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PREPARATION AND PHYSICOCHEMICAL ANALYSIS OF ECO-ENZYME FROM *CITRUS LIMON* L. BURM. F. (SHAUK) FRUIT PEELS WASTE

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Abstract

Eco-enzyme, an organic solution derived from the fermentation of fruit waste, brown sugar, and water in a 3:1:10 ratio, offers a sustainable alternative to chemical-based solutions. In this study, *Citrus limon* L. Burm. f. (Shauk) peels were collected and fermented for three months. The resulting eco-enzyme exhibited a dark brown colour, a fermented aroma, and physicochemical properties including pH 3.9, total acidity 1.74 g/L, density 0.953 g/mL, and alcohol content 2.50%. Alkaloids, organic acids, glycosides, flavonoids, saponins, and phenolic compounds were present in the prepared eco-enzyme, contributing to its antibacterial and cleaning properties. The prepared eco-enzyme was successfully applied as a cleaning agent for ceramics and whiteboards. Wastewater samples from Nantawgone Quarter, Meiktila Township, were treated with the eco-enzyme at concentrations of 5%, 10%, and 20% (v/v). The treatment effectively improved water quality parameters, including pH, total alkalinity, hardness, total dissolved solids, and chloride and sulphate contents. This study highlights the potential of eco-enzymes as an eco-friendly, cost-effective solution for environmental hygiene and wastewater treatment while also promoting the recycling of organic waste.

Keywords: *Citrus limon* L. Burm. f., eco-enzyme, fermentation, physicochemical properties, cleaning agent, wastewater treatment

Introduction

Eco-enzyme is a type of vinegar made by fermenting fruit waste with sugar to form organic acid and alcohol. Eco-enzymes are also called bio-enzymes, garbage enzymes, fruit enzymes, flower enzymes, etc. It is a type of homemade vinegar, reduced from alcohol by fermentation of kitchen waste as substrate with sugar. The liquid eco-enzyme contains protease, lipase, and amylase, all of which could be employed as cleaning agents (Indraloka *et al.*, 2023). It is a universal, natural cleaner produced from vegetable/fruit peels or waste. These organic substances are capable of breaking down chemical and other organic waste, thus helping us in removing stains, odours, getting rid of other harmful microbes, etc. Organic waste is frequently disposed of directly into the environment without treatment, causing negative environmental effects such as pollution, disruption of environmental aesthetics, and disruption of public health (Rusdianasari *et al.,* 2021). They also greatly neutralize toxins and pollutants. Eco-enzyme acts as an antifungal, antibacterial, and insecticidal agent (Pawar *et al.,* 2022).

Almost a fifth of the total citrus cultivars are subjected to industrial processes, mainly for the production of their juices, which represent the most consumed fruit juices worldwide, thus generating a large amount of processing waste (about 120 million tons per year) (Zema *et al.*, 2018). The high rate of pollution given by citrus waste depends on its easy fermentability, since it is abundant, and chemically complex, biodegradable, thus requesting high oxygen demand (Russo *et al.,* 2021). One method for reducing organic waste is to use fruit peel and vegetable waste to make eco-enzyme. An eco-enzyme is a complex organic liquid that contains a number of organic enzymes and acids that developed from waste lemon, lime, cabbage, pineapple, orange, and mango peels (Arun and Sivashanmugam, 2015).

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The main aim of this research is to prepare and analyze some physicochemical properties of eco-enzyme from peels of citrus fruits and to apply it as a cleaning reagent for ceramic products and wastewater treatment.

Materials and Methods

Sample Collection and Preparation

Citrus limon L. Burm. f. (Shauk) fruit peels samples were collected from the local market, Meiktila Township, Mandalay Region. *Citrus limon* L. Burm. f. (Shauk) fruit peels were cut with a knife into small pieces. Then 600 g of sample were mixed with 200 g of brown sugar as a carbon source and 2 L of water inside an airtight plastic bottle with a 5 L capacity and left standing overnight. After overnight, 0.3% yeast culture solution was added to the above bottle and stirred thoroughly. This was kept in a dark place at room temperature. Fermentation was conducted for 90 days in sun-protected conditions at room temperature (Figure 1). After 90 days, the content in the plastic bottle was filtered (Suliestyah *et al.,* 2022).

Figure 1. Fermentation vessel of eco-enzyme from *Citrus limon* L. Burm. f. (Shauk) fruit peels

Determination of Physicochemical Properties of Prepared Eco-enzyme Sample

During the fermentation period (15, 30, 45, 60, 75 and 90 days), the pH, total acidity, density, total dissolved solids and alcohol contents were determined by the AOAC method (AOAC, 2000). For the determination of the properties of the prepared eco-enzyme, pH values were determined by a pH meter, the total acidity contents by titrimetric method, density by a density bottle, total dissolved solids by an oven drying method, and alcohol contents by a hydrometer.

Qualitative Tests for Metabolite

Qualitative tests were carried out on eco-enzyme with a view to determine the presence of active constituents namely, alkaloids, flavonoids, phenolic compounds, saponins and glycosides (Trease and Evans, 1980; Harborne, 1993; Marini-Bettolo *et al*., 1981).

Application of Prepared Eco-enzyme

Application of eco-enzyme as a cleaning agent

In this study, 50% eco-enzyme has been used as a cleaning agent for whiteboard marker residue and ceramic items (dishes and basins).

Application of eco-enzyme in wastewater treatment

Wastewater sample was collected from the Nantawgone quarter, Meiktila Township, Mandalay Region. In this study, 5%, 10%, and 20% (v/v) of eco-enzyme dosage with the wastewater samples were selected. Four beakers were filled with 100 mL of wastewater sample and the respective dosage of eco enzyme solution. These beakers were covered and were left for a 1-day digestion period. Some physicochemical properties of wastewater samples before and after treatment with eco-enzyme were determined by AOAC method. In this study, pH values were measured by a pH meter, the total alkalinity, total hardness, and chloride contents were determined by titrimetric method, total dissolved solids were determined by an oven drying method, and sulphate contents were determined by UV-Visible spectrophotometer.

Results and Discussion

Fermentation Process in Preparation of Eco-Enzyme

In this study, eco-enzyme is a fermentation product of *Citrus limon* L. Burm. f. (Shauk) fruit peels, brown sugar, and water in a proportion of 3:1:10. During the fermentation process of making eco-enzyme, naturally occurring microorganisms on fruit scraps break down the organic material, producing enzymes, organic acids like acetic acid, and carbon dioxide, resulting in a liquid with cleaning and potentially fertilizing properties due to the breakdown of complex organic compounds into smaller, readily usable components; essentially, the microbes "digest" the waste, lowering the pH level and creating a beneficial enzyme-rich solution. After fermentation of 90 days, the final product of eco-enzyme is in the form of suspended residue and fermented liquid. The residue can be used as organic fertilizer residue. The total volume of eco-enzyme was found to be 2000 mL.

Physicochemical Properties of Eco-enzyme

pH and total acidity

Microorganisms, such as yeast and bacteria, play a crucial role in fermentation. The growth and multiplication of these organisms are also influenced by pH levels. In this research work, the pH of the eco-enzyme was within the acidic range throughout the period of fermentation. After 90 days of fermentation, the pH of the eco-enzyme was decreased from 6.3 to 3.9. The low pH of the eco-enzyme is caused by the high content of organic acids. The longer the fermentation time, the lower the pH of the eco-enzyme (Table 1 and Figure 2).

Eco-enzyme contains organic acids in the form of acetic acid and lactic acid. The organic acids contained in eco-enzymes are the result of the fermentation process (Natasya *et al.*, 2023). In this study, the total acidity of eco-enzyme was found to increase from 0.58 g/L to 1.74 g/L (Table 2 and Figure 3). This is due to one of the organic acids, acetic acid, which is produced through the fermentation process, is obtained when the sucrose contained in the fermentation solution turns into alcohol and continues to become acetic acid (Kamaliya and Lusiani, 2023).

Table 1. pH Values of Eco-enzyme Sample at Different Fermentation Periods

Fermentation period (day)	pH
0	6.3
15	5.9
30	5.3
45	4.8
60	4.5
75	4.1
90	3.9

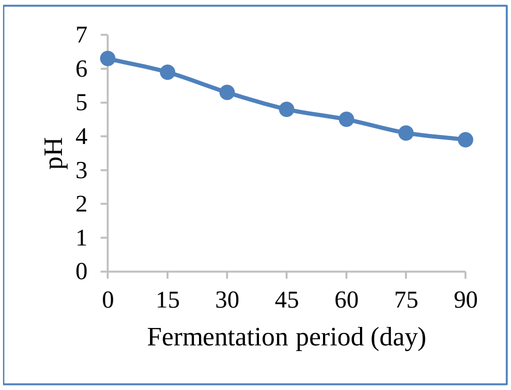

Figure 2. Changing in pH of eco-enzyme during fermentation

Table 2. Total Acidity of Eco-enzyme Sample at Different Fermentation Periods

Fermentation period (day)	Total acidity (g/L)
0	0.58
15	1.35
30	1.49
45	1.58
60	1.65
75	1.70
90	1.74

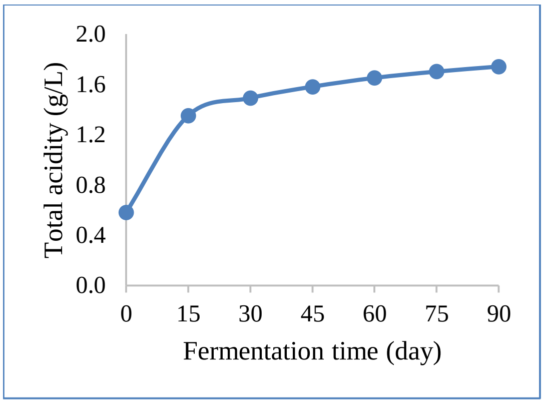

Figure 3. Changing in total acidity of eco-enzyme during fermentation

Density and total dissolved solids

The changes in density of the eco-enzyme sample during fermentation at different periods are shown in (Table 3 and Figure 4). Throughout the fermentation, the density values of the prepared eco-enzyme sample were found to decrease slightly. The density of eco-enzyme was found to be 0.953 g/mL after 90 days of fermentation.

The decrease in total dissolved solids of eco-enzyme was observed throughout the period of fermentation. In this study, the total dissolved solid of eco-enzyme was found to be 1357 mg/L after 90 days of fermentation (Table 4 and Figure 5). The total dissolved solid will vary depending on the type and amount of fruit waste used in the eco-enzyme through fruit waste. Generally, the total dissolved solid level of the eco -enzyme through fruit waste will be relatively low, as most of the components in the waste will break down during the fermentation process, typically ranging between 200 and 400 ppm. However, certain types of fruit waste, such as citrus fruits, may contain higher levels of minerals, resulting in a higher TDS level (Benny *et al*., 2023). Total Dissolved Solids (TDS) decrease as microorganisms break down complex organic compounds in fruit waste into smaller, soluble molecules like citric and acetic acids. These are absorbed by the water, reducing TDS levels.

Table 3. Density of Eco-enzyme Sample at Different Fermentation Periods

Fermentation period (day)	Density (g/mL)
0	1.023
15	1.010
30	1.003
45	1.000
60	0.990
75	0.959
90	0.953

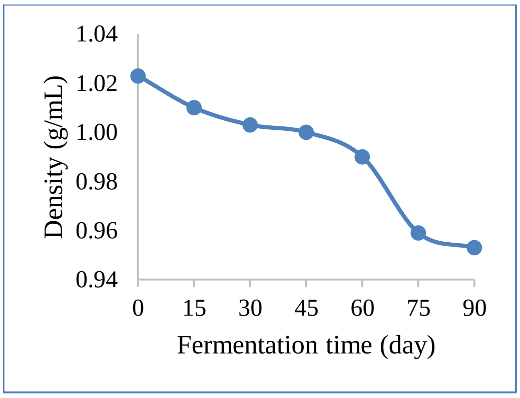

Figure 4. Changing in density of eco-enzyme during fermentation

Table 4. Total dissolved solids of Eco-enzyme Sample at Different Fermentation Period

Fermentation period (day)	Total dissolved solids (mg/L)
0	2200
15	1988
30	1710
45	1655
60	1490
75	1362
90	1357

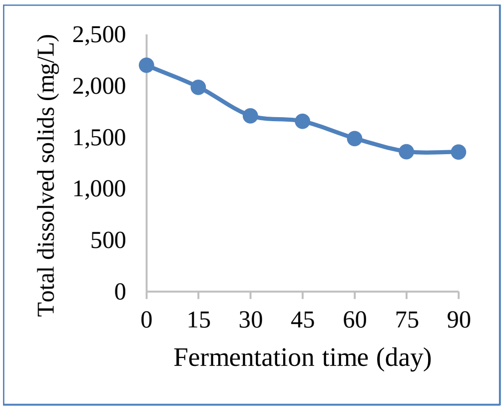

Figure 5. Changing in total dissolved solids of eco-enzyme during fermentation

Alcohol contents

Fermentation is a metabolic process in which organisms convert carbohydrates, like starch or sugars, into either alcohol or acids. Alcoholic fermentation is mainly carried out by yeast. In this study, the alcohol content of eco-enzyme was found to increase from 0.0% to 2.50%. This is because naturally occurring yeasts on the fruit peel break down sugars present in the fruit, converting them into ethanol (alcohol) as a byproduct of their metabolic process during anaerobic fermentation. It was found that, the longer the fermentation time, the higher the alcohol content (Table 5 and Figure 6).

Table 5. Alcohol Contents of Eco-enzyme Sample at Different Fermentation Periods

Fermentation period (day)	Alcohol content (%)
0	0.00
15	0.96
30	1.83
45	1.95
60	2.25
75	2.45
90	2.50

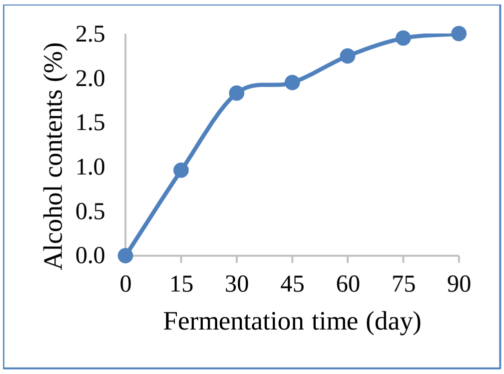

Figure 6. Changing in alcohol contents of eco-enzyme during fermentation

Physicochemical Properties of Eco-enzyme After 90 Days of Fermentation

After 90 days of fermentation, the eco-enzyme developed a dark brown colour and a distinct fermented aroma, influenced by the composition of the ingredients used. At the end of the fermentation process, the eco-enzyme exhibited a pH of 3.9, total acidity of 1.74 g/L, density of 0.953 g/mL, total dissolved solids of 1,357 mg/L, and an alcohol content of 2.50%. These physicochemical parameters agree with the ranges reported in the literature, indicating the preparation process yielded expected and consistent results.

Table 6. Some Physicochemical Properties of Eco-enzyme after 90 Days of Fermentation

No.	Properties	Prepared eco-enzyme	Literature values of eco-enzyme*
1.	colour	dark brown	-
2.	odour	fermented	-
3.	pH	3.9	2.9 - 6.5
4.	total acidity (g/L)	1.74	1.63-5.30
5.	density (g/mL)	0.953	0.999-1.035
6.	total dissolved solids (mg/L)	1357	1000-2000
7.	alcohol contents (%)	2.50	-

*(Abdullah *et al.*, 2023; Ismali, 2024; Lakra *et al.*, 2022; Kamaliya and Lusiani, 2023)

Metabolites in Prepared Eco-enzyme

The prepared eco-enzyme contained alkaloids, organic acids, glycosides, flavonoids, saponins and phenolic compounds (Table 7). Alkaloids have potent antibacterial activity. Organic acids such as acetic acid, lactic acid, and their mixtures possess strong antimicrobial activity and have been used as cleaning reagents and hand sanitizers. Phenolic compounds and flavonoids may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration**.** Saponins and glycosides could be used for several biological effects, such as antimicrobial, antibacterial etc. Saponin is also an effective cleaning agent. It is a natural detergent and foaming substance that is effective at lifting grease, dirt and grime from ceramic products.

Table 7. Test of Eco-enzyme after 90 days of Fermentation

No.	Tests compounds	Test reagents	Observation	Remark
1	alkaloids	Mayer's reagent	white ppt	+
		Dragendorff's reagent	brown ppt	+
		Wagner's reagent	brown ppt	+
2	organic acids	bromocresol green indicator	green colour solution	+
3	glycosides	10% lead acetate	white ppt	+
4	flavonoids	conc. HCl solution and Mg turnings	pale yellow colour solution	+
5	saponins	distilled water	frothing	+
6.	phenolic compounds	1 % $FeCl_3$ and $K_3[Fe(CN)_6]$	blue-black colour solution	+

(+) = presence, (-) = absence, ppt = precipitate

Application of Eco-Enzyme

Cleaning agent

In households, eco-enzyme cleaners are an eco-friendly alternative to traditional chemical cleaning products. They are equally effective at removing stains, disinfecting surfaces, and cleaning drains.

In this study, 50% eco-enzyme has been used as a cleaning agent for ceramic products (basins and dishes) and whiteboard marker residues. Figures 8, 9 and 10 show the before and after cleaning of the ceramic basin, oily dishes, and whiteboard marker residues. When the prepared eco-enzyme was applied to ceramic basins, it effectively removed calcium deposits and organic residues. Proteases break down protein-based stains, including food residues, eggs, blood, urine, feces, wine, and other beverages. Amylases break down starch-based residues, such as those from sauces, ice cream, and gravy. On greasy, oil-coated dishes, the lipase enzymes efficiently dissolve fats, making it easier to rinse away residues (Aartheeswari and Kirthiga, 2021). The prepared eco-enzyme was capable of removing whiteboard marker residues by breaking down the organic compounds in the ink, leaving the surface clean and residue-free.

Figure 7. Before and after cleaning of ceramics basin with 50% eco-enzyme

(a) dirty dish with grease (b) dirty dish with oil (c) clean dish

Figure 8. Before and after cleaning of ceramic dishes with 50% eco-enzyme

Figure 9. Before and after cleaning of whiteboard with 50% eco-enzyme

Wastewater treatment with eco-enzyme

Water quality parameters were evaluated to study the efficiency of the eco-enzyme solution in wastewater treatment. In this study, the collected wastewater sample was treated with different concentrations of eco-enzyme (5%, 10%, and 20%) (v/v), and some physicochemical parameters (pH, total alkalinity, hardness, total dissolved solids, chloride, and sulphate) were determined before and after treatment (Table 8). The results were compared with the standard values recommended by the World Health Organization (WHO, 1984).

The pH values of water treated with 10% and 20% eco-enzyme (7.2 and 6.6, respectively) fall within WHO standards, while that treated with 5% eco-enzyme (8.5) is at the upper limit but still acceptable. In terms of total alkalinity, the 10% eco-enzyme treatment is closest to the desirable limit (240 mg/L vs. 200 mg/L) (Singh and Simgh, 2008). Both 10% and 20% eco-enzyme treatments meet the acceptable range for total hardness, but only the 10% treatment meets the WHO standard for total dissolved solids (TDS) at 1000 mg/L. Chloride contents in all treated samples are within acceptable limits. Although sulphate contents exceed WHO standards in all cases, the 10% eco-enzyme treatment shows the lowest level, making it the closest to the standard. It was found that different concentrations of eco-enzyme treatment reduced the values of pH, total alkalinity, total hardness, total dissolved solids, chloride contents, and sulphate contents of the wastewater. However, the 10% eco-enzyme treatment demonstrates the optimum condition, meeting pH, total alkalinity, total hardness, TDS, and chloride standards, with sulphate slightly above the recommended limit but lower than other treatments.

Table 8. Some Physicochemical Properties of Wastewater Samples before and after Treatment with Prepared Eco-enzyme Sample

No.	Wastewater Sample	pH	Total alkalinity (mg/L)	Total hardness (mg/L)	Total dissolved solids (mg/L)	Chloride contents (mg/L)	Sulphate contents (mg/L)
1.	before treatment	9.4	390	339	1500	256	497
2.	5 % eco-enzyme	8.5	290	290	1350	230	431
3.	10 % eco-enzyme	7.2	240	235	1000	213	423
4.	20 % eco-enzyme	6.6	110	202	1300	210	460
5.	WHO Standards	6.5-8.5	200	300	1000	250	400

Conclusion

This study focused on producing eco-enzyme from *Citrus limon* peels through fermentation with brown sugar and water. Over 90 days, the fermentation process altered its properties, increasing acidity and alcohol content while reducing pH, density, and total dissolved solids. The prepared eco-enzyme contained alkaloids, flavonoids, and organic acids, contributing to its antibacterial properties. It proved effective as a natural cleaning solution for ceramics and whiteboards. It is also effective in wastewater treatment, offering a sustainable, low-cost alternative to synthetic chemicals. The prepared eco-enzyme not only reduces organic waste but also supports environmental hygiene and agricultural applications.

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