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Role of enzymes in fruit juice processing and its quality enhancement

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ABSTRACT

The demand of fruit juices is increasing day by day as a result of increasing health awareness among the people. Most of juice extraction processes are not producing satisfactory quantity and quality of juices. Enzymatic extraction of juices results in higher yield. The enzymes used are mainly pectinase, cellulase, hemicellase etc. Apart from promoting juice extraction, enzyme addition increases the release of various phenolics and other nutritionally important components in the juice. Juice appearance is also improved by enzymatic clarification. Juice quality in terms of reduced viscosity, decreased turbidity, and improved filterability is also improved. Enzymes are also used for debittering of citrus fruit juices and in preventing darkening of juices.

Key words: enzymes, juices, extraction, pectic, clarification.

INTRODUCTION

The post-harvest shelf life of maximum of fruits and vegetables is very limited due to their perishable nature. In India more then 20–25 percent of fruits and vegetables are spoiled before utilization. Despite being the world's second largest producer of fruits and vegetables, in India only 1.5 percent of the total fruits and vegetables produced are processed [1].

Fruits are mainly water (75–90%), most located in vacuole causing turgor to the fruit tissue, and fruit juice is prepared by mechanically squeezing or macerating fresh fruits without the application of heat or solvents. Fruit cell wall consists of crystalline cellulose microfibrils embedded in an amorphous matrix of pectin and hemicelluloses.

Juice is composed of water; soluble solids (sugars and organic acids); aroma and flavor compounds; vitamins and minerals; pectic substances; pigments; and, to a very small degree, proteins and fats. During ripening, fruits decrease in acidity and starch and increase in sugars. Juices are products for direct consumption and are obtained by the extraction of cellular juice from fruit; this operation can be done by pressing or by diffusion [2].

The fruit juice consumption has been associated to a healthful diet. Beyond its nutritional potential, these juices contain different compounds that show biological activities. Currently, the interest on phenolic compounds lies on its antioxidant capacity, which contributes to the protection of the harmful effects of oxidation stress on human health [3-4].

In the mid 1930s, when the fruit industry began to produce juices, the yield was low and the industry underwent difficulties to filtrate the juice and attain a stable clarification. From then, these difficulties were overcome through research using enzymes such as pectinases, cellulases and microorganisms that produce hemicellulases, and as the knowledge about fruit components increased. The mix of these enzymes is known as maceration enzymes [5].

Mechanical crushing of the pectin rich fruits results in a highly viscous fruit puree from which it is difficult to extract juice directly by pressing, due to the fact that mechanical crushing of the tissues gives juice that remains bound to the pulp to form a jellified mass. The pectin divides itself between the liquid phase and the pulp particles, causing an increase in the viscosity of the juice and facilitating water retention [6]. Thus in order to improve yield of juice with high aromatic quality in a short processing time, to increase its nutritional quality and to reduce the amount of waste, there is a need to degrade the pectin. For this reason, addition of pectinolytic enzyme preparations to the fruit pulp prior to pressing is a prerequisite for obtaining a satisfactory juice yield. The pectic enzymes help in degrading the pectin, structurally important support substances found in the middle lamellae and primary walls of plants, especially fleshy fruits and vegetables [7]. Pectins have high capacity for binding water because of the presence of hydrophilic (-OH) groups. Pectic enzymes disrupt these bonds enabling the water in the form of juice to be released during fruit juice extraction.

Apart from promoting juice extraction, enzyme addition increases the release of various phenolics and other nutritionally important components in the juice. [8] presented evidence for the occurrence of flavonol-glucosides in cell wall of flower petals. The presence of cell wall localized flavanoids and other phenolics has also been reported in Norway spruce needles [9]. [10] demonstrated that enzyme catalysed degradation of the cell wall polysaccharides in the skin fraction of black current juice press residues enhanced the extraction of antioxidant phenols. They indicated that various phenolics, including some flavonoids, are located within or at least tightly associated to cell wall material. They hypothesized that it might be possible that plant cell wall degrading enzymes release phytochmicals. Besides the enzyme maceration of the pulp, they results in a more cloud stable juice with lower viscosity and turbidity [11].

Fruit juices contain colloids that are mainly polysaccharides (pectin, cellulose, hemicellulose, lignin and starch), protein, tannin, metals etc [12]. These juices are the most important products of a number of fruits; they are well accepted by consumers due to its taste and nutritional properties. According to the physical and chemical properties of each fruit, their juices present difference degrees of natural turbidity. Juice turbidity is due to the presence of insoluble materials such as cell fragments that originate from its pulpy tissue and/or components that are not totally dissolved. These insoluble materials, which account for many of the juice taste, aroma and colour characteristics, vary in size, ranging from micro to larger pulp fragments. One of the major problems encountered in the preparation of fruit juices is cloudiness due primarily to the presence of pectins. The pectin can be associated with plant polymers and the cell debris which are fiber-like molecular structure. The cloudiness that they cause is difficult to remove except by enzymatic depectinization. The use of enzymes can enhance yield and help in the clarification of a wide range of juices such as apple, pear, orange and peach. Enzymes degrade pectin or cell wall, allowing the more juice for extraction per ton of fruit. In some cases, the addition of enzyme complex can result in improved yield for the juice and in a better extraction for colour, with the outcome being a high-end product [13]. Therefore in this review various aspects of enzymatic processing of juices have been discussed.

ENZYMES

In all cells reactions occur non-stop; due to their complexity, they should be slow at the temperature these reactions occur (approximately 37 ^{o}C). However, these reactions are very rapid, thereby indicating that in live cells there are catalysing substances which differ from inorganic catalysts in that they are much more complex substances [14]. These catalytic substances are enzymes which catalyse biochemical reactions at much faster rates.

Enzymes are biocatalysts of tertiary or quaternary globular protein structure [15] and are formed by long chains of aminoacids with peptide bonds [16]. They may react under moderate conditions of temperature and pH [17], and are present in microorganisms, animals and plants. Actually enzymes are applied in different areas: medical, food, textile, chemical, pulp and paper, to name a few [18].

Almost 4000 enzymes are currently known and from these approximately 200 are used commercially, most of them of microbial origin. The worldwide demand for enzymes is met by twelve key producers and by 400 smaller players. Approximately 60% of enzymes are produced in Europe [16].

Enzymatic synthesis in industrial scale is difficult to achieve due to the low operational life of biocatalysts and due to the negative effects of excess substrate and product over the catalytic activity [19].

The main advantages of using microbial cells as a source of enzymes are the high enzyme concentration that can be obtained through genetic manipulation and the adjustment of planting culture, the easy and quick screening of super-producer microorganisms, short fermentation cycles, low cost fermentation means, and the diversity of enzymes that catalyse the same eaction, thereby allowing flexibility in the conditions of usage [20].

USE OF ENZYMES IN JUICE PROCESSING

The presence of enzymes in food is very common, with oxireductases and hydrolases being the most important ones. In the oxireductase group, the enzymes that are of interest for the food industry are phenolases, peroxidases, catalases, peroxidases and lipoxygenases [21].

The use of enzymes in food processing is one of the greatest impacts of biotechnology on the sector [22]. Hydrolases and oxirecdutases are the most popular enzyme groups in the food industry and are involved in new product development and in the development and enhancing of organoleptic characteristics (aroma, flavour and colour) and in increasing yield [5].

In the agroindustrial sector the use of enzymes led to a process optimisation, reducing process-related energy costs, improving nutritional safety and quality of food, the development of new products and new applications for a number of farming products [23].

Overall in the food industry enzymes are used as components in food processing. Although they may remain in the final product they do not have a function. Therefore, they are not considered as food additives, thickening agents, sweeteners, antioxidants and others [24].

There are mainly two groups of enzyme which are used in fruit juice industry i.e. pectinases and amylases.

Amylases are very important biotechnologically with applications in industries such as textile, pulp and paper, leather, detergents, beer, spirits, bread production, cereals for children, starch liquefaction and saccharification, animal feed, and the chemical ⁄ pharmaceutical industry [25]. From 1970 onwards the juice industry started to process edible fruits in large quantities; such fruits are picked while unripe and stored for relatively long periods of time at low temperature. Under these conditions fruit pulp contain starch in sufficient amounts to cause turbidity or even gelatinize during processing, which makes productive procedures difficult. For this reason the demand for amylolytic enzymes, especially glucoamylase has increased in the sector [26].

Pectinases were one of the first enzymes to be used and are commercially applied on the processing of juices and wines [27. Its usage in the juice industry is related to a number of factors, especially in the clarification, maceration and extraction, stabilisation of the juice colour during storage and increased yield. Currently these enzymes account for approximately 20% of the worldwide enzyme market and are naturally produced by plants, fungi, yeast and bacteria [28]. These enzymes cause the degradation of long, complex molecules called pectins; today they have a key role in the juice industry [27].

Pectinases are classified in to three groups, according to the following criteria: Hydrolytic (hydrolases) or trans-eliminative (lyases) cleavage of the glycosidic bonds; endo (randomic) or exo (from the molecule end) mechanism of action and preference for substrates, pectic acid or pectin [5]. The three main types of pectinases are pectinesterase, depolymerising enzymes and protopectinases.

Sathyanarayana and Panda [29] had examined that pectinases are a complex group of enzymes that degrade various pectic substances present in plant tissues. Pectinases have potential applications in fruit, paper and textile industries. Apart from these industrial applications, these enzymes possess biological importance in protoplast fusion technology and plant pathology. Since applications of pectinases in various fields are widening, it is important to understand the nature and properties of these enzymes for efficient and effective usage. For the past few years, vigorous research has been carried out on isolation and characterization of pectinases. New affinity matrices with improved characteristics and affinity-precipitation techniques have been developed for purification of pectinases. Recently much attention has been focused on chemical modification of pectinases and their catalytic performance by various researchers. These studies are helpful in determining key amino acid residues responsible for substrate binding, catalytic action, and physico-chemical environmental conditions for maximum hydrolysis. This short review highlights progress on purification and understanding the biochemical aspects of microbial pectinases.

In addition to pectinases, other enzymes such as hemicelullases and celullases are used in the production of fruit juices, with the objective of optimising the processing of these products [24]. These enzymes are part of maceration enzymes acting on soluble pectin hydrolysis and on cell wall components,lower viscosity and maintenance of texture [5]. In accordance with the principles of the council of the European Union, hemicelulases and celulases are forbidden in Fruit Juice Directive (COUNCIL DIRECTIVE 2001/112/EC).

The enzymatic liquefaction technology is used to degrade cell wall polysaccharides, thereby releasing soluble compounds, especially the D-glacturonic acid and neutral sugars [30]. In pulp liquefaction, pectin and cellulose hydrolysis is due to the activity of polygalacturonases, pectin lyases, pectinesterases and cellulases. Upon the effect of these enzymes on the cell wall, neutral sugars such as D-arabinose, D-galactose, L-rhamnose and D-xylose, which are bound to pectic substances, are released and become soluble [31].

Other enzymes of interest on juices, which application has been described recently, are phenolases related to darkening juice, laccase related to phenolic compounds interfering in juice colour and turbidity; and naringinase and limoninase acting on compounds that cause bitterness in citrus juices.

Phenolases, also known as polyphenol oxidases, are intracellular enzymes that are present in plants, animals and fungi [32]. Its importance for the food industry is related to products of plant origin as this enzyme causes fruits and vegetables to darken [33].

Laccase is a polyphenoloxidase (p-diphenoloxydase, EC 1.10.3.2) which oxidises polyphenols, methoxylated phenols, diamines and a large number of other compounds, but it does not oxidise tyrosine. The typical reaction of laccase occurs with a phenolic compound undergoes oxidation to form a free radical. This radical may be transformed into quinone in the second step of the reaction. Quinone, which is originated from this free radical, may undergo polymerisation. Laccase may be obtained from bacteria, fungi or plants [23].

Naringinase is a complex enzyme consisting of α-rhamnosidase (EC 3. 2.1.40) and flavonoid-β-glycosidase (EC 3. 2.1.21). Naringinase can be produced by submerged culture or by a solid state fermentation by a number of microorganisms such as *Aspergillus niger, Aspergillus oryzae, Penicilium decumbens, Phanopsis citri, Aspergillus usamii, Cochiobolus miyabeanus, Rhizotonia solani and Rhizopus nigricans.* Commercially available nariginase is obtained from *A. niger*; its optimum pH ranges from three to seven for rhamnosidase activity and from four to six for β-glycosidase activity [34].

EFFECT OF ENZYMATIC TREATMENT ON JUICE YIELD AND JUICE CLARIFICATION

High juice yield is an important goal for juice manufacture. Many modern processes for fruit and vegetable juice production employ enzymes as important processing aids to obtain higher yields and clarity [35].

The use of maceration enzymes increases extraction yield and improves processing without extra costs. These enzymes are used after the cut of the raw material, in order to macerate the pulp to total or partial fruit liquefaction, thereby decreasing processing time and improving the extraction of fruit components. Upon the extraction, pectinases are added for clarification and decreased viscosity in order to facilitate filtration and concentration [36]. Total or partial liquefaction are not allowed on European market (COUNCIL DIRECTIVE 2001/112/EC).

Authors such as Downes [37] and Mikeladze & Kandelaki [38] reported that in fruits such as strawberries, cherries, raspberries and plums the juice is retained within the cell structure and must be released. When added to the fruit, enzymatic preparations promote the breakdown of cell structure and dissolve pectin compounds, thereby allowing the juice to flow easier. Kashyap *et al*. [27] reported that the production of these juices requires enzymatic depectinisation because the juice of these fruits has high pectin content, which remain as dissolved colloidal residues, thereby generating a viscous juice, making it difficult to clarify, filtrate and concentrate these juices. The addition of enzymes during the maceration step facilitates these processes.

Research conducted by Granada *et al*. [39] used pectinolytic enzymes in the extraction by cold pressing of clarified blackberry juices (*Rubus* spp. L.) of three different cultivars – Guarani, Tupi and Brazos, in search of increase yield, comparing it with the juice without the addition of enzymes. After clarification, the juices were analysed as to their chemical and organoleptic characteristics. The authors found that the use of the enzymatic preparation increased substantially juice extraction which, in average, for the three cultivars, was 81.73% and 53.79% for the control arm,

with statistically significant differences, and that the use of the enzyme was rather effective, resulting in an increase in extraction of approximately 52% in average for the three cultivars.

There are many research efforts involving the application of enzymes in apple juice. Apple juice is the most popular juice worldwide and it ranks second to orange juice in the USA, according to Bump [40]. The latter conducted research to determine yield in the extraction and determination of the physical, chemical and organoleptic characteristics of clarified apple juice in order to increase juice extraction, using three different treatments: with pectinolytic enzymes; with rice husk – fining agents in pressing and the association of both. The results indicated that the juice treated with pectinolytic enzymes were in all cases more effective than the treatment with rice husk.

Other authors [41-42] also used rice husk as pressing fining agent associated to the pectinolytic enzymes, obtaining a good yield when extracting apple juice.

Potential benefits of apple phenolic compounds for human health have been recognized in recent years. The application of enzyme treatment may be the key to increase phenolic compounds content in juices. Markowski *et al.* [43] applied two pectinolytic enzymes Rohapect MA PLUS and Panzym MK in apple juice processing and get to increase the phenolic compounds content.

Pectinolytic enzymes are used in the preparation of apple juice to facilitate the pressing process or juice extraction, helping in the separation of flocculent precipitates by sedimentation, filtration of centrifugation. Apple juice clarification is affected by pH, temperature, enzyme contact time with the pulp and enzyme concentration. Overall the time required to obtain clarification is inversely proportional to the concentration of the enzyme used at a constant temperature in the range of 5–50 ^{o}C, with treatment time of 2–16 h [27].

Yamaski *et al*. [44] showed that apple juice clarification can be obtained from a mixture of polygalacturonase and polymethylesterase only, without the presence of apple contaminant enzymes. The authors also mentioned that clarified apple juice can develop sediments during storage, especially refrigerated storage. This defect is known as late sediment and results from juices that are processed at temperatures that are higher than the storage temperature. This defect may also stem from the polymerisation of polyphenols and oxidation of proanthocyanins during pressing and grinding. Differently from the apple pectin, which is highly methylated, the orange pectin is only partially methylated. This happens because orange juice contains a large quantity of pectin esterase, which degrades the methoxy groups in the pectin molecules. In the process of extracting orange juice, pectinases can be used to obtain a higher yield of sugar and soluble solids, resulting in a higher juice yield and, therefore, lower viscosity.

Wang *et al*. [35] investigated the enzymatic maceration of blackberries with eight different pectinolytic enzyme preparations. Juice yields were increased greatly when macerated blackberries were treated with enzyme preparations, but no significant difference in yield was found among different enzymes. The amounts of anthocyanins and polyphenols in the juices as well as clarity of the juices were greatly varied because of different enzyme treatment. Juice prepared with Klerzyme 150 showed better clarity and greater amount of anthocyanins than the juices prepared with other enzyme preparations.

Ribeiro *et al.* [45] had examined that the use of enzymes in juice industry has contributed in increasing the yield and production of various types of juices. The addition pectinases aims in particular to degrade the pectic substances in the cell wall and middle lamella of the cells of plants, aiming to minimise the impacts of these compounds on the characteristics of the final product, such as colour, turbidity and viscosity. Enzymes able to remove bitterness of citrus juice, extract pigments, among other applications, have also had great interest in the juice industry

In the study conducted by Oliveira *et al*. [46] the apple juice processing by liquefaction yielded 83.5% with 16.5% of bagasse, while processing by pressing resulted in a yield of 64.5% with 35.5% of bagasse; therefore there is a 19 bp difference in favour of the enzymatic process, thereby demonstrating that the enzymatic liquefaction process presents practical advantages over the process of extraction by pressing, especially in connection with a high juice yield and a lower bagasse release. In the production of white grape and red grape juice, enzymes have an important role in depectinisation, in yield increase and in clarification. For red grapes, especially the Concord variety (*V. labrusca*), the use of an enzymatic preparation acts as auxiliary in the process to obtain the highest extraction of the pigment that is naturally present in the fruit [47].

Pectinases may always be used in production of fruit juice whereas cellulases are allowed only in some countries, not in the EU (COUNCIL DIRECTIVE 2001 / 112 / EC). Pectinases and cellulases may be added to grinding in order to improve yield in apple juice obtained in pressing, which results in an increase in the extraction of phenolic compounds approximately twice as much in the juice, as polyphenols procyadinin B_2, catechin and epicatechins, quercetins and floridzins may be associated to the cell wall [48]. The presence of enzymes during treatment before pressing in order to verify the effect of processing in the content of phenolic compounds on apple juice resulted in an additional yield of 90% in phenols than the control (340 ppm catechin) with no treatment [48]. The authors concluded that the use of pectinolytic enzymes in maceration improves juice extraction yield. The yield in the treatment of peeled fruit juice is low, even in the presence of maceration enzymes, thereby indicating that there are more phenols in the peel than in the pulp. The peel works as a backup that helps juice extraction when pressing the apples, which can explain the low values. With the peel, yields were higher in the presence of the pectinolytic enzyme (75%) and a longer maceration time (8 h), as the enzyme degrades pectin and facilitates the extraction of juice from cytosol. The application of an enzymatic system to extract apple juice also contributes to minimize significantly the elimination of solid residues of pectic nature, the main factor that accounts for the high water retention in the conventional extraction systems.

OPTIMIZING ENZYMATIC HYDROLYSIS PARAMETERS FOR MAXIMIZING JUICE YIELD

There is great potential of enzyme based extraction technology with the selection of appropriate enzymes with optimized operating conditions. Various enzyme combinations are used to loosen structural integrity of botanical material thereby enhancing the extraction of various components [49]. The enzymatic treatment for hydrolysis of pectic substances are influenced by several factors such as incubation time, incubation temperature and enzyme concentration [50-51].

Landbo *et al.* (52) had examined that the effects of different, statistically designed, enzymatic maceration treatments on juice yield, turbidity and phenol yield (total phenols and total anthocyanins) in experimentally produced elderberry juice. Increased pectinolytic enzyme dose, longer maceration time and elevated reaction temperature all had significantly positive effects on the juice yield. Increased enzyme dose and maceration temperature also increased the yields of anthocyanins in the elderberry juice, while none of the reaction parameters affected the juice turbidity. With the optimal treatment with a pectinolytic enzyme preparation, Pectinex BE 3L, produced by a cloned Aspergillus strain, a maximal juice yield of 77% w/w of the berry mash, an anthocyanin yield of 2380 mg/kg fresh berry mash, and a turbidity level of 128 formazin nephelometric units (FNU) were obtained. The results demonstrated that juice yields and phenolic yields in elderberry juice could be improved with enzyme treatment and that the optimal reaction conditions for obtaining the best juice yield, highest phenolics, and lowest turbidity levels could be rationally identified via statistical factor level optimization.

Sin *et al.* [51] in his study used Sapodilla juice and treated juice with pectinase enzyme at different incubation times (30–120 min), temperatures (30–50^{o}C) and enzyme concentrations (0.03–0.10%). These three factors were used as independent variables whose effects on turbidity, clarity, viscosity and colour (L values) were evaluated. Significant regression models describing the changes of turbidity, clarity, viscosity and colour (L values) with respect to the independent variables were established, with the coefficient of determination, R^2, greater than 0.8. The results indicated that enzyme concentration was the most important factor affecting the characteristics of the juice as it exerted a significant influence on all the dependent variables. The recommended enzyme clarification condition was 0.1% enzyme concentration at 40 ^{o}C for 120 min.

Abdullah *et al.* [53] had employed response surface methodology (RSM) for simultaneous analysis of the effect of enzymatic treatment conditions of incubation time, incubation temperature and enzyme concentration on physical characteristics such as turbidity, clarity, viscosity and color. In this study, a two-factor central composite design was used to establish the optimum conditions for the enzymatic treatment for clarification of carambola fruit juice. Carambola fruit juice was treated with pectinase enzyme at different incubation time (20–100 min), incubation temperature (30–50°C) and enzyme concentration (0.01–0.10 v/v %). These three variables were used as independent variables, whose effects on turbidity, clarity, viscosity and color were evaluated. Significant regression models describing the changes on turbidity, clarity, viscosity and color with respect to the independent variables were established with coefficient of determination, R^2, greater than 0.70. The results indicated that the enzyme concentration was the most important factor affecting the characteristics of the carambola fruit juice as it exerted a significant influence on most of the dependent variables. The recommended enzymatic treatment condition from the study was at 0.10% enzyme concentration at 30 °C for 20 min.

Kaur *et al.* [54] had proposed the effect of enzyme concentration (0.16–0.84 mg/100 g guava pulp), incubation temperature (36.6–53.4°C) and incubation time (0.95–11 h) on juice yield. A central composite rotatable design was used to establish the optimum conditions for enzymatic hydrolysis of guava to obtain maximum juice yield. Significant regression model describing the changes of juice yield with respect to hydrolysis parameters were established with the coefficient of determination, $R^2 = 0.85$. Enzyme concentration was the most significant variable affecting the juice yield. The recommended enzymatic treatment condition from the study was at the enzyme concentration 0.70 mg/100 g guava pulp, incubation time 7.27 h and incubation temperature 43.3 °C.

Dzogbefia and Djokoto [22] had proposed that pectic enzymes are widely used in the food industry for fruit juice extraction as well as in the clarification of cloudy juices. The enzyme was produced in the laboratory by culturing the yeast in papaya juice supplemented with 1% pectin for 6 days. Known amounts of enzyme preparation (0–40 mg protein) were added to a measured weight of papaya mash for varying reaction periods (30–90 min) and the amount of free-run juice obtained in each treatment compared with a control sample. Treatment of 200 g of papaya mash with different dosages of the pectic enzyme extract resulted in rapid increases in flow rate of free-run juice. Mash treated with 32 mg of total protein extract with a 30-min reaction time was the optimum for a maximum rate of juice flow (25 ml/min) when initial rates were measured. There was no significant difference ($P \leq 0.05$) between the red- and yellow-fleshed varieties of the papaya used. When juice flow was monitored over 6 min, the treated samples gave a flow rate that was more than twice those of the untreated samples. This biotechnological approach could be adopted to enhance papaya juice production by local fruit juice processors when parameters for scale-up processes are established.

Landbo and Meyer [55] had studied pre-press maceration treatments with 10 different pectinolytic enzymes in experimental black currant juice production using response surface design templates. Enzyme dosage, maceration time, reaction temperature and degree of berry crushing were varied, and the juice yields, anthocyanins, total phenols, and turbidity in the resulting juices were compared for a total of 250 different enzymatic treatments. The yields of anthocyanins and total phenols in the juices ranged from 900 to 2200 and 3050 to 5100 mg/kg wet weight black currant mash, respectively. Juice yields ranged from 66.4% to 78.9% by wet weight of mash. Turbidity levels ranged from 25 to 916 formazan nephelometric units (FNU). The reaction parameters induced larger variations in the responses than the different enzyme preparations, but the cloned *Aspergillus niger/Aspergillus aculeatus* preparation Pectinex BER consistently tended to be among those giving the best responses regarding anthocyanin yields, phenols, and low juice turbidity. The optimal maceration was achieved using an enzyme dosage of 0.18% by wet weight of berries with a reaction at 60.8^{o} C for 30 min on the most finely crushed berry mash. This treatment gave similar profiles of anthocyanins in the juices with all the 10 enzyme preparations. The same 10 juices all exhibited antioxidant activity against human low-density lipoprotein oxidation *in vitro*, but the antioxidant potency varied depending on the enzyme preparation used in the pre-press maceration.

Lee [50] had examined that raw banana juice is turbid, viscous and gray in colour. This work was initiated to optimize the enzymatic clarification process of banana juice using response surface methodology. Banana juice was treated with pectinase at various enzyme concentrations (0.01– 0.1%), temperatures (30–50^{o}C) and times (30–120 min) of treatment. The effect of these enzyme treatments on filterability, clarity, turbidity and viscosity of the juice were studied by employing a second order central composite design. The coefficient of determination, R^2 values for filterability, clarity, turbidity and viscosity were greater than 0.900. Statistical analysis showed that filterability, clarity, viscosity and turbidity were significantly ($p < 0.05$) correlated to enzyme concentration, incubation temperature and incubation time. Enzyme concentration was the most important factor affecting the characteristics of the banana juice as it exerted a highly significant influence ($p < 0.01$) on all the dependent variables. An increase in time and/or concentration of enzyme treatment was associated with an increase in filterability and clarity, and decrease in turbidity and viscosity. Based on response surface and contour plots, the optimum conditions for clarifying the banana juice were: 0.084% enzyme concentration, incubation temperature of 43.2^{o}C and incubation time of 80 min.

Kashyap *et al.* [27] had studied that pectinases are one of the upcoming enzymes of fruit and textile industries. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. The role of acidic pectinases in bringing down the cloudiness and bitterness of fruit juices is well established. Recently, there have been a good number of reports on the application of alkaline pectinases in the textile industry for the retting and degumming of fiber crops, production of good quality paper, fermentation of coffee and tea, oil extractions and treatment of pectic waste water.

Isabella *et al*. [56] had demonstrated that pink guavas from Ibiapaba plateau (Serra Grande) in Ubajara country, CE, Brazil, were mashed 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 filtration, a clear juice with a light yellow colour was obtained. This clear juice was preserved by the Hot-pack method. During the extraction and clarification of the juice, some of the important physical and chemical changes were followed by measuring changes in total soluble solids (^{o}Brix), acidity, viscosity, total phenolics content, colour, turbidity and ascorbic acid retention.

Vaidya *et al*. [57] had worked on kiwi fruit and determined that Kiwi fruit is nutritionally rich with high ascorbic acid content (193 mg/100g) but the extraction of its juice is difficult due to slimy pulp. To overcome this problem a combination of enzyme (pectinase 0.025g/kg + amylase 0.025g/kg + mash enzyme 0.05g/kg) were used to macerate pulp (2 hour at 50^{o}C) and thus facilitating the extraction of juice. The treatment enhanced the juice recovery (78.46%) compared to the control (58.44%) and the treatment did not affect the TSS, titrable acidity, pH, reducing and total sugar of the clarified juice. This treatment showed a drastic decrease in pectin content and consequently, decrease in the viscosity of the juice. The outstanding feature of the juice was its high acidity and high concentration of ascorbic acid which however, decreased by 21% after clarification. The recovered juice was ameliorated with sugar (22 ± 1 ºB), 100 ppm SO_2 and 0.1% DAHP, and was fermented by pure culture of *Saccharomyces cerevisiae* at 22 ± 1 ºC. After fermentation, a wine of 9.7% alcohol and 7- 8 ºB residual total soluble solids was obtained. Blending with sucrose syrup made the wine palatable. Since, in present study the enzymes combinations were used for a period of 2 h, high yield and clarity of juice were recorded. The sensory panel adjudged the wine with good ranking for colour, aroma, body and overall acceptability.

REMOVAL OF BITTERNESS OF JUICE

The phenomenon of late bitterness in some citrus fruit juices represents an important economic hindrance for citriculture worldwide, especially in connection with consumer acceptance [58].

The bitter taste is mainly attributed to the formation of limonin, a highly oxygenated derivative of triterpene (a compound of a furan and an epoxyde group) and belongs to the class of limonoids. The application of microorganisms as a tool to convert bitter citrus juices into non-bitter products requires enzymes of bacterial cells that are capable of metabolising limonin [58].

Limonoate dehydrogenase is an enzyme that can be found in a number of species of microorganisms and can prevent the formation of limonin as it catalyses the oxidation of limonoate A-ring lactone, 17-dehydrolimonoate, a non-bitter derivative that cannot be converted
into limonin. Limonoate dehydrogenase can be isolated from *Arthrobacter globiformis*, *Pseudomonas* sp. strains, and from *Rhodococcus fascians* drolimonoate, a non-bitter derivative than cannot be converted into limonin [59].

Another key substance for bitterness in juices is naringin, a bitter compound found in grapefruits and in sour oranges. Its threshold in water is approximately 20 ppm, but it can be detected at 1.5 ppm. Naringin is abundant in green fruits and its concentration declines with ripening. Processed grapefruit juices contain approximately 50 ppm of naringin. The limonin in processed juices acts synergistically with naringin to cause bitterness. An adequate procedure to remove bitterness from juices is naringin hydrolysis by naringinase. In the standard process, nariginase converts naringin into naringenin in two steps. The substrate naringin is hydrolysed by the component rhamnosidase to produce prunin, which is in turn converted by the flavonoid-β-glucosidase into naringenin. The optimal activity pH for α-rhamnosidase is 4.5 and for β-glucosidase is 3.0 [34].

PREVENTING DARKENING OF JUICES

The chemical or enzymatic darkening process suffered by juices is the one defect in beverages. Co-oxidation and polymerisation reactions result in changes in aroma and colour, and in stains. The presence of polyphenols in juices increases the susceptibility of enzymatic darkening. In apple products such as juice and cider, phenols have been of considerable interest due to their influence on organoleptic characteristics such as colour, bitter and adstringent taste, in the formation of certain aromas and in the clarity of beverages. Giovanelli & Ravasini [60] conducted a research on the use of an enzyme combination and filtration with membranes in the stabilisation of apple juice. The enzyme used was laccase. The activity of the enzyme was measured in total phenolic regression, changes in colour and in turbidity. The intensification of colour occurred more rapidly than the formation of polymers, as evaluated in terms of turbidity. Thus, 2–3 h of treatment with 50 mg /L of enzyme would be sufficient to produce adequate oxidation

effects. Comparative tests with standard treatments and with laccase to remove phenols demonstrated the high efficacy of the enzyme when compared to more extreme treatments such as the use of activated coals. The use of membrane filtration solved a critical problem in enzymatic treatment, i.e. the removal of the products of both the reaction and the enzyme itself. According to the authors, active filtration has been used successfully as a method to treat grape juice to produce white wine. Stability testes have shown that juices treated with laccase and then submitted to active filtration are more stable in terms of colour, although these samples presented turbidity. They concluded that the juice treated with ultrafiltered laccase with 15 kDa membrane is a very stable product, with colour values that are lower than their counterparts in non-treated juices.Thus, it was concluded that laccase produces a significant reduction in the phenolic content of juices, associated with an amazing addition of colour [60]. According to Minusse *et al*. [23] research conducted by Giovanelli & Ravasini [60] and Gokmen *et al*. [61], have shown that ultrafiltered samples treated with laccase increased the susceptibility to darkening during storage.

CONCLUSION

The above study clearly indicates that the enzymes are very beneficial to fruit juice industry. Their use results in higher yield of fruit juice and improves physical quality characteristics such as clarity, viscosity, filtrabilty, colour etc. Enzymatic extraction of juices adds nutraceutical nutritional and improves organoleptic properties that is reduces bitterness and prevents darkening of juices

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