Volume 4 (December 2024)

# Journal of Biobased Chemicals

Department of Chemical Engineering
Universitas Jember

# PREFACE

We want to present our journal's 4$^{th}$ volume and edition, Journal of Biobased Chemicals, published by the Department of Chemical Engineering, University of Jember, Indonesia. This volume is expected to enhance the findings and research aboutnatural product and their derivatives, mainly in energy, chemicals, and materials. We present articles on biobased chemical products, processes, and management.

This new journal was envisioned and founded to represent the growing needs of biobased chemicals research as an emerging and increasingly vital field, now widely recognized as an ideal substitution for fossil-based chemicals. The journal aimsto deliver and provide notable and standardized research and findings through journal reporting. The journal is intended as a window or a library for practitioners and researchers to share their works, identify new issues, and organize further research. At the same time, industrial users could apply the invention for scale-up, problem-solving, and application.

Hopefully, this edition will contribute valuable thought for the readers and enhance future research on biobased chemical products. Finally, we thank all participants, including authors, reviewers, and editors, for contributing.

December 2024

Boy A. Fachri

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## Journal of Biobased Chemicals

Journal of Biobased Chemicals implements a regular system for uploading, reviewing, and accepting the journal. Moreover, the journal is supported by an expert team in their field to maintain the quality of the publication.


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# Biosynthesis of Zinc Oxide Nanoparticles with Horned Banana Peel Waste Extract (*Musa paradisiaca fa. corniculata*)

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**Abstract.** The high amount of banana consumption in Indonesia has caused banana peel waste to increase, which can cause environmental pollution. One of the utilizations of banana peel waste is as a metal nanoparticle synthesis. This study aims to synthesize ZnO nanoparticles with banana peel extract as a capping agent and determine the effect of solvent volume variation on the characterization of ZnO particles using variable volumes of ethanol-water solvent with a ratio of 2:1 and 1:1 (v/v). This research uses the maceration extraction method for 24 hours. ZnO particles were characterized, including Fourier Transform Infrared Spectroscopy (FTIR) and Particle Size Analyzer (PSA). In this study, the total polyphenol and flavonoid content in ethanol-water 1:1 (v/v) horn banana peel extract was higher at 4.944 % and 5.940 % than in ethanol-water 2:1 (v/v) at 4.114% and 4.131%. Based on the results of FTIR testing, both samples have ZnO peaks where in ethanol-water 1:1 (v/v), 441 cm-1, and 619 cm-1, while ethanol-water 2:1 (v/v) is 428 cm-1. Then, from the PSA test results, the ethanol-water 1:1 (v/v) sample has a smaller average nanoparticle diameter of 135.6 nm than the ethanol-water 2:1 (v/v) sample, which is 153.6 nm. ZnO nanoparticles were successfully synthesized using the natural capping agent banana peel extract. Different levels of secondary metabolites in each extract influence the diameter of the synthesized ZnO nanoparticles.

**Keywords:** *capping agent, banana horn skin, ZnO nanoparticles*

## 1. Introduction

Banana plants are one of the tropical fruit commodities that are widely found in Indonesia. Indonesia is one of the world's banana-producing countries, with banana tons in 2020 of 8,182,756 tons, an increase of around 12.39% compared to 2019. As the population in Indonesia increases and the public's nutritional awareness increases, the demand for bananas also increases [1]. This is proven by the increasing amount of banana production in Indonesia in 2022 of 9,245,427 tons [2]. Banana peels are often overlooked and even considered waste, accounting for 35-50% of the total mass of banana fruit [3]. The high production and

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consumption of bananas in Indonesia has led to an increase in banana peel waste. If banana waste is not processed correctly, it will cause environmental pollution. One study showed that plant waste such as orange peel, lemon peel, mango peel, rice husk, and banana peel can be utilized as a metal nanoparticle synthesis. So, banana peel waste can be used as a nanoparticle synthesis agent [4].

Nanoparticles are one of the nanotechnologies that are experiencing rapid development. This is due to the high utilization of nanoparticles in human life, such as biomedicine, energy, industry, environment, electronics, and textiles [5]. Metal oxide nanoparticles are one of the promising types of nanoparticles due to their unique biological, chemical, and physical properties. Based on the research that has been done, some metals such as ZnO, $TiO_2$, CaO, and MgO have anti-microbial abilities, and these compounds are considered harmless to humans [6]. Economically, ZnO has a production cost of 75% cheaper than TiO2 in industrial-scale production. Therefore, ZnO nanoparticles are widely used in various interests, one is an anti-microbial agent because ZnO nanoparticles have a longer life than organic-based disinfectants [4].

Zinc oxide (ZnO) is a nanometer-sized material with photocatalytic ability and has been tested to slow the development of gram-negative or gram-positive bacteria [7]. In addition, ZnO is classified as a non-toxic, biocompatible, and bio-safe material [8]. The antibacterial performance of ZnO is highly dependent on size and morphology. The smaller the diameter of ZnO particles, the better the antibacterial performance [9].

The synthesis of ZnO nanoparticles faces serious challenges in the form of agglomeration that can reduce the effectiveness and unique properties of the particles. Using capping agents is a potential solution to prevent aggregation, with natural capping agents from secondary metabolite compounds (such as tannins, alkaloids, polyphenols, and flavonoids) offering a more environmentally friendly and sustainable alternative [10], [11]. Agglomeration is the clumping of particles that causes non-uniform size and reduces the concentration of ZnO in the nanoparticles. Capping agents coat ZnO particles during synthesis to prevent accumulation [10]. The synthesis of ZnO nanoparticles also provides the potential to provide new insights into the interactions between organic compounds and inorganic nanoparticles. Based on the research, secondary metabolite compounds can be found in banana peel extracts, such as saponins, alkaloids, flavonoids, and polyphenols [12]. In addition, the determination of solvents in the extraction process influences the yield of secondary metabolites. The solvent

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used in the extraction must have low toxicity, be non-volatile, and not damage the extract components [13], the content of saponins, flavonoids, glycosides, and phenols. [14] Obtained reducing sugars from research on banana peel extraction with ethanol and water solvents. Ethanol and water solvents were used to determine the quality of ZnO nanoparticles produced with volume variations. A mixture of ethanol and water solvents can increase the extraction of secondary metabolite compounds soluble in solvents [15].

Research on nanoparticles using various natural materials, such as star apple leaf, water hyacinth leaves, tin leaf, and pectin, has been explored through nanoparticle synthesis. For star apple leaf, extract volumes (2-10 mL) and pH variations (7-9) were tested, with an FTIR result showing a ZnO peak at 405-768 $cm^{-1}$ [16]. Water hyacinth leaves had volume variations (20-40 mL), and PSA tests revealed particle sizes between 15.6 and 76.9 nm [17]. Tin leaf showed volume variations (30-40 mL), and PSA tests recorded an average ZnO nanoparticle size of 49.62 nm [18]. Pectin synthesis with temperature variations (60-100°C) resulted in an FTIR wavelength peak of around 435-566 $cm^{-1}$ [19].

Based on the description above, this study aims to synthesize ZnO nanoparticles with the essential ingredients of horned banana peel extract as a capping agent and determine the effect of variation in the volume of ethanol-water solvent on the characterization of ZnO particles produced from horned banana peel on ZnO particle synthesis. ZnO particles were characterized, including Fourier Transform Infrared Spectroscopy (FTIR) and Particle Size Analyzer (PSA). Through this research, it is expected to reduce banana peel waste and become literature for further studies related to this research.

## 2. Research Method

### 2.1 Materials

Banana horn peel waste, 96% ethanol ($C_2H_5OH$) (Sigma Aldrich, USA), distilled water ($H_2O$), zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) (The Emsure, Germany), and sodium hydroxide (NaOH) (The Emsure, Germany).

### 2.2 Equipment

The tools used in this research include 100 mL Pyrex beaker, 50 mL Pyrex measuring cup, Hot plate stirrer Thermo Scientific, Thermolyne 30420C-33-80 Muffle Furnace, OHAUS CP214 analytical balance, Panasonic oven, blender, Pyrex measuring pipette, dropper pipette,

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Digital Photo Tachometer pH meter, 250 mL Pyrex Erlenmeyer, 50 mL Pyrex glass funnel, and 8 cm watch glass.

## 2.3 Methods

### 2.3.1 Horn Banana Peel Extraction

The banana peels were washed, cut into small pieces, and dried in the sun for ± 15 hours. The banana peels were pulverized with the help of a blender. The powder of the horned banana peel was weighed in the amount of 15 grams, and the weighing was repeated 2 times. The extraction process began using the maceration method. Divided the sample into two, namely ethanol-water 2:1 (v/v) with a ratio of 96% ethanol totaling 60 mL and water totaling 30 mL and ethanol-water 1:1 (v/v) with a ratio of 96% ethanol totaling 30 mL and water totaling 30 mL. The two samples were allowed to stand for 1x24 hours, then filtered using filter paper to obtain a solution of ethanol-water extract of banana horn skin [20].

### 2.3.2 Assay of Total Flavonoid and Polyphenol Content of Horn Banana Peel Extract

#### 2.3.2.1 Total Flavonoid Content

The procedure that needs to be done in determining the total flavonoid content is to prepare 1 mL of horn banana peel extract and then put it in a 10 mL measuring flask. Added methanol in the amount of 3 mL, $AlCl_3$ 10% in the amount of 0.2 mL, $CH_3COOK$ 1M in the amount of 0.2 mL, and included distilled water until the limit mark of 10 mL. Incubated the solution for 30 minutes and measured the absorbance with the help of a UV-Vis spectrophotometer at a wavelength of 432 nm [21]. Determination of total flavonoid content using the help of a 1-5 ppm quercetin standard curve [22].

#### 2.3.2.2 Total Polyphenol Content

In testing the total polyphenol content using the help of a standard curve of 1-5 ppm gallic acid solution. Prepared 0.25 mL of horned banana peel extract, then Folin-Ciocalteu 10% reagent was added as much as 500 µL and 4 mL of 7.5% $Na_2CO_3$ and distilled water until the limit mark of 10 mL. Shaken until homogeneous, then incubated the solution for 60 minutes at room temperature. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm [21], [23].

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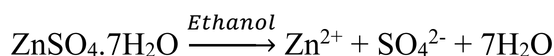
2.3.3 Synthesis of ZnO Particles with Horn Banana Peel Extract Material

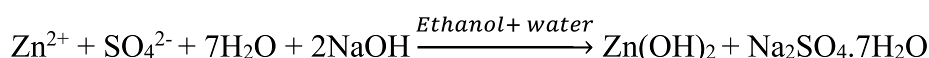
Synthesized ZnO particles through the reaction process between $ZnSO_4.7H_2O$ and banana horn skin extract solution [24]. In the ethanol-water 2:1 (v/v) sample, 3.28 grams of $ZnSO_4.7H_2O$ was reacted with 40 mL of banana horn skin extract solution. In the ethanol-water 1:1 (v/v) sample, 1.64 grams of $ZnSO_4.7H_2O$ was reacted with 20 mL of banana peel horn extract solution and stirred each sample with the help of a hotplate stirrer for 2 hours at 70 °C with a speed of 450 rpm. After stirring, 2 M NaOH was added slowly until pH 12. Closed the glass beaker using plastic wrap and aluminum foil, then allowed to stand for 24 hours at room temperature. Filtered the resulting precipitate using filter paper. Washed with demineralization until pH 7. Dried the washed precipitate using an oven for 4 hours at 80 °C. Calcined the precipitate using a furnace for 3 hours at 500 °C. The calcination process is an endothermic decomposition reaction that aims to remove gases from hydroxides or carbonates to obtain high-purity powders in the form of oxides [25]. Below is the reaction mechanism in the formation of ZnO particles [26]:

- First stage (Solvation stage):



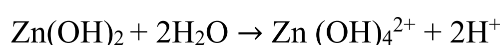
$$ZnSO_4.7H_2O \xrightarrow{Ethanol} Zn^{2+} + SO_4^{2-} + 7H_2O$$

- The second stage (Hydrolysis stage):



$$Zn^{2+} + SO_4^{2-} + 7H_2O + 2NaOH \xrightarrow{Ethanol+\ water} Zn(OH)_2 + Na_2SO_4.7H_2O$$

- Third stage (Polymerization stage):



$$Zn(OH)_2 + 2H_2O \rightarrow Zn\ (OH)_4^{2+} + 2H^+$$

- Fourth stage (Transformation stage):

$$Zn(OH)_4^{2+} \leftrightarrow ZnO + H_2O + OH^-$$

2.3.4 Characterization of ZnO Nanoparticles

Fourier Transform Infrared (FTIR) is useful as a determinant of the presence of functional groups based on wave numbers when vibrations occur. FTIR analysis is performed in wave numbers around 600 to 4000 $cm^{-1}$ [27]. The study observed several peaks and functional groups where the Zn-O functional group occurred at wave numbers 400-600 $cm^{-1}$ [28].

Particle Size Analysis (PSA) is one of the nanoparticle analyses that aims to determine the size of nanoparticles dispersed in colloidal nanoparticles. Based on the results of previous research, the particle size that meets the requirements to be classified as nanoparticles is 10-

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1000 nm [29]. The principal PSA uses is Dynamic Light Scattering (DLS), where light is scattered during measurement. When particles are irradiated with light, light scattering occurs, fluctuating with a speed that depends on the particle size. The smaller the particle size, the faster the particle distribution fluctuates. Particle distribution and size are key characteristics in nanoparticle systems because they affect the targeting ability of the nanoparticle [30].

## 3. Result and Discussion

### 3.1 Extraction of Secondary Metabolites of Horn Banana Peels

**Figure 1** shows the results of the extraction of horned banana peel using various ethanol-water ratios. The extraction process produced a brownish-yellow solution with different levels of color concentration. As shown in **Figure 1**, (a) 1:1 (v/v) ethanol-water extract has a more intense color compared to (b) 2:1 (v/v) ethanol-water extract.

**Figure 1.** Horn Banana Peel Extract with Ethanol-Water Variation (a) (1:1) and (b) (2:1)

This study uses the maceration method because it has an easy process and limits the degradation of secondary metabolite compounds due to high heat exposure. The choice of solvent used must be appropriate to attract metabolite compounds. Solvents attract extracts more quickly if they have the same polarity properties [31]. Ethanol has a high polarity, extracting polar compounds such as phenolic compounds [32]. This study uses a combination of ethanol and water as solvents to extract secondary metabolite compounds from samples. Ethanol and water were chosen because they can dissolve secondary metabolite compounds soluble in organic solvents and water. The ethanol-water solvent volume ratio aims to find the ideal solvent volume ratio that can extract more secondary metabolite compounds, especially

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flavonoids, and polyphenols, which serve as capping agents for forming nanometer-sized materials.

3.2 Analysis of Total Polyphenol and Flavonoid Content of Horn Banana Peel Extract

The results showed that the total polyphenol content in ethanol-water 1:1 (v/v) was more significant than with ethanol-water 2:1 (v/v), namely 4.944% and 4.114%, as shown in **Table 2**. Furthermore, the total flavonoid content test showed that ethanol-water 1:1 (v/v) was more significant than ethanol-water 2:1 (v/v), namely 5.940% and 4.131%, as in **Table 2**. These two tests show that the content of secondary metabolite compounds in ethanol-water 1:1 (v/v) is more significant than in ethanol-water 2:1 (v/v). This is because, in the variation (1:1), the ethanol used is less, namely 30 mL, compared to the variation (2:1), which is 60 mL, so that in the variation (1:1), the hydrogenation bond that occurs is lower. Based on previous research, hydrogenation bonds can break some bonds in the structure of polyphenols and flavonoids, thereby reducing their levels [38].

**Table 1.** Total Polyphenol and Flavonoid Content of Horn Banana Peel Extract

| Sample Extract | Total Polyphenol Content | Total Flavonoid Content |
|---|---|---|
| (1:1) | 4.944 % | 5.940 % |
| (2:1) | 4.114 % | 4.131 % |

Based on previous research explains that the more concentrated the color of the extract, the more secondary metabolite content [24]. According to the research that has been done, the total polyphenol and flavonoid content in the ethanol-water variation 1:1 (v/v) is more significant than ethanol-water 2:1 (v/v) as in **Table 2,** where the variation (1:1) has a more concentrated according to extract according to color according than according to the variation (2:1).

Polyphenols are compounds found in plants with more than one phenol group, divided into several parts, such as flavonoids, tannins, stilbenes, and phenolic acids. Flavonoids are one part of polyphenols that have the basic structure of the flavon ring and are divided into several parts, such as flavones, flavonols, flavones, isoflavones, and anthocyanins. Polyphenols and flavonoids have several similarities, namely having a hydroxyl group (-OH) that can bind to the surface of nanoparticles, where this bond can prevent nanoparticle agglomeration. In addition, polyphenols and flavonoids have antioxidant properties that can protect nanoparticles from free radical damage, thus extending the life of nanoparticles and increasing their efficiency [33]. Free radicals are unstable molecules that can damage the surface of nanoparticles, resulting in

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decreased stability and function [34]. In synthesizing ZnO nanoparticles using natural capping agents, testing the total polyphenol and flavonoid content is needed because it determines the effectiveness of the capping agent and understands the interaction between nanoparticles and the resulting capping agent.

In determining the total polyphenol content, Folin-Ciocalteau reagent was added to the sample, which aims to oxidize phenolic compounds into blue, as shown in **Figure 2.** The density of the blue color formed is proportional to the amount of phenolic compounds in the sample [35]. However, the reaction process between the Folin-Ciocalteau reagent and phenolic compounds runs slowly in an acidic atmosphere, so adding sodium carbonate is needed to create an alkaline atmosphere, and the reaction can run faster [36].

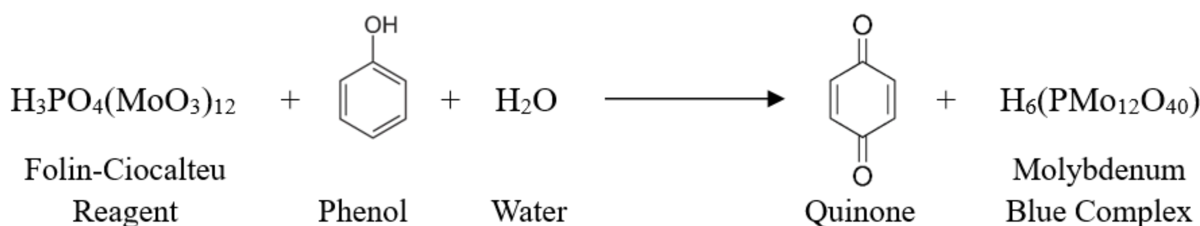



**Figure 2.** Reaction of phenol with Folin-Ciocalteu reagent

The total flavonoid content of horned banana peel extract was determined using colorimetry. $AlCl_3$ solution is used to form colored complex compounds with flavonoids so that there is a shift in wavelength towards visible, marked by the solution changing color to yellow [22]. The purpose of adding $CH_3COOK$ solution is to stabilize and maintain the wavelength in the visible part.

The principle of determining the total flavonoid content using $AlCl_3$ is the formation of complexes between $AlCl_3$ with keto groups at C-4 atoms and with hydroxyl groups at adjacent C-3 or C-5 atoms of flavones and flavonols, as in **Figure 3**. Quercetin is a standard solution because quercetin is a flavonoid of the flavonol group with a keto and a hydroxyl group [37].

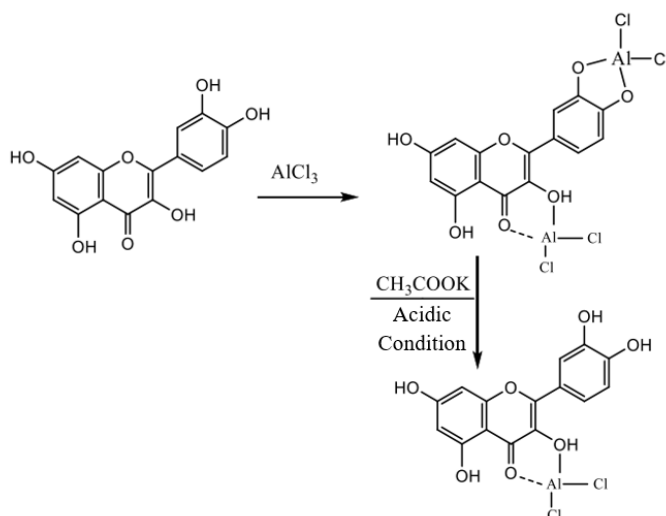
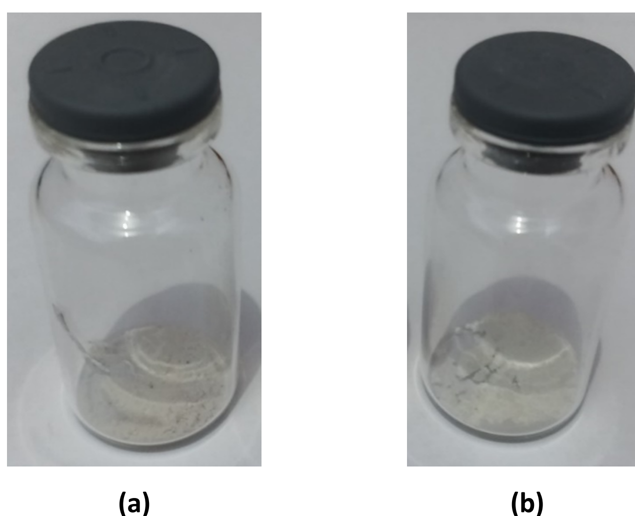



**Figure 3.** Reaction of Flavonoids with $AlCl_3$

### 3.3 Synthesis of ZnO Particles Using Horned Banana Peel Extract

The formation of ZnO particles from zinc sulfate heptahydrate typically involves four stages: solvation, hydrolysis, polymerization, and transformation. Zinc sulfate heptahydrate is initially dissolved in a banana peel extract and ethanol mixture. The ethanol slows hydrolysis, removing sulfate ions to form $Zn(OH)_2$ [26]. This $Zn(OH)_2$ then undergoes polymerization, forming Zn-O-Zn bonds that eventually transform into ZnO compounds. The final step is calcination, where the mixture is heated at high temperatures. This process results in smaller, more crystalline ZnO nanoparticles, which appear white, as shown in **Figure 4**.



(a) (b)

**Figure 4.** ZnO Synthesis Results Using Horned Banana Peel Extract with Ethanol-Water Variation (a) (1:1) and (b) (2:1)

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Secondary metabolite compounds of banana peel extract, especially flavonoids and polyphenols, function as capping agents that interact with the surface of $Zn^{2+}$ metal ions and bind $Zn^{2+}$ metal ions to form a stable structure [24]. This interaction occurs at the polar head of secondary metabolite compounds, namely functional groups such as hydroxyl. The interaction between metabolite compounds and $Zn^{2+}$ metal ions is to prevent aggregation. Agglomeration can cause the particle size to become non-uniform, with some particles being more significant than others. Therefore, increasing the amount of secondary metabolite compounds in ZnO synthesis produces particles with better morphology and size. The reaction equation occurs when synthesizing ZnO particles using a capping agent made from banana horn skin extract.

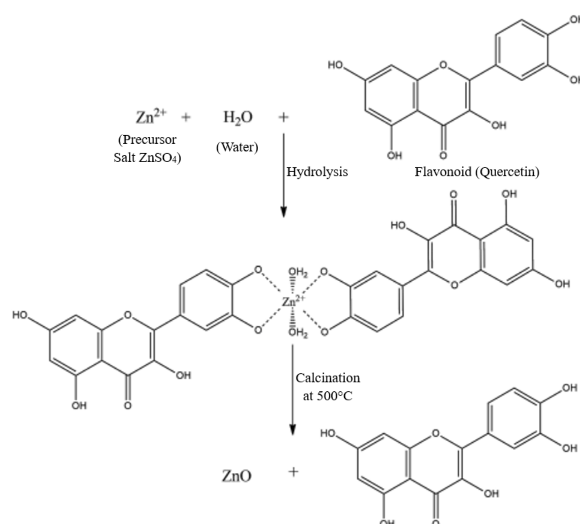



**Figure 5.** Mechanism of Interaction between ZnO Particles and Flavonoid Capping Agent [39]

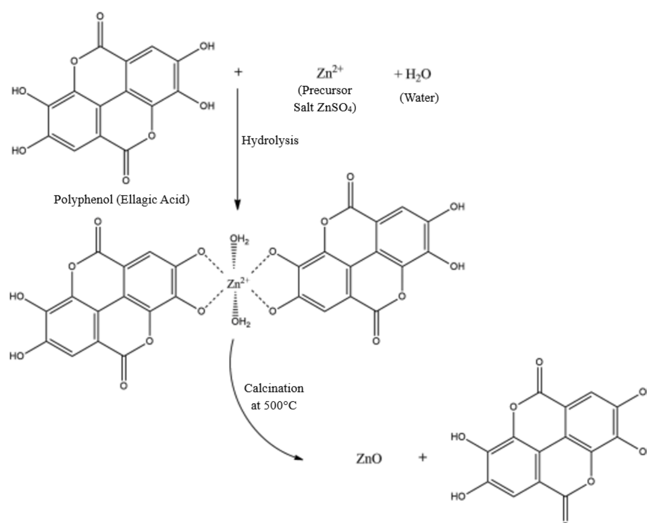



**Figure 6.** Mechanism of Interaction between ZnO Particles and Polyphenol Capping Agent [40]

## 3.4 Characterization of ZnO Particles

### 3.4.1 *Fourier Transform Infrared* (FTIR)

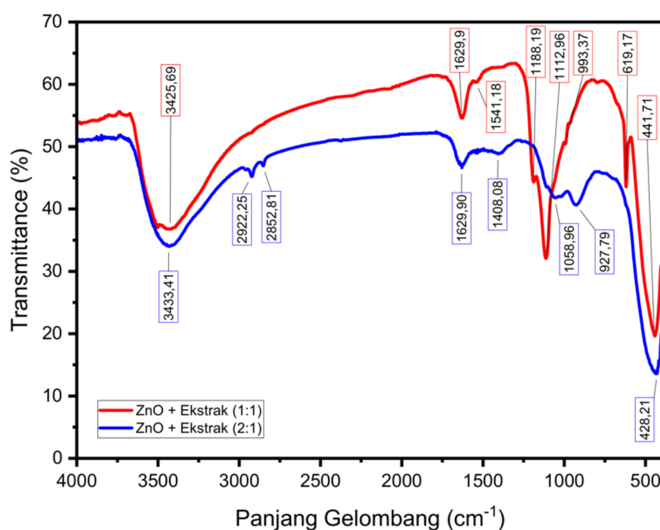



**Figure 7.** FTIR Spectrum of Horn Banana Peel Extract at Ethanol-Water Variation (1:1) and (2:1)

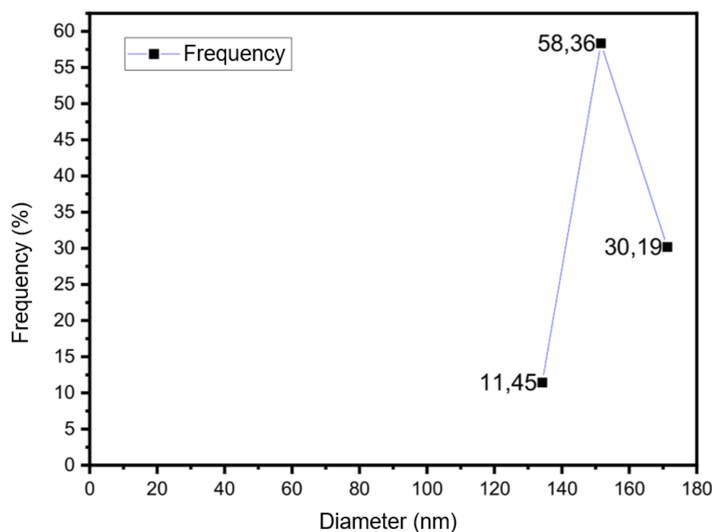
The FTIR analysis identified that the ZnO 1:1 and 2:1 sample extract shows a distinctive broad peak at wave numbers 3425cm$^{-1}$ and 3433cm$^{-1}$, respectively. In the variation (1:1), several peaks appear, including 3425 cm$^{-1,}$ which is the O-H group on polyphenols, 1629 cm$^{-1,}$ which is the C=C group of aromatic rings, 1541 cm$^{-1,}$ which is the C-C stretching group, 1188 cm$^{-1,}$ and 1112 cm$^{-1}$ shows the C-O-C or C-O polysaccharide group, 993 cm$^{-1}$ shows the C-O-C stretching group, and 441 cm$^{-1}$ and 619 cm$^{-1}$ which are ZnO groups [28], [41]–[44].

Meanwhile, the variation (2:1) has several peak points, namely 3433 $cm^{-1}$, the O-H group on polyphenols. 2922 $cm^{-1}$ and 2852 $cm^{-1}$ are C-H stretching, 1629 $cm^{-1}$ is an aromatic ring C=C group, 1408 $cm^{-1}$ is a deformed $CH_2$ group, 1058 $cm^{-1}$ is C-N stretching, 927 $cm^{-1}$ is a C-O-C stretching group and 428 $cm^{-1}$ is a ZnO peak [28], [41], [42], [45].

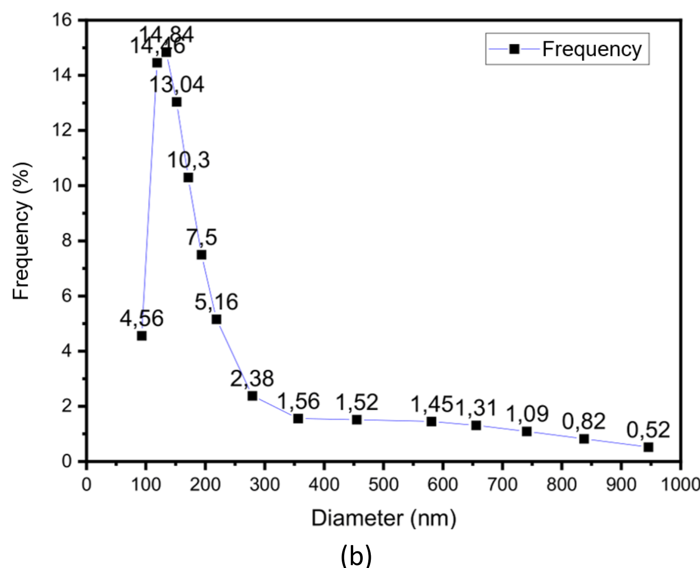
Functional groups such as CO-C, C-O, and C=C are derivatives of proteins contained in banana horn peel extract and serve as capping agents in the synthesis of nanoparticles [41]. From the curve seen in **Figure 7**, there is a prominent difference in the variation (1:1) at a wavelength of 1112 $cm^{-1}$, namely the C-O-C or C-O group, the difference seen is at a wavelength of 1112 $cm^{-1}$ diving sharply down, which means the concentration of the C-O-C or C-O group is lower. This is because, in the variation (1:1), the amount of ethanol used is less than the variation (2:1), so the concentration of C-O-C or C-O groups is lower, and hydrogenation bonds as well [46].

3.4.2 *Particle Size Analyzer* (PSA)

ZnO synthesis results through the PSA characterization process, which aims to determine the average particle diameter and PI (Polydispersity Index) value, which indicates the uniformity of particle size. The results of PSA test data analysis of ZnO samples are shown in **Figure 8** and **Table 3**.




(a)




(b)

**Figure 8.** Diameter Distribution Chart of Synthesized ZnO Using Horn Banana Peel Extract (a) Ethanol (96%)-water 2:1 (v/v) (b) Ethanol (96%)-water 1:1 (v/v)

**Table 2.** PSA Test Result Data of Synthesized ZnO

| PSA Analysis Result Data | ZnO + Extract 1:1 | ZnO + Extract 2:1 |
|---|---|---|
| Average Diameter (nm) | 135.6 | 153.6 |
| Polydispersity Index (PI) | 0.33 | 0.221 |
| Dispersity | Polydisperse | Monodisperse |

The PSA test showed that the particles obtained were 135.6 nm for the 1:1 sample and 153.6 nm for the 2:1 sample. The particle size obtained met the requirements of a nanoparticle size of 10-1000 nm [29]. The polydensity index (PI) value is used to estimate a sample's particle size distribution range and determine the aggregation's presence or absence. A low PI value indicates higher particle size homogeneity. The polydispersity index value is divided into three, namely monodisperse (less than 0.3), polydisperse (0.3 to 0.7), and superdipers (more than 0.7). A polydispersity index value below 0.3 indicates that the particle size has a narrow distribution, while a polydispersity index above 0.3 indicates that the particle size has a broader distribution [47]. The study's results on the sample of ZnO nanoparticles ethanol-water 1:1 classified as polydisperse with a PI value of 0.33, while the sample of ZnO nanoparticles ethanol-water 2:1 classified as monodisperse with a PI value of 0.221. The difference in PI value is due to the amount of ethanol used in the ethanol-water variation (1:1) being less than the ethanol-water variation (2:1), so particle aggregation is more inhibited, and solvent-polymer interactions are weaker, so the particle size distribution is more expansive. However, the average diameter of the particles is smaller in the ethanol-water variation (1:1) [47].

## 4. Conclusions

The ratio of ethanol and water in the extraction solvent affects the number of secondary metabolites extracted from the horned banana peel in synthesizing ZnO nanoparticles. At a 1:1 ratio, the color of the solution was more intense than the 2:1 ratio, indicating higher metabolite levels. FTIR results showed ZnO peaks at 428 $cm^{-1}$, 441 $cm^{-1}$, and 619 $cm^{-1}$. PSA test showed the diameter of nanoparticles at a 1:1 ratio was smaller (135.6 nm) than at 2:1 (153.6 nm) due to higher flavonoid and polyphenol content at a 1:1 ratio, reducing agglomeration. Both meet the classification of nanoparticles (10-1000 nm).

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RESEARCH ARTICLE

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# Kinetics of Extraction of Arabica Coffee Bean (*Coffea arabica*)

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**Abstract.** Coffee beans are the seeds of the coffee plant and a source of coffee beverages. Arabica coffee beans contain oil, which has many benefits. Extracting oil (solute) from coffee can be done through solvent extraction (leaching). The coffee bean oil extraction process uses n-hexane as a solvent because it can dissolve compounds with the same properties. This research aimed to study the extraction of essential oil from Arabica coffee beans and determine the kinetic model of extraction of essential oil from Arabica coffee beans by leaching. The process of separating coffee oil and solvent using thermogravimetric analysis with a temperature of 80 °C until the sample mass is constant. The variables used were temperature and time. The extraction time is 2 hours, with retrieval time every 10 minutes. The temperature used is 30, 40, and 50 °C with a ratio of material and solvent 1:10. The results showed that the kinetics of arabica coffee extraction followed the second-order extraction kinetics model. The second-order kinetic parameter values of arabica coffee essential oil extraction, namely extraction capacity (Cs) at 30, 40, and 50 °C were 5.45836, 5.46, and 5.46001 g $L^{-1}$, respectively, the rate of the initial extraction (h) was 0.00762718, 0,00756716, and 0,0104452 $gL^{-1}minute^{-1}$, the extraction rate constant (k) was 0.000256, 0.000254, and 0.00035 $g^{-1}L^{-1}minute^{-1}$, and determination values (R square) of 0.9965, 0.9967 and 0.9983.

**Keywords:** *coffee oil, extraction kinetics, and solvent extraction*

## 1. Introduction

Coffee is a reasonably high-value commodity among other plantations and is essential as a foreign exchange through exports. The taste and quality of coffee beans are determined by the processing method [1]. Coffee serves as the primary source of income for coffee farmers in Indonesia [2]. The success of coffee plantation agribusiness depends on coffee production, processing methods, and marketing strategy [3]. Indonesia has the most extensive coffee plantations, namely Arabica coffee beans and Robusta coffee beans [4][5]. Arabica coffee has a strong, slightly sour taste and distinctive aroma [6]. The oil content in fresh coffee beans ranges from 8% to 18%, depending on the type of coffee bean [7]. Arabica coffee beans contain

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oil that can be used as a source of triglycerides for biodiesel production, materials for making polyurethane membranes, antibacterial, anti-depressant, antioxidant, anti-cellulite, anti-hyperglycemic, anti-inflammatory, aromatherapy, relieve nausea, help the respiratory system, health soap, and others commercial things [8][9][10]. This is because coffee has complex chemical components such as caffeine, linoleic acid, palmitic acid, stearic acid, tocopherols, phosphatides, sterols, ceramides, and diterpenes which have been proven in biological activity. The content of roasted green coffee beans is 7-17% fat, consisting of 75% triacylglycerol and free fatty acids, similar to the composition of oils in other plants [11]. Green coffee bean oil contains a non-saponification fraction of phosphatides, sterols, ceramides, and diterpenes [12]. The two main diterpenes commonly observed in coffee are *kahweol* and *cafestol.* These two main diterpenes can increase Glutathione S-Transferase (GST) activity in rats' small intestine and liver. In animal models, these diterpenes have been shown to exert biological effects such as anticancer and chemo-preventive properties [13][14].

Extracting oil (solute) from coffee can be done through solvent extraction (leaching) [15][16][17]. Extracting oil from seeds using a solvent is preferred because the yield reaches 99% of the total oil content. Essential oils generally contain aromatic compounds such as terpenes (monoterpenes and sesquiterpenes), aldehydes, ketones, esters, alcohols, and phenols. Although diterpenes are a type of terpene, essential oils are typically richer in monoterpenes and sesquiterpenes than in diterpenes. The basic principle of extraction with solvents is that compounds with the same properties will dissolve with the properties of the solvent used. Extracts must have high solubility in solvents to obtain maximum extract content [18][19]. The selection of the type of solvent in the coffee oil extraction process needs to be considered because it affects the amount of oil and the yield of free fatty acids produced [20]. It is known that the highest extraction results with the highest amount of free fatty acids were obtained using hexane solvent compared to other non-polar solvents. The highest oil yield was produced in extraction with hexane solvent compared to ethanol and methanol solvents [21]. N-hexane, petroleum ether, carbon disulfide, carbon tetrachloride, benzene, and corn oil [22]are usually used to extract the nonpolar phase. Soxhletation is a type of extraction that uses solvents. The results obtained from this extraction are almost the same as the screw pressing technique since some of the polar fractions are also extracted [23] [24].

The kinetics of extracting essential oils from plants has been studied several times. According to the researchers, the mathematical model of steam extraction of basil essential oil

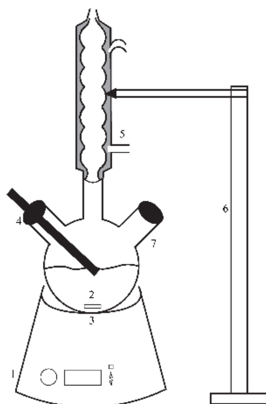
is based on the diffusion transfer and simultaneous convection of the vapor phase. The kinetics of essential oil extraction uses the first-order kinetics**.** However, the kinetic model for sandalwood oil extraction uses a second-order equation. The rapidly increasing amount can be seen in the second-order characteristics of essential oil at the beginning, and it will decrease slowly if the time is longer. Kinetic studies are critical studies that need to be done in the coffee bean extraction process. This study aims to determine the steps of the reaction rate controller and its kinetic model. In solvent extraction, it is important to remember that knowledge of the kinetics of oil extraction is essential as it helps determine the highest oil yield in the time interval studied. Therefore, it is necessary to conduct extensive studies on oil extraction kinetics from Arabica coffee beans [25] [26]. During the oil extraction process, the rate of extraction (the rate at which equilibrium is reached) is affected by several factors, such as the diffusing capacities of the solute and solvent, size, shape, internal structure of the seed particles (matrix), and the rate of dissolution of the solvent to the oil-soluble substance (solute). In other words, the extraction kinetics involves releasing oil from the porous or cellular matrix into the solvent through a mass transfer mechanism. The oil (solute) that binds to the solid matrix of the kernel particles both physically and chemically must be transferred to the solvent phase through the dissolution process [27]. The novelty of this study compared to previous research lies in its focus on the kinetic study of essential oil extraction from Arabica coffee beans. This topic has yet to be extensively explored in prior studies. Most previous research has concentrated on coffee oil's chemical composition and biological benefits. Still, it has yet to profoundly investigate the kinetic models of extraction that could be used to improve the efficiency of the extraction process. This study compares first-order and second-order kinetic models in the extraction process, providing a better understanding of the extraction rate mechanism and selecting the most appropriate model to describe the coffee oil extraction process. This approach differs from previous studies, which typically used only one kinetic model without a comprehensive comparison.

## 2. Materials and Methods

### 2.1 Materials

The materials used in this study were arabica coffee beans from Andungsari Village, Pakem District, Bondowoso Regency, and n-hexane PA.

## 2.2 Equipment



1. Heating mantle+stirrer
2. Three neck flasks
3. Stirrer
4. Thermometer
5. Ball condenser
6. Stative
7. Stopper

**Figure 1.** One Set of Leaching Extraction Tools

The tools used in this study were one set of leaching extraction equipment, oven (Samsung), measuring cup, measuring pipette, pipette, beaker glass, analytical balance, ball pipette, 80-mesh size sieve, glass funnel, watch glass, spoon, Erlenmeyer, rotary evaporator (IKA RV 8), membrane filtration.

## 2.3 Variables

Variables used in this study were time and temperature. The oil extraction in this process was done every 10 minutes for 2 hours with temperature variations of 30, 40, and 50 °C.

## 2.4 Methods

### 2.4.1 Determination of Soxhlet Extraction

The experiment in this study used arabica coffee beans. The first step was to roast Arabic coffee beans in an oven at 225 °C for 15 minutes. Roasting at 225 °C causes the change in color of the coffee beans, accompanied by the release of a distinctive coffee aroma. The color of coffee beans turns dark brown (blackish brown), accompanied by a powerful coffee aroma typical. This temperature produces maximum coffee bean oil. Then, it was cooled, mashed, and sieved using an 80-mesh sieve. When coffee beans are heated, the volatile compounds responsible for the aroma and taste of coffee are released. If coffee beans are ground directly

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without cooling, these compounds can evaporate quickly and reduce the quality of the aroma and taste of the coffee. Cooling helps stabilize these compounds, so they remain intact during extraction. High temperatures can cause thermal degradation of several essential compounds in coffee beans. This degradation process can be reduced by cooling the coffee beans so that the quality of the resulting extract remains optimal. From a practical perspective, cooling the coffee beans after heating will make subsequent handling easier and safer. Coffee beans that are still hot can cause equipment such as grinders or sieves to become damaged or compromise user safety. Cooling also helps ensure that the coffee beans can be crushed and sifted consistently, resulting in uniform particle sizes. This is important to ensure the extraction process runs effectively and efficiently. Next, the materials and solvents were prepared with a 1:10 ratio for each, namely 20 grams of material and 200 mL of solvent. Furthermore, setting up the extraction tools. The extraction method used was the Soxhlet method. The extraction was carried out until the results of the solvent inside the round flask became apparent, with a total time of 20 hours. The results were then put into the rotary evaporator to separate the solvent and oil to obtain the total oil yield in the arabica coffee beans. The operating conditions when using a rotary evaporator are as follows: the temperature is between 40-60 °C because n-hexane has a boiling point of approximately 69 °C at atmospheric pressure. The water bath temperature is typically between 40-60 °C to ensure efficient evaporation without approaching the standard boiling point. The vacuum pressure is maintained between 100-150 mbar to lower the boiling point of n-hexane, allowing the solvent to evaporate at a lower temperature. The rotation speed is set between 100-200 rpm to increase the surface area contact between the solvent and the water bath, thereby accelerating evaporation. The coolant must have a sufficient flow rate to effectively condense the n-hexane vapor, with the coolant temperature usually set around 0-10 °C to ensure effective condensation. The operational requirements for the rotary evaporator include ensuring no leaks in the vacuum system before starting. Any leaks can cause the solvent to evaporate inefficiently, requiring higher temperatures and increasing risk. It is essential to continuously monitor the water bath temperature and vacuum pressure during operation. Uncontrolled fluctuations in temperature or pressure can lead to inconsistent evaporation results or even damage to the sample.

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2.4.2 Determination of Extraction Kinetics

Studies by researchers on the kinetics of essential oil extraction are brought closer by two simultaneous processes, diffusion and convection, which represent the first-order and second-order extraction kinetics models. First-order kinetics of extraction can be interpreted by the change in the concentration of essential oils each time between the concentration of essential oils in a saturated state (Cs) and the concentration of essential oils in the material (Ct) when 't' is time (minutes) which is expressed in the equation below:

$$\frac{dCt}{dt} = k_1(Cs - Ct) \tag{1}$$

Where $k_1$ is the first-order essential oil extraction rate constant ($min^{-1}$), Cs is the oil concentration in a saturated state. It is the extraction capacity ($gL^{-1}$), and Ct is the concentration of essential oil ($gL^{-1}$) when t (minutes). The value of the rate constant and extraction capacity of first-order essential oils is obtained by integrating equation (1) in the boundary conditions Ct = 0 when t = 0 and Ct = Ct when t = t, so the following equation is obtained:

$$\ln\frac{Cs}{Cs - Ct} = k_1 t \tag{2}$$

Equation (2) can be stated if the values of the extraction rate constants and first-order extraction capacities, namely $k_1$ and Cs are obtained from the linear regression equation between log data (Cs-Ct) to t.

$$\log(Cs - Ct) = \log Cs - \frac{k_1}{2{,}303}t \tag{3}$$

Second-order kinetics of extraction involves two simultaneous processes, beginning with an increase in the amount of essential oil in the extraction process quickly and with a decrease in the rate of formation of essential oil when an equilibrium state is reached in the volume of essential oil produced. Second-order kinetics can be shown in the equation below:

$$\frac{dCt}{dt} = k_2(Cs - Ct)^2 \tag{4}$$

With $k_2$ being the second-order essential oil extraction rate constant ($g^{-1}$ L $menit^{-1}$). Equality (4) integrated with the boundary conditions Ct = 0 when t = 0 and Ct = Ct when t = 0, so it is obtained:

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$$\frac{t}{Ct} = \frac{1}{k_2 Cs^2} + \frac{t}{Cs} \quad (5)$$

The initial rate of extraction can be stated by h, namely $k_2Cs^2$ where Cs is the extraction capacity of the essential oil ($gL^{-1}$). The h, $k_2$, and C values are obtained from the slope and intercept values of the linear regression line between the t and t/Ct data [28].

The experiment used arabica coffee beans. The coffee beans were roasted in an oven at 225℃ for 15 minutes. Then, it was cooled, mashed, and sieved using an 80-mesh sieve. Next, prepared materials and solvents with a ratio of 1:10 for each, namely 20 grams of material and 200 mL of solvent. After that, the extraction equipment was set up. The extraction was done for 2 hours, using coffee bean oil every 10 minutes. The extraction results were then analyzed using thermogravimetry to see the amount of oil produced. This analysis aimed to determine the presence of solvents in the oil. The solvent in the extracted oil must be removed to obtain pure coffee bean oil. This analysis was carried out using an oven at 80 ℃ until the mass obtained was constant.

## 3. Result and Discussion

### 3.1 The Total Oil Yield

The total oil obtained from Bondowoso Arabica coffee beans was 5.46 grams. The yield at the end of the extraction of arabica coffee bean essential oil was 27.3%. The yield value of Bondowoso arabica coffee bean essential oil is higher than that of Aceh and Lampung, respectively, at 3.43% and 18.69% [10][23]. The yield of essential oils is influenced by plant species, planting location, harvesting age, and distillation method. In addition, the operating conditions used in this study differed from those for Arabica coffee beans from Aceh and Lampung. Research conducted by Lamona and Numan (2018) used arabica coffee from Aceh. It implemented the soxhlet extraction method, using 40 grams of material and 200 mL n-hexane at a temperature of 80 ℃ for 180 minutes. Research on the extraction of arabica coffee beans was also carried out by Berghuis and Maulana (2023) with arabica coffee from Lampung using the soxhlet extraction method, which used 10 grams of material and 250 mL of n-hexane at a temperature of 60 ℃ for 6 hours.

The influence of different conditions in this study can be observed through the variables used to extract essential oil from Arabica coffee beans. Differences in plant species, cultivation location, harvest age, and distillation methods significantly impact the extraction results.

Operational conditions such as temperature and extraction time also play an important role in different studies, such as those conducted by Lamona and Numan (2018) and Berghuis and Maulana (2023). Variations in temperature and extraction duration yielded different results. The experimental results showed that the essential oil yield from Bondowoso Arabica coffee beans was higher (27.3%) compared to those from Aceh (3.43%) and Lampung (18.69%). This difference is attributed to the varying extraction conditions, such as the higher temperature and longer extraction time used in the Bondowoso Arabica coffee beans study. Increasing the extraction temperature can cause the diffusion rate of the solvent into the cell walls to rise. As a result, the cell walls will rupture, resulting in the release of essential oils. In addition, the extraction time also influences the results obtained. The longer the extraction time, the greater the results obtained until it reaches equilibrium [25]. Increasing the extraction temperature can affect the extraction yield by enhancing the diffusion rate of the solvent into the cell walls, resulting in the release of more essential oil. Therefore, operational conditions, particularly temperature and extraction time, significantly impact the yield of essential oil obtained.

### 3.2 The Extraction Kinetics

Based on the research results shown in Table 1, the yield of arabica coffee bean essential oil increased rapidly in the 30$^{th}$ to 60$^{th}$ minute and slowed down after the 60$^{th}$ minute of the extraction process. In Table 1, it can be seen that the higher the temperature and the longer the extraction time, the yield of arabica coffee bean essential oil will increase and be constant when it reaches equilibrium.

**Table 1.** The Results of Arabica Coffee Bean Essential Oil Weight at Different Temperature and Time

| Time (minutes) | Weight (gram) at | | |
|---|---|---|---|
| | 30 °C | 40 °C | 50 °C |
| 10 | 0.049 | 0.051 | 0.057 |
| 20 | 0.050 | 0.052 | 0.059 |
| 30 | 0.051 | 0.055 | 0.062 |
| 40 | 0.054 | 0.058 | 0.065 |
| 50 | 0.058 | 0.063 | 0.068 |
| 60 | 0.061 | 0.066 | 0.070 |
| 70 | 0.063 | 0.068 | 0.072 |
| 80 | 0.064 | 0.069 | 0.074 |
| 90 | 0.065 | 0.070 | 0.074 |
| 100 | 0.066 | 0.071 | 0.075 |
| 110 | 0.066 | 0.072 | 0.076 |
| 120 | 0.066 | 0.072 | 0.076 |

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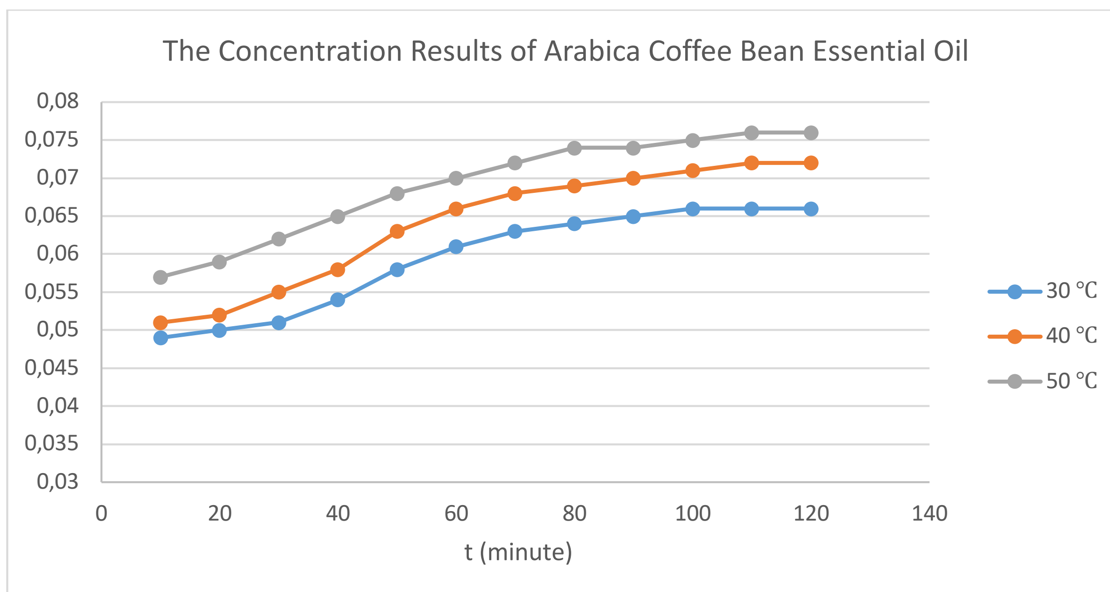


**Figure 2.** The Results of Arabica Coffee Bean Essential Oil Weight (gram)

Arabica coffee bean essential oil reached its maximum value at 120 minutes, as shown in Figure 2; this indicates that the essential oil reached a saturated state. The phenomenon of high extraction rates at the beginning of the process, followed by the diffusion of essential oils from the interior to the surface of the solid, is a characteristic of the extraction of essential oils from plants. The results of the coffee bean essential oil above are by the Arrhenius equation below:

$$k = A.e^{\frac{-E}{RT}} \quad (6)$$

$$k = 27{,}287.e^{-1520{,}9/T} \quad (7)$$

Information:

k = extraction rate constant

A = collision factor $((L/mol).s)$

E = activation energy $(J/mol)$

R = gas constant $(8{,}314\ Jmol^{-1}K^{-1})$

T = temperature (K)

From Figure 2 and Equation 7, it can be seen that the relationship between k and temperature is that the higher the temperature, the greater the value of k and the yield of arabica coffee bean essential oil, and with increasing time, the extraction rate increases.

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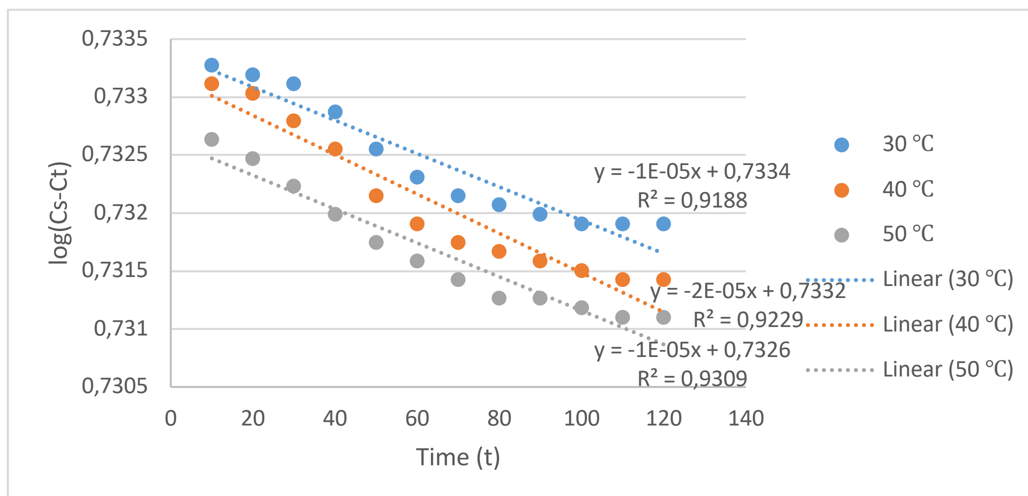


**Figure 3.** The Kinetic Model Curve of First-Order Coffee Bean Oil Extraction

Extraction kinetics is a combination of extraction and diffusion rates. A first-order and second-order kinetic model can approximate the extraction rate. The results of the log plot (Cs-Ct) against t, as shown in Figure 3, and using a first-order reaction using equation (3), the intercept value is obtained, which states the magnitude of the log Cs value, with Cs being the extraction capacity. The extraction rate constant value is obtained from the slope value of the graph in Figure 3. The extraction rate values at temperatures of 30, 40, and 50 ℃ are 0.00002303, 0.00004606, 0.00002303, respectively, and the first-order extraction capacity is 5.41253, 5.41003, and 5.40256 ($g.L^{-1}minute^{-1}$) with the R-square values at temperatures of 30, 40, and 50 ℃ are 0.9188, 0.9229, and 0.9309 respectively.

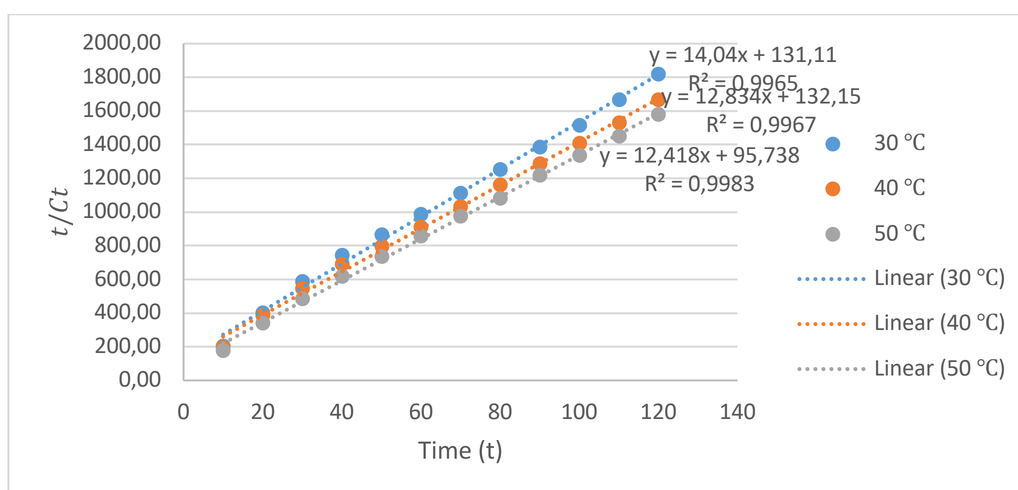


**Figure 4.** The Kinetic Model Curve of Second-Order Coffee Bean Essential Oil Extraction

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From equation (5), the extraction capacity of Arabica coffee bean essential oils obtained using the second-order kinetic model is 5.45836, 5.46, and 5.46001 $gL^{-1}$, respectively. Meanwhile, the initial extraction rate (h), namely $k_2Cs^2$, was obtained from the intercept values 0.00762718, 0.00756716, and 0.0104452 g $L^{-1}menit^{-1}$. The value of the second-order extraction rate constant ($k_2$) at temperatures of 30, 40, and 50 °C is 0.000256, 0.000254, and 0.00035 $g^{-1}$ $L^{-1}minute^{-1}$, respectively, and the R-square values are 0.9965, 0.9967, and 0.9983 respectively.

**Table 2.** Kinetic Parameters for First-Order and Second-Order Reactions at Different Temperatures

| Temperature (°C) | Order Reaction | Extraction Capacity (Cs) ($g.L^{-1}$) | Extraction Rate (k) ($g.L^{-1}.minute^{-1}$) | Initial Rate (h) ($g.L^{-1}.minute^{-1}$) | R-square |
|---|---|---|---|---|---|
| 30 | First Order | 5.41253 | 0.00002303 | - | 0.9188 |
| | Second Order | 5.45836 | 0.000256 | - | 0.9965 |
| 40 | First Order | 5.41003 | 0.00004606 | - | 0.9229 |
| | Second Order | 5.46 | 0.000254 | 0.00762718 | 0.9967 |
| 50 | First Order | 5.40256 | 0.00002303 | 0.00756716 | 0.9309 |
| | Second Order | 5.46001 | 0.00035 | 0.0104452 | 0.9983 |

Based on Table 2, it is evident that the $R^2$ values for the second-order kinetic model (0.9965, 0.9967, 0.9983) are higher than those for the first-order kinetic model (0.9188, 0.9229, 0.9309) at all temperatures tested. This indicates that the second-order kinetic model is more appropriate for the data in this study, suggesting that the extraction rate of Arabica coffee bean essential oil is better described by the second-order kinetic model than the first-order model.

**Table 3**. Kinetics of Essential Oil Extraction of Various Plants

| Plants | Methods | Order | Cs ($gL^{-1}$) | k | h ($gL^{-1}min^{-1}$) | $R^2$ | Ref. |
|---|---|---|---|---|---|---|---|
| Sandalwood | *Microwave Hydrodistilation* | 2 | 0.6015 | 0.0642 | 0.0232 | 0.9597 | [29] |
| Vertiver | *Microwave Hydrodistilation* | 2 | 6.2189 | 0.0007 | 0.029 | 0.9427 | [30] |
| Black Pepper | *Hydrodistilation* | 2 | 4.9 | 0.0086 | 0.206 | 0.997 | [25] |
| Arabica Coffee | *Leaching* | 2 | 5.46001 | 0.00035 | 0.0104452 | 0.9983 | The present study |

Table 3 shows the kinetics of the extraction of essential oils from several plants by following the second-order kinetic model. The plant species influence the extraction rate constant (k) and the concentration of essential oils in the saturated state (Cs). The k value in the extraction of Arabica coffee beans using the leaching method has the smallest k value because the temperature used in this study is lower than in other studies. The higher the temperature, the greater the k obtained [25][29][30].

## 4. Conclusion

The extraction of Bondowoso arabica coffee bean essential oil using the solvent extraction (leaching) method produced an experimental yield of 27.3% with the extraction kinetics following the second-order kinetics model. The kinetic parameters of the essential oil extraction of Bondowoso arabica coffee beans, namely extraction rate constants, k was 0.000256, 0.000254, and 0.00035 $g^{-1}$ $L^{-1}minute^{-1}$, with determination values (R square) of 99.65%, 99.67%, and 99.83% and using second order reactions.

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RESEARCH ARTICLE

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# Extraction of Bioactive Compounds from Coffee Husk with Acetone Using Microwave Assisted Extraction Method and Analysis of Phenolic Compounds

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**Abstract.** Nowadays, coffee husks are limited to animal feed and fertilizer. Therefore, a study used Robusta coffee husk waste as a raw material. The purpose of this study was to determine the effect of several variables and to determine the optimum conditions in the process of extracting bioactive compounds from coffee husk waste. The coffee husk contains bioactive compounds, including anthocyanins and polyphenol compounds, such as flavonols, flavan-3-ols, hydroxycinnamic acids, and caffeine. Coffee husk waste will be extracted using the Microwave-Assisted Extraction (MAE) method with acetone solvent, with several variables: the ratio of material to solvent, microwave power, and extraction time. Analysis of the identification of phenolic compound content was then also carried out using UV-Vis spectrophotometry. The variables of material ratio, extraction time, and microwave power were proven to be interrelated so that they could produce total phenol at optimum conditions. The optimum conditions for extracting bioactive compounds from coffee husk waste were obtained at a material ratio of 0.04 g/mL, 9 minutes, and power of 300 watts, with a total phenol yield of 8.65 GAE/g sample.

**Keywords***: coffee husk, acetone, extraction, MAE, total phenol*

## 1. Introduction

Indonesia is one of the largest coffee-producing countries in the world, ranking 4th after Colombia, Vietnam, and Brazil, with coffee production reaching 762.38 thousand tons in 2020 [1]. Coffee is one of the plantation commodities with a relatively high economic value among other plantation plants, and it plays a vital role as a source of foreign exchange for the country and a source of income for coffee farmers in Indonesia. In coffee processing, several stages are carried out, from peeling the coffee husk until the coffee becomes the final product of ground coffee. Coffee husks are the most significant waste (40% - 45%) from coffee processing [2].

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Nowadays, coffee husks are limited to animal feed and fertilizer. Lack of public attention and minimal information obtained regarding the use of coffee husk waste are the causes of the lack of utilization and processing of coffee husk waste [3]. Coffee husk waste contains ferulic acid, caffeic acid, gallic acid, and p-coumaric acid. In addition, coffee husk also contains active secondary metabolite compounds, including anthocyanins and polyphenol compounds such as flavonols, flavan-3-ols, hydroxycinnamic acids, and caffeine [4].

Natural phenolic compounds are generally polyphenols that form ether, ester, or glycoside compounds, including flavonoids, tannins, tocopherols, coumarins, lignins, cinnamic acid derivatives, and polyfunctional organic acids. The total phenolic content determines an extract's potential for free radical scavengers [5]. A study reported that dry coffee husk contains phenolic compounds of around 1.8-8.56% of the total primary and secondary metabolite content [6].

These bioactive compounds can be obtained by extraction. Several developments have emerged from conventional methods of extracting a sample, one of them being the microwave-assisted extraction method. MAE is an extraction that utilizes microwave radiation to accelerate selective extraction through a rapid and efficient solvent heating [7].

Based on these potentials, a study utilized Robusta coffee husk waste as a raw material for research. Coffee husk waste will be extracted using the MAE method with acetone solvent. This study uses several variables to determine optimal results: the ratio of material to solvent, microwave power, and extraction time. An analysis was also carried out to identify the presence of phenolic compounds using UV-Vis spectrophotometry. The objectives of this study include determining the effect of variations in the ratio of material to solvent, microwave power, and time in the extraction process, as well as determining the optimum conditions in the extraction process of bioactive compounds from coffee husk waste.

## 2. Material and Methods

### 2.1 Equipment

The equipment used in this study includes a set of MAE method tools, namely a microwave (Samsung MS23K3515AS-SE), condenser, and measuring flask. Some glass equipment such as measuring cups, beakers, Erlenmeyer flasks, stirring rods, measuring pipettes, test tubes, cuvettes, funnels, and supporting tools such as analytical balance (Pioneer), hoses, clamps, stands, micropipettes, and UV-Vis spectrophotometers 752AP.

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### 2.2 Materials

The materials used in this study were robusta coffee husk waste, raw materials obtained from coffee farmers in Tanah Wulan Village, Bondowoso Regency, East Java, Indonesia. There are also distilled water, acetone (technical), gallic acid (p.a. Merck), Folin-Ciocalteu reagent (p.a. Merck), $Na_2CO_3$ (p.a. Merck), aluminum foil, and filter paper.

### 2.3 Methods

#### 2.3.1 Design Expert

This study used the Design Expert application (software) with CCD type (Central Composite Design) to obtain experimental design. The results of running through Design Expert used several variables such as extraction time (3 min, 6 min, 9 min), microwave power (100, 200, 300 Watt), and the ratio of material to solvent (0.2 (10 g: 0 mL), 0.12 (6 g:50 mL); 0.04 (2 g:50 mL)).

#### 2.3.2 Preparation of Coffee Husk Waste Simplicia

Robusta coffee husk waste simplicia was taken from robusta coffee farmers in the Tanah Wulan, Bondowoso Regency. Simplicia's robusta coffee husk waste was dried in the sun until the coffee husk waste was dehydrated. The dried simplicia was ground using a grinding machine and then sieved with a sieve size of 80 mesh.

#### 2.3.3 Coffee Husk Simplicia Water Content Test

The water content of coffee husk waste is determined using the oven method [8]. A total of 5 grams of sample is put into the oven for 2 hours at a temperature of 100 °C and then weighed. After that, the sample is put into the oven for 10 minutes and weighed again. The drying process is repeated 3 times until a constant weight is obtained, with a water content result of ± 10%. The water content can be calculated using Equation 1 [8].

$$Water\ Content = \frac{initial\ mass(g) - final\ mass(g)}{initial\ mass\ of\ coffee\ husk}\ x\ 100\% \qquad (1)$$

#### 2.3.4 Coffee Husk Extraction Using the MAE Method

The extraction process in this study used the MAE method because it was considered more effective. This method has the advantage that the process takes a short time, and the total phenol content produced is higher [9]. The extraction process begins by dissolving the sample in acetone solvent using a beaker glass. The ratio of materials and solvents used in this study is 0.2 (10 g:50 mL), 0.12 (6 g:50 mL), 0.04 (2 g:50 mL). The sample solution is then put into

an extraction container (measuring flask) and extracted using a microwave with time variations of 3 min, 6 min, and 9 min and power variations of 100, 200, and 300 watts. The extracted sample solution is filtered with filter paper to separate the filtrate.

### 2.3.5 Analysis of Total Phenol Content with UV-Vis Spectrophotometry

According to research by Ayuchecaria et al. (2020), the analysis of phenolic compound content using UV-Vis spectrophotometry has several stages, namely [10] :

#### 2.3.5.1 Preparation of Gallic Acid Stock Solution (100 ppm)

A total of 0.01 grams of gallic acid is dissolved in 1 mL of acetone. Then, the solution is diluted with distilled water to 100 mL.

#### 2.3.5.2 Determination of Maximum Wavelength of Gallic Acid

A total of 0.2 mL of gallic acid stock solution was put into a test tube, and 1 mL of Folin-Ciocalteu reagent was added and then shaken until homogeneous. The solution was left for 5 minutes at room temperature. After being left to stand, 2 mL of 10% $Na_2CO_3$ was added to the solution, and distilled water was added until the volume became 10 mL, then shaken until homogeneous and left for 8 minutes. The solution was then analyzed using UV-Vis spectrophotometry with a 600-800 nm wavelength interval.

#### 2.3.5.3 Preparation of Gallic Acid Standard Curve

A 100 ppm gallic acid stock solution was taken, each 1, 3, 5, and 7 mL. The solution was then diluted with distilled water until the final volume was 10 mL, and a concentration of 10, 30, 50, and 70 ppm was obtained. Each solution was taken in 0.2 mL and put into a test tube. The next step was to add 1 mL of Folin-Ciocalteu reagent, shake until the mixture of the two solutions became homogeneous, and then leave for 5 minutes at room temperature. The maximum wavelength absorption that had been obtained previously was measured. Then, a calibration curve was made with the regression equation $y = ax+b$.

#### 2.3.5.4 Determination of Total Phenolic Content

Take 0.2 mL of diluted extract (0.01 mL of sample diluted to a volume of 10 mL), add 6.8 mL of distilled water, and add 1 mL of Folin-Ciocalteu reagent then shake until the solution mixture becomes homogeneous and left for 5 minutes. Then, 2 mL of 10% $Na_2CO_3$ is added to the mixture, shaken again until the solution becomes homogeneous, and then the solution is

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left for 8 minutes at room temperature. The absorbance is measured using a UV-Vis spectrophotometer at the maximum wavelength that has been obtained.

## 3. Results and Discussion

### 3.1 *Design Expert*

Based on the running results using CCD, 20 samples were obtained. The CCD type Design Expert was used because it has the advantage that the design basis is a factorial design, so it can be used for order one or order 2. The running results obtained 20 designs using variations in extraction time, the ratio of material to solvent, and the microwave power used.

### 3.2 Raw Material Preparation

The raw material in the form of coffee husk obtained from coffee farmers is first dried using sunlight for 3 days to remove the water content contained in the coffee husk. The dried coffee husk is then ground using a grinding machine to make it powder. The raw material in the form of powder aims to facilitate the extraction process so that it is easier to mix. The coffee husk powder is then sieved using an 80-mesh sieve. The finer the coffee husk powder used, the larger the surface area, and the easier it is to penetrate by microwaves, so the higher the solubility level is, the greater the yield produced [11]. The determination of the water content of the coffee husk powder is then carried out using the oven method. The raw material's high or low water content affects the process and extraction results [12]. This water content determination was repeated 3 times until the results were stable to obtain a valid water content percentage. The water content of the sample used was 7.15%; this shows that the percentage of the water content of the robusta coffee husk powder has met the simplex standard, where the water content should not be more than 10% [13].

**Table 1.** Water Content Test Results

| Sample weight (g) | Sample weight after drying (g) | Water content (%) |
|---|---|---|
| 5 | 4.65 | 6.96 |
| 5 | 4.64 | 7.12 |
| 5 | 4.63 | 7.38 |
| Average | 4.64 | 7.15 |

### 3.3 Coffee Husk Extraction Using MAE Method

The finely ground robusta coffee husk powder is then weighed to be extracted using the MAE method. The robusta coffee husk powder is then dissolved in acetone solvent according

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to the ratio obtained through the Design Expert software. The selection of acetone solvent is based on its polarity, which can dissolve secondary metabolite compounds that are polar to non-polar, including phenolic compounds in robusta coffee husk. The extraction process produces sediment and filtrate, which are filtered using filter paper and a funnel. The filtration of the extraction results aims to obtain maximum separation results so that the filtrate is not mixed with the coffee husk powder [14].

3.4 Analysis of Total Phenol Content with UV-Vis Spectrophotometry

The determination of wavelength aims to determine the absorption area that can produce the absorbance value of the parent solution whose absorbance is measured. The wavelength used is the wavelength that has maximum absorbance so that it shows maximum sensitivity. The spectrophotometric tool is set to the wavelength range used, which is around 600-800. Based on research conducted Ayuchecaria et al. (2020), the maximum wavelength is 600-800 nm, and it states that this range obtains ideal wavelength results in determining the presence of bioactive compounds in plants [10]. Septiani et al. (2018) Said that the maximum wavelength is 765 nm [13]. The graph in Figure 1 shows the wavelength measurement with a range of 600-800 nm, and the maximum wavelength is obtained at 760 nm, which has the highest absorbance value. To be more specific, then the wavelength measurement was carried out again with a range of 762-768 nm. Table 2 shows the maximum wavelength is 766 nm.

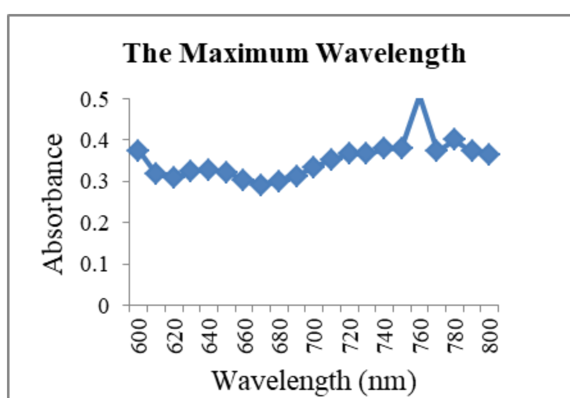


**Figure 1.** The Measurement of Maximum Wavelength

**Table 2.** The Measurement of Maximum Wavelength

| Wavelength (nm) | Absorbance |
|---|---|
| 762 | 0.477 |
| 764 | 0.488 |
| **766** | **0.568** |
| 768 | 0.422 |

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### 3.4.1 Gallic Acid Standard Curve

The standard curve was made using a gallic acid solution that had previously been made with several concentrations, namely 10, 30, 50, and 70 ppm. The standard curve was created based on the determination of the absorbance values of several concentrations and measured based on the maximum wavelength that had been obtained. The calibration curve states the relationship between the concentration of gallic acid and the absorbance value expressed by a linear line. The standard curve results obtained a linear regression equation $y = 0.0098x + 0.1222$ with a correlation coefficient $R^2$ of 0.9914. According to Miller & Miller (2005), in the study Arikalang et al. (2018), based on the $R^2$ results obtained, the correlation coefficient gave linear results because it met the acceptable criteria, namely 0.99 [15]. These results meet the requirements, so they can be used to determine the total phenol content of coffee husk extract.

### 3.4.2 Analysis of Total Phenol Content

The extraction process used the MAE technique with influential variables in time, solvent ratio, and microwave power. The selection of variables is based on factors that affect the extraction process using the microwave technique: time, the ratio of material to solvent, and the amount of power [16]. The extraction results will be filtered using filter paper to obtain maximum separation results so the filtrate is not mixed with robusta coffee husk powder.

The extraction results were analyzed for total phenol content using visible spectrophotometry. Total phenol analysis was carried out with the help of the Folin reagent, which aims to show that the coffee husk extract contains phenolics, which is indicated by a change in the color of the solution to blue. The blue color indicates the presence of bioactive compounds in the coffee husk extract. The results of this analysis are expressed in gallic acid equivalents (mg GAE/g sample).

The results of the absorbance value and total phenol content of coffee husk extract based on the research that has been carried out are presented in Table 3. Table 3 below shows that the highest total phenol results were produced by sample 17 with an extraction time variable of 9 minutes, a material-to-solvent ratio of 0.04 g/mL, and a microwave power of 300 watts. The total phenol produced was 8.65 mg GAE/g sample with an absorbance value of 0.97. The results of this study are higher when compared to previous research conducted Rahayu et al. (2022),Simlipi wherewith the same raw materials and extraction methods but using ethanol solvent, the highest total phenol was obtained at a ratio of 1:30 and a time of 10 minutes, namely 8.55 mg GAE/g [17].

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### 3.3 Effect of Microwave Power, Material Ratio, and Extraction Time on Total Phenol Content

**Table 3.** Total Phenolic Content in Coffee Husk Extract

| Run | Time (minutes) | Ratio (g/mL) | Power (watt) | Absorbance | Total phenol (mg GAE/g sample) |
|---|---|---|---|---|---|
| 1 | 6 | 0.12 | 200 | 0.62 | 1.69 |
| 2 | 6 | 0.2 | 200 | 0.89 | 1.58 |
| 3 | 6 | 0.2 | 200 | 0.89 | 1.58 |
| 4 | 3 | 0.04 | 100 | 0.59 | 4.82 |
| 5 | 9 | 0.12 | 200 | 0.75 | 2.14 |
| 6 | 9 | 0.2 | 100 | 0.91 | 1.62 |
| 7 | 6 | 0.12 | 300 | 0.70 | 1.97 |
| 8 | 9 | 0.2 | 300 | 0.93 | 1.65 |
| 9 | 3 | 0.04 | 300 | 0.60 | 4.89 |
| 10 | 3 | 0.2 | 300 | 0.92 | 1.63 |
| 11 | 3 | 0.12 | 200 | 0.59 | 1.59 |
| 12 | 9 | 0.04 | 100 | 0.89 | 7.86 |
| 13 | 6 | 0.2 | 100 | 0.97 | 1.73 |
| 14 | 3 | 0.2 | 100 | 0.86 | 1.51 |
| 15 | 6 | 0.2 | 200 | 0.89 | 1.58 |
| 16 | 6 | 0.2 | 200 | 0.89 | 1.58 |
| **17** | **9** | **0.04** | **300** | **0.97** | **8.65** |
| 18 | 6 | 0.04 | 200 | 0.59 | 4.84 |
| 19 | 6 | 0.2 | 200 | 0.89 | 1.58 |
| 20 | 6 | 0.2 | 200 | 0.89 | 1.58 |

Based on the results of the research that has been done, the effect of several variables used on the total phenol produced can be seen in Figures 2(a), (b), and (c). Figure 2(a) shows the effect of time and power variables on the total phenol produced. The highest total phenol results were produced at 9 minutes and 300 watts of microwave power of 8.65 GAE/g sample, while the lowest total phenol content was at 3 minutes and 100 watts of power, which was 1.51 GAE/g sample. It can be concluded that the longer the extraction time and the higher the microwave power used, the higher the total phenol produced.

Figure 2(b) shows the effect of the ratio of materials and time variables on the total phenol produced. The highest total phenol results were obtained at a ratio of 0.04 g/mL and an extraction time of 9 minutes, resulting in a total phenol of 8.65 GAE/g sample. It can be concluded that the smaller the ratio of materials to solvents but with a longer extraction time, the higher the total phenol produced. The use of a large ratio will result in a decreasing total phenol. This is because there is a decrease in microwave absorption in the material, so more energy needs to be absorbed by the solvent [17].

Figure 2(c) shows the effect of power variables and material ratio on the total phenol produced. The highest total phenol yield was obtained at a ratio of 0.04 g/mL and 300 watts of microwave power, which was 8.65 GAE/g sample. It can be concluded that the smaller the ratio of material to solvent but with higher microwave power, the higher the total phenol will be produced.

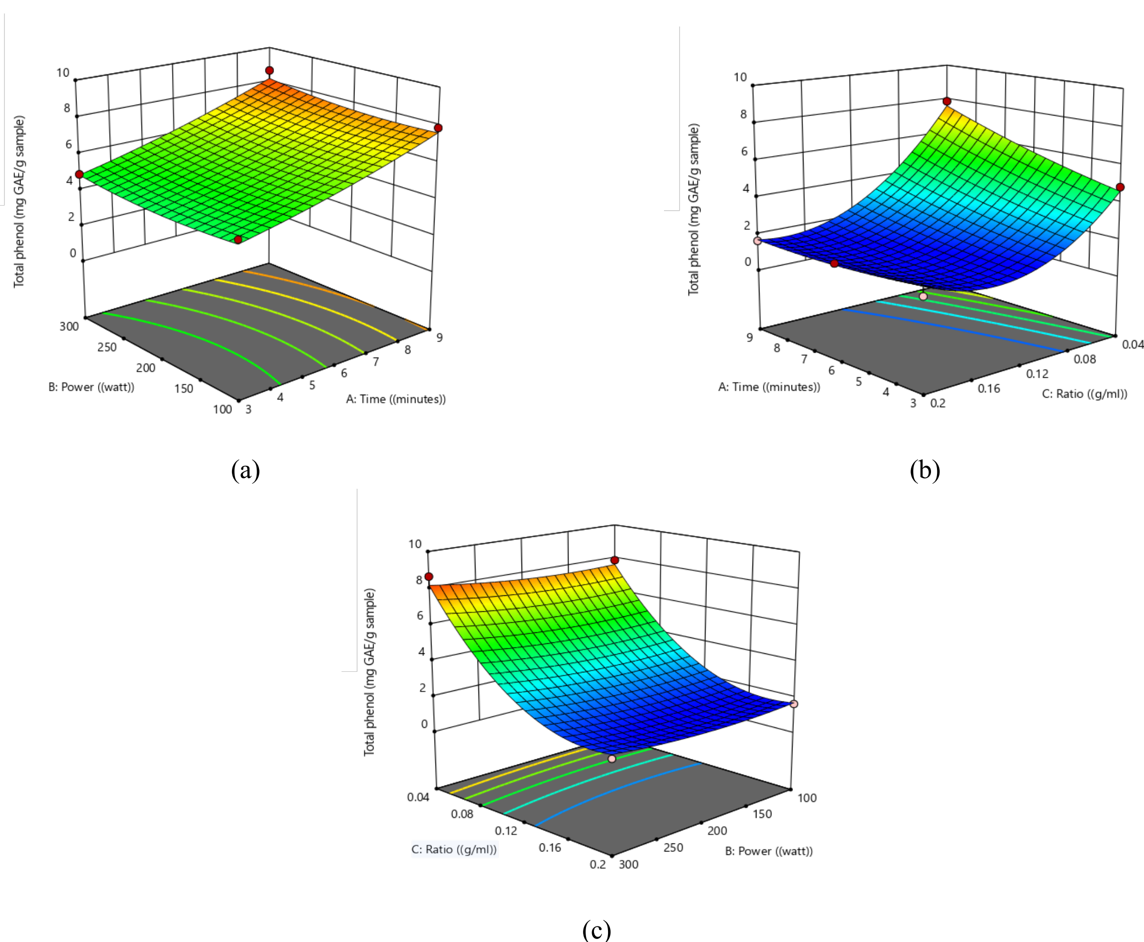


**Figure 2.** Response Surface Analysis on Total Phenol (a) Effect of Time vs Power Variable, (b) Effect of Ratio vs Time Variable, (c) Effect of Power vs Ratio Variable

The ratio of material to solvent is a reasonably necessary parameter because it affects the total phenol results of robusta coffee husk extract. A ratio of 0.04 (2 g: 50 mL) gives a significant average total phenol value because there is more effective contact between the material and the solvent when compared to other solvent ratios. The ratios of 0.2 and 0.12 are less effective because the weight of the material used is too much, so the material only partially dissolves into the solvent. The greater the ratio of material extracted to the solvent used, the more optimal the results obtained [18].

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The time variable in the extraction process is also a parameter that significantly influences the total phenol produced. Extraction time is the contact time between microwaves and the material to be extracted. Extraction time that is too short causes the solubility of phenolic compounds to be less than optimal, so the material is not extracted perfectly and vice versa. The longer the extraction time, the more analytes are extracted because the contact between the solvent and the solute will be longer, so the dissolution of phenolic compounds will continue [19]. This research produced the highest total phenol at the optimum time, namely 9 minutes.

Microwave power variables also affect the total phenol yield, so selecting the power used is necessary. The use of power that is too high, namely above 300 Watt, produces temperatures above 60 °C so that it can cause degradation of the target compound structure and excess pressure in the extraction process and affect the quality of the resulting extract, so a power of 100-300 watt is selected [18]. At low power usage, the cell wall breaks down gradually so that the solvent can be selective to the target compound. In the principle of MAE extraction, the heat generated by particle friction due to microwave energy can cause the cell matrix to break down, and the target compound will come out. So, if the temperature is too low, the cell matrix will not break down completely, and the amount of dissolved target compound is small [18]. Microwave power and extraction time are closely related, where the combination of high power below 400 and longer time becomes the variable with the best results in the MAE method. This study obtained the highest total phenol results at 300 watts of power and 9 minutes, while the lowest were produced at 100 watts of power and 3 minutes.

### 3.4 ANOVA Analysis

Analysis of research results using the ANOVA method aims to show that the variables used in this study affect the resulting research product. The response value used in this study is total phenol. The results of the ANOVA analysis have several parameters shown in Table 4. The F-value is a specific value used to compare whether the test is significant or not, and the p-value is the magnitude of the observed probability from the statistical test. Several parameters observed include extraction time (A), ratio of material to solvent (B), and microwave power (C).

**Table 4.** The Results of ANOVA Analysis

| Source | Sum of Squares | Df | Mean Square | F-value | p-value |
|---|---|---|---|---|---|
| Model | 89.75 | 3 | 9.97 | 42.43 | 0.0001 |
| A-Time | 5.57 | 1 | 5.57 | 23.71 | 0.0007 |
| B-Ratio | 58.11 | 1 | 58.11 | 247.24 | 0.0001 |
| C-Power | 0.1140 | 1 | 0.1140 | 0.4853 | 0.5019 |
| *Pure Error* | 0.0062 | 5 | 0.0012 | | |

Parameters with a P-value smaller than 5% (0.05) can be significant or have a real effect. Based on Table 4 above shows that the models and variables A, B, and C used are substantial. A value greater than 0.1 indicates an insignificant model. The F value of the model of 42.43 suggests that the model is significant.

Based on the ANOVA analysis, the $R^2$ value was obtained at 95.16%, as shown in Table 5. This value indicates that the model used is according to the research results. The $R^2$ value is stated to be in according to the model if the resulting percentage is more significant than 75% [20]. This can indicate that the equation used to predict the influential variables is appropriate and can be used to predict the study's actual results.

**Table 5.** The $R^2$ Value in ANOVA

| | |
|---|---|
| $R^2$ | 0.9516 |
| Adjusted $R^2$ | 0.9345 |
| Predicted $R^2$ | 0.7934 |

Based on the ANOVA equation obtained, it can be concluded that the influence of process parameters, namely extraction time, microwave power, and the ratio of material to solvent, can affect the total phenol produced. The ANOVA equation is as follows:

$$\text{Total Phenol} = 7.10 + 0.33A + 78.62B + 0.0095C \tag{2}$$

Description:

A: extraction time

B: ratio of material to solvent

C: microwave power

The equation shows that extraction time, material-to-solvent ratio, and microwave power are directly proportional to the total phenol value. Total phenol increases with increasing extraction time, material-to-solvent ratio, and microwave power. A positive constant value indicates this.

Figure 3 shows a plot of actual data with model-predicted data, which is close to accurate; there is a strong correlation between actual and predicted data. The expected data line is the result of computer calculations using the Design Expert application based on the variables that have been inputted, and the points around the line are the actual data from the experiments that have been carried out. Based on Figure 3, the data plot touches a line that shows that the actual data is close to the predicted data, supported by an $R^2$ value of 95.16%.

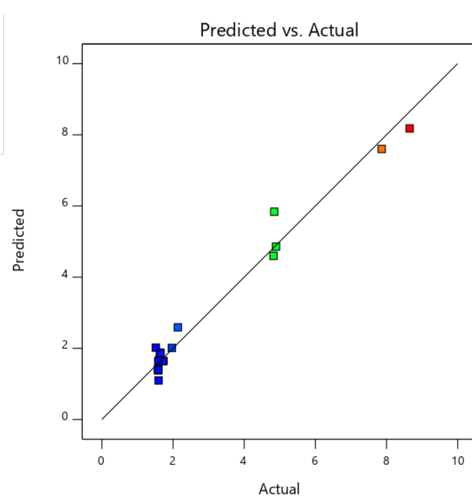


**Figure 3.** Predicted Data vs Actual Data Plot

## 4. Conclusion

The variables used in the study of the extraction of bioactive compounds from coffee husk waste using the MAE method affect the total phenol produced. The variables of the ratio of material to solvent, extraction time, and microwave power are related to specific combinations that can result in total phenol at optimum conditions. The optimum conditions in the process of extracting bioactive compounds from coffee husk waste using the MAE method were produced at a variable ratio of material to solvent of 0.04 g/mL, a time of 9 minutes, and a power of 300 Watt with a total phenol yield of 8.65 GAE/g sample.

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RESEARCH ARTICLE

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# Kinetic Extraction of *Moringa oleifera* Leaves using the Microwave Assisted-Extraction (MAE) Method

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**Abstract.** Moringa oleifera leaves are known for their distinctive leaf shape and offer numerous health and nutritional benefits. They are rich in vitamins, minerals, proteins, and bioactive compounds and possess antioxidant and anti-inflammatory properties. This study aimed to explore the extraction process, identify the presence of tannin compounds in Moringa leaves, and determine the appropriate kinetic model for the extraction of Moringa leaf extract using the Microwave-Assisted Extraction (MAE) method. The extraction was performed using 96% ethanol as the solvent and the MAE technique, which is known to enhance extraction efficiency. Variables such as power (150 watts), a material-to-solvent ratio of 1:15 (b/v), and extraction times of 2, 4, 6, 8, and 10 minutes were tested. The results indicated that the highest yield was achieved after 10 minutes, with a value of 10.25%. The extraction time was extended at most 10 minutes due to time limitations and diminishing returns as extraction time increased. The study concluded that the second-order kinetic model ($R^2 = 0.9897$) was the most suitable for describing the extraction of tannin compounds from Moringa leaves, outperforming the first-order model with a value closer to 1.

**Keywords:** *extraction, Moringa oleifera, flavonoids, and microwave-assisted extraction*

## 1. Introduction

Moringa leaves, or *Moringa oleifera,* are tropical plants from South Asia known for their high nutritional content, especially in the leaves used in traditional medicine [1]. Moringa leaves are rich in essential nutrients such as protein, calcium, iron, and vitamins. Moringa leaves also contain bioactive compounds, including flavonoids, tannins, and saponins, which have antioxidant and anti-inflammatory benefits [2]. In Indonesia, Moringa leaves are used as a vegetable to accompany rice. Moringa leaves have many benefits, including being anti-diabetic, anti-hepatitis, a medicine for heart problems, and reducing cholesterol levels in the body.

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According to research by Rohyani et al. (2015), the results of phytochemical screening from Moringa leaves contain flavonoids, alkaloids, steroids, tannins, saponins, anthraquinones, and terpenoids [3]. The content per 100 grams of dried Moringa leaves contains 10% flavonoids, 0.075% water, 2.05% calories, 0.382% carbohydrates, 0.271% protein, 0.023% fat, 0.192% calcium, 3.68% magnesium, 2.04% phosphorus, 0.006% copper, 0.282% iron, and 8.7% sulfur [4].

Extraction is a key step in utilizing bioactive compounds from natural materials, such as plants, for various industrial and medical applications [5]. Optimal extraction allows maximum retrieval of valuable compounds with high efficiency, optimizing the final product yield. Factors such as temperature, time, solvent type, and extraction method affect the results. Extraction methods generally used are maceration, percolation, soxletation, and ultrasonic. Factors that need to be considered in selecting an extraction method are the nature of the material, the type of solvent, and the purpose of the extraction [6]. The duration of tannin extraction is often due to the chemical nature of tannin, which is easily soluble in water or other solvents, as well as the speed of diffusion of plant tissue into the solvent. Tannin is a polyphenolic compound with a high affinity for water, so the transfer process from the plant matrix to the solution can occur efficiently and quickly. In addition, modern extraction methods such as microwave-assisted extraction (sonication) speed up this process by increasing contact between the solvent and plant material, reducing diffusion barriers, and accelerating the breakdown of plant cell walls [7].

Extracting bioactive compounds from plants is a key step in developing vegetable products, including from Moringa leaves [8]. Microwave-assisted extraction (MAE) utilizes microwave energy to speed the extraction process. It allows the extraction of active compounds more efficiently and in a shorter time than conventional methods. In this research, we will explore the influence of three key parameters in the extraction method, solvent type, extraction temperature, and extraction time, on the tannin content in Moringa leaves extract using the MAE method [9]. MAE is an innovative technique that utilizes microwaves to speed up extracting active compounds from plant materials. This method has demonstrated significant advantages in extraction efficiency compared to conventional methods, which often require longer extraction times and more amounts of solvent [10].

This research aims to study extraction, determine the presence of tannin content in Moringa leaves, and determine the kinetic model of extraction of Moringa leaves extract yield using the MAE method. Tannin has antioxidant properties that can be beneficial for humans.

By knowing and understanding the variations in extraction, we can optimize the process to maximize the potential of bioactive compounds from Moringa leaves, providing benefits for the food industry, pharmaceuticals, and human health.

Based on research conducted by Handayani et al. 2020. The maceration method was used with 96% ethanol solvent, and qualitative testing was done using Alkaloid, Flavonoid, Saponin, and Tannin tests using reagents appropriate to the test parameters. Qualitative test results show that Moringa seeds contain positive alkaloids, indicated by an orange precipitate, flavonoids, m a r k ed by the formation of an orange-yellow color, saponins characterized by a stable foam, and tannins, indicated by a black color [17]. Research by EA Koesnadi et al. 2021 used rambusa leaves extract, which was extracted using the MAE method with an extraction time ratio of 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, and 6 minutes with 300 watts microwave irradiation power. The results showed that an extraction time of 4 minutes was able to produce rambusa leaves extract which had the highest antioxidant activity based on the percentage of free radical inhibition, namely 25.29% with an IC50 value of 196.17 mg/L, yield of 17.40%, total phenol 75.07 mg GAE/g extract, total flavonoids 33.05 mg QE/g extract and total tannins 2.76 mg TAE/g [18].

The problems in obtaining Moringa leaf extract using the MAE method are rarely solved. The exact ratio of ingredients to solvent and extraction time on total tannin content is unknown. Previous research on the extraction of Moringa leaves regarding total flavonoids and antioxidant activity can be seen in Table 1.

**Table 1.** Research on tannin extraction

| Material | Method | Result | Reference |
|---|---|---|---|
| Garlic Peel (*Allium sativum* L.) | Microwave Assisted Extraction (MAE) | In this study, variable raw material ratios of 1:10, 1:15, 1:20, 1:25, 1:30 were used, power of 10% and 50% of the maximum power of the tool (399) watts, and time of 5, 10, 15 minutes. The research results showed that the total yield value obtained was 2.04% from a solvent raw material ratio of 1:15, extraction time of 10 minutes, and 50% power. | [9] |
| *Psidium guajava* leaves | Soxhlet | This research uses ethanol solvent with a solid-liquid ratio of 1/20 and 1/60 (w/v). The extraction temperature was 80°C and extraction times were 30, 45, 60, 75, and 90 minutes. The highest tannin content was obtained at 60 minutes and a solid-liquid ratio of 1/20 (w/v), approximately 17% and 12%, respectively. The highest percentage of tannin content was for a solid-liquid ratio of 1/60 (w/v) at an extraction time of 60 minutes. If compared, the tannin content at a solid-liquid ratio of 1/20 (w/v) is greater than 1/60 (w/v). These results indicate that increasing the amount of solvent has no significant | [12] |

| Material | Method | Result | Reference |
|---|---|---|---|
| | | effect on the tannin diffusion rate of Psidium guajava leaves. | |
| Acacia Wood | Microwave Assisted Extraction (MAE) | This research used distilled water and ethanol solvents with microwave power of 100, 180, 300, 450 watts and extraction times of 1, 3, 5 minutes. The extraction results were analyzed using a UV-Vis spectrophotometer. This research obtained the highest tannin yield of 26,606 mg/g at 100 watts of power and 3 minutes with ethanol solvent. | [15] |
| Moringa leaves (*Moringa oleifera*) | Microwave Assisted Extraction (MAE) | Ethanol solvent, solvent concentration (60, 70, and 80%), raw material mass (25 and 50 grams), extraction time 10 minutes with a solvent volume of 500 mL and a power of 380 watts. The best yield value produced by the Microwave Assisted Etraction (MAE) method produces the largest yield, namely 9% for a material mass of 50 grams, time of 10 minutes and ethanol solvent concentration of 80%. | [ 16] |
| Moringa Seeds (*Moringa oleifera* Lam.) | Maceration | This research used the maceration method with 96% ethanol solvent, qualitative testing using Alkaloid, Flavonoid, Saponin and Tannin tests using reagents in accordance with the test parameters. Qualitative test results showed that Moringa seeds were positive for containing alkaloids, indicated by the presence of an orange precipitate, flavonoids indicated by the formation of an orange yellow color, saponins indicated by a stable foam, and tannins indicated by a black color. | [17] |
| leaves (*Passiflora foetida* L.) | Microwave Assisted Extraction (MAE) | % ethanol solvent with material ratio (1:10), extraction time ratio of 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes and 6 minutes with 300 watts microwave irradiation power. The results showed that an extraction time of 4 minutes was able to produce rambusa leaves extract which had the highest antioxidant activity based on the percentage of free radical inhibition, namely 25.29% with an IC50 value of 196.17 mg/L, yield of 17.40%, total phenol 75.07 mg GAE/g extract, total flavonoids 33.05 mg QE/g extract and total tannins 2.76 mg TAE/g. | [18] |

## 2. RESEARCH METHODS

### 2.1 Tools and materials

This research needs a series of MAE extraction tools, a 60 mesh sieve, analytical scales/balance, filter paper, a glassware set, a test tube rack, and a rotary evaporator. The ingredients used are Moringa leaves, distilled water, 96% ethanol, concentrated hydrochloric acid (HCl), anhydrous acetic acid, and 1% $FeCl_3$.

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## 2.2 Sample preparation

### 2.2.1 Making Simplisia Powder

Raw materials are made from Moringa leaves from Kaliwates District, Jember Regency. The Moringa leaves are sorted and washed clean, then dried at an oven temperature of 60℃ until the Moringa leaves are dry. The dried Moringa leaves are then blended and then sieved using a 60 mesh sieve to obtain Moringa leaves simplicia powder [23].

### 2.2.2 Simplicia and Water Content Test

Determination of simplicia water content was carried out using the gravimetric method. A total of 2 grams of simplicia powder was used to determine water content using an oven at 105℃ for 3 hours. The water content requirement for simplicia, according to the applicable standard parameters, is no more than 10%. Determining the water content of simplicia is very important to provide a maximum limit on the water content in simplicia, because the amount of water that tends to be high can become a medium for the growth of bacteria and fungi which can damage the compounds contained in simplicia [24]. The calculation of % water content can be seen in the equation below [23]:

$$\%\,Water\ content = \frac{(mass\ of\ empty\ cup + ingredients\ before\ drying) - (mass\ of\ cup + ingredients\ after\ drying)}{mass\ of\ simplicia}\ x\ 100\%$$

### 2.2.3 Moringa Leaves Extraction

The third stage of the procedure is extracting Moringa leaves. The variable ratio of material to solvent is 1:15 b/v [22], using the lowest microwave power, 150 watts. The solvent used in the extraction process is ethanol, with a concentration of 96%. The extraction process will take 2, 4, 6, 8, and 10 minutes [19]. The raw materials are put into the Erlenmeyer according to the predetermined ratio variations and then into the microwave with power according to the variables. The solution is irradiated in the microwave periodically to maintain a temperature of at least 80℃. The solution is then left to cool to room temperature and filtered, and the filtrate is concentrated using a rotary evaporator until it becomes a thick extract.

### 2.2.4 Yield Analysis

The resulting extract is then weighed in a container, and the weight of the concentrated extract is divided by the initial weight using the following formula [25]:

$$\%\,Yield = \frac{thick\ extract\ mass}{mass\ of\ simplicia\ powder}\ x\ 100\%$$

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### 2.3 Tannin Phytochemical Analysis

The extract that has been put into the test tube, then 1-2 drops of 1% $FeCl_3$ solution are added as a reagent. If the extract contains tannin compounds, there will be a color change in the filtrate, indicating the presence of tannins with a color change to green or bluish-black. This color change occurs due to the interaction between the $Fe^{3+}$ ion from $FeCl_3$ with the phenolic group in tannin, forming a complex that produces the color [25].

**Figure 1.** Color Change of Tannin Compound Extract

### 2.4 Extraction Kinetics

To determine the optimal operating conditions in the Moringa leaves extraction process, it is essential to calculate the extraction kinetics using the Lagergren equation, a widely used model for describing the rate of adsorption or extraction processes. The Lagergren equation, often applied to first-order kinetic reactions, provides insights into how quickly the active compounds in the Moringa leaves are extracted under varying conditions, such as time, temperature, and solvent concentration. By applying this equation, researchers can evaluate how different factors affect the extraction rate, including solvent type, extraction time,

temperature, and the ratio of solvent to plant material. The results help to identify the reaction rate constant ($k_1$), which quantifies the speed of the extraction process, and the equilibrium concentration (Cs), which indicates the maximum extractable concentration under specific conditions.

Using this model, we can minimize the number of variables by focusing on the most influential factors, such as temperature and extraction time, which are likely to have the most significant impact on the efficiency and yield of the extraction process. This allows for a more streamlined and controlled approach to optimize the extraction, ensuring the process is efficient and cost-effective. Moreover, understanding the kinetics can aid in determining the exact point at which the extraction process reaches equilibrium, ensuring that no resources are wasted and maximum yields are achieved without over-extraction.

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2.4.1 First Order Kinetic Model

The first-order kinetic equation, as proposed by Lagergren, can be expressed in its differential form as follows:

$$\frac{dCt}{dt} = k_1(C_s - C_t) \quad (1)$$

In this equation, t (min) represents the extraction time, and $k_1$ ($min^{-1}$) is the extraction rate constant for the first-order reaction. To integrate equation (1), the boundary conditions are applied: Ct = 0 at t = 0 and Ct = Ct at t = t:

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t \quad (2)$$

The equation (1) that has been obtained can be converted into linear form as follows:

$$\log(C_s - C_t) = \log(C_s) - \frac{k_t}{2{,}303}t \quad (3)$$

Then, a plot is made between $(C_s - C_t)$t and t are used to get the slope and intercept, which can be used to determine the value of the first-order extraction rate constant $k_1$ and the value of extraction capacity $C_s$.

2.4.2 First Order Kinetic Model

The second-order kinetic equation that describes the extraction rate can be expressed as follows:

$$\frac{dCt}{dt} = k_2(C_s - C_t)^2 \quad (4)$$

Where $k_2$ ($Lg^{-1}\ min^{-1}$)is the extraction rate constant for the second order. By grouping the variables in equation (4), we obtain:

$$\frac{dC_t}{(C_s - C_t)^2} = k_2 dt \quad (5)$$

A further equation in (8) can be obtained by integrating equation (5) using boundary conditions $C_t = 0$at $t = 0$and $C_t = C_t$at $t = t$and by rearranging as follows:

$$\frac{1}{(C_s - C_t)} - \frac{1}{C_s} = k_2 t \quad (6)$$

$$C_t = C_s - \frac{C_s}{1 + C_s k_2 t} \quad (7)$$

$$C_t = \frac{C_s^2 k_2 t}{1 + C_s k_2 t} \quad (8)$$

Equation (8) is an integrated extraction rate law for the second order and can be converted back into linear form as follows:

$$\frac{T}{C_t} = \frac{1}{k_2 C_s^2} + \frac{t}{C_s} \quad (9)$$

The extraction rate $C_t/t$can be obtained from equation (9), as follows

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{k2C_s^2}\right)+\left(\frac{t}{C_s}\right)} \tag{10}$$

And the initial extraction rate h, as $C_t = $ tit t approaches 0, can be defined as follows:

$$H = k_2C_s^2 \tag{11}$$

Equation (8) can be changed again so that finally, it can be found:

$$\frac{t}{C_t} = \frac{t}{C_s} + \frac{1}{h} \tag{12}$$

Given the initial extraction rate h, extraction capacity $C_s$and extraction rate constant $k_2$, the second order can be determined experimentally from the slope and intercept by plotting between $t/C_t$with t. [19].

## 3. RESULT AND DISCUSSION

The Moringa leaves extraction process requires an efficient approach to ensure optimal results. Several process stages are carried out to obtain the yield of Moringa leaf extract.

### 3.1 Sample preparation

The samples used in this research were *Moringa oleifera* L. plants in the Kaliwates sub-district, Jember Regency. The sample used was fresh Moringa leaves picked from the leaf's stalks that were still green. A total of 1 kg of Moringa leaves was washed with running water to remove impurities that could affect the extraction process results. If the sample has been cleaned, the drying process is then carried out using an oven at 60°C to remove the water content in the sample so that it avoids microbial growth and can be stored for a long time. The dried samples were then ground using a blender and then sifted using a 60 mesh sieve, which aims to limit variations in sample size so that a fine powder that is relatively the same size and uniform is obtained.

### 3.2 Water Content Analysis

Analysis of the water content in dry samples of Moringa leaves aims to determine the water content in the sample. The high water content in the sample can affect the extract concentration process. Low water content will inhibit the growth of microorganisms so that samples can be stored longer.

Water content analysis was carried out by heating an oven at 105°C for 3 hours, using a temperature higher than the water boiling point to maximize evaporation of the water in the sample. The results of the analysis of water content in dry samples of Moringa leaves were

4.58%, the results of the water content test were almost the same as in research conducted by Febriani et al. [24] namely 4.6% and were following the requirements for simplicia water content according to standard parameters. What applies is no more than 10%.

3.3 Extraction of Moringa Leaves

MAE is a modern method that utilizes microwave radiation to heat solvents and materials quickly and evenly. This technique increases solvent diffusion into plant cells and accelerates the release of active compounds, including tannins, from the cellular matrix. The advantages of MAE compared to conventional methods include shorter extraction time, less solvent use, and higher extraction efficiency.

The extract yield obtained was derived from the Moringa leaves extraction treatment using 96% ethanol solvent with a ratio of 1:15 w/v and with time variations of 2, 4, 6, 8, and 10 minutes using the MAE method. The yield results are obtained in the table below based on the treatment that the specified variations have carried out.

**Table 2.** Effect of Extraction Time with the MAE Method

| w/v (g/ml) | Extraction Time (minutes) | Yield (%) |
|---|---|---|
| 1:15 | 2 | 4.1 |
| 1:15 | 4 | 6.34 |
| 1:15 | 6 | 8.52 |
| 1:15 | 8 | 9.66 |
| 1:15 | 10 | 10.25 |

Evaluation of the effect on the extraction time variable was carried out for 2 minutes to 10 minutes with a mass and solvent ratio and using a constant power of 1:15 b/v, respectively, using 96% ethanol solvent, and the power used was 150 watts. The experimental results can be seen in Table 2. Based on the table above, along with increasing extraction time, there is a significant difference in yield. It can be seen in the table that the 2nd minute is the lowest yield when compared to the yield at other time variations. It is suspected that the ability of the ethanol solvent to extract the material has not been maximized, so only a small number of components are taken from the material.

Whether or not the extraction process takes a long time significantly influences the resulting extract yield. From the table above, it can be seen that the yield of the extract produced has different results over various changes in time. An increase in the duration of the extraction time used will increase the yield value. Likewise, the length of the extraction time will improve

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the ability of the solvent to penetrate a material. The solubility of components in the material progresses slowly as time increases; however, if the optimal time has been reached, the number of components taken from the raw material will experience a stable value or decrease. This is because the components contained in the material have a limited amount, and the solvent used has a limited ability to dissolve the existing material, so even if the extraction time is extended, the dissolved substances in the material are no longer there [22].

### 3.4 Identification of Tannins in Moringa Leaves Extract

The tannin content in Moringa oleifera leaves has significant potential in various industrial applications, from food to pharmaceuticals. Tannin is a phenolic compound known for its antioxidant and antimicrobial properties. Identifying the tannin content in Moringa leaves is an important first step in understanding the potential benefits of the extract.

The extract obtained was subjected to qualitative tests on tannins. The aim of conducting qualitative tests on tannins is to determine the presence of tannins in Moringa leaves. The phytochemical test carried out in this research was by adding tannin extract with 1% $FeCl_3$. This can be seen in Figure 1, which shows the qualitative test results of the extract solution with 96% ethanol solvent.

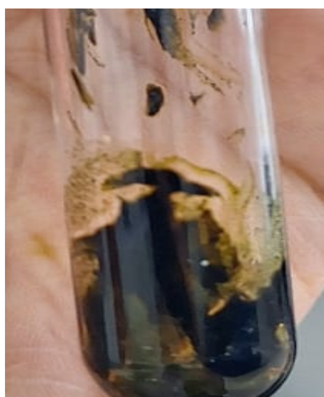


**Figure 2.** Results of Phytochemical Identification of Tannin Content.

Moringa oleifera L. leaves extract showed positive results. To determine whether the sample contained a phenol group, the researchers tested it by placing Moringa leaf extract into a test tube and adding 1% $FeCl_3$. Then, observe the color change in the sample; the color of the sample will change to blackish green or blackish blue; this indicates that the sample is positive for containing phenol groups, and it is possible that one is tannin. A blackish-green or blackish-

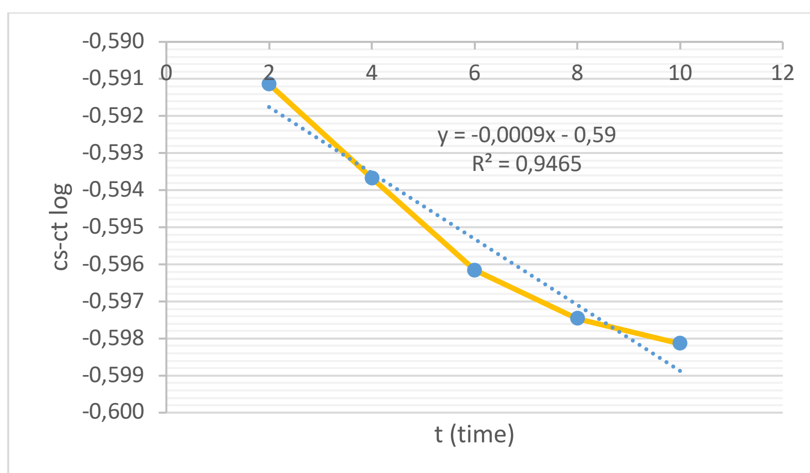
blue color is formed in the extract after adding $FeCl_3$ because tannins will form complex compounds with Fe ions [20].

Tannins are included in the phenolic group of active plant compounds, meaning they have an astringent taste. Tannin compounds are distributed in almost all plant species and have a role in protection from predators. They may also act as natural pesticides and regulate plant growth. Tannin compounds also function as antioxidants and inhibitors of tumor growth, and tannin compounds are also polyphenolic compounds found in plants [21].

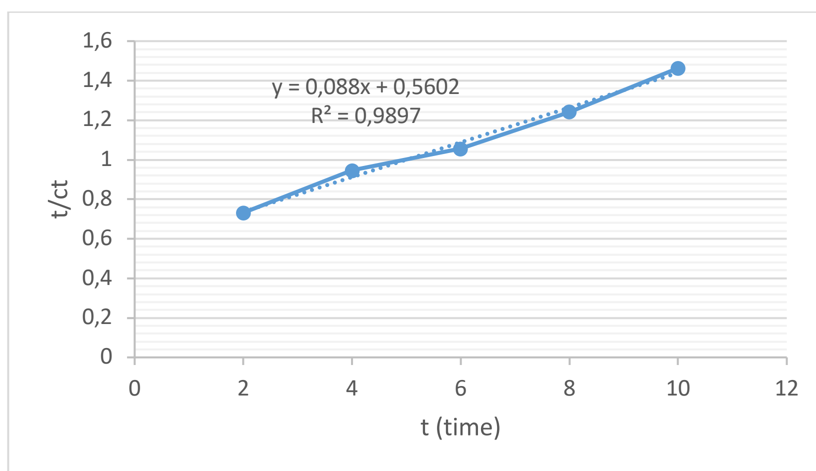
### 3.5 Extraction Kinetics

In studying extraction kinetics, it is important to understand how changes in extraction conditions affect the rate of tannin release from Moringa leaves. Parameters such as temperature, extraction time, and microwave power are significant variables. Kinetics studies are often carried out by monitoring tannin concentrations in extracts at different intervals, allowing researchers to identify appropriate kinetic models, such as first- or second-order models. These results help in optimizing extraction conditions to obtain maximum results.

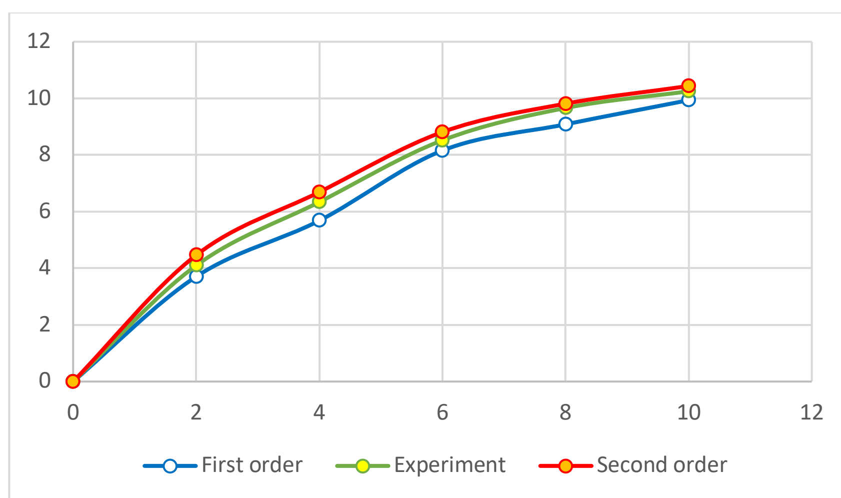
The results of Moringa leaf extract are greatly influenced by the operational conditions used in the MAE process. For example, increasing the extraction temperature usually increases yields, but too high a temperature can cause the degradation of tannin compounds. Likewise, excessively long extraction durations can reduce process efficiency due to thermal or oxidative degradation. Therefore, finding the optimal balance between temperature, time, and microwave power is a step in obtaining high-quality extract yields.




(a)




(b)




(c)

**Figure 3**. Reaction kinetics in the extraction of Moringa leaves tannin compounds using the MAE method: (a) Zero order model, (b) First order model, and (c) Comparison between extraction kinetic models

The results of this study follow research carried out previously using different materials [26]. The extraction process using 96% ethanol as a solvent is partly controlled by desorption kinetics because the desorption force from the matrix into the fluid is the concentration gradient from the soil to the extraction fluid, which can influence the kinetic rate of extraction, which creates two regions in the kinetic rate curve, namely the "fast region" ($k_1$) and the "slow region" ($k_2$). This curve agrees with the second-order kinetic model [27]. The extraction rate constants for order 1 and order 2 modeling can be seen in Table 3.

**Table 3.** Rate constants of 1st-order and 2nd-order extraction kinetic models

| Material | Kinetic Model | | | |
|---|---|---|---|---|
| | Order 1 | | Order 2 | |
| Moringa leaves (*Moringa oleifera* L. | Slopes | -0.0009 | Slopes | 0.088 |
| | $k_1$ | 0.0009 | $k_2$ | 0.088 |
| | Intercept | 0.59 | Intercept | 0.5602 |
| | $R^2$ | 0.9465 | h | 0.0059 |

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| Material | Kinetic Model | | | |
|---|---|---|---|---|
| | Order 1 | | Order 2 | |
| | | | $R^2$ | 0.9897 |

Based on Table 3, the kinetic model is in order 1 and 2. This table shows the value of the reaction rate constant and the value of Cs. The 2nd-order extraction kinetics model for the extraction of Moringa leaves with 96% ethanol solvent, and the MAE method has a coefficient of determination ($R^2$ = 0.9897), which is higher than the 1st-order extraction kinetics model ($R^2$ = 0, 9465). The $R^2$ value or coefficient of determination is a value that shows the level of conformity of the equation to the research conducted. The value of $R^2$ is getting closer to 1, meaning that the equation obtained is closer to the actual research results, so it can be said that modeling the kinetics of Moringa leaves extraction is more suitable using 2nd-order kinetics compared to 1st-order kinetics. The kinetic rate constant value of the 2nd order extraction model is 0.088 mL/g.min, and the Cs value in the 2nd order is 0.2591 mg/mL.

## 4. CONCLUSION

Moringa oleifera L. leaves using the MAE method have shown that the extraction process using this method is efficient and effective in optimizing extraction results. Identification of tannins using 1% $FeCl_3$ showed positive results by showing the presence of tannin content in Moringa leaves undergo a characteristic color change, namely, the sample changes color to blackish blue. The yield results show that the extraction time variable can influence the extract results. The lowest yield was obtained in the 2nd minute of extraction, namely 4.1%, and the highest yield was obtained in the 10th minute of the extraction process, 10.25%. The extraction kinetic model suitable for extracting tannin compounds from Moringa leaves is order 2, where the R2 0.9897 value is higher than order 1, and the value is almost close to 1.

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# The Effect of Time, pH, and Starter Concentration on Bioethanol Content in the Tobacco Stem Fermentation Process

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**Abstract.** The depletion of fossil fuels is occurring in various parts of the world while fuel energy needs continue to increase. This condition encourages the search for alternative fuels with high availability of raw materials. Bioethanol, an environmentally friendly renewable energy from biomass, can be a solution to replace fuel oil. Tobacco stems, with high cellulose and hemicellulose content, can be used as raw materials for bioethanol. This study aims to optimize bioethanol production from tobacco stems through the influence of fermentation time, pH, and starter concentration. The research method involves base pretreatment, hydrolysis, fermentation, and distillation, with the results analyzed using the Response Surface Methodology (RSM) approach and the Central Composite Design (CCD) model. The independent variables used during fermentation include fermentation time (72 – 168 hours), pH (4 – 5), and starter concentration (0.1% – 0.3% w/v). Based on the Analysis of Variance (ANOVA), the variables that significantly affect ethanol content are fermentation time and starter concentration. The results of the CCD analysis showed optimum conditions at a fermentation time of 120 hours, pH 4.5, and starter concentration of 0.2% (w/v), producing a bioethanol content of 23.007% (v/v). This study shows the potential of tobacco stems as a sustainable source of bioethanol.

**Keywords:** *bioethanol, fermentation, hydrolysis, tobacco stem, renewable energy*

## 1. Introduction

The need for fossil fuels in industry, transportation, and households increases yearly [1]. Fossil-based fuels are non-renewable fuels that can run out if used continuously [2]. Meanwhile, the demand for energy in various countries is growing. Even the Environmental Impact Assessment (EIA) estimates global energy demand will increase by 25% by 2050 [3]. Dependence on and depletion of fossil-based fuels has prompted researchers to look for

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alternative fuels with abundant availability [4]. Bioethanol is a renewable fuel that is adequate as a substitute for fossil fuels[5].

Bioethanol, with the chemical formula $C_2H_5O$, is a single-chain alcohol with an octane number of 108, does not evaporate quickly, has a low calorific value, and is flammable [6]. Bioethanol has advantages such as being biodegradable, non-toxic, has a higher oxygen content and octane number compared to other fuels, and is environmentally friendly because it can produce lower $CO_2$ gas [7]. Bioethanol has several categories that are differentiated based on the raw materials used for production, some of which are first-generation and second-generation [8]. First-generation bioethanol is a conventional biofuel made from raw materials from food crops such as barley, wheat, corn, and sugar cane [9]. First-generation bioethanol is unsuitable for commercial and large-scale production because raw materials are essential as Indonesia's main food commodity [10]. This is supported by the Republic of Indonesia Law No. 18 of 2012, which states that using food commodities as raw materials for energy sources is not permitted if it threatens food security [11]. Second-generation bioethanol comes from non-edible lignocellulosic biomass [12]. Second-generation bioethanol is appropriate for large-scale and commercial development because it comes from highly available biomass and does not compete with food needs [13].

One of the biomass that can potentially be used as raw material for bioethanol production is tobacco (*Nicotiana tabacum* L.) [14]. Tobacco biomass is abundantly available in Indonesia [15]. This is supported by data stating that in 2022, 96.6% of tobacco was produced through smallholder plantations in Indonesia, with a harvest of 225.7 thousand tons [16]. High tobacco production, not accompanied by optimal processing, can cause environmental pollution [17]. Lack of knowledge, innovation, and technology results in farmers not processing tobacco stalks but burning them directly or burying them in the environment [18]. Meanwhile, tobacco stems have a high cellulose and hemicellulose content, namely 56.10% and 22.44% so that they can be processed into products such as bioethanol [19].

Bioethanol is produced in three basic stages: pretreatment, hydrolysis, and fermentation [20]. Pretreatment is the initial step in breaking down lignocellulose to make it more accessible to polymers in biomass and reduce cellulose crystallinity, porosity, and surface area of the material [21]. Hydrolysis, as the second stage, aims to minimize cellulose and convert it into sugar to ferment it [22]. The primary sugars biomass hydrolysis produces are glucose and xylose [23]. The next stage, fermentation, is carried out to convert sugar from raw materials

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into ethanol [24]. Fermentation is the third step in the second-generation ethanol production process [25]. In fermentation, sugar, glucose, and xylose are metabolized by microorganisms in the previous stage, resulting in metabolism, namely ethanol [26]. The fermentation stage of bioethanol production generally uses the help of yeast [27].

Yeast or khamir is a fungus that consists of one cell, does not form hyphae, and is included in the Ascomycotina fungus group [28]. Bread yeast can be used in fermentation because it is stable, not dangerous or toxic, and easy to obtain and maintain [29]. One of the bread yeast species, namely *Saccharomyces cerevisiae*, is the right choice for bioethanol production fermentation because it can produce large amounts of alcohol and has a tolerance for high alcohol levels (12% - 18% abv) [30]. The characteristics of *Saccharomyces cerevisiae* are that it is facultatively anaerobic, grows well at a temperature of 30 ºC and pH 4.0-4.6, is resistant to high temperatures, and can tolerate a wide range of pH, so the process is less susceptible to infection [31]. In addition, this microbe has a very economical price and is easy to find compared to other types of yeast [32].

Several studies have been conducted on bioethanol from tobacco raw materials. Yuan et al. [33] produced tobacco-based biobutanol using the base and acid pretreatment method, enzymatic hydrolysis, fermentation with *Saccharomyces cerevisiae* to obtain an alkaline yield of 2.75 kg/10 kg of tobacco stem (72.7%), acid-catalyzed yield = 2.69 kg of ethanol/10 kg of tobacco stem (70.6%). Sophanodorn et al. [34] produced a tobacco-based bioethanol yield of 12.47 g/L using pretreatment and enzymatic hydrolysis, yeast culture, and fermentation using *Saccharomyces cerevisiae*. Saccharomyces cerevisiae's concentration affects the fermentation time length [35]. The longer the fermentation time, the higher the bioethanol content produced [36]. However, the bioethanol levels decrease when they reach the optimal point because the productivity of *Saccharomyces cerevisiae* decreases, and the nutrients start to run out [37]. The addition of yeast can affect the ethanol content. Guo et al. [38] conducted bioethanol production from tobacco with operating conditions of 5 grams of starter, 100 mL of 2% glucose solution, pH 4.8 sodium acetate buffer solution, obtained simultaneous saccharification and ethanol fermentation results of 106.6 mg/g ethanol, which increased by 138.0% compared to tobacco stems that were not given additional.

Based on this background, an innovation in making bioethanol with optimal levels and a cheap process is needed. Although research on making bioethanol has been conducted, there has never been a discussion on the effect of time, pH, and Starter concentration on bioethanol

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levels simultaneously. These three variables need to be discussed regarding optimal conditions so that the bioethanol levels obtained are promising [39]. Therefore, this study will discuss "The Effect of Time, pH, and Starter Concentration on Bioethanol Levels in the Tobacco Stem Fermentation Process," with a focus on the fermentation process to determine the effect of time, pH, and starter concentration on tobacco stem fermentation. By knowing the operating conditions of these three variables, this research method is expected to be more efficient and economical, and the bioethanol levels obtained are more optimal.

## 2. Research Methods

### 2.1 Materials

The materials used in this research included alkaline hydrolysate, sulfuric acid ($H_2SO_4$, analytical grade, obtained from Merck), baker's yeast (*Saccharomyces cerevisiae*, food grade, from Sigma-Aldrich), urea ($(NH_2)_2CO$, analytical grade, from Merck), distilled water, and filter paper (Whatman Grade 1, from Sigma-Aldrich). Sample preparation began with tobacco stems collected from PTPN 10, Ajung, Jember Regency, which were subsequently processed for use in bioethanol production.

### 2.2 Pretreatment

The sample is dried tobacco stems cut into small pieces (3-4 cm) and dried in the sun for 3 days, then oven-dried at a temperature of 130 – 190°C for 20 minutes with an interval of 10 minutes [40]. The dried stems are ground using a blender until they become a fine powder, then sieved using a 120 mesh sieve to reduce the size and increase the surface area.

### 2.3 Hydrolysis

The sample used in the fermentation process was a base hydrolysate produced through an initial pretreatment. This pretreatment involved treating the sample with 6% NaOH at a temperature of 140°C and an agitation speed of 150 rpm for 1 hour. Following this, the base hydrolysis process was conducted under optimal conditions: using 2% NaOH solution, microwave power of 350 watts, and a treatment time of 15 minutes. This process yielded a base hydrolysate with a 7.40 mg/mL reducing sugar concentration.

In the fermentation stage, 20 bottles of base hydrolysate samples were prepared according to variable data generated by the Design Expert 11 Software and the Response Surface Methodology (RSM) Central Composite Design (CCD) model. The pH of the hydrolysate was adjusted to the range of 4-5 using diluted H2SO4. Then, baker's yeast was

added as the starter at concentrations of 0.1%–0.3% (w/v) along with urea ($((NH_2)_2CO)$) at 2% of the hydrolysate volume to support fermentation.

2.4 Fermentation

Fermentation was carried out for 72–168 hours. The fermented hydrolysate sample is shown in Figure 2.1. After fermentation, the sample was distilled at 78°C to separate bioethanol. The results of the distillation process in the form of bioethanol were stored in vials for each experiment, as shown in Figure 2.2. The distillation results obtained were then analyzed for bioethanol content using an alcohol meter. After that, the bioethanol content was analyzed using an alcohol meter. In addition, to further characterize the obtained bioethanol, infrared spectroscopy was used to analyze its chemical composition.

**Figure 1.** Fermentation Hydrolysate Sample

**Figure 2.** Bioethanol From Tobacco Stems

2.5 Results Analysis

Analysis of the results was carried out with the stages of sample preparation, where pH was set according to the treatment, bread yeast was added, and mixing was performed. The fermentation process was carried out for 72 - 168 hours with starter concentrations varying from (0.1% to 0.3% w/v). Afterward, the distillation process was conducted to produce the distillate

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product, and the bioethanol content was analyzed. The results of this study were analyzed using the Response Surface Methodology (RSM) approach with the Central Composite Design (CCD) model. The Derringer method was applied to optimize the experimental results, determining the optimal conditions for fermentation time, pH, and starter concentration to maximize bioethanol content while reducing costs and experimental time.

2.6 Data Analysis

This study was conducted in 20 runs with variations in data obtained from the Design Expert 11 Software, adjusted to the Response Surface Methodology (RSM) approach of the Central Composite Design (CCD) model, as seen in Table 1.

**Table 1**. Fermentation Data Variation

| Standard Deviation | Run | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|---|
| | | pH | Time (hour) | Concentration (%) |
| 5 | 1 | 4.00 | 72.00 | 0.30 |
| 17 | 2 | 4.50 | 120.00 | 0.20 |
| 7 | 3 | 4.00 | 168.00 | 0.30 |
| 12 | 4 | 4.50 | 200.72 | 0.20 |
| 9 | 5 | 3.60 | 120.00 | 0.20 |
| 15 | 6 | 4.50 | 120.00 | 0.20 |
| 4 | 7 | 5.00 | 168.00 | 0.10 |
| 13 | 8 | 4.50 | 120.00 | 0.03 |
| 11 | 9 | 4.50 | 39.27 | 0.20 |
| 16 | 10 | 4.50 | 120.00 | 0.20 |
| 3 | 11 | 4.00 | 168.00 | 0.10 |
| 19 | 12 | 4.50 | 120.00 | 0.20 |
| 2 | 13 | 5.00 | 72.00 | 0.10 |
| 8 | 14 | 5.00 | 168.00 | 0.30 |
| 14 | 15 | 4.50 | 120.00 | 0.36 |
| 6 | 16 | 5.00 | 72.00 | 0.30 |
| 10 | 17 | 5.35 | 120 | 0.20 |
| 18 | 18 | 4.50 | 120 | 0.20 |
| 1 | 19 | 4.00 | 72 | 0.10 |
| 20 | 20 | 4.50 | 120 | 0.20 |

Actual Data Analysis of ethanol content from sample fermentation variation data will be calculated for accuracy using Mean Absolute Percentage Error (MAPE), which measures the prediction error percentage. The formula used can be seen in Equation 1:

$$MAPE = \sum_{t=1}^{n} \left| \frac{y - \hat{y}}{y} \right| \times 100\% \quad (1)$$

Each formula element has the description n as the amount of data, y as the actual result value, and ŷ as the predicted result value. MAE measures the average prediction error on the same scale as the observed variable. The lower the MAE value, the better the model's prediction performance [41].

## 3. Result and Discussion

### 3.1 Analysis of Variance (ANOVA) Bioethanol Content

Ethanol content test data were processed using ANOVA with Software Design Expert 11 software, following the Response Surface Methodology (RSM) approach of the Central Composite Design (CCD) model. This analysis aims to determine the effect of independent variables (pH, time, and concentration) on the dependent variable (ethanol content). Testing was carried out randomly on 3 independent and 1 dependent variable with 20 runs. The results of the ANOVA analysis are shown in Table 2.

**Table 2**. ANOVA Results of Ethanol Content

| *Source* | *Sum of Squares* | *df* | *Mean Square* | *F-value* | *P-value* | |
|---|---|---|---|---|---|---|
| ***Model*** | 25.60 | 9 | 2.84 | 5.32 | 0.0076 | *significant* |
| A-pH | 0.0019 | 1 | 0.0019 | 0.0035 | 0.9542 | |
| B-Time | 13.08 | 1 | 13.08 | 24.48 | 0.0006 | |
| C-Concentration | 3.59 | 1 | 3.59 | 6.72 | 0.0269 | |
| AB | 0.1250 | 1 | 0.1250 | 0.2340 | 0.6390 | |
| AC | 0.0000 | 1 | 0.0000 | 0.0000 | 1.0000 | |
| BC | 3.13 | 1 | 3.13 | 5.85 | 0.0361 | |
| $A^2$ | 1.37 | 1 | 1.37 | 2.56 | 0.1404 | |
| $B^2$ | 4.74 | 1 | 4.74 | 8.87 | 0.0138 | |
| $C^2$ | 0.0268 | 1 | 0.0268 | 0.0502 | 0.8272 | |
| ***Residual*** | 5.34 | 10 | 0.5342 | | | |
| *Lack of Fit* | 4.34 | 5 | 0.8684 | 4.34 | 0.0665 | *not significant* |
| *Pure Error* | 1.0000 | 5 | 0.2000 | | | |
| ***Cor Total*** | 30.94 | 19 | | | | |

ANOVA data in Table 2 shows a Lack of Fit value of 0.0665. This value is more significant than α, and hypothesis H1 is accepted, indicating the suitability of the selected model. If the P-value in Lack of Fit is minor than α, the data is significant and suggests the model is unsuitable. Conversely, if the P-value is more significant than α, the data is insignificant, indicating the model is suitable [42]. After the results of the ANOVA test of ethanol content were obtained, the optimum response variable value was calculated using the Derringer method. This method determines the optimal conditions for the optimum value of ethanol content from the variables of time, pH, and starter concentration. The Response Surface Methodology (RSM) optimization in Table 3 shows the optimum value.

**Table 3**. Derringer's Desirability

| pH | Time | Starter Concentration | Etanol Content | Desirability |
|---|---|---|---|---|
| 4.5 | 120 | 0.2 | 23 | 1 |

Table 3 shows the Desirability (D) value reaching 1 at optimum conditions with pH 4,5, 120 hours, and a starter concentration of 0.2%, producing ethanol content of 23%. Next, the Cook's Distance Plot is carried out to determine the influence of data points in the least squares regression, check validation, and show the optimal experimental design space. The Cook's Distance vs Response Plot plot is shown in Figure 1 [43], and the results show that most values are between 0 and 1.

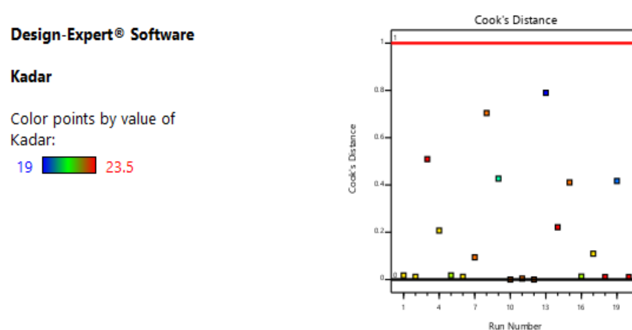


**Figure 3.** Cook's Distance Plot

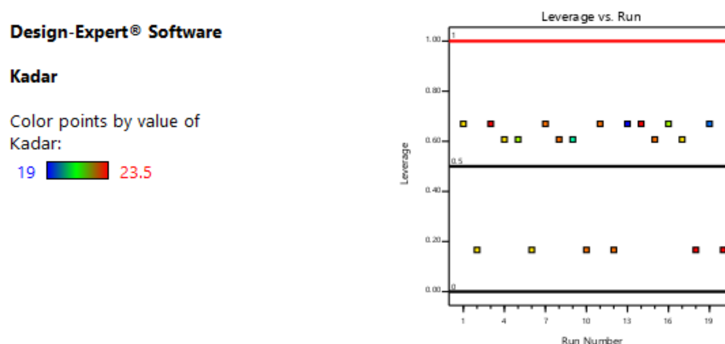


**Figure 4.** Leverage vs. Run Plot

The Leverage Test measures the influence of each point on model fit, with a leverage of 1.00 indicating a complete fit [43]. The average leverage is the number of terms in the model divided by the number of trials, shown in Figure 2. ANOVA analysis also presents the mathematical equations between the independent and dependent variables. Two equations from Software Design Expert 11, namely the coded and the actual equations, are used to predict the dependent variable [44]. The coded equation (equation 2) shows the relative impact of factors, while the actual equation (equation 3) is used to predict the response at a specific factor level.

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Equation 2 (Coded):

$$Y = 23,01 - 0,0117A + 0,9785B + 0,5126C + 0,1250AB + 0,0000AC - 0,6250BC - 0,3083A^2 - 0,5735B^2 - 0,0432C^2 \quad (2)$$

Equation 3 (Actual):

$$Y = -9,40335 + 10,45124A + 0,082728B + 22,47685C + 0,005208AB - 6,55977E\text{-}14AC - 0,130208BC - 1,23328A^2 - 0,000249B^2 - 4,31554C^2 \quad (3)$$

3.2 Effect of Variables on Ethanol Content

Three independent variables were used in fermentation: 72-168 hours, pH 4-5, and starter concentration 0.1%-0.3%. The actual data of ethanol content from running 1-20 are shown in Table 4.

**Table 4.** Actual Data on Ethanol Content

| Run | Time (hour) | pH | Starter Concentration (%) | Etanol Content (%) |
|---|---|---|---|---|
| 1 | 39.3 | 4.5 | 0.2 | 20.5 |
| 2 | 72 | 4 | 0.3 | 22.5 |
| 3 | 72 | 5 | 0.1 | 19.0 |
| 4 | 72 | 5 | 0.3 | 22.0 |
| 5 | 72 | 4 | 0.1 | 19.5 |
| 6 | 120 | 4.5 | 0.2 | 22.5 |
| 7 | 120 | 3.7 | 0.2 | 22.0 |
| 8 | 120 | 4.5 | 0.2 | 22.5 |
| 9 | 120 | 4.5 | 0.03 | 23.0 |
| 10 | 120 | 4.5 | 0.2 | 23.0 |
| 11 | 120 | 4.5 | 0.2 | 23.0 |
| 12 | 120 | 4.5 | 0.4 | 23.0 |
| 13 | 120 | 5.3 | 0.2 | 22.5 |
| 14 | 120 | 4.5 | 0.2 | 23.5 |
| 15 | 120 | 4.5 | 0.2 | 23.5 |
| 16 | 168 | 5 | 0.1 | 23.0 |
| 17 | 168 | 4 | 0.1 | 23.0 |
| 18 | 168 | 5 | 0.3 | 23.5 |
| 19 | 168 | 4 | 0.3 | 23.5 |
| 20 | 200.8 | 4.5 | 0.2 | 22.5 |

Table 4 shows that increasing fermentation time and starter concentration increases ethanol content. Ethanol content (%v/v) can be known after the distillation results are analyzed for bioethanol content using an alcohol meter. Then, a content test will be carried out by running 20 times. Then, the bioethanol content will be obtained, as shown in Table 4. At a fermentation time of 200,726 hours, ethanol content decreased because nutrient concentrations decreased and microbial growth slowed [45]. The optimal activity of *Saccharomyces cerevisiae* was achieved at the right fermentation time, but fermentation that was too long depleted nutrients

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and reduced the ethanol produced [46]. In ethanol fermentation, by-products such as lactic acid, acetic acid, and glycerol were produced, which could affect the final ethanol yield. The actual ethanol content data from Table 4 will be calculated for accuracy using the Mean Absolute Percentage Error (MAPE) using the formula in Equation 1. The results are obtained from the difference between the actual and predicted data divided by the actual data and expressed in absolute value so that the MAPE is always positive. The MAPE results that match the actual and predicted values from the RSM CCD method approach are shown in Table 5.

**Table 5**. Mean Absolute Percentage Error (MAPE) Results

| Run | Actual Value | Prediction Value | Error Value $\|(y - \hat{y})/y\|$ |
|---|---|---|---|
| **1** | 20.5 | 19.74 | 0.037073171 |
| **2** | 22.5 | 22.38 | 0.005333333 |
| **3** | 19 | 19.83 | 0.043684211 |
| **4** | 22 | 22.1 | 0.004545455 |
| **5** | 19.5 | 20.1 | 0.030769231 |
| **6** | 22.5 | 23.01 | 0.022666667 |
| **7** | 22 | 22.15 | 0.006818182 |
| **8** | 22.5 | 23.01 | 0.022666667 |
| **9** | 23 | 22.02 | 0.042608696 |
| **10** | 23 | 23.01 | 0.000434783 |
| **11** | 23 | 23.01 | 0.000434783 |
| **12** | 23 | 23.75 | 0.032608696 |
| **13** | 22.5 | 22.11 | 0.017333333 |
| **14** | 23.5 | 23.01 | 0.020851064 |
| **15** | 23.5 | 23.01 | 0.020851064 |
| **16** | 23 | 23.29 | 0.012608696 |
| **17** | 23 | 23.06 | 0.002608696 |
| **18** | 23.5 | 23.06 | 0.018723404 |
| **19** | 23.5 | 22.83 | 0.028510638 |
| **20** | 22.5 | 23.03 | 0.023555556 |
| | | **MAPE Value** | **0.06058458** |
| | | **MAPE Value (%)** | **6%** |

Table 5 shows that the MAPE value in this study is 6%. The calculation of MAPE involves subtracting the actual and predicted values, the absolute value of the difference, then dividing it by the actual value for each run and summing the results. The lower the MAPE value, the better the ability of the prediction model, with MAPE having a range of values to measure the accuracy of the prediction model [47].

### 3.3 3D Modeling Visualization

#### 3.3.1 Effect of Time and pH on Bioethanol Content

Figure 3 shows the three-dimensional Response Surface curve for time and pH factors on bioethanol content, with five colored regions indicating bioethanol content. The deeper red color indicates higher bioethanol content. The contour plot's circular line shows the bioethanol

content's quadratic response value, indicating optimal conditions. Although pH has an interaction with time, the interaction is not significant on bioethanol content (P = 0.6390 > α = 0.05), as seen in Table 1, where the effect of time on bioethanol content is significant (P = 0.0006). Still, the effect of pH is not (P = 0.9542).

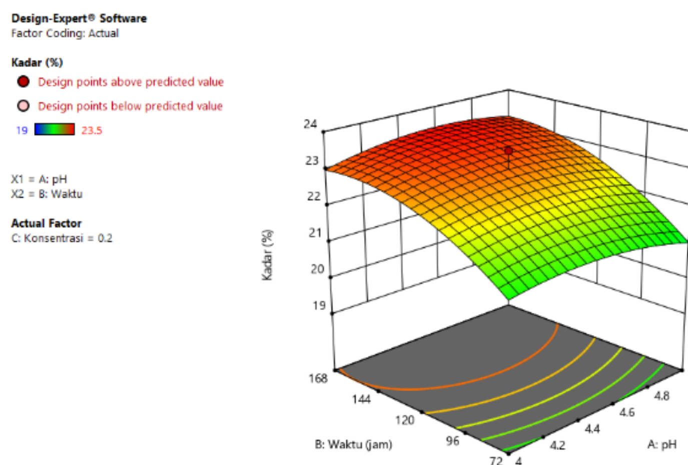


**Figure 3.** Effect Curve of Time and pH on Bioethanol Content

Supportive environmental conditions, including pH, influence high bioethanol levels. *Saccharomyces cerevisiae* grows optimally at pH 4-6, where pH below 3 reduces the speed of alcohol fermentation [48]. At a pH of 4.0-4.5, fermentation and growth of baker's yeast are optimal, while pH below 4.0 produces low ethanol levels. In addition, the length of fermentation time also affects the bioethanol content. Longer fermentation times increase ethanol levels, but after a specific time, nutrients are depleted, and the growth of microorganisms reaches the stationary phase, reducing ethanol production [49]. For example, at a fermentation time of 120 hours with a pH of 4.5, the ethanol content reaches 23.5%. Still, at a fermentation time of 200.726 hours, the ethanol content drops to 22.5% due to lack of nutrients and cessation of microorganism growth.

### 3.3.2 The Effect of Starter Concentration and pH on Bioethanol Content

Figure 4 shows the three-dimensional Response Surface curve for starter concentration and pH factors on bioethanol content, with five colored regions depicting bioethanol content. The deeper red color indicates higher bioethanol content. The contour plot's circular line shows the bioethanol content's quadratic response value, indicating optimal conditions. Although pH interacts with starter concentration, the interaction is insignificant on bioethanol content (P =

1.0000 > α = 0.05), as seen in Table 1. The effect of starter concentration on bioethanol content is significant (P = 0.0269), while pH's effect is insignificant (P = 0.9542).

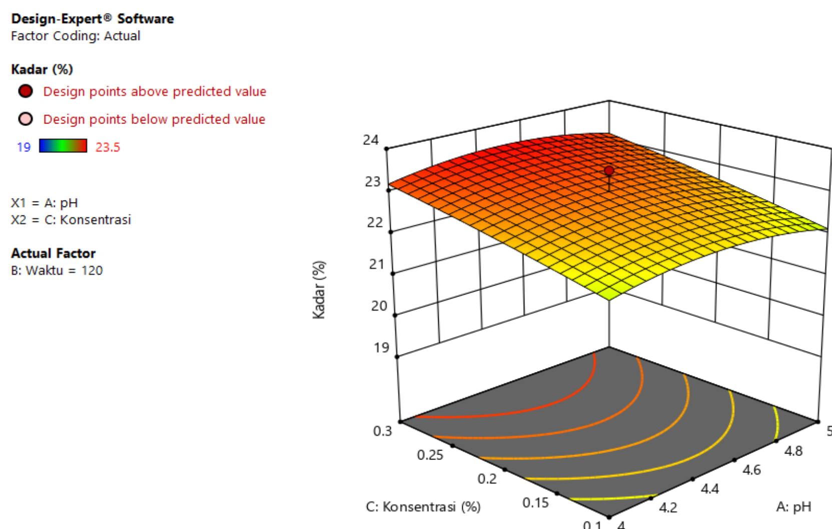


**Figure 4.** Effect Curve of Starter Concentration and pH on Bioethanol Content

Table 4 shows that the bioethanol content with pH 4 and a starter concentration of 0.1% at a fermentation time of 72 hours produces 19.5%, while pH 4.5 with a starter concentration of 0.368179% and a fermentation time of 120 hours produces 23%. Increasing the starter concentration accelerates fermentation due to the higher substrate, but pH 5.3409 with a starter concentration of 0.2% only produces 22.5% bioethanol because *Saccharomyces cerevisiae* is not optimal at that pH. At pH 5, glucose tends to be low, resulting in yeast being unable to decompose into ethanol and producing by-products such as acetic acid [45].

## 3.3. The Effect of Starter Concentration and Time on Bioethanol Content

Figure 5 shows the three-dimensional Response Surface curve for starter concentration and fermentation time factors on bioethanol content, with five colored regions depicting bioethanol content. The deeper red color indicates higher bioethanol content. The contour plot's circular line shows the bioethanol content's quadratic response value, indicating optimal conditions. The effect of fermentation time on bioethanol content was significant (P = 0.0006) and starter concentration (P = 0.0269), as seen in Table 1. There was a significant interaction between fermentation time and starter concentration on bioethanol content (P = 0.0361 <α = 0.05), which was observed in Figure 5.

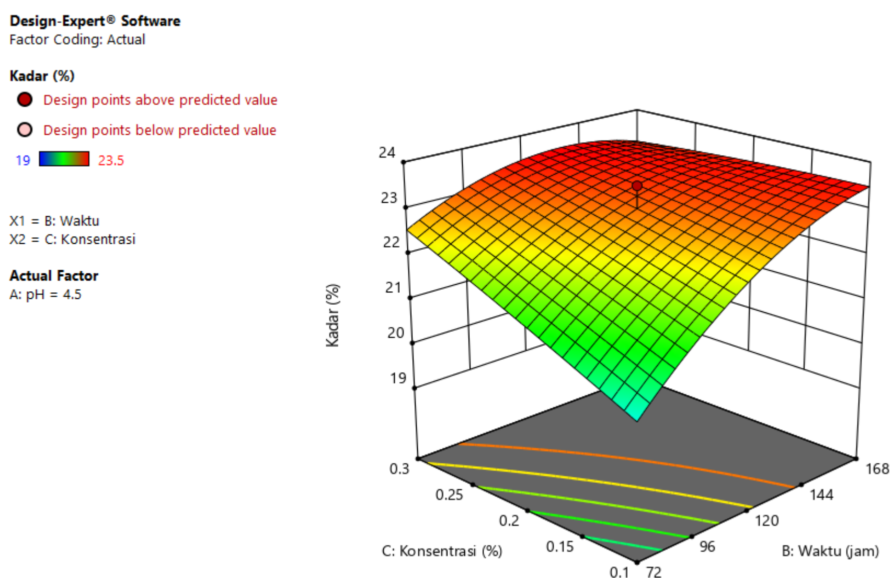


**Figure 5.** Effect Curve of Starter Concentration and Time on Bioethanol Content

The data in Table 4 shows that the longer the fermentation time and the higher the starter concentration, the higher the ethanol content. Increasing the starter concentration accelerates fermentation and increases the straightforward sugar content, allowing baker's yeast (*Saccharomyces cerevisiae*) to work optimally in producing ethanol [50]. However, the fermentation time has a maximum limit of 120 hours, after which the yeast activity decreases, especially at pH 5, where fermentation reaches the death phase. The decrease in ethanol levels is also caused by the depletion of the substrate and the reaction of changing to acetic acid. [49]. This study used a separate fermentation method than SSF because it used the results of alkaline pretreatment. Previous studies showed differences, where the highest ethanol content occurred in fermentation for 168 hours, but this study found the optimum at 120 hours. The results also showed that the optimal pH for *Saccharomyces cerevisiae* was 4.5, and the best starter concentration was 0.2%. Although there were differences with previous studies, this study recorded higher ethanol levels.

3.4 Parity Plot Analysis

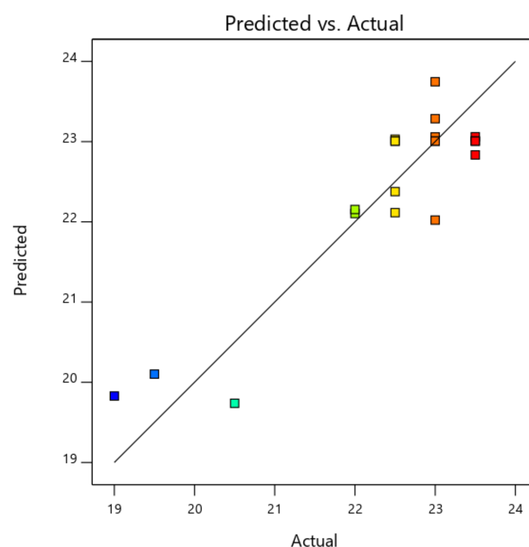


**Figure 6.** Predicted vs Actual Graph of Ethanol Levels

The straight lines on the graph are the predicted data, while the dots represent the actual data from each run. In Figure 6, the expected and actual ethanol content graphs show fairly good similarity with moderate scattering, indicating a significant approximation in the model analysis.

## 4. Conclusions

Based on this study, bioethanol content is mainly influenced by fermentation time and starter concentration, with optimal conditions at 120 hours of fermentation time, pH 4.5, and starter concentration of 0.2%. Fermentation time and starter concentration significantly affect ethanol yield, with an interactive effect between the two variables. pH also plays a role, although its impact is more minor. This study shows the potential of tobacco stems as a sustainable and renewable source of bioethanol, with a maximum ethanol content of 23.007% under optimized conditions. This information is essential for optimizing bioethanol production.

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