International Journal of Multidisciplinary Approach and Studies

ISSN NO:: 2348 – 537X

Juice Extraction from Fruits and Vegetables and their Enzymological Aspects

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ABSTRACT:

Fruits and vegetables is the most precious items in the food processing and technology sector to minimize or prevent the various illness related to our body system. India has huge source of fruits and vegetables because of large production. India stand 2nd position for their production globally, but processing is still low (limited to 2.2%). Due to modern life style people doesn't want to take fruits in fresh form so that juice is become more popular with time span as the generation is growing. This study includes the juice extraction process as well as enzymatic aspects including different types of enzymes which play important role to change the sensory attributes of juices during their extraction. Processing of fruits and vegetables will also help to improve the economic level of India.

Key-words: *Fruits, Vegetables, Processing of Fruits, Processing of Vegetables, juice extraction, enzymatic aspect of fruits and vegetables etc.*

1. INTRODUCTION

Fruits and vegetables both fresh and processed are vital in improving the nutritional quality of our diet. India is the second largest producer of fruits & vegetables in the world, after China with the 71.516 million metric tonnes of fruits and 133.738 million metric tonnes of vegetables (National Horticulture Board, during 2009-10 India). The area under cultivation of fruits stood at 6.329 million hectares while vegetables were cultivated at 7.985 million hectares. Amongst fruits, the country ranks first in production of bananas (31.24%) papayas (42.11%), mangoes (39%) lemons and limes. Production has been steadily increasing due to advancement in production technology, but losses have remained static at 30-35%

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(http://www.apeda.gov.in). This means that the loss of fruits and vegetables is also increasing with the increase with their production.

India has the distinction of producing almost all-tropical and exotic fruits and vegetables because of varied climatic conditions. Due to the short shelf life of these crops, most of fruits and vegetables perish during harvest, storage, grading, transport, packaging and distribution. Only 2.2% of these crops are processed into value-added products (www.nhb/2009-2010). During 2010-11, India exported fruits and vegetables worth Rs.3856 crores which comprised of fruits worth Rs.2635 crores and vegetables worth Rs.1221 crores (http://www.apeda.gov.in).

Hence, there is a need for maximum commercial utilization of fruits and vegetables and to adapt production and marketing activities to the requirements of the world market and to cater to domestic demand which, over the past few years, has been increasing because of various socio-economic factors. If the nutritive value of the processed food products could be maintained, this sector will emerge as a major value-added food industry (http://www.apeda.gov.in). Processing is very important for fruits having the largest production (for minimize the post harvest losses).

2. HISTORICAL BACKGROUND

The Indian have used green juice (extracted from green fruits and vegetables e.g. green mango juice) in ceremonial practice for more than two thousand years. 1930's - The Norwalk Juicer, the worlds' first juicer was invented by Dr Norman Walker (www.en.wikipedia.org).1954 - Champion Juicer, the worlds' first masticating juicer, was invented. The Champion juices almost every type of vegetable, even leafy ones. 1993 - The worlds' first twin gear juice extractor developed by Mr. Kim.

JUICE AND THEIR TYPES: Juice is the liquid that is naturally contained in fruits and vegetables. Juice is rich in vitamins and minerals (www.en.wikipedia.org). Fruit juices and squashes are popular products in both rural and urban areas. Most fruits can be used for the production of juice. When pasteurized properly and stored in sealed containers, fruit juices have a shelf life of several months. Juice is categorized in to forms (i) Natural/Pure Juice- It is the juice, as extracted from ripe fruits and containing only natural sugars; (ii) Sweetened Juice- It is a liquid products which contains at least 85% juice and 10% TSS.

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The juice recovery from pulp varies with the variety and maturity of fruit and method of juice extraction. Generally three methods of juice extraction are employed viz. cold, hot and enzymatic methods (Joshi *et al.*,1991, Soleha *et al.*,1994).

Most of the Guava produced around the world is consumed fresh. Marketing of processed products such as puree, paste, canned slices in syrup or nector is limited (Jatiani *et al.*,1988). Clarified and cloudy guava juices are currently produced and may have greater market potential, but optimal process conditions for these products have not been determined.

The use of enzymes to maximize the yield of cloudy juice and promote clarification is uncommon in the production of guava juice. Commercial preparatios containing pectinases, arabinase and cellulase may benefit guava juice production. Pectinase assists in pectin hydrolysis, which causes a reduction in pulp viscosity and a significant increase in juice yield. Pectin methyl esterase (PME) and polygalacturonase (PG) are pectinases which release carboxylic acids and galacturonic acid during enzyme treatment, which may lead to a decrease in the pulp pH (Zoghbi *et al.*,1992).

Guava juice can be used in the manufacture of a clear guava jelly or in various drinks. A clear juice may be prepared from guava puree that is depectinesed enzymatically. About 0.1 % pectin – degrading enzyme is mixed into the puree at room temperature; heating of the product at approximately 120^{0}F will greatly speed the action of the enzyme. After 1 hr. clear juice is separated from the red pulp by centrifuging or by pressing in a hydraulic juice press. A batch type or continuous flow centrifuge can be used on the depectinised puree with no further treatment. The clear juice after centrifuge or after press can be preserved by freezing or pasteurization in hermetically sealed cans.

A significant portion of the population prefers a grit-free, clear, haze-free guava juice. Clarified guava juice may be more acceptable to the general population, and may be used in the manufacturing of clear guava nector or jelly, clear guava powder or a mixed fruit juice blend. There is also potential for use of an instant guava powder in formulated /drinks, baby foods and other products. Transportation costs would be reduced significantly when shipping this product to distant markets. However, information about guava powders does not exist in the literature. Guava has delicate color and flavor properties and drying operations must be carefully designed to maintain these (Chopda and Barrett, 2001). Specific pasteurization conditions are required to produce high quality juice in terms of nutrients content and taste, and also to make sure it is safe for consumption (Zainal, *et al.*,1999).

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International Journal of Multidisciplinary Approach and Studies ISSN NO:: 2348 – 537X

With this in view and need to develop satisfactorily and techno-economical method for the preparation of clarified juice from guava, the present investigation was planned to optimize an appropriate enzyme concentration, incubation time and temperature for pectinolytic liquefaction of guava pulp for higher yield of clarified guava juice. Some review on important fruits and vegetables are given below -

Table 1: Research findings by various researcher related to juice extraction and enzymatic aspects

S. No.	Research findings	References
1	Effects of carbonation and sonication on the quality of guava juice for selected physicochemical properties such as colour, cloud stability, pH, acidity, total soluble solids, polyphenoloxidase activity acid content and microbial stability. Ascorbic acid content was found to be significantly ($P < 0.05$) higher in samples treated with carbonation and sonication than in the control. Nevertheless, sonication coupled with carbonation was not an efficient treatment for microbial inactivation at room temperature.	Cheng *et al.*, (2007)
2	Highly concentrated guava juice was prepared, which involves addition of Aloe arborescens to the juice in order to reduce astringency and concentration using a conventional plat type heat exchanger and a filling nozzle. The method avoids problems associated with clogging of the equipment with concentrate juice, heat induced deterioration of juice quality and yield losses due to inclusion of air during filling.	Yasusato and Nakazato (2006)
3	Evaluated the kinetics of cooked flavour development (CFD) during thermal treatment of a guava beverage within a temperature range of 85^0C to 90^0C. A trained sensory panel and R-index test were used to determine the temperature dependence of CFD. At 90^0C, the treatment time required for the panel to detect a change in flavour was 119 s, whereas 184 s and 248 s	Alvaro *et al.*,(2005)

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	were needed for detecting the flavour change at 87.5^{0}C and 85^{0}C respectively.	
4	In enzymic processing of banana, litchi and guava pulps was standardized for production of completely clarified juice, which is free from sediment after bottling.	Shamala *et al.*,(2005)
5	Optimization of conditions for pectinolytic liquefaction of guava (Var. Lucknow 49) pulp to obtain higher yields of clarified juices. Effects of pectinase (polygalacturonase) concentration (0.5-2.5%), incubation time (14-22 hrs), pH (4.0-6.0) and temperature (27-39^{0}C) were studied. Guava juice yield varied from 72 to 94%. Levels of pectinase, incubation time and pH had significant effects on guava juice yield. Maximum juice yield (94%) was obtained at 2% enzyme concentration, 20 hrs incubation time, pH 4.5 and at 30^{0}C temperature. the multiple response optimization showed optimum juice yield to be 95.1%.	Diwan and Shukla (2005)
6	Thermo-physical properties were determined at concentrations between 10 and 40 ^{0}Brix and temperatures between 30 and 80^{0}C. The apparent viscosity and density decreased with increase in temperature. While the specific heat capacity increased with increase in temperature. However the thermal conductivity was not influenced by temperature. The apparent viscosity and density increased as concentration increased.	Shamsudin *et al.*,(2003)
7	The vacuum concentration method was found to give to a superior product in terms of tannin and total sugars contents, retention of ascorbic acid and extent of non-enzymic browning. During storage, decrease in total acidity tannin contents and levels of ascorbic acid were observed.	Harsimrat *et al.*, (2003)
8	Clarity of juices extracted in the presence of pectinase was also higher than those extracted in its absence. Ascorbic acid content increased with pectinase treatment in guava juice, but decreased in banana and plum juices. Pectinase increased TSS and	Ali and Essa (2002)

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	decreased total insoluble solids in all juices. pH was unaffected by enzyme treatment, but titratable acidity was greater for guava and plum juices processed using pectinase, it decreased on pectinase treatment of banana juice. Conversely, the TSS/acid ratio, an important measure of juice quality, of banana juice increased while that for guava and plum juices decreased with enzyme treatment. Enzyme treatment reduced colour values for all juices. Addition of pectinase reduced viscosity and improved filtration properties of all juices. Viscosity of pectinase treated juices decreased with increase of temperature and viscometer spindle speed (30-70^{0}C and 0.6-60 rpm, respectively).	
9	It was found that the juice exhibits pseudo-plastic behavior in the range of shear rate between 40 to 160 s^{-1} and within pasteurization temperature of 60-90^{0}C. While the flow behavior index and density increased. Consistency coefficient, thermal conductivity and specific heat capacity decreased with increasing temperature. The correlation coefficients ranging from 0.75 to 1.00. The linear regression equations or models for consistency coefficient, flow behavior index, density, specific heat capacity and thermal conductivity were determined with correlation coefficient ranged from 0.8078 to 0.9597.	Zainal *et al.*,(2001)
10	Application of pectinex ultra SP-L was optimal using 700 ppm enzyme for 1.5 h at 50^{0}C. Clarified guava juice was clearer (89.6 %) when prepared using ultrafiltration (MW cut off 40-60 kDa) rather than plate and frame filtration (82.8 %), however the latter was higher in both soluble solids and ascorbic acid. Clarified guava juice powders were made using freeze-drying, spray drying and tunnel drying. The freeze dried product had superior quality. However, the spray-dried product was stable and may be more economical.	Chopda and Barrett (2001)

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11	An RTS beverage having 15 % pulp, 14^0Brix total soluble solids and 0.3% acidity (as citric acid) was prepared, packed in cleaned pasteurized bottles and heat processed at 90^0C for 20 min. Physicochemical and sensory analyses of the RTS beverage were performed initially (0) and after 6 months of storage at room temperature (20-30^0C). After storage at room temperature, vitamin C content was highest (28.1 mg/100 g) in pure guava beverage, while carotene content was maximum (444.6 mg/100 g) in pure papaya beverage was acceptable after 6 months of storage at room temperature.	Tiwari (2000)
12	Effects of high-pressure treatment on guava juice, pectic substances and related juice characteristics with heat treatment. The viscosity and turbidity of guava juice pressurized at 600 MPa and 25^0C for 10 min. increased slightly, whereas the viscosity of juice heated at 95^0C for 5 min. decreased while turbidity increased. Heat treatment of juice decreased water and alkali soluble pectins and slightly increased oxalate soluble pectin.	Yen and Lin (1998)
13	Operating conditions of high pressure treatment (pressure 100-500 MPa), pressurizing time (5-60 min) and temperature (10-60^0C), repeated pressure treatment (1-3 cycles) and depressurizing time (rapidly, < 1 s or slowly approximately 30 S) on the inactivation of enzyme and increased by increasing the pressurizing time and repeated pressure treatment in a pressure range of 200-400 MPa and at 10 or 60^0C. Pressure treatment with 500 MPa at 10 or 25^0C for one cycle showed than did other pressurizing conditions. Depressurizing time had no significant ($P > 0.05$) effects on the inactivation of microorganisms and enzymes. Microorganisms in guava juice (12^0Brix, pH 3.9) were completely inactivated by pressurizing at 500 MPa and 10^0C for 15 min. polyphenoloxidase activity was inactivated.	Lin and Yen (1998)

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14	Clarification of guava juice using four pectinases (polygalacturonases, i.e. Rohament, PC, Rohapect, Al, Rohapect Bl and Rahapect D5L), Rohapect D5L was chosen for further studies due to its clarification properties. Volatile compounds in guava juices were isolated by steam distillation and solvent extraction and then identified by GC and GC MS. 53 compounds were identified, the major one being 3-carene, n-hexanal, trans-3-hexen-l-ol, beta-caryophyllene, alpha-copaene, alpha-humulene and beta-bisabolene. Effects of heating (45 ± 1^{0}C, 1 h) or enzyme treatment on volatile constituents of guava juice were also examined. Enzyme treatment decreased vitamin C content of guava juice and increased reducing sugar and total soluble solids contents and acidity, all changes were relatively small.	Pong *et al.*,(1996)
15	In the case of tomato, there must be a high polysaccharide content (cellulose and pectic substances), a considerable thickness of pulp, no discoloured parts and easy detachment of the skin and few seeds.	(Castaldo et al., 1995)
16	Mashed pink guavas from Ibiapaba plateau (Serra Grande) in Ubajara country, CE, Brazil, and the pulp treated with 600 ppm of a pectic enzyme at 45°C for 120 min. The pulp so-treated was pressed to give an average juice yield of 84.70%. The pressed juice was cloudy and pink in colour but, after addition of fining agents and filtrationn, a clear juice with a light yellow colour was obtained. This clear juice was preserved by the Hot-pack method.	Brasil *et al.*,(1995)

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17	Juices, nectors and fruit juice beverages from 11 types of fruit (papaya, guava, sour-sop, passion fruit, banana, orange, grapefruit, mandarin, tangelo, pineapple and mango) grwon in the Ivory Coast. Data are given for pulp yield, screened or centrifuged juice yield, nector yield, chemical composition of the juices, composition of fruit juices beverages and the juice content of juice based beverages. Yields and compositions of the individual juices and the derived beverages are discussed.	Mosso *et al.*,(1994)
18	Lye peeling caused reduction in pectin, tannins and ascorbic acid in both the pulp and juice and it also reducted the yellow index slightly in the juice. However lye peeling improved the sensory quality of guava juice and nector.	Khuradiya and Srivastava (1994)
19	Strawberries are soft fruit with a high pectin content of around 0.5 to 0.7% of fruit weight. Arabinose and galactose are the major neutral sugars, linked in arabino-galactan type II as pectin hairy-region side chains (Will et al., 1992). Strawberry pectin methoxylation ranges from 20% to 60% depending on raw material, justifying the use of PME for LM pectin formation *in situ*. Calcium content is relatively high (on average 300 ppm) but can also vary and can be in the fruit as bound or free form, depending on the ionic environment. Free calcium is only available for pectate formation. For this reason calcium is added in the Firmfruit process to complement the natural calcium, resulting in a more extensive pectate formation.	
20	Partially clarified guava juice concentrate was prepared from single strength guava puree (5.5^0Brix) of juice treatment with pectinase (2 h at 50^0C), extraction of juice with a rack-and-cloth press, and vacuum concentration to 23^0Brix. The concentrate had the following characteristics : Density 1.10, pH, 3.16, Total acids, 4.7%, ash 1.51%, moisture 72.4%, ascorbic acid, 867 mg% and viscosity, 4.4 cp.	Hodgson *et al.*, (1990)

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21	Combined effect of cold and hot method of pulp extraction in three varieties of guava fruit namely Chittidar, Allahabad Safeda and Lucknow-49 on preparation of nector and subsequent processing and storage change therein. Although hot method of pulp extraction did increase pulp recovery by 5 to 8 per cent but developed pink brown pigment, which further increased during storage. Pulp extracted without application of heat apparently did not show development of pink brown discoloration and nectors prepared from such pulp remained acceptable. Among the various Lucknow-49 showed least development of pink brown discoloration and scored superior marks.	Murari and Verma (1989)

3. JUICE EXTRACTION PROCESS

3.1 Selection Of Fruit: Only fully ripe fruit are selected. Over ripped and green fruits, if used, adversely affect the quality of juice (Srivastava and Kumar, 2005).

3.2 Sorting And Washing: Diseased, damaged or decade fruits are rejected or trimmed. Dirt and spray residues of arsenic, lead etc. are removed by washing with water or dilute hydrochloric acid (1 part acid: 20 parts water).

3.3 Juice Extraction: Generally juice is extracted from fresh fruit from crushing and pressing them. Screw-type juice extractors, basket presses or fruit pulpers are mostly used.

3.4 Juice Extraction Methods

(i) Manually

(ii) Mechanical: (a) Semi continuous process; (b) Continuous process

3.5 Fruit Juice Extractor: These machines are suitable for both fruits and vegetables such as mango, orange, apples, pineapple, tomato, aloevera, and more.These fruit juice machines and extractor suitable for crushing hard seedless fruits before pulping or juice extraction.

3.6 Deaeration: Fruit juices contains some air, most of which present on the surface of the juice and some is dissolved in it. Most of the air as well as other gases are removed by subjecting the fresh juice to a high vacuum. This process is called deaeration and the equipment used for the purpose is called deaerator. Being a very expansive method, it is not used in India at present.

3.7 Straining Or Filtration: Fruit juices always contain varying amount of suspended matter consisting of broken fruit tissue, seed, skin, gums, pectin substances and protein in colloidal suspension. Seeds and pieces of pulp and skin are removed by straining through a thick cloth or sieve. It is increase the appearance of the juice.

3.8 Clarification: Complete removal of all suspended from juice, as in lime juice cordial.

Methods of clarification-

- Settling
- Filtration
- Freezing
- Cold storage
- High temperature
- Chemicals
    1. Gelatin
    2. Albumen
    3. Casein
    4. Mixtures of tannin and gelatin
- Enzymes

3.9 Addition Of Sugar: All juices are sweetened by adding sugar, excepts those of grapes and apples. Sugar also act as preservative for the flavor, color and prolongs the keeping qualities.

Sugar based products classification-

a)	Low sugar	-	30% or below
b)	Medium Sugar	-	> 30% and < 50%
c)	High Sugar	-	50% and above

3.10 Fortification: Juices, squashes, syrup etc. are sometimes fortified with vitamins to enhance their nutritive value to improve taste, texture or color to replace nutrients loss in processing (Srivastava and Kumar, 2005).

Ascorbic acid=250-500gm per litre juice

(Ascorbic acid act as antioxidant).

Beta carotene =7-10 mg per litre juice

(Beta carotene imports attractive orange color).

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3.11 Preservation: Fruit juices and nectars are preserved by pasteurization but sometimes chemical preservatives are used (Srivastava and Kumar, 2005). Squashes, crushes and cordial are preserved only by chemicals in the case of syrup, the sugar concentration is sufficient to prevent spoilage.

3.12 Bottling: Bottles are thoroughly washed with hot water and filled leaving 1.5 to 2.5 cm head spaced.

3.13 Sealing: Sealing is done either with crown corks (by crown corking machine) or with caps (by capping machine).

3.14 Storage: Product should stored in cool and dry place.

4. ENZYMOLOGICAL ASPECTS OF FRUIT JUICE

- Enzymes are proteins with important catalytic activity, being involved in metabolic activity, being involved in metabolic reactions and obtained from different sources.
- Enzymes are either integrate part of the juice or added to them, providing number of advantages in the processes.
- The food and beverage enzyme market makes up 57 % of the overall enzyme market.

4.1 Uses Of Enzyme In Juice Processing Industry:

The main reasons for the use of enzymes are the following: -

- To improve the yield juice production
- To liquefy the entire fruit for maximal utilization of raw material
- To improve color and aroma
- To clarify juice
- To break down all insoluble carbohydrates such as pectins, hemicellulose and starch.

4.2 Types Of Enzymes In Fruit Juice Production: There are various enzymes using widely throughout the globe are given below -

- **Oxido-Reductases-** glucose oxidase and catalase (Reed and Underkofler, 1966).
- **Glucose oxidase-** used for removal of glucose or O_2 from fruit juice, wine and beer (Panesar et al., 2010).

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- **Catalase**- The shelf life of citrus fruit juices can be prolonged with the combination of the enzymes, glucose oxidase and catalase; another applications of catalase is the removal of O_2 from the air present in head space of canned/bottled.
- **Hydrolases-** It is the major enzyme used in the fruit juice industries. Generally hydrolytic enzymes, e.g., cellolases, xylanases, pectinases.
- **Amylases & pectinases** used due to high temperature stability. They are helpful to liquefying of starch (Reed and Underkofler, 1966).
- **Polygalacturonase** - It help to decrease the submerged feremention.
- **Xylanases** – This stains also showed the best production of pectinolytic enzymes during growth on citrus pectin or sugar beet pulp.
- **Cellulases** – It help to partial removal of hemicellulose, lignin and pectin.
- **Naringinase** enzymes may used to remove bitterness compound (naringin).
- **Naringinase** enzyme is responsible for reduce the bitterness in in the citrus fruits (Naringnin, the primary bittering water-soluble component found in the fruit membrane) (Reed and Underkofler, 1966).
- **Prunin**, which is 33 % as bitter as naringin, in turn is further hydrolyzed to naringenin and D-Glucose (Reed and Underkofler, 1966).
- Some workers have reported the use of immobilized naringinase in the removal of bitterness in kinnow juice (Panesar et al., 2010).
- The higher naringin conversion (95 %) was attained with naringinase concentrations higher than 800 mg/l and temperature higher than 30 °C.
- **Polyphenol oxidase and O-Methyltransferase:-**The browning reaction take place in the presence of o-diphenols, oxygen, and the enzyme polyphenol oxidase.
- **Pectic enzyme (**Srivastava and Kumar, 2005**)**: They are used widely in the disintegration of fruit pulps and for the clarification of juices and wines. Use of enzymes for clarification fruit juices was introduced in 1930. It had been originally assumed that pectic enzymes plat a dominant part in the reaction which leads to the clarification of fruit juices. For Thompson seedless grapes, pectic enzyme gives 12 % increase in the total yield.
- **Pectinolytic enzymes**: Commercial pectinolytic enzyme preparations produced from *Aspergillus niger* can be used in the clarification of fruit juices (Reed and Underkofler, 1966). Pectinolytic enzymes are used for the fruits-processing industry to increase

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yields, improve liquefaction, clarification and filterability of juices, maceration, and extract of plant tissues (Srivastava and Kumar, 2005). It not only remove the cloudiness in the final product, but also raise the juice yield by 15 % in the processing of white grapes and brings about beneficial changes in the flavour (Panesar et al., 2010).

- **Commercial enzymes:** Tailor-made industrial enzyme preparation can improve the economy of the process by reduction of the costs, increasing yields, improving the filtration rates with easier cleaning processes. Enzyme can reduce waste water production.

4.3 Applications Of Enzymes: Enzymes have many applications in fruit juices processing, including pre-peeling, pulp washing, peel juice extraction, juice clarification (Panesar et al., 2010). The largest industrial application of pectinases is in fruit extraction and clarification. A mixture of pectinases and amylases is used to clarify fruit juices decreasing filtration time up to 50 %. Treatment of fruit pulp with pectinases also showed an increase in fruit juice volume from banana, grapes and apples (Panesar et al., 2010). Pectinase in combination with other enzymes, celluloses, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction.

4.4 Future Prospects Of Enzyme Use In Fruit Processing Sector: Future studies on pectic enzymes should be devoted to the understanding of the regulatory mechanism of the enzyme secretion at the molecular level and the mechanism of action of different pectinolytic enzymes on pectic substances. Clarification of fruits, vegetables and sugar juices by microfiltration or ultra filtration allows the flow sheets to be simplified or the processes made cleaner and the final product quality improved. Novel enzyme immobilization technique will increase the yield in citrus juice processing.

5. CONCLUSION:

India is the second largest producer of fruits and vegetables in the world after China, still the juice produced have poor quality due to lack of sanitation practices; as well as the production of juice is also very low quantitatively in comparison to other countries. For coming out the worst condition, we should select the emerging technologies developed world wide for the enhancement of juice production as well as the best quality juice in all aspects like nutritional quality, sensory attributes and storage life/shelf life with the help of appropriate enzymes.

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