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The potential of local orange peel-derived ecoenzymes in producing indole acetic acid

Siska Alicia Farma[1,2*], Nurfa Dewiza Luzik[1], Salma Sakina[1], Irma Leilani Eka Putri[1,2], Linda Advinda[1], Azwir Anhar[1,2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, West Sumatera, Indonesia
[2]Center of Research on Recycling Organic Waste Management, Universitas Negeri Padang
[*]Corresponding author: Email: siskaalicia@fmipa.unp.ac.id

ABSTRACT

Background: Ecoenzymes, created from the fermentation of organic citrus waste, offer a sustainable method to produce Indole Acetic Acid (IAA), a phytohormone vital for plant growth. This study investigates the potential of these ecoenzymes in promoting sustainable agriculture.

Objective: This study aims to evaluate the capacity of ecoenzymes derived from local citrus organic waste to synthesize IAA hormones.

Methods: The ecoenzyme was extracted from fruit powders and centrifuged to separate the supernatant. One ml of ecoenzyme supernatant was then mixed with 2 ml of Salkowski reagent and incubated for 12 hours at room temperature in the dark to facilitate reaction. The presence and concentration of IAA were determined using spectrophotometry at a wavelength of 530 nm, while total protein levels were measured using the Warburg-Christian method.

Results: ecoenzymes from local citrus sources contain IAA, with the highest concentration observed in sample 7A (30.26 µg/ml). The ecoenzyme exhibited favorable characteristics, including an average degree of acidity of 3.55, and the highest total protein content was found in sample 2A (144.277 mg/mL).

Conclusion: Ecoenzymes from local orange peels successfully produce IAA, supported by fermentation-induced microbial activity and acidic conditions. This highlights their potential in sustainable agriculture.

Keywords: ecoenzyme, indole acetic acid, phytohormones, plant

Introduction

Ecoenzymes are fermented products derived from organic waste [1]. The fermentation process required to produce an ecoenzyme solution spans approximately three months [2]. As biocatalysts, ecoenzymes expedite natural biochemical reactions, generating enzymes that are beneficial for processing fruit or vegetable waste [3]. These enzymes represent a viable waste management strategy, contributing to the global objective of achieving zero waste [4]. Notably, orange peel is commonly utilized in the production of ecoenzymes. In West Sumatera, citrus fruits, particularly oranges, are prevalent. The ecoenzymes derived from organic orange peels contain amylase, protease, and lipase. Furthermore, ecoenzymes can produce nitrate (NO_3) and carbon trioxide (CO_3), essential nutrients for soil health [5].

Phytohormones play a critical role in plant growth and development, with five primary groups identified: gibberellins, ethylene, cytokinins, abscisic acid, and auxin. Among these, auxin is particularly significant, influencing various physiological processes in plants, such as cell elongation, water absorption, abscission inhibition, lateral bud growth suppression, root formation, and cambium activity.

One of the factors that play an important role in plant growth and development is phytohormones. Among the five groups of phytohormones—gibberellins, ethylene, cytokinins, abscisic acid, and auxin—auxin stands out for its critical contributions to physiological changes in plants [6].

Table 1. Organic ingredients orange peel variations

No	Local oranges waste code	Amount of orange peel variations (g)	Types of oranges
1	1A	225 g, 225 g, 225 g, 225 g	Mixed
2	2A	450 g, 450 g	Sweet
3	3A	900 g	Sweet
4	4A	900 g	Sweet
5	5A	450 g, 450 g	Sour
6	6A	900 g	Sour
7	7A	900 g	Sour

These changes include promoting cell elongation, enhancing the cells' water absorption capacity or facilitating abscission, inhibiting the growth of lateral buds, and supporting root formation and cambium activity [7].

Indole Acetic Acid (IAA), a vital component of the auxin group, is naturally produced in plant meristems and is crucial for cell elongation. However, the endogenous production of IAA may not always meet the plant's needs, necessitating external (exogenous) sources of IAA [8]. Exogenous IAA can be synthesized through bacterial activity, for example, by *Pseudomonas fluorescens*, which has been shown to enhance stem growth in chili plants [9]. The effect of exogenous IAA on plant growth varies with concentration; high concentrations promote the development of lateral and adventitious roots, while low concentrations encourage primary root growth [10].

Exogenous IAA production by microorganisms requires tryptophan as a precursor [11,12]. Given the potential for ecoenzymes to produce IAA, further research into this capability could significantly increase the value of ecoenzymes, offering benefits for subsequent studies and broader impacts, notably in plant growth enhancement.

Methods

Ecoenzyme production

This study was conducted at the Plant Physiology Laboratory, Padang State University, West Sumatera, Indonesia. Ecoenzyme was produced using local orange peels from three categories: sweet oranges, sour oranges, and a mixture of both. Specifically, the orange varieties used included Pasaman orange, Gunung Omeh orange, lime, and kaffir lime. A total of 900 g of local orange waste and 300 g of molasses were fermented in 3 liters of water within an airtight container for three months. The orange peels were categorized into seven distinct variations (Table 1).

Physical characteristic of the ecoenzyme

The parameters measured included the degree of acidity (pH) and total protein content. The total protein content was quantified using the Warburg-Christian method.

Measurement of IAA

IAA levels were quantitatively assessed using spectrometry and the Salkowski reagent test [13]. The Salkowski reagent was prepared by combining 2 mL of 0.5 M $FeCl_3$ solution with 98 mL of 35% $HClO_4$, and the mixture was stored in a dark container.

To prepare the standard curve, a stock solution of IAA at 100 ppm was diluted to obtain standard solutions ranging from 0.10, 20, 30, 40, 50, to 60 ppm.

The ecoenzyme solution was separated from the fruit residues through filtration. Each filtered sample, amounting to 10 mL, was placed into two test tubes and then centrifuged at 4,000 rpm for 30 minutes. The clear supernatant was carefully transferred to a sterile test tube for further analysis.

For the IAA assay, 1 mL of the supernatant was combined with 2 mL of Salkowski's reagent in each test tube. This mixture was incubated

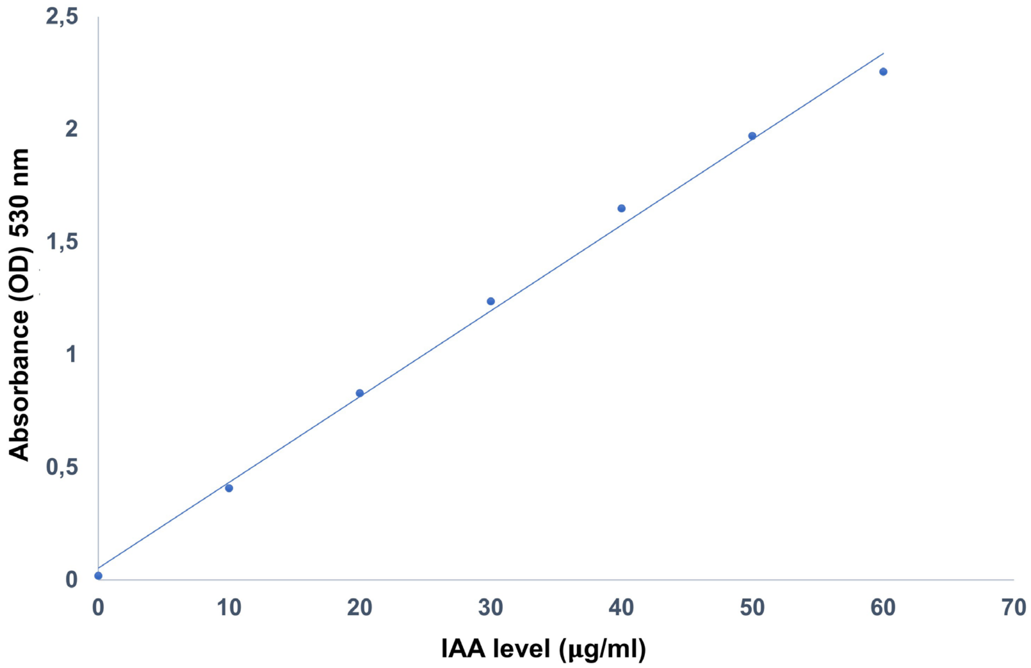

Figure 1. IAA standard curve

for 12 hours at room temperature in the dark to allow for color development. The absorbance of the resulting solution was measured at 530 nm using a spectrophotometer.

Data analysis

The absorbance values obtained were plotted against the IAA standard curve (ranging from 0 to 60 ppm) to determine the final concentration. This process allowed for the quantification of IAA activity present in the ecoenzyme solutions [10].

Results

Physical and environmental characteristic

Based on the measurement results characteristics of ecoenzyme, it was found the average degree of acidity of ecoenzyme is 3.55. While the highest total protein content was found in sample A2 the sweet local oranges, which was 144.277 (mg/mL) (Table 2). This proves that ecoenzyme has good characteristics to stimulate the formation of the auxin IAA hormone.

The ecoenzyme's physical characteristics were quantitatively assessed, revealing an average acidity (pH) of 3.55. Notably, the highest total protein content was observed in sample 2A, derived from sweet local oranges, with a concentration of 144.28 mg/mL (Table 2). These findings indicate that the ecoenzyme possesses favorable properties conducive to stimulating IAA formation.

Table 2. Protein total in samples

Sample	Protein total level (mg/mL)
1A	111.90
2A	144.28
3A	138.63
4A	134.86
5A	128.84
6A	93.45
7A	105.87

IAA levels in ecoenzyme

IAA concentration within the ecoenzyme was determined through spectrophotometric absorbance measurements, which were then compared to a standard IAA curve. The creation of this standard curve was aimed at deriving a regression equation to facilitate the calculation of ecoenzyme IAA concentration (Figure 1). The derived regression equation was $y = 0.038x + 0.053$, with a regression

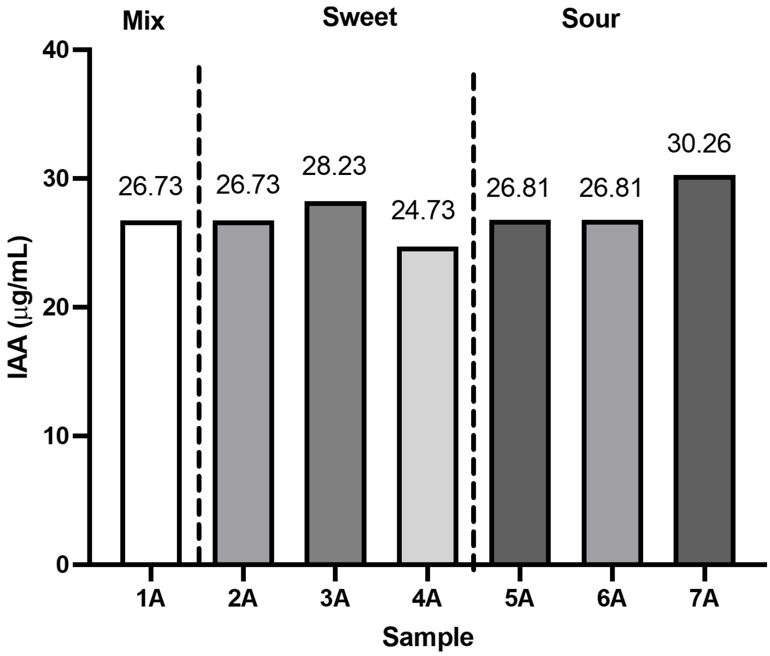

Figure 2. IAA ecoenzyme concentration

value of 0.996, indicating a high level of correlation. The measured ecoenzyme samples exhibited IAA concentrations ranging between 26.73 µg/mL and 30.26 µg/mL. Among these, sample 7A displayed the highest level of IAA (Figure 2).

Discussion

The ecoenzyme, derived from local orange peels, demonstrated favorable physical and environmental properties. Utilizing three variants of local oranges—sweet, sour, and a mixed category—the successful production of ecoenzymes is evidenced by their characteristic brown liquid form and acidic nature, with a pH below 4 [6]. This acidic condition is particularly conducive to the formation of hormones such as auxin, gibberellins, and cytokinins [14].

Hormones are broadly classified into three categories: protein hormones, steroids, and amines. Proteins, as macromolecules composed of amino acids linked by peptide bonds, serve various functions including enzymatic activity, transportation, and hormone production. The fermentation duration [15,16], impacting the proliferation of microorganisms and their proteolytic enzyme activity, influences the ecoenzyme's protein content [17,18]. Our findings suggest that the high total protein content in the ecoenzyme could facilitate the production of hormones scuh as auxin.

The biosynthesis of the hormone IAA is notably influenced by the availability of tryptophan as a precursor. The hydrolysis of tryptophan by tryptophanase results in the production of indole and pyruvic acid [19]. Moreover, certain endophytic bacteria, which inhabit plant tissues such as roots, stems, and fruits, possess the capability to synthesize tryptophan [2]. This synthesized tryptophan subsequently facilitates the production of IAA, highlighting the symbiotic relationship between plants and their endophytic microbial communities.

Ecoenzymes host a diverse microbiome influenced by the nature of the substrate. Specifically, ecoenzymes derived from organic orange peels demonstrate significant activity of lactic acid bacteria [20]. This beneficial microbial activity is also observed in ecoenzymes created from organic mango peels [9], suggesting a consistent pattern of lactic acid bacteria involvement across different substrates. The fermentation process employed in ecoenzyme production not only yields a distinctive sour aroma, attributed to acetic acid, but also establishes an environment conducive to the microbial synthesis of IAA. This synthesis occurs through bacterial metabolic processes that are naturally supported by the nutrient-rich environment of fruit and vegetable waste.

The IAA present within ecoenzymes is a product of bacterial metabolism, utilizing tryptophan available in the ecoenzyme solution. The fermentation, an anaerobic metabolic process, enables bacteria to extract energy from carbohydrates in the absence of oxygen, producing by-products such as alcohol and acetic acid. The carbohydrates necessary for this fermentation are supplied through sugars—such as brown sugar, palm sugar, or molasses—incorporated during the ecoenzyme production. Brown sugar, in particular, is rich in free amino acids including lysine, tryptophan, glutamic acid, aspartic acid, alanine, and glycine, further supporting the microbial synthesis of IAA [7].

IAA synthesis in ecoenzymes can proceed via multiple pathways, one route being the indole-3-pyruvate pathway. In this process, tryptophan is converted by the enzyme aminotransferase, and in final step, IAAId is oxidized into IAA [21]. The dynamic of microbial population and the total protein content in ecoenzymes derived from local orange peels show an upsurge at fermentation's onset [20]. This phase facilitates an increase in amino acids [22], among which tryptophan serves as a crucial precursor for microbial synthesis of IAA.

Analysis of ecoenzyme supernatant samples revealed IAA production across all tested samples, with concentrations ranging from 26.73 µg/mL to 30.26 µg/mL (Figure 2). Notably, sample 7A exhibited the highest IAA concentration, attributed to its optimal pH facilitating accelerated bacterial activity and enhanced enzyme production. A 24-hour incubation period yielded lower IAA levels, ascribed to the logarithmic phase of bacterial growth where tryptophan-to-IAA conversion enzymes are less abundant [23]. Conversely, the peak production of IAA was observed at 48 hours of incubation, coinciding with a surge in enzymes crucial for IAA synthesis, such as tryptophan monooxygenase, IAM hydrolase, indole-pyruvate decarboxylase, and IAAld dehydrogenase. Beyond 72 hours, the microbial community enters a decline phase, significantly diminishing IAA production.

The concentration of IAA within the ecoenzyme impacts plant growth, with specific ranges promoting stem cell elongation, notably around 0.9 g/L [24]. Concentrations exceeding this threshold, however, can inhibit elongation. Ecoenzymes sourced from parenchyma-rich tissues are superior to those from epidermal tissue [25]. The application of ecoenzyme treatments to soil has demonstrated significant growth benefits for chili and aloe vera plants [26]. The effective concentration range for enhancing primary root growth lies between 10^{-9} and 10^{-12} M, while higher IAA concentrations may inhibit this growth [10]

Conclusion

The study established that ecoenzymes produced from various local citrus peels are capable of generating IAA, with an average concentration of 27.18 µg/mL. The highest IAA production was recorded in sample 7A, sourced from sour local oranges. The ecoenzymes' high protein content and average acidity of 3.55 underscore their potential as effective plant growth-promoting agents.

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Declaration of interest

None.

Author contributions

Conceptualization, SAF; Methodology, ILEP, AA; Investigation, NDL, SS; Writing – Original Draft, NDL, SS, ILEP; Writing – Review & Editing, SAF, AA; Funding Acquisition, SAF; Supervision, SAF.

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