

Stability and effectiveness of cocoa husk liquid smoke in varied storage times as a control agent for *Phytophthora palmivora* infection

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ABSTRACT. Pod rot disease, caused by *Phytophthora palmivora*, is a major factor contributing to the decline in cocoa productivity. The growth of this fungus can be inhibited by using liquid smoke from cocoa husks. However, the stability and effectiveness of cocoa husk liquid smoke at different storage times have not been studied, even though these parameters are critical for its potential use as a fungicide. The purpose of this study was to test the stability and effectiveness of cocoa husk liquid smoke, at various storage time, against *P. palmivora*. Cocoa husk liquid smoke was obtained from the pyrolysis of cocoa husks at a temperature of 500 °C. The treatments consisted of storage times of 1 day, 3 days, 6 days, and 9 days. The test consisted of a stability test, in vitro test, and in vivo test. Sterile distilled water was used as a negative control and chemical fungicides (antracol) as a positive control. Data were analyzed using analysis of variance with a significant level of 5% and continued with Tukey's further test. The results showed that cocoa husk liquid smoke remained chemically stable up to 6 days of storage based on color, aroma, and pH and meet the SNI 8985:2021 quality criteria 2. The results of the in vitro test showed that storage times of up to 9 days still showed inhibitory ability against *P. palmivora*, while the results of the in vivo test showed the best performance at storage times of 6 days. The active compounds in cocoa husks liquid smoke at storage 6 days show the fenol and organic acid which able to inhibit the fungus growth.

Keywords: cocoa husk; liquid smoke; *Phytophthora palmivora*; pod rot disease; storage time

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INTRODUCTION

Indonesia, as an agricultural country with abundant biodiversity, plays a key role in the international agricultural sector. Indonesia has successfully secured the third-largest cocoa (*Theobroma cacao*) production position in the world at 2022 (Okt & Myk, 2023). However, national cocoa production continued to decline in 2023. The Indonesian Central Bureau of Statistics (2024) reported that Indonesian cacao production in 2023 decreased by approximately 2.84% compared to 2022, from 650,610 tons to 632,120 tons. One of the main causes of this decline is the attack of stem and fruit rot caused by the fungus *Phytophthora palmivora* (Zikri *et al.*, 2022). This fungus can damage 50% of the fruit and infect the stems, roots, flower stalks, and young shoots of cocoa plants (Buleleng Regency Agriculture Office, 2024).

In response to this problem, most farmers rely on synthetic chemical pesticides to control it (Misman *et al.*, 2022). Excessive use of pesticides can have detrimental impacts, such as soil and water pollution, damage to beneficial soil microorganisms, and a reduction in biodiversity (Hashimi *et al.*, 2020; Kumar *et al.*, 2023; Naz *et al.*, 2023). Furthermore, long-term use triggers pathogen resistance and increases the risk of chronic diseases such as cancer and endocrine system disorders, which can lead to decreased effectiveness and serious health impacts (FAO & WHO, 2020). Therefore, a shift in approaches to plant disease control is needed to be safer and more sustainable. One option that can be developed is the use of natural pesticides (biopesticides), which are more environmentally friendly and do not leave toxic residues (Fenibo *et al.*, 2022). One source of natural

biopesticides is liquid smoke from cocoa husks, which has been largely an organic waste product and has not been optimally utilized.

The use of cocoa husk liquid smoke as a natural fungicide has been reported by Mashuni *et al.* (2018) with the same effectiveness as synthetic fungicides as an antifungal agent on cocoa seeds. Cocoa husk extracts contain active compounds, namely flavonoid, tannin, alkaloid, saponin and triterpenoid (Surbayanti *et al.*, 2023; Wijayanti *et al.*, 2023), while liquid smoke cocoa husks contains phenol and organic acids (Wijaya *et al.*, 2017), which play a role in inhibiting bacterial growth (Mashuni *et al.*, 2018). Liquid smoke cocoa husks with concentration of 20%, can reduce fungal growth in cocoa seeds (Mashuni *et al.*, 2024). However, these studies only examined the ability of liquid smoke from cocoa husk as a fungicide against *P. palmivora*. Meanwhile, the stability and effectiveness of liquid smoke from cocoa husks stored for a specific period against *P. palmivora* have not been previously reported.

This study examines the stability and effectiveness of liquid smoke from cocoa husks over varying storage periods. This study aims to determine the stability and effectiveness of liquid smoke from cocoa husks over varying storage periods against *P. palmivora*. This research is important for providing information on the expiration date of liquid smoke from cocoa husks as a fungicide. Information regarding this will serve as a reference for the public in the production and marketing of liquid smoke from cocoa husks. This research also supports environmental conservation efforts through the concept of zero-waste agriculture, which utilizes waste from cocoa plantations, in the form of cocoa husks, as an environmentally friendly fungicide for cocoa plants.

MATERIALS AND METHODS

Study area. Cocoa husk waste was obtained from the community cocoa plantations in North Luwu Regency, Luwu Regency, and East Luwu Regency. The drying, pyrolysis, in vitro and in vivo testing processes were conducted at the Cell and Tissue Laboratory, Faculty of Science, Universitas Cokroaminoto Palopo. Identification of bioactive compounds in liquid smoke from cocoa husks using Gas Chromatography-Mass Spectrometry (GC-MS) was carried out at the PNUP Makassar Laboratory.

Preparation of liquid smoke samples from cocoa husks. Fresh cocoa husks were thoroughly washed to remove dirt and contamination, then dried in the sun for 7-10 days. Liquid smoke from cocoa husks was obtained by pyrolyzing 1,000 g of dried cocoa husks at a temperature of approximately 500 °C for 2-3 hours (Mashuni *et al.*, 2018). The liquid smoke from cocoa husks was stored in brown bottles, then placed on shelves with controlled temperature (30 ± 1 °C), humidity (80-90%), and no light exposure according to the storage durations: 1 day (P0), 3 days (P1), 6 days (P2), and 9 days (P3).

Liquid smoke stability test. Liquid smoke stored for the specified storage period was observed for changes in quality, including color, aroma, and pH (Sahrum *et al.*, 2021). The results were compared with SNI 8985:2021.

In vitro test. The in vitro test procedure used the food poisoning method with three repetitions. A total of 2 mL of cocoa husk liquid smoke from each treatment was homogenized with 18 mL of sterile potato dextrose agar (PDA) in a Petri dish (Novriyanti *et al.*, 2010). The control treatment used sterile distilled water (negative control) and the fungicide antracol (positive control) as a substitute for the cocoa husk liquid smoke. *P. palmivora* colonies (5 mm in size) were inoculated onto the surface of the solidified media (Novriyanti *et al.*, 2010) and incubated at 25 °C for 7 days. Antifungal activity (AFA) was analyzed using the following formula (Mori *et al.*, 1997):

$$\text{AFA (\%)} = \frac{\text{GC} - \text{GT}}{\text{GC}} \times 100$$

Notes:

GC = hyphal growth on the control medium (mm)

GT = hyphal growth on the test medium (mm)

Antifungal (AFA) values were further classified into antifungal activity levels (Mori *et al.*, 1997) as shown in Table 1 below.

Table 1. Antifungal activity level

AFA (%)	Level
AFA $\geq 75\%$	++++ (Very strong)
$75\% \leq \text{AFA} < 50\%$	+++ (Strong)
$50\% \leq \text{AFA} < 25\%$	++ (Moderate)
$25\% \leq \text{AFA} < 0\%$	+ (Weak)
0%	- (Not active)

In vivo test. Whole, healthy cocoa pods were thoroughly washed and dried. The pods were sterilized by spraying the entire surface with a 70% concentration of alcohol and allowed to dry. Liquid smoke from each treatment was applied to the surface of the pods and left for 15 minutes (to allow the liquid smoke to dry). Sterile aquadest served as a negative control, and antracol fungicide solution served as a positive control. The pods were punctured with a sterile needle and *P. palmivora* colonies (Ginting *et al.*, 2022) were applied. They were left for 5 days until lesions (rot symptoms) appeared. Observations were made by measuring the area of symptoms (Wartono & Taufiq, 2021), pod skin color, and bead damage.

Analysis of active compounds in liquid smoke from cocoa husks. The cocoa husk liquid smoke, which demonstrated the best results of all treatments in both in vitro and in vivo tests, was further analyzed for its active compound content. This testing was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) (Tegang *et al.*, 2020; Suryani *et al.*, 2023).

Data analysis. Data from the stability test and in vivo tests on fruit skin color and bean damage were analyzed using descriptive analysis. Data from the in vitro test and the extent of rot symptoms (in vivo test) were analyzed using analysis of variance at a 5% significance level (Tegang *et al.*, 2020) using the SPSS program version 20.0. A further Tukey test was conducted to determine the best treatment.

RESULTS AND DISCUSSION

Stability of cocoa husk liquid smoke. Color changes in the storage duration of liquid smoke indicate changes during storage (Fig. 1 and Table 2). The liquid smoke from cocoa husks stored for 1 day showed a yellowish color and became increasingly brown with increasing storage time. Meanwhile, the liquid smoke from cocoa husks darkened after 9 days of storage. These results indicate that, based on color parameters, the liquid smoke from cocoa husks stored for 1 day, 3 days, and 6 days met the quality criteria 1 and 2 of SNI 8985:2021. The color of liquid smoke from cocoa husks are caused by active compounds. According to GC-MS results of liquid smoke from cocoa husks showed that the light brown color of liquid smoke from cocoa husks stored for 6 days is caused by several compounds that produce a yellow color, namely 2-cyclopenten-1-one, 2-methyl, and creosol. Meanwhile, 2,3-dimethoxyphenol produces an orange to brownish color. Phenol and organic acid affected to the color, aroma, and antibiotics activity of wood liquid smoke (Suryani *et al.*, 2023). The color changes on the 9th day storage be caused by changes in chemical compounds or tar precipitation. The chemical transformation, compounds decompose, or react with others can be occur during storage (Xin *et al.*, 2022).

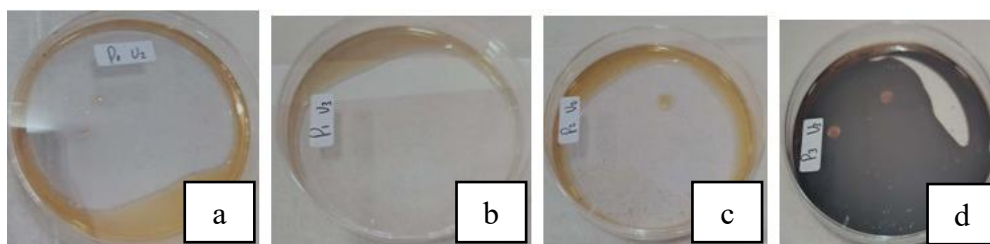


Fig. 1. Changes in color of cocoa husk liquid smoke during storage: a. 1 day, b. 3 days, c. 6 days, d. 9 days

Changes in the liquid smoke from cocoa husks also affected its aroma parameters (Table 2). This parameter is determined by variations in the organic components in the liquid smoke, particularly phenols and acids (Saputra, 2021). In the initial stages after pyrolysis, the aroma of liquid smoke from cocoa husks tends to be sharp and pungent. However, with increased storage time, changes in the composition of volatile compounds occur due to oxidation and evaporation, resulting in a milder and less intense smoke aroma. According to GC-MS results at 6th day storage, the sour aroma of liquid smoke from cocoa husks is caused by the presence of organic acids, as previously described. The pungent aroma is produced by phenol,3-methyl-. The stinging and burning sensation in the eye caused by the compound phenol,4-ethyl-2-methoxy and (2-methyl-cyclohex-2-enylidene)-acetaldehyde. Meanwhile, the smoky and burning aroma is caused by the compound creosol, 2-hydroxy-3-propyl-2-cyclopenten-1-one, phenol,4-ethyl-2-methoxy-, 4-ethyl-2,6-dimethoxyphenol, and 2,6-dimethoxy-4-propylphenol. Similar findings were also reported by Xue *et al.* (2022) found that during storage, wood vinegar decreased the phenolic and organic acid components that contribute to the distinctive aroma of liquid smoke. Research by Susianti *et al.* (2020) also showed that purification and storage methods reduced the pungent odor due to the degradation of unstable volatile fractions. The evaporation of volatile compounds in the liquid smoke of cocoa husks, causing the compounds remain the organic acids, so that the odor becomes sour and nor pungent in 9th day storage.

Table 2. Changes in the quality of liquid smoke from cocoa husks during storage

Treatment	Parameter Quality		
	Color	Aroma	pH
P0 (1 day)	Slightly yellow	Very strong smoke and sour aroma	4.00
P1 (3 days)	Yellow	Strong smoke and sour aroma	4.00
P2 (6 days)	Light brown	Less intense smoke and sour aroma	4.00
P3 (9 days)	Blackish brown	Sour aroma	4.00

The pH parameter test results for liquid smoke from cocoa husks (Table 2) showed no change in acidity (pH) and met the quality criterion 2 of SNI 8985:2021. This indicates that pH stability was quite good during storage. These results align with research by Aminah *et al.* (2024), who found that liquid smoke made from biomass waste remained stable and showed no significant pH changes for up to 14 days of storage. The pH of liquid smoke from cocoa husks caused by organic acid i.e: formic acid, propionic acid, methyl palmitate, decyl palmitate, myristic acid, oleic acid, and methyl oleate which detected in GC-MS. This pH stability indicates that the degradation of the organic acid compounds within it is very minimal (Rahmawati *et al.*, 2022). This stability is related to the presence of phenolic and flavonoid compounds in liquid smoke, which can potentially raise the pH, thereby maintaining the consistency of the chemical properties and quality of the liquid smoke during storage (Mahardani & Yuanita, 2021).

The stability of the liquid smoke is also supported by the storage container, which is a brown, airtight bottle. Strong liquid smoke in a tightly closed container at room temperature can reduce direct contact with oxygen, thereby inhibiting the oxidation process of organic compounds that can increase the pH (Nuriman *et al.*, 2021). The results of the stability test of cocoa shell liquid smoke with a storage period of up to 6 days (P2) still meet the SNI quality criteria 2 based on color, aroma, and pH parameters.

In vitro test. In vitro test results showed that the antifungal activity of cocoa husk liquid smoke against *P. palmivora* growth was very strong after storage for up to 9 days. This indicates that 1 until 9 days of storage are effective as fungicides against *P. palmivora*. However, the fungal activity (Tabel 3) at 3 days (P1), 6 days (P2), and 9 days (P3) was higher than that of the chemical fungicide (antracol). Table 3 also shows that *P. palmivora* did not grow in treatments P1, P2, and P3. This is indicated by the percentage of antifungal activity at all three storage periods reaching 100%. Whereas, AFA of P0 (1 days) demonstrated only 80.4%, accompanied by a large standard deviation (16.20%), which indicating substantial variability in the early storage stage.

Table 3. Antifungal activity of cocoa husks liquid smoke in various time storage against *Phytophthora palmivora*

Storage times	AFA (%)	Activity level
K+	93.45 $\pm$ 4.47 ^{ab}	Very strong (++++)
P0 (1 day)	80.40 $\pm$ 16.20 ^a	Very strong (++++)
P1 (3 days)	100.00 $\pm$ 0.00 ^b	Very strong (++++)
P2 (6 days)	100.00 $\pm$ 0.00 ^b	Very strong (++++)
P3 (9 days)	100.00 $\pm$ 0.00 ^b	Very strong (++++)

Notes: a-d= The means on the same row with different supercripts are significantly different ($P < 0.05$)

The results show increased antifungal activity in liquid smoke from cocoa husks appears to indicate that storage of liquid smoke from cocoa husks affects the bioactive components within them. Similar results were also reported by Sheikhjan *et al.* (2025), who showed an increase in the concentration of bioactive compounds during storage, increasing antifungal activity. The increased antifungal activity may be due to: (1) an *aging effect* may occur, in which continued chemical transformations during storage increase the concentration or availability of phenolic compounds, which are known contributors to antifungal activity; (2) the early storage stage (P0) may still contain volatile compounds that interfere with or mask the antifungal action of phenolics; their gradual volatilization could remove these inhibitory components, resulting in greater bioactivity at days 3, 6, and 9; (3) storage may facilitate reactions such as esterification, oxidation, or polymer transformation, leading to the formation of new phenolic derivatives or other oxidative products with stronger antifungal properties. Xin *et al.* (2022) explain that chemical transformations in liquid smoke can occur during storage, where the initial compounds decompose or react with each other, resulting in different chemical compound profiles over time. Another factor that may influence the increase in antifungal activity in liquid smoke after 3-9 days of storage is the evaporation of water during storage, which causes the chemical compound composition to become more concentrated. Fauziah *et al.* (2014) found that the water content of pindang layang fish decreased by 20-21% during 6 days of storage. A decrease in water content during storage of smoked skipjack tuna at cool temperature was also reported (Alinti *et al.*, 2018).

In vivo test. Testing the antifungal activity of liquid smoke from cocoa husks at various storage times showed that the highest area of cocoa pod rot symptoms caused by *P. palmivora* was observed after 9 days of storage (P3), at 20.80 cm. Meanwhile, 6 days of storage (P2) showed high effectiveness, with no pod rot symptoms appearing on the surface of the cocoa pods or the inner tissue of the cocoa pods (Fig. 2). The effectiveness of liquid smoke from cocoa husks stored for 6 days is comparable to that of chemical fungicides (antracol). Sari *et al.* (2024) suggested that intermediate storage periods yielded the best compound stability. Short-term storage still maintained strong phenolic concentrations in liquid smoke (Xue *et al.*, 2022). These results indicate that applying liquid smoke to cocoa husks stored for 6 days optimally inhibited *P. palmivora* infection with an effectiveness equivalent to synthetic pesticides. The ability of liquid smoke to inhibit the growth of *P. palmivora* is thought to be caused by changes in chemical content such as changes in phenolic compounds, organic acids, or other bioactive components, or reactions with each other, which may enhance antifungal activity at 3 and 6 days (Xin *et al.*, 2022; Sheikhjan *et al.*, 2025). Meanwhile, reducing the water content during storage (Fauziah *et al.*, 2014) causes the extract to become more

concentrated, resulting in saturation of the extract and difficulty in diffusing into the fungal cells (Putri *et al.*, 2019b).

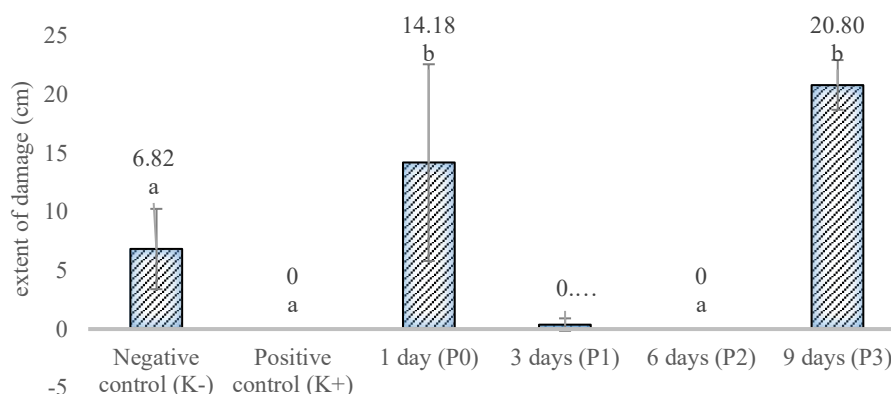


Fig. 2. Extent of damage in cocoa pod. Notes: a-d= The means on the same row with different supercripts are significantly different ($P < 0.05$)

In contrast to the results shown by other treatments, liquid smoke cocoa husks stored for 1 day showed a greater area of symptoms than the negative control treatment (sterile distilled water). The surface of the cocoa pods smeared with liquid smoke cocoa husks, with a storage period of 1 day, showed blackened fruit color and the presence of fungal hyphae. However, the results of observations on the beans showed that *P. palmivora* fungal infection in P1 affected some of the beans, unlike the negative control, which had infected almost half of the total beans. Similar results were also shown by P2 and P3. Figure 3 also shows that the surface of the cocoa pod skin in P2 was dark or blackish. However, this color was not caused by symptoms of major *P. palmivora* infection. This color appeared approximately 15 minutes after the liquid smoke cocoa husks were applied to the cocoa pods and darkened further over the observation day, so it is suspected that the blackish color is the effect of the absorption of the liquid smoke compounds in the cocoa pods.

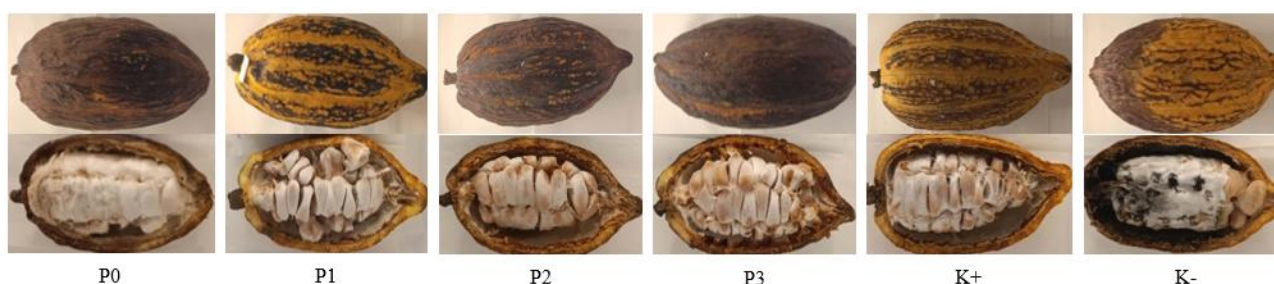


Fig. 3. Morphology of skin pods and bean in each treatment

Active compounds in cocoa husk liquid smoke. In vitro and in vivo test results showed that cocoa husk liquid smoke stored for 6 days (P2) provided the best results for controlling *P. palmivora*. Therefore, the cocoa husk liquid smoke stored for 6 days was further analyzed for active compounds using Gas Chromatography-Mass Spectrometry (GC-MS). Figure 4 shows that 45 compounds were detected by GC-MS. This indicates that cocoa husk liquid smoke has a complex chemical composition that includes dominant compounds such as formic acid, 2-methylpropyl ester (32.77%) with potential as an aroma and flavoring, mequinol (10.71%) with potential as an antioxidant and antifungal, as well as various aromatic compounds (phenols and their derivatives), fatty acid esters, aromatic compounds, heterocyclic compounds (pyrazole and furan derivatives from thermal decomposition), and oxygenate compounds (such as furfural which contributes to the caramel/ sweet aroma).

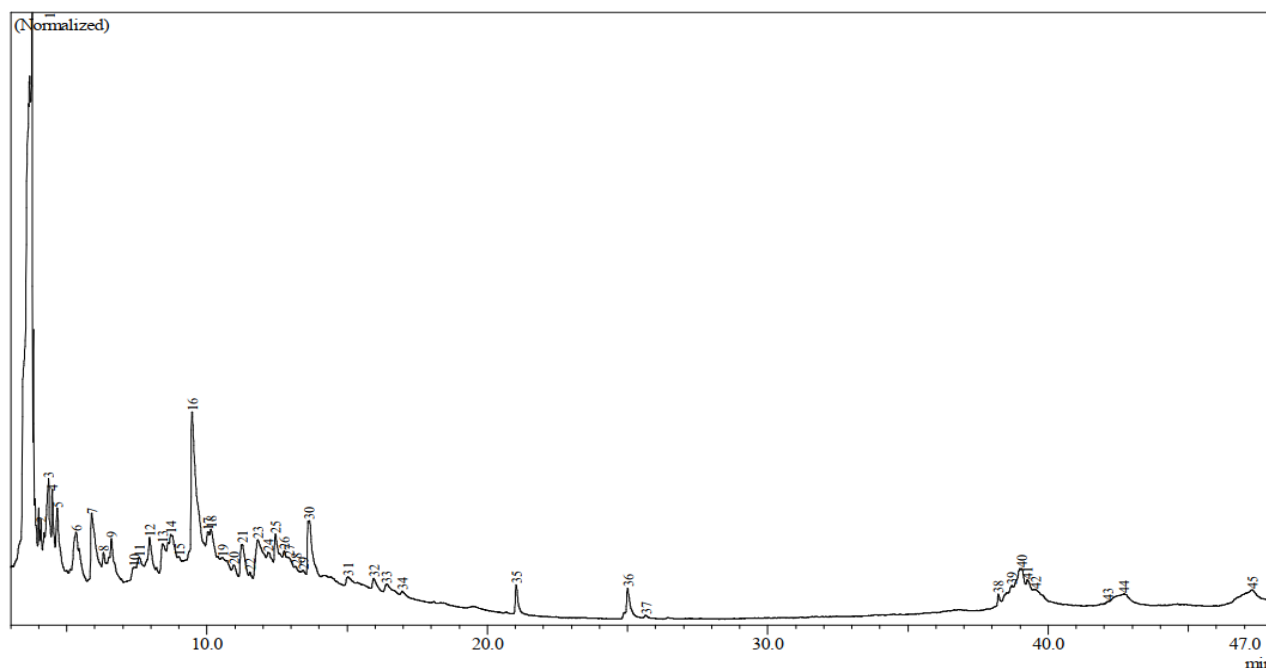


Fig. 4. Chromatogram of GC-MS 6th day storage result

Phenolic compounds include mequinol, fenol, creosol, phenol,3-methyl, **phenol,4-ethyl-2-methoxy-**, **2,3-Dimethoxyphenol**, **3,5-Dimethoxy-4-hydroxytoluene**, **4-Ethyl-2,6-dimethoxyphenol**, **Guaiacol**, **4-butyl-**, **2,6-Dimethoxy-4-propylphenol**. Phenolic compounds were the most diverse compounds in the liquid smoke from cocoa husks stored for 6 days. Phenolic compounds are known to have antioxidant and antifungal properties. Phenolic compounds can damage membrane, denature membran's protein, and lyse fungal cell walls which result in inhibit the cell growth (Rachmawaty *et al.*, 2018).

Table 4. Antifungal content in liquid smoke from cocoa husks stored for 6 days based on *Gas Chromatography-Mass Spectrometry* (GC-MS)

Active compound name	Peak number	Percentage area (%)	Similarity (%)
Organic acid			
Formic acid, 2-methylpropyl ester	1	32.77	94
Propanoic acid	3	3.24	94
Hexadecanoic acid, methyl ester	35	0.69	97
9-Octadecenoic acid (Z)-, methyl ester	36	1.18	94
Hexadecanoic acid, decyl ester	39-41	5,25	80
6-Octadecenoic acid	45	1.69	85
Phenolic			
Mequinol	16	10.71	94
Phenol, 3-methyl-	17	1.26	92
Creosol	21	1.41	94
Phenol, 4-ethyl-2-methoxy-	25	1.92	88
2,3-Dimethoxyphenol	30	2.45	91
3,5-Dimethoxy-4-hydroxytoluene	31	0.33	89
4-Ethyl-2,6-dimethoxyphenol	32	0.36	93
Guaiacol, 4-butyl-	33	0.43	91
2,6-Dimethoxy-4-propylphenol	34	0.13	80
Others			
1,2-propanediol butyrate	12	2.25	86
2-Hydroxy-3-propyl-2-cyclopenten-1-one	22	0.11	89

The analysis of the active compounds in the liquid smoke from cocoa husks stored for 6 days also identified organic acids, which are responsible for determining the pH of the liquid smoke from

cocoa husks. These organic acid compounds are formic acid, propionic acid, methyl palmitate, decyl palmitate, myristic acid, oleic acid, and methyl oleate. These acid compounds can also act as antifungals. Propionic acid has significantly minimum fungicidal concentration to inhibited mycelial growth of *macrophomina phaseolina*, *Botrytis cinereal*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, and *Rhizoctonia solani* (Sehirli & Saydam, 2016). Active compounds known as antifungals and not belonging to the phenol, flavonoid, or organic acid groups include 1,2-propanediol 1,2-propanediol butyrate and 2-hydroxy-3-propyl-2-cyclopenten-1-one.

In previous study, cacao husk liquid smoke contains acetic acid (Putri *et al.*, 2019a; Wijaya *et al.*, 2024), but in this study, acetic acid was absence. The absence of acetic acid in cacao husk liquid smoke caused by pyrolysis temperature. Acetic acid detected at 112-512 °C, but no acetic acid detected at 300-400 °C (Devita *et al.*, 2021; Wijaya *et al.*, 2024). Acetic acid is decomposition of hemicellulose and cellulose (Wijaya *et al.*, 2020) which found in cocoa husk. Meanwhile, derived of lignin degradation is the phenol group in high temperature (Putri *et al.*, 2019a). Wijaya *et al.* (2017) reported that cocoa husks contain 17.27 % cellulose, 19.56 % hemicellulose, and 52.02% lignin. These compounds gradually degraded in different temperature. Hemicellulose starts to be degraded at 200 °C, cellulose and lignin degraded at 240 °C, and aromatic layer formation at 400 °C (Putri *et al.*, 2019a).

The carcinogenic compound as reported by Desvita *et al.* (2021), namely 1,3-benzodioxole, 4-methoxy-6- (2-propenyl) 1,3 benzodioxole was not found in cocoa husk liquid smoke. The absence of this particular carcinogenic compound suggests a potentially lower risk; however, further toxicological assessment and comprehensive profiling of PAHs and other hazardous compounds are required before the liquid smoke can be considered safe for food preservation.

CONCLUSION

The storage time of liquid smoke from cocoa husk which shows the best stability at 1-6 days storage in color, aroma, and pH parameters. The liquid smoke from cocoa husks meet the SNI 8985:2021 quality criteria 2. The liquid smoke from cocoa husks effective to inhibit the growth of *P. palmivora* fungus at 6th day storage according to in vitro test with 100% AFA value and in vivo test with no extent of damage to cocoa pods. This effectiveness caused by fenol and organic acids compounds in liquid smoke from cocoa husks. This study shows that liquid smoke from cocoa husks at 6th day storage is effective in inhibiting the growth of *Phyrophthora palmivora* even without prior purification treatment. However, this study is stil limited laboratory testing. In addition, the composition of active compounds at each storage period has not been detected. Therefore, this study can be continued by analyzing the fluctuation of compounds at each storage periode and further testing on a large scale or for other uses.

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