

1.Projects ID and Title:NSF-SCH-2025-137 & : **“Preparing clear  
apple juice by pectinase enzyme”**

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## **National Science Fair Research Paper**

**Project Name: "Preparing clear apple juice by pectinase enzyme"**

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Melvisharam.**

**Category: Life science**

**Level: Higher level**

## **ABSTRACT**

The experiment was conducted to prepare clear apple juice using the enzyme pectinase. Fresh apple pulp was treated with pectinase to break down pectin, which is responsible for the cloudiness in juice. The enzyme-treated juice was treated with different concentration of pectinase to evaluate clarity, yield, and colour. The results showed that pectinase treatment significantly increased juice clarity and yield by effectively breaking down the pectin substances. Thus, the use of pectinase proved to be an efficient method for producing clear apple juice with improved quality

and appearance. The colour and transparency of the enzyme-treated juice were also enhanced, resulting in an improved appearance and consumer acceptability. The experiment demonstrated that pectinase plays a vital role in the fruit juice industry for producing clear, high-quality apple juice efficiently and economically.

## **INTRODUCTION :**

Apple juice had been one of the most popular fruit beverages consumed worldwide due to its pleasant flavour, nutritional value, and refreshing nature. However, freshly extracted apple juice often appeared cloudy and viscous because of the presence of suspended pectin, starch, and other colloidal particles. To obtain a clear and visually appealing juice, enzymatic clarification had been commonly employed in the juice industry.

Pectinase enzyme played a vital role in this process as it hydrolysed the pectin substances present in the apple pulp, thereby reducing turbidity and increasing juice yield. The enzyme broke down complex polysaccharides into simpler molecules, which facilitated the separation of solid particles from the liquid portion. The use of pectinase had been considered an efficient, natural, and eco-friendly alternative to mechanical or chemical clarification methods.

In this study, clear apple juice had been prepared using pectinase enzyme treatment. The effectiveness of the enzyme in improving juice clarity, yield, and filtration efficiency had been analyzed to understand its potential application in fruit juice processing industries.

## **STATEMENT OF THE PROBLEM:**

During the preparation of apple juice, the extracted juice was often found to be cloudy due to the presence of pectin and other suspended particles. This turbidity reduced the visual appeal, market quality, and consumer acceptability of the juice. Traditional clarification methods were not always effective and could lead to nutrient loss or require additional processing steps. Therefore, there was a need to investigate the use of pectinase enzyme to break down pectin and enhance the clarity of apple juice. The study aimed to determine how effectively pectinase enzyme treatment improved the transparency and yield of apple juice compared to untreated samples.

## **Hypothesis**

It was hypothesized that pectinase produced from orange peel and jaggery would effectively break down pectin in apple mash and produce a clearer apple juice with higher transmittance and lower turbidity compared with untreated control juice.

## **Design of the study**

Study type: Experimental, controlled, laboratory-based.

Independent variable: Presence and concentration of pectinase produced from orange peel and jaggery (e.g., crude enzyme vs. Control).

Dependent variables: Clarity of apple juice (measured as % transmittance or turbidity in NTU), yield of clear juice (mL per 100 g apples), viscosity, and pH.

**Controls:** (1) Negative control — apple mash without added pectinase (incubated under same conditions). (2) Positive control — commercial pectinase (if available) to benchmark activity.

**Replicates:** Each treatment and control was replicated at least three times to allow statistical comparison.

**Sample size:** Replicates were chosen to provide minimal  $n=3$  per group; sample size was adjusted based on lab capacity.

**Randomization & blinding:** Samples were randomized for treatment assignment; measurements were taken by a technician blind to treatment where feasible.

**Outcome measurement & analysis:** Clarity was quantified using a spectrophotometer (percent transmittance at 660 nm) or a turbidity meter (NTU). Juice yield was recorded. Data were analyzed using mean  $\pm$  SD and compared by t-test or ANOVA with significance set at  $p < 0.05$ .

**Materials & equipment:** Fresh apples, orange peel (waste), jaggery (as carbon source), distilled water, blender, muslin/cheesecloth, turbidity meter, pH meter, Beakers, testtubes, balance, glassware, and personal protective equipment.

## Procedure

1. **Raw material preparation:** Orange peels were washed thoroughly, cut into small pieces, and oven-dried at low temperature ( $\sim 50\text{--}60^\circ\text{C}$ ) until constant weight was achieved; dried peels were then ground to a powder. Jaggery was powdered or dissolved as required. Apples were washed, peeled (if desired), cored, and cut into pieces for mashing.
2. **Preparation of fermentation medium:** The powdered orange peel (substrate) was mixed with jaggery as carbon source and distilled water to reach desired solid-to-liquid ratio (e.g., 5% w/v substrate). The pH was adjusted to the optimal range for the chosen organism/enzyme production. The medium was sterilized if a pure culture was used. Fermentation for pectinase production: The medium was inoculated with the prepared culture and incubated under controlled conditions (temperature  $\sim 28\text{--}30^\circ\text{C}$  for fungi; 24–72 hours) with periodic shaking for submerged fermentation, or spread on solid substrate for solid-state fermentation, until maximal pectinase activity was expected.
3. **Crude enzyme extraction:** After incubation, the culture broth or fermented substrate was filtered through muslin or cheesecloth and centrifuged to remove solids. The clear supernatant (crude pectinase extract) was collected and, if necessary, concentrated (e.g., by ammonium sulphate precipitation or ultrafiltration) and dialyzed to remove small impurities.
4. **Preparation of apple mash:** Apple pieces were homogenized in a blender to prepare a uniform mash. Mash was weighed (for example, 100 g batches) and divided into treatment groups (enzyme-treated, controls).
5. **Enzymatic treatment of apple mash:** A measured volume of crude pectinase (equivalent to a chosen activity, e.g., X U/g mash) was added to the apple mash.
6. **Separation of clear juice:** Mash was pressed or centrifuged and the juice was collected. Juice yield (mL) per batch was recorded.
7. **Clarification and measurement:** Collected juice was allowed to settle or was centrifuged and then filtered through fine cloth or filter paper. Clarity or turbidity were measured by Turbidity meter.

8. Data recording & analysis: All measurements were recorded. Graphs and tables were prepared to present results.

**Preparation of pectinase using orange peel and Jaggery:**

Orange peels were washed, chopped, and sun-dried/oven-dried until moisture content was low. They were then ground into a fine powder. Jaggery was powdered or dissolved in warm distilled water to make a concentrated jaggery solution. Left for 24 hrs, pectinase enzyme prepared. The medium composition was chosen to promote pectinase production. Juice yield increased in enzyme-treated batches due to pectin breakdown.







CLEAR APPLE JUICE



# PREPARIN G









	Blue	Green	Red
Date:		04/11/2025	
Time:		1 5:48:42	Local
Lat:		0.00000	Deg.
Lon:		0.00000	Deg.
Turbidity:		80 ± 29	NTIJ
SPM:		80 ± 30	mg/L
bb Red:		2.70 ± 1.11	l/m
Reflec Red:		0.069 ± 0.01	l/sr
Reflec Green:		0.066 ± 0.01	l/sr
Reflec Blue:		0.061 ± 0.009	l/sr

Blue                      Green                      Red

Date:                      04/11 /2025

Time:                      1 5:47:57                      Local

Lat:                      0.00000                      Deg.

Lon:                      0.00000                      Deg.

Turbidity:                       $80 \pm 29$                       NTIJ

SPM:                       $80 \pm 30$                       mg/L

bb Red:                       $1.69 \pm 0.69$                       I/m

Reflec Red:  $0.055 \pm 0.008$ . I/sr

Reflec Green:  $0.067 \pm 0.01$                       I/sr

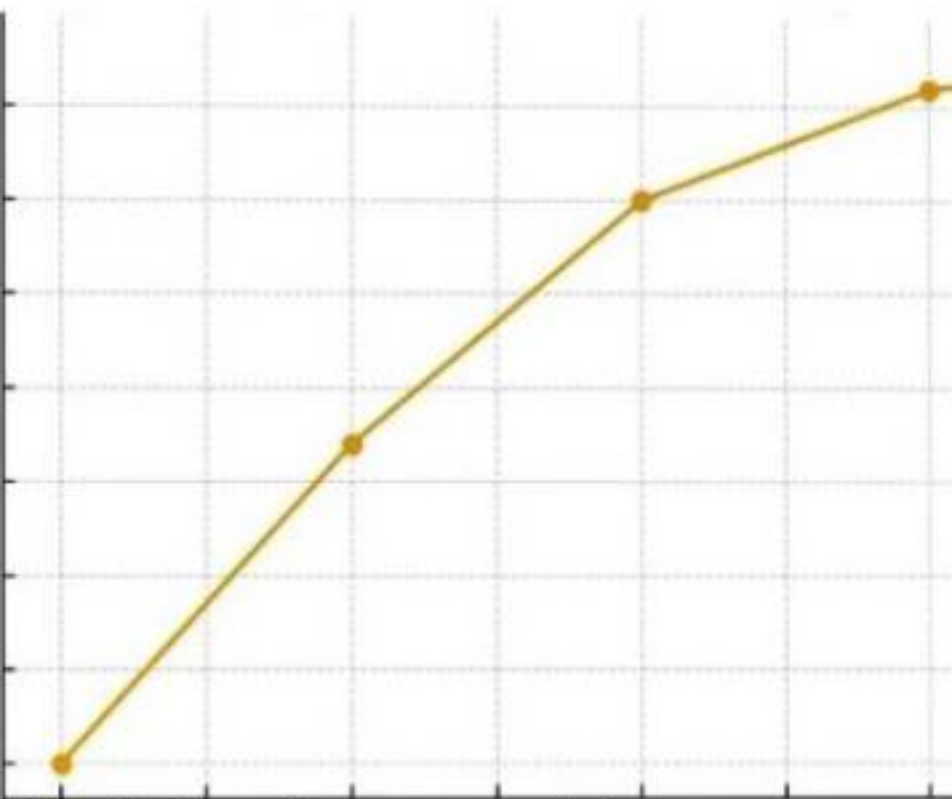
Reflec Blue:  $0.071 \pm 0.011$                       I/sr

Tabulation:

Sample no.	Volume of juice (ml)	Pectinase enzyme (ml)	Juice yield (ml) Clarity
1	10	5	20

2	10	10	30
3	10	20	40
4	10	30	50
5	10	40	70
6	10	50	80
7	10	60	110

**Graphical Representation :**



Clarity vs Enzyme concentration

#### Results and Discussion:

The results revealed that the addition of pectinase enzyme significantly improved both the yield and clarity of apple juice. The control sample, which did not contain any enzyme, produced a lower yield and appeared turbid due to the presence of suspended pectin substances.

Samples treated with increasing concentrations of pectinase exhibited greater clarity and higher juice yield. The optimum enzyme concentration was found to be 0%, which gave a clear juice with minimal turbidity.

The enzyme acted on the pectin present in apple pulp, breaking it down into simpler soluble compounds, thus reducing viscosity and facilitating the release of more juice. These findings were consistent with earlier reports on enzymatic clarification of fruit juices.

#### **In a Nutshell:**

The study had successfully demonstrated that pectinase enzyme could be effectively used to produce clear apple juice. The enzyme treatment increased juice yield and clarity, making the product more appealing and suitable for commercial use.

#### **Conclusion:**

It was concluded that the use of pectinase enzyme enhanced the clarity and extraction efficiency of apple juice. The enzyme broke down the pectin responsible for turbidity, resulting in a higher yield of clear juice. The optimum concentration of enzyme was found to be 0%, which gave the best clarity and volume.

#### **Application:**

- Used in fruit juice industries for large-scale clarification of apple, grape, and citrus juices.
- Applicable in wine production, where clarity and flavour are important.
- Useful in food processing to improve texture and appearance of fruit-based products.

#### **Future Enhancement:**

The study could be extended to optimize temperature and incubation time for maximum enzyme efficiency. Different natural sources of pectinase, such as microbial fermentation, could be explored for better yield. The process could be scaled up for industrial-level production of clear apple juice.

#### **References:**

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