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Industrial application of glutaraldehyde activated immobilized xylanase for clarification of tomato juice

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Abstract

Xylanase is being utilized in many industries and fruit juice clarification is one of them. The industrial use of a free xylanase enzyme is finite because it is expensive and is structurally unbalanced in free form and not stable at high pH and temperature, which can be reduced by entrapment of xylanase upon an insoluble support. Immobilization of xylanase enzyme can be done within sodium alginate beads using entrapment technique. In this study amalgamation of sodium alginate and calcium chloride (0.44 M) was used for the preparation of immobilized beads. After the enzyme is immobilized it is applied on tomato juice which can be obtained by simple extraction .The turbidity and viscosity of juice is due the presence of polysaccharides like cellulose, hemicelluloses, starch, pectin etc. Therefore, juices needs be clarified before commercialization in market. Treatment of tomato juice with xylanase under optimized conditions resulted in an increase in reducing sugars, juice clarity, filterability, pH and but a decline in turbidity and viscosity. Immobilized xylanase was more effective in improving tomato juice overall acceptability and quality as compared to its soluble counterpart. The results showed xylanase, in immobilized form, is a potential candidate for use in fruit juice clarification. The clarity of juice is measured by comparing the transmittance of both samples that is tomato juice treated with xylanase and juice not treated with xylanase.

Keywords: Xylanase; Immobilization; Juice clarification; Matrix entrapment; Algonac acid; Transmittance.

Introduction

Xylanase (EC 3.2.1.8) is a hydrolytic enzyme which catalyzes the breaking of the glycosidic bonds in xylan (a hemicellulosic polysaccharide, After cellulose, xylan is the second most abundant natural structural polysaccharide in plants, accounting for one-third of all renewable organic carbon on earth.) to form mainly xylobiose, xylotriose, and a small fraction of xylooligosaccharides with a higher degree of polymerization [1].The enzyme has gained a massive interest due to its potential contribution in pulp and paper industry, production of xylooligosaccharides, texture promotion of bakery products, textile industry, development in nutritional value of pig and poultry feed, fruit softening and clarification of juices and wines, detergents, bioconversion of lignocellulosic material and agro-wastes to fuels, extraction of coffee, plant oils and pigments, retting of plant fibers, and so on [2]. An increase in the no of articles and reports partnering to the isolation of newer microorganism species for xylanase production xylanase reveal an ever increasing interest by the scientific community in this field [3, 4]. Xylanases are produced in fungi as well as bacteria and it catalyzes the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan yielding various 1,4-β D-xylo-oligosaccharides.

Xylanase has a lot of application in industries like paper and pulp , animal feed, bio-ethanol production, baking, fruit and vegetable processing etc. In combination with enzymes like lipase and amylase, xylanases have also been used in biological deinking of paper. As the ability of enzyme to work properly at higher temperature become a common desirable property of enzymes for many industries , there has been an increasing focus on thermostable acidophilic xylanases in combination with pectinase and cellulase for their usage in juice clarification and improved yield [5]. Cloudiness in tomato fruit juice is due to formation of a colloidal suspension where the continuous medium is a solution of sugars, pectin, malic acid, and xylan, and the dispersed particles are mainly produced by cellular tissue comminuted at the time of fruit processing. For obtaining a clear juice these pre-unsterilized suspended particles have to be removed in a process known as clarification. The method is very useful to remove active haze precursors providing a more clear transparent juice and decreasing the potential for haze formation during storage. Therefore, addition clarifying agents is intended to improve clarity, color and/or stability of juices. Use of cell wall degrading enzymes as clarifying agents for cloud removal appears to be a critical processing step [6]. Most of the enzymes used in industry now days are of extracellular in nature i.e. the target enzymes are secreted from the microbes into the growth media. Compared to intracellular enzymes; extra cellular enzymes can be produced more economically since the purification is straight forward. Concurrently with cellulases and pectinases, xylanases are used in clarification of juices, the preparation of dextran for use as food thickeners, and the production of fluids and juices from plant material. Acidic xylanases are generally used for extraction and clarification of fruit juices. Xylanases also improves the yield of juice by means of partial or total liquefaction of fruit and vegetables, stabilization of fruit pulp increased recovery of vitamins, mineral salts , pigments , essential oils, aromas etc., reduction of viscosity, hydrolysis of substances that hinder the physical and chemical clearing of juice and cause cloudiness in the concentrate [6]. Due to their several applications in industries and wide high stability at wide range of reaction conditions, xylanases are being studied extensively. For commercial realization and economic viability of xylanase production, it is of utmost importance to identify microbes that are able to produce high secretive level of xylanase. Though xylanases have been extracted and purified from many different fungi [7, 8], bacteria [9] and yeasts [10].

Materials and Methods

Chemical & Reagents: Alginic acid, sodium salt**,** Food grade Xylanase, Glutaraldehyde**,** 3, 5 –Di nitro salicylic acid**,** Beech wood xylan**,** Xylose. All chemicals and rents used in this research were of the highest purity and of the analytical grade. All Food grade Xylanase Enzyme was procured from Sigma Aldrich.

Sample collection and processing: Ripe tomato fruits (1 kg) was purchased from kalyanpur local market and washed in tap water.Extraction of juice was carried out by selecting fresh tomatoes and washing them thoroughly with water and cutting the washed tomato in four large parts after that Macerated using a blender to get a smooth textured fleshy tissue (pulp).then filtered through 2–4 layers of muslin cloth to separate juice from the pulp. The pH of the juice obtained was 5.2.

Enzyme Assay: Xylanase activity was assayed by measuring the amount of reducing sugar Xylose formed from xylan. 1 mL of 1% Birchwood xylan solution was mixed with 0.1 mg enzyme solution and incubated for 15 min at 60 °C. The reaction was stopped by adding 1 mL of 1% 3,5-dinitrosalicylic acid (DNS) reagent. The mixture was heated for 5 min at 100 °C in a boiling water bath and cooled. Absorbance of sample was measured at 550 nm against the substrate blank. A standard curve of xylose ranging from 0 to 1mg/ml was constructed and used to estimate the released xylose. One unit (U) of enzyme activity is defined as the amount of enzyme liberating 1 micro mol of xylose equivalents in 1 min under the assay conditions.

Immobilization Procedure:

A. Preparation of sodium alginate solution:

1. Dissolve completely 6.0 g of sodium alginate in 180 ml of distilled deionized water.
2. Centrifuge the solution or apply vacuum to remove air bubbles.

B. Immobilization of xylanase: 10 mg of xylanase enzyme was dissolved in 20 ml of distilled water at room temperature. The solution of distilled water and xylanase enzyme was mix properly. After the xylanase enzyme is dissolved it is mix with sodium alginate solution. After that the mixture is extruded drop wise into a stirred 8% CaCl2 solution. (The syringe was clamped at a height of 14-15 cm above and solution was dropped)(Fig 1). The resulting bead was soaking in cold 8% $CaCl_2$ solution to increase the strength of the beads. The beads were wash using citric-NaOH buffer (50 mM, pH4.5) and activated with glutaraldehyde solution and then filter from the washing solution and dried. the beads and it is ready to be used.

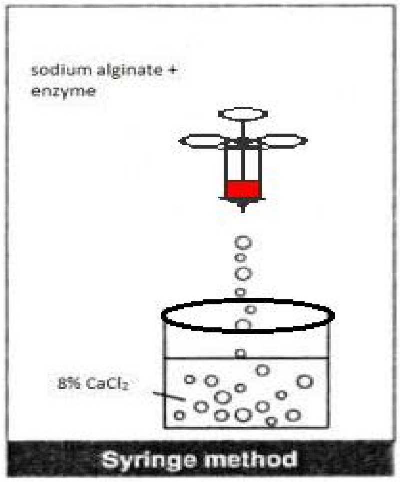

Fig.1 Immobilization of xylanase by Syringe method

Notes:

Sodium alginate solution is best prepared by adding the powder to agitated water, rather than vice versa, to avoid the formation of clumps. After sodium alginate is completely dissolved, air bubbles are eliminated by leaving the solution stable for 30 minutes.

Although not necessary, the beads may be hardened by mixing some amines in the sodium alginate solution and cross-linking with glutaraldehyde.

One bead of Sodium alginates contain around 5.0 microgram of Xylanase enzyme trapped in it.

Processing of Tomato juice by immobilized xylanase:

Tomato juice was treated with Immobilized xylanase for clarification of tomato juices. The enzyme dose, incubation temperature and time of treatment were optimized to enhance clarity and other quality characteristics of juice. 160 Sodium Alginate beads were filtered and wash with distilled water and place into a 500 ml sterilized glass cylindrical tank.160 Sodium Alginate beads occupied around 48 ml volume of the reactor leaving 452 ml effective available volume in the reactor. The juices from the tomato preparation were pumped into the cylindrical tank with a flow rate of 8.33 ml/sec from the bottom. As the juice passes through the immobilized xylanase beads present in the tank, the xylan present in the juice is broken down in xylo oligosaccharides and xylose by Immobilized xylanase. The treated juice is collected from the top of the cylinder in a flask. The immobilized enzyme is removed from the suspension and remaining enzymes impurity present in the treated juice removed by heating the filtrate in a boiling water bath for 5 min. The juice was analyzed for clarity by measuring its transmittance and reducing sugar .The conditions for treatment of fruit juice with xylanase are optimized. Treatment of fruit juice with xylanase results in an improvement in the physico-chemical characteristics of the fruit juices.

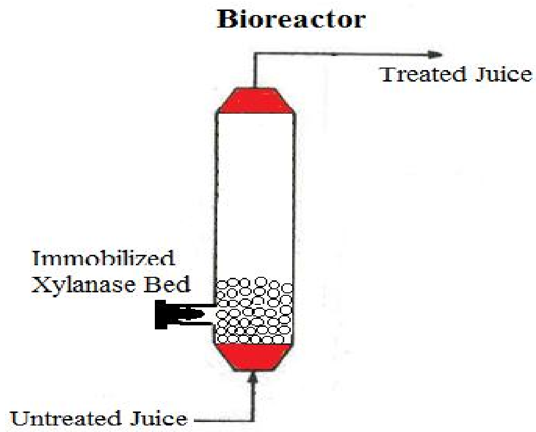

Fig.2 Processing of Tomato juice by immobilized Xylanase

Results and Discussion

Immobilization of xylanase:

During immobilized of xylanase, 3% sodium alginate and 0.44 M $CaCl_2$ solution was suitable for achieving the best result and after immobilization it activated in glutaradehyde solution (Data not shown).

Reusability of immobilized xylanase: Xylanase enzyme is very costly and in many industries it is imperative for full-scale process commercialization. Immobilized enzyme are favored as they can be reused leading to a low cost of the product. Reusability of purified xylanase immobilized on Sodium alginate was studied for four back to back cycles, particularly in one cycle 500 ml of tomato juice is passed through the reactor with a flow rate of 8.33 ml/sec. the result of which are shown in Fig 3.The residual activity of immobilized xylanase was more than 90 %, 85%, 79 % and 55 % after 1, 2, 3 and 4 response cycles, individually. The reduction in residual activity of enzyme with no of cycle has been accounted by a few scientists [11, 12]. A decrease in residual activity of enzyme may happen because of inactivation of enzyme as recommended before [13].The considerable stability observed during immobilized xylanase in this study would make the process economical. These results implied that xylanase immobilized on sodium alginate beads could be used for containment and utilization of enzyme over and over to a limit. The stability of xylanase immobilized on sodium alginate beads at high pH and temperature can also be useful during the processes that are being operated usually at high pH and temperature.

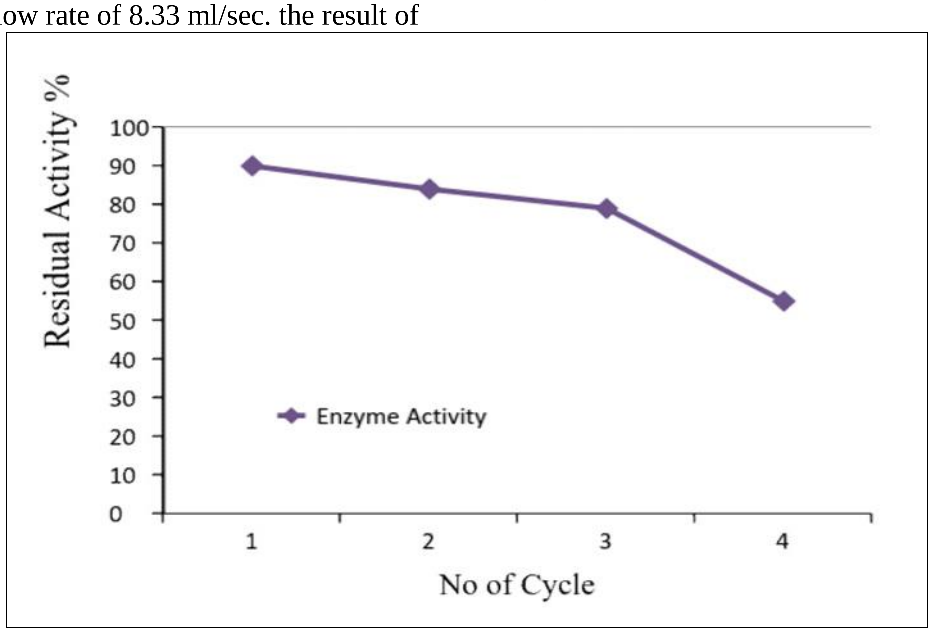

Fig.3 Change in residual activity with no of cycle.

Optimization of enzyme dose: Treatment of tomato juice with purified xylanase (0–30 IU/ml) at 30 °C for 30 min revealed maximum increase in, clarity (22 %) and reducing sugars (12 %) at 20 IU/ ml as compared to untreated juice. As the enzyme dose was increased or decreased from 20 IU, there was a decline in juice clarity indicating that 20 IU/ml was the optimum enzyme dose for treatment of tomato fruit juice. The optimum enzyme dose for treatment of tomato juice also was 20 IU/ml because maximum clarity (12 %) and reducing sugars (7 %) were recorded at this concentration (Fig.3). The optimum enzyme dose is likely to depend on its characteristics as well as the nature of fruit juice. Some researchers reported maximum juice yield and clarification by 15–20 IU/ml of xylanase [14]. Sin et al. suggested that increase in initial xylanase concentration will cause aggregation and settling of cloud particles thereby increasing the clarity of juice. However, after a certain enzyme concentration, substrate in the fruit jucie will become limited preventing the aggregation of cloud particles. Increase clarity of juice may be due to the release of water resulting from reduction in water holding capacity of xylan (the major hemicellulosic component of fruit cell wall) after its degradation by xylanase [15]. The release of xylose and short chain oligosaccharides following treatment of fruit juice with xylanase might account for enhancement in reducing sugar level of the juice.

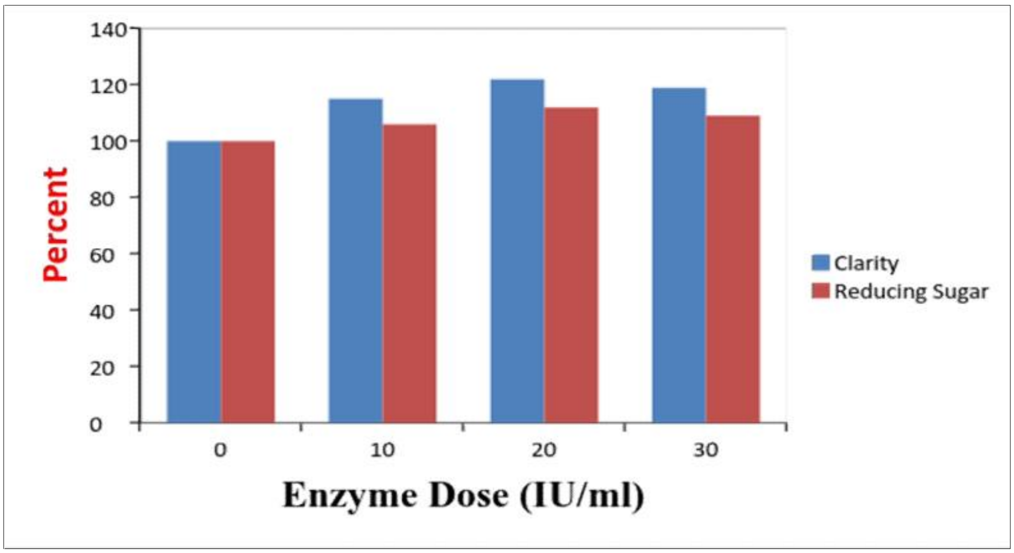

Fig.4 Optimization of enzyme dose

Effect of temperature on immobilized xylanase: Enzyme activity recorded at different temperatures from 30°C to 90°C revealed that the optimum temperature for both free and immobilized enzyme is 60-65°C where maximum hydrolyzing activity of the enzymes was found. The activity of free enzyme increased up to 60 °C and decreased there after exhibiting 78 %, 50 % and 37 % relative activity at 60, 70 and 80 °C, respectively whereas, the activity of immobilized enzyme increased with rise in temperature up to 60 °C followed by a decline exhibiting 82 % and 74 % activity at 70 and 80 °C, respectively (Fig.5). The optimum temperatures of free and the immobilized enzyme were taken as 60 to 65 °C, respectively as maximum enzyme activity was observed at these temperatures. A similar increase in optimum temperature for immobilized xylanase was reported by other researchers [4, 5, 16] and this could be due to enhanced stability or conformational rigidity on immobilization. although, the effect of temperature may vary depending on the substance from which the matrix used for immobilization is made of .

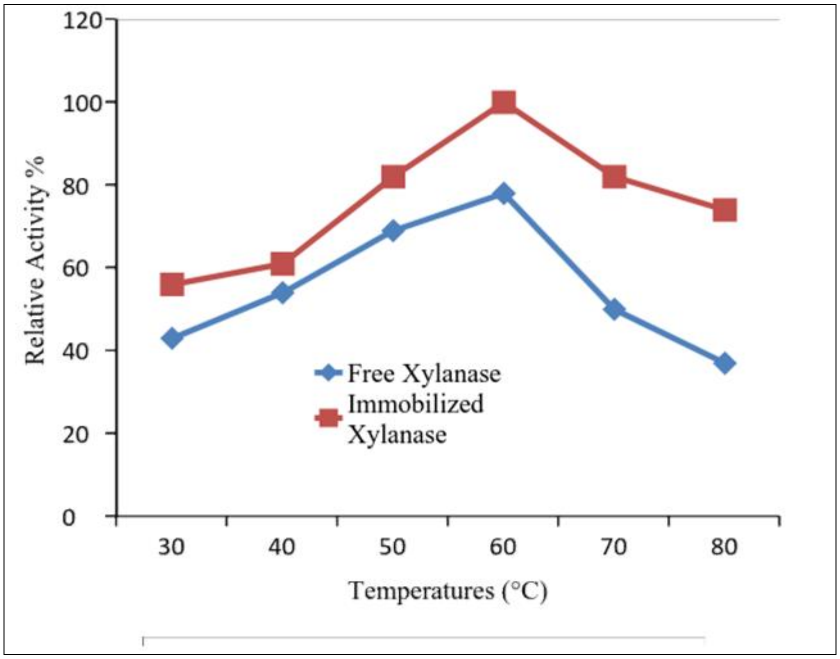

Fig.5 Effect of temperature on immobilized xylanase

Effect of pH on immobilized xylanase: The pH stability data revealed significant stability of both free and immobilized xylanase in the pH range 6.0–10.0 after 24 h pre-incubation at these pH values, however, the bound form exhibited better stability (Fig. 5.4). At pH 9 , free enzyme exhibited 83 % residual activity, while immobilized enzyme showed 98 % activity. Hence, it can be concluded from above data that the pH stability of xylanase was significantly enhanced after immobilization. Xylanase entrapped in calcium-alginate beads was found to be stable over a much wider pH range as compared to soluble enzyme [4]. Enhanced stability xylanase after immobilization on aluminum oxide pellets has also been documented by Nagar et al [17]. The increase in stability of immobilized xylanase over a wide pH range is likely to be advantageous during its application

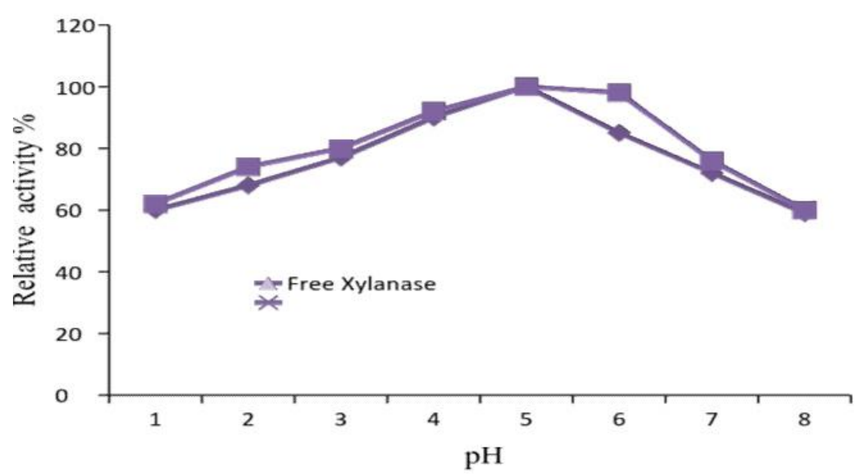

Fig.6 Effect of pH on immobilized xylanase

Sensory analysis: Through numerous methods, the purification and characterization of an enzyme may be a prerequisite for its successful industrial application. Currently research on xylanase garners much attention due to its broad biotechnological and industrial applications. In this study the purified enzyme was applied for clarification of tomato juices and its effectiveness was studied after the process optimization. All the experiments were performed twice and a mean value was recorded.

Tomato juice and pulp are the major primary processed products of tomato which may be utilized for the production of high value added products like puree, sauce, ketchup, chutney, powder etc. All varieties of tomato are not suitable for processing point of view. The varieties which are used for juice manufacture must possess following characteristics.

1. Deep red colored varieties are preferred as yellow colored pigments not only mask the red colour in processed tomato products but these are also susceptible to oxidation resulting in brown colouration.
2. Firm but ripe fruits should always be used as they contain sufficient amount of pectin which is essential for the consistency of the finished products like puree, sauce, ketchup etc.
3. Green colored and sour varieties should not be used as they will affect the flavour and color of the resultant products.
4. Tomatoes are also susceptible to microbial decay, hence any infected or diseased fruit should never be used for the manufacture of products as they may pose health hazards.

Untreated Juice in which no enzyme treatment was done and Juice in which Xylanase activity was involved were evaluated for organoleptic characteristics (average smell and taste, body and consistency, and color and flavor) by a team of 15 judges. A modified 20 point scale was used to determine physical characteristics, out of which 50% point (10 out of 20) were used for smell and taste analysis, 30% points (6 out of 20) were used for body and consistency and and 20% points (4 out of 20) were used for colour and texture[18].

Fig.7 Picture of treated (Right) and untreated juice (Left) sample.

Smell and Taste Analysis: All Juice samples were analyzed by a team of 15 judges and points were given out of 10 for smell and taste. Results obtained are shown in table 5.1

Body and consistency analysis: All Juice samples were analyzed by a team of 15 judges and points were given out of 6 for body and consistency. Results obtained are shown in table 1.

Color and texture analysis: All Juice samples were analyzed by a team of 15 judges and points were given out of 4 for color and texture. Results obtained are shown in table 5.1

Table 1: Sensory analysis

	Smell and Taste Analysis		Body and consistency analysis		Color and texture analysis	
People	Treated Juice	Untreated Juice	Treated Juice	Untreated Juice	Treated Juice	Untreated Juice
1	10	8	6	5	4	3
2	10	8	5	5	4	4
3	9	8	5	4	4	4
4	10	7	6	5	3	3
5	9	8	6	5	4	2
6	9	8	5	4	3	3
7	8	8	5	4	3	4
8	9	8	5	5	4	3
9	10	7	6	5	4	4
10	10	8	5	5	3	3
11	9	8	5	5	4	2
12	9	8	6	4	3	3
13	8	7	5	4	4	4
14	9	8	5	4	4	3
15	10	8	6	5	4	3
Total	141	117	81	69	55	48
Average	9.4	7.8	5.4	4.6	3.7	3.2

Discussion and analysis of Physical characteristics

Judges rate juice for different physical properties and their average was calculated which is summarized below.

Table 2: Physical characteristics

Characteristics	Treated Juice Avg. Score	Untreated Juice Avg. Score
Smell and Taste	9.4	7.8
Body and Consistency	5.4	4.6
Colour and Texture	3.7	3.2
Total Score	18.5	15.6

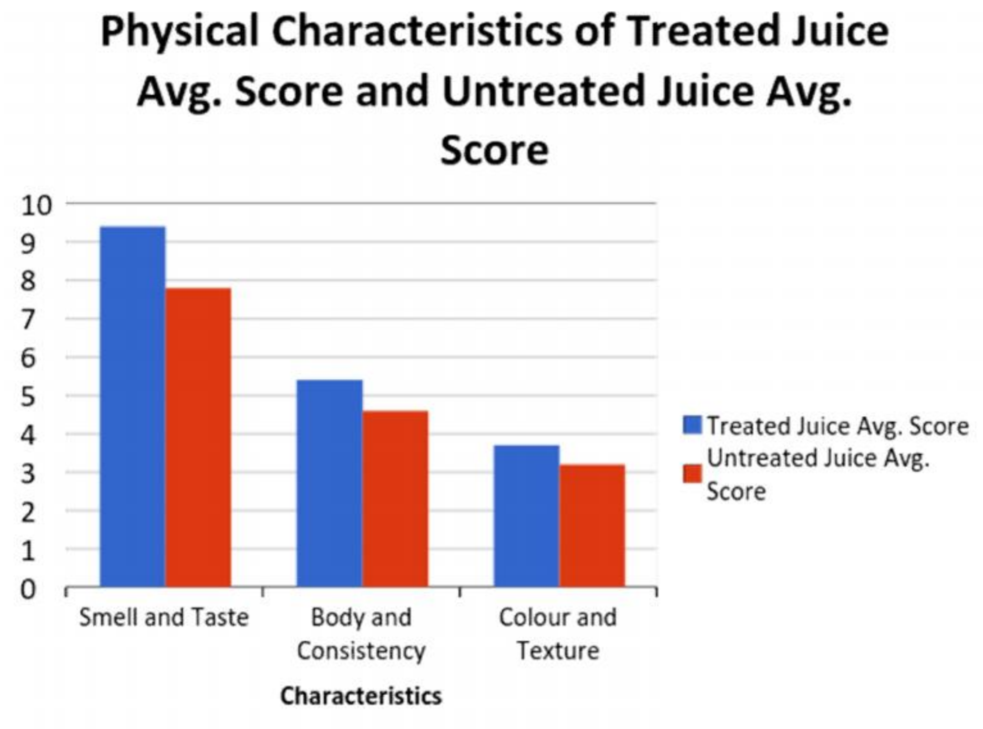

Fig.8 Physical characteristics of treated juice

The original scale was developed for wine quality analysis. Wines having average point below 9 were not acceptable and similarly wines that have average score above 19 were outstanding. In case of juice same scale was used by Ahmad S. Gad and similar result was observed by Ebtisam I et al. in Evaluation Yogurt Fortified with Vegetable and Fruit Juice as a Natural Sources of Antioxidant [19]

Rating scale is given below

Below 9- Unacceptable
9-19- Standard Juice
19-20- Outstanding Juice

Qualitative analysis of the chemical characteristics

pH Determination: pH was measured using pH meter and readings were taken before and after the treatment with immobilized xylanase following readings were obtained.

Table 3: pH Determination of treated and untreated juice

Treated Juice	Untreated Juice
6.2	5.8

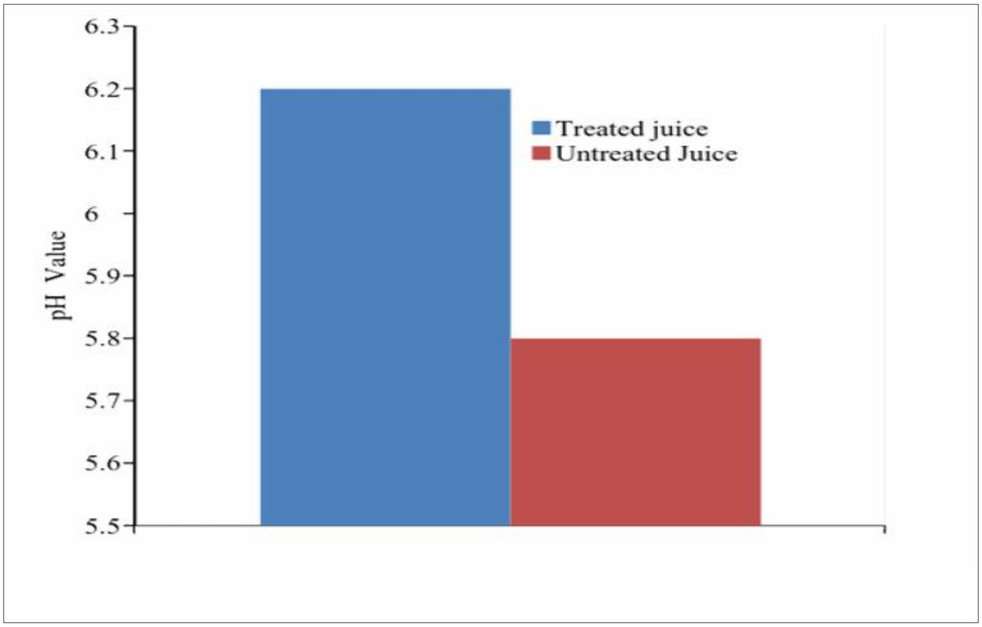

Fig.9 pH comparison of treated and untreated juice.

Transmittance Analysis: Absorbance analysis was done using spectrophotometer present in lab 125 of Biochemical Engg HBTU kanpur and reading obtained were as follows. Transmittance readings were taken before treatment with xylanase and after treatment with immobilized xylanase. The treated jucie obtained from the first time treatmet cycle was again treated with xylanase by second time treatment cycle. The maximum transmittance was observed after the second treatment cycle.

Table 4: Transmittance Analysis of treated and untreated juice

Wavelength	Untreated Juice	Treated Juice
650 nm	0.49	0.697

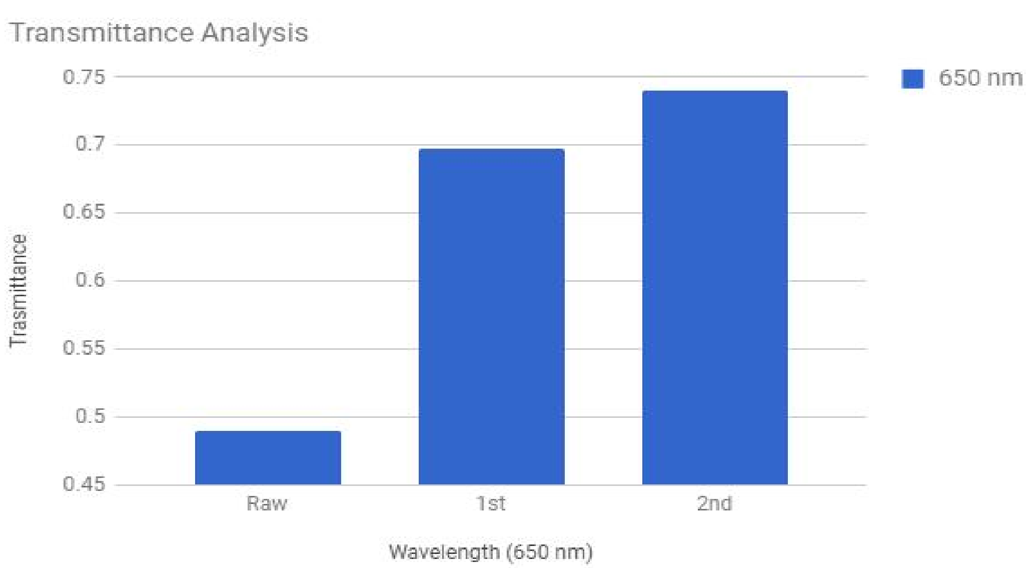

Fig.10 Transmittance Analysis of treated and untreated juice.

Conclusion:

During this experiment we can conclude that xylanase enzyme has some very important key characteristics such as reusable activity at high temperatures and acidic pH, which are very useful at various stages in juice clarification. Entrapment of xylanase with Sodium alginate beads improves its thermal stability and active efficiency. Use of sodium alginate for immobilization of xylanase enzyme in the form of beads which can be used for improved xylanolytic activity and stability at high pH and temperature. The immobilized xylanase showed considerable advantage over its soluble counterpart for application in clarification of fruit juices which include reusability of enzyme and low downstream processing costs because the product is not contaminated with enzyme.This study also gives us a view of how immobilized xylanase can also be used for tomato juice clarification. In addition to that during our experiment we have also found that results of organoleptic properties were quite good, which is indicates that the Tomato juice quality was considerably improved after the clarification.

Result of this research work point out that Immobilized Xylanase enzyme shows high effeciency in tomato juice clarification under following conditions:

Optimized condition	Data
No of response cycle	1
Enzyme dose	20 IU/ml
Temperature	60 °C
pH	9

Treated juice recover after Xylanase treatment is better than untreated juice by comparing analysis with it's following characteristic:

Characteristic	Treated juice	Untreated juice
Smell and Taste	9.4	7.8
Body and Consistency	5.4	4.6
Colour and Texture	3.7	3.2
pH value	6.2	5.8
Transmittance (650 nm)	0.697	0.49

From the current study it can be concluded that the immobilization of xylanase into Na-alginate beads improved its thermal and storage stabilities and its reusability. the treatment of tomato juice with immobilized xylanase improve its overall acceptability as we can conclude from sensory analysis. In conclusion i can say that the process is effective and can also be used at industrial level for larger scale production.

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