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Garbage enzyme: A study on compositional analysis of kitchen waste ferments

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Abstract
Garbage enzyme (GE) refers to Kitchen waste ferments which is an organic solution produced by the simple fermentation of fresh kitchen waste (fruit and vegetable peels), sugar (brown sugar, jaggery or molasses) in water. It is vinegar like liquid, multi-component in nature and has been reported to be a multipurpose domestic preparation. Garbage enzymes have been reported to have applications as cleanser, deodoriser, insect repellent, soil conditioner, pesticide and fertilizer. Not much has been said about bioactive constitution of garbage enzyme which could be responsible for their multiple roles. In the present study, some of the physicochemical, enzymatic and microbial characteristics of indigenous laboratory preparations of Garbage Enzyme were analysed. Main findings indicated that GE was acidic in nature, acetic acid being a major component. Biochemical analysis of GE preparations revealed the presence of acetic acid, sugars, proteins, alcohol, enzyme activities like protease, amylase, lipase and papain. Heterogeneous microbial flora was found to be present. Therefore, the present study provides evidence for the application of GE as a multipurpose liquid. Garbage enzyme is eco-friendly alternative to commercial cleaning agents that cause water pollution and subsequently affect the environment adversely.

Keywords: Garbage enzyme, kitchen waste, characteristics

Introduction
Garbage Enzyme is a concentrated, complex liquid, a product of a 3-month fermentation process made from kitchen waste (fruits and vegetables peels), sugar (black sugar/ jaggery and molasses) in water. Garbage Enzyme was first reported by Dr. Rosukon Poompanvong, an alternative health-care practitioner from Thailand [1]. It is claimed to be a multipurpose solution for household and agricultural uses and is reported to act as household cleanser, insect repellent, air purifier, soil conditioner, fertilizer, pesticide etc. [2]. A product after 3 months of fermentation reported to contain acetic acid, alcohol, Propionic acid as major components [3].
Fruit and vegetables wastes are generated in huge amounts from both households, industrial sources and large scale GE production could put these wastes to better use. Waste materials may have additional application through GE and be composted into organic fertilizer. This may provide a viable and low cost environment friendly alternativeto many other improper solid waste management strategies. The present study therefore was carried out to investigate into the biochemical, enzymatic and microbiological composition of laboratory preparations of Garbage Enzymes using different types of organic waste.

Material and Methods
a. Method for GE preparation: A Ratio of 1:3:10 of sugar (jaggery/Molasses), vegetable/fruit peels and water were mixed and allowed for expansion in air tight plastic bottles. These bottles were then placed in cool, dry, well-ventilated area dark area. Direct sunlight was avoided. Gases were released daily from bottles. After 3 months, the concentrated liquid from the residues was filtered and filtrate was stored for further use [4]. Residues were used as fertilizer after drying. In the present study, two types of GE were prepared depending upon different kitchen waste.

1. **GE-1:** Filtrate obtained from different fruits peel (papaya, banana, Sapodilla (chicku) and pomegranate).
2. **GE-2:** Filtrate obtained from vegetables peels (potato, gourd, egg plant (brinjal) and turnip).

b. Biochemical characterization of GE

1. **Estimation of Protein (laboratory manual of biotechnology):** Protein was estimated in

the test samples using standard Biuret method. BSA was used as standard protein [5].

2. **Estimation of Carbohydrate:** Carbohydrates were estimated using anthrone method and glucose was used as standard [6].
3. **Acetic acid Estimation:** Concentration of acetic acid in GE samples was determined by an acid- base neutralization titrimetric method in which an acid and a base reacted to produce water and a salt [7]. Sample was titrated with NaOH solution using phenolphthalein as indicator. For each titration, gram of acetic acid per litre of the sample was calculated.
4. **Estimation of Alcohol:** Acidified potassium dichromate solution was used as test reagent. Followed by boiling for 10 minutes and absorbance was recorded at 620nm [8].
5. **Qualitative Enzyme Analysis (Agar Plate Diffusion Assay)**

a. **Protease Activity:** Agar (1.5%) was prepared along with 1% (w/v) Casein as substrate. Samples were loaded into wells cut in agar plates [9]. Plates were incubated at 37 ºC for 24 hrs. Plates were stained with methylene blue and zone of clearance was observed. Proteinase K was used as positive control (0.03gm/5ml).

b. **Amylase activity:** For estimation of Amylase activity, Starch was used as substrate in agar diffusion assay [10]. After incubation, plates were flooded with iodine solution and zone of clearance was measured.

c. **Lipase Activity:** Tributyrin act as a substrate in Agar plate diffusion assay for lipase determination [11]. After incubation, plates were stained with methyl red dye and zone of clearance was visualized.

6. **Quantitative Analysis:**

a. **Amylase Activity:** Enzyme assayed was performed on the basis of reduction in blue colour intensity resulting from enzymatic hydrolysis of starch [12, 13]. Enzyme control, substrate control and tests were prepared. To prepare test, 0.25 ml of test sample (enzyme) and 0.25 ml of substrate were mixed and Iodine was used as test reagent. Absorbance was recorded at 690 nm. One unit of enzyme is equivalent to that amount of enzyme which causes 10% reduction in OD test as compared to that of substrate control under the assay conditions. Enzyme activity was calculated as follows: Factor A= OD of substrate control - OD of test/OD of substrate control Enzyme Unit= Factor A× 4×100× dilution factor/10

b. **Lipase Activity:** A spectrophotometric method was used for the measurement of lipase activity using p-Nitrophenylpalmitate (pNPP) as the substrate [14,15]. After incubation at 25ºC for 21 hours, 1ml of ethanol and acetone (chilled) (1:1) mixture was used as stopping reagent. Absorbance was read at 410 nm using spectrophotometer.

c. **Papain Activity:**

- 5 ml of Casein was measured and transferred into test tubes and warmed for 5 min at 37 ± 0.5ºC.1 ml of sample solution was added and kept for 10 at 37 ± 0.5 ºC to react after shaking the mixture. 5 ml of TCA was added and allowed to stand for 30 min at 37 ± 5 ºC. Then centrifuged the mixture at 10000 rpm at 9 ºC for 10 minutes to get the clear solution. Read the absorbance (Ab) of the filtrate at 275 nm using water as reference [16].
- In test tubes, measured 1 ml of the sample solution and added 5 ml of TCA and shook the mixture well. 5 ml of casein was added and shook the mixture well and allowed to stand for 30 min at 37 ºC. Read the absorbance (Ab) of the solution at 275 nm.
- Separately, measured the absorbance (As and As_0) of Tyrosine standard solution and 0.1 mol/l HCl at 275 nm using water as reference.
- One unit of the enzyme activity is the quantity of enzyme which increases an absorbance equivalent to 1µgof tyrosine per minute when the test was performed. Enzyme activity was calculated as follows:

$$\text{Enzyme Activity (Unit/ml)} = \frac{(A_t - A_b) \times 11 \times 5}{(A_s - As_0)}$$

7. Test for Microbial composition

a. **Bacterial composition:** 100µl of GE was spread on Nutrient agar plate with Amphotericin B as an anti-fungal agent. Plates were incubated at 37 °C for 24 hours to check the growth [17]. Gram positive and negative bacteria were identified by using Gram staining. Biochemical tests including Catalase test [18], Methyl red test, Voges-Proskaeur test [19] were done for bacterial identification.

b. **Fungal Composition:** YEPD Agar plates were prepared with Ampicillin as an anti-bacterial agent [20]. 100µl of GE was spread on agar plate and incubated at 27 °C for 24-48 hours. Fungal strains were identified using Lactophenol Cotton Blue Stain.

Results and Discussion

Physical characterization of garbage enzyme

GE used in the present study was prepared from kitchen waste which includes fruits and vegetable peels and was allowed to ferment for 3 months in the presence of brown sugar (Table1).

Table 1: Physical characteristics of both GE preparations

Physical characteristic	GE-1 Preparation	GE-2 Preparation
pH	4.3±0.4	3.3±0.2
Odour	Sweet sour	Pungent
Colour	Dark brown	Light brown
Turbidity	less viscous	more viscous

Physical parameters including pH, odour, colour and turbidity of GE preparations were analysed. As shown in above table1, pH was found to be about 4.3 and 3.3, indicating acidic nature which could be correlated with the presence of high content of acetic acid. GE-1 -preparation was found to be less viscous as compare to GE-2 preparation. Generally, the colour of GE was dark brown but the type of raw material used could be responsible for the characteristic variation in this physical parameter. If more vegetables were used than fruits, it smelled pungent and fragrant if more fruit peels were used in the preparation of GE [21].

Biochemical Analysis of Garbage Enzyme

- **Qualitative analysis:** Preliminary experiments were performed for the analysis biochemical constitution. As these enzymes are being obtained by the fermentation of vegetable and fruit peels, it indicates the presence of organic molecules. Both preparations of GE tested positive for carbohydrates, lipids, proteins, amino acids and acetic acid content whereas acetic acid and lipids were found to be present in vinegar.
- **Quantitative analysis**

a. **Acetic acid estimation:** Acetic acid concentration of both the garbage enzymes was determined by titrimetric

method. Preliminary test of both GE preparations showed the presence of acetic acid in GE although much higher concentration of acetic acid was present in vinegar (Table2).

Table 2: Estimation of acetic acid

Sample	Concentration (g/ml)
Vinegar	56.4±2.12
GE-1	4.2±0.42*
GE-2	5.4±0.28*

Acetic acid essentially, was a product of complete fermentation process and it has been earlier proposed to be associated with the preparation of similar kind [3]. As discussed earlier, the proposed action of GE as cleaner, deodorizer and disinfectant might be, partly due to the presence of acetic acid.

b. Estimation of proteins: Estimation of proteins was done by using by biuret method for GE preparations. The result obtained confirmed the presence of considerable levels of proteins (Table 3).

Table 3: Estimation of protein

Sample	Concentration (mg/ml)
Vinegar	2.575 ± 0.275
GE-1	4.225±0.261
GE-2	4.47±0.480

GE-2 was found to have higher concentration of protein compared to GE-1 preparation.

c. Estimation of carbohydrates: Estimation of carbohydrates was done by using by Anthrone method. Result obtained indicated the presence of significant levels of carbohydrates (Table 4).

Table 4: Estimation of carbohydrates

Sample	Concentration (mg/ml)
GE-1	14.295±0.219
GE-2	13.855±0.487
Vinegar	11.51±0.969

d. Estimation of alcohol: Result obtained indicated the presence of significant levels of alcohol in both preparations of GE (Table 5).

Table 5: Estimation of alcohol

Sample	Concentration (ml/ml)
GE-1	0.18
GE-2	0.13
Vinegar	0.09

Enzyme analysis

a. Qualitative Analysis: As the test preparations were obtained by the fermentation of vegetable and fruit peels, it indicated towards a possibility of the preparations being enzymatic in composition.

1. Protease Activity: Agar (1.5%) was prepared along with 1% (w/v) Casein as substrate. Samples were loaded into wells cut in agar plates [9]. Plates were incubated at 37ºc for 24 hrs and zone of clearance was recorded (Table 6, Figure 1).

Table 6: Protease activity of test GE preparations in agar diffusion method

Test Samples	Zone of clearance (in cm)
GE-1	1.6±0.01
GE-2	1.4±0.05
Positive control (proteinase k)	3.3±0.05
Negative control (water)	ND

Fig 1: Showing Protease activity in positive control (Proteinase k) which is observed as zone of clearance.

2. Lipase Activity: Analysis of lipase activity was carried out using tributyrin as substrate in the agar plate diffusion experiment. Zone of clearance was observed following flooding of plates with methyl red dye as indicator (Table 7, Figure 2).

Table 7: Lipase activity of test GE preparations in agar plate diffusion method

Samples	Zone of clearance (cm)
GE-1	2.35±0.1
GE-2	2.45±0.05
Negative Control (water)	ND

Results are Mean ±SD OF 3-5 independent observations.

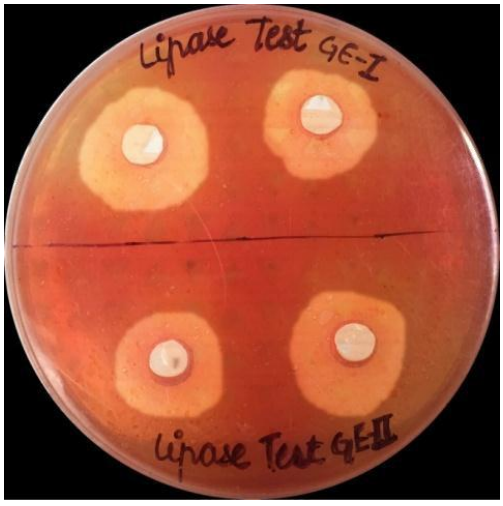


Fig 2: Showing the zone of clearance for lipase activity of GE 1 and GE 2 preparations.

3. **Amylase Activity:** Qualitative analysis for amylase activity was done using iodine flooding in agar plate diffusion and results observed as zone of clearance indicated the presence of amylase compared to α amylase as positive control (Table 8, Figure 3).
Zone of clearance indicates towards the presence of amylase in garbage enzymes preparations.

Table 8: Amylase activity of test GE preparations in agar plate diffusion method

Samples	Zone of clearance (cm)
GE-1	1.13±0.1
GE-2	1.43±0.5
Positive control (α amylase)	2.85±0.2
Negative control (water)	ND

Results are Mean ±SD OF 4-5 independent observations

A. B. C.

Fig 3: Showing the Amylase activity in (a) positive control (b) GE 1 and (c) GE 2 preparation

4. **Papain analysis:** Papain analysis was carried out by agar plate diffusion method using raw papaya juice as positive control and water as negative control showed a visible zone of clearance with GE-1 and GE-2 and the diameters of the test zones (1.49±0.1, 1.23±0.5) were comparable to that obtained with positive control (2.75±0.2) (Table 9).

Table 9: Papain activity of test GE preparations in agar plate diffusion method.

Samples	Zone of clearance
GE-1	1.49±0.1
GE-2	1.23±0.5
Positive control (Papaya juice)	2.75±0.2
Negative control (water)	ND

b. Quantitative analysis: Quantitative analysis was conducted for the estimation of enzymatic composition of both garbage enzyme preparations. Results obtained confirmed the presence of significant levels of amylase, protease, lipase and papain. Papain had been found to be significantly higher in the preparation of garbage enzymes which was produced using papaya peels as the major source. Spectrophotometric method was used for estimating papain activity in test preparations of GE and as seen, GE-1 and GE-2 were found to have considerable levels of papain activity which was of the order of 153.57±11.73 U/ml and 78.38±22.35 U/ml respectively (Table 10).

Table 10: Amylase and Papain activity in test garbage enzyme preparations.

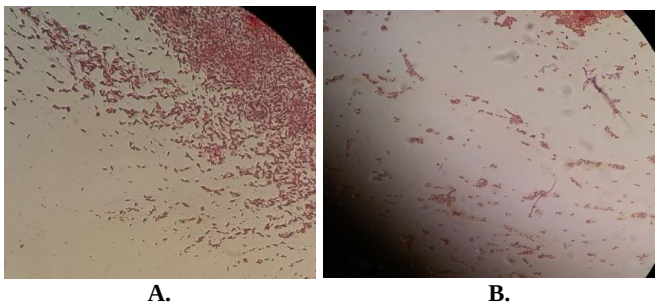
Sample	Amylase Activity (U/ml)	Papain activity (U/ml)
GE-1	151.6±25.02	153.57±11.73
GE-2	198.5±36.6	78.38±22.35
Vinegar	39.96± 0.212	47.68±0.180
Negative control	ND	ND

Results are Mean ±SD OF 3-5 independent observations.

The observed difference in the results clearly indicates that the enzyme composition of the preparations could be influenced and determined by the type of raw material employed. As reported by Tang and Tong (2011), fermentation broths obtained from orange peels and fermented for variable time periods ranging from 15 days to 5 months was found to contain α-amylase, protease and lipase as analyzed by HPLC and SDS-PAGE analyses confirm the existence of these three enzymes in fermentation broths. The study stands as a supporting evidence for the present work [21].

Microbial Composition

Microbial composition of garbage enzymes was examined by spread plate method and the bacterial and fungal growth was observed. Figure 4 and 5 showed microscopic examination following Gram's stain for bacteria and fungal staining with Lactophenol cotton blue.

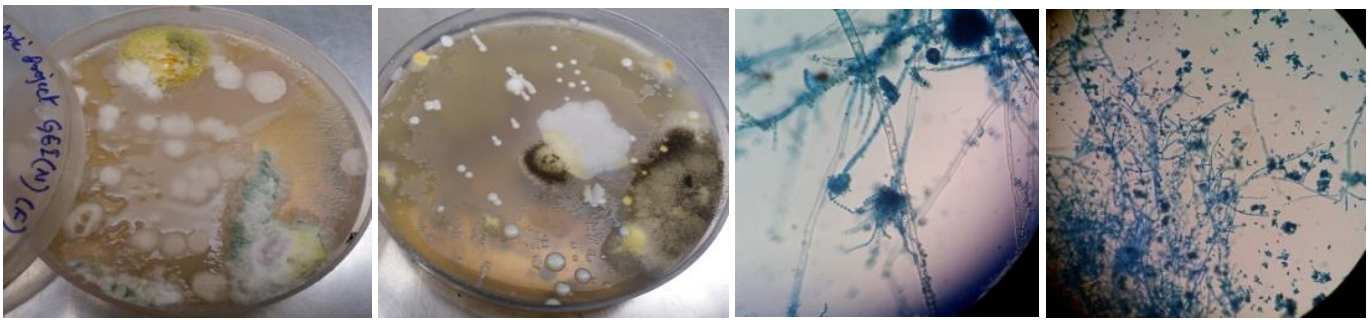


A. **B.**

Fig 4: Showing the presence of gram negative rod shaped bacteria

Microscopic examination of growth revealed the presence of gram negative rod shaped bacteria. The result is suggestive of the presence of heterogeneous bacterial populations in garbage enzymepreparations, which are possibly stable at room temperature. Biochemical test profiles for microbe identification indicated Catalase+, Methyl red +/-, lactose fermenting (acid/gas), Voges-proskaeur negative growth. Microorganism suspected are *Yersinia, Pseudomonas sp.*

Fungal Characterisation:



A. B. C. D.

Fig 5: Showing (a. and b.) plates having fungal growth (c and d) microscopic examination of fungal growth.

The presence of fungal growth was observed after 72hrs incubation. By microscopic examination, the presence of fungal hyphae was observed. Following Lactophenol cotton blue staining, fungi tentatively identified are *Aspergillus niger*, *Trichoderma viride*, *Saccharyomyces cerevisiae and Rhizopuss tolonifer*. In earlier report by Prakash (2011), it had been observed that white, black or brown layer, suggestive of yeast, was formed on top of the liquid [3].

It can be summarized that Garbage enzyme preparations were acidic in nature with pH range of 3.3-4.5; being light to dark brown in colour and had slight turbidity which varied with preparations. The preparation was found to have various organic substances like protein, carbohydrates, acetic acids and alcohol.

Quantitative analysis indicated towards the presence of enzymes like Amylase, Lipase, Protease and Papain in substantial amount in these preparations. Preparations were found to be positive for the presence of different types of microbes which are possibly stable at room temperature. Results indicated probable presence of microorganisms like Pseudomonas, Yersinia, A. niger, Trichoderma viride, Saccharomyces cerevisiae and *Rhizopuss tolonifera*.

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