

Enzyme Bio-Cleaners Production from Fruit Waste

Vaishnavi H Gowda¹, Deepak Kumar²

^{1,2}Student, R V College of Engineering

Abstract:

In recent years, garbage enzymes derived from fruit waste have emerged as eco-friendly and sustainable solutions for waste management and environmental protection. This literature review delves into the production, analysis, and versatile applications of garbage enzymes. Their role in composting, landfill leachate treatment, soil improvement, agricultural production, wastewater, and soil remediation is explored. The findings showcase the significant potential of garbage enzymes in addressing pressing environmental challenges. Through optimization of enzyme parameters and large-scale implementation, garbage enzymes hold promise for a greener future. This review highlights the transformative impact of these bio-cleaners on the path towards a cleaner and more sustainable planet.

Keywords: Garbage Enzymes, Fruit Waste, Bio-Cleaners, Waste Management, Environmental Sustainability, Enzymatic Activity, Antimicrobial Properties

1. Introduction:

In the face of escalating environmental concerns, the exploration of sustainable and eco-friendly solutions has become imperative. Garbage enzymes, derived from fruit waste fermentation, have emerged as a promising option in waste management and environmental preservation. These multi-enzyme preparations, comprising proteases, amylases, cellulases, lipases, and more, exhibit remarkable capabilities in breaking down diverse organic compounds.

Background:

The ever-increasing generation of organic waste and its adverse impact on the environment has prompted researchers to seek innovative approaches for waste treatment and utilization. Garbage enzymes, also known as kitchen waste enzymes or eco-enzymes, offer a novel pathway to convert fruit waste into valuable bio-cleaners. The concept of garbage enzymes was introduced in Taiwan and has since gained global attention due to its low-cost production and sustainable nature.

2. Objectives

The objective was to identify a bio-cleaner that offers optimal and highly efficient performance at room temperature while being environmentally friendly. It was essential to find a bio-cleaner that does not pose any harm to the environment, ensuring its sustainability. Additionally, we aimed to select a bio-cleaner that is safe for children and pets, prioritizing their well-being and avoiding any potential risk. The objectives of the project are drawn accordingly and a systematic approach needs to be adopted in order to achieve the project's objectives.

The main objective of the project is the green synthesis (as environmentally friendly as possible) of a biocleaner from fruit waste and to analyze them for their efficiency as a bio cleaner. For this, the first objective was the production of bio-cleaners, which according to the literature review can be done by fermenting the required fruit waste for 30+ days, and then performing a comparative study. For this we divided our objectives into three parts.

Green synthesis of enzyme bio-cleaners from mosambi, pineapple, pomegranate, lime, papaya, orange and banana waste and their comparative study.

Fermentation of fruit waste with Neem, Tulsi and mint to determine the change in their antimicrobial and enzymatic activity. Testing efficacy of products obtained from different waste material on different parameters like lipase activity, amylase activity, protease activity, antimicrobial activity and antimicrobial activity.

3. Methodology

For this project, we opted to collect five different fruit waste samples, namely mosambi, pineapple, pomegranate, lemon, papaya, orange and lime. Prior to fermentation, each sample was thoroughly washed twice with distilled water.

To investigate the antimicrobial activity on the fermented fruit samples, we incorporated Neem (*Azadirachta indica*) Tulsi (*Ocimum sanctum*) and Mint (*Mentha spicata*), three highly regarded medicinal plants known for their antimicrobial, anti-inflammatory, and antioxidant properties. Our approach involved creating four batches, with one batch containing only neem, another batch containing only tulsi, a third batch with both neem and tulsi, and a fourth batch without any additional additives. In this a total of 16 containers (4 x 4) were prepared. for the samples mosambi, pineapple, pomegranate, lemon.

Another type of batch containing with mint and other without any additional additives in this a total of 6 containers were prepared for the sample papaya, banana and orange.

Each container consisted of 1 part fruit, 3 parts jaggery, and 10 parts water. The container with only neem and Tulsi contained 150g of each plant, while the container with both neem and tulsi had 75g of each.

Once the mixing process was complete, we added 15mL of starter culture to each container and allowed the samples to ferment for a duration of 4 weeks. At the end of this fermentation period, we extracted the liquid from the containers and proceeded with the required experiments.

Overall, our project involved the careful selection of fruit waste samples, the addition of neem and Tulsi for their antimicrobial properties, the appropriate mixing of ingredients, and the subsequent fermentation process lasting 4 weeks. The resulting liquid was then collected for further analysis and experimentation.

4. Experiments Performed

4.1 Amylase Activity

Amylase activity refers to the enzymatic capability of amylases, a group of enzymes that play a fundamental role in carbohydrate metabolism. Amylases are responsible for the hydrolysis of starch and glycogen, which are complex polysaccharides, into simpler sugars such as maltose and glucose. This process is known as amylolysis. The activity of amylases is influenced by several factors, including temperature, pH, substrate concentration, and the presence of cofactors. Amylases have an optimal temperature and pH range in which they function most efficiently.

The activity of extracellular amylase was estimated by determining the amount of reducing sugars released from starch, using the 3,5-dinitrosalicylic acid (DNSA) method [17] with some modifications. Four 20 mL plug test tubes were filled with 1 mL of GE or CHGE; then 2 mL of DNSA reagent was added to two of these tubes, followed by incubation at 40 °C for 10 min, and then 1 mL of 1% starch was added to the tubes, which were then incubated at 40 °C for 5 min. Reaction was stopped by the addition of 2 mL DNSA chromogenic reagent, which was boiled for 5 min, to the two remaining test tubes. A total of 16 mL of distilled water was added after the tubes were cooled. The absorbance at 540 nm was read with a spectrophotometer and used to determine the amount of reducing sugars. The maltose was used as a standard. One unit of enzymatic activity was defined as the amount of enzyme that produces 1 mol of reducing sugar as maltose per minute at 540 nm.

$$\text{Amylase activity [mg/(min} \cdot \text{g)]} = \frac{(\text{mg of maltose released} \times \text{Dilution factor})}{(\text{Volume of solutions} \times \text{Enzymatic reaction time})}$$

4.2 Lipase Activity

Lipase activity refers to the enzymatic capability of lipases, a group of enzymes that play a significant role in lipid metabolism. Lipases are responsible for the hydrolysis of triglycerides, which are the primary components of dietary fats and oils, into fatty acids and glycerol. This process is known as lipolysis.

The lipase activity was determined by using the following method [19]. A total of 2.50 mL of ultra-pure water, 1 mL of Tris HCl buffer and 3 mL of olive oil were added to conical flasks and 1 mL of GE or CHGE was added to the flasks. The GE or CHGE solutions were mixed, followed by a 15 min incubation at 37 °C, and then 3 mL of 95% ethanol solution and 3–4 drops of phenolphthalein indicator were added to the reaction mixtures. The final reactions were titrated with NaOH until a light pink color appeared. One unit of lipase activity was expressed as the amount of enzyme that releases 1 mol of fatty acids per minute.

$$\text{Lipase activity } (\mu\text{/g}) = \frac{(\text{Volume of NaOH used for test} - \text{Volume of NaOH used for blank}) \times \text{Dilution factor}}{\text{Volume of solution}}$$

4.3 Protease Activity

Protease activity refers to the enzymatic capability of proteases, a group of enzymes that play a vital role in protein metabolism. Proteases, also known as proteolytic enzymes or peptidases, catalyze the hydrolysis of peptide bonds within proteins, resulting in the breakdown of proteins into smaller peptides or individual amino acids.

A total of 1 mL of GE or CHGE was added to 2.5 mL 1% (w/v) casein solution dissolved in 50 mM PBS (pH 7.5). A total of 2.5 mL 0.4 M trichloroacetic acid solution was added into the mixture after incubation at 40 °C for 10 min. Then, 1 mL of 3× diluted Folin reagent was added to the reaction. The absorbance values were recorded at 660 nm with a spectrophotometer after incubation at 40 °C for 20 min. Protease activity was defined as the amount of enzyme that releases 1 μg tyrosine per gram of plant per minute .

$$\text{Protease activity}(\mu\text{/g}) = \frac{(\text{mg of tyrosine released} \times \text{Dilution factor})}{(\text{Volume of solutions} \times \text{Enzymatic reaction time})}$$

4.4 Antimicrobial Activity

To perform the experiment, begin by preparing a nutrient agar media, which will serve as the growth medium. Once prepared, carefully pour the media into a sterile petri dish. Next, take the microbe to be tested and spread it evenly across the surface of the solidified agar media using a spread plate technique. Afterward, carefully place paper disks containing ampicillin and the sample on the agar surface. The petri dish should then be incubated at a temperature of 37°C for a duration of 24 hours. Following the incubation

period, observe and measure the extent of growth restriction exhibited by the ampicillin and sample-treated paper disks on the agar medium.

5. RESULTS

5.1 Fermented Product

The following figure shows the fermented products of all samples after a time period of 1 month with the use of starter culture obtained from Fine Elements :



Fig 5.1.1 Post fermentation product of all the samples (orange+mint, orange, banana+mint, banana, papaya+mint, papaya, pomegranate, lime, mosambi, pineapple)

These fermentation products contain the bio-enzyme that can be extracted and used as a cleaner. The extraction can be done through simple filtration or centrifugation techniques. We centrifuged the samples at 5000 rpm for 5 minutes on a table top centrifuge to collect the bio enzymes and then run the tests on them to compare the efficiency of each sample.



Fig 5.2.2 : Centrifuge tubes (left), centrifuge bin (middle), Centrifuge (right) and Antimicrobial activity .

5.2 Enzymatic Activity

We conducted 3 tests for enzymatic activity following the literature review.

5.2.1 Amylase testing

The following table shows the results for Amylase testing on the samples we fermented and the ones we received from Fine Elements.

[Tab 5.2.1.1: Amylase activity table]

Sl. no.	Sample	Sample volume	Absorbance	Maltose released in mg/ml	Amylase activity
1	mosambi	1	0.1	2.2747	0.020679
2	Pineapple	1	0.122	2.7002	0.024547
3	Rose+Marigold	1	0.106	2.3907	0.021734
4	Pineapple	1	0.147	3.1838	0.028944
5	Pineapple+Neem	1	0.191	4.0348	0.03668
6	Pineapple+Tulsi	1	0.227	4.7311	0.043009
7	Pineapple+Neem+Tulsi	1	0.197	4.1509	0.037735
8	Pomegranate	1	0.678	13.4545	0.122314
9	Pomegranate+Neem	1	0.459	9.2186	0.083805
10	Pomegranate+Tulsi	1	0.44	8.8511	0.080465
11	Pome+Neem+Tulsi	1	0.47	9.4313	0.085737
12	Mosambi	1	0.176	3.7447	0.034043
13	Mosambi+Neem	1	0.219	4.5764	0.041604
14	Mosambi+Tulsi	1	0.202	4.2476	0.038615
15	Mosambi+N+T	1	0.193	4.0735	0.037033
16	Lime	1	0.168	3.5899	0.032635
17	Lime+Neem	1	0.203	4.2669	0.038789
18	Lime+Tulsi	1	0.226	4.7118	0.042835
19	Lime+N+T	1	0.234	4.8665	0.044241
20	Papaya	1	0.437	8.79	0.079909
21	Papaya + mint	1	0.412	7.92	0.072
22	Banana	1	0.659	12.93	0.117545
23	Banana + Mint	1	0.626	12.22	0.111091

24	Orange	1	0.756	14.66	0.133273
25	Orange + mint	1	0.612	11.74	0.106727

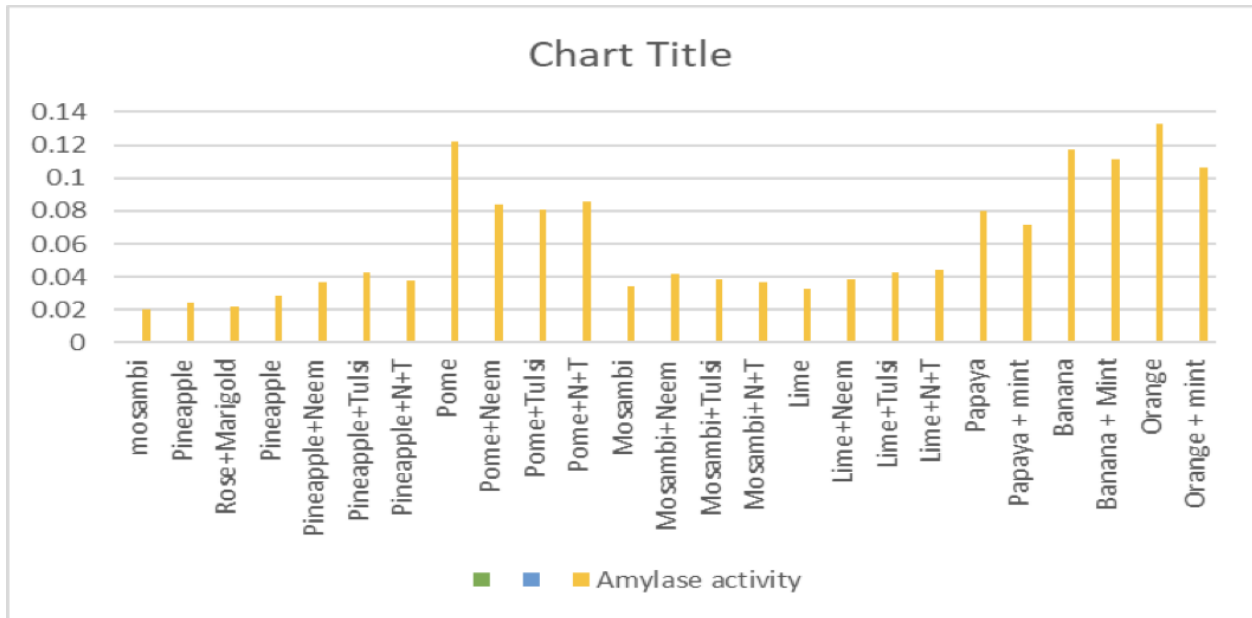


Fig 5.2.1.1: Amylase activity bar graph

5.2.2. Lipase Testing

The following table shows the results for the lipase activity tests run on the samples we fermented and the ones we received from Fine Elements:

[Tab 5.2.2.1: Lipase Activity Table]

Sample	Volume of NaOH Rundown (ml)	Lipase Activity (μ /g)
Blank	2.5	0
Mous	10.3	0.7429
Rose + Marigold	13.4	1.0381
Pineapple	12.4	0.7048
Pineapple + Neem	11.2	0.5905
Pineapple + Tulsi	12.2	1.1619
Pineapple + Neem + Tulsi	9.2	0.6286
Pome	11.2	0.8381
Pome + Neem	7.7	0.7333
Pome + Tulsi	13.2	1.2571
Pome + Neem + Tulsi	11.4	1.0857
Mous	12.9	0.7714

Mous + Neem	10.4	0.7524
Mous + Tulsi	10.7	0.7809
Mous + Neem + Tulsi	10.9	0.8
Lime	12.3	0.8476
Lime + Neem	13.2	1.1143
Lime + Tulsi	12.8	1.219
Lime + Neem + Tulsi	13.1	1.2476
Papaya	9.7	0.6857
Papaya + Mint	8.4	0.7524
Banana	8.5	0.8095
Banana + Mint	11.9	0.8952
Orange + Mint	10.3	0.8857
Orange	9.4	0.8476

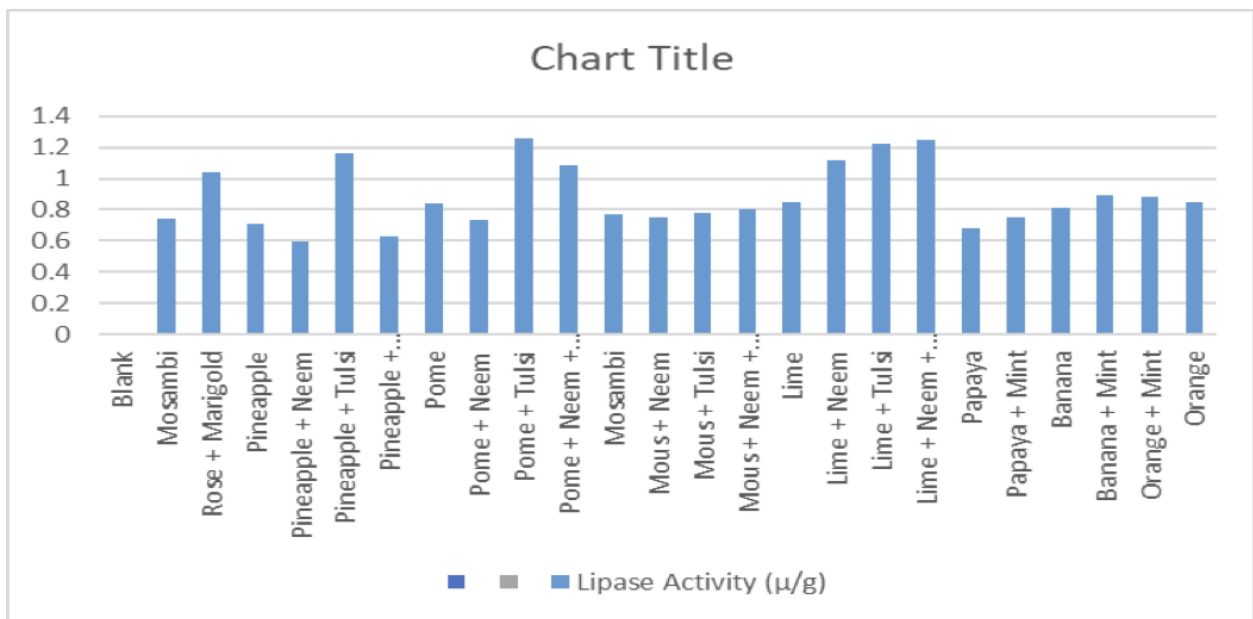


Fig 5.2.2.1: Lipase Activity of Different Samples

Sample	Lipase Activity (µ/g)	Standard Deviation	Significance
Blank	0	0	No data variation
Mosambi	0.75715	0.01424	Low variation
Rose + Marigold	1.0381	0	No data variation

Pineapple	0.7048	0.03029	Low variation
Pineapple + Neem	0.5905	0.04538	Low variation
Pineapple + Tulsi	1.1619	0.23448	Moderate variation
Pineapple + Neem + Tulsi	0.6286	0.19905	Moderate variation
Pome	0.8381	0.18716	Moderate variation
Pome + Neem	0.7333	0.03028	Low variation
Pome + Tulsi	1.2571	0.20748	Moderate variation
Pome + Neem + Tulsi	1.0857	0.16173	Moderate variation
Mous + Neem	0.7524	0.02095	Low variation
Mous + Tulsi	0.7809	0.04575	Low variation
Mous + Neem + Tulsi	0.8	0.01575	Low variation
Lime	0.8476	0.17226	Moderate variation
Lime + Neem	1.1143	0.12627	Moderate variation
Lime + Tulsi	1.219	0.17929	Moderate variation
Lime + Neem + Tulsi	1.2476	0.17084	Moderate variation
Papaya	0.6857	0.02752	Low variation
Papaya + Mint	0.7524	0.02466	Low variation
Banana	0.8095	0.09392	Low variation
Banana + Mint	0.8952	0.05756	Low variation
Orange + Mint	0.8857	0.03361	Low variation
Orange	0.8476	0.15649	Moderate variation

- Variability Levels:** The samples' standard deviations are categorized into three levels of variability—low, moderate, and no data variation. These levels help categorize the consistency of lipase activity within each sample group.

2. **Consistent Results:** Several samples, such as “Blank”, Rose + “Marigold”, and “Mous + Neem + Tulsi,” exhibit no variation, indicating consistent lipase activity with all values either being the same or very close to each other.
3. **Influence of Plant Combinations:** Samples like “Pome + Neem” and “Pome + Neem + Tulsi” display low variability, suggesting that the inclusion of Neem in these combinations might contribute to a more stabilized enzyme activity.
4. **Moderate Variability Patterns:** Samples like “Pineapple + Tulsi” and “Lime + Neem + Tulsi” show moderate variability, indicating potential interactions among the combined plants that lead to some variability in lipase activity.
5. **Potential Synergy:** The moderate variability in certain samples like “Pome + Tulsi” and “Lime + Tulsi” could point toward the possibility of synergistic effects between plant extracts on lipase activity.
6. **Stability vs. Variation:** The samples with low variability suggest stable interactions between enzymes and plant extracts, whereas those with moderate variability indicate complex interactions that can influence lipase activity.
7. **Optimization Insights:** Plant combinations displaying moderate variability could offer avenues for further research into optimizing lipase activity in specific contexts, such as enhancing enzymatic reactions in various applications.
8. **Factors Influencing Enzyme Activity:** Variability arises from factors like varying plant concentrations, enzyme-substrate interactions, and potential additive or inhibitory effects among the plant compounds.
9. **Application Relevance:** Understanding variability in enzyme activity is essential for industries like food processing, where consistent enzyme performance is critical for maintaining product quality.
10. **Research Directions:** The observed variability underscores the need for deeper investigations into the mechanisms underlying enzyme-plant interactions, potentially leading to novel strategies for controlling enzyme activity.

In summary, the table's standard deviations reveal intriguing patterns of consistency and variability in lipase activity across different samples. These insights can guide further research into understanding the underlying mechanisms driving these variations and leveraging them for practical applications.

5.2.3 Protease Testing

For Protease activity, Absorbance was read at 660 nm wavelength using a UV-Vis Spectrophotometer. The standard curve was drawn by taking a known concentration of Tyrosine and recording the optical density of different concentrations of tyrosine to create a standard curve. Then the recorded absorbances of the samples we fermented and the ones received from Fine Elements, i.e., 1 mL were plotted on the extrapolated standard curve to give the tyrosine concentration which in turn was used to calculate the protease activity.

[Tab 5.2.3.1: Protease activity Table for different samples]

Sample	Volum e (ml)	Absorbance at 600nm	mg of Tyrosine Released	Enzymatic Reaction	Protease Activity
Pineapple	1	0.467	1.116	30	0.005314
Mosambi	1	0.424	1.012	30	0.004819

Rose+Mari old	1	0.677	1.618	30	0.007705
Pomegranate	1	0.58	1.391	30	0.006629
Pomegranate+Neem	1	0.622	1.491	30	0.0071
Pomegranate+Tulsi	1	0.599	1.438	30	0.006848
Pomegranate+Neem+ Tulsi	1	0.57	1.365	30	0.0065
Pineapple+Neem	1	0.519	1.236	30	0.005886
Pineapple+Tulsi	1	0.492	1.173	30	0.005586
Pineapple+Neem+ Tulsi	1	0.453	1.08	30	0.005143
Lime	1	0.478	1.142	30	0.005438
Lime+Neem	1	0.52	1.24	30	0.005905
Lime+Tulsi	1	0.492	1.173	30	0.005586
Lime+Neem+Tulsi	1	0.463	1.105	30	0.005262
Mosambi+Neem	1	0.57	1.365	30	0.0065
Mosambi+Tulsi	1	0.546	1.304	30	0.00621
Mosambi+Tulsi + Neem	1	0.51	1.218	30	0.0058
Papaya	1	0.54	1.280192	30	0.0061
Papaya+Mint	1	0.5921	1.410979	30	0.006723
Orange+Mint	1	0.465	1.113126	30	0.0053
Orange	1	0.413	0.984253	30	0.004687
Banana+Mint	1	0.437	1.041529	30	0.004955
Banana	1	0.41	0.965629	30	0.004599

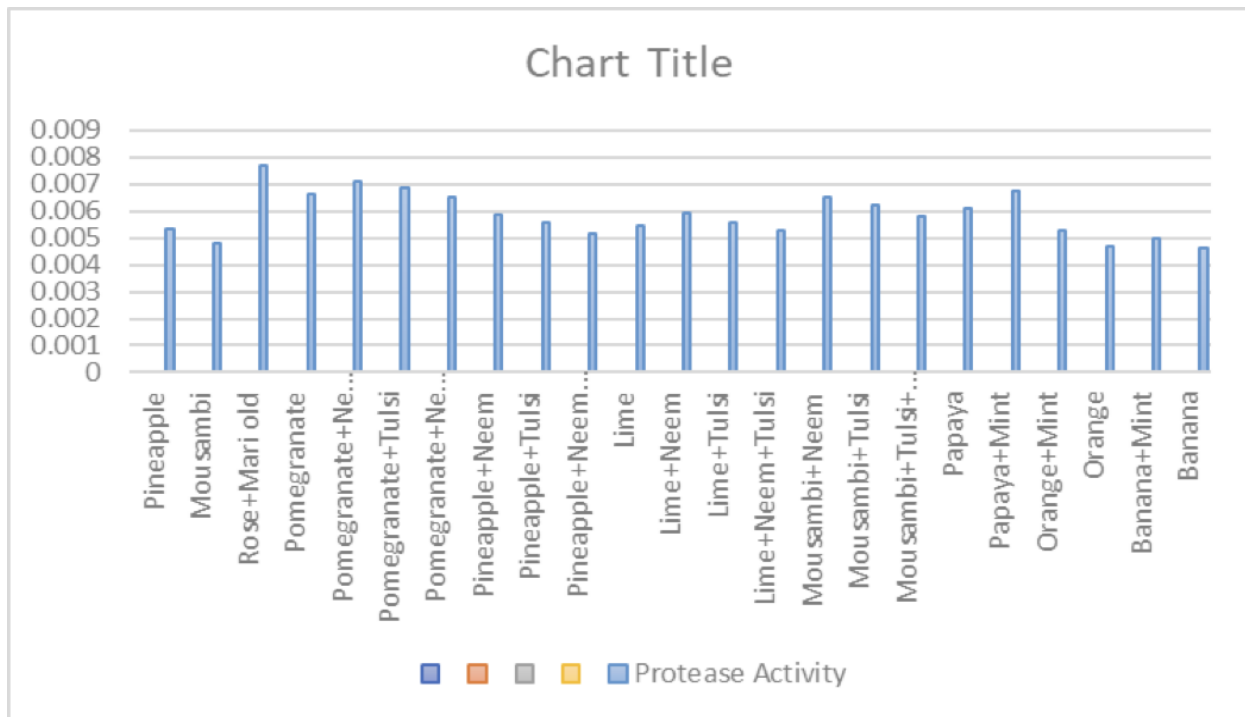


Fig 5.2.3.1 Protease activity bar graph of different samples

[Tab 5.2.3.2: Standard deviation of different samples]

Sample	Protease Activity	Standard Deviation
Pineapple	0.005314	0.000448
Mosambi	0.004819	0.000229
Rose+Marigold	0.007704	0.000858
Pomegranate	0.006628	0.000605
Pomegranate+Neem	0.0071	0.000625
Pomegranate+Tulsi	0.006848	0.000603
Pomegranate+Neem+Tulsi	0.0065	0.000565
Pineapple+Neem	0.005886	0.000464
Pineapple+Tulsi	0.005586	0.000408
Pineapple+Neem+Tulsi	0.005143	0.000335
Lime	0.005438	0.000387
Lime+Neem	0.005905	0.00045
Lime+Tulsi	0.005586	0.000408
Lime+Neem+Tulsi	0.005262	0.000301

Mosambi+Neem	0.0065	0.000565
Mosambi+Tulsi	0.00621	0.000495
Mosambi+Tulsi+Neem	0.0058	0.000424
Papaya	0.0061	0.000506
Papaya+Mint	0.006723	0.000616
Orange+Mint	0.0053	0.000434
Orange	0.004687	0.000286
Banana+Mint	0.004955	0.000329
Banana	0.004599	0.000236

1. There are significant variations in protease activity among the different samples.
2. "Rose+Marigold" and "Pomegranate+Neem" samples exhibit higher protease activity, suggesting potential enzymatic differences compared to others.
3. The presence of additives (Neem, Tulsi, Mint) doesn't show a consistent impact on protease activity across all combinations.
4. Some samples, such as "Papaya+Mint," display higher variability in protease activity measurements, indicating potential instability or variability in experimental conditions.

5.2.4 Antimicrobial Testing



Fig 5.2.4.1 Antimicrobial activity of the enzyme sample from pomegranate

The antimicrobial testing was done by preparing the paper disks dipped in the samples and placing them on petri plates which contain Nutrient Agar Media, spread plated with *E. coli*. Ampicillin disks were also added to each petri dish as the control for testing.

6. Conclusion

The report shows that from the samples we fermented, the most preferred would be Pomegranate based bio-cleaner as it has a better enzymatic activity and the other aspects of Pomegranate based GE do not fall too short compared to the other two samples. The Tests performed gave an insight to how GE can be used

as bio-cleaners. The report also touched upon how effective GE produced from different fruit wastes are and how to produce them.

In conclusion, the production and analysis of enzyme bio-cleaners from fruit waste offer a promising and sustainable solution to address the challenges of waste management. Through the fermentation process of organic waste, such as fruit peels and vegetable scraps, powerful enzyme-based liquids can be obtained, which demonstrate excellent cleaning, odor elimination, and pest control properties. The addition of additives like neem, tulsi and mint further enhances the antimicrobial activity of these bio-cleaners. However, there is a literature gap in terms of comprehensive evaluation and comparative studies that assess the effectiveness of enzyme bio-cleaners in various waste management applications, including odor control, drain unclogging, and pesticide activity. Additionally, further research is needed to explore the synergistic effects of different additives and their impact on the overall performance of bio-cleaners. Addressing these gaps will contribute to optimizing the production process and developing more efficient and versatile enzyme bio-cleaner formulations. Embracing the utilization of enzyme bio-cleaners derived from fruit waste can lead to a greener future by reducing the reliance on chemical-based products and promoting sustainable waste management practices.

Scope for future work

GE is a growing field, which means that the future scope would be endless. It can range from basic floor cleaners or table cleaners but can even be modified into handwashes, soil improvement, etc. while it's still possible to make them at home without any industrial equipment or training.

The production and analysis of enzyme bio-cleaners from fruit waste hold immense potential for future advancements and applications. Several areas of future research and development can be explored to