Preliminary study on the antibacterial activity of liquid smoke from cacao pod shells (Theobroma cacao L)

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Abstract. Cacao pod shells (Theobroma cacao L) form biomass waste that can be used as raw material for liquid smoke because this biomass contains lignin, cellulose, and hemicellulose. This research studied the antimicrobial activity of liquid smoke from cacao pod shells on several common food-borne pathogens, such as Salmonella choleraesuis, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis. The liquid smoke used was obtained from cacao pod shells that were pyrolyzed at 300 °C (T1), 340 °C (T2), and 380 °C (T3). Liquid smoke concentration varied from 1% to 5%. The antibacterial activity test was conducted using the Kirby–Bauer method. The results showed that liquid smoke produced from T1 and T2 (and at liquid smoke concentrations of 4% and 5%) could inhibit the growth of all the tested bacteria. At T3 and 1–5% of liquid smoke, only E. coli was consistently inhibited (inhibition zone = 6–7.05 mm), while the growth of S. choleraesuis and S. aureus was inhibited at 3–5% liquid smoke with the inhibition zone ranging from 6 mm to 7.2 mm. B. subtilis was inhibited by a 2–5% liquid smoke concentration. All the tested bacteria showed sensitivity to liquid smoke, but E. coli was the stronger resistant compared to others. The results of this study show that liquid smoke from cacao pod shells could be used as a preservative agent to inhibit microorganisms in food.

1. Introduction

As an agricultural country, Indonesia is a major producer of cacao (Theobroma cacao L). In the plantation sector, cacao is one of the most important export commodities, with a total planting area of 1.7 million hectares and 651,000 tons of cacao beans produced [1]. Cacao pod shells are produced in almost the same volume but are not widely used. Some studies report that cacao pod shells contain lignin (51.98%), cellulose (20.15%), and hemicellulose (21.06%) [2]. These cacao pod shells have potential as raw materials to produce liquid smoke (LS), which is usually produced from raw materials that contain cellulose, hemicellulose, and lignin. Liquid smoke is usually produced using the pyrolysis method. Pyrolysis of cellulose and hemicellulose results in acetic acid and aldehydes. Decomposition of lignin results in phenols and their derivatives. Different types of biomass produce different levels of acetic acid, phenols, and carbonyls [3]. These components indicate the liquid smoke is of good quality [4]. Phenol and acetic acid components possess antioxidant and antibacterial properties that can be applied in pharmaceutical, food, medical, and other industries. Previous research has reported that liquid smoke contains an antibacterial agent that can inhibit microorganism growth and is effective against Pseudomonas fluorescens [5], Streptococcus mutans [4], Listeria monocytogenes [6], black pod disease [7], Escherichia coli, Saccharomyces cerevisiae [8], and Salmonella typhimurium [9].
Liquid smoke has an acidic pH that can inhibit the growth of pathogens and spoilage bacteria. Phenolic compounds can damage cell wall membrane formation and denature proteins. Raw materials that have been used to create liquid smoke include coconut shells [5], palm kernel shells [4,7,10-12], durian peel waste [13,14], and oil palm empty fruit bunches [3]. However, little information is known about the antibacterial activity of liquid smoke from cacao pod shells. The goal of this study is to investigate the antibacterial activity of liquid smoke from cacao pod shells on *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Bacillus subtilis*.

2. Methodology

2.1. Preparation of liquid smoke
The raw cacao pod shells used in this study were obtained from several cities in Aceh: Takengon, Langsa, and Saree. The research methodology to produce liquid smoke follows the same methodology used in previous research [11]. Liquid smoke was produced in a stainless steel pyrolysis reactor consisting of a 50-cm-long cylindrical chamber with a diameter of 32 cm. The raw material (Cacao pod shells) was then dried for five days in the sun. Three kg of the dry cacao pod shells underwent pyrolysis at 300 °C (T1), 340 °C (T2), and 380 °C (T3) for about 1.5 hours. Pyrolysis of the cacao pod shells results in crude liquid smoke, tar, and charcoal. The crude liquid smoke was purified using the distillation method at 190 °C to produce grade 1 liquid smoke.

2.2. Antibacterial assessment of liquid smoke
The Gram-negative bacteria used *Escherichia coli* ATCC (American Type Culture Collection) 25992 and *Salmonella choleraesuis* ATCC 14028, and the Gram-positive used *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. The antibacterial activity was determined using the agar disk diffusion method (Kirby–Bauer test) measuring the clear zone formed. An antibacterial activity test has already been given to several concentrations (K) of liquid smoke: 1% (K1), 2% (K2), 3% (K3), 4% (K4), and 5% (K5). The positive controls used were amoxicillin (Gram positive) and tetracycline (Gram negative). Sterile aquades were used for the negative controls. A bacterial suspension was applied to the Mueller-Hinton agar media (Oxoid CM0337B). Sterile paper disks soaked in various concentrations of liquid smoke were placed on the agar media containing bacteria (standardized 0.5 Macfarland) and incubated at 37 °C for 24 hours. The diameter of the clear zone was calculated, and if it was resistant to antibacterial agents, then no clear zone formed.

2.3. Statistical analyses
A one-way ANOVA was conducted for a statistical analysis. It was followed by a least significant difference (LSD) procedure for significant difference treatments, for which α was less than 0.05 with a mean ± a standard deviation. The software SPSS (version 22) for Windows was used.

3. Results and discussion

3.1. The effects of liquid smoke on gram negative bacteria
An antibacterial activity test was conducted that used two strains of bacteria, namely *E. coli* and *S. choleraesuis*, which were gram negative bacteria. The structures of the gram negative bacteria cell membranes comprised lipopolysaccharides, lipoproteins, and some macromolecules that could inhibit the penetration of antibacterial liquid smoke into the cell membranes [8,15]. The antibacterial activity test was performed using the Kirby-Bauer method, and antibacterial power strengths were categorized as very weak (an inhibitory zone diameter of less than 5 mm), medium (an inhibitory zone diameter of 5 mm–10 mm), strong (an inhibitory zone diameter of greater than 10 mm–20 mm), and very strong (an inhibitory zone diameter of greater than 20 mm–30 mm), as per Tripsila et al. [16].
cial liquid smoke could inhibit bacteria. Diameters belonged to the medium category for their antibacterial properties against gram positive bacteria. Tables 3 and 4 show that the inhibitory zone diameters of this bacteria comprised peptidoglycan and was coated by tough and hard mesh cell walls. Two species of gram positive bacteria were used for this study: *Streptococcus aureus* and *Listeria monocytogenes*. Further, research by Faisal, found that liquid smoke from oil palm kernel shells that experienced pyrolysis at 340°C could inhibit *Streptococcus choleraesuis*. A previous study found that the use of commercial liquid smoke could inhibit *Listeria monocytogenes* [6]. Further, research by Faisal, found that liquid smoke from oil palm kernel shells that experienced pyrolysis at 340°C could inhibit *Streptococcus choleraesuis* [14].

### 3.2. The effects of liquid smoke on gram positive bacteria

Two species of gram positive bacteria were used for this study: *S. aureus* and *B. subtilis*. A total of 90% of this bacteria comprised peptidoglycan and was coated by tough and hard mesh cell walls [8,17]. Tables 3 and 4 show that the inhibitory zone diameters for the smokes were 6 mm–7.36 mm, and these diameters belonged to the medium category for their antibacterial properties against gram positive bacteria. The negative control (Aquadest) had no inhibition zone.

#### Table 1. Data for the *E. coli* inhibitory zone diameters that were tested.

| Pyrolysis Temperature (°C) | K1  | K2  | K3  | K4  | K5  | Control |
|---------------------------|-----|-----|-----|-----|-----|---------|
| T1                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.17±0.00 bA | 17.92 |
| T2                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.03±0.01 b/i | 18.39 |
| T3                        | 6.07±0.01 aA | 6.07±0.01 aA | 6.13±0.01 aA | 7.05±0.01 bA | 18.54 |

Note: that the combination of lowercase (liquid smoke concentration) and uppercase (pyrolysis temperature) data shows the relationship between the two variables, which was significantly different with an α of less than 0.05.

#### Table 2. Data for the *S. choleraesuis* inhibitory zone diameters that were tested.

| Pyrolysis Temperature (°C) | K1  | K2  | K3  | K4  | K5  | Control |
|----------------------------|-----|-----|-----|-----|-----|---------|
| T1                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.13±0.02 bA | 7.22±0.02 bA | 16.28 |
| T2                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.03±0.01 b/i | 7.12±0.03 b/i | 17.04 |
| T3                        | 6.07±0.01 aA | 6.07±0.01 aA | 6.13±0.01 aA | 7.05±0.01 bA | 7.17±0.06 bA | 17.96 |

Note: that the combination of lowercase (liquid smoke concentration) and uppercase (pyrolysis temperature) data shows the relationship between the two variables, which was significantly different with an α of less than 0.05.

The antibacterial activity of the liquid smoke showed the presence of various concentrations and temperatures of pyrolysis in different inhibitory zone diameters. As aforementioned, and as shown in Tables 1 and 2, the inhibitory zone diameters of 6 mm–7.22 mm were categorized as medium in regards to their antibacterial properties against *E. coli* and *S. choleraesuis*. Liquid smoke *K1–K3* had treatments *T1–T3*, and these liquid smoke did not have significantly different inhibitory diameters. However, liquid smoke *K5*, which had treatment *T1*, was the most effective at inhibiting the growth of *E. coli* bacteria. As the concentration of liquid smoke increased, the inhibitory zone grew; it was the largest and showed an increase in antibacterial activity. However, the LSD procedure showed that in regards to temperature pyrolysis was not significantly different, although liquid smoke *K4* was significantly different for *E. coli*.

The negative control (Aquadest) had an inhibition zone of 0 mm for each bacteria. The greatest inhibition zone was found for the positive control, which had strong diameter values of 16.28 mm–18.54 mm. A previous study found that the use of commercial liquid smoke could inhibit *Listeria monocytogenes* [6]. Further, research by Faisal, found that liquid smoke from oil palm kernel shells that experienced pyrolysis at 340°C could inhibit *Streptococcus choleraesuis* [14].

#### Table 3. Data for the *S. aureus* inhibitory zone diameters that were tested.

| Pyrolysis Temperature (°C) | K1  | K2  | K3  | K4  | K5  | Control |
|---------------------------|-----|-----|-----|-----|-----|---------|
| T1                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.01±0.01 bA | 7.02±0.00 bA | 28.74 |
| T2                        | 6±0.00 aA | 6±0.00 aA | 6±0.00 aA | 7.02±0.00 bA | 7.02±0.02 bA | 28.73 |
| T3                        | 6±0.00 aA | 6±0.00 aA | 6.53±0.12 bA | 7.01±0.01 bA | 7.20±0.06 bA | 28.81 |

Note: that the combination of lowercase (liquid smoke concentration) and uppercase (pyrolysis temperature) data shows the relationship between the two variables, which was significantly different with an α of less than 0.05.
Comparisons of the inhibitory zone diameters, liquid smoke concentrations, and pyrolysis temperatures are shown in Tables 3 and 4. The efficacies of the liquid smoke from cacao pod shells at inhibiting bacteria growth were different. Liquid smokes K\textsubscript{1}-K\textsubscript{5}, which had treatments T\textsubscript{1} and T\textsubscript{2}, were not effective against \textit{S. aureus} and \textit{B. subtilis}. However, the largest inhibition of \textit{S. aureus} and \textit{B. subtilis} was noted at temperature T\textsubscript{3} with liquid smoke K\textsubscript{5}. This result indicated that this liquid smoke concentration had an antibacterial ability.

The one-way ANOVA test showed statistically significant differences (\(\alpha = 0.05\)) for treatments. Further, the LSD test showed that the highest inhibition zone diameter was obtained with treatment T\textsubscript{3} (for smoke K\textsubscript{5}). Treatments T\textsubscript{1} and T\textsubscript{2} were not significantly different. The differences in diameter and their different abilities to inhibit liquid smoke from penetrating gram negative and gram positive bacteria may have occurred due to differences in the structures of the cell walls of the bacteria.

4. Conclusions
Liquid smoke from cacao pod shells has potential antibacterial properties against \textit{E. coli}, \textit{S. choleraesuis}, \textit{S. aureus}, and \textit{B. subtilis}, which are gram negative and gram positive bacteria, respectively. Indeed, this study found that the most effective liquid smoke concentrations against the bacteria were K\textsubscript{4} and K\textsubscript{5} with inhibitory effects of 4\% and 5\%, respectively.

Acknowledgements
The authors would like to appreciate Universitas Syiah Kuala and The Ministry of Education and Culture for supporting this study.

References
[1] Badan Pusat Statistik 2017 \textit{Indonesia Cocoa Statistics} (Jakarta: BPS)
[2] Wiharto M. 2017 \textit{JPKP} (Jurnal Kimia dan Pendidikan Kimia) 21 66
[3] Faisal M, Gani A and Mulana F, Desvita H and Kamaruzzaman S 2020 \textit{Rasayan J.Chem.} 13 514
[4] Faisal M, Gani A, Husni and Daimon H 2017 \textit{Int. J. GEOMATE}. 13 116
[5] Saloko S, Darmadji P, Setiaji B and Pranoto Y 2014 \textit{Food Biosci}. 7 71
[6] Martin E M, O’Bryan C A, Lary Jr R Y, Griffis C L, Vaughn K L, Marcy J A and Crandall P 2010 \textit{Meat. Sci.}, 85 640
[7] Faisal M, Chumzurni T and Daimon H 2018 \textit{Int. J. GEOMATE} 14 36
[8] Milly P J, Toledo R T and Ramakrishnan S 2005 \textit{J. Food. Sci} 70 M12
[9] Van Loo E J, Babu D, Crandall P G and Ricke S C 2012 \textit{J. Food. Prot.} 75 1148
[10] Faisal M, Gani A, Husni and Hiroyuki D 2016 \textit{J. Eng. Appl. Sci}. 11 2583
[11] Faisal M and Gani A 2018 \textit{Int. J. GEOMEAT} 15 145
[12] Faisal M, Gani A and Husni 2018 \textit{Rasayan J.Chem}. 11 1120
[13] Faisal M, Yelvia Sunarti A. R and Desvita H 2018 \textit{Rasayan J.Chem}. 11 871
[14] Faisal M, Gani A and Mulana F 2019 \textit{F1000Res}. 8 240
[15] McDonnell G and Russell A D 1999 \textit{Clin. Microbiol. Rev}. 12 147
[16] Triprisila L F, Suharjono S, Christianto A and Fatchiyah F 2016 \textit{Mater Sociomed}. 28 244
[17] Kapoor G, Saigal S and Elongavan A 2017 \textit{J. Anaesthesiology Clin Pharmacol}. 33 300

Table 4. Data for the \textit{B. subtilis} inhibitory zone diameters that were tested.

| Pyrolysis Temperature (°C) | K\textsubscript{1} | K\textsubscript{2} | K\textsubscript{3} | K\textsubscript{4} | K\textsubscript{5} | Control + |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------|
| T\textsubscript{1}         | 6±0.00          | aA              | 6±0.00          | aA              | 6±0.00          | bA         |
| T\textsubscript{2}         | 6±0.00          | aA              | 6±0.00          | aA              | 6.04±0.01       | bA         |
| T\textsubscript{3}         | 6±0.00          | aA              | 6.06±0.01       | dB              | 6.04±0.02       | dB         |

*Note: that the combination of lowercase (liquid smoke concentration) and uppercase (pyrolysis temperature) data shows the relationship between the two variables, which was significantly different with an \(\alpha\) of less than 0.05.*