Research Article

Application of Xylanase Enzyme Produced by *Bacillus substilis* in Clarification of Fruit and Vegetable Juices

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ABSTRACT

The enzyme xylanase belongs to group of enzymes that can degrade complex xylan present on the lignocelluloses biomass in to xylan and xylose molecules. The present study focused on application of xylanase obtained from bacterium *Bacillus substlis* from dense forest soil of Western Ghats on fruit and vegetable juice clarification. The use of purified *Bacillus subtilis* xylanase (soluble form) for treatment of fruit pulps of tomato, pineapple and apple improved the physico-chemical characteristics of the resulting juices. Confirmation of application was performed by UV-Spectrophotometry using DNS method. Tomato juice has the highest clarified activity and degrades in maximum amount after 4 hrs of incubation compared to pineapple and apple juice. Activity was tested every 1 hour interval and gradually increased when the amount of degradation occurred.

Keywords: Xylanase, Lignocelluloses, Spectrophotometry, *Bacillus subtilis*, UV-Spectrophotometry.

INTRODUCTION

In recent times, as the consumption of natural fruit juice continues to rise, there's a growing focus on leveraging modern technologies to enhance the quality of the fruit juice industry[1]. Nonetheless, one of the primary challenges in enriching and clarifying raw juices stems from the presence of polysaccharides like pectins, starch, and hemicellulosic components. These substances have a tendency to settle during storage, leading to a decline in juice quality. Hence, clarification is imperative before commercialization[2]. Recently, various methods such as gelatine treatment, bentonite application, chemical processes, ultrafiltration, and microfiltration membranes have been employed to clarify fruit juices. Additionally, the utilization of enzymes to enhance juice yield and produce superior quality products has gained significance. Enzymes offer notable advantages including high efficiency, selectivity, specificity, operation under mild reaction conditions, and low toxicity[3].

Juice clarification is a vital process in the production of high-quality fruit juices. It involves the removal of suspended particles, cloudiness, and impurities from the juice to enhance its clarity, color, flavor, and shelf life[4]. Various methods are employed for juice clarification, including physical processes such as filtration, centrifugation, and sedimentation, as well as chemical treatments like fining agents and enzymes[5]. These methods help to separate solids and undesirable compounds from the juice, resulting in a clear, visually appealing product that meets consumer expectations. Juice clarification is essential not only for aesthetic reasons but also for maintaining the nutritional value and sensory characteristics of the juice[6].

Xylan constitutes a significant portion of hemicellulose, the use of xylanase in breaking down xylan into shorter sugar residues holds paramount importance in industrial operations. Xylanase (1,4-β-xylan xylanohydrolase; EC 3.2.1.8), a pivotal biocatalyst, finds widespread application across various industries including food and beverage, feedstock enhancement, paper pulp bleaching, wastewater treatment, and the bioconversion of lignocellulosic waste into valuable products[7].

The xylanase enzymes are obtained from micro organisms principally from fungi and bacteria. One of the major producer is *Bacillus substilis*[8]. The organism can be isolated from soils, decay materials, etc. Xylanase plays a crucial role in juice clarification processes by breaking down xylan, a component of hemicellulose present in fruit juices. By degrading xylan into shorter sugar residues, xylanase helps to reduce the viscosity of the juice and facilitates the removal of polysaccharides that can cause cloudiness or haze[9]. This enzymatic action enhances the clarity and stability of the juice, improving its overall quality and shelf life[10]. Thus, the application of xylanase in juice clarification is instrumental in achieving desired clarity and maintaining product integrity[11].

The present study involves application of xylanase produced from *Bacillus substilis* in the clarification of fruit and vegetable juices.

MATERIALS AND METHODS

Tomato, Pineapple and Apple were purchased from local markets. They were washed thoroughly with water, and macerated using a blender to form a smooth textured pulp. The pulps obtained were then clarified using the xylanase enzyme obtained as under: Pineapple juice (P): 10g pulp + 10 U/g of enzyme. Tomato juice (T): 10g pulp + 20 U/g of enzyme. Apple juice (A): 10g pulp + 20 U/g of enzyme and it undergoes as triplicates. The enzyme and pulp were incubated for 4 hrs at 37°C. After incubation, the

enzyme was inactivated by heating the suspension in a boiling water bath for 5 min. The pulp was then cooled and filtered through muslin cloth. The filtrate was centrifuged at 10,000rpm for 15 min. The supernatant (juice) was used for determining juice clarity. The untreated pulp for each of the fruit was kept as control. Juice clarity was measured by measuring the absorbance of juice. The activities of enzymes were measured in terms of UV-Visible spectrophotometer at 620 nm [12].

Confirmation of application by UV-Spectrophotometry using DNS method

Xyloglucan or xylan is the major hemicellulosic polysaccharide in primary plant cell walls of fruits and vegetables. Xylan can be hydrolysed by xylanase into xylose and xylo oligosaccharides, which can be further converted to ethyl alcohol. Clarification of juice by xylanase is confirmed by DNS Assay measured for fruit samples after xylanase treatment. The presence of metabolites xylan, xylose and xylo oligosaccharides is studied.

The xylanase enzyme activity was done by measuring the reducing sugar released by the reaction on the birch wood xylan. Thus, the xylanase assay was done according to 3, 5 – dinitro salicylic acid (DNS) method. Xylose standards were prepared and 1 mL of sample was added into 2.5 ml of DNS reagent and then kept in a boiling water bath for 5 minutes. After cooling for a few minutes, the released xylose was quantified at 620 nm using UV- Visible spectrophotometer against a reagent blank. A reagent blank was made by addition of 1 ml deionised water and 2.5 ml DNS reagent. An enzyme blank was also made in which the reagent was added before the addition of enzyme so that only the reducing sugar is estimated[13].

RESULTS

The use of purified *Bacillus subtilis* xylanase (soluble form) for treatment of fruit pulps of tomato, pineapple and apple improved the physico-chemical characteristics of the resulting juices.

Table 1: Xylanase Activity in clarified juice using DNS reagent

Juice clarified sample	Xylanase activity (U/ml)
T1	142.5
T2	148.6
T3	158.06
T4	160.9
P1	132.5
P2	136.9
P3	140.3
P4	144.6
A1	101.2
A2	117.4
A3	121.3
A4	133.8

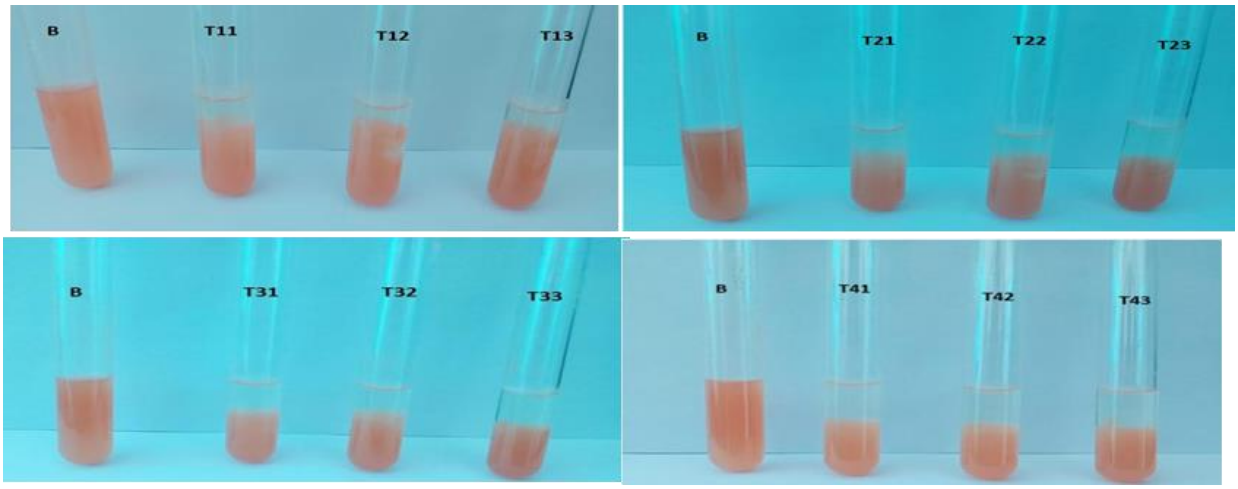


Figure 1: Juice Clarification (Tomato) of Xylanase enzyme

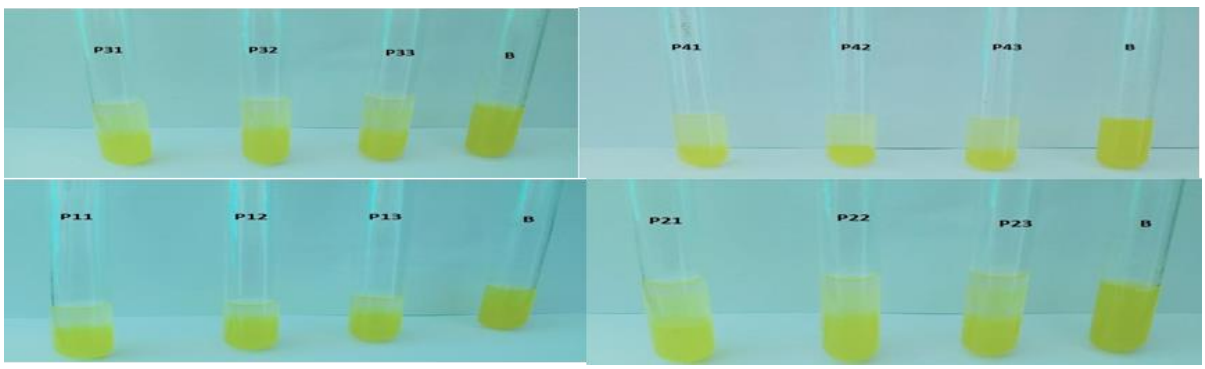


Figure 2: Juice Clarification (Pineapple) of Xylanase Enzyme

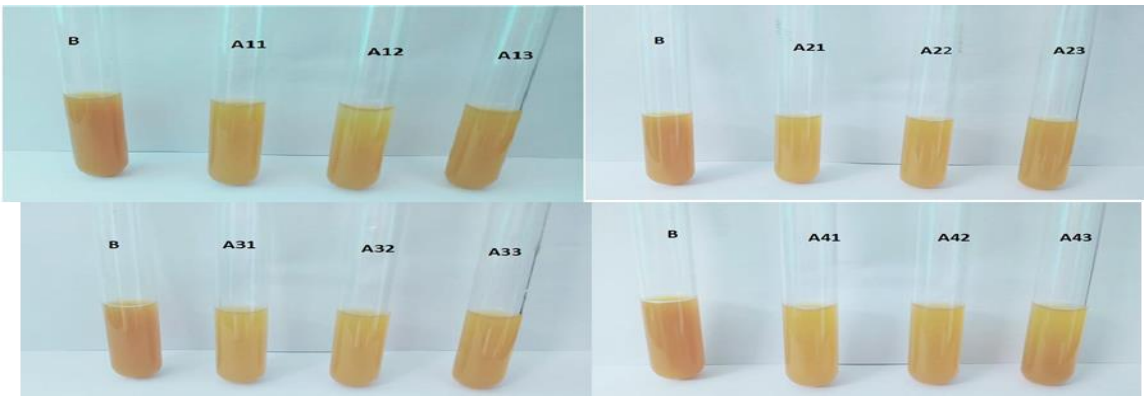
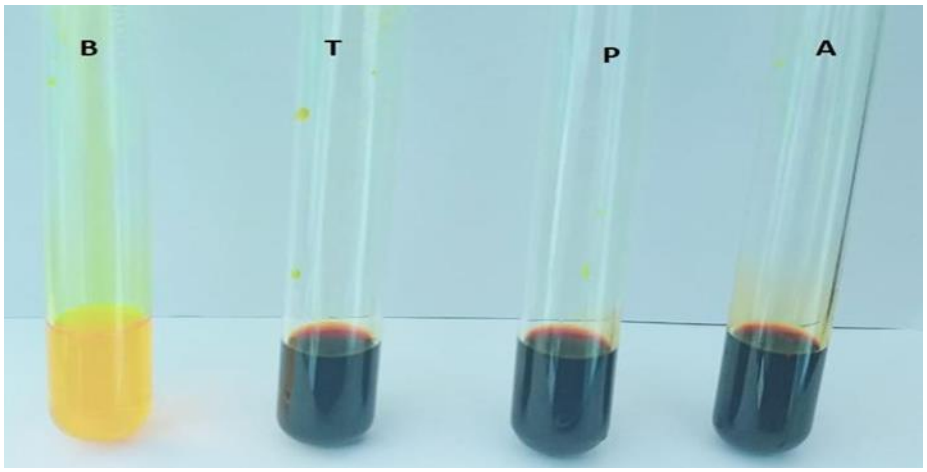


Figure 3: Juice Clarification (Apple) of Xylanase Enzyme



B: Blank; T: Tomato; P: Pineapple; A: Apple

Figure 4: Clarified juice used for confirmation by biochemical test using DNS method

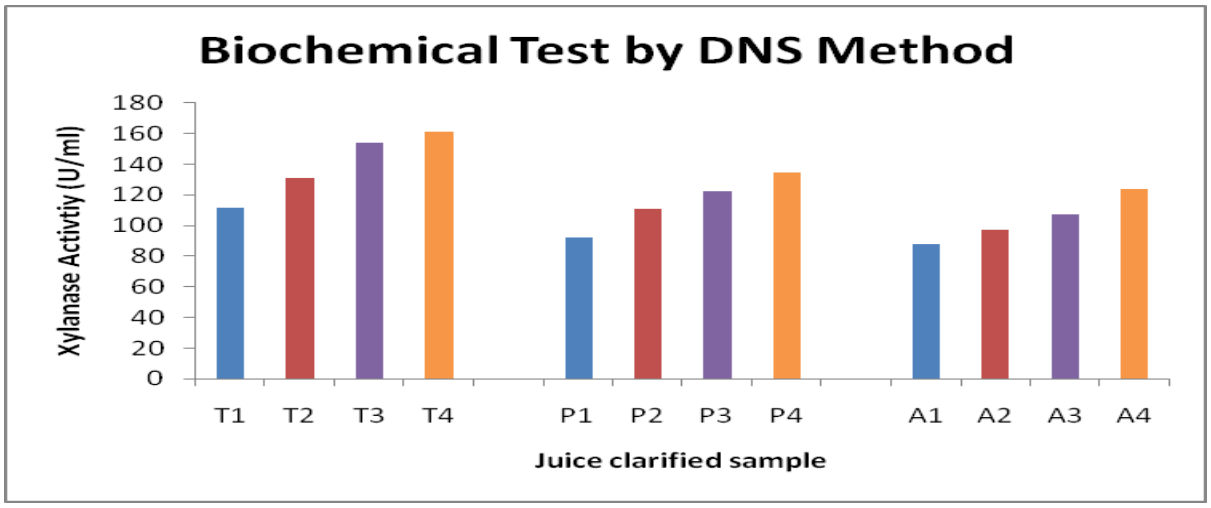


Figure 5: Clarified Xylanase Activity in clarified juice using DNS reagent

Confirmation of application by UV-Spectrophotometry using DNS method

Reducing sugar xylose was present in clarified juice treated with purified xylanase enzyme. Tomato juice has the highest clarified activity and degrades in maximum amount after 4 hrs of incubation compared to pineapple and apple juice. Activity was tested every 1 hr interval and gradually increased when the amount of degradation occurred.

CONCLUSION

From the study it can be concluded that xylanase enzyme produced from *Bacillus substilis* can be used in clarifying fruit juices and vegetable juices. As the modern era is depended on new enzyme technologies, using xylanase enzyme for these purposes will be advantageous as its production are of low cost. This also does not create any environmental problem. How ever huge production of enzymes are needed for commercial application. therefore,

additional research is essential to fully unlock the enzyme production potential of this organism.

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