

The Pyrolysis Reactor Design and The Effect of Liquid Smoke from Coconut Shell on Microbial Contamination of Tofu

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ABSTRACT

Liquid smoke is a natural food preservative which can be made of coconut shells through the pyrolysis process. This study aimed to design a pyrolysis reactor and utilize the coconut shell waste to produce liquid smoke as a natural preservative of tofu. 1.5 kg of chopped coconut shell was pyrolyzed at 400°C for 5 hours and produced 488 mL of grade 3 liquid smoke with a yield of 34.23%. The liquid smoke was then purified by extraction using ethyl acetate (1: 1 ratio) solvent and 70°C temperature for 2 hours. The extract was then distilled at 80°C and produced grade 1 liquid smoke. This liquid smoke had an acid content of 12.26% and a phenol content of 0.73%. This liquid smoke was then applied to tofu for 3 days and analyzed the microbial contamination. The smallest amount of microbial contamination was found in the samples of yellow tofu and white tofu coated with liquid smoke and stored in the refrigerator for 1.4 \times 105 CFU / mL and 8 \times 103 CFU/ml.

KEYWORDS

Coconut shell Tofu Liquid smoke Microbial Contamination

INTRODUCTION

Tofu is a perishable food product. This is due to the high protein and water content which reach 70% -85%, and water activity of 0.98-0.99 (Harmayani et al., 2009). The low durability of tofu makes the shelf life of tofu is very short. Tofu can last for 1-2 days. More than that tofu is not suitable to be consumed because of the changes in the aroma, texture, and color of the tofu (Koswara, 2011). Many tofu makers still use a lot of formaldehyde as the preservative. National Agency of Drug and Food Control (2006) conducted laboratory tests on 76 samples of tofu sold in the city of Bandung. The results showed that 1.32% of the samples contained formalin. A research related to the study of formaldehyde content in tofu with quantitative and qualitative methods showed that 4.58% of tofu sold in Bandung was positive with formalin content of 0.084% (Khoerudin, 2017). Formalin is a prohibited food ingredient because it is toxic and is not included in food additives issued by the Ministry of Health (Winarno, 2004). One alternative that can be used as a natural preservative for tofu is liquid smoke. Liquid smoke has good potential to be applied to tofu because it contains antimicrobial compounds (Yunus, 2011). The dominant compounds of liquid smoke are phenol and acid compounds, where the two compounds are

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antimicrobial and will be stronger if the two compounds are present together (Darmadji, 1996). The advantages of using liquid smoke as a preservative are the quick and easy preservation process by applying the liquid smoke to food or by dipping the food until the surface is coated with liquid smoke (Pearson & Tauber, 1973).

Liquid smoke can be produced from various raw materials including sawdust, nutmeg shells, mangrove stems, cloves twigs, nutmeg twigs, coconut shells, coconut tree trunks, walnut tree trunks, and walnut shells. Coconut shells produced the largest yield of liquid smoke than others as much as 50% with a phenol content of 5.06% and acid number of 13.33% (Yusnaini and Rodianawati, 2014). The lignin content in coconut shells was 33.3%, while the cellulose content was lower at 27.31% (Joseph and Kindagen, 1993). The 400° C of combustion temperature in the pyrolysis process can decompose the contents of coconut shells, namely hemisolulose, cellulose, and lignin (Girard, 1992). A research on the effect of coconut shell pyrolysis time on the manufacture of liquid smoke with a temperature susceptibility of 350° C-400° C concluded that the longer the pyrolysis time, the greater the quantity of liquid smoke was obtained, but after 4 hours the quantity of liquid smoke would decrease (Pamungkas, 2007). Coconut shell could be pyrolyzed at a temperature of 400° C so that the components in the coconut shell could be decomposed into organic acids and several phenolic compounds (Nasruddin, 2015).

There are 3 levels (grades) of liquid smoke, namely grade 1, grade 2, and grade 3. Grade 3 liquid smoke is liquid smoke produced from wood pyrolysis and cannot be used for food preservatives because it still contains a lot of tar or PAH (Policylic Aromatic Hydrocarbon) which is carcinogenic to the body. This grade 3 liquid smoke is usually used in rubber processing, deodorizing, and wood preservatives to make it resistant to termites. Grade 2 liquid smoke is liquid smoke resulted from pyrolysis that has been left for 1 day and being filtered. This liquid smoke usually being used as food preservatives with smoked taste as the substitution of formaldehyde (for example: smoked meat, smoked fish / smoked milkfish). Fish or meat that has used this liquid smoke is usually transparent brown, has a medium sour taste, and has a weak smoke aroma. Fish or meat preserved in grade 2 liquid smoke will last for 3 days. Liquid smoke that has passed the pyrolysis process is left for 1 day then a filtration process is carried out, after that fractionation process is carried out to obtain grade 1 liquid smoke. This liquid smoke is used as a food preservative such as meatballs, noodles, tofu, and barbeque spices. The character of this liquid smoke has a clear color, slightly sour taste, neutral aroma, is liquid smoke of the best quality and does not contain harmful compounds anymore to be applied to food products. In general, the pyrolysis method is used to produce this liquid smoke.

Pyrolysis is a method which can be used to produce liquid smoke by decomposing wooden materials (cellulose, hemicelluloses, and lignin) through thermochemistry (heating) without or with a little oxygen and other reagent. The decomposition of cellulose and hemicellulose occurs at 180° C - 350° C, while lignin decomposes at 300° C - 500° C. The pyrolysis process which occurs above 500° C will generate a lot of unwanted PAH compounds (Simko, 2005).

The design of the pyrolysis reactor determines the quality and quantity of the liquid smoke. The heat generated from fossils fuel in pyrolysis reactor resulted in unevenly spread of heat. Moreover, fossils fuel creates combustion gas emission like CO and CO₂. The pyrolysis reactor with ceramic heating element in the wall of the reactor will create the distribution of heat can be spread evenly and also easy to be controlled so that the pyrolysis process can run optimally. In addition, the ceramic heating element has the advantages of corrosion resistance, high temperature resistance, long service life, high efficiency, energy save, identical temperature, good thermal conductivity, fast thermal compensation, does not contain harmful substances such as lead, cadmium, mercury, hexavalent. chromium, double polybrominated, polybrominated diphenyl, and meet the environmental protection requirements of European RoHS, etc. (International Thermal Co., Ltd-Indonesian,). Therefore, it is necessary to conduct research related to the design of the pyrolysis reactor and the utilization of coconut shell waste into liquid smoke as the preservative for tofu.

METHODS

The Pyrolysis Reactor Design and Test

The pyrolysis equipment scheme consisted of a pyrolysis reactor with a fixed bed cylinder shape, a heater on the reactor wall connected to the smoke condenser to liquefy the smoke generated from the pyrolysis process. The schematic of the equipment could be seen in Figure 1. The design of the pyrolysis reactor had 1.5 kg capacity with the temperature of 400°C. The parts of the pyrolysis included:

- a. Pyrolysis reactor with stainless plate, 15 cm diameter and 40 cm high.
- b. Connecting pipe between the pyrolysis furnace and the condenser. The pipe was made of stainless steel with the diameter of 4 inches and 90 cm length.
- c. Helical coil type condenser.
- d. Ceramic heater attached to the wall of the reactor with the maximum heating temperature of 700° C.

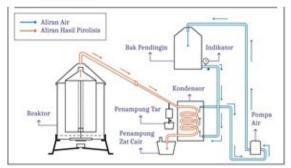


Figure 1. The Overview of Pyrolysis Process (Siddiqui, 2009)

The performance test of the pyrolysis device was carried out to the heater to find out whether the burner could reach the desired temperature and the pyrolysis reactor could burn the coconut shells.

Liquid Smoke Production

Liquid smoke used chopped coconut shell as the raw material in the pyrolysis process. The temperature used in the pyrolysis process was 400° C for 5 hours. The smoke from pyrolysis was

then condensed, cooled, and deposited for 24 hours. Then, liquid smoke was filtered using filter paper. The filtrate produced from the process was then extracted using ethyl acetate as a solvent with the ratio of 1: 1. The extraction method used liquid smoke mixed with solvent then stirred using a magnetic stirrer at a speed of 250 rpm while heated at 70° C for 10 minutes. After that it was transferred into separating funnel and left for 2 hours. The extract and raffinate were then separated. The next method was distillation of the extract to separate the ethyl acetate solvent from liquid smoke forming compounds. The distillation was carried out at 80° C for 2 hours.

The Application of Liquid Smoke on Tofu

The final product of liquid smoke was then applied to the tofu sample to test the anti-microbial compounds based on the level of microbial contamination. The application was done by coating the two types of tofu, namely yellow milk tofu and white milk tofu. The application was done by dipping the tofu in the liquid smoke until the entire surface of the tofu wet. After that, the tofu was stored in two places; the open space and the refrigerator (showcase). Tofu samples were left for three days to be analyzed and compared to the control samples; tofu without liquid smoke applied.

Analytical Method

There were several methods of liquid smoke analysis such as the determination of the acid number by the acid-base titration method and the determination of phenol content by the spectrophotometric method,

The Determination of the Acid Number

The acid number was determined by using the acid-base titration method. 10 ml of the sample was diluted to 100 ml with distilled water. 10 ml sample solution was put to Erlenmeyer and added 2-3 drops of phenolphthalein indicator then titrated with 0.1 N NaOH solution until the end point of the titration which changes the color of the sample to purple red and stable. The total titrated acid was expressed as percent acetic acid (SNI, 01-3207-1992). Furthermore, analysis by Gas Chromatography-Mass Spectroscopy (GC-MS) was used to determine the acid compounds contained in the liquid smoke more specifically.

The Determination of Total Phenol Content

The determination of total phenol content was carried out with gallic acid (GAE) as the standard (Orak, 2006) using the spectrophotometric method with the concentrations of 100, 200, 300, 400, and 500 ppm at a wavelength of 640 nm with folin ciocalte reagent (Folin & Ciocalteu, 1994) and Na2CO3. GC-MS analysis was also used to see the phenol compounds and their derivatives contained in liquid smoke.

The Analysis of Microbial on Tofu Samples

The method of analyzing tofu which has been coated with liquid smoke was by calculating the microbial contamination using the Total Plate Count (TPC) method (Djide, 2007). The tofu sample

was then ground, then 1 g was taken to be dissolved in 9 ml of sterile water. After that, the dilution process was carried out to 10-7 dilutions. The solution was then added to Nutrient Agar (NA) media and incubated for 3 days. The number of microbial colonies that grew was then counted.

RESULT AND DISCUSSION

The Design and Performance Test of the Pyrolysis Reactor

The design of the pyrolysis equipment made in this study can be seen in Figure 2.



Figure 2. The Design of Pyrolysis Reactor

Overall, this pyrolysis reactor had two main components; the combustion tube and the condenser. The function of the combustion tube was as a place for pyrolyzed the raw material (coconut shell) for making liquid smoke. This tube contained a heater which functioned to heat the raw material with the power of 3000 W and a net made of wire as a storage area for the raw materials. The combustion tube was made of stainless plate with inner diameter of 15 cm, a height of 40 cm, and a thickness of 0.3 cm. The combustion tube was also equipped with a glass wool as the heat protector so that the heat generated was not wasted into the environment.

The condenser was made of stainless with the diameter of 20 cm and a height of 30 cm. There was a pipe in the shape of a coil inside the condenser which later be passed by the smoke from the burning of the coconut shell. Flowing water was used as the coolant so that the smoke generated from the pyrolysis process could be condensed. The combustion tube and the condenser were connected by a stainless steel pipe with the diameter of 2 inches and a length of 90 cm. Other components in pyrolysis reactor were presented in Table 1.

No	Component	Function				
1	Combustion Tube	The place where the pyrolysis process occurs. This tube is equipped with				
		a lockable cylinder cover and a gasket to prevent the resulting gas from				
		escaping between the covers				
2 <i>Heater</i> The source of heat		The source of heat generated from electrical energy. The heater is				
		covered with glasswool so that the heat generated is not wasted into the				
		environment				

No	Component	Function		
3	Condenser	The place for cooling the smoke where it will change its phase into liquid		
4	Thermocouple	The temperature sensor which is used to measure the temperature in the combustion tube		
5	Contactor	As the power button		
6	Temp indicator	To measure the temperature in the combustion tube		

The pyrolysis device that has been designed was then tested to determine whether the temperature reached by the heater could heat the raw materials to the desired temperature or not. In this test, the pyrolysis device was tried to be operated by filling with 1.5 kg coconut shell raw material. Water was used as the condenser coolant. The results of this test showed that the heater was able to heat the coconut shell up to 400° C. In one hour of combustion, the output from the condenser was still in the form of smoke, but after 20 minutes the product had turned into a liquid phase. During the process, the smoke was still coming out between the combustion tube covers. This was due to the uneven structure of the cover so that a gasket was added between the tube and the cover so that the smoke did not come out and only got into the condenser connecting pipe.

The Pyrolysis of Coconut Shell

The pyrolysis process with the temperature of 400° C produced grade 3 liquid smoke with a yield of 32.23% of liquid smoke and 58% of charcoal formed. The yield of the liquid smoke was lower than other studies of 52.85% at 350° C - 400° C (Tranggono et al., 1996) and 50% at 400° C (Yusnaini and Rodianawati, 2014). The difference in yield value of the liquid smoke obtained was due to the differences of water content in the coconut shell. The higher the water content in the raw material the higher yield of liquid smoke produced (Sutin, 2008). In this study, the coconut shell raw material was drying for 2 days so that the water content contained in the coconut shell was reduced.



Figure 3. Grade 3 Liquid Smoke at 400° C

The color of the liquid smoke which came out as the condensate from the pyrolysis process of coconut shell at 400° C was dark black. This indicated that the liquid smoke still contained a lot of tar. The tar produced in the coconut shell pyrolysis process was quite a lot so that the resulting tar deposits stuck in the walls of the pyrolysis reactor and also in the liquid smoke container. There was a decrease of the weight of liquid smoke as 9.77% due to the escape of the pyrolysis smoke from between the reactor covers so that it did not condense and the tar deposits stuck to and became a crust on the walls of the pyrolysis reactor.

Liquid Smoke Purification

Grade 3 liquid smoke which came out as the condensate from the pyrolysis was left then filtered to separate the tar deposits in liquid smoke. In this process, the resulting filtrate was grade 2 liquid smoke where this liquid smoke was free from tar deposits but still contained dissolved tar. It could be seen from the color of grade 2 liquid smoke was still black but clearer than grade 3 liquid smoke.

Grade 2 liquid smoke was then extracted using ethyl acetate solvent at the temperature of 70°C for 2 hours. The extract obtained was liquid smoke with the desired ingredients such as acid and phenol compounds. In this study, 514 mL of extract was produced which was grade 1 liquid smoke. Grade 1 liquid smoke had a clear brownish color which indicated that the liquid smoke had separated from the tar. Grade 1 liquid smoke resulting from the extraction still contained ethyl acetate as a solvent, so a distillation process was carried out to separate the ethyl acetate from the desired compounds in liquid smoke. The distillation was carried out at 80°C for two hours. The distillate was a condensed ethyl acetate while the raffinate was grade 3 liquid smoke which was already pure because it did not contain ethyl acetate solvent. It produced 50 mL of liquid smoke with a brownish yellow and clear color.



Figure 4. Grade 1 Liquid Smoke

The Characteristics of Liquid Smoke

After the liquid smoke passed through the fractionation process by extraction and distillation, then it was tested to determine the quality of the liquid smoke obtained. The tests carried out included determining the levels of the desired compounds in liquid smoke; acid and phenol compounds, measuring pH, determining specific gravity, and determining the yield of the resulted liquid smoke.

Acid Number

Acid compound is one of the compounds that make up the desired liquid smoke. The role of acidic compounds in liquid smoke is as an antimicrobial. Antimicrobial properties are what is needed to preserve food ingredients. The acid content identified in liquid smoke is the amount of acetic acid calculated as the amount of acetic acid. This acid level determination is done by calculating the total acid that is titrated (Sutin, 2008).

In this study, the acid content of the liquid smoke that had been fractionated by extraction and distillation was 12.26%. The results of the acid levels obtained were not much different from the results of the previous studies as 13.33% (Yusnaini and Rodianawati, 2014). Other researchers conducted pyrolysis of liquid smoke at the temperature of 300°C, then the liquid smoke was fractionated by extraction using ethyl acetate as the solvent and resulted in the acid levels of 2.806% and 6.982% (Sutin, 2008). This was due to the acidic compounds such as acetic acid which had polar properties and when extracted using a polar ethyl acetate solvent, the acidic compound would be distributed in the solvent so that the value decreased when titrated to determine the acid content. The distillation process carried out in this study after the extraction was aimed to remove the ethyl acetate solvent so that it would increase the acid number. In addition, the difference of pyrolysis temperature also affected the acid content of the liquid smoke produced. Pyrolysis with low temperature of 300 C produced liquid smoke with lower acid number compared to liquid smoke with the pyrolysis process at the temperature of 400°C. This indicated that the pyrolysis process at the temperature of 300°C was not optimal. Furthermore, the results of the analysis of liquid smoke that had been fractionated using GC-MS showed that the dominant component of the acid compound was acetic acid ethyl ester with an area of 37.59%.

Phenol Level

Phenolic compounds are one of the dominant compounds contained in liquid smoke. Phenol is a compound that acts as an anti-oxidant in liquid smoke. Antioxidants function to minimize the oxidation of fats and oils so that they can extend the shelf life of food and reduce the risk of food damage (Hernani and Raharjo, 2005).

In determining phenol levels, gallic acid as a standard solution was measured for its absorbance with a wavelength of 640 nm. In a grade 1 liquid smoke sample, the absorbance of the sample was also measured with the same wavelength. From the results of this analysis, the phenol concentration in the grade 1 liquid smoke sample was 784 ppm with a total phenol content of 0.73%. This result was lower compared to the previous studies 0.9751-1.052% (Sutin, 2008). This difference in results could occur due to several things, including differences in the lignin content of the coconut shells used and the extraction process using a separating funnel for a short time, causing the extraction of phenol compounds from grade 3 liquid smoke to be less effective. The extraction process should be carried out by maceration with a larger solvent ratio. The results of the liquid smoke analysis using GC-MS showed the presence of two phenolic compounds, namely phenol with spread area of 7% and phenol 2-methoxy with spread area of 3.5%.

Phenol levels that can be consumed by humans are in the susceptible range from 0.06 to 0.2% per day (World Health Organization & International Program on Chemical Safety, 1994). However, the phenol content obtained from this study was 0.73% and did not meet the requirements for consumption. This was because the raw material used was coconut shell, where

coconut shell contained more lignin than cellulose. Phenol is the result of the decomposition of lignin (Diatmika et al, 2019). This was what underlies the high phenol levels in this study. The application of liquid smoke to food will need to be adjusted to the phenol content that can be consumed. The process of diluting liquid smoke to adjust its phenol content as a food preservative will be carried out in a further study.

Acidity of Liquid Smoke

pH is an important parameter as a determinant of the quality of the liquid smoke produced. This pH determination can identify the formation of organic acids and phenols resulting from the decomposition of coconut shells in the pyrolysis process (Diatmika et al, 2019). In this study, the pH value of liquid smoke was 3. Other studies also provide a relative acidity level with a pH value ranging from 2.7 (Yusnaini and Rodianawati, 2014), and 3.55 (Sutin 2008). Liquid smoke which is used as a food preservative has high quality if the pH of the liquid smoke is low (Haji et al, 2007). The value obtained identified that the resulting liquid smoke had high acidity so it was suitable to be used as a food preservative since preservation process with a low pH made the microbes difficult to reproduce.

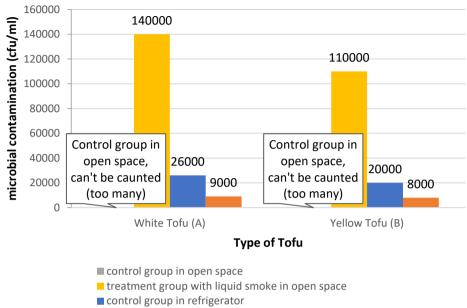
The Application of Liquid Smoke on Tofu

Fractionated liquid smoke was applied to two different types of tofu; yellow and white milk tofu obtained from Cihanjuang. The two samples of tofu were then immersed in liquid smoke until the entire surface was coated. The samples were then stored in two different places; in the open space and in the showcase. The control of each sample was yellow and white tofus which were not immersed in the liquid smoke. The observation of tofu was carried out in 3 days.

The Analysis of Total Plate Count of Tofu Samples

In the control sample where the tofu was not coated with liquid and kept in the open space, the amount of microbial contamination could not be calculated because of the dilution factor was still low so that the concentration of bacteria in the suspension was still high. Based on the SPC (Standard plate count) rules, the colony, which amounted from 30-300, could be counted. This was intended to minimize the possibility of errors in the analysis process, especially statistical errors. If the colony was less than 30 or more than 300 then it might be counted the closest. However, the sample of tofu in the open space covered with liquid smoke showed a smaller value of microbial contamination, indicated by the amount of microbial contamination which could still be calculated using the same dilution factor. The total number of microbes (CFU / mL) by calculating the SPC in the tofu sample applied with liquid smoke and the control conditions, both stored in the open and in the refrigerator was shown in Figure 5. Tofu stored in the refrigerator had the contamination value of microbes which relatively smaller compared to tofu left in open space. Low temperature in the refrigerator could inhibit microbial growth in tofu. Tofu samples with liquid smoke had lower microbial contamination when compared to control samples, both stored in open spaces and in refrigerators. This was because the liquid smoke superimposed on tofu contains acid and phenol compounds which function as antimicrobials and antioxidants so

that it could inhibit the growth of microbes and the oxidation process on the tofu surface which could reduce the quality of the tofu.



treatment group with liquid smoke in refrigerator

Figure 5. Diagram of Microbial Contamination of Tofu Samples

Tofu samples with various treatments then be compared with the Quality Requirements of Tofu according to SNI 01-3142-1998 and SII No.0270-1990 where the maximum total plate microbial contamination rate (total plate) is 1.0 x 106 cfu/ml. The value of microbial contamination of tofu samples showed whether the tofu was suitable for consumption or not. The results of this comparison were shown in Table 2.

Table 1. Microbiological Quality of Tofu Products Based on SNI 0270-1990 (Badan Standarisasi Nasional, 1998)

Storing Method	Treatment	Type of Tofu	SPC (cfu/ml)	Maximum Limit Contamination	Note: Consumable/ Not Consumable
Open Space	Control	Yellow Tofu	TBUD	1 X 10 ⁶	Not Consumable
		White Tofu	TBUD	1 X 10 ⁶	Not Consumable
	With Liquid	Yellow Tofu	1,1 X 10 ⁵	1 X 10 ⁶	Consumable
	Smoke	White Tofu	1,4 X 10 ⁵	1 X 10 ⁶	Consumable
Refrigerator	Control	Yellow Tofu	2 X 10 ⁴	1 X 10 ⁶	Consumable
		White Tofu	2,6 x 10 ⁴	1 X 10 ⁶	Consumable
	With Liquid	Yellow Tofu	8 x 10 ³	1 X 10 ⁶	Consumable
	Smoke	White Tofu	9 X 10 ³	1 X 10 ⁶	Consumable

Table 2 showed that not all treatment combinations obtained an SPC value below 1×106 cfu / ml, which indicated tofu was safe for consumption. The types of tofu that are no longer safe for

consumption such were tofu which were stored in an open space and did not coated with liquid smoke. This was occurred due to microbiological damage which could lead to the process of product decomposition, either by bacteria or mold.

CONCLUSION

The pyrolysis device was designed with 2 main components; combustion tube with a heater in it and a helical coil type condenser. This equipments were able to evenly burn coconut shells at 4000C for 5 hours and produced liquid smoke with a yield of 32.23% with phenol and acid levels after purification of 0.73 & and 12.26% (w / v). The analysis of microbial contamination of tofu through the Total Plate Count method showed that tofu which was applied with liquid smoke, both stored in open spaces and refrigerators, had microbial contamination below the maximum value of microbial contamination (1.0 x 106 cfu/ml) according to SNI 01-3142- 1998 and SII No. 0270-1990 and consumable.

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