

ANALYSIS AND EVALUATION OF BIOENZYMES DERIVED FROM FRUIT PEELS OF LEMON, ORANGE AND PINEAPPLE

Abstract

Several toxic chemicals are being used as fertilizer, pesticide, disinfectant, deodorizing agents etc. which is hazardous to both mankind and the environment. One of the ways by which the use of these chemicals can be reduced is by opting for bioenzymes. Bioenzyme, a liquid made by fermenting kitchen waste, jaggery and water can be used as a fertilizer and cleaning agent. Here, bioenzymes made from peels of pineapple, orange, and lemon were analyzed. The protein, sugar and acetic acid content in the liquids were quantified. The bacterial and fungal composition was studied. Further biochemical analysis of the liquids indicated the presence of enzymes (proteases, amylases, and lipases) and secondary metabolites (tannins, flavonoids, saponins) which can help in the breakdown of toxic chemicals and as a healthy, organic, eco-friendly and cheap alternative to their synthetic chemical counterparts.

Keywords: bioenzymes; kitchen waste; eco-friendly; organic; cheap alternative.

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I. INTRODUCTION

Cleaning agents that are used in day-to day life can cause irritation and discomfort because of inhalation of the constituents that are suspended during cleaning activity. It also may form secondary pollutants due to the reaction of unsaturated organic compounds with oxidants such as ozone [17]. A study was conducted including four thousand Spanish women aged 30-65 years revealed that 25% of asthma cases experienced by them were due to the exposure of cleaning agents [11]. It was also reported by them that among the cleaning workers, lower respiratory tract issues were more common on working days and mostly contributed by the exposure to both diluted and undiluted forms of bleach, ammonia, air freshers, glass cleaning sprays etc. [10].

Solid waste management (SWM) has proved to be another severe problem for several urban local bodies in India, where urbanization and industrialization have resulted in increased Municipal Solid Waste (MSW) generation per person [8]. Landfill, incineration and composting are common options for waste disposal. However, they are not satisfactory for treating organic waste, thanks to the generation of toxic methane gas and bad odour, high energy consumption and slow degradation into harmless products [1]. Open dumps release methane from decomposition of biodegradable waste under anaerobic conditions along with that they also form breeding grounds for various pests like mosquitoes and worms which will cause infectious diseases and therefore the odour could also be a big issue, especially during summer when daily temperature in India can go up to 45°C [8].

A solution that reduces not only the growing dependency on toxic chemicals for cleaning, fertilizing etc but also deals with the kitchen waste that is generated daily in every household is given by researcher Dr. Rosukon from Thailand, who has been working in the making of garbage enzyme also called as bioenzymes since the last few decades. She developed garbage enzyme by fermenting kitchen waste with jaggery and water in the ratio 3:1:10 for three months. The garbage enzyme is understood to contain various sugars, proteins, ethanoic acid, and helpful bacterial population that makes it a multipurpose liquid. The bioenzyme, hence can be used as a cleaning agent, deodorizer or fertilizer, due to its rich compositions of biologically important compounds [14]. It possesses protease, amylase and lipase activity and has reduced 37.2% of total solids, 38.6% of suspended solids and 99% of pathogens in industrial waste activated sludge [2]. Thus, the crude enzymes present within the garbage enzymes are often utilized in waste water treatment because the enzymes can breakdown the toxic compounds present within the industrial effluents [3]. In this study, the composition of bioenzymes made by fermenting pineapple, orange and lemon peels were studied.

II. MATERIALS AND METHOD

1. Preparation of Bioenzyme: The fruit peels (Pineapple, Orange, Lemon separately) collected from local juice vendors were mixed with jaggery and water in the ratio 3:1:10 in separate air-tight plastic containers. A pinch of yeast was added to fasten the fermentation process. The containers were opened on alternate days to release the gases formed due to fermentation. After a period of 45 days, all the three liquids were filtered [14].

2. Preliminary Screening and Quantitative Analysis of the Extracts Table 1: The pH, colour and odour of the extracts were recorded.

- **Protein Estimation:** Protein in the sample was estimated using Lowry-Bronsted method. Bovine Serum Albumin was used as standard. A series of protein solutions in increasing concentrations (0, 2, 4, 6, 8, 10 mg/ml) were prepared, their absorbance was measured at 660nm for making the standard graph. The value of the protein content present in the samples were calculated using the standard graph [9].
- **Sugar Estimation:** Sugars present in the sample were estimated using DNSA reagent. Dextrose was used as the standard. A series of sugar solutions in increasing order (0, 0.125, 0.25, 0.375, 0.5, 0.625, 0.75 mg/ml) of their concentrations were prepared, their absorbance was recorded at 520nm, for making the standard graph. 1ml of each sample were diluted with 9 ml of distilled water and 0.1ml of this extract was tested. The values of the sugar content present in the samples were calculated using the standard graph and then were multiplied by the dilution factor [12].
- **Acetic Acid Estimation:** Acetic acid present in the samples were estimated with the help of acid-base titration. The sample were titrated against NaOH using phenolphthalein. The volume of NaOH required to neutralize the acetic acid present in the sample was noted. Normality equation ($N_1V_1=N_2V_2$) was used to calculate the acetic acid content in the sample [5].

3. Qualitative Screening of Phytochemicals and Enzymes Table 2: tests were carried out to check the presence of alkaloids, saponins, tannins, steroids, quinones, sugar etc. present in each of the solutions [15].

- **Test for Alkaloids:** 2 ml of samples were treated with a few drops of Dragendroff's reagent and checked for the presence of red precipitate.
- **Test for Saponins:** 3 ml of samples were mixed with 10 ml of distilled water and shaken vigorously and then allowed to settle. And then the foam layer was noted.
- **Test for Quinones:** 1 ml of sample was treated with conc. HCl and checked for the presence of green colour.
- **Test for Tannins:** 1 ml of the sample was treated with a few drops of 5% ferric chloride and was observed for the presence of reddish black colour.
- **Test for Sugars-** 1 ml of sample solution treated with 1 ml of Fehling's A and B solution each and heated in a hot water bath and checked for the presence of red precipitate.
- **Test for Flavonoids:** In 1 ml of the sample a few drops of dil. NaOH were added, then a few drops of HCl were added. The colour change from intense yellow to colourless was checked. The presence of enzymes was tested by investigating their

activity on their respective substrates and thereby recording the digestion of the substrate.

- **Test for Proteases:** The activity of the protease present in the extract was checked indirectly by investigating the presence of its substrate that is protein. Here, (Bovine Serum Albumin) BSA was taken as the substrate. The presence of BSA was revealed by the colour change in the presence of Biuret method. Thus, the disappearance of the purple colour in the sample treated with the extract indicated the presence of proteases [6].
- **Test for Amylases:** Here, starch was taken as the substrate and Lugol’s iodine was used as an indicator for its presence. The digestion of starch was revealed by the disappearance of the dark colour in the samples treated with the extracts, indicating the presence of amylase [7].
- **Test for Lipases:** Here, an alkaline solution of milk was taken as the substrate and phenolphthalein was used as an indicator of change in the pH. The presence of lipases was indicated by the change in the colour of the treated samples from pink to colourless as the lipase breaks down the fats into fatty acids which increase the pH of the solution giving rise to the colour change [13].

4. **Microbial Composition:** The microbial composition of the extracts was carried out in two parts namely the bacterial composition and the fungal composition.

- **Bacterial Composition:** 100 microlitres of diluted (10^{-4}) samples were spread on Nutrient Agar plates. All the three plates were kept in 37°C for 24 hours. The bacterial colony characters were noted. Gram staining was done to check for the presence of gram positive and gram-negative bacteria [16].
- **Fungal Composition:** 100 microlitres of diluted (10^{-4}) samples were spread on YEPD (Yeast Extract Peptone Dextrose) Agar plates. The plates were incubated at 27°C for 72 hours. The fungal colony characters were noted. The fungus was taken on a clear slide and observed under 40 x power of the compound microscope [4].

III.RESULTS

1. Preliminary Screening and Quantitative Analysis of the Extracts

Table 1: Preliminary Screening of the Extracts and Quantitative Estimation Of Extracts

Characters	Extracts		
	Pineapple	Orange	Lemon
pH	5.20	5.60	5.35
Odour	Pungent wine-like	Citrusy sweet	Lemony

Colour	Reddish orange	Orange	Yellowish orange
Protein content (mg/ml)	1.151	3.295	0.822
Sugar content (mg/ml)	16.676	12.168	13.670
Acetic acid Content (mg/ml)	9.6186	7.3554	9.4339

2. Qualitative Screening of Phytochemicals and Enzymes

Table 2: Qualitative Screening of Phytochemicals and Enzymes

Test	Extracts		
	Pineapple	Orange	Lemon
Alkaloids	Absent	Absent	Absent
Saponin	Present	Absent	Present
Quinone	Absent	Absent	Absent
Tannin	Absent	Present	Absent
Steroids	Absent	Absent	Absent
Sugar	Present	Present	Present
Flavonoids	Present	Present	Present
Proteases	Present	Present	Present
Amylases	Present	Present	Present
Lipases	Present	Present	Present

3. Microbial Composition: The microbial content in the extracts was investigated using spread plate method. The bacterial colonies and the fungal colonies were allowed to grow in the appropriate media in agar and then after the period of incubation, the colony characters were recorded and the microbes were tentatively identified based on their colony characters and microscopic structure. Given in the figures 1, 2, 3, 4.

- Bacterial colonies were first observed on orange extract agar plate after 18 hours. Bacterial growth on the lemon and pineapple agar plate was visible after 36 hours. Gram staining revealed the presence of gram-positive bacteria in all the three enzyme extracts [16].
- Fungal colonies were also first observed in orange extract agar plate after 48 hours of incubation. The fungal growth on lemon and pineapple extract agar plate was seen after 72 hours of incubation. It was observed that many fungal colonies were seen in orange agar plate compared to the lemon and pineapple agar plates. It was observed that only one colony was seen in plate spread with lemon bioenzyme and none in plate spread with pineapple Bioenzyme. The fungus was then observed under 40x power of the compound microscope. The fungal colony suspected based on the colony characters and the microscopic examination are *Aspergillus*, *Rhizopus* etc. Many cells of *Saccharomyces cerevisiae* were also visible [4].

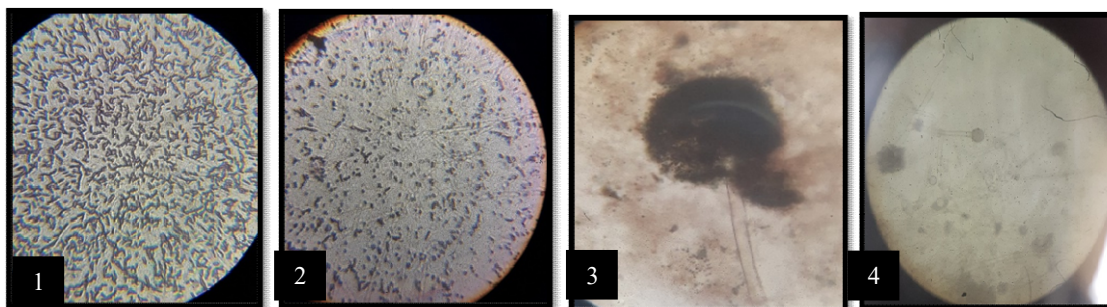


Figure 1 and 2: Gram positive bacteria ;
Figure 3 and 4: Fungus suspected to be *Aspergillus*, *Rhizopus*

IV. DISCUSSION

The findings of this study can be concluded as that all the three enzyme extracts are acidic in nature and appear orangish in colour with citrusy, fruity smell. The qualitative analysis revealed that all the three extracts possessed various concentrations of sugar, protein, and acetic acid. The presence of secondary metabolites like saponins, flavonoids, and tannins were also established. The test for screening of enzymes confirmed the presence of proteases, amylases and lipases in all the three extracts. The study of bacterial composition revealed the presence of gram positive rod-shaped and coccus bacteria. The study of the fungal composition of the extracts indicated the presence of Yeast, *Rhizopus* and *Aspergillus*. One reason why the microbes may not be flourishing in the plates spread with Pineapple and Lemon Bioenzyme might be that acetic acid content in them is higher than the Orange Bioenzyme. The presence of acetic acid and saponins in the solutions prove to be useful in disinfecting and cleaning process. The presence of enzymes like proteases, amylases and lipases can prove helpful in breaking down the substrate and making it less harmful and also effective in removing stains. The presence of flavonoids indicate that the solutions indicate their ability to detoxify toxic compounds, thereby justifying its use in sewage water treatment. Thus, the use of bioenzymes can deal with two purposes at the same time, one, it can be useful in solid waste management, at the same time reducing the greenhouse gases being formed, the other is that it can minimize the dependency on the synthetic and toxic chemicals that are used in daily household purposes. Future research should consider the possible use of these extracts as fertilizers, antimicrobial agents and also in the use of waste water treatments..

REFERENCES

- [1] Arancon RA, Lin CS, Chan KM, Kwan TH, Luque R., "Advances on waste valorization: New horizons for a more sustainable society," in Energy science & engineering, vol.I, 2013, pp. 53–71.
- [2] Arun C, Sivashanmugam P. , "Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge," in Process Safety and Environmental Protection, 2015, pp. 471-478.
- [3] Arun C, Sivashanmugam P., "Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from pre-consumer organic waste," in Bioresource technology, 2017, pp. 200-210.
- [4] Dymond J., "Methods of Enzymology (eds Lorsch J)," Elsevier, Baltimore, 2013, pp. 191-204.

- [5] Haque S.M., Ahmad A., “Development and validation of analytical method for quantification of acetic acid content in amlodipine besylate,” *International Journal of Pharmacy and Pharmaceutical Sciences*, 2019, pp. 8-11.
- [6] Itzhaki RF, Gill DM., “A micro-biuret method for estimating proteins,” in *Analytical biochemistry*, 1964, pp. 401-410.
- [7] Jayaraman J. , “ *Laboratory manual in Biochemistry*, Wiley Eastern,” New Delhi, 1981, pp 48.
- [8] Kumar S, Smith SR, Fowler G, Velis C, Kumar, SJ, Arya S, Kumar R, Cheeseman C., “ Challenges and opportunities associated with waste management in India,” in *Royal Society open science*, 2017, pp. 60764.
- [9] Lowry OH, Rosebrough NJ, Farr AL, Randall, RJ., “ Protein measurement with the Folin phenol reagent,” in *The Journal of biological chemistry*, 1951, pp. 265–275.
- [10] Medina-Ramón M, Zock JP, Kogevinas M, Sunyer J, Antó JM. , “Asthma symptoms in women employed in domestic cleaning: A Community Based Study,” in *Thorax.*, 2003, pp. 950-954.
- [11] Medina-Ramón M, Zock JP, Kogevinas M, Sunyer J, Torralba Y, Borrell A, Burgos F, Antó JM., “ Asthma, chronic bronchitis, and exposure to irritant agents in occupational domestic cleaning: a nested case-control study,” in *Occupational & environmental medicine*, 2005, pp. 598-606.
- [12] Miller GL., “Use of Dinitrosalicylic acid reagent for determination of reducing sugar,” in *Analytical chemistry*, 1959, pp. 426-428.
- [13] Rice FE, Markley AL., “ Proof of the presence of lipase in milk and a new method for the detection of the enzyme;” in *Journal of dairy science*, 1922, pp. 64-82.
- [14] Samriti, SS, Arya A., “Garbage enzyme: A study on compositional analysis of kitchen waste ferments,” in *The Pharma Innovation Journal*, 2019, pp. 1193-1197.
- [15] Shaikh JR, Patil MK., “ Qualitative tests for preliminary phytochemical screening: An overview,” in *International Journal of Chemical Studies*, 2020, pp. 603–608.
- [16] Tiwari RP, Hoondal GS and Tewari R. , “ *Laboratory techniques in microbiology & biotechnology*,” Chandigarh, 2009, pp. 63-66.
- [17] Wolkoff P, Nielsen GD., “Organic compounds in indoor air—their relevance for perceived indoor air quality,” in *Atmospheric environment*, 2001, pp. 4407- 4417.