International Journal of GEOMATE, July, 2022, Vol.23, Issue 95, pp.89-96
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2022.95.7522
Geotechnique, Construction Materials and Environment

ANTIBACTERIAL ACTIVITY OF LIQUID SMOKE POWDER FROM RICE HUSK

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ABSTRACT: The technological trend toward waste reduction has attracted the attention of researchers using rice husk as a value-added material. Rice husk, which mostly consists of lignocellulose, is a potential feedstock for generating liquid smoke powder, which can be used to preserve food. The present work investigates the antimicrobial activity of liquid smoke powder from rice husk on Gram-negative (*Salmonella choleraesuis* and *Escherichia coli)* and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)*. Liquid smoke was prepared through the pyrolysis of rice husk at 300°C, 350°C, and 400°C. After purification by distillation, the liquid smoke was prepared into liquid smoke powder using the spray-drying method. The Kirby–Bauer method was used to test antibiotic activity. Gas chromatography-mass spectrometry (GC–MS) analysis shows that rice husk liquid smoke contains various chemicals, such as acetic acid, 2-propanone, butanal, propanoic acid, 2-cyclopentene, and phenolic compounds. The liquid smoke powder inhibited the growth of all the tested bacteria, with various inhibition zones (6–9 mm). The findings of this study suggest that rice husk liquid smoke powder can be used as a natural alternative to synthetic food preservatives.

Keywords: Rice husks, Liquid smoke powder, Kirby–Bauer method, Antimicrobial activity, Inhibition zone

1. INTRODUCTION

The use of agricultural waste to produce value-added materials is a way to achieve a lifestyle based on the “go green” concept. Rice husk, a byproduct of the rice milling industry, is one of the agricultural wastes that interest researchers, as it can be processed into various types of products. Depending on the rice variety, the amount of rice husks produced from the rice milling industry ranges from 20%–to 33% [1]. Like other biomass, rice husk has a high content of organic constituents, with a total carbon content of 45.28% (w/w) [2]. Rice husk is organic waste that is difficult to compost because of its hard texture, strong material (i.e., not easily broken down), and high silica content. However, contents including cellulose, hemicellulose, and lignin in rice husk make this biomass a suitable raw material to produce liquid smoke. The pyrolysis of materials with high carbon content generates liquid smoke. Liquid smoke contains components derived from the thermal degradation of lignin, such as phenols that act as antioxidants, acids that act as antimicrobials, guaia,col and its derivatives, syringes and their derivatives, and alkyl aryl. Making liquid smoke from rice husks provides many benefits, particularly because it reduces the size of the husk waste pile. Liquid smoke from various biomasses, including palm kernel shell [3,4], durian husk [5,6], rice husk [7,8], bamboo [9], oil palm empty bunches [10,11], sawdust [12], and cacao bark [13], has been used as a natural preservative for various foodstuffs. Moreover, liquid smoke can also be used in agriculture for biological pest control, for example, to anticipate anthracnose in chilies [10]. With its various compositions, liquid smoke is also thought to have a positive effect on plants in terms of improving soil quality and neutralizing soil acidity, warding off pests and plant pathogens, and stimulating plant growth at the roots, stems, tubers, leaves, flowers, and fruit.

Liquid smoke is frequently used in its liquid condition, which is inconvenient, particularly in the distribution and shipping process, because it requires special containers and handling. Phenolic compounds in liquid smoke are also prone to damage (oxidation). Therefore, a method is needed to protect the active components in liquid smoke and facilitate its handling, such as by transforming liquid smoke into powder. Generally, smoke powders are made using the freeze-drying method with the addition of maltodextrin. Smoke powder also has antioxidant and antibacterial properties, just as liquid smoke does. To date, research and application of smoke powder to preserve food have not been extensively carried out, so the antibacterial properties of smoke powders from various liquid smoke raw materials have not been well described. This paper aims to study the antimicrobial activity of smoke powder derived from rice husk on three types of bacteria commonly found in food, namely, *S. choleraesuis*, *E. coli*, *S. aureus*, and *B. subtilis*.

2. METHODOLOGY

2.1 Liquid Smoke Preparation

A total of 2 kg of rice husk was placed in a pyrolysis reactor and then pyrolyzed at 300, 350, and 400°C to produce smoke, which was then condensed using a condenser to produce liquid smoke. The liquid smoke was purified in a distillation unit at 190°C so that it was free from tar and other toxic compounds. A schematic diagram for liquid smoke powder preparation is shown in Figure 1. The detailed procedure for making liquid smoke followed other studies [14-16].

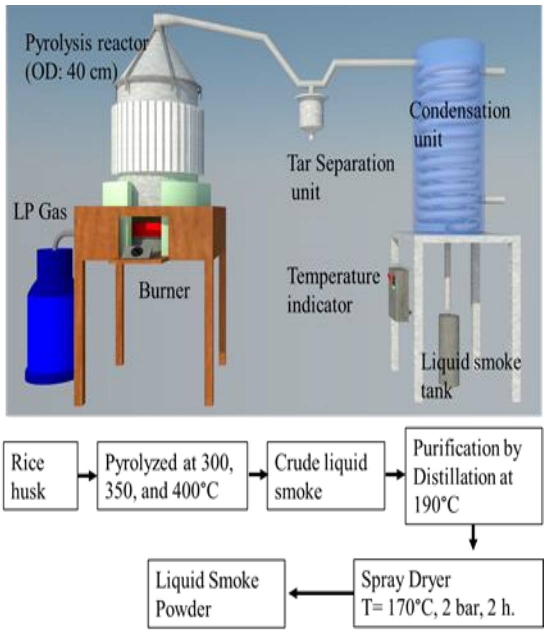

Fig. 1 Schematic diagram of liquid smoke powder preparation from rice husk

2.2 Preparation of Liquid Smoke Powder

The preparation of smoke powder from liquid smoke derived from rice husk was carried out using a spray dryer. A 1 liter aliquot of liquid smoke was added with maltodextrin (30% of the liquid smoke volume), then stirred thoroughly. After it was well blended, the mixture was placed in a spray dryer, and the powdering process was carried out at 170°C and 2 bar for 2 h.

3. RESULTS AND DISCUSSION

3.1 GC-MS Test

A GC–MS test determines the composition of liquid smoke at each pyrolysis temperature. Pyrolysis temperature plays an important role in the composition of the liquid smoke [17], which is one of the parameters for determining the quality of the liquid smoke produced. Liquid smoke has several components, including phenol, acetic acid, ethanol, cyclopentanone, and propionic acid [6]. Tables 1–3 present the various chemical compounds obtained from rice husk pyrolysis at 300°C–400°C.

Table 1 Rice husk liquid smoke chemical compounds (pyrolyzed at 300°C)

Retention time (min)	Component	Concentration (%)
9.193	Acetic acid (CAS) ethylic acid	40.50
9.622	2-Propanone, 1-hydroxy- (CAS) acetol	13.99
9.942	Butanal, 3-hydroxy- (CAS) 3-hydrocybutanal	1.23
10.616	Propanoic acid (CAS) propionic acid	1.44
12.017	2-Cyclopenten-1-one (CAS) cyclopentenone	0.64
14.109	3-Pentanone, 2-methyl- (CAS) 2-methyl-3-pentanone	1.12
14.476	phenol (CAS) izal	7.48
14.817	2-Buten-1-ol, 3-methyl- (CAS) prenol	4.17
15.618	Phenol, 2-methoxy- (CAS) guaiacol	8.52
16.026	Phenol, 2-methyl- (CAS) o-cresol	1.27
16.800	2-Methoxy-4-methyl phenol	2.78
17.092	Phenol, 3-ethyl- (CAS) m-ethylphenol	1.66
18.543	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) eugenol	6.66
18.718	trans-caryophyllene	7.29
28.875	Cyclohexanecarboxylic acid, 1-phenyl-, methyl ester (CAS)	1.25

Table 2 Rice husk liquid smoke chemical compounds (pyrolyzed at 350°C)

Retention time (min)	Component	Concentration (%)
8.305	Acetic acid (CAS) ethylic acid	40.54
8.708	2-hydroxymethyl-3-methyl-oxirane	4.43
8.927	Propanoic acid (CAS) propionic acid	17.29
9.475	N-nitroso-2-methyl-1,3-oxazolidine	2.02
9.922	3-Pentanone, 2-methyl- (CAS) 2-methyl-3-pentanone	1.96
12.609	Acetic acid, anhydride (CAS) acetic oxide	1.00
14.214	2-Butanone, 1-(acetyloxy)- (CAS) 1-acetoxy-2-butanone	0.63
14.587	2-Buten-1-ol, 3-methyl- (CAS) prenol	1.96
14.888	Phenol (CAS) izal	8.82
15.342	Ethane, 1,1,1	2.09
15.696	Phenol, 2-methoxy-(CAS) guaiacol	5.30
16.157	Phenol, 2-methyl-(CAS) o-cresol	3.47
16.950	2-Methoxy-4-methyl phenol	0.69
18.735	Phenol, 2-methoxy-4-(2-propenyl)-(CAS) eugenol	7.91

GC–MS analysis showed that after distillation, the composition of liquid smoke that had been degraded at a pyrolysis temperature of 300°C was dominated by acetic acid content, which was 40.50%. Likewise, the acetic acid content of the liquid smoked pyrolyzed at 350°C and 400°C was 41.54% and 43.87%, respectively. The acetic acid content tended to increase with the pyrolysis temperature. Acetic acid has antibacterial properties [18,19]. In a very low pH environment, acetic acid can cause enzyme denaturation and destabilize the cell membrane permeability of bacteria, thus inhibiting their growth and decreasing the viability of bacterial cells. In addition, acidic compounds also determine the taste, aroma, and product shelf life of smoked products [20,21]. The content of chemical compounds in rice husk after pyrolysis is similar to the results in a previous study on durian peel by Faisal et al. [6], where the composition of durian peel pyrolyzed at 300°C was dominated by an acetic acid content of 36.37%.

Table 3 Rice husk liquid smoke chemical compounds (pyrolyzed at 400°C)

Retention time (min)	Component	Concentration (%
5.500	Bicyclo [2.2.1] hepane,-5-(ethyl-1-amie)	0.98
8.125	Acetic acid (CAS) ethylic acid	43.87
12.677	2-Cyclopenten-1-one, 2-methyl- (CAS) 2-methyl-2-cyclopentenone	1.62
14.095	2-methylene-7-oxabicyclo[4.1.0]heptane	3.65
14.477	Phenol, 2-methyl- (CAS) o-cresol	1.27
14.792	5-Hydroxy-2-heptanone	7.42
15.320	phenol (CAS) izal	7.48
15.716	Phenol, 2-methoxy-(CAS) guaiacol	8.52
16.408	Bicyclo [4.1.0] heptane, 7-(1-methylethylidene)-(CAS) 7-isopropylenyl bic 11	1.11
16.811	Phenol, 3-ethyl- (CAS) m-ethylphenol	1.66
17.527	2,5-Dimethoxytoluene	1.95
18.362	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) eugenol	6.66
18.645	trans-caryophyllene	9.44
5.500	Bicyclo [2.2.1] heptane ,-5-(ethyl-1-amine)	0.98

Tables 1–3 also show that phenolic compound content also increases with the pyrolysis temperature. The phenolic content obtained at 300, 350, and 400°C was 21.13, 26.19, and 28.37%, respectively. Phenol is a pyrolysis product of lignin compounds [19,22]. Thus, the high phenol content in liquid smoke prepared from rice husk is due to the high content of lignin compounds in the rice husk. The identification results also show that phenol is the second-largest component in liquid smoke. The high phenolic content at a pyrolysis temperature of 400°C is probably due to the lignin decomposition process at 300°C–450°C, so the

higher the pyrolysis temperature, the higher the phenol concentration will be [23]. Phenols are the main antioxidants in liquid smoke [24,25]. The anti-oxidative role of liquid smoke is indicated by phenolic compounds with high boiling points, particularly 2,6-dimethoxyphenols: 2,6 dimethoxy-4-methylphenol and 2,6-dimethoxy-4-ethylphenol act as hydrogen donors to the free radicals and inhibit chain reactions. Liquid smoke can be used as a preservative since it has a pH of 2.8–3.1, which inhibits the growth of pathogenic microorganisms. The growth of rotting bacteria and pathogens such as *E. coli, B. subtilis, Pseudomonas,* and *Salmonella* is inhibited by liquid smoke [26,27].

3.2 Effect of Liquid Smoke Powder on Gram-positive Bacteria.

The antibacterial activity of liquid smoke powder is shown by the clear zones around a colony. Two Gram-positive bacteria (*S.aureus and B.subtilis*) have different sensitivities to the liquid smoke powder. The diameter inhibition zone in each treatment is shown in Tables 4–5 and Figure 2, with the antibacterial activity test results performed using the Kirby–Bauer method.

Table 4 Inhibition zone of *S.aureus* by liquid smoke powder.

Liquid smoke	Inhibition zone diameter (mm)	Control + (mm)
LP1	6.67 ± 0.03^{A}	16.81
LP2	7.84 ± 0.12^{B}	
LP3	9.09 ± 0.34^{C}	

Note: The different superscripts within a column show the actual difference with α = 0.05

LP1= Rice husk liquid smoke produced through pyrolysis at 300°C.
LP2= Rice husk liquid smoke produced through pyrolysis at 350°C
LP3= Rice husk liquid smoke produced through pyrolysis at 400°C

Table 5 Inhibition zone of *B. subtilis* by liquid smoke powder

Liquid smoke	Inhibition zone diameter (mm)	Control + (mm)
LP1	6.53 ± 0.0^{A}	24.84
LP2	6.58 ± 0.0^{A}	
LP3	6.69 ± 0.0^{A}	

Note: The different superscript letter within a column shows the actual difference with an α=0.05

Comparisons of the inhibition zone diameters and pyrolysis temperatures are presented in Tables 4–5 for Gram-positive bacteria. The antibacterial properties against *S.aureus* and *B.subtilis* were intermediate, with an inhibition zone diameter of 6.67–9.09 mm and 6.53–6.69 mm, respectively, for each treatment (LP1–LP3).

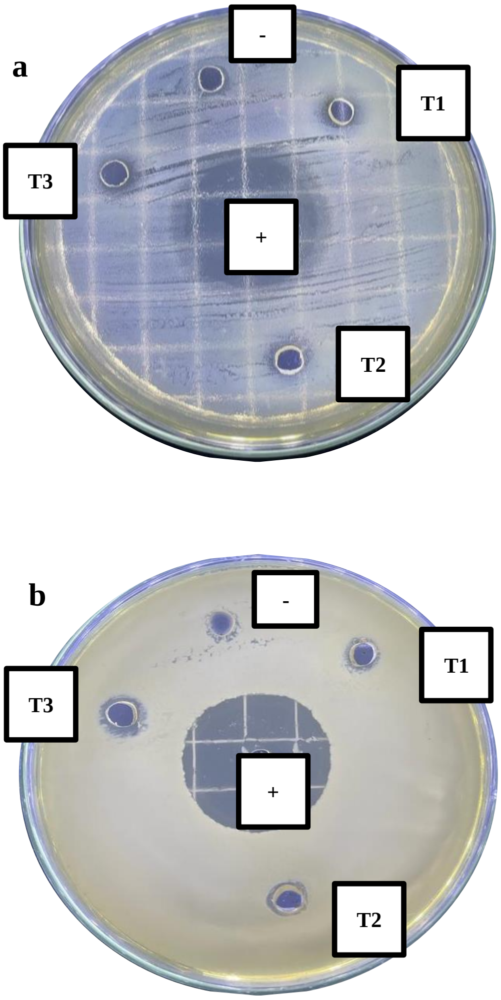

Figure 2. Inhibition zone of [a] *S.aureus* and [b] *B. subtilis* by liquid smoke powder.
Note : - = Negative control, + = Positive control, T1 = Liquid smoke powder from pyrolysis at 300°C; T2 = Liquid smoke powder from pyrolysis at 350°C and;
T3 = Liquid smoke powder from pyrolysis at 400°C

Sample LP3 was the most effective against bacteria. The pyrolysis temperature affected the composition of the liquid smoke and the liquid smoke powder, which, in turn, affected the antibacterial properties. The LSD test showed that the pyrolysis temperature was not significantly different for *B.subtilis*, although *S.aureus* was quite different. A previous study showed that liquid smoke from oil palm kernel shells inhibited

Streptococcus bacteria [4]. Liquid smoke from different biomass types has different compositions (such as phenol, acetic acid, and carbonyl contents) that influence an antibacterial agent. Previous research has reported that liquid smoke from durian peel waste [6], oil palm empty bunches [15], cacao pod shells [13], rice hulls [7,28], palm kernel shells [3], coconut shells [30], and corncob [31] can inhibit the growth of bacteria.

3.3 Effect of Liquid Smoke Powder on Gram-negative Bacteria

This study used two strains of Gram-negative bacteria, namely, *E.coli and Salmonella*. The Gram-negative bacteria cell wall is surrounded by two membrane bilayers [32-35]. The cell wall (approximately 5–10 nm) contains high contents of lipid, lipoprotein, and lipopolysaccharide and is less susceptible to anionic detergent [33]. Based on the inhibition zone diameter, the antibacterial power strengths were categorized as very weak (<5 mm), intermediate (5–10 mm), strong (10–20 mm), and very strong (20–30 mm) [36].

Table 6 Inhibition zone of *E.coli* by liquid smoke powder

Liquid smoke	Inhibition zone diameter (mm)	Control + (mm)
LP1	6.58 ± 0.03^{A}	18.10
LP2	6.99 ± 0.25^{A}	
LP3	7.58 ± 0.04^{B}	

Note: The different superscripts within a column show the actual difference with α = 0.05

Table 7 Inhibition zone of *Salmonella* by liquid smoke powder

Liquid smoke	Inhibition zone diameter (mm)	Control + (mm)
LP1	6.48 ± 0.0^{A}	27.58
LP2	6.65 ± 0.06^{A}	
LP3	7.36 ± 0.06^{B}	

Note: The different superscript letter within a column shows the actual difference with α=0.05

The distilled water (negative control) was tested on Gram-positive bacteria, and harmful bacteria did not inhibit the growth of bacteria (inhibition zone = 0 mm). Tables 6–7 and Figure 3 show that the inhibition zone diameter for the liquid smoke powder was 6.48 mm to 7.58 mm, and the antibacterial properties were intermediate. However, as the pyrolysis temperature increased (at LP3), the inhibition zone grew, which means increasing antibacterial ability. Antibacterial activity in Gram-positive and Gram-negative bacteria increases with the pyrolysis temperature. Furthermore, research by Desvita et al. [37] showed that liquid smoke from cacao pod shells can inhibit Gram-positive and Gram-negative bacteria, with an inhibition zone diameter of 6–7.36 mm.

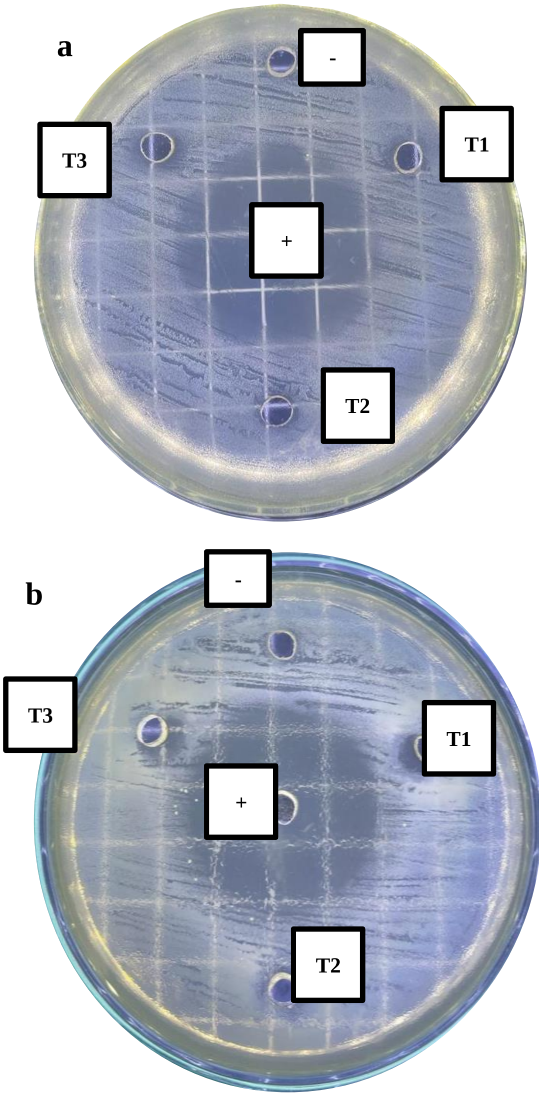

Figure 3. Inhibition zone of [a] *E.coli* and [b] *Salmonella* by liquid smoke powder.
Note : - = Negative control, + = Positive control, T1 = Liquid smoke powder from pyrolysis at 300°C; T2 = Liquid smoke powder from pyrolysis at 350°C and;
T3 = Liquid smoke powder from pyrolysis at 400°C

In a study by Desvita et al. [7], using 5% liquid smoke inhibited the growth of *Salmonella* and *E.coli*, with inhibition zones of 7.75 mm and 7.07 mm, respectively. Previous research by Martin et al. [38] found that commercial liquid smoke inhibited the growth of *L. monocytogenes*. Furthermore, research by Mansur et al. [39] found that liquid smoke from cajuput pyrolyzed at 500°C inhibited *P. aeruginosa, E. coli, S. typhimurium, B. subtilis, S. aureus,* and *L. monocytogenes.*

Statistical analysis was applied by using a one-way ANOVA test with α = 0.05 (significant differences) for each treatment. However, the LSD procedure showed that the largest diameter inhibition zone was obtained using LP3, with no significant difference between treatments LP1 and LP2. The different structures of the cell walls of each bacterium (Gram-positive and Gram-negative) may have affected the ability to inhibit bacterial growth.

4. CONCLUSIONS

Liquid smoke from rice husk contains various organic compounds, including acetic acid and carbonyl and phenolic compounds, which can potentially inhibit some common bacteria in food. The pyrolysis temperature affected the number of chemical components produced. Liquid smoke powder from rice husk was found to inhibit the growth of *S.aureus, B.subtilis, E.coli,* and *Salmonella* bacteria. The best results for inhibiting the growth of bacteria are achieved by liquid smoke produced at a pyrolysis temperature of 400ºC. The liquid smoke powder was most effective at inhibiting *S.aureus.*

5. ACKNOWLEDGEMENTS

We are grateful to the Universitas Syiah Kuala (Grant No. 166/UN11/ SPK/PNBP/ 2021) for financial support. We also thank Hera Desvita for technical assistance.

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