



Effect of Pyrolysis Temperature on Chemical Characteristics and Toxicity of Liquid Smoke from Teak Sawdust

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ABSTRACT

Liquid smoke derived from lignocellulosic biomass has attracted increasing attention as a natural preservative due to its antimicrobial and antioxidant properties. The chemical composition and toxicity of liquid smoke are strongly influenced by pyrolysis temperature. This study investigated the effect of pyrolysis temperature (320–400 °C) on the chemical characteristics and toxicity of liquid smoke produced from teak (*Tectona grandis*) sawdust. The product was characterized through pH and total acidity measurements, FTIR and GC–MS analyses, and toxicity evaluation using the Brine Shrimp Lethality Test (BSLT). The liquid smoke exhibited acidic properties with pH values ranging from 3.5 to 4.5 and total acidity between 0.3–0.6% (w/v). FTIR spectra confirmed the presence of phenolic, carbonyl, and methoxy functional groups originating from lignin and cellulose degradation. GC–MS analysis revealed dominant phenolic compounds, particularly phenol and 2-methoxyphenol, along with acid esters and carbonyl derivatives contributing to antioxidant and antimicrobial potential. The LC₅₀ values ranged from 981.84 to 1819.57 ppm, indicating non-toxic to low toxicity levels. Increasing pyrolysis temperature enhanced chemical stability and reduced toxicity. No polycyclic aromatic hydrocarbons (PAHs) were detected. Teak sawdust is a promising biomass source for producing environmentally friendly and food-grade liquid smoke, with higher pyrolysis temperatures improving product safety and quality.

Keywords: biomass, food, liquid smoke, pyrolysis, toxicity test.

1. INTRODUCTION

The use of lignocellulosic biomass as a source of renewable chemicals continues to grow due to its potential to produce environmentally friendly and economically valuable natural products. One product of the thermochemical conversion of biomass is liquid smoke, produced by the pyrolysis of organic materials such as wood and sawdust [1,2]. Liquid smoke contains various bioactive compounds, especially organic acids, phenols, guaiacol, and carbonyl compounds, which play an important role in antimicrobial activity, antioxidant, and natural preservative properties [3,4]. Studies show that the chemical characteristics and toxicity levels of liquid smoke are highly

dependent on the pyrolysis temperature, heating time, and the type of raw material used [5,6].

Teak (*Tectona grandis*) is a lignocellulosic material rich in lignin and extractives, making it a potential candidate for producing high-quality liquid smoke. Unutilized teak sawdust waste from the wood-processing industry can be used as a high-value biomass [7]. Previous studies have shown that higher pyrolysis temperatures lead to greater decomposition into phenols and aromatic compounds, and can affect the toxicity level of liquid smoke [6,8]. However, a comprehensive study on the relationships among pyrolysis temperature, chemical composition, and the toxicity of liquid smoke

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from teak sawdust still requires further research.

Analysis of general chemical properties such as pH, total acidity, and functional groups, as well as identification of the main constituent compounds using Gas Chromatography–Mass Spectrometry (GC–MS), is important to gain an in-depth understanding of the profile of bioactive compounds in liquid smoke [9,10]. The GC–MS test is capable of detecting and identifying volatile compounds in high concentrations, such as phenol, acetic acid, and furfural, which can be correlated with toxicity test results to determine the dominant compounds that are potentially toxic or biologically beneficial [4,11]. The combination of FTIR analysis, GC–MS, and toxicity tests is expected to provide a more comprehensive picture of the effect of pyrolysis temperature on the chemical composition and toxicity of liquid smoke [12].

This study aims to analyze the effect of pyrolysis temperature on the chemical characteristics and toxicity levels of liquid smoke from teak sawdust using a multidisciplinary approach through pH, total acid, FTIR, GC–MS, and toxicity tests. The research results are expected to contribute to the development of safer, higher-quality, and environmentally friendly liquid smoke production technology, as well as encourage the use of teak wood waste as a raw material for economically valuable biomass.

Compared with other commonly studied lignocellulosic biomasses such as coconut shells, rice husks, and palm empty fruit bunches, teak sawdust possesses a relatively high lignin content and unique extractive compounds that may influence the formation of phenolic derivatives during pyrolysis. These compositional differences are expected to affect not only the chemical stability and bioactivity of the resulting liquid smoke but also its toxicity profile. Despite the growing interest in biomass-derived liquid smoke, systematic studies that simultaneously evaluate the relationship between pyrolysis temperature, detailed chemical composition, and toxicity

characteristics of teak sawdust-based liquid smoke remain limited. Therefore, this study aims to address this research gap by providing an integrated assessment of physicochemical properties and toxicity behavior to support the development of safer and higher-value biomass utilization.

2. RESEARCH METHODS

2.1. Materials

The tools used in this research were a pyrolysis tool and a distillation tool set (Figure 1). The liquid smoke product obtained from this process was characterized to determine some of its properties using the toxicity test using Brine Shrimp Lethality Test (BSLT) method, Fourier Transform Infra-Red Spectroscopy (FTIR) Shimadzu IR prestige 21, and Gas Chromatography–Mass Spectrometry (GC–MS) Ultra QP-2010 Shimadzu was used to study the chemical content and toxicity level in the product.

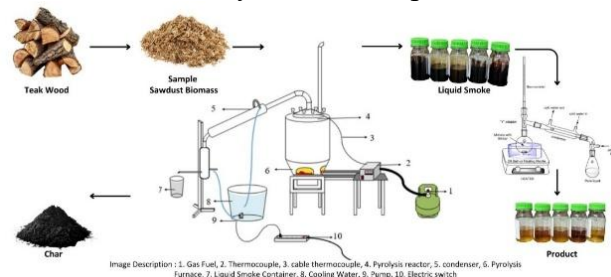


Figure 1. Research tools and process design.

2.2. Experiments

Teak wood sawdust was first dried naturally under sunlight for three days until it reached an even dry condition. After the drying process, the sawdust was weighed as much as 1 kg for each pyrolysis experiment, then put into a pyrolysis reactor and carbonized for 1 hour at various temperatures of 320°C, 340°C, 360°C, 380°C, and 400°C. The liquid smoke obtained from pyrolysis was then distilled twice to obtain a grade 1 liquid smoke product, which was then characterized to determine the level of toxicity [8,13].

2.3. Product characterization

2.3.1. pH and Total Acid

The pH measurement of the liquid smoke sample was performed using universal

indicator paper. The indicator strip was immersed in the sample until a color change was observed, and the resulting color was compared with the standard pH color scale to determine the pH value. The obtained pH values were evaluated against the Indonesian National Standard for liquid smoke (SNI 8985:2021).

The total acidity of the liquid smoke was determined using the acid–base titration method. A 10 mL aliquot of the sample was diluted with 90 mL of distilled water and mixed thoroughly to obtain a homogeneous solution. The diluted sample was then transferred into an Erlenmeyer flask, and three drops of phenolphthalein indicator were added. Titration was carried out using 0.1 N NaOH until a persistent pale pink colour appeared, indicating the titration endpoint. The acid content was calculated based on the total titratable acidity formula and expressed as a percentage (% w/v). All measurements and interpretations were conducted in reference to SNI 8985:2021 for liquid smoke quality standards.

$$\% \text{ Total Acid} = \frac{(V \times N)_{\text{NaOH}} \times \text{Eq. Wt} \times 100}{\text{Sample weight or volume}} \dots\dots (1)$$

2.3.2. FTIR (Fourier Transform Infrared Spectroscopy) Testing

The liquid smoke sample was prepared for FTIR analysis using the KBr pellet method. In this procedure, the sample was thoroughly mixed with spectroscopic-grade potassium bromide (KBr) powder at a ratio of 1:10 (sample:KBr, w/w). The resulting mixture was then dried and finely ground to obtain a homogeneous powder. Subsequently, the mixture was compressed using a pellet press to form a thin, transparent pellet suitable for infrared measurement. The prepared pellet was immediately placed in the FTIR sample compartment, and the infrared spectrum was recorded over the relevant wavenumber range.

2.3.3. Gas Chromatography Analysis

The analysis was performed by introducing 0.1 mL of the sample into a 100 mL beaker, followed by the addition of 5 mL of methanol. The mixture was covered with aluminum foil and subjected to ultrasonic extraction at 70°C for 20 minutes. After extraction, the solution was filtered through a syringe filter and transferred into a GC vial for analysis. Gas chromatography-mass spectrometry (GC–MS) analysis was conducted using a Shimadzu GC–MS Ultra QP 2010 instrument equipped with an SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm i.d.). The injector temperature was maintained at 250°C and operated in split less mode with a pressure of 76.9 kPa, carrier gas flow rate of 1.4 mL/min, and a split ratio of 1:10. The ion source and interface temperatures were set at 200°C and 280°C, respectively. The solvent cut time was 3 min, and data were acquired over a mass range of 40–700 m/z. The oven temperature program began at 70 °C (held for 2 min), increased to 200 °C at 10 °C/min, and then raised to 280 °C (held for 9 min) at 5 °C/min, resulting in a total analysis time of approximately 30 min. The resulting chromatograms were interpreted using the NIST 20 mass spectral library for compound identification.

2.3.4. Toxicity test using the Brine Shrimp Lethality Test (BSLT) Method

The brine shrimp lethality test (BSLT) was conducted to evaluate the toxicity of the liquid smoke sample using *Artemia salina* larvae as the bioindicator. Seawater (500 mL) was filtered and aerated for 2 hours to serve as the hatching medium. Approximately 0.5 g of *A. salina* eggs were disinfected with 2% sodium hypochlorite (NaOCl) for 5 minutes, rinsed thoroughly with seawater, and transferred into the hatching medium. The medium was continuously aerated under yellow light illumination for 48 hours until the eggs hatched into nauplii. A stock solution of the liquid smoke sample was prepared at 500 ppm by dissolving 0.005 g of the sample in 10 mL of seawater. This stock

was serially diluted to obtain concentrations of 100, 400, 600, and 800 ppm, with a total volume of 5 mL for each concentration. Ten nauplii of *A. salina* were transferred into each test tube containing the respective concentrations and incubated for 24 hours under controlled conditions. After incubation, the number of dead larvae was counted, and the mortality percentage was used to determine the toxicity level (LC_{50} value) of the liquid smoke sample.

2.3.5. Toxicity Test on Aquatic Animals

The acute aquatic toxicity test of the liquid smoke sample was conducted using mosquito larvae (*Aedes* spp.) as the test organism. Groups of 5, 10, and 15 larvae were introduced into separate containers containing test water. Liquid smoke was then added to achieve final concentrations of 1%, 5%, and 10% (v/v). The test containers were maintained under controlled laboratory conditions for 24 hours without feeding. After the exposure period, the numbers of dead and surviving larvae were recorded. The larval mortality rate was subsequently used to assess the toxicity level of the liquid smoke sample. The aquatic larvae bioassay was conducted to provide complementary ecological toxicity information and to observe biological responses of living organisms exposed to liquid smoke under controlled conditions. This test was not intended to establish insecticidal application but to broaden the understanding of environmental safety aspects.

3. RESULTS AND DISCUSSION

3.1. Liquid Smoke Toxicity Test

3.1.1. Results of pH and Total Acid tests

Based on the results of the tests that have been carried out, as shown in Figure 2, the pH of grade-1 liquid smoke ranged from 3.5 to 4.5, indicating its acidic nature. Meanwhile, the total titratable acidity calculated from the titration results ranged from 0.3 to 0.6% (w/v), depending on the applied pyrolysis temperature. According to the Indonesian National Standard for liquid smoke (SNI 8985:2021), the required pH range for Grade

1 quality is 1.5–2.75, while Grade 2 quality is specified within the range of 2.76–4.50.

Numerically, the pH values obtained in this study (3.5–4.5) fall outside the Grade 1 requirement but remain fully within the acceptable limits for Grade 2 liquid smoke. This indicates that although the product does not meet the more stringent acidity criteria of Grade 1, it still complies with the national quality standard for Grade 2. The relatively higher pH values may be associated with the partial degradation of organic acids at elevated pyrolysis temperatures, which contributes to improved chemical stability and reduced corrosiveness of the liquid smoke.

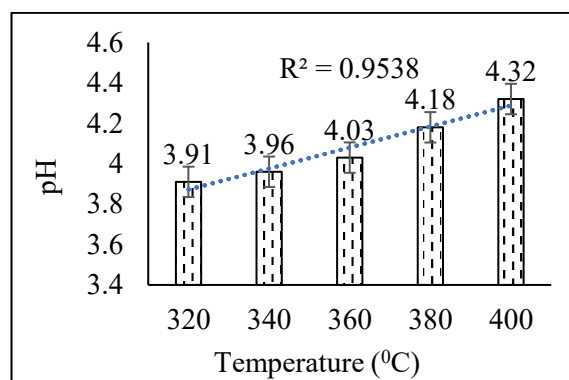


Figure 2. pH test result.

The quality of liquid smoke produced from teak wood shows a pH ranging from 3.5 to 4.5, which indicates the acidic nature of the product; while the total acid measured ranges from 0.3 to 0.6. These data reflect that as pH increases; there is a tendency for the total acid content in liquid smoke to decrease. Pyrolysis temperature also plays a significant role in the quality of liquid smoke; at 400°C, the total acid content was recorded as low as 0.3963, while at 360°C, the figure increased to 0.6305. Although the pH value obtained from this study does not fully meet the Japanese standard which sets limits between 1.5 to 3.7, the results are in accordance with the Indonesian National Standard (SNI) 8985:2021, where the pH quality for category 1 is between 1.5 to 2.75 and for category 2 reaches 2.76 to 4.5, with all results from the tested pyrolysis temperatures falling within the quality category 2 [14].

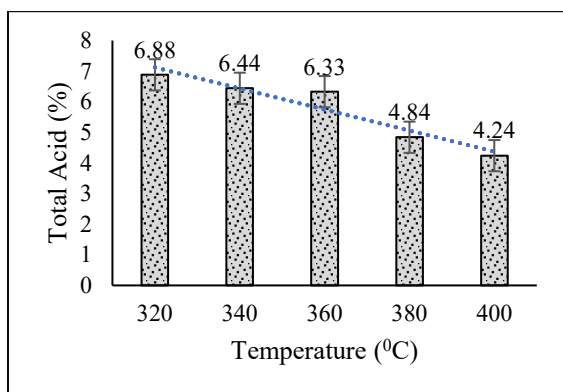


Figure 3. NaOH consumption during acid–base titration used to calculate total acidity of liquid smoke at different pyrolysis temperatures.

Figure 3 presents the NaOH consumption during the titration process, which was used to determine the total acidity values. The variation in NaOH volume reflects differences in acid content at each pyrolysis temperature. Higher pyrolysis temperatures tended to produce liquid smoke with lower total acidity, indicating progressive thermal degradation of organic acids.

Based on the analysis conducted, the acid content in liquid smoke is relatively low, ranging from 0.3 to 0.6%, which does not meet the Japanese standard set between 1% and 18% [10]. This low acid content can be attributed to the titration process, which creates high dilution, resulting in a lower acid concentration [15]. In general, the acid content in liquid smoke is influenced by several variables, such as the type of wood, the moisture content of the raw material, and the pyrolysis and distillation temperatures applied. Research shows that with increasing distillation fraction temperature, the formation of organic acids tends to increase, and conversely, lower pyrolysis temperatures produce relatively higher acid levels [3]. In addition, phenolic compounds also play an important role in determining acidity; the more phenols in liquid smoke, the higher the concentration of acids formed [16].

3.1.2. FTIR (Fourier Transform Infrared Spectroscopy) Testing

The results of the FTIR (Fourier Transform Infrared Spectroscopy) analysis in Figure 4

showed that liquid smoke (grade 1) from teak sawdust contains various functional groups typical of biomass pyrolysis. The absorption spectrum at wavenumbers 3442–3445 cm^{-1} indicates the presence of O–H groups from phenol, guaiacol, and alcohol compounds that play a role in antimicrobial and antioxidant activities [17,18]. The dominant phenolic compounds in bio-oil are known to have significant antimicrobial properties, and this antioxidant potential makes it very attractive for applications in the health and materials fields [19,20]. Absorption at 2958–2966 cm^{-1} , 2922–2931 cm^{-1} , and 2875–2877 cm^{-1} indicates the presence of C–H groups from alkyl, methyl, and methylene as a result of the degradation of cellulose and hemicellulose, which are the main components of biomass residues [21]. The peaks around 1710–1714 cm^{-1} indicate C=O groups from organic acids such as acetic acid, while the 1645–1650 cm^{-1} indicates the presence of aromatic C=C double bonds, which are commonly found in the aromatic structures of lignin-derived compounds such as guaiacol [22].

The methoxy group ($-\text{OCH}_3$) of the guaiacol compound was detected at 1375–1398 cm^{-1} , and the C–O–C absorption of secondary alcohol or ester appeared at 1030–1270 cm^{-1} , indicating the potential of this compound to function as a chemical raw material [23]. Additional peaks at 730–733 cm^{-1} , 545–550 cm^{-1} , and 376–422 cm^{-1} indicate the presence of furan compounds and aromatic vibrations, which are generally associated with bioactive compounds in bio-oil, contributing to the chemical functional properties of liquid smoke [24]. Overall, the FTIR results confirm that liquid smoke contains major bioactive compounds such as phenol, acetic acid, guaiacol, and aromatic compounds that play an important role in its antimicrobial, antioxidant, and natural preservative properties, thus demonstrating its applicability in various industrial sectors, including food preservation and pharmaceuticals [20].

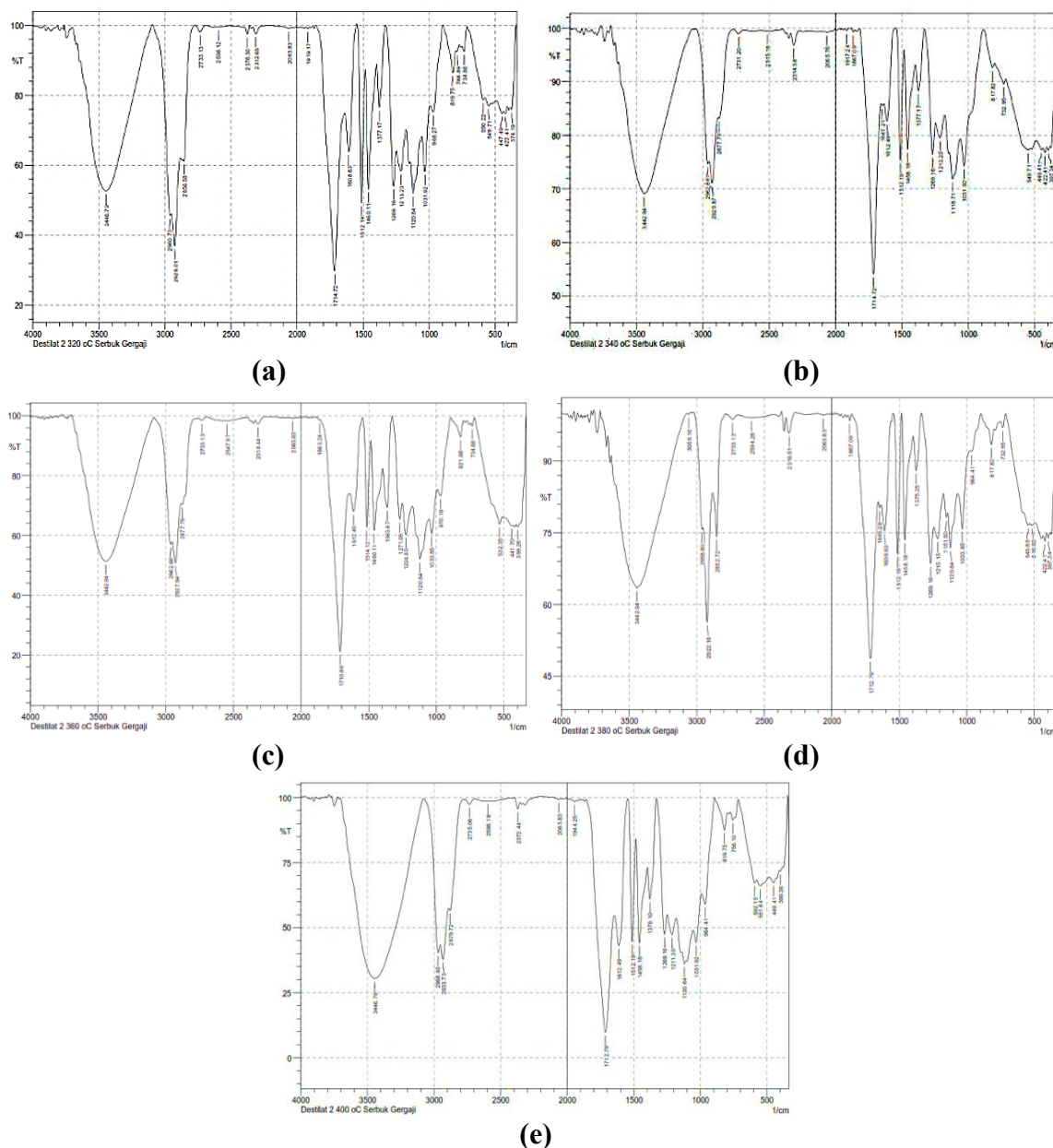


Figure 4. FTIR test at various temperatures 320°C (a), 340°C (b), 360°C (c), 380°C (d), and 400°C (e).

3.1.3. Gas Chromatography Analysis

The GC-MS analysis of liquid smoke produced from teak wood sawdust reveals a rich chemical composition comprising various pyrolysis products, prominently featuring phenolic compounds (Figure 5). Among these, phenol and its derivative 2-methoxyphenol are noted as dominant components, contributing significantly to the bioactivity, including antimicrobial and antioxidant activities of the smoke [15,25]. The presence of these phenolic compounds enhances the efficacy of liquid smoke in

preserving food and necessitates monitoring due to their potential irritant effects on human health [26,27]. Additionally, the analysis identified significant quantities of acid esters, such as formic acid, alongside carbonyl compounds like 1-hydroxy-2-butanone, which are critical in influencing the aroma, chemical stability, and sensory attributes of the product [25,28]. These compounds serve as markers for thermal degradation during pyrolysis, highlighting their role in

determining the quality and functional characteristics of the liquid smoke.

The GC–MS chromatogram presented in Figure 5 corresponds to the liquid smoke sample produced at a pyrolysis temperature of 340 °C. The identified compounds listed in Table 1 were classified into several major chemical groups to facilitate scientific interpretation.

Phenolic compounds, including phenol, 2-methoxyphenol, and various dimethoxyphenol derivatives, were found to be dominant components. These compounds are well known for their antimicrobial and antioxidant properties, which contribute significantly to the preservative function of liquid smoke.

The second major group consists of esters and organic acid derivatives, such as formic acid esters and hexanoic acid, which influence the aroma profile and chemical stability of the product. Carbonyl compounds, including ketones and aldehydes such as 1-hydroxy-2-butanone and butanal, were also detected and are typically associated with thermal degradation reactions during pyrolysis.

Minor compounds belonging to hydrocarbon and nitrogen-containing groups were present in smaller proportions and contribute to the overall chemical complexity and sensory characteristics of the liquid smoke. The predominance of phenolic and acid-derived compounds indicates that teak sawdust liquid smoke possesses strong potential as a natural preservative with functional bioactive properties [29]. Notably, the absence of polycyclic aromatic hydrocarbons (PAHs) underscores the safety of using teak wood sawdust-based liquid smoke for human consumption, as it minimizes toxicological risks [26]. The quantitative insights provided by relative peak areas in the GC-MS analysis are vital for quality control, enabling producers to refine processes to ensure compliance with food-grade standards [30]. Overall, the composition of teak wood-derived liquid smoke, rich in phenolic and acid compounds while devoid of harmful

PAHs, represents a promising avenue for safe food preservation and enhancement.

Table 1. GC-MS test result.

Chemical Compound	Composition
Ammonium acetate	5.01
1-Hydroxy-2-butanone	0.78
1,5-Cyclooctadiene, 1,2,3,3,8,8-hexadeutero-	1.22
1,2-Ethanediol, diformate (CAS)	1.16
3-Hexene, (E)-	0.30
Hexanoic acid (CAS)	0.28
Norvaline, 3-hydroxy-	0.53
4-Cyclohexene-1,2-diol	0.79
Cyclopropyl carbinyl-D2-methyl ether	4.23
Formic acid, 2-methylpropyl ester	16.48
1-Hydroxy-2-butanone	12.98
1,2,3-Thiadiazole, 5-methyl- (CAS)	4.08
Butanal (CAS)	1.87
2-Butenoic acid (CAS)	0.18
N-(n-Butoxymethyl)acrylamide	2.64
2-Cyclopenten-1-one, 3-methyl-	4.75
1-Penten-1-one, 2-methyl- (CAS)	0.73
Phenol (CAS)	5.01
2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS)	5.02
Phenol, 2-methoxy- (CAS)	2.78
Phenol, 2-methoxy- (CAS)	13.05
Ethylcyclopentenolone	0.73
Ethanone, 1-cyclohexyl-	0.47
2-Methoxy-4-methylphenol	1.66
2-Methoxy-4-methylphenol	5.47
Phenol, 4-ethyl-2-methoxy- (CAS)	0.46
Phenol, 4-ethyl-2-methoxy- (CAS)	2.05
1,2-Benzenediol (CAS)	0.61
Phenol, 2,6-dimethoxy- (CAS)	3.51
3,5-Dimethoxy-4-hydroxytoluene	0.25
2,6-Dimethoxy-4-propylphenol	0.14
Total	99.99%

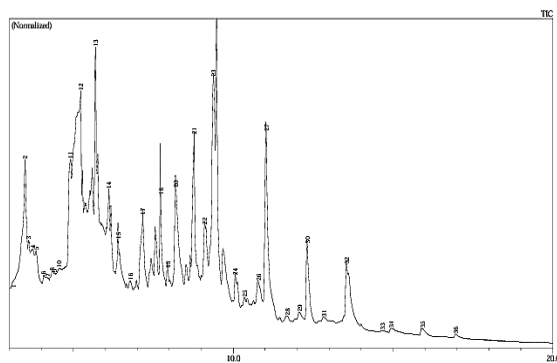


Figure 5. Chromatogram of GC-MS analysis of liquid smoke of sawdust at 340°C.

3.2. Liquid Smoke Toxicity Test

3.2.1. Toxicity test using the Brine Shrimp Lethality Test (BSLT) method.

Toxicity testing using the Brine Shrimp Lethality Test (BSLT) method on liquid smoke produced from teak sawdust showed that its toxicity level varies depending on the pyrolysis temperature. In a recent study, the LC_{50} values obtained ranged from 981.84 to 1819.57 ppm (Figure 6), indicating a tendency for toxicity to decrease as the pyrolysis temperature increases. Higher pyrolysis temperatures contribute to the degradation of toxic compounds such as phenols, organic acids, and carbonyls into simpler, less harmful compounds [31]. The results of the study showed that the related assessors, which involved the log concentration and probit value, produced an R^2 value of more than 0.96 (Figure 7), indicating that there was a strong relationship and a good regression model in describing the mortality of *Artemia salina* larvae [32]. Based on existing toxicity criteria, an LC_{50} value above 1000 ppm indicates that grade 1 liquid smoke can be categorized as non-toxic to low toxicity, so it is relatively safe for further use [33]. Research shows that increasing the pyrolysis temperature not only improves the aroma quality and chemical properties of liquid smoke, but also reduces the potential toxicity, making it more suitable for development as a safe and environmentally friendly natural preservative [31].

The LC_{50} values obtained from the BSLT assay ranged from 981.84 to 1819.57 ppm, indicating that the toxicity of liquid smoke decreased with increasing pyrolysis temperature. This trend can be associated with changes in chemical composition identified through GC-MS analysis. At higher temperatures, the relative proportion of reactive carbonyl and low-molecular-weight volatile compounds tends to decrease due to further thermal degradation, while more stable phenolic derivatives become dominant. The reduction of these reactive compounds may contribute to the lower toxicity observed at elevated pyrolysis temperatures.

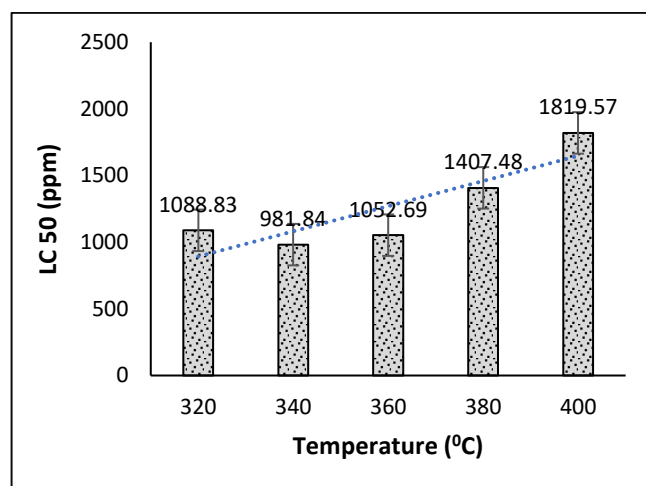


Figure 6. Toxicity test using the BSLT (Brine Shrimp Lethality Test) method.

3.2.2. In Vivo Toxicity Test on Aquatic Animals

At low pyrolysis temperatures of around 320°C, the resulting liquid smoke contains high concentrations of volatile compounds such as acetaldehyde, phenol, and eugenol, which have toxic activity and natural insecticidal properties in Figure 8. Studies have shown that these compounds have toxic effects on insect larvae, but the exact mortality rate of larvae cannot be ascertained from existing references [34,35]. In contrast, at higher temperatures, around 380°C, there is significant degradation of these active compounds into more stable and less reactive molecules, which can lead to changes in toxicity activity [36]. This increase in

pyrolysis temperature triggers chemical transformations in various components of liquid smoke, where phenolic and aliphatic compounds contribute to toxicity, while more reactive aldehyde and ketone compounds, such as acetaldehyde, undergo decomposition [37,38]. These results indicate that pyrolysis temperature control is a critical

factor in determining the chemical characteristics and toxicity of liquid smoke. Temperature control not only affects the chemical quality of liquid smoke but also determines its biological effectiveness, whether as a natural preservative or an environmentally friendly insecticide.

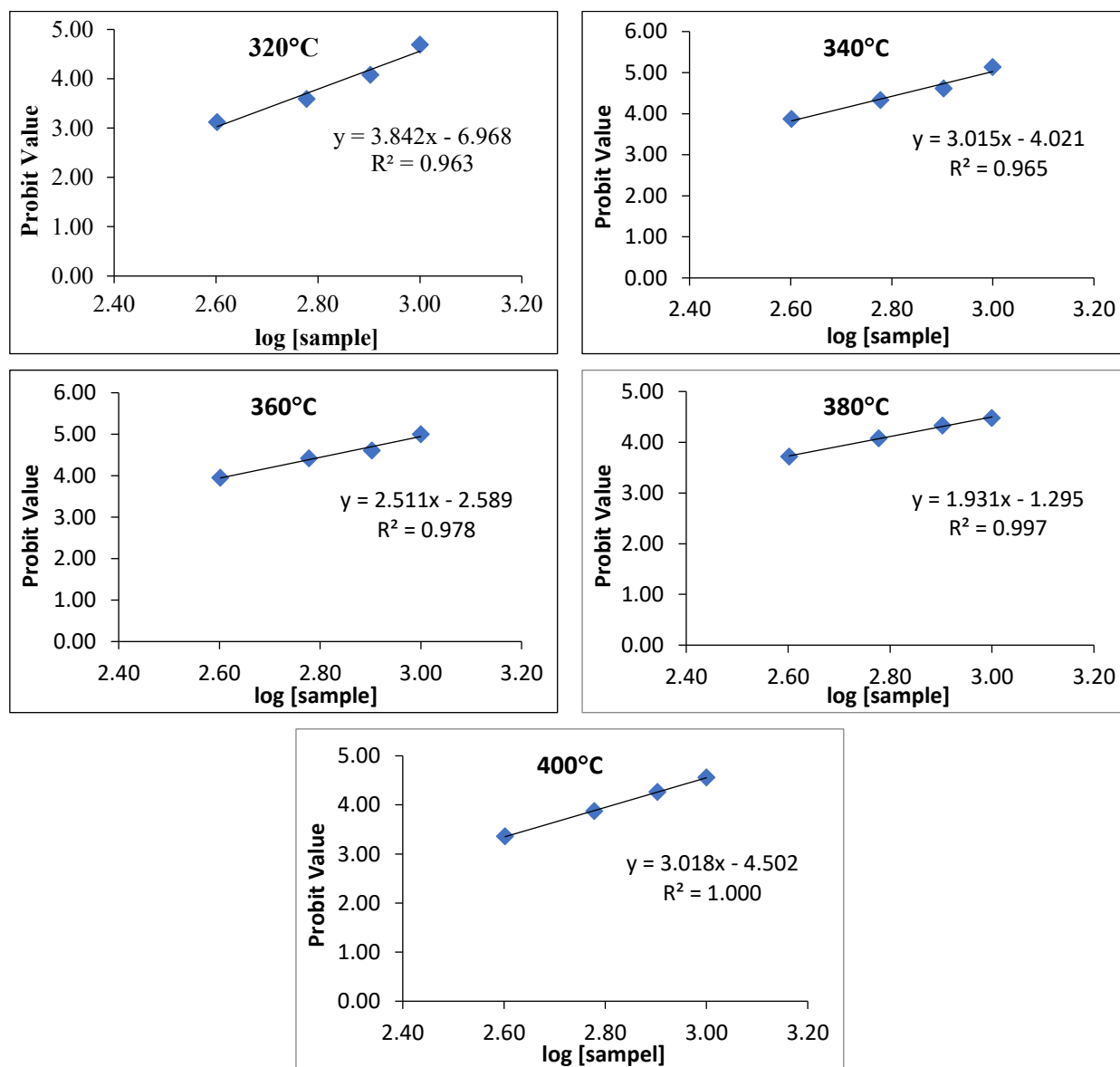


Figure 7. Graph of the relationship between the log of sample concentration and the probit value of sawdust liquid smoke at various pyrolysis temperatures.

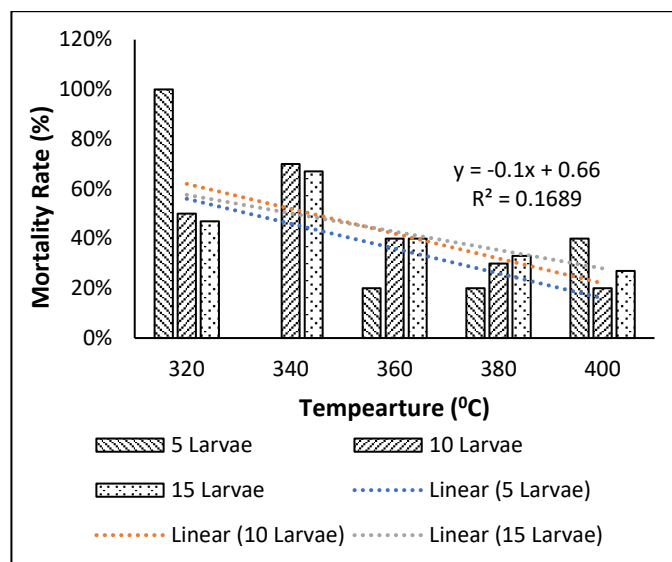


Figure 8. In vivo aquatic animal test results.

4. CONCLUSION

This study demonstrates that pyrolysis temperature significantly influences the chemical characteristics and toxicity of liquid smoke derived from teak sawdust. Higher pyrolysis temperatures promote the formation of chemically stable phenolic and ester compounds while reducing the presence of reactive components associated with toxicity. The resulting liquid smoke exhibited low toxicity and favorable physicochemical properties, indicating its potential for environmentally friendly utilization. Although the findings suggest promising prospects for food-related applications, further studies involving detailed toxicological assessment, purification optimization, and long-term safety evaluation are necessary before practical application can be recommended.

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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