



Efficacy of Pectinase purified from *Bacillus* VIT sun-2 and in combination with xylanase and cellulase for the yield and clarification improvement of various culinary juices from South India for Pharma and Health Benefits

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Abstract: Big bio business is going on enzymes because of wide variety of industries using commercial biocatalysts, in addition to the feed industry. Three-quarters of the market is for enzymes involved in the hydrolysis of natural polymers. Food processing enzymes including pectinases in juice industries account for about 45 percent of enzyme usage. Mostly in primary cell walls of plants a complex set of polyssachrides, pectin is present. It is also present in middle lamella where it help to bind cells together. Pectin's chemical composition, amount and structure will be different among variety of plants and over time. In our study we carried out the studies to determine pectinolytic activity from *Bacillus* VIT sun-2 and in combination with xylanase and cellulase to improve yield and clarification of culinary juices.

Keywords: Juice, pectinase, xylanase, cellulase, viscosity.

Introduction:

Fruit juices behave as a nutritious beverage and play a significant role in healthy diet because of the natural content which provides variety of nutrients. The main advantage of fruit juice is that it mixes and flush out the accumulated toxins present in the system. They also help in shedding off excess weight. It is to make sure that it should be consumed as soon as possible after preparing it because the shelf life of any fruit juice is very less. Fruit juice loss its vital elements if you keep them for long time. During digestion process, every food item that we take in must be transformed as glucose. Now fruit juice becomes fructose and transformation process from fructose to glucose during digestion requires only less energy from the body. It is Fruit juices are helpful in giving some amount of health benefits when they are taken in required amount. Apart from this, antioxidants present in the fruit juices were help in decreasing a risk of developing diseases. Insoluble particles which are more in raw press juice is due to the presence of pectic substances and they are known as 'cloud particles'. They consists of positively charged protein nucleus in which the negatively charged protein is coated to it ¹. Biotechnological potential of food, brewery and wine, animal feed, textile and laundry, pulp and paper industries were increasing due to the continuous research on pectinase, cellulase enzymes ². Pectinases have variety of application in fruit juice extraction and clarification process ³. The pressing efficiency of the fruit is also increased by this enzyme in combination with other enzymes like xylanase, cellulase and amylase⁴. Fruit juices namely apple, orange, pomegranate and grapes were taken in this study. Application of Pectinase purified from *Bacillus* VIT sun-2 and in combination with xylanase and cellulase to improve yield and juice clarification process was carried out and the effect was measured in terms of viscosity.

Methodology:

Chemicals:

All the reagents, chemicals and enzymes used in the study were from Hi Media chemicals, Mumbai and Sigma chemicals, Bangalore.

Collection of soil sample:

Soil samples were collected from various fruit industrial waste yard in Chittoor, Andhra Pradesh, and fruit waste yard in Vellore district, Tamil nadu India. Soil samples were collected using gloves in a sterile container to reduce the contamination caused by the environment during transport. The soil samples were stored in refrigerator for further analysis⁵.



Figure 1: Soil samples from Mango waste yard



Figure 2: Soil samples from Tomato waste yard

Microorganism:

Microorganism were screened from the soil sample and enzymatic assays were carried out followed by purification of enzyme through ammonium sulphate precipitation followed by ion exchange chromatography and SDS-PAGE. The partially purified enzyme is used for the experiment along with commercial cellulase and xylanase.



Figure 3: Pure Culture of Bacillus VIT sun-2

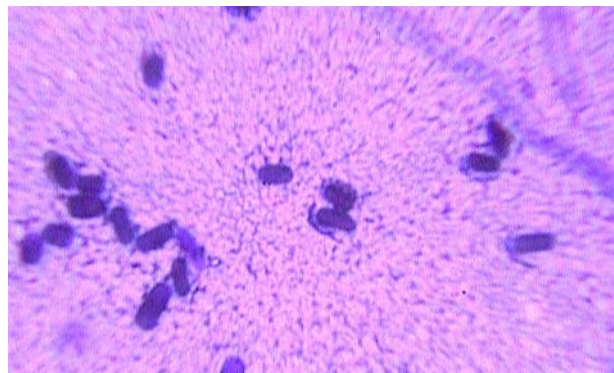


Figure 4: Gram's Stain Image of Bacillus VIT sun-2 strain

Sample collection:

We collected fruit samples from the food court in VIT. The fruit samples which are used for continuing the experiment are apple, orange, pomegranate and grapes. Although the equipment which is used for the extraction of juices is well sterilized. I.e. it maintains the proper aseptic condition so; that there are no chances of contamination. We selected those fruits which were in good quality for our experimentation.

Juice extraction:

Juice making process involved various steps such as crushing, pressing and filtration. Ripened fruit were selected based on the colour and conditions and were deseeded, peeled and blended using a blender.

Viscosity determination:

The viscosity data were obtained for 20 milliliters of the fruit juice along with different concentration of partial purified enzyme were determined using Ostwald viscometer ⁶ (Sigma- aldrich). The viscosity was determined in Pascal second units. Viscosity of the fruit juice is determined using the formula given below.

$$\eta_2 = \frac{\eta_1 \rho_2 t_2}{\rho_1 t_1}$$

[Note: η_1 = Viscosity of water ; ρ_1 = Density of water ; t_1 = time of flow of water;
 η_2 =Viscosity of Sample; ρ_2 =Density of Sample; t_2 = time of flow of Sample

Results and Discussion:

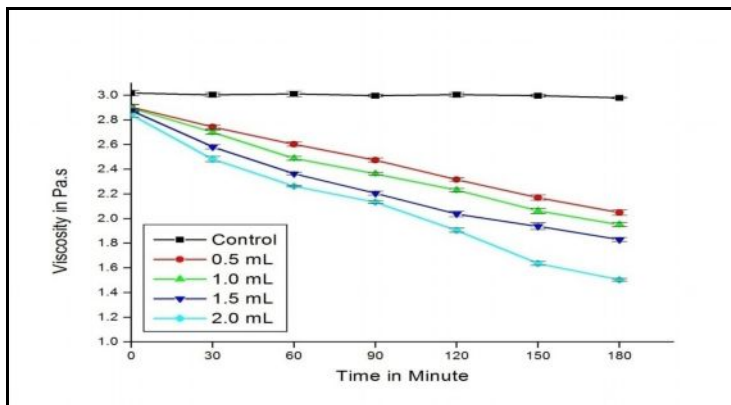


Figure 5 : Viscosity of apple juice with various concentrations of enzymes

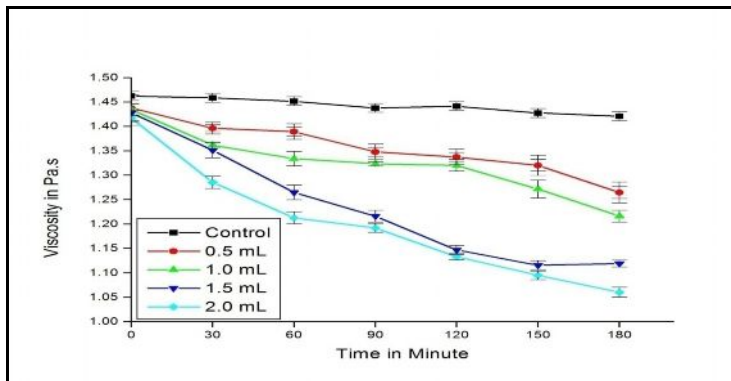


Figure 6: Viscosity of orange juice with various concentrations of enzymes

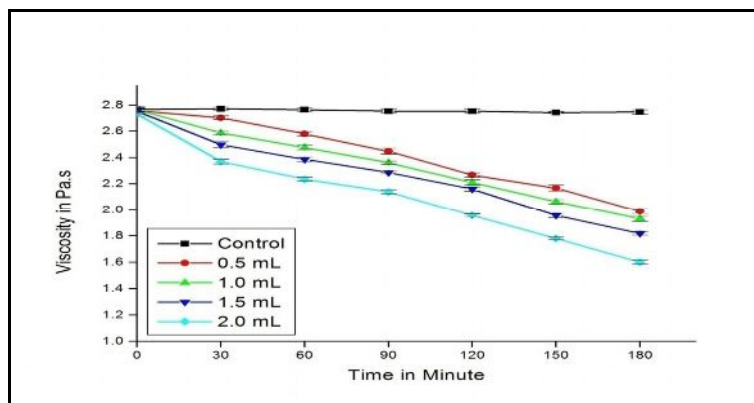


Figure 7: Viscosity of pomegranate juice with various concentrations of enzymes

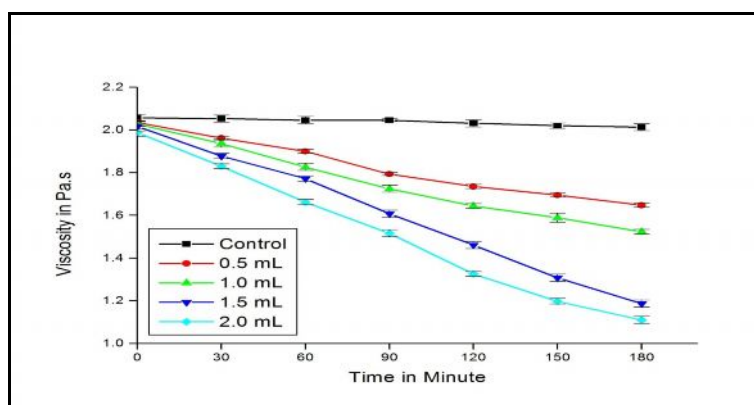


Figure 8: Viscosity of grape juice with various concentrations of enzymes

From the Observation, it has been shown that fruit juices such as apple, orange, pomegranate and grape were taken in this study. Figure 5 shows the Viscosity of apple juice with various concentration of enzyme. Viscosity of orange juice with various concentration of enzyme was given figure 6. In figure 7 the viscosity of pomegranate juice with various concentration of enzyme in terms of viscosity was plotted. The effect of various concentration of enzyme in grape was given Figure 8. The effect of partially purified enzyme in juice clarification process increased with incubation time and enzyme concentration. Thus, the purified enzyme has more efficacy in yield and clarification of apple juice followed by grape, orange and pomegranate juice. Thus, it shows the partially purified enzyme from *bacillus* VIT sun-2 in combination with cellulase and xylanase have more efficacy in juice clarification and yield of apple juice which can be used industrial sector and is more useful in beneficial of human health.

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