Pyrolysis of cajuput (*Melaleuca leucadendron*) twigs and rice (*Oryza sativa*) husks to produce liquid smoke-containing fine chemicals for antibacterial agent application

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Abstract

Pyrolysis of cajuput (*Melaleuca leucadendron*) twigs and rice (*Oryza sativa*) husks to produce liquid smoke and antibacterial activities of the liquid smoke fractions were investigated. The liquid smoke was produced by pyrolysis at 500 °C for 8 h and contained fine chemicals, such as acetic acid, carbonyl, cyclic ketones, and phenolic compounds with pH 2.1–2.9. The liquid smoke was separated by vacuum evaporation under vacuum conditions at low temperatures (40 °C, 50 °C, and 60 °C) to recover three fractions. The composition of each fraction influenced its antibacterial activities. Antibacterial activities of the liquid smoke fractions were tested against Gram-positive bacteria (*Listeria monocytogenes*, *Bacillus subtilis*, and *Staphylococcus aureus*) and Gram-negative bacteria (*Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Escherichia coli*). Whole fractions of the liquid smoke inhibited the six pathogenic bacteria, with the inhibition zone larger or smaller than the positive control. Among the liquid smoke fractions, the liquid recovered at 60 °C for the cajuput twigs and rice husks demonstrated a stronger inhibitory effect on bacterial growth than the other fractions.

Keywords Pyrolysis · Liquid smoke · Fine chemicals · Vacuum evaporation · Antibacterial agent

1 Introduction

Liquid smoke is produced by condensing smoke created by the pyrolysis of biomass, dark brown, free-flowing organic liquids, and highly oxygenated compounds [1, 2]. Other terms of liquid smoke include bio-oil, bio-crude oil, biofuel oil, wood vinegar, wood liquid, wood distillates, wood oil, tar, pyrolytic tar, pyrolysis liquid, pyrolysis oil, pyroligneous tar, and pyroligneous acid [1, 3]. Liquid smoke produced from bio-oil refineries through water extraction is used in the food industry as preservative, coloring, and flavoring of

meat, cheese, and sausages because of its antimicrobial and antioxidant activities.

Linkbeck et al. reviewed the use of liquid smoke as a natural antimicrobial against common foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella*, *Escherichia coli*, and *Staphylococcus*, in food preservation and its desired smoke flavor [4]. The antimicrobial activity of redistilled liquid smoke derived from oil-palm shells was also tested against *Staphylococcus aureus* and *E. coli*. Redistillation of liquid smoke was carried out at 80 °C. The liquid smoke was applied as a fresh catch preservative and fly repellent in fish salting–fermentation [5]. The antimicrobial effect of liquid smoke was also tested against *L. monocytogenes* in frankfurters as ready-to-eat meat and poultry products [6]. Liquid smoke as an antibacterial against *Clostridium perfringens* and *S. aureus* is effective in bacon processing [7]. The commercial liquid smokes derived from several types of wood are also effective as natural antimicrobials against foodborne pathogens, such as *Salmonella enteritidis*, *S. aureus*, and *E. coli* [8]. In preserving proteinaceous foods, such as fish balls, coconut shell liquid smoke has antibacterial activity against *Pseudomonas aeruginosa* and *S. aureus* [9]. Liquid smoke can also inhibit the growth of *Vibrio vulnificus*,

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Yersinia enterocolitica, *Bacillus subtilis*, *S. aureus*, *L. monocytogenes*, *Listeria innocua*, *Brochothrix thermosphacta*, and *Lactococcus lactis* [10]. Therefore, liquid smoke can extend product shelf life by preventing damage caused by spoilage and pathogenic bacteria [11].

Liquid smoke as a food preservative and smoke flavoring has several advantages over traditional smoking. For instance, liquid smoke is easier to apply, needs less time, is more environmentally friendly, eliminates potentially toxic compounds, more readily controls the concentration of smoke being applied, and produces more consistent results than the traditional one [3, 4, 12, 13]. Commercial crude liquid smoke from the pyrolysis of hardwoods and softwoods is commonly used for food preservation. Crude liquid smoke can potentially be a carcinogen because pyrolysis during liquid smoke production can induce the formation of potentially carcinogenic polycyclic aromatic hydrocarbons (PAHs). PAHs have limited solubility in aqueous solutions and are mostly removed from the liquid smoke during aging or purification [13]. Therefore, innovation in the purification process was carried out by separating crude liquid smoke into several clear colored fractions to remove PAHs.

Several types of lignocellulosic biomass that can be used to produce liquid smoke by pyrolysis include woodchips, sawdust, coconut shells, oil palm empty fruit bunch, and rice husks (RH) [4, 13–17]. Pyrolysis is a process of thermal decomposition of biomass and occurs in four stages: water evaporation, decomposition of hemicelluloses and cellulose (180–350 °C), and decomposition of lignin (300–500 °C) into liquid smoke, charcoal briquettes, and gaseous products. Pyrolysis occurs in the absence of oxygen supply during the process, except in certain cases where additional oxygen is needed for partial combustion to increase thermal energy [1, 18].

Decompositions of cellulose produce carbonyl and furans compounds, hemicellulose produces acetic acid and carbon dioxide, and lignin produces phenolic compounds [19]. Low-water-content biomass produces high-quality liquid smoke and high amounts of phenols, acids, and carbonyls [20]. By contrast, high water content results in mixing the condensation of water vapor with liquid smoke [21]. Therefore, liquid smoke contains numerous oxygenated compounds, which are a complex mixture of carboxylic acids, water, alcohols, esters, anhydrosugars, furans, phenolics, aldehydes, and ketones with a wide range of molecular weights and functionalities influenced by biomass feedstock composition, water content, pyrolysis temperature, biomass particle size, heating rate, residence time, pyrolysis reactor type, and condensation systems [1, 2, 12]. The rapid quenching during condensation produces many reactive species that would further react (degrade, cleave, or condensate with other molecules) and make the liquid smoke chemically unstable [1]. Therefore, the storage of liquid smoke needs attention

to maintain its stability. Instability increases with heating. Liquid smoke should be stored at or below room temperature because changes occur slowly at room temperature. The changes in liquid smoke properties include increasing viscosity [22] and decreasing volatility, phase separation, and deposition of gums [1]. Changes in molecular weight, water content, viscosity, and solid contents of liquid smoke during storage were also determined [23].

The use of biomass as a raw material to produce liquid smoke depends on its availability in a location. Cajuput and RH are biomass widely available in Indonesia. Cajuput has been planted in Sulawesi, Java, Moluccas, and East Nusa Tenggara. Based on AgroIndonesia, the yearly production of cajuput oil in Indonesia is approximately 600–700 tons [24]. After the extraction of the twigs and leaves, cajuput oil is produced and used as a traditional essential oil for medicinal purposes. Then, the process retains a huge amount of leaves and twigs as by-products. Moreover, the rice production in Indonesia in 2020 was 55.16 million tons of paddy, and the harvested area was 10.79 million hectares [25]. The paddy production should contain 20–25% RH. Therefore, the availability of RH is approximately 13.79 million tons. The utilization of non-wood materials, especially agricultural residue such as cajuput twigs (CT) and RH, as a raw material for liquid smoke production benefits the nation's economy and environment.

Although many previous studies analyzed the antibacterial properties of liquid smoke, most of them used crude liquid smoke derived from the pyrolysis of biomass [4, 26–29] that potentially contains carcinogenic PAHs. An efficient approach for producing natural food preservatives from liquid smoke fractions needs to be developed. This study aimed to determine the important chemical characteristics and antibacterial potential of the liquid smoke fractions after their separation from cajuput (*Melaleuca leucadendron*) twigs and rice (*Oryza sativa*) husks. The antibacterial activity was investigated against six foodborne pathogenic bacteria to ensure the applicability and safety of liquid smoke for food. We reported the antibacterial activity of the liquid smoke fractions based on their volatility for the first time. We also used CT as the source of liquid smoke for the first time. Thus, countries with abundant sources of CT or RH can use these materials as a potential source of natural food preservatives.

2 Materials and methods

2.1 Materials

Cajuput twigs (CT) as a by-product of oil extraction in Yogyakarta, Indonesia, and rice husks (RH) were used as raw materials. The cellulose, hemicellulose, and lignin contents

in CT and RH are shown in Table 1. The lignocellulose composition test of the biomass was carried out using the Chesson method [30]. The ash contents in RH and CT were $22.98\% \pm 0.04\%$ and $5.41\% \pm 0.08\%$, respectively, as determined in accordance with the national quality standard (SNI No. 01–2891-1992). The water contents of CT and RH were 13.65% and 8.13%, respectively, as measured using a moisture analyzer (AND Mx 50, USA). Mueller Hinton Agar (MHA) was purchased from Oxoid.

2.2 Methods

2.2.1 Pyrolysis of biomass

CT samples were pretreated by reducing the size to $\pm 1\text{--}5\text{ cm}$ with a grinder and then dried in direct sunlight for approximately a day to reduce moisture content. Low moisture content can accelerate the heating rate during initial pyrolysis. Liquid smoke was produced by pyrolysis on a 30 L-capacity reactor at $500\text{ }^{\circ}\text{C}$ for 8 h, and the schematic of this process is shown in Fig. 1. The reactor was heated by an LPG stove and accompanied by temperature and gas flow controllers. Approximately 5 kg of each sunlight-dried CT and RH were fed into the reactor. The smoke generated from the reactor entered a cyclone to separate heavy tar and small particulates carried away. Then, the smoke was condensed in a condenser using water as a coolant to obtain liquid smoke [16]. The residue left in the reactor was charcoal. Liquid smoke

resulting from the pyrolysis was collected in a container and deposited for 1 day.

2.2.2 Separation of liquid smoke into three fractions

Approximately 50 mL of liquid smoke was introduced into an Erlenmeyer flask to separate into three fractions using a rotary vacuum evaporator (Rotavapor R-100, Buchi) with the water bath temperature set at $40\text{ }^{\circ}\text{C}$, $50\text{ }^{\circ}\text{C}$, and $60\text{ }^{\circ}\text{C}$ for 1 h at each temperature and vacuum condition at 200 mbar. The liquid smoke fraction products were coded according to the biomass source and water bath temperatures, namely, “CT 40,” “CT 50,” and “CT 60” for CT with bath temperatures of $40\text{ }^{\circ}\text{C}$, $50\text{ }^{\circ}\text{C}$, and $60\text{ }^{\circ}\text{C}$, respectively. Meanwhile, liquid smoke fractions derived from RH with water bath temperatures of $40\text{ }^{\circ}\text{C}$, $50\text{ }^{\circ}\text{C}$, and $60\text{ }^{\circ}\text{C}$ were denoted as “RH 40,” “RH 50,” and “RH 60,” respectively. Liquid smoke was separated into three fractions to obtain a clearer product containing chemicals with a near boiling point and was free from large molecular weight chemicals, such as PAHs, which are generally carcinogenic. All fractions were stored in a freezer at $-18\text{ }^{\circ}\text{C}$ to retain their stability. The properties, including chemical composition, pH, and antibacterial activity, of all fractions were identified. A schematic of the separation of the liquid smoke fractions is illustrated in Fig. 2.

2.2.3 Determination of the antibacterial activity of liquid smoke

The antibacterial activity of the liquid smoke fractions was determined using the agar well diffusion method as previously described by Balouiri et al. (2016) with some modifications [31]. The well diffusion test was carried out using three strains of Gram-positive bacteria, i.e., *B. subtilis* (ATCC19659), *L. monocytogenes* (ATCC7644), *S. aureus* (ATCC25923), and three strains of Gram-negative bacteria,

Table 1 Composition of cajuput twigs and rice husks

Biomass	Cellulose, wt. %	Hemicellulose, wt. %	Lignin, wt. %
Rice husks	34.86 ± 3.31	21.13 ± 0.58	30.46 ± 3.05
Cajuput twigs	31.94 ± 0.44	17.99 ± 0.66	27.51 ± 0.26

Fig. 1 A schematic pyrolysis process

Fig. 2 A schematic of the separation process using a rotary vacuum evaporator to produce liquid smoke fractions

i.e., *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), and *Salmonella typhimurium* (ATCC14028). The agar plate surface of MHA (Oxoid) on a sterile Petri dish was inoculated by spreading 20 μ L of bacterial inoculum. Furthermore, a hole with a diameter of 6 mm had punched aseptically with a sterile cork borer. In each Petri dish, three wells were made, one for the positive control and the other two for the liquid smoke samples. Each of the three wells was added with 60 μ L of positive control (0.01% chloramphenicol) and 60 μ L of liquid smoke. Then, the agar plates were incubated for 24 h at 37 °C under aerobic conditions. After 24 h, a clearer area was formed around the hole as a zone of inhibition for bacterial growth. The antibacterial activity of each liquid smoke sample was determined by measuring the diameter of the formed zone of inhibition in millimeter.

2.2.4 Degree of acidity and chemical composition analysis

The liquid smoke fractions derived from CT and RH were analyzed to determine their degree of acidity using a pH meter (SevenExcellence, Multiparameter) coupled with an InLab® Science Pro-ISM electrode (Mettler Toledo). Chemical composition was analyzed using gas chromatography–mass spectrometry (GC–MS). The GC–MS instrument (GC-2010/QP2010S, Shimadzu) was equipped with a DB-624 column (Agilent Technologies, Inc. 30 m $\times$ 250 μ m $\times$ 1.40 μ m). The injector temperature was

250 °C. The carrier gas was helium. The initial oven temperature of the column was 40 °C, which was maintained for 5 min, raised to 190 °C at 4 °C/min, and then maintained for 17.5 min at 190 °C. The ion source temperature and interface temperature were 240 °C. Ionization energy was 70 eV, and the mass ranged from m/z 28 AMU to 600 AMU. The total flow was 36 mL/min, the column flow was 0.85 mL/min, and the linear velocity was 33.2 cm/s. The chemicals in the samples were identified by comparing the spectra and retention time of individual compounds with those of authentic reference compounds stored in the mass spectral data library.

3 Results and discussion

3.1 Separation of the liquid smoke

Liquid smoke derived from CT and RH was separated using a rotary vacuum evaporator by setting the water bath temperature to 40 °C, 50 °C, and 60 °C. The yield of the liquid smoke fractions is displayed in Table 2.

As shown in Table 2, the RH products (RH40, RH50, and RH60) had higher yield than the CT products (CT40, CT50, and CT60) because the liquid smoke fractions from RH contained more volatile compounds at the temperature range of 40–60 °C compared with

Table 2 Yield of liquid smoke fractions derived from cajuput twigs (CT) and rice husks (RH)

Fraction of liquid smoke	RH		CT	
	Yield (wt. %)	Std. Dev	Yield (wt. %)	Std. Dev
Liquid product, bath 40 °C	22.2	0.0339	15.8	0.026
Liquid product, bath 50 °C	30.1	0.0004	25.8	0.035
Liquid product, bath 60 °C	41.4	0.0323	37.6	0.029
Residue	3.3	0.0016	20.8	0.012

those from CT, as evidenced by the fewer residue that remained in the RH products than the CT products. The residue was a fraction that can no longer evaporate after the liquid smoke fraction with a water bath temperature of 60 °C was produced. Moreover, the yield of the liquid smoke fraction produced from the separation process was a liquid product with a water bath temperature of 60 °C > 50 °C > 40 °C. This result indicates less volatile compounds at 40 °C than at 50 °C and 60 °C. At the same water bath temperature, the yield of the liquid smoke fractions from RH was higher than that of the liquid smoke fractions from CT. Thus, the light fraction of liquid smoke derived from RH was greater than that of liquid smoke from CT, and the composition of the chemical compounds depend on the biomass used. All obtained liquid smoke fractions were clearly water soluble. However, the residue was black, contained chemical compounds with low volatility and high molecular weight, and was not dissolved in water. The residue of liquid smoke derived from CT was higher than that of liquid smoke derived from RH because of differences in biomass composition. The residue was not used in this study because it might contain carcinogenic substances, such as PAHs formed from incomplete burning and pyrolysis temperatures between 500 °C and 900 °C. The level of PAHs formation is influenced by biomass source [4]. PAHs have low water solubility and easily separate using phase separation and filtration techniques [4, 13]. Therefore, the liquid smoke fractions used for the antibacterial test were RH 40, RH 50, RH 60, CT 40, CT 50, and CT 60.

3.2 Chemical compositions and pH in each liquid smoke fraction

On the basis of GC–MS analysis, chemical compounds of liquid smoke fractions derived from RH and CT are shown in Table 3. Each fraction derived from CT and RH contained acetic acid, phenol, guaiacol, and m-cresol. 2-Propanone, 1-hydroxy- was detected in RH 40, RH 50, and RH 60. Furfural; 2-cyclopenten-1-one, 2-methyl-; ethanone, 1-(2-furanyl)-; phenol, 2-ethyl-, and some of phenol, 4-ethyl-2-methoxy- were detected in the RH 40 fraction because of their high volatility and small quantity, allowing them to be separated at a temperature of 40 °C and a vacuum of 200 mbar. Some quantity of phenol, 2-methoxy-4-methyl- was detected in the RH 50 fraction. Meanwhile, 1-hydroxy-2-butanone was only detected in the RH 60 fraction. On the basis of the boiling point data (Table 3), the chemical compounds detected in each fraction had a boiling point of less than 240 °C. Others were unknown compounds. Conversely, the chemical compounds of the CT fractions were less those of the RH fractions. 2-Propanone, 1-hydroxy- was only detected in CT 60, furfural was detected in CT 40 and CT 50, and 2-cyclopenten-1-one, 2-methyl was detected in CT 40. The identified compounds varied widely, and the compounds responsible for the antimicrobial activity were unclear. Thus, the safety of the liquid smoke needs to be evaluated before it was recommended for use. The differences in the composition of chemical compounds in each fraction caused differences in antibacterial activities.

As shown in Table 3, the acetic acid content of the CT fractions was higher than that of the RH fractions. In the CT

Table 3 Chemical compositions in separation fractions of liquid smoke derived from rice husks and cajuput twigs

Chemical	Boiling point (°C) *	Area (%)					
		RH 40	RH 50	RH 60	CT 40	CT 50	CT 60
Acetic acid	118.05	19.27	34.35	41.13	20.64	39.51	65.52
2-Propanone, 1-hydroxy-	145.55	4.32	8.61	22.25	-	-	12.42
1-Hydroxy-2-butanone	160.05	-	-	9.13	-	-	-
Furfural	161.55	10.07	-	-	20.41	9.17	-
2-Cyclopenten-1-one, 2-methyl-	159.55	2.87	-	-	5.55	-	-
Ethanone, 1-(2-furanyl)-	172.85	1.99	-	-	-	-	-
Phenol	181.85	18.15	20.31	12.24	15.38	19.9	11.25
Phenol, 2-methoxy (guaiacol)	205.05	20.34	20.12	6.73	22.12	18.26	4.61
Phenol, 3-methyl- (m-cresol)	202.05	7.71	6.08	3.66	9.64	13.16	6.20
Phenol, 2-methoxy-4-methyl- (creosol)	221.05	6.51	5.23	-	6.26	-	-
Phenol, 2-ethyl-	204.85	3.85	-	-	-	-	-
Phenol, 4-ethyl-2-methoxy-	236.5 #	3.06	-	-	-	-	-
Others	-	1.84	5.31	4.86	-	-	-

* <https://webbook.nist.gov/>

<https://pubchem.ncbi.nlm.nih.gov/>

fractions, the acetic acid content was CT 60 > CT 50 > CT 40, and the RH fractions showed the same order as RH 60 > RH 50 > RH 40. The acetic acid content affected the pH of each fraction, as displayed in Fig. 3. The separating fractions of liquid smoke were highly acidic (pH 2.1–2.9) because of acetic acid contents. The pH of each fraction followed the acetic acid content trend in Table 3. The higher the percentage of acetic acid, the lower the pH.

3.3 Antibacterial activity of liquid smoke fractions

In this study, the antibacterial activities of the CT and RH liquid smoke fractions were identified using various pathogenic bacteria to support the possible future application as a preservative in food, especially meat and fish. The selection of test bacteria focused on contaminants in food products

Fig. 3 pH of the separating fractions of liquid smoke

that cause food spoilage and several foodborne infections in humans. The pathogenic bacteria include Gram-positive strains *L. monocytogenes*, *B. subtilis*, and *S. aureus* and Gram-negative strains *S. typhimurium*, *P. aeruginosa*, and *E. coli*. *Salmonella*, *E. coli*, and *L. monocytogenes* are pathogenic bacteria widely found in fish together with human non-pathogenic bacteria species and natural microflora of aquatic environments. These bacteria are responsible for human foodborne diseases [32]. Apart from being pathogenic, *Bacillus* spp., *Salmonella*, *E. coli*, and *Pseudomonas* cause food spoilage [33]. The susceptibility of different strains of organisms to the liquid smoke fractions can be influenced by the compounds contained. The antibacterial activity of the liquid smoke fractions derived from RH and CT against Gram-positive and Gram-negative bacteria is displayed in Figs. 4, 5, 6, 7, 8, 9, 10, and 11.

The inhibitory effects of the RH and CT fractions against *L. monocytogenes* are shown in Fig. 4a, b. The whole fractions of liquid smoke (RH 40, RH 50, RH 60, CT 40, CT 50, and CT 60) inhibited the growth of *L. monocytogenes* (Fig. 4a). The inhibition diameters of RH 40, RH 60, and CT 60 were greater than that of the positive control, whereas the inhibition diameters of RH 50, CT 40, and CT 50 were smaller than that of the positive control. The inhibition zone diameter ranged from 10.38 to 17.05 mm for RH, 9.16 to 15.28 mm for CT, and 10.86 mm for the positive control (chloramphenicol). The RH 40–60 and CT 50–60 fractions exhibited antibacterial activity in a strong category based on the diameter of the inhibition zone. Meanwhile, CT 40 demonstrated antibacterial activity in the moderate category. The strength of antibacterial activity was designated as follows:

(a)

(b)

Fig. 4 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Listeria monocytogenes*: **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

Fig. 5 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Staphylococcus aureus*: **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

Fig. 6 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Salmonella typhimurium*: **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

inhibition area > 20 mm, very strong; inhibition area of 10–20 mm, strong; inhibition area of 5–10 mm, moderate; and inhibition area < 5 mm, weak [5].

Figure 4 displays that the type of biomass and liquid smoke fraction affected the antibacterial properties against *L. monocytogenes*. *L. monocytogenes* is a potentially dangerous foodborne pathogen that causes listeriosis, particularly in high-risk groups, namely, young, old, pregnant, and immune-compromised individuals [34]. *L. monocytogenes* can cause septicemia and meningitis with high mortality rates. Infections of this bacterium are currently associated

with a fatality rate of approximately 17%, which is the highest rate observed among foodborne pathogens [35]. The transmission of *L. monocytogenes* from foods to humans is mostly related to products that do not receive any heat treatment, so-called “ready-to-eat (RTE)” products, including cheeses and raw food such as vegetables, milk, meat, and seafood. *Listeria* can grow at low-temperature, high-salt, acidic, and microaerophilic conditions. Therefore, its growth is difficult to control in many RTE and refrigerated foods. The infective dose of *L. monocytogenes* can be as low as 1000 bacteria [4].

Fig. 7 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Pseudomonas aeruginosa*: **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

Fig. 8 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Bacillus subtilis*: **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

As shown in Fig. 4b, RH 60 and CT 60 had larger diameters of inhibition zone than the other fractions that were influenced by the chemical composition of the liquid smoke, especially the contents of phenol, carbonyl, and acetic acid. Morey et al. studied the effect of liquid smoke against *L. monocytogenes* on the shelf life and quality of frankfurters and reported that phenols and carbonyl compounds influence the antimicrobial properties of liquid smoke. Organic acids could enhance the antimicrobial efficacy of liquid smoke [6]. Compared with CT 60, RH 60 contained higher carbonyl and phenol contents, including 2-propanone, 1-hydroxy-

1-hydroxy-2-butanone; and phenol. Therefore, the diameter of the inhibition zone against *L. monocytogenes* was also larger. In addition, the acetic acid content increased the efficacy at certain concentrations. Morey et al. used liquid smoke from Zesti Smoke (Kerry Ingredients and Flavors, TN) that was produced by distilling sawdust from hardwood (hickory wood) sawmills to form a liquid [36]. The addition of 2.5–10% wt/wt liquid smoke as a frankfurter ingredient effectively suppresses the growth of *L. monocytogenes* [6].

Faith et al. (1992) reported that specific phenol (i.e., isoeugenol) found in liquid smoke possesses anti-listerial

Fig. 9 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) and cajuput twigs (CT) against *Escherichia coli*, **a** photograph of inhibition zone and **b** data of inhibition diameter compared to control. K control (chloramphenicol), U1 and U2 Duplo sample

activity, whereas other phenolic ingredients (e.g., eugenol, vanillin, and coniferyl alcohol) do not possess potential anti-listerial activity. Moreover, the combination of low pH (pH 5.8) and isoeugenol (100 ppm) is more effective in inhibiting *L. monocytogenes* than either treatment alone. The synergistic effect of acidic (particularly acetic acid) and phenolic constituents may result in anti-listerial activity potential [37]. Further study by Guilbaud et al. (2008) demonstrated that liquid smoke strongly affects the metabolic pathways of Lmo355 and Lmo2829 proteins, which are involved in the membrane bioengineering and lipid metabolism of *L. monocytogenes*. The study suggested

that liquid smoke affects the synthesis of the bacterial cell membrane and reduces the virulence of *Listeria* by decreasing the hemolytic activity [38].

Staphylococcus is a Gram-positive spherical-shaped pathogenic bacteria. *S. aureus* causes staphylococcal foodborne disease (SFD) as a common foodborne disease worldwide. It is caused by food contamination [39] and consumption of foods contaminated with toxins produced by the bacteria as opposed to the consumption of the bacteria itself. Symptoms of SFD include nausea, vomiting, and abdominal cramps with or without diarrhea. Foods commonly contaminated with *S. aureus* include meat and meat products; poultry

Fig. 10 Inhibitory effects of liquid smoke fraction derived from cajuput twigs (CT) against pathogenic bacteria

Fig. 11 Inhibitory effects of liquid smoke fraction derived from rice husks (RH) against pathogenic bacteria

and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products; sandwich fillings; milk; and dairy products [4, 40]. Antibacterial activity of liquid smoke against *S. aureus* is shown in Fig. 5. All liquid smoke fractions had inhibition zones that were larger than that of the positive control. The inhibition zone diameters ranged from 16.02 to 17.67 mm (RH), 14.05 to 20.26 mm (CT), and 14.02 mm (chloramphenicol). On the basis of inhibition zone diameter, CT 60 had very strong antibacterial activity, and the other fractions had strong antibacterial activity [5].

As shown in Fig. 5, CT 60 had the greatest diameter inhibition zone because of the high content of acetic acid, although other chemical compounds were also influenced. This result was consistent with the finding of a study conducted by Taonima and Bartholomew (2005), who investigated the use of liquid smoke in bacon processing as an antibacterial against *S. aureus* growth. They used concentrated liquid smoke (Charsol Supreme, Red Arrow, Manitowoc, Wis.) diluted to 75% of sterile deionized water before application. The concentrated liquid smoke contained 24.0–30.0% carbonyls with pH 2.2–2.8, 14.0–16.0% of total acidity (as acetic acid), and 15.0–23.0 mg/mL of smoke flavor compounds. The use of 1.25% (mL/g-pork belly) of the liquid smoke solution inhibited the growth of *S. aureus* with undetectable staphylococcal enterotoxins in the bacon. They stated that the liquid smoke exhibits antibacterial activity because smoke phenols, carbonyls, and organic acids penetrate the whole belly pieces and effectively inhibit the pathogens [7].

S. typhimurium is a Gram-negative foodborne bacterium that is usually found in raw poultry and eggs. It can contaminate various foods, such as dairy, meat, raw vegetables, fruits, and animal feeds [4]. *Salmonella* spp. are common and widely distributed foodborne pathogens in the European Union. In 2012, 91,034 *Salmonella* cases and 347 strong-evidence outbreaks related to *Salmonella* were reported [41]. Possible sources of human infection with *S. typhimurium* range from the consumption of beef, pork, poultry, and dairy products [42]. *S. typhimurium* causes nontyphoidal

salmonellosis infections relative to human illness, such as acute gastroenteritis [43]. *S. typhimurium* infection can also cause severe illness involving diarrhea, chills, abdominal cramps, fever, head and body aches, nausea, and vomiting [44].

The antibacterial activity of the RH 60 fraction against *S. typhimurium* was stronger than those of the other fractions, which may be influenced by its higher carbonyl content than the other fractions. Carbonyls could absorb nutrients, deactivate extracellular bacterial enzymes for metabolism, and modify the substrate to not be available for bacterial enzyme action [6]. The inhibition zones of CT 60 and RH 60 were larger than that of the positive control, whereas the inhibition zones of the other fractions were almost the same or lower than that of the positive control. The inhibition zone diameter ranged from 17.92 to 21.36 mm (RH) and 11.75 to 20.62 mm (CT). Moreover, the inhibition zone diameter for the positive control against *S. typhimurium* was 18.71 mm (Fig. 6). RH 60 and CT 60 had very strong antibacterial activity based on the diameter of the inhibition zone, and the other fractions had strong antibacterial activity [5]. Liquid smoke derived from the pyrolysis of RH at 450–500°C [13] was tested for its antibacterial activity, and results showed that the liquid smoke inhibited *S. typhimurium* with the minimum inhibitory concentration (MIC) of 0.822% (v/v) [45]. Loo et al. also tested commercial and pecan shell-extracted liquid smokes as a natural antimicrobial against *S. enteritidis* and *S. typhimurium* with MIC values of 0.5–8.0% and 0.5–12.0%, respectively [8].

P. aeruginosa is a common bacterial species that produces toxins and other metabolites that induce human gastrointestinal diseases [43]. *P. aeruginosa* can be found in meat, milk, and drinking water [46]. *P. aeruginosa* is an opportunistic pathogen causing nosocomial infections among immunocompromised persons. *P. aeruginosa* infections can be classified as either acute or chronic. Acute infections, such as ventilator-associated pneumonia, are invasive, cytotoxic, and frequently result in systemic infection, septic shock, and mortality. By contrast, the chronic respiratory

infections associated with cystic fibrosis are minimally invasive, noncytotoxic, and rarely progress to systemic infection [47].

Antibacterial activity of liquid smoke against *P. aeruginosa* is shown in Fig. 7. All liquid smoke fractions had larger inhibition zones than the positive control, with the liquid smoke inhibition zone diameter ranging from 16.46 to 20.46 mm (RH), 10.35 to 19.91 mm (CT), and 9.60 mm (chloramphenicol). RH 60 had very strong antibacterial activity on the basis of the diameter of the inhibition zone, and the other fractions had strong antibacterial activity [5]. The inhibition ability of liquid smoke against *P. aeruginosa* was also tested using the broth dilution method to determine the MIC. The liquid smoke derived from coconut shells obtained from the Indonesian small-scale industry had 0.22% MIC [9].

B. subtilis is a commensal bacterium and can be considered a pathogen of nosocomial infection, which subsequently causes secondary infections [48]. *B. subtilis* has occasionally been isolated from cases of food-associated illness, but its roles are usually uncertain. Several traditional fermented foods based on leaves and seeds, such as cocoa, coffee, and vanilla, are processed by an enzyme produced by *B. subtilis*. *B. subtilis* has been implicated in some cases related to food-borne illness, with vomiting as the commonest symptom, but accompanying diarrhea is frequently reported. *B. subtilis* can be found in foods such as chicken, cereal products, and a contaminant in nutritional supplements [49].

The antibacterial activity of the liquid smoke fraction against *B. subtilis* is shown in Fig. 8. All liquid smoke fractions from RH had a larger inhibition zone than the positive control, whereas the liquid smoke from CT did not entirely have a larger inhibition zone. CT 40 resulted in a smaller inhibition zone than the positive control. The inhibition zone of RH liquid smoke against *B. subtilis* ranged from 15.20 to 17.03 mm, whereas that of CT liquid smoke ranged from 6.89 to 18.19 mm and 16.22 mm for chloramphenicol. CT 40 had moderate antibacterial activity on the basis of the diameter of the inhibition zone, and the other fractions had strong antibacterial activity [5]. Commercial liquid smokes used in the Spanish food industry were also tested with the preparation of stock solution in Tris–HCl pH 7.5 and the pH adjusted to neutrality against *B. subtilis*, with MIC values of 0.6–0.8%, respectively. The liquid smoke tested contained total phenol derivatives 11,758.2 mg/kg, total carbonyl derivatives 31,017.7 mg/kg, and acids 880.4 mg/kg. Several components such as aldehydes, ketones and diketones, esters, furan and pyran derivatives, phenol derivatives, guaiacol derivatives, and syringol derivatives were also found [10].

E. coli is a Gram-negative bacterium [4] and recognized as non-pathogenic normal cohabitant commonly found in the intestinal flora of human and animals. However, certain

strains might induce disease, and *E. coli* is a potentially pathogenic organism [50]. *E. coli* can contaminate salads containing raw vegetables, pork, poultry meat, beef, and milk products. Pathogenic strains of *E. coli* can cause distinct disease syndrome as different diarrheal diseases, enteritis, urinary tract infection, septicemia, and other clinical infections, such as neonatal meningitis [51–53].

The antibacterial activity of liquid smoke against *E. coli* is displayed in Fig. 9. Among the liquid smoke fractions, CT 60 had the largest inhibition zone. The inhibition zone of CT 60 was also larger than that of the positive control. RH 50, CT 50, and CT 40 had smaller inhibition zones than the positive control. The diameter of the liquid smoke inhibition zone against *E. coli* ranged from 12.68 to 16.50 mm (RH), 10.59 to 19.70 mm (CT), and 14.29 mm (chloramphenicol). All fractions had strong antibacterial activity on the basis of the diameter of the inhibition zone [5]. Previous studies have been shown that the presence of *E. coli* inhibitory activity is associated with the compounds that play a role in inhibitory mechanisms. The liquid smoke fraction with phenol level 1.4–4.0 mg/mL, carbonyl level 2.0–7.0 g/100 mL, and pH 2.0 demonstrated an inhibitory effect on *E. coli* O157:H7 growth [54].

Figure 10 shows the effect of the liquid smoke fractions derived from CT on the inhibition zone diameters of six types of pathogenic bacteria. The inhibition zone of the liquid smoke fractions against *S. typhimurium*, *E. coli*, *P. aeruginosa*, *L. monocytogenes*, *B. subtilis*, and *S. aureus* was in the order of CT 60 > CT 50 > CT 40. The large inhibition zone of CT 60 was caused by the high content of acetic acid, which reached 65.5%. As a result, the pH of CT 60 was lower than those of CT 40 and CT 50 (Fig. 3). Carbonyl in the form of 2-propanone-1-hydroxy- (12.4%) (Table 3) also affected the antibacterial activity of CT 60, which was not detected on CT 40 and CT 50. The composition of CT 60 compared with CT 40–50 (Table 3) showed that the contents of phenol, guaiacol, m-cresol, and creosol exerted less effect on antibacterial activity. CT 40–60 had a weaker effect on *L. monocytogenes* and *B. subtilis* than on the other pathogenic bacteria. Moreover, CT 60 exerted the most effect on *S. typhimurium*, *E. coli*, *P. aeruginosa*, and *S. aureus*.

The liquid smoke fractions that originated from RH had different profiles than those from CT. RH 50 had a smaller inhibition zone than RH 40, and the largest inhibition zone against all tested pathogenic bacteria, except for *E. coli*, was observed with RH 60 (Fig. 11). The large inhibition zone of RH 60 was caused by its higher contents of acetic acid (41.1%), 2-propanone, 1-hydroxy- (22.3%), and 1-hydroxy-2-butanone (9.1%) (Table 3) and lower pH of 2.1 compared with RH 40 and RH 50 (Fig. 3). Inhibition zone of RH 40 > RH 60 > RH 50 against *E. coli* was predicted influenced by the total detected composition of the liquid smoke fractions that originated from RH. RH 60

had the most effect on *S. typhimurium* and *P. aeruginosa*. As shown in Figs. 4, 5, 6, 7, 8, 9, 10, and 11, the antibacterial activity of liquid smoke affected pathogenic organisms with varying degrees of sensitivity to the ingredients of the liquid smoke. The tested pathogenic bacteria showed different responses to the liquid smoke fractions because of their different cell wall compositions. Gram-negative bacteria have a cell wall with a thin peptidoglycan layer and are surrounded by an outer membrane composed of lipopolysaccharides. The walls of Gram-negative bacteria cannot retain the initial Gram stain reagent. The outer membrane of Gram-negative bacteria acts as a barrier against hydrophobic compounds. Meanwhile, Gram-positive bacteria do not have an outer membrane but have a cell wall with a peptidoglycan layer many times thicker than that found in Gram-negative bacteria, in which the initial Gram stain reagent can be retained [55]. The peptidoglycan cell wall is mechanically and chemically strong to protect the bacterial cell, and the cell walls of Gram-positive bacteria are more resistant to mechanical or chemical stresses than those of Gram-negative bacteria [56]. McDonnell and Russell (1999) stated that peptidoglycan and teichoic acid on the cell walls of gram-positive bacteria do not act as an effective barrier against the entry of antimicrobial compounds (antiseptics and disinfectants) because substances with high molecular weights can easily cross the cell wall of *Staphylococcus* [57]. The plasticity ability of Gram-positive bacterial cells determines the sensitivity of cells to antimicrobial compounds, where the thickness and degree of cross-linking of peptidoglycan can be modified as a form of cell defense. Organic acids include acetic acid, an anionic surfactant acting as a disinfectant that disturbs membrane stability. The general mechanism of inhibition of microbial growth by organic acids is through acidification of the cell cytoplasm caused by the release of excess protons after acid dissociation [58].

Food spoilage is a complex condition in which biological and physicochemical activities may interact and make the product unacceptable for human consumption [59]. The various preservation techniques used in the food industry can be categorized into physical, chemical, and biopreservation methods. Smoking is a means of chemical preservation generally applied to meat foods and fish. Varlet et al. (2007) reported that the combined effect of phenolic compounds formed during smoking and the high-temperature exposure result in microbial growth reduction [60]. Liquid smoke fractions derived from CT (CT 40, CT 50, and CT 60) and RH (RH 40, RH 50, and RH 60) had strong and moderate antibacterial effects, indicating their potential application in food smoking.

The results of this study showed that organisms that played an important role in the deterioration of foods

and human health were effectively inhibited by the liquid smoke fractions derived from CT (CT 40, CT 50, and CT 60) and RH (RH 40, RH 50, and RH 60). However, these results need to be tested in real systems because the necessary levels to inhibit microbial growth could be considerably different for foods and laboratory culture media.

4 Conclusion

Liquid smoke produced from the pyrolysis of cajuput twigs and rice husks contained fine chemicals, such as organic acids, carbonyl, and phenolic compounds. The liquid smoke was separated into three fractions under vacuum conditions and a low temperature of 40–60°C to ensure the safety of foodstuff from large molecular weight PAHs that are carcinogenic. The antibacterial activity of the liquid smoke fractions against pathogenic bacteria, including *L. monocytogenes*, *B. subtilis*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli* that cause food spoilage and several foodborne infections in humans, was investigated to promote the possible future application of liquid smoke fractions as a natural preservative for food products, especially for meat and fish. The method to determine the antibacterial activity was tested by measuring the inhibition zone formed after adding liquid smoke to the agar plate previously grown with bacteria. On the basis of the inhibition zone formed, the liquid smoke fractions effectively inhibited the six pathogenic bacteria. Among the fractions, the liquid smoke recovered at 60°C originated from CT and RH had a larger inhibition zone than the other fractions because of the high contents of acetic acid and carbonyl with low pH. The results prove that all the liquid smoke fractions can be applied in the future as food preservatives in fish and meat that are often damaged by *L. monocytogenes*, *B. subtilis*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *P. aeruginosa*, and *E. coli*.

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Author contributions All authors contributed equally to this article. DM separated the liquid smoke into three fractions, interpreted the GC–MS and pH analyses, drafted the manuscript, and handled the major reviews of the article. SS separated the liquid smoke into three fractions and analyzed and interpreted the antibacterial activities. WAR collected and pretreated the raw biomass, conducted pyrolysis, and drew the schematic process related to the pyrolysis methods. RS interpreted the data of the antibacterial activity. RM separated the liquid smoke into three fractions and drafted the “Introduction” section. The authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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