CHARACTERISTICS OF LIQUID SMOKE FROM YOUNG COCONUT SHELLS AT VARIOUS PYROLYSIS TEMPERATURES

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Abstract: Young coconut shells contain wood components, such as hemicellulose, cellulose, and lignin. These compounds can be used as raw materials for liquid smoke. The physical and biological characteristics of liquid smoke from young coconut shells pyrolysed at various temperatures were investigated in this study. Specifically, young coconut shells were pyrolysed at 300°C–420°C in a slow pyrolysis reactor. To eliminate tar, the liquid smoke was distilled at 190°C. Further, the chemical content of the liquid smoke was quantified using Gas Chromatography-Mass Spectrometry (GC-MS). Acetic acid and phenol compounds were identified using high performance liquid chromatography (HPLC) and ultraviolet-vis spectrophotometry. The gas chromatography-mass spectrometry (GC-MS) data revealed that liquid smoke contains over 15 chemical components, including phenolic acid, carboxylic acid and its derivatives. Antibacterial, minimum inhibitory concentration (MIC) and minimum killing concentration (MKC) tests were performed to analyse the antimicrobial properties of liquid smoke in inhibiting the growth of *Escherichia coli* and *Salmonella enterica* sv Typhimurium. The pyrolysis temperatures affected the composition of the produced liquid smoke. The highest phenol and acetic acid content were found at 340°C and 380°C, where lignin is degraded into phenolic compounds and cellulose is degraded to produce acetic acid. The results of the antibacterial test showed that the maximum inhibition zone was obtained at 420°C, and at 340°C and 380°C the bacteria were inhibited and died.

Keywords: Young Coconut Shell; Pyrolysis; Liquid Smoke

Abstrak: Tempurung kelapa muda mengandung komponen kayu seperti hemiselulosa, selulosa, dan lignin. Senyawa ini dapat digunakan sebagai bahan baku asap cair. Karakteristik fisik dan biologis asap cair dari tempurung kelapa muda yang dipirolisis pada berbagai suhu diselidiki dalam penelitian ini. Tempurung kelapa muda dipirolisis pada suhu 300 °C - 420 °C dalam reaktor pirolisis lambat. Untuk menghilangkan tar asap cair didistilasi pada suhu 190 °C. Selanjutnya, kandungan senyawa kimia asap cair dikuantifikasi menggunakan *gas chromatography-mass spectrometry* (GC-MS). Senyawa asam asetat dan fenol diidentifikasi menggunakan *high performance liquid chromatography* (HPLC) dan Spektrofotometri UV-Vis. Data *gas chromatography-mass spectrometry* (GC-MS) menunjukkan bahwa asap cair mengandung lebih dari 15 komponen kimia, termasuk asam fenolik, asam karboksilat dan turunannya. Uji

antibakteri, *Minimum Inhibitory Concentrantion* (MIC) dan *Minimum Killing Concentrantion* (MKC) dilakukan untuk menganalisis sifat antimikroba asap cair dalam menghambat pertumbuhan *Escherichia coli* dan *Salmonella enterica* sv Typhimurium. Temperatur pirolisis mempengaruhi komposisi asap cair yang dihasilkan. Kandungan fenol dan asam asetat tertinggi ditemukan pada suhu 340°C dan 380°C, dimana pada suhu tersebut lignin terdegradasi menjadi senyawa fenol dan selulosa terdegradasi menghasilkan asam asetat. Hasil uji antibakteri menunjukkan bahwa zona hambat maksimum diperoleh pada suhu 420 °C, pada suhu 340 °C dan 380 °C bakteri ditemukan terhambat dan mati.

Kata kunci: Tempurung Kelapa Muda; Pirolisis; Asap Cair

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Introduction

Due to its tropical climate, Indonesia is one of the largest coconut producers in the world. In 2018, coconut trees covered 3.4 million hectares in Indonesia (Direktorat Jenderal Perkebunan, 2020). In general, people use young coconuts for consumption, both their meat and water, and roadside stalls selling young coconuts are common. Unlike coconut shells, which have been utilised to make various types of products such as handicrafts and activated charcoal due to their solidity, young coconut shell waste has not been optimally utilised. However, failure to manage young coconut shell waste properly can cause environmental problems. Because young coconut shell waste contains high levels of lignin (33.30%) and cellulose (30.58%), it can be used as a raw material for liquid smoke production. Liquid smoke, which is generally obtained from the pyrolysis of wood biomass (Nunes et al., 2020), has been used as a preservative in various foodstuffs, including rabbit meat (Sasongko et al., 2014), fish balls (Faisal & Gani, 2018), tofu (Ginayati et al., 2015) and mackerel (Faisal, Gani, et al., 2018).

Liquid smoke contains organic components, such as phenol and acetic acid, which have antimicrobial and antioxidant properties. These components can inhibit the activity of spoilage microbes in food, thus extending the shelf life of food products (Desvita et al., 2020). Several studies have shown that liquid smoke can be produced from various kinds of biomass waste, such as palm oil shells (Faisal, et al., 2018), sawdust (Suryani et al., 2022), Mabang wood (*Shorea pachyphylla*) (Oramahi et al., 2022) and durian husks (Faisal et al., 2020; Faisal et al., 2018). However, the use of young coconuts as a raw material for making liquid smoke has not been explored, especially at different pyrolysis temperatures. Desvita et al. (2021) found that there was an effect of pyrolysis temperature on the composition of chemical compounds in liquid smoke, and pyrolysis at 300, 340, 380 and 400°C resulted in 9, 14, 11 and 18 compounds, including phenols, ketones, furans and esters. This study aims to characterise the components of

liquid smoke from young coconut shells pyrolysed at various temperatures.

Material and Instrument

The materials used in this research were young coconut shells, distilled water, plate count agar (Merck KGaA, ISO 4833, ISO 17410 and FDA-BAM), peptone water, 0.25% ciprofloxacin, *Escherichia coli* and *Salmonella enterica* sv *Typhimurium* (bacterial culture obtained from a fundamental science laboratory, Aceh Besar).

The instruments used in this research were a slow pyrolysis reactor unit (40 cm diameter, 60 cm height and 5 kg capacity) equipped with a condenser unit, a tar trap unit and a temperature controller, a distillation unit, gas chromatography-mass spectrometry (GC-MS) (Shimadzu GCMS-QP2010 Ultra, Agilent column Type 19091S-433: 93.92873 DB-5MS UI), UV-Vis Spectrometry, and high performance liquid chromatography (HPLC) (Hitachi L-4200H).

Methods

Young coconut shell pretreatment

Young coconut shells were dried in sunlight for five days to remove the moisture content. To check the amount of moisture content in the coconut shell, a shell sample was baked at 105°C for about three hours in an oven, and the moisture content was calculated according to SNI 06-3730-1995. The dried coconut shells were crushed into smaller pieces prior to pyrolysis.

Pyrolysis

Three kilograms of dried young coconut shells were pyrolysed using a slow pyrolysis reactor. Young coconut shells were pyrolysed at 300°C (A1), 340°C (A2), 380°C (A3) and 420°C (A4) and condensed using a condenser to produce liquid smoke, tar and char (Desvita et al., 2020). Depending on the temperature, the pyrolysis process lasted approximately 2–3 hours.

Liquid smoke distillation

The liquid smoke produced from the pyrolysis was purified again to separate the liquid smoke from the tar (Desvita et al., 2021). The temperature commonly used in the distillation process is 180–200°C. In this study, a temperature of 190°C was used.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Liquid smoke component analysis was carried out using gas chromatography-mass spectrometry (GC-MS) (Shimadzu GCMS-QP2010 Ultra, Agilent column Type 19091S-433: 93.92873 DB-5MS UI. This instrument can be used to identify chemical compounds such as acetic acid, phenol and their derivatives and non-carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) contained in liquid smoke.

Analysis of phenol and acetic acid

A UV-vis spectrometry test was conducted to determine the concentration of phenolic compounds in the samples of liquid smoke from young coconut shells. This test was carried out with the total phenol equivalent of gallic acid as the test parameter. Meanwhile, High performance liquid chromatography (HPLC) (Hitachi L-4200H) was used to analyse the concentration of acetic acid compounds (as a percent). High performance liquid chromatography (HPLC) analysis was carried out based on the separation of a compound into two phases: (1) a stationary phase in the form of a column and (2) a mobile phase in the form of an organic solvent. The separation held in the column was then quantified by comparing the sample response to the standard response, whose concentration is known.

Antibacterial Test

An antibacterial activity test was carried out using the disc diffusion method (Kirby–Bauer test). A sterile inoculation loop was inserted into a test tube containing a bacterial suspension and then smeared onto an agar (NA) medium. After the spread of dry bacteria, the paper disks that had been soaked in liquid smoke were drained and placed on a medium that contained bacteria with slight pressure so that the paper disks stuck to the media surface. It was then incubated at 37°C for 24 hours. Antibacterial activity is said to be positive if inhibition or a clear zone appears around the paper disk. For the positive control, 0.25% ciprofloxacin was used, while distilled water was used for the negative control.

Minimum Inhibiton Concentration and Minimum Killing Concentration Test

The minimum inhibiton concentration (MIC) and minimum killing concentration (MKC) tests were carried out using the stratified dilution series method and The minimum inhibiton concentration (MIC) and minimum killing concentration (MKC) results were obtained from the total plate count (TPC). The bacterial cultures used were *Escherichia coli* and *Salmonella enterica* sv *Typhimurium*. The 0.25% Ciprofloxacin was used as positive control while distilled water was for negative control.

Results and discussion

Results of Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Identification of the chemical compounds in liquid smoke from young coconut shells pyrolysed at different temperatures was conducted using gas chromatography-mass spectrometry (GC-MS). The results revealed different components in each liquid smoke sample at various pyrolysis temperatures The degradation of lignin, hemicellulose and cellulose in the pyrolysis of young

coconut shells produced chemical compounds from the phenol group, carboxylic acids, sterol compounds and furans. Phenyl propane units contained in lignin form chains with crosslinks in an amorphous three-dimensional structure to bind cellulose and hemicellulose. An increase in the pyrolysis temperature results in the bond being broken, forming free radicals that re-polymerise and create side chains to produce new compounds (Kawamoto, 2017).

According to Börcsök and Pásztory (2021), lignin is a component of wood that is so stable thermally that it is hard to degrade at low temperatures and decomposes in a high temperature range of 200–500°C, whereas cellulose starts to decompose in the range of 200–250°C. In the initial stage, hemicellulose degraded through acid hydrolysis and dehydration, which started at temperatures above 100°C, but the weight loss of hemicellulose occurred in the temperature range of 220–315°C. In this temperature range, mono-oligosaccharides turned into furfural and their derivatives. Increasing the temperature caused the phenyl propane unit to decrease, in turn causing furfural and its derivatives resulting from hemicellulose degradation to react with lignin to form new lignin carbohydrates, such as acetic acid and aldehydes. Cellulose degradation produced carbonic acid and ketone compounds. At higher temperatures, lignin degraded and produced phenolic compounds and phenolic ethers and their derivatives (Faisal et al., 2020). This is confirmed by the gas chromatography-mass spectrometry (GC-MS) (% area) results of the young coconut shell-derived liquid smoke, where different pyrolysis temperatures produced different compounds (see Tables 1 and 2). In sample A1, the main components contained in liquid smoke included gamma-sitosterol (32.5%), phenol (26.95%), methylene chloride, 2H-3,9a-methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl- and acetic acid (2.46%) (Figure 2). In sample A2, the phenol content increased (45.36%), and phenol derivatives such as phenol, 2-methoxy-3-(2-propenyl)- (CAS) eugenol, phenol, 2-methoxy, phenol, 4-ethyl-2-methoxy and furfural (18.35%), 4,4-dimethyl-2-cyclopenten-1-one (CAS) ethanone, 1-(2-furanyl)-, acetic acid, phenyl ester (4.07%), acetic acid (CAS) 1,8-nonadien-3-ol (1.31%), carbonic acid (0.81%) and 2-furancarboxaldehyde, 5-methyl (0.96%) were identified. In sample A3, the percentage of acetic acid decreased (3.35%), while phenol continued to increase (53.5%). In addition, compounds such as tetracosamethyl-cyclododecasiloxane (9.23%), methylene chloride (12.26%), thymol, TMS derivative (7.78%), 2,4-cyclohexadien-1-one,3,5-bis(1,1-dimethylethyl)-4-hydroxy (3.7%), 7,9-di-tert butyloxaspiro (4,5) deca-6,9-diene-2,8-dione (3.17%), N-ethyldiethanolamine, 2TMS derivative (2.47%), silane, methylvinyl (2-methylpent-3yloxy) (methylvinyltridecyloxysilyloxy) (1.15%) and cyclononasiloxane, octadecamethyl (0.96%) were also formed. The main components of the liquid acid in sample A4 were phenols (42.37%) and their derivatives, such as phenol, 2-methoxy (7.35%), phenol, 2-methoxy- (7.35%) and other components in smaller amounts (see Table 2 and Figure 2).

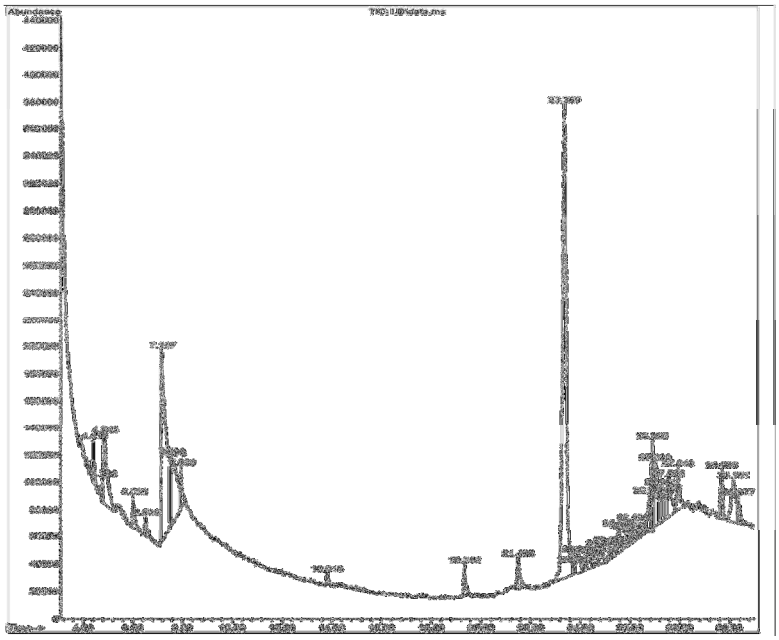

Figure 1. Liquid smoke chromatogram results on sample A1

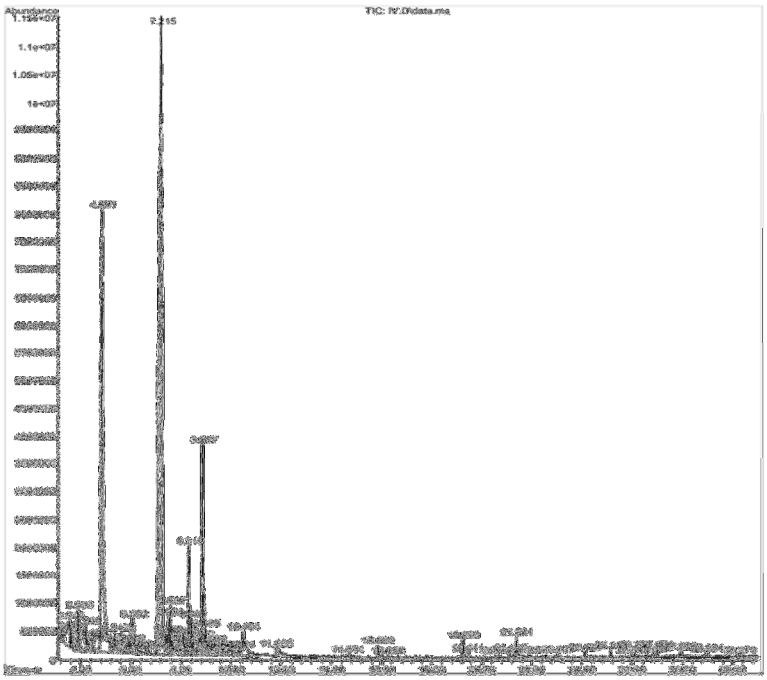

Figure 2. Liquid smoke chromatogram results on sample A4

The differences in the chemical contents of liquid smoke from young coconut shells could be due to differences in pyrolysis temperatures, resulting in variations in lignin degradation (López-Beceiro et al., 2021). Adiningsih and Priatni (2021) found that the chemical decomposition process of wood biomass pyrolysed at high temperatures took place in stages. In the temperature range of 100–150°C, water molecules evaporated. At a temperature of 200–240°C, hemicellulose and cellulose decomposed into pyrolignate solution (low-boiling-point organic acids, such as acetic acid, formic acid, methanol, CO, CO_2 and a small amount of tar), and at a temperature of 240–400°C, depolymerisation and bond breaking between C–O and C–C occurred. Within this temperature range, cellulose degraded. Lignin began to decompose and produced tar. In addition, the amount of pyrolignite solution and CO gas decreased, while CO, CH_4 and H_2 increased. At a temperature higher than 400°C, aromatic compounds formed, and the lignin continued to decompose up to 500°C.

Table 1. Results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis on sample A1

Component	%Area
Methylene chloride	7.71
Phenol	26.95
gamma.-Sitosterol	32.5
Carbonic acid, monoamide	1.27
1,12-dibromododecane-, 5-Chlorovaleric acid	3.89
Acetic acid	2.46
Tetracosamethyl-cyclododecasiloxane	1.16
Silicic acid, diethyl bis(trimethylsilyl) ester	1.75
1-(4-Hydroxy-3-methoxyphenyl)tetradecan-3-one	1.88
Squalene	1.12
(2E)-4-(4-Hydroxy-3-methoxyphenyl)-2-butanone oxime	1.66
1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)	1.1
1-Dimethyl(phenyl)silyloxypentane	3.32
(5α)-4,4-Dimethylcholestan-3-one	3.19
2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-,)	3.37
Thymol, TBDMS derivative	2.7

Table 2. Results of Gas Chromatography-Mass Spectrometry (GC-MS) analysis on sample A4

Component	%Area
Methylene chloride	5.38
Acetic acid (CAS) 1,8-Nonadien-3-ol	1.31
Acetic acid, phenyl ester (CAS) Phenol, 2-methyl	4.07
Cyclopentanone (CAS) 6-Hydroxy-2,6-dihydropyran-3-one	1.33
Furfural	18.35
Tetrahydropyrrole-3-ol-5-carboxylic acid, 1-acetyl-, methyl ester	1.24
4,4-Dimethyl-2-cyclopentene-1-one (CAS) Ethanone, 1-(2-furanyl)-	2.42

Component	%Area
Phenol	45.36
Phenol, 2-methoxy	6.39
Phenol, 2-methoxy-3-(2-propenyl)- (CAS) Eugenol	2.36
Creosol	1.52
Carbonic Acid	0.81
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.71
Phenol, 4-ethyl-2-methoxy	0.76
2-Cyclopenten-1-one, 2-methyl	0.8
2-Furancarboxaldehyde, 5-methyl	0.96

Phenol and Acetic Acid Analysis Result

Phenol and acetic acid are important components of liquid smoke due to their useful applications. Phenols have antioxidant, antiseptic and antibacterial properties. Meanwhile, acetic acid can exhibit an antimicrobial effect by denaturing proteins and splitting lipids, thereby damaging fungal cell membranes and inactivating enzymes secreted by fungi (Winarni et al., 2021). Acetic acid is obtained from the hemicellulose degradation process, while phenol is obtained from the lignin degradation process (Faisal et al., 2020). Table 3 shows the phenol and acetic acid levels in the liquid smoke produced from the pyrolysis of young coconut shells based on UV-vis spectrometry (phenol) and HPLC (acetic acid) analyses. Phenol levels increased in samples A1 and A2 and decreased in samples A3 and A4. The same was true for the levels of acetic acid, where the levels of acetic acid continued to increase in samples A1, A2 and A3 but decreased in sample A4. This decrease in phenol and acetic acid levels could be due to an increase in temperature in the lignin and cellulose degradation process, which resulted in the proportion of C–C and C–H bonds is reduced, while the C–OH, C–O–C, C=O and O–C–O bonds increased, causing the degradation of other compounds.

Table 3. Phenol and acetic acid in liquid smoke from young coconut shells at various pyrolysis temperatures

Sample	Phenol (%)	Acetic acid (%)
A1	2.04	4.16
A2	2.10	6.93
A3	2.06	9.23
A4	2.02	7.33

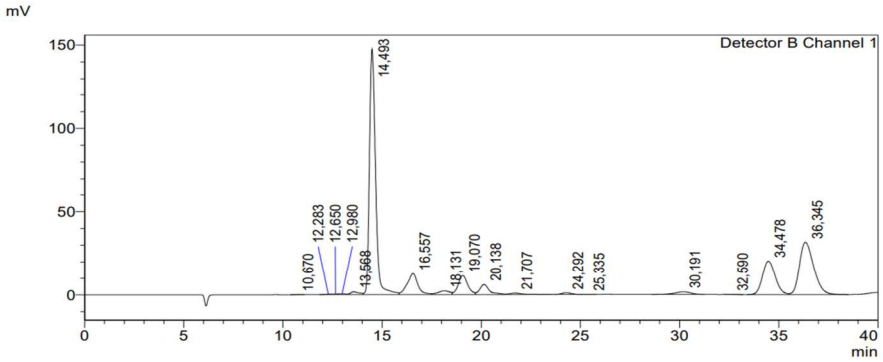

Figure 3. HPLC chromatogram results on sample A1

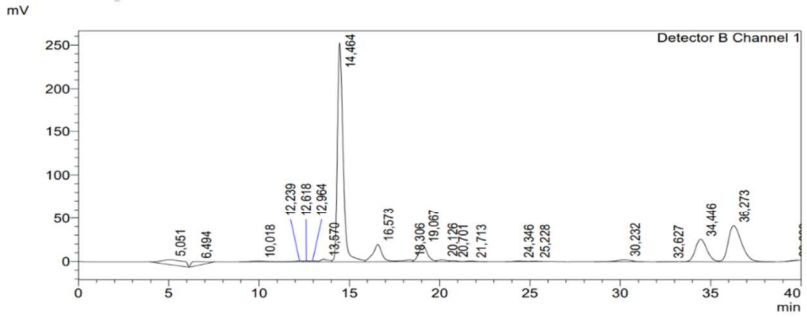

Figure 4. HPLC chromatogram results on sample A4

Results of Antibacterial Analysis

The antibacterial test was carried out using *Escherichia coli* and *Salmonella enterica* sv *Typhimurium* as bacterial cultures. These two pathogenic bacteria were chosen because they are widely found and act as food spoilage agents. Distilled water was used as the negative control and 0.25% ciprofloxacin as the positive control. The test results showed that the pyrolysis temperature affected the resulting inhibitory power. Specifically, an increase in the pyrolysis temperature in the preparation of liquid smoke produced compounds that could inhibit bacterial growth, such as carboxylic and phenolic acids. The diameter of the inhibition zone was categorised as weak (inhibitory diameter < 5mm), moderate (5–10 mm), strong (10–19 mm) and very strong (> 20 mm) (Faisal et al., 2017). Table 4 shows that liquid smoke from young coconut shells has a good ability to inhibit bacterial growth (inhibition zone diameter > 15 mm). Of the two types of bacteria used in the test, young coconut shell liquid smoke was able to inhibit the activity of *Escherichia coli* bacteria more effectively than that of bacteria, as the diameters of the liquid smoke inhibition zone in *Escherichia coli* bacteria were larger than those in *Salmonella enterica* sv *Typhimurium* bacteria. The activity of phenolic compounds in liquid smoke resulted in the formation of complex bonds with bacterial cell walls, thereby reducing cell wall permeability and damaging bacterial cell membranes due to their lipophilic properties (Rachmawaty et al., 2018).

Table 4. Results of antibacterial test of liquid smoke from young coconut shells at various pyrolysis temperatures

Bacteria	Sample	Inhibition Zone (mm)
Escherichia coli	A1	16.63
	A2	17.93
	A3	20.59
	A4	23.23
	Ciprofloxacin 0.25%	38.26
	Distilled water	0
Salmonella enterica sv *Typhimurium*	A1	15.38
	A2	16.49
	A3	18.34
	A4	20.41
	Ciprofloxacin 0.25%	36.25
	Distilled water	0

Results of MIC and MKC Tests

MIC is the minimum concentration of antibacterial agent needed to inhibit the growth of *Escherichia coli* and *Salmonella enterica* sv *Typhimurium* bacteria, while MKC is the minimum concentration of antibacterial agent capable of killing the bacteria (Kowalska-Krochmal & Dudek-Wicher, 2021). Table 5 shows the results of the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC) tests using liquid smoke as an antibacterial agent. The results indicated that liquid smoke could be used as an antibacterial agent to inhibit and kill *Escherichia coli* and *Salmonella enterica* sv *Typhimurium* bacteria. The higher the pyrolysis temperature of the liquid smoke, the better the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC). In *Escherichia coli* bacteria, minimum inhibitory concentration (MIC) began to appear in sample A2, as seen by the decrease in the total plate count, which indicated that the growth of the bacteria was being inhibited. Meanwhile, minimum killing concentration (MKC) was seen in sample A3, where the total plate count was 0, indicating that the bacterial killing had started. In *Salmonella enterica* sv *Typhimurium* bacteria, minimum inhibitory concentration (MIC) was seen in sample A3, and minimum killing concentration (MKC) was seen in sample A4. Thus, these results confirmed the results of the antibacterial test, as liquid smoke was able to inhibit and kill *Escherichia coli* bacteria better than *Salmonella enterica* sv *Typhimurium*.

Table 5. Total Plate Count in Minimum inhibitory concentration and Minimum killing concentration tests

Bacteria	Total Plate Count					
	A1	A2	A3	A4	Ciprofloxacin 0.25%	Distilled water
Escherichia coli	6	1	0	0	0	40
Salmonella enterica sv *Typhimurium*	12	3	1	0	0	66

Conclusion

Temperature variations in the pyrolysis process resulted in liquid smoke with different chemical and biological characteristics. Based on the results of the acetic acid and phenol analyses using High performance Liquid Chromatography (HPLC) and UV-vis spectrometry, liquid smoke produced at temperatures of 380°C and 340°C had the highest acetic acid (9.23%) and phenolic content (2.10%). Further, the presence of phenol and acetic acid content inhibited the growth of *Escherichia coli* and *Salmonella enterica* sv *Typhimurium* bacteria, with an inhibition zone of 23.23 mm at 420°C.

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