The Chemical Compounds Analysis of Bio-oil and Char from Cocoa Pod Husks Waste Pyrolysis by GC-MS/FTIR and its Potential as Biofungicide

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Abstract. Cocoa pod husk (CPH) is a lignocellulosic waste that can be processed for producing chemical compounds. It can be used antifungal active substances which have the potential to become promising biofungicide. The purpose of this study was to obtain active material of bio-oil and char from CPH pyrolysis as an antifungal. This research include CPH pyrolysis, compounds analysis of bio-oil and char using Gas Chromatography-Mass Spectrometry/Fourier transform infrared (GC-MS/FTIR) further testing effectiveness as antifungal with the Total Plate Count (TPC) method. The spectrogram of GC-MS shows content of major chemical compounds of bio-oil are 2-methoxy-phenol; 2,3-dimethyl-pyridine; 3-methyl-1,2-cyclopentanedione; 2,6-dimethoxy- phenol; 2-butanol; 5-hydroxy-2,7-dimethyl-4-octanone; maltol; 3,4-dihydroxyaceto-phenone; 3-methyl-phenol; 2-methoxy-5-methylphenol; 2,3-dimethyl-cyclohexanol. The FTIR spectrum of char shows vibration at functional groups O-H, C≡C, C=O and CH3. The concentration of bio-oil 30% (v/v) and char effectively inhibit 100% fungus like control + (synthetic fungicide). Therefore, it is very promising to be applied as biofungicide.

Keywords: Antifungal, Bio-Oil, Char, Cocoa pod husk, Pyrolysis.

1 Introduction

Cocoa pod husk (CPH) are lignocellulosic waste and it is the main byproduct of the chocolate industry [1]. Lignocellulose materials are naturally designed composites that play crucial roles in the survival of plants [2]. Lignocellulosic materials are mainly composed of cellulose (35-50 %), hemicellulose (15-35 %), and lignin (10-35 %). The concentration of the mentioned components varies with plant types [3]. Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The long-chain cellulose polymers are linked together by hydrogen and Van Der Waals bonds, which cause the cellulose to be packed

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into microfibrils. Hemicelluloses and lignin cover the microfibrils. Cellulose in biomass is present in both crystalline and amorphous forms [4]. The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars [5]. Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack. Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol) [6]. The CPH biomass consists primarily of fibrous materials including 19.7-26.1% cellulose, 8.7-12.8% hemicellulose, 14-28% lignin and 6.0-12.6 % pectin so that it can be used as raw materials for the production of bio-oil and char by the pyrolysis method [7, 8].

The pyrolysis is a technology for thermal treatment of biomass to recover a new material and energy [9-11]. The pyrolysis process consists of two main sections, the furnace/reactor, which converts biomass to products volatile vapour bio-oil, non-condensable gas and char [12-17]), and a condensing system, which recovers the condensable gases as a liquid product. Bio-oil is a complex mixture of chemicals including acids, ketones, furans, phenols, hydro sugars and other oxygenates [18, 19].

The CPH could be made into a value-added material because they are rich in phenolics and are therefore a promising source of these compounds [20]. The CPH is a good source of phenolic compounds that can function as antioxidants and could be used as ingredients in functional food. Epidemiological studies indicate that phenolic compounds have the potential effect to prevent chronic diseases and also have anticarcinogenic, antiinflammatory, antimicrobial, antifungal and biofungicide [21-23]. Some researchers have tested the effectiveness of bio-oil from organic waste as a natural pesticide [24, 25]. The bio-oil and char from CPH pyrolysis can be used as biofungicides because they contain active compounds which are effective as inhibitors of fungal and bacterial growth.

The first step in cocoa cultivation in supporting the development of cocoa plants is by providing seeds that are suitable to be planted in the field so as to produce cocoa plants that are able to produce optimally [26]. Cocoa seed have properties that are not resistant to moisture, temperature and humidity. This causes the cocoa seed to be easily contaminated with fungi during the storage process [27]. Efforts to maintain seed quality can be done using seed storage media [28]. Storage media that can be used include using char which is able to maintain the ideal moisture content of the seed during storage. The use of char storage media acts as a buffer of moisture during storage, which is to provide water if the cocoa seed is lacking in water and otherwise absorbs water if the cocoa seed is excessive, so char can play a role in improving the quality of cocoa seed during the storage period.

2 Material and Methods

2.1 Materials

The cacao pod husks (CPH) were obtained from a cacao plantation in Lambandia, East Kolaka, Southeast Sulawesi Province, located in Eastern Indonesia. The CPH is dried under the sun for 6-7 days then cut them into pieces up to 2-3 cm in size.

2.2 Pyrolysis Method

Dried CPH samples (1000 g) was put into a pyrolysis reactor which was equipped with a series of condensers and thermocouples. Pyrolysis was carried out at 500 °C for ± 2-3 hours [9]

with a heating flow rate of 6 °C/minute. Pyrolysis was stopped after no more bio-oil dripped into the reservoir. The results of pyrolysis were bio-oil, tar and char. Bio-oil from pyrolysis was filtered using whatman filter paper coated with gauze and activated carbon to obtain clear bio-oil. Other pyrolysis product, namely char, was mashed up to 80 mesh in size for cocoa seed storage media.

2.3 The Chemical Compound Analysis of Bio-oil and Char by GC-MS/FTIR

The chemical compound analysis of bio-oil content was carried out using GC-MS instrument. Each of bio-oil sample, as much as 1 µL was injected into Thermo Scientific GC-MS Trace 1300 GC/ISQ with ionizing type EI (Electron Impact) 70 ev, injector temperature and detector 290°C, column temperature 70°C to 280°C, 30 m column length, 25 mm diameter in column, 5°C temperature rise per minute, 100 kPa helium carrier gas, flow rate of 60 ml/min. Compounds were identified by comparing retention times to well-characterised materials. The analysis of char using FTIR Thermo Scientific Nicolet iS 10 with beam splitter KBr/Ge mid-infrared and detector type Deuterated Tri-Glycine Sulfate (DTGS) to determine the functional group contained. The scanning range of the FTIR was set to 4000-650 cm^{-1} at 8 cm^{-1} resolution with OMNIC FTIR software.

2.4 The Effectiveness Test of Bio-oil and Char as Biopesticides

The supply of cocoa seed was prepared by removing the cocoa pod to take the middle part of the fruit. The cocoa beans were collected and cleaned up from the pulp using sawdust, then the skin of the beans were removed. Cocoa beans are washed thoroughly with water then immersed into bio-oil, synthetic fungicide (control+) and water (control-) for 10-15 minutes. Soaked cocoa beans are dried in an open room without sunlight then some are covered with char. Futhermore, they were stored for 12 days [29]. Observation of the antifungal effectiveness of bio-oil and char used the TPC method.

3 Results and Discussion

The CPH pyrolysis produces bio-oil, tar and char. It also obtained the gases which can not be condensed by cooling, so it could not be accommodated in the liquid reservoir. Most of these gases are trapped in the container while others apart from the reservoir through the conduit of smoke and escape into the atmosphere [10]. The rendemen of CPH pyrolysis was 35 ± 5% for bio-oil, 5 ± 1% for tar and 30 ± 2% for char. The CPH bio-oil has a density of 1.085 ± 0.003 g ml^{-1}. The volume of bio-oil produced is influenced by decomposition and depolymerization of cellulose during pyrolysis [30].

3.1 The Chemical Compound Content of Bio-oil and Char from CPH Pyrolysis

Figure 1 shows the analysis from GC/MS of CPH bio-oil, and the corresponding identified compounds are listed in Table 1. The compounds' content was determined by using standard NIST MS software. The percentage peak areas correspond to the relative yields of the products among all of the identified components.

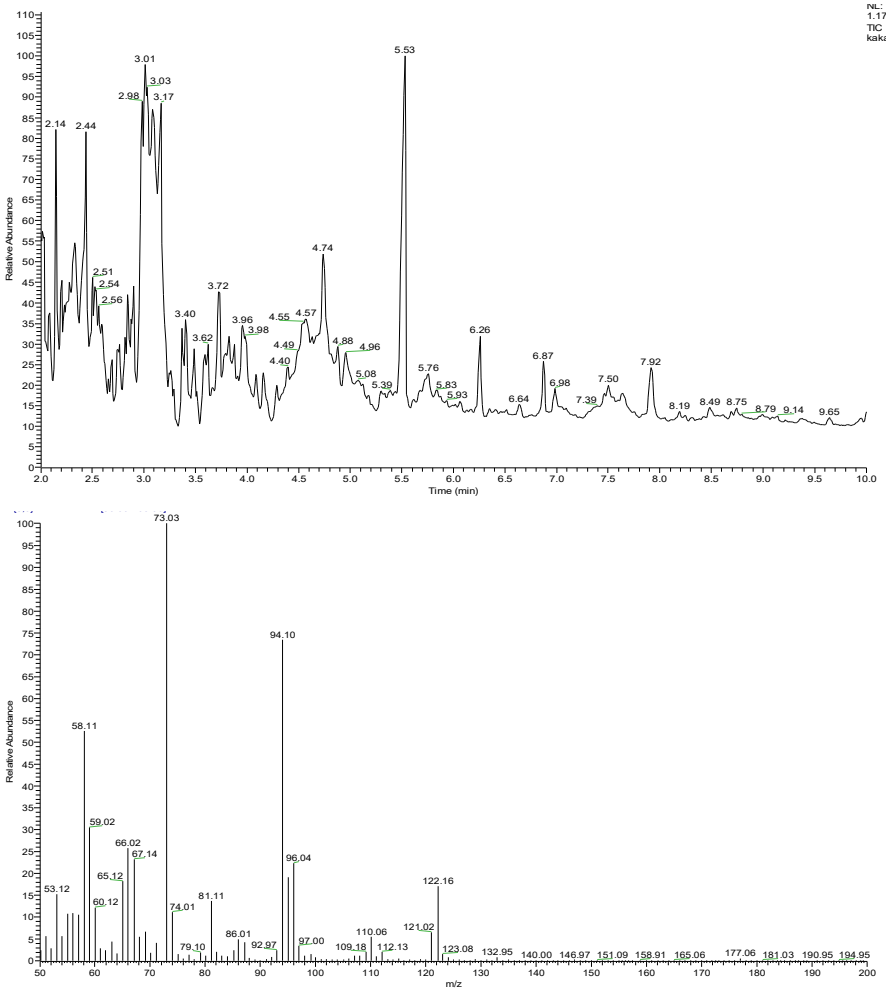

Fig. 1. GC-MS spechtrogram of CPH bio-oil

Table 1 . The chemical compound of CPH bio-oil

Retention time (minute)	Compound Name	Chemical formula	Area (%)	Molecular Weight
2.14	3-methyl-pyridine	C_6H_7N	2.08	93
2.33	2-Butanol	$C_4H_{10}O$	6.88	74
2.44	2,3-dimethyl-pyridine	C_7H_9N	9.29	107
3.01	2-methoxy-phenol	$C_7H_8O_2$	24.19	124
3.17	3-methyl-1,2-cyclopentanedione	$C_6H_8O_2$	8.04	112
3.40	3-methyl-phenol	C_7H_8O	3.86	108
3.62	1,2,3-trimethyldiaziridine	$C_4H_{10}N_2$	1.85	86
3.72	Maltol	$C_6H_6O_3$	6.15	126
3.96	2-methoxy-5-methylphenol	$C_8H_{10}O_2$	3.20	138

4.74	3,4-dihydroxyacetophenone	$C_8H_8O_3$	5.21	152
5.53	2,6-dimethoxy- phenol	$C_8H_{10}O_3$	7.18	154
5.76	2,3-dimethyl- cyclohexanol	$C_8H_{16}O$	2.63	128
6.26	Dehydroacetic Acid	$C_8H_8O_4$	1.08	168
7.92	4-(ethoxymethyl)-2-methoxy-phenol	$C_{10}H_{14}O_3$	1.20	182

Figure 1 and Table 1 show that CPH bio-oil predominantly contains phenolic compounds such as 2-methoxy-phenol (24.19%); 2,6-dimethoxy- phenol (7.18%); 3-methyl-phenol (3.86%); 2-methoxy-5-methylphenol (3.20%); 4-(ethoxymethyl)-2-methoxy-phenol (1.20%) and ketone i.e. 3,4-dihydroxyacetophenone (5.21%). Phenolic and ketone compounds have antifungal and antimicrobial effectiveness [24]. In addition, CPH bio-oil contains pyridine compounds, i.e. 2,5-dimethyl-pyridine (9.29%) and 3-methyl-pyridine (2.08%). Pyridine compound is an integral part of DNA and RNA that has pharmacological properties and effective as bactericide and fungicide [31].

In this study FTIR was used for the analysis of char from the result of CPH pyrolysis. This was intended to determine the functional group contained in char, which was used as a protective media for seed from fungal attack. The FTIR spectrum of CPH char was presented in Figure 2.

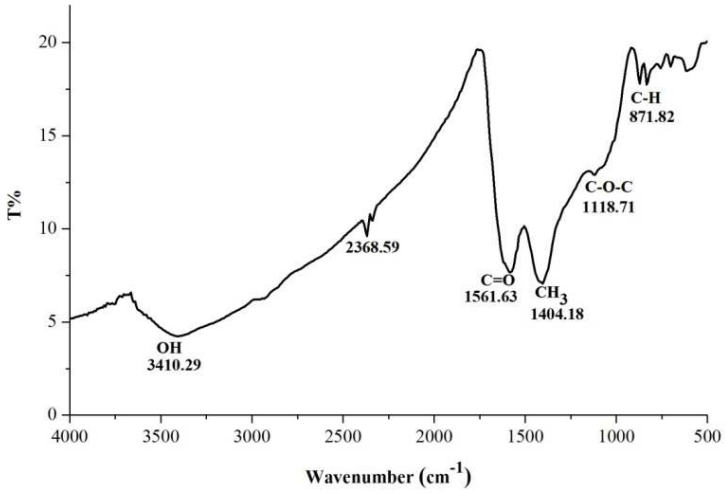

Fig. 2. The FTIR spectrum of CPH char

Tabel 2. The spectrum region of CPH char

No	Wavenumber (cm^{-1})	Functional group
1	3410.29	O-H
2	2368.59	C≡C
3	1561.63	C=C
4	1404.18	CH_3
5	1118.72	C-O-C
6	871.81	C-H

Adsorption in the infrared (IR) region takes place (4000–400 cm^{-1}) due to the rotational and vibrational movement of the molecular groups and chemical bond of a molecule [32]. The FTIR spectrum was obtained to evaluate qualitatively the chemical structures of CPH char. The FTIR

analysis of CPH char (**Figure 2**) shows the absorption of wavenumbers 3410.29 cm^{-1}, this indicates the presence of a hydroxyl functional group (O-H). The spectrum at wavenumber 2368.59 is identified as a functional group of C≡C. The region of the spectrum of 1561.63 cm^{-1} is attributed to deformation of aromatics groups C=C stretching, whereas the peak around 1404.18 cm^{-1} could correspond to the CH_3 deformation of the CPH char. The C-O-C stretching band is typical of the peak observed at 1118.72 cm^{-1} and the peak at 871.81 cm^{-1} could also be attributed to the C-H out-of-plane bending. The results of FTIR analysis reported by previous researchers showing functional groups contained in CPH char are hydroxyl O-H groups (phenol and alcohol), C=CH aromatic, CH and CH_2 aliphatic and other carbon atom compounds [33-35] which have potential as biofungicides and moisture buffer.

3.2 The Analysis of Characteristics and Effectiveness of Antifungals on Cocoa Seeds

Improving of the quality of cocoa seeds is influenced by several factors that can cause overall changes in seeds including physically, physiologically and chemically. Changes in seed quality can occur during storage periods and it is usually caused by fungal attacks, high water content and changes in pH [36].

3.2.1 The pH and Water Content Analysis of Cocoa Seeds

Efforts to improve the quality of cocoa seeds have several inhibiting factors, one of which is the acidity of the seed. If the pH of cocoa seeds is ≤ 5 or ≥ 6, the quality of seed sprouts will decrease. The results of the measurement of pH on cocoa seeds from each treatment during a storage period of 12 days with a measurement interval of 3 days ranged from 5-6. This result is in accordance with SNI 01-2323-2000 standards regarding cocoa seeds. The pH of the seed approaching the base is likely to result in absorption of nutrients and the activity of microorganisms running fast. While the low pH can affect microbial activity so that the mechanism of providing nutrients through the process of decomposition of organic matter on the soil will be difficult [37].

The quality of cocoa seeds is strongly influenced by the water content in the seeds, because the high and low water content of the seeds can cause damage or even death of cacao seeds [38]. Based on the results of the research on the water content of cocoa seeds in each treatment for 12 days the storage period, with an interval of 3 days the test time decreased i.e. on the early day the water content of 75% decreased to 27% on the 12th day. Based on the results of the study of water content until the 6th day in all treatments, it still met the quality standards of cocoa seeds while those on day 9 to day 12 were below the minimum threshold of seed provisions, namely 30-40% [39] (**Figure 3**).

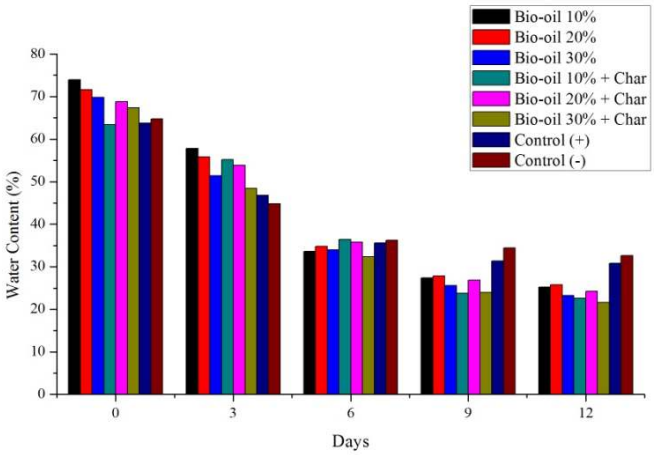

Fig. 3. The water content of cocoa seeds

3.2.2 The Antifungal Effectiveness of Bio-oil and Char on Cocoa Seeds

Bio-oil and char are media that are used as active ingredients to inhibit the growth of fungi and other microbes in seeds during the storage period [40]. The ability of bio-oil and char to inhibit the growth of mold on seeds during the storage period can be seen the TPC in Table 3.

Tabel 3. The TPC (CFU/g) on cocoa seeds after 2 days of incubation and 10 days of storage

Treatment	TPC (CFU/g)
Bio-oil 10%	37×10^2
Bio-oil 20%	7.5×10^2
Bio-oil 30%	-
Bio-oil 10% + char	18.5×10^2
Bio-oil 20% + char	1×10^2
Bio-oil 30% + char	-
Control (+)	-
Control (-)	72.5×10^2

Table 3 shows that cacao seeds treated with a bio-oil 10% concentration have a low power to inhibit fungal growth with TPC is 37×10^2 CFU/g and the addition of char covering, it can reduce fungal growth characterized by TPC 18.5×10^2 CFU/g. At bio-oil concentration 20% gives a greater inhibition of fungal growth compared to the 10% bio-oil with TPC 7.5×10^2 CFU/g and the addition of char covering causes a further increase of inhibitory ability characterized by the TPC 1×10^2 CFU/g. The 30% bio-oil concentration showed the greatest antimicrobial activity compared to other concentrations because it was able to kill fungi characterized by the absence of fungi growing on the media, the same effect that was shown by synthetic fungicides. The results of this study are in accordance with SNI 7388: 2009 stating that the cacao seeds given bio-oil and carbon-covered did not exceed the maximum limit of the number of fungal colonies on cacao seeds, which was equal to 1×10^4 CFU/g [41].

4 Conclusion

The GC-MS spectrogram of bio-oil from CPH pyrolysis shows the presence of chemical compounds: 2-methoxy-phenol; 2-methoxy-phenol; 2,6-dimethoxy- phenol; 3-methyl-phenol; 2-methoxy-5-methylphenol; 4-(ethoxymethyl)-2-methoxy-phenol; 3,4-dihydroxyaceto-phenone; 2,5-dimethyl-pyridine and 3-methyl-pyridine. Furthermore, the FTIR spectrum of char from CPH pyrolysis shows functional groups of the substances are O-H hydroxyl (phenol and alcohol), C=CH aromatic, CH and CH_2 aliphatic. The presence of phenolic, ketone, alcohol and pyridine compounds make bio-oil and char can be used as antifungals during preparation of cocoa seeds. Characteristics of cacao seeds during the storage period were: pH ranged from 5 to 6 for 12 days of storage period and water content of 30% -40% for 6 days of storage period. The ability of bio oil and char from CPH pyrolysis in inhibiting fungal growth in the preparation of cocoa seeds is comparable to the synthetic fungicides so that they can be applied as biofungicides.

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