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Role of Enzymes in Fruit juices Clarification during Processing: A review

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Abstract

Consumption of natural fruit juice as an alternative to the traditional caffeine-containing beverages such as coffee, tea or carbonated soft drinks has increased in recent years very promptly which endeavour to promote and improve its production to be competitive for both domestic demand and export markets. Enzyme is an essential tool in juice processes, both in terms of quality improvement and cost saving. These can be obtained from plants, animals and microorganisms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Fruit and vegetable juice production is nowadays unthinkable without the use of enzymes. The degradation of plant cell walls by enzymatic treatment results is easier release of the components contained in cells. The cloudiness in the juices is mainly caused by the presence of polysaccharides such as pectin and starch. Commercial pectic enzymes and other enzymes like amylases, cellulases, glucose oxidase etc. are now an integral part of fruit juice technology. They are used to extract, clarify, and modify juices from many fruits including berries, stone, and citrus fruits, grapes, apples, pears. Therefore, enzymatic treatment is an effective way to reduce the cloudiness in the fruit juices.

Keywords: fruit juice, cloudiness, clarification, enzyme

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1. Intruduction

Over the years, juices have garnered sufficient amount of attention as a healthy drink. Mirroring the fact, per capita consumption of juices exhibited robust growth over the past few years. The major problem associated with it is haziness in fruit juices. Fruit juices appear cloudy, though depending on fruits, especially due to occurrence of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (Vaillant *et al.*,, 2001). Clarity of juice is a determinant factor for marketing, the fruit juice industry has been investing to optimize this feature (Tribess and Tadini, 2006).

The production of clarified and stable juice is a critical factor for the beverage industries. Clarification is associated with removal of pectic substances and other polysaccharides as well as carbohydrates (Vaillant *et al.*,, 1999; Kashyap *et al.*,, 2001; Landbo and Meyer, 2007). Generally, centrifugation, membrane filtration or addition of clarifying agents such as gelatin, bentonite, silica sol and polyvinyl pyrrolidone are the keys applied for clarification (Chatterjee, 2004). However, the drawbacks of these methods are time-consuming, inefficiency and requirement of laborious efforts. Besides, addition of fining agents and filter aids may lead in slight change in flavor which is unacceptable (Alvarez, 2000). The development of membrane separation processes to replace the traditional approach has enabled the automation of the whole production resulting in lower labor requirement and a considerably shorter process time than the traditional process. But membrane fouling is a major drawback associated with it. An alternative procedure in this chain is enzymatic treatment of fruit juices (Grzeskowiak-Przywecka and Slominska, 2007).

Enzymes are proteins that act as biological catalysts. A catalyst is a substance that speeds up the rate of a chemical reaction, but is not itself changed by the reaction (Neet, 2008). Enzymes act on colloids yielding a crystal clear juice with the appearance, stability, mouth-feel, taste and texture characteristics preferred by consumers. Enzymes mainly used for clarification purposes include pectinase, amylase, cellulase, hemicellulase, xylanase, glucanase, glucosidase, amyloglucosidase and arabanase, etc. In 1995, the estimated market value of enzymes was more than 1 billion US dollars which was expected to be double in 2005 (Godfrey and West, 1996). Till now, the enzymes have been used for clarification of juices of many fruits like orange, apple, banana, lemon, grape, carombola, sapodilla, berries, etc.

2. Types of enzymes

Out of 4000 known enzymes approximately 200 are used commercially. Europe contributes around 60% of the world enzymes. They are used to extract, clarify and modify juices from many fruits, including berries, stone and citrus fruits, grapes, apples, pears and even vegetables. There are so many enzymes used to clarify fruit juices include pectinases, amylases, cellulases, arabanase, fructozyme, dextranase, protease, amyloglucosidase, etc. The available commercial pectinase preparations used in fruit processing generally contain amylases, cellulases and a mixture of pectinesterase (PE), polygalacturonase (PG), and pectinlyase (PL) enzymes. Some of these are described below:-

2.1. Amylases

Amylases are digestive enzymes hydrolyzing glycosidic bonds of starch. Amylases are among the most important enzymes and are of great significance for biotechnology. They contribute around 25% of the world market enzymes (Rao *et al.*,, 1998). Several sources are used for the manufacturing of amylases including plants, animal and chemicals. But the microbial enzymes have proved the most significant benefits to the food and beverage industries (Tanyildizi, 2005). Table 1 shows the list of micro-organisms used generally. In beverage industry, these have potentially been used in clarification of fruit juices. They provide a wide range of temperatures of operations required for various industrial applications (Haki and Rakshit, 2003). Amylases can be divided into two categories, endoamylases and exoamylases. Endoamylases randomly cleave the interior of the starch molecule whereas exoamylases act from the non-reducing end successively resulting in short end products (Gupta *et al.*,, 2003). There are three types of

enzymes namely: α-amylase, β-amylase, γ-amylase. Amongst all α-amylase are one of the most popular and important form of industrial amylases.

Table 1: Production of α-amylase enzyme using specific micro-organisms.

Name of micro-organism	**Type of fermentation**	**pH**	**Tempe-rature**	**Reference**
Bacteria				
Bacillus amyloliquefaciens	SMF	7.0	33ºC	Tanyildizi *et al.*, (2007)
Bacillus subtilis DM-03	SSF	6.0-10.0	50ºC	Mukherjee *et al.*, (2009)
Bacillus subtilis KCC103	SMF	6.5	37ºC	Rajagopalan *et al.*, (2008)
Bacillus subtilis JS-2004	SMF	7.0	135ºC	Asgher *et al.*, (2007)
Bacillus sp. KR-8104	NA	4.0-6.0	70-75ºC	Sajedi *et al.*, (2005)
Bacillus sp. PS-7	SSF	6.5	60ºC	Sodhi *et al.*, (2005)
Bacillus subtilis DM-03	SSF	6.0-10.0	50ºC	Mukherjee *et al.*, (2009)
Fungi				
Aspergillus niger UO-1	SMF	4.95	50ºC	Hernández e al. (2006)
Aspergillus niger ATCC	SMF	5.0/6.0	30ºC	Dakhmouche *et al.*, (2006)
Aspergillus oryzae	SSF	7.0	35ºC	Rahardjo *et al.*, (2005)
Thermomyces lanuginosus	SSF	6.0	50ºC	Kunamneni *et al.*, (2005).
Thermomyces lanuginosus		6.0	50ºC	Jensen *et al.*, (2002).

SMF-submerged fermentation, SSF- solid state fermentation, NA-not available

Table -2: Specific conditions required for production of pectinase using micro-organisms

Microorganism	Substrate	Type of fermentation	pH	Temperature	Time (hrs)	Reference
Bacillus sp. MFW7.	Cassava waste		6.5	35ºC	96	Mukesh kumar *et al.*, (2012)
Aspergillus niger	soil	SMF	6.8	30ºC	24-48	Reddy and Sreeramulu (2012)
Aspergillus Niger	Wheat bran	SMF	3.8	30 ºC	24-48	Khairnar *et al.*, (2009)
A. niger	wheat bran	SMF	5.5	30 ºC	48	U. A. Okafor *et al.*, (2010)
A. niger	pectin	SCF	4.5	35 ºC	4 days	Abbasi & Fazaelipoor (2010)
A. japonicus	NA	NA	4.0-5.5	50ºC	NA	Hasunuma *et al.*, (2003)
Penicillium sp EGC5	Orange-sugar cane bagasse and wheat bran (1:1:1)	SSF	4.5-5.5	40ºC	NA	Martin *et al.*, (2004)

SCF- Surface culture fermentation, SSF- solid state fermentation, SMF- submerged fermentation

Table 3: Micro-organisms used for the production of cellulase

Micro-organism	Substrate	Type of fermentation	References
Bacillus pumilus eb3	Carboxymethyl cellulose	NA	Ariffin *et al.,* (2006)
Aspergillus spp.	Wheat Straw, Wheat Bran, Rice Straw and Corn Cob for diff sp.	SSF	Abo-State *et al.,* (2010)
Chaetomium sp.	Cotton seed	SSF	Ravindran *et al.,* (2010)
Chaetomium sp.	CMC	SMF	Ravindran *et al.,* (2010)
Paenibacillus species)	CMC		Maki *et al.,* (2009)
Aspergillus niger	Rice straw	SMF	M. Charitha Devi and M. Sunil Kumar(2012)

Table- 4: Micro-organisms used for the production of amyloglucosidase

Microorganisms	**Type of fermentation**	**Substrate**	**pH**	**References**
A. Oryzae	SSF	Wheat bran and sugar cane bagasse) at the ratio of 1:1	5.0	Parbat and Singhal (2011)
Aspergillus niger	SSF	Wheat bran	4.8	Imran *et al.,* (2012)
Rhizopus sp.	liquid culture	yeast extract and polypepton	4.5	Nahar *et al.,* (2008)
Aspergillus niger UV-60	SMF	Food waste (FW)	–	Wang *et al.,* (2008)
Aspergillus oryzae	Wheat bran supplemented with 1%, (w/w) starch, 0.25%, (w/w) urea	Wheat bran supplemented with 1%, (w/w) starch, 0.25%, (w/w) urea	6.0	Vasudeo Zambare, 2010
Aspergillus awamori	SSF	Wheat bran containing 25% starch and 14% protein (w/w, d b	4.0	Bertolin *et al.,* (2003)
Aspergillus awamori		Molasses and wheat flour		Al-Turki *et al.,* (2008)

2.1.1. α-Amylase

α-amylases (endo-1,4-a-D-glucan glucanohydrolase), are extracellular endo enzymes that randomly cleave internal α-1,4-glycosidic linkages in starch and ultimately generate low molecular weight products, such glucose, maltose and maltotriose units (Kandra, 2003; Rajagopalan, 2008; Ghorai, 2009). These are very specific catalytic groups act neither on α-1,6-linkages nor terminal glucose but are able to produce a mixture of short products containing α-1,6-linkages and α-1,4-linkages (Whitcomb, 2007). These are becoming very popular because of their widespread applications. They are utilized in starch saccharification textile, food, fermentation and distilling industries (Pandey *et al.,*, 2000). These are Ca metelloenzymes requiring calcium to act on the substrate. (Manelius and Bertoft, 1996).

Table- 5: Application of various enzymes for fruit juices

Fruit juice	Enzymes	Optimum pH	Operating conditions	Results	References
Banana juice	0.084% pectinase and 0.02% amylase	4.6	60 C for 1 hr for amylase and 43.2ºC for 80 min for pectinase	Clarity (0.006 Abs), turbidity (0.92 NTU) and viscosity (1.89 cps).	Lee *et al.*, (2005)
Mausambi juice	.0004 % pectinases from Aspergillus niger	3.6	41.89ºC	Apparent viscosity (1.11 Mpa s), Clarity (83.97%)	Rai *et al.*, (2004)
Sapodilla juice	0.1% crude pectinase along with hemicellulase and cellulase	4.7	40ºC for 120 min	lowest turbidity, absorbance value and viscosity with highest L value	Sin *et al.*, (2006)
Carombolla juice	0.10 % crude pectinase along with b-galactosidase, chitinase and transgalactosidase	4.02	30ºC for 20 min	minimum turbidity, absorbance, viscosity and maximum L* value.	Abdullah *et al.*, (2007)
Apple juice, butia palm fruit juice blueberry juice and grape juice	919 U/ml pectinase	5.0	30ºC for 60 min	Clarity of juice increased. Blueberry juice showed 40% decrease in turbidity	Ivana *et al.*, (2011)
Kiwifruit juice	0.025 % Amylases+ 0.025 % pectinases+ 0.05% mash enzyme	3.52 ±.02	50ºC for 2 hr	Clarity increased and haziness reduced.	Devina *et al.*, (2009)
Apple juice	3.4 U/ml low-temperature-active endo-polygalacturonase	3.5	40ºC for 60 min	Reduced the intrinsic viscosity of apple juice by 4.5%, and increased the transmittance by 71.8%	Peng *et al.*, (2011)
White grape juice	.048 % Commercial pectinase	NA	27-30ºC for 30 min	Clarity increased up to 98-99%, viscosity and total phenols were reduced by 25% and 32% respectively.	Hassan *et al.*, (1992)
Peach juice	Pectinase	NA	25ºC for 60 min	Viscosity (68%) reduction	Santin *et al.*, (2008)

Guava juice	Pectinase 0.11 %	NA	41.30C incubation time of 115.98 min	Clarity (1.34 Abs), turbidity (1.07 Abs), titrable acidity (0.525%), viscosity (2.4 cps)	Sevda *et al.*, (2012)
Guava juice	Commercial fungal enzymes pectinase 0.7%	NA	*45.35C, incubation time 7.23 h*	*Clarity (34.54%) and viscosity 1.24 cps*	Sawinder *et al.*, (2011)
Passion Fruit Juice	.001 % Amylase + Celluclast+ Pectinex (in equal quantitities)	NA	50ºc for 30 to 120 min	53 % reduction in viscocity	Rui *et al.*, (2012)
Orange juice	pectinase produced by Rhizopusoryzae 2 %	3.54	40°c for 1h	Least turbidity of 36 NTU and 51% reduction in viscosity	Kareem and Adebowale (2007)
Papaya puree	Pectic enzymes produced from Saccharomyces cerevisiae (ATCC 52712) 32 mg of total protein per 200 g of mash	5.5 -6.0	30 min	Maximum rate of juice flow (25 ml/min	Dzogbefia and Djokoto (2006)
Carrot juice	Pectinase produced from Aspergillus aculeatus, 100 ml/t	4.5-5.0	40-45ºC. The incubation time is 80 min	decline in Turbidity from 240.33 to 187.33 NTU and viscosity from 2.54 to 2.09 mpa·s	Liao *et al.*, (2007)
Passion fruit pulp	0.0136 % Pectinase and 0.0024% amylase w/w passion fruit pulp	3.8	49ºC for 25 min	increase in turbidity and decease in viscocity was found from 550 to 728 NTU and 2.4 to 1.82 cp	Mugwiza and Qian (2009)

2.2. Pectinases

Pectin is a high molecular weight polysaccharide consisted of linear chains of α-1, 4-linked D-galacturonic acid residues present in middle lamella of plant cell wall. (Torres-Fanela *et al.*,, 2003). The presence of pectic substances is a major factor responsible for cloudiness in juices (Borrego, 1989). The impact of increase in viscosity due to these substances is in-efficiency of filtration during juice processing (Rombouts, 1980). Pectic enzymes are used to degrade such pectins. They have a considerable effect in increasing the juice yield and in clarification of fruit juices (Whitaker, 1990). The hydrolyzation of pectins by pectinase results in pectin-protein complex which further flocculates. Thus fruit juice of lower viscosity and turbidity is obtained. These are broadly classified in three groups. They are Pectin methyl esterase (PME), Polygalacturonase (PG) and Pectate lyase`s (PAL) (Haidar Abbasi, 2010; Ranveer Singh, 2005). Microbial pectinase share 25% in global food enzymes market (Table 2). These are obtained from fungal resources especially A. niger (Naidu, 1998; Kotzekidov, 1991).

2.3. Cellulases

Cellulose is the most abundant constituent of the plant (Saha, 2002). It is an acrystalline polymer of D-glucose residues linked by β-1, 4 glucosidic

linkages associated in a highly ordered manner (Ghose, 1984). Thus break down of cellulose becomes difficult and needs additional effort. Cellulases are the enzymes used for degradation of cellulose and are grown in cellulosic materials (Lee and Koo, 2001). Complete cleavage of cellulose requires synergistic effect of all componenets of cellulases. Cellulase is made up of three major components as: cellobiohydrolase (exoglucanases or exo-β-glucanase), endoglucanase (carboxy methyl cellulase (CMCase) or endo-β-glucanase) and β-glucosidase (Bhat, 2000). The combined actions of endo- and exo-glucanase cleave the amorphors region of cellulase into glucose units whereas β-glucosidase acts on the crystalline region giving the same end product as glucose. Amorphous region is hydrolyzed more easily than that of crystalline region (Muhlenthaler, 1967). The exoglucanase and endoglucanase cleave the β-1, 4 glucosidic bonds from the chain ends and internally respectively (Davies and Henrissat, 1995). β-glucosidase breaks down cellobiose and short cello-oligosaccharides from non-reducing end to remove glucose (Bashir, 1989). These enzymes act synergistically to cleave the glycosidic bonds of cellulose. Table 3 shows the Microorganisms used for the production of the enzyme.

2.4. Amyloglucosidase

Amyloglucosidase is a hydrolyzing enzyme which belongs to the amylase family. It consecutively hydrolyzes α-1, 4 glycosidic bonds from the non-reducing ends of substrate starch releasing the glucose as end product (Fogarty, 1983). It also has the ability to hydrolyze α-1, 6 glycosidic bonds and α-1, 3 glycosidic bonds but to a lesser extent resulting in the same end product glucose (Mertens and Skory, 2006). Hence it can completely break down starch into glucose. It is also known as 1, 4-α-glucosidase, γ-amylase, lysosomal α-glucosidase, acid maltase; exo-1, 4-α-glucosidase, glucose amylase; γ-1, 4-glucan glucohydrolase, acid maltase and 1,k-D-glucan. It plays an important role in starch processing. Its importance is next to protease in the usage and marketing among all the industrial enzymes across the world (Parbat and Singhal, 2011). It is majorly produced by fungi like Aspergillus awamori, A. saitoi, A. oryzae. Rhizopus sp, Mucor sp, Penicillium sp., and yeast (Sen and Chakarabarty, 1984) (Table 4). Among these, Rhizopus spp. shows the best results for production (Jin *et al.*,, 1999). It can also be used for starch liquefaction as well as high fructose and glucose syrup production (Nguyen *et al.*,, 2002). Whole grain can be used as substrate for alcohol industry for production of alcohol. It is utilized in baking, juice, beverages pharmaceuticals (Raimbault, 1981) and potentially in textiles and paper industries (Quang *et al.*,, 2002).

2.5 Application of enzymes in clarification of fruit juices

The application of enzymes will depend on kind of polysaccharides present in different fruit juices. Pectin is the major obstacle in citrus juices as well as in apple juice (Prathyusha and Suneetha, 2011). Pectinase plays a major role in clarification at this condition. Whereas amylase is preferred where the difficulties are encountered due to the presence of starch. Sometimes these enzymes are used in combination with cellulose and hemicellulase to effectively reduce haziness in juices. The major factors influencing the activity of enzyme are temperature, incubation period, pH and concentration. These parameters vary for different fruits. The incorporation of enzymes in fruit juice processing has been proved cost effective and efficient method. Till now, these have been applied in many fruit juices. Table 5 demonstrates the few of the studies conducted on fruit juices using various enzymes.

3 Conclusion

Enzymes are becoming a basic need for the beverage industries now-a-days. The production of enzyme will depend on various factors including type of strain. Strains useful for the continuous process need to be discovered. Substrate present in the fruit juice is the deciding factor for type of enzyme to be added. There is a great potential for application of enzyme kinetics in the future era.

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