



Initial Screening of Physical and Biochemical Characteristics of Ecoenzyme Solutions from Fruits and Spices

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ABSTRACT

Ecoenzymes are known as multipurpose solutions. This study aims to conduct initial screening of the physical and biochemical characteristics of ecoenzyme solutions made from fruit and spices with different sugars. Analysis of physical characteristics was carried out by analyzing color and odor as well as the acidity (pH) value. Biochemical character analysis was carried out by observing the presence or absence of primary and secondary metabolites in the tested ecoenzyme solutions. The results showed that the tested ecoenzyme solution had a brown color with a fresh, pungent odor and an acidic pH. Analysis of the primary metabolite content showed positive results for the presence of reducing sugars and fats, while analysis of the secondary metabolite content showed the presence of alkaloids and flavonoids in the tested ecoenzyme solutions. The results of this study are expected to add scientific information related to the further utilization of ecoenzyme solutions made from fruit and spices.

INTRODUCTION

Since its introduction in the 1980s, the multipurpose fermentation solution known as ecoenzyme has been widely used for human benefit. Ecoenzyme is a fermented product typically made from organic waste such as fruits and vegetables, sugar, and water. The fermentation process can last 3-6 months, during which physical changes occur, such as odor, color, and pH. Furthermore, during the fermentation process, in addition to physical changes, biochemical processes also occur, including the breakdown of sugars by microorganisms due to the sugar added to the ecoenzyme solution. Furthermore, protein hydrolysis into simple amino acids also occurs during this process, which can increase the biological value of the ecoenzyme solution. In the lipid domain, fat breakdown has also been detected during the fermentation process, which can affect the physical and chemical characteristics of the ecoenzyme solution (Elhalis et al., 2020).

Organic materials such as vegetables and fruit are often initially used solely for the purpose of reducing organic waste. However, as their utilization increases, it turns out that the secondary metabolite content in organic waste makes ecoenzyme solutions suitable for various purposes, including potential applications in health and sustainable agriculture. Several studies have shown that the fermentation process of organic materials can increase the number of bioactive compounds due to the activity of microorganisms that break down complex compounds into simpler ones (de Oliveira et al., 2020).

Fruits and spices have been shown to contain secondary metabolites such as alkaloids and flavonoids. The use of more than one type of organic material in ecoenzyme production is considered superior due to the complexity of secondary metabolites compared to using a single type. The fermentation process has been reported to increase the content of secondary metabolites, making this process a valuable way to add value to organic waste (Leonard et al., 2021).

Initial screening of physical and biochemical characteristics can be two important steps: identifying the quality of the resulting ecoenzyme solution and qualitatively identifying the primary and secondary metabolites present in the organic material. Physical tests include color, odor, and pH, followed by biochemical tests, which examine primary and secondary metabolites using special solutions. These tests rely on color changes or morphological changes, such as the formation of gas or sediment, that occur before and after the reaction. This initial screening is typically used to provide an initial overview of the quality of the ecoenzyme solution and the primary and secondary metabolite content of an organic material, before the material is further tested using more sensitive qualitative and quantitative methods, thus providing an overview of its further utilization (Sabater et al., 2020).

Although research on ecoenzymes continues to develop, preliminary screening of the physical and biochemical components of ecoenzyme solutions made from fruits and spices remains relatively limited. Most reported studies have focused on initial screening of ecoenzyme solutions made from fruits and/or vegetables. Therefore, research on the initial screening of the biochemical characteristics of ecoenzyme solutions made from fruits and spices is deemed

necessary to determine the physical condition and primary and secondary metabolite content of these ecoenzyme solutions, which are the result of the fermentation process. The results of this research are expected to be the basis for the use of ecoenzyme solutions, especially those made from fruit and spices, as a source of bioactive compounds in the fields of agriculture, food, and health.

METHODS

The materials used in this study included an ecoenzyme solution based on fruit and spices: dragon fruit peel, soursop seeds, avocado seeds, and nutmeg. This ecoenzyme solution used three different sugar sources: molasses, Javanese sugar, and palm sugar. In addition, several reagents for secondary metabolite testing were used in this study, such as Dragendorff reagent, FeCl_3 , HCl , CuSO_4 , and NaOH .

Methods

This study conducted an initial screening for physical characteristics, namely color, odor, and pH. Subsequently, an initial screening for biochemical characteristics, namely the content of primary and secondary metabolites, was conducted.

Physical Character Screening

The physical character screening was carried out using color, odor, and pH parameters. Color and odor analysis was carried out by directly observing and smelling the ecoenzyme solution. The pH analysis was performed using pH paper (MQuant™) by dipping the paper into the paper and comparing the resulting color with the standard color on the pH paper package.

Biochemical Character Screening

a. Reducing sugar test

The reducing sugar test is conducted using the Benedict's method. 2 mL of ecoenzyme solution is placed in a test tube and 2 mL of Benedict's solution is added. The solution is then homogenized and heated for 5 minutes. A positive test is indicated by a color change from green, yellow, orange, to brick red.

b. Protein test

The protein test is conducted using the Biuret method. 2 mL of ecoenzyme solution is placed in a test tube and 2 mL of 10% NaOH solution is added. The solution is then stirred and 2-3 drops of 1% CuSO_4 solution are added and homogenized. A positive test is indicated by the appearance of a purple color.

c. Fat test

The fat test is conducted using oil paper. The ecoenzyme solution is spotted onto the oil paper and allowed to dry. A positive test is indicated by the appearance of an oil trail on the paper.

d. Organic acid test

The organic acid test is conducted using the sodium bicarbonate method. 5 mL of ecoenzyme solution is placed in a test tube and 1/2 tablespoon of baking soda is added. A positive test is indicated by the formation of gas bubbles.

e. Alkaloid test

This test is performed by adding 2 mL of ecoenzyme solution to a test tube and adding 2-3 drops of Dragendorff's reagent. A positive test is indicated by the formation of a brick-red precipitate.

f. Saponin test

This test is performed by adding 2 mL of ecoenzyme solution to a test tube and shaking for 1 minute. A positive test is indicated by the formation of foam that persists after 3 minutes of shaking.

g. Tannin test

This test is performed by adding 2 mL of ecoenzyme solution to a test tube and adding 2-3 drops of 1% FeCl_3 solution. A positive test is indicated by a color change to blackish green or dark blue.

h. Flavonoid test

This test is performed by adding 2 mL of ecoenzyme solution to a test tube and adding 2-3 drops of concentrated HCl. A positive test is indicated by a color change to yellow, orange, or red.

i. Coumarin Test

This test is performed by adding 2 mL of ecoenzyme solution to a test tube and adding 5 drops of 10% NaOH. A positive test is indicated by a color change to yellow.

RESULTS

Table 1. Physical Character Screening

Parameter	Ecoenzyme with molasses sugar	Ecoenzyme with brown sugar	Ecoenzyme with palm sugar
Color	Dark brown	Brown	Brown
Odor	Fresh and pungent	Less fresh but pungent	Less fresh but pungent
pH	3	4	34

Table 2. Primary Metabolites Screening

Parameter	Ecoenzyme with molasses sugar	Ecoenzyme with brown sugar	Ecoenzyme with palm sugar
Reducing sugar	+	+	+
Protein	+	-	-
Lipid	+	+	+
Organic acids	+	-	-

Table 3. Secondary Metabolites Screening

Parameter	Ecoenzyme with molasses sugar	Ecoenzyme with brown sugar	Ecoenzyme with palm sugar
Alkaloids	+	+	+
Saponins	-	-	-
Tannins	+	+	-

Flavonoids	+	+	+
Coumarins	-	-	-

DISCUSSION

Enzyme solutions, an example of a fermentation solution, exhibit distinctive characteristics when successfully prepared. One characteristic is a pungent, fresh odor. Research shows that three ecoenzyme solutions with different sugar sources exhibit the same pungent, fresh odor. This pungent odor originates from acidic compounds such as acetic acid, lactic acid, and alcohol, combined with volatile gases such as ammonia and hydrogen sulfide, which are produced during the fermentation process (Algiffary & Sutabri, 2023).

Furthermore, the color of the three ecoenzyme solutions with different sugars was brown. This indicates the degradation of organic compounds during fermentation (Santosa et al., 2023). Furthermore, the color change to dark brown is also related to the length of fermentation. The brown color begins to appear after approximately three months (Hamada, 2025), and the longer the fermentation process, the deeper the brown color becomes (Okto Supratman, Mualimah Hudatwi, 2023).

In testing the physical characteristics, the results showed that the three ecoenzyme solutions with different sugars exhibited different fermentation pH values. Based on the results, the ecoenzyme solution with molasses added had a pH of 3, while the treatments using Javanese sugar and palm sugar each had a pH of 4. These results indicate that the ecoenzyme solutions with different sugars had an acidic pH, a key factor in confirming the successful ecoenzyme production process. These results indicate that microorganisms are decomposing organic matter into simple organic compounds, particularly organic acids such as acetic acid and lactic acid. This acidic condition indicates the formation of metabolites resulting from the degradation of organic matter (Sambaraju & Sree Lakshmi, 2020). These results align with numerous previous studies, such as those on ecoenzyme production using vegetables and fruit (Gumilar et al., 2025).

Regarding the analysis of primary metabolite characteristics, the results showed that the reducing sugar and fat tests yielded positive results in all three ecoenzyme solutions. This indicates the process of sugar and fat degradation, indicated by the presence of amylase and lipase enzyme activity (Retnowati et al., 2023). Conversely, in the protein and organic acid tests, only the ecoenzyme solution containing molasses sugar showed positive results, and this is closely related to the much more complex composition of molasses compared to Javanese or palm sugar. Furthermore, molasses contains simple sugars, nitrogen, minerals, and organic compounds, compared to Javanese or palm sugar, which are primarily composed of sucrose (Zhang et al., 2021). These results align with research (Karimah & Lusiani, 2024) that used brown sugar, resulting in a negative protein test.

Initial screening for secondary metabolite content showed that the three ecoenzyme solutions containing different sugars contained alkaloids and flavonoids. This suggests that secondary metabolites are already present in the organic material, and over time, some secondary metabolites may be released

from the plant tissue due to microbial activity (Bawanda et al., 2025). The results of this study align with several previous studies, such as those by Rusdianasari et al. (2021) on ecoenzyme solutions using pineapple, papaya, and orange peels.

Tannin tests showed that ecoenzyme solutions containing molasses and Javanese sugar contained tannins, indicated by a color change to blackish green or dark blue. However, this did not occur in ecoenzyme solutions containing palm sugar. This is likely due to the very high tannin content in molasses, which may also be present in Javanese sugar, but not in palm sugar. The difference in sap between Javanese and palm sugar is also suspected to be the cause of the higher tannin content in Javanese sugar. Furthermore, the longer heating process results in a higher tannin content in Javanese sugar than in palm sugar (Saputro et al., 2020).

Finally, the three ecoenzyme solutions containing different types of sugar did not contain saponins or coumarins. This is likely caused by the fermentation process due to the degradation of saponins and coumarins due to the activity of microorganisms which are ultimately broken down into other compounds so that both are not detected in the three types of ecoenzyme solutions tested (Huang et al., 2022), (Sariri & Yakin, 2020) even though the basic ingredients of the fruit and rhizomes used are proven to contain saponins and coumarins.

CONCLUSIONS AND RECOMMENDATIONS

The physical characteristics of the three ecoenzyme solutions tested showed good ecoenzyme solutions. Furthermore, for biochemical characteristics, especially in the qualitative testing of primary metabolites, it showed that only reducing sugar and fat content were detected in the three ecoenzyme solutions tested, and in the qualitative testing of secondary metabolites, it showed that alkaloid and flavonoid content were detected in the three ecoenzyme solutions tested.

FURTHER STUDY

Further research, both qualitative and quantitative, is needed to confirm the results of this study.

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