). Only in the 1960s the chemical nature of plant tissues becomes apparent and with this knowledge, scientists began to use a greater range of enzymes more efficiently. As a result, pectinases are one of the important upcoming enzymes of the commercial sector specially for fruit juice industry as a prerequisites for obtaining well clarified and stable juices with higher yields (Dupaigne,1974; Tocchini and Lara,1977; Jaleel *et al*., 1978; Viquez *et al*., 1981; Girard and Fukumoto,1999; Lee *et al*., 2006; Sandri *et al*., 2012). Primarily, these enzymes are responsible for the degradation of the long and complex molecules in the fruit pulp called pectin that occur as structural polysaccharides and responsible for turbidity in pulp. With the addition of pectinases the viscosity of the fruit juice drops, the press ability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained with higher yields. Pectinases are now an integral part of fruit juice industries as well as having various biotechnological applications. The main emphasis of this article is on the types of pectinases and their applications in food industries.

Nature of pectic substances

Chemically, pectic substances are complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by α (1-4) linkage. The side chains of the pectin molecule consist of L-rhamnose, arabinose, galactose and xylose. The carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions. Based on the type of modifications of the backbone chain, pectic substances are classified into protopectin, pectic acid, pectinic acid and pectin (Be Miller, 1986). A committee appointed by the American Chemical Society in 1944 has defined pectic substances (Kertesz *et al*., 1944) as follows:

Protopectin: This is a parent pectic substance and upon restricted hydrolysis yields pectin or pectinic acid. Protopectin is occasionally a term used to describe the water-insoluble pectic substances found in plant tissues and from which soluble pectic substances are produced (Kilara, 1982).

Pectic acids: is the name applied to pectic substances composed of colloidal polygalacturonic acid and is essentially free of methyl ester groups. Normal or acid salts of pectic acid are called pectates.

Pectinic acids: are those colloidal polygalacturonic acids containing various amounts of methyl ester groups. Pectinates are normal or acid salts of pectinic acids (Kilara, 1982).

Pectins: A generic name for the mixture of widely differing compositions containing pectinic acid as the major component.

Pectin in native form is located in the cell

*Corresponding author.
Email: *tapreajay@gmail.com*

Table 1. Pectin content (%) of some fresh fruits and dried plant parts

Material	% pectin as calcium pectate	
	Fresh fruits	Dried plant parts
Apples	0.5-1.6	---
Apricots	0.7-1.3	---
Bananas	0.7-1.2	----
Citrus peel	----	30-35
Currants	0.9-1.5	----
Guavas	0.7-1.5	---
Grapes	0.2-1.0	---
Lemon peel	----	35.5
Lemon pulp	----	32.0
Lemon rind	----	20.0
Pineapple	0.3-0.6	---
Peas	0.5-0.8	---
Peaches	0.3-1.2	---
Potatoes	---	2.5
Strawberries	0.6-0.7	---
Sugar beet	---	20-30
Tomatoes	0.2-0.5	---

wall and it may be interlined with other structural polysaccharides and proteins to form insoluble protopectin. In most succulent plant tissues, the total pectic substances, on fresh weight basis, amount to approximately 0.5 to 1%. Pectin content of some fresh fruits and of some dried plant parts as reported by Fogarty and Ward (1972) are given in Table 1.

Mechanical crushing of pectin-rich fruit yields a fruit juice with high viscosity, which remains bound to the pulp in the form of a jellified mass. It is difficult to extract this juice by pressing or using other mechanical methods. With the addition of enzymes such as pectinases the viscosity of the fruit juice drops, the pressability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained and with higher yields.

Classification of pectic enzymes

Pectinases are classified under three headings according to the criteria: whether pectin, pectic acid or oligo-D-galacturonate is the preferred substrate, whether the cleavage is random (endo-, liquefying of depolymerizing enzymes) or endwise (exo- or saccharifying enzymes) whether pectinases act by trans elimination or hydrolysis (Whitaker, 1990; Sakai, 1992; Blanco,1999).

The three major types of pectinases are as follows:

Pectinesterases (PE)

Pectinesterases also known as pectinmethyl hydrolase, catalyzes deesterification of the methoxyl group of pectin forming pectic acid. The enzyme acts preferentially on a methyl ester group of galacturonate unit next to a non-esterified galacturonate units (Cosgrove, 1997).

Depolymerizing enzymes

Depolymerases catalyze the hydrolytic cleavage of the α(1, 4)-glycosidic bonds in the D-galacturonic acid moieties of the pectic substances (Rexova, 1976).

These are the enzymes:

Hydrolyzing glycosidic linkages

They include:

Polymethylgalacturonases (PMG): Catalyze the hydrolytic cleavage of α(1,4) glycosidic bonds. They may be:

Endo-PMG: causes random cleavage of α(1,4) -glycosidic linkages of pectin, preferentially highly esterified pectin.

Exo-PMG: causes sequential cleavage of α (1,4)-glycosidic linkage of pectin from the non-reducing end of the pectin chain.

Polygalacturonases (PG): Catalyze hydrolysis of α (1,4)-glycosidic linkages in pectic acid (polygalacturonic acid). They are also of two types:

Endo-PG: also known as poly (1,4-α-D-galacturonide) glycanohydrolase, catalyzes random hydrolysis of α (1,4) -glycosidic linkages in pectic acid.

Exo-PG: also known as poly(1,4-α-D-galacturonide) galacturonohydrolase, catalyzes hydrolysis in a sequential fashion of α-1,4-glycosidic linkages on pectic acid.

Cleaving

Cleaving α (1,4) -glycosidic linkages by trans-elimination, which results in galacturonide with an unsaturated bond between C4 and C5 at the non-reducing end of the galacturonic acid formed. These include:

Polymethylegalacturonate lyases (PMGL). Catalyze breakdown of pectin by trans-eliminative cleavage. They are:

Endo-PMGL: also known as poly (methoxygalacturonide) lyase, catalyzes random cleavage of α-1,4-glycosidic linkages in pectin.

Exo-PMGL: catalyzes stepwise breakdown of pectin by trans-eliminative cleavage.

Polygalacturonate lyases (PGL). Catalyze cleavage of α-1,4-glycosidic linkage in pectic acid by trans-elimination. They are also of two types:

Endo-PGL: also known as poly (1,4-α-D-galacturonide) lyase, catalyzes random cleavage of α-1,4-glycosidic linkages in pectic acid.

Exo-PGL: also known as poly (1,4-α-D-galacturonide) exolyase, catalyzes sequential cleavage of α-1,4-glycosidic linkages in pectic acid.

Protopectinase

This enzyme solubilises protopectin forming highly polymerized soluble pectin.

On the basis of their applications, pectinases are mainly of two types: acidic pectinases and

Table 2. Characterization of microbial pectinases

Producer	Type of pectinase	Opti. pH for activity	Optimum Temp. for activity (°C)	Reference
Acidic pectinases				
Aspergillus niger CH4	Endo-pectinase,	4.5-6.0	Below 50	Acuna-Arguelles *et al.,* 1995
	Exo-pectinase	3.5-5.0		
Penicillium frequentans	Endopoly galacturonase (Endo-PG)	4.5-4.7	50	Borin *et al*., 1996
Sclerotium rolfsii	Endo-PG	3.5	55	Channe and Shewal, 1995
Rhizoctonia solani	Endo-PG	4.8	50	Marcus *et al*., 1986
Mucor pusilus	PG	5.0	40	Al-Obaidi *et al*., 1987
Cloctridium thermosaccharolyticum	Polygalacturonate Hydrolase	5.5-7.0	30-40	Rijssel *et al*., 1993
Alkaline pectinases				
Bacillus sp. RK9	PGL	10.0	---	Fogarty and Kelly, 1983
Bacillus sp. NT-33	PG	10.5	75	Cao *et al*., 1992
Bacillus polymyxa	PG	8.4-9.4	45	Nagel and Vaughn, 1961
Bacillus pumilis	PATE	8.0-8.5	60	Dave and Vaughn, 1971
Amucola sp.	Pectate lyase (PAL)	10.25	70	Bruhlmann *et al*., 1994
Xanthomonas compestris	PATE	9.5	25-30	Nasumo and Starr, 1967
Bacillus No. P-4-N	PG	10-10.5	65	Horikoshi, 1990
Bacillus stearothermophillus	PATE	9.0	70	Karbassi and Vaughn, 1980
Penicillium italicum CECT 22941	Pectin lyase	8.0	50	Alana *et al*., 1990
Bacillus sp. DT 7	Pectin lyase	8.0	60	Kashyap *et al*., 2000
Bacillus subtilis	PAL	8.5	60-65	Chesson and Codner, 1978

alkaline pectinases. The important producers of these pectinases as reported in the literature are given in Table 2.

Application of pectinolytic enzymes

Pectin degrading enzymes are used extensively for the extraction and clarification of fruit juices and wines (Tressler and Joslyn, 1971). Its importance in fruit juice technology and in wine making has been excellently brought out by Hickinbotham and Williams (1940).

Extraction of juice from soft fruits

Tropical fruits are usually too pulpy and pectinaceous to yield juice by simple pressing or centrifugation. Such techniques involve expenditure of excessive amounts of energy and results in meagre juice yield. Juices can be easily expressed from fruits like lemons, oranges, tomatoes, pineapple etc. In soft fruits like guava, banana, papaya, mangoes the expression of juice is little bit difficult by the conventional methods. In certain other fruits like grapes, apples etc. extraction of juice is incomplete since some quantity of juice is retained in the pomace after expression. In the conventional process of preparing juices from soft fruits the pulp is boiled with water and the extract is further processed (Waldt and Mahoney, 1967).

An enzyme process developed at CFTRI (Sreekantiah *et al*., 1971) enables not only expression of juice from soft fruits without drastic treatments, but also aid in the clarification of juices. The process consists of pulping the fruits (after peeling wherever necessary) and warming to 60-65^{o}C for 15 minutes to inactivate the innate enzymes. The pulp is then cooled and calculated quantities of pectinolytic enzyme is added and mixed well. After sufficient incubation period, the juice is separated out by using basket centrifuge or pressing through cheese cloth in a filter press. The juice thus obtained is racked at 3 to 5^{o}C for 24 to 48 hrs during which period all the suspended particle settle down at the bottom. The clear supernatant is then clarified, if necessary, by using filter aids and stored after pasteurization. Though there is use of certain mechanical methods juices may differ in certain characteristics and composition.

Pectic enzymes are used in apple juice preparation to facilitate pressing or juice extraction and to aid in the separation of a flocculent precipitate by sedimentation, filtration or centrifugation. Schols *et al.* (1990) described a new pectinase called rhamnogalacturonase and its role in the maceration of apple tissue. This enzyme was found in *Aspergillus aculeatus* initially, but it seems also to be produced by other *Aspergillus* spp. Treatment with pectinase takes anything from 15 min to 2 h depending upon the exact nature of the enzyme and how much is used, the reaction temperature and the variety of apple chosen (Kilara, 1982). By pectinase enzyme treatment, there is increase in the yield of juice from grapes upto 30%. The juice yield increases as concentration of pectinase increases from 0.05 to 1.5% (Villettaz, 1993).

Clarification of fruit juices and wine

The practice of fruit juice clarification by the use of enzymes was introduced in Germany and the United States of America in the early thirties. Application of pectic enzymes in fruit juice technology has been dealt in detail by Neubeck (1959). The enzymatic clarification is influenced by a number of variables including concentration of the enzyme, temperature and incubation time of the treatment (Neubeck, 1975; Baumann, 1981; Lanzarini and Pifferi, 1989). Before

the technique of enzyme clarification; fining, heat coagulation, clarification by freezing were some of the methods adopted previously for obtaining clear juices.

The commercial pectinolytic enzymes are used as processing aids for pectin degradation which settled down organic particles in suspension. The use of pectinolytic enzymes not only resulted in higher yield and clarity of juice but also preserves the nutrients, original color and flavour. The mixture of pectinase, cellulase and hemicellulase enzymes which were effective in viscosity reduction and filterability improvement in the preparation of clarified juices (Jaleel *et al*.,1978; Koffi *et al*., 1991; Shahadan and Abdullah 1995).

Application of pectinase enzyme have improved the clarification process for apple juice with 35% viscosity drop (Girard and Fukumoto,1999; Mondor *et al*., 2000), tangerine juice (Chamchong and Noomhorm, 1991), pineapple juice (Carneiro *et al*., 2002) and plum, peach, pear and apricot juice prior to ultrafiltration. Also the juice recovery of enzymatically treated pulps increased significantly from 52-72% in plums, 38-63% in peach, 60-72% in pear and 50-80% in apricot (Joshi *et al*., 2011). Brasil *et al*. (1995) reported a remarkable reduction of 62.9% in the guava juice viscosity when Clarex-L super-concentrate (Miles-Brasil, Brazil) was applied. The results obtained by Sharma *et al.* (2005) also indicated that not only temperature, but also enzyme concentration and incubation time affected carrot juice viscosity when Pectinex Smash XXL was employed achieving up to 41% reduction.

The turbidity and viscosity of banana juice are caused mainly by the polysaccharides in the juice such as pectin and starch. Pectins make the clarification process harder because of their fibre-like molecular structure. Several researchers have reported that depectinization using pectinase could effectively clarify banana pulp (Viquez *et al*.,1981; Koffi *et al.*, 1991; Yusof and Ibrahim, 1994; Brasil *et al*., 1995; Alvarez *et al*., 1998; Ceci and Lozano, 1998; Vaillant *et al.*, 1999; Lee *et al*., 2006). Pectinase hydrolyzes pectins and cause pectin–protein complexes to flocculate. The resulting juice has a much lower amount of pectins and a lower viscosity, which facilitates the subsequent filtration process.

A modified method for producing apple juice has been developed at CFTRI (Jaleel *et al*., 1978) wherein by judicious use of pectinolytic enzymes not only a sparkling juice is obtained but also 20% of the pectin is recovered. Enzyme treated juices are ideal for preparing concentrates.

The use of pectic enzymes for wine clarification was first recommended by Bensone and Cruess (1941). It is better if the enzyme is added to the must itself before fermentation. This help in greater yield. The enzyme-treated banana must used to produce banana wine led to an enhanced clarification of the wine product without affecting any other characteristics (Cheirsilp and Umsakul, 2008). Pollard and Kieser (1951) have advocated use of pectinolytic enzymes in cider manufacture.

Preparation of fruit cordials

Pectinolytic enzyme preparations have also been used to hasten the process of lime juice (Sreekantiah *et al.*, 1968) and orange juice cordial production (Alkorta *et al*., 1998). It has been found that when pectinolytic enzyme is added to lime juice at 1% level and allowed to react for 48hr. At room temperature (25-35^{o}C), it could be easily filtered and sparkling clear product is obtained. The process not only reduces the processing time to 4 to 6 days from 4 to 6 months, but also the cost of storing the juice over long periods is eliminated.

Other uses

Other exceptional uses of pectinases in fruit processing are: in the manufacture of better quality purees from prunes, peaches, apricots, strawberries and other fruits. The enzymatic process makes the use of enzymes to soften skins and tissues. The pectinolytic enzymes are also used in canning of orange segments. In the customary procedure, sodium hydroxide solution is used in deskinning of the segments. This causes considerable loss of soluble solids and the percentage of broken segments is also high. When pectinolytic enzymes are used deskinning is completed within 90 to 120 minutes. Pectinases are also used in sugar extraction process from date fruits (Bahramian *et al*., 2011). Enzymes can be used economically if proper strategies are used for their optimization during clarification of fruit pulp (Vaillent *et al*., 2001).

Other important processes where pectic enzymes are utilized are: in the preparation of hydrolysed products of pectin (Altermatt and Deuel, 1952), in the retting of textile fibres, in the refinement of vegetable fibres during starch manufacture, in the curing of coffee, in cocoa and tobacco (Kertesz, 1951), as an analytical tool in the estimation of certain plant products and to degrade plant cells in cytological and tissue culture work (Hohl, 1948).

Conclusion

Increased awareness in health issue leads to increased in consumption of fruit juices and other

natural products as an alternative to the traditional caffeine containing beverages such as coffee, tea or carbonated soft drinks. Accompanying the increase in quantity of consumption there has been a parallel increase in the demand of variety fruit juices including exotic and tropical fruit juices in the market. But tropical fruits are usually too pulpy and pectinaceous to yield juice by simple pressing or centrifugation. Such techniques involve expenditure of excessive amounts of energy and results in meagre juice yield. As an alternative recently enzymes particularly pectinolytic enzymes are effectively utilized in fruit processing industry. These enzymes are utilized for a variety of manufacturing processes wherever plant material is involved.

Enzymatic processing makes the juice not only clear by breaking down the pectin and allowing the suspended particles to settle down, but also eliminate undesirable changes in colour, bouquet and stability. Pectic enzymes are also helpful in other processes such as in the manufacture of fruit purees, wine clarification, deskinning of orange segments. The costs of producing clarified juice would also be highly competitive, compared with other established processes and would have a higher production yield. For tropical fruit juice industries, it represents a real alternative method to diversify production and increase market share.

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