Study of Phenol and Acetic Acid Components in Liquid Smoke of Palm and Test of Microbial Activities Used The Kirby-Bauer Method

D. Ardilla1[1,], Thamrin[1], B. Wirjosentono[1], Eddyanto[1]*

[1] *Department of Food Technology, UMSU. USU Graduate Faculty of Chemistry, Medan 20155, Indonesia;ardila.desi@gmail.com*

Abstract. The aim of this study is to determine the content of phenol and acetic acid in palm shell liquid smoke (ACCKS) and anti-microbial activity against *E. coli* and *S. aureus* by using Kirby–Bauer method. Pyrolysis process was carried out using a tool refractory covered by white cement so that the smoke does not escape into the air (not in contact with O_2). The temperature used: 500, 700, and 900°C using a spiral condenser type with coil number of 30 windings. The ACCKS obtained presipited in let stand for 7 d, and then centrifuged by 3800 rpm for 60 min, filtered and distilled at temperature of 125°C for 60 min. ACCKS analyzed by using GC-MS and identified phenol and acetic acid for each temperature was phenol 35.09, 27.92 and 14.49%, while for acetic acid content of 44.97, 63.51 and 78.26%. Test anti-microbial activity of *E. coli* and *S. aureus* using the Kirby-Bauer method at each temperature inhibitory power 16, 17.9 and 18 mm, while for *S. aureus* at each temperature inhibitory power 25, 20 and 30 mm. The inhibition of the positive control for each microbe 22.5 mm and 38 mm.

Keywords: ACCKS redestilasi; phenol; acetic acid; microbial test

Introduction

Palm shells can be classified into hardwood due to the lignin content is quite high at 29.4%, so the researchers used a palm shells as raw material in the manufacture of liquid smoke. The manufacture of activated charcoal also has potential as an alternative energy or as a source of renewable energy (power plant). Pyrolysis is irregular decomposition of organic materials due to warming without dealing with the outside air [1]. This implies if the heated oil palm shell without dealing with the outside air and high temperatures deberi there will be a decomposition reaction of the compounds that make up the shell and produce substances in three forms, namely solids, liquids and gases. In the liquid smoke contains a complex chemical compound, for the separation of several chemical compounds have been carried out by various methods, the separation of the components of the compound based on polarity, acidity level and volatility [2]. Various can act as an anti-oxidant and anti-microbial effect and give the typical color of the smoke flavor in food

products [3]. Pyrolysis process in the fieldwork using an average temperature above the usual temperature pyrolysis.

It is reported that a regular increase of 150°C (350-500°C) does not change the composition of the smoke condensate, but there was a slight increase on anti-oxidative effect and no effect on their anti microbia, maximum levels of phenol, carbonyl and acid reached at pyrolysis temperature of 600°C [4].

Table 1 shows the lignin content of the shell, which is high enough to be categorized into hard wood. In addition, liquid smoke has good quality when produced from hardwood than softwood. However, liquid smoke produced from burning hardwood will be different in composition to those produced from softwood combustion [5]. In general, hardwoods will produce superior aroma, aromatic content richer and more acidic than softwood. Liquid smoke produced from hardwood asapan when applied to products with the aim of extending the shelf life will be superior to both the aroma and color because the content of phenols and organic acids that act as anti-microbial and anti-oxidant. The chemical composition of the liquid smoke is water (11-92%), phenol (0.2 - 2.9%), acid (2.8 - 9.5%), carbonyl (2.6-4%), tar (1-7%) [6].

Water content in the raw material liquid smoke gives variation to the composition of the smoke, the level of water increases cause low levels of phenols and carbonyl compounds increased. The smoked flavor in these conditions are more acidic combustion temperature also affects the composition of the smoke. The maximum levels of phenolic compounds, carbonyl and acid pyrolysis temperature is reached at 600^{o}C [7]. Liquid smoke is produced at a temperature of 400^{o}C when applied to asapan products will provide superior organoleptic value.

Experimental

Palm shells used were obtined from RISPA Plantation, Indonesia. Pure cultures of *E. coli* and *S. aureus* microbial MHA media (Muller Hilton Agar), a blank disc and cloro fenikol blank discs. Equipments used were pyrolysis reactor, petridis, ose needles, pipettes clinic, calipers, label stickers, GC-MS.

Liquid smoke was produced using pyrolysis reactor at temperature 500, 700, and 900^{o}C with refractoryand condenser. The resulting liquid smoke accommodated until liquid smoke does not drip anymore. Palm shells were dried for 3 d before used and then weighed 15 kg and placed inside the pyrolysis reactor. ACCKS deposited for 7 d,

centrifuged at a speed 3800 rpm for 60 min then filtered and distilled at 125^{o}C for 60 min, redestiled ACCKS characterized by GC-MS and tested microbial activity against *E. coli* and *S. aureus*.

Microbial activity assay was used by Kirby-Bauer method, the media used was MHA (Muller Hilton Agar), poured in until silenced petridis solidified, pure cultures of microbes that have been rejuvenated incorporated into solid media by means of using a needle ose smeared. Paper disc (blank disc) which has been spilled with 10 ml ACCKS placed on solid medium using a pipette clinic. Each 1 petridis used 2 paper disc, and then incubated at 37°C for 24 h. Inhibition zone measured using calipers in millimeter. Then, the components of liquid smoke was identified by GC-MS.

Results and Discussion

Identification of Liquid Smoke Components

Identify the components of liquid smoke using a GC-MS, volatile components in dichloro methane liquid smoke can be seen in Table 1. The amount of each component is shown semi-quantitatively by determining the peak area (%). Table 1 shows the 16 components identified from ACCKS and visible components of acetic acid at 500°C has a peak water area of 44.97% and a retention time of 20.386 followed by 35.63% phenol peak area and retention time of 37.461. For temperature 700°C showed 9 components are identified, acetic acid component peak area 63.51% with the highest retention time 20.202 while the phenol peak area 27.92% with a retention time of 37.462. At 900 °C for 8 components also are identified high peak area the acetic acid is 78.26% with a retention time of 21.491 while the phenol peak area 14.49% with a retention time of 37.494. Components shown in Table 1 produced by thermal degradation of cellulose, hemi cellulose, lignin also present in commercial liquid smoke. In view of the percentage (%) both peak area at 500, 700, and 900^{o}C acetic acid has the highest peak area indicates that this oil shell liquid smoke potential as food preservatives [8,9].

On the results of the analysis are shown in Table 1, there are no compounds polycyclic aromatic hydrocarbons (PAHs), including benzo (a) Pirene, this was due to undergo treatment ACCKS generated precipitation for one day, centrifuged, filtered and distilled. Above treatments is a way to reduce or even eliminate the PAH compounds contained in liquid smoke product.

According to Guillen *et al.*[10] and Stolyhwo & Sikorski [11], factor PAH and PAH curing temperature is not formed if the pyrolysis temperature is below 425°C. Meanwhile, according to Trend Analytic Chemistry (2006) PAHs are formed when fuel is maximum in the temperature range 500-700°C, the higher temperature pyrolysis reactor is the lower the resulting compounds.

Judging from the nature of PAH are soluble in water and volatile showed that PAH compounds can be removed from liquid smoke ACCKS be produced at a temperature of 500, 700, and 900^{o}C can still be used and are safe for food preservation.

Table 1. Liquid smoke component.

Temperature (^{o}C)	Component	Area (%)	Retention time (min)
500	Aseton	4,04	5,604
	Methyl alkohol	4,36	6,057
	Phyridin	0,47	12,425
	Methyl	0,80	17,913
	Methyl 2 cyclopenten	0,43	18,120
	Acetic acid	**44,97**	**20,386**
	Furan carboxaldehid	1,72	21,476
	Etanon	0,35	22,884
	Propiorit acid	4,32	23,646
	Butirad acid	0.68	26,512
	Funfuril alkohol	0,21	27,748
	Phenol 2 metoxy	0,63	33,765
	Phenol	**35,63**	**37,461**
	Phenol 4 methyl	0,30	39,472
	Phenol 2 methyl	0,28	39,569
	Tugenol	1,04	41,516
700	Aseton	0,59	5,611
	Methanol	0,54	6,075
	Pyridin	0,19	12,479
	Aseton	0,07	12,583
	Acetic acid	**63,51**	**20,202**
	Propanoic acid	3,58	12,656
	Butirad acid	0,37	26,526
	Butanoid acid 4 cloro	0,41	27,390
	Phenol	**27,92**	**37,494**
900	Aseton	1,28	5,608

	Methanol	1,06	6,072
	Acetic acid	**78,26**	**20,203**
	2 furan carboxaldehid	0,86	21,491
	Propanoid acid	3,08	23,665
	Butyrol aceton	0,70	7,328
	Phenol 2 methoxy	0,28	33,679
	Phenol	**14,49**	**37,494**

Test Microbes Activity

Bacteria associated with food poisoning such as *E. coli* and *S. aureus.* Related to the ultimate goal is to use this ACCKS food preservation it is necessary to test microbial activity. Analysis of microbial activity test can be seen in Table 2, the inhibition zone on 3 cultures for each microbe.

Tabel 2. A (test Activitity Liquid smoke).

No	Temperature ACCKS (^oC)	Diameter expressed *E.Coli* (mm)		Median
		Chapter 1	Chapter 2	
1	500	8.31	7.69	16.0
2	700	8.25	9.65	17.9
3	900	9.63	8.87	18.5

Tabel 2. B (Test Activity Liquid smoke).

No	Temperature ACCKS (^oC)	Diemeter expressed *S.Aureus* (mm)		Median
		Chapter 1	Chapter 2	
1	500	12.8	12.2	25
2	700	10.0	10.0	20
3	900	15.5	14.5	30

From the results of the analysis are shown in the table above can be seen on the bacteria *S. aureus* inhibition zone larger than the zone of inhibition *of E. coli,* it is due to *E. coli* bacteria are classified as Gram (-) and *S. aureus* gram (+). And Gram (+) are more sensitive to phenol than Gram (-) as layer-saccharides lipopoli specifically surrounds the cell wall of gram (-) it may prevent the entry of fatty acids that prevent the buildup fat bacterial cell membrane (-). Anti-microbial activity of inhibitors can be called effective if dimeter inhibiting more than 15 mm. Microbial activity inhibitor that produces 30-35 mm

diameter expressed high microbial activity, while the 20-30 mm diameter inhibiting the activity of low and anti microbial activity ineffective or resistant. Specific growth rate (LPS) LPS divided microbial control is smaller than 1. In this study the researchers used a positive control is a sheet fenikol on average *E. coli* and *S. aureus* 21.75 mm 28.23 mm. Pszcola (1995) states that the two main compounds in liquid smoke is phenol and organic acids (acetic acid) it is evident from the research that has been done [12-14].

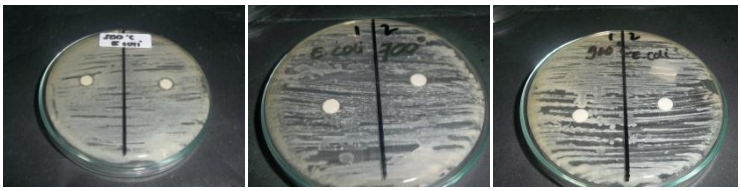

Figure 1. Diemeter expressed bacteri *E.Coli.*

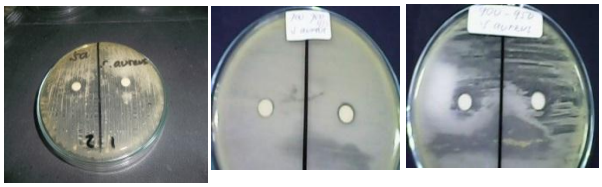

Figure 2. Diameter expressed bacteri *S.Aureus.*

Conclusion

The compound of liquid smoke shells palm oil produced at high temperatures is still safe to use for food preservatives. At high temperatures or fuse gas, liquid smoke produced is still exist even in small amounts. Based on GC-MS spectra the dominant compound is acetic acid and phenol and no PAH compounds after distilled. To test microbia activity of ACCKS in this themperature show that effective as an anti-microbial and can be used as a food ingredient.

[1] A. Awaluddin, Direct Liquefaction Process Biomass into Bio-oil by using Thermo-Oil, Proposals I-MHERE Project. HEI-IU University of Riau, 2007.
[2] K. P. Putnam, D. W. Bombick, J. T. Avalos, D. J. Doolittle, *Food Chem. Toxicol* **1999**, *37*, 1113.
[3] Karseno, Darmadji P., Rahayu K., *Agritech* **2002**, *21(1)*, 10.
[4] Frethem *et al*, *J. Food Sci.,***1980**, 45, 999–1002.
[5] J. P. Girard, in : "Technology of Meat and Meat Products". Ellis Horwood. New York 1992, pp. 165-201.
[6] Fatimah, Components Analysis of Liquid Smoke from Coconut Shell, *Thesis Post-graduate Program, Gajah Mada University*, Yogyakarta. **1998**
[7] Potthast. K., 1976: Determination of phenols in smoked meat products. *Advances in Smoking of Foods,* , **1976**, p. 39.* (wie unter 14)
[8] Guillen *et al*, L., *J. Agric. Food Chem*., **1995**, 43, pp. 463-468.
[9] Guillen *et al*, *J. Agric. Food Chem*, **2001** 49: 2395-2403.
[10] Guillen MD, Sopelana P, Partearroyo MA. *J. Agric. Food Chem.* **2000**, *48*, 5083.
[11] Stolyhwo A, Sikorski ZE. *Food Chem.* **2005**, *91*, 303.
[12] Cardinal M, Cornet J, Serot T, Baron R. *Food Chem.***2006**, *96*, 137.

[13] Kirby-Bauer. "Antimicrobial Susceptibility Testing by CLSI (NCCLS) reference Disk Diffution Method", **2008** Eddition, Shockwarc Flash Object.
[14] Pszocola D.E., *J. Food Tech.* **1995**, *49*, 70.
[15] Soldera S, Sebastianutto N, Bortolomeazzi R.., *J. Agric. Food Chem*. **2008**, *56*, 2727.