Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 13 (3): 401-408, 2013
ISSN 1812-5654 / DOI: 10.3923/jas.2013.401.408

Characterization of Redistilled Liquid Smoke of Oil-palm Shells and its Application as Fish Preservatives

[1]S.S. Achmadi, [2]N.R. Mubarik, [1]R. Nursyamsi and [1]P. Septiaji
[1]Department of Chemistry,
[2]Department of Biology, Bogor Agricultural University, Kampus Dramaga, Bogor 16680, Indonesia

Abstract: Oil-palm shells as an agricultural waste can be pyrolysed producing gaseous material that can be condensed into liquid smoke. Redistilled liquid smoke has been proven effective as fresh fish preservative due to its antibacterial activity. In the present study, the redistilled liquid smoke is evaluated as fish preservative of fresh catch and as fly repellent in fish salting-fermenting process. The tests included total volatile base-nitrogen levels in fish muscle and antibacterial activity using agar diffusion method. The content of various organic acids in liquid smoke reached 9.2%, mainly consisting of acetic acid and phenolics, at pH of 3.2. There were no harmful compounds such as tar and polycyclic aromatic hydrocarbons. The redistilled liquid smoke concentrations of 8, 9, and 10% were able to maintain the freshness of pomfret fish up to 24 h. In these levels of redistilled liquid smoke solution, diameters of clear zone formed on the antibacterial test ranged 10-20 mm, meaning that the liquid smoke fell into strong antibacterial category. It is suggested that the content of carboxylic acids, phenolic compounds and carbonyl serve as a natural preservative to replace the use of synthetic preservatives in salting fermented fish.

Key words: Antimicrobe, fish preservative, flies repellant, liquid smoke, oil-palm shells

INTRODUCTION

Currently, charcoal industries in Indonesia use charcoal as the main product, and the rest of approximately 70-80% is in the form of wasted gas that is released to the atmosphere as air pollutant. Attempt to increase added value of the smoke to be an environmentally friendly has been done, in various studies on the liquid smoke, which is commonly called as wood vinegar or liquid smoke. The production of liquid smoke can be integrated with charcoal making (Nurhayati and Sofyan, 2005). The liquid smoke is widely used as antibacterial and antioxidant by many food technologists.

Pyrolysis of coconut shells has been reported to produce liquid smoke with phenolic level of 9.36%, carbonyls 8.34%, and organic acids 6.38%. The distilled liquid smoke at lower than 100°C yields lower quantity of phenolics (3.90%) and lower tar (0.29%) as compared with the distilled products at higher temperatures (Darmadji, 2002). Solution of 2.5% liquid smoke made of coconut shell extends the shelf life of fish ball from 16 to 32 hours at room temperature (Zuraida *et al*., 2011).

Liquid smoke of oil-palm shells has been stated to inhibit the growth of *Psedoumonas fluorescens*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* bacteria. The activity of biopreservative toward *S. aureus* is exhibited at 0.6% concentration and toward *E. coli* at 0.8% (Halim *et al*., 2006). Tamaela (2003) and Yanti and Rochima (2009) also found that liquid smoke of coconut shells is potential as an alternative in fish preservation. The potency can be used for various processing and storage technology for fish products, so that the potency of liquid smoke derived from oil-palm shells, which is abundantly found in oil palm plantation, should be evaluated as fish preservative of fresh catch and as fly repellent in fish salting-fermenting process.

MATERIALS AND METHODS

Materials: Materials used were crude liquid smoke of oil-palm shells extracted from pyrolysis product at the temperature of 400°C (provided by PT Global Deorub Industry), *Staphylococcus aureus* and *Escherichia coli* (available from the Laboratory of Microbiology Culture Collection, the Department of Biology, Bogor Agricultural University), fresh water pomfret fish, patin fish (*Pangasius hypophthalmus*), *Chrysoma megalocephoda* flies and brine shrimp larvae *Artemia salina*. The experiment was done in 2011-2012. Chemicals used were

Corresponding Author: S.S. Achmadi, Department of Chemistry, Bogor Agricultural University, Kampus Dramaga, Bogor 16680, Indonesia

Folin-Ciocalteau reagent, Tashiro indicator solution, chloramphenicol in powder form, liquid media of Tryptic Soy Broth (TSB) and solid medium of Nutrient Agar (NA).

Preparation of liquid smoke: Crude liquid smoke of oil-palm shells (20 L) was placed in a large container and distilled using a concentration boule type TA62D at 80±5°C. The distillate in the form of liquid smoke was then collected in a closed container. Total Acid Assay was measured by titration (AOAC, 2005). Phenolics Determination was carried out using Folin-Ciocalteau reagent (Waterhouse, 2002).

GC-MS analysis: Chemical compounds in the redistilled liquid smoke were identified using gas chromatography-mass spectrometry (GC-MS) instrument equipped with 60-meter HP5 column. Detector temperature, initial column temperature and final column temperature were 250, 280 and 290°C, respectively. The carrier gas was helium with flow rate of 23.7 mL min^{-1}. at 17.56 psi pressure. The liquid smoke sample injected was 1 μL. Toxicity assay was performed on *A. salina* and calculated based on the percent mortality using probit scale (Manilal *et al*., 2009).

Level of TVB-N Assay (Standar Nasional Indonesia 2354.8:2009): Samples of fresh-cut pomfret fish (10 g each) that had been immersed in various concentrations of liquid smoke were placed in beaker glass. To each beaker glass was added 90 mL perchloric acid 6%. The content of the beaker glass was stirred for 2 min and filtered. The filtrate (50 mL) was transferred into a distillation tube, added a few drops of phenolphthalein indicator and antifoaming silicon. The distillation tube was mounted on a steam distillation apparatus and 10 mL NaOH 20% was added. A collecting erlenmeyer was prepared, containing 100 mL H_3BO_4 3% and 3-5 drops of Tashiro indicator (violet solution). Steam distillation was performed for approximately 10 min until 100 mL distillate was collected and the final volume was approximately 200 mL (green solution). Distillation of blank solution was performed in a similar way using 50 mL perchloric acid 6%. The distilled samples as well as the blank were titrated using solution of HCl 0.02 N. The end point of titration was indicated by reforming the violet color. The analysis was carried out every 8 h until the permissible total volatile base nitrogen (TVB-N) value was reached. The experiment was repeated twice.

$$\text{TVB} - \text{N}\left(\frac{\text{mg}}{\text{100g}}\right) = \frac{(\text{Vc} - \text{Vb}) \times \text{N} \times 14.007 \times 100}{\text{w}}$$

where, V_c is the volume of HCl for titration on sample, V_b is the volume of HCl for blank titration, N is the normality of HCl solution, W is sample weight (g), 14.007 is the atomic weight of nitrogen and 2 is the dilution factor.

Inhibition Assay on Bacteria was carried out as reported by Fitrial *et al*. (2008). The NA medium containing bacteria (*S. aureus* and *E. coli*, ±15 mL) was poured into the petri dish that already contained solid NA medium (the first layer). The mixture was homogenized and the second layer was left to solidify. In the first step, five wells were conditioned aseptically, 6 mm diameter each. Into each well, the liquid smoke solution (8, 9 and 10% concentrations), a positive control (solution of 100 ppm chloramphenicol) and negative control (sterilized distilled water) were transferred, 60 μL each. Each of the bacteria, i.e. were tested with five replications. Diameters of the measurable inhibition zone (replicated three times) were converted to inhibition index. Preparation of Dried-fermented Fish followed Ariyani *et al*. (2007) method. Patin fish was used in this experiment. In the salting process, 30% of salt (based on the fish weight), was applied. Some salts were put into the fish abdominal cavity and the rest of salts were dissolved into a saturated solution and fermented for 48 h.

Assay for Repellency toward *Chrysoma megalocephoda* Flies (Yuliani *et al*., 2005). The testing was performed using 25 flies that were caged in a 60×40×30 cm glass tank. The treated fish were put into this glass tank. Observation was started after 1 min. by counting the number of fly perch on the dried-fermented fish every minute until 60 min. The experiments were replicated three times for each concentration of 5, 10, 15 and 20% and the percent repellency was calculated.

Statistical analysis: The observed data on inhibition index and fly repellency were subjected to analysis of variance (ANOVA). If there was any significant difference, the difference among treatment means was determined by Duncan's multiple range test at $p = 0.05$.

RESULTS

The redistilled liquid smoke of oil-palm shells at 80°C gave clear acidic liquid, containing 63% of simple carboxylic acids and to a lesser extent were phenolics, with no indication of toxic polyaromatic hydrocarbons. The liquid was also a strong antibacterial toward *S. aureus* and *E. coli*, which would be a beneficial property for fish preservative. The low TVB-N values of fish muscles immersed in the liquid indicated good inhibition of protein putrefaction until 24 h and 10% concentration of the gave significant effect on avoiding fly infestation in salting-fermentation process.

Physical and chemical characteristics of the redistilled liquid smoke: Oil-palm shells were initially pyrolyzed at 400°C, producing dark-color liquid smoke with tar or Polycyclic Aromatic Hydrocarbons (PAHs). Preparation of liquid smoke by redistillation at 80±5°C yielded clear

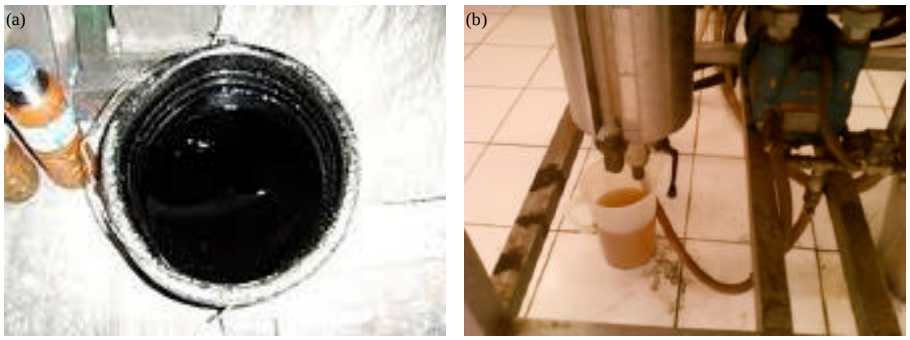

Fig. 1(a-b): (a) Appearance of crude liquid smoke and (b) Redistilled liquid smoke of oil- palm shells at 80°C

Table 1: GC-MS analysis on liquid smoke of oil-palm shells with 90% similarity

Retention time (min)	Area (%)	MW	Compound	Similarity (%)
5.05	41.47	60	Acetic acid	90
5.31	1.00	74	Propanoic acid	90
6.24	3.34	96	Furfural	91
7.03	0.66	82	2-Cyclopenten-1-on	91
7.99	26.13	94	Phenol	91
8.73	1.33	108	2-Methylphenol	97
8.95	1.06	109	3-Methylphenol	96
9.11	5.50	124	2-Methoxyphenol	97
9.72	0.32	122	3,5-Dimethylphenol	97
10.04	0.21	138	2-Methoxy-3-methyl phenol	91
10.19	2.42	138	2-Methoxy-4-methyl phenol	95
11.04	1.59	152	4-Ethyl-2-methoxy phenol	95
11.72	0.43	154	2,6-Dimethoxyphenol	93
11.89	0.16	166	2-Methoxy-4-propyl phenol	91

MW = molecular weight

yellowish brown liquid (Fig. 1). The liquid smoke of oil-palm shells has specific odor and volatile. There is an indication that the redistillation of the liquid smoke at 80±5°C is able to reduce PAHs content that has boiling point of more than 300°C.

Three parameters of liquid smoke were observed: organic acid content, total phenolics and pH. The redistilled liquid smoke of the oil-palm shells exhibits organic acids of 9.14%, total phenolics of 2.6% and pH 3.2. The GC-MS analysis on the redistilled liquid smoke identified some chemicals as shown in Table 1. These compounds have more than 90% similarity with those in the GC-MS instrument's library. The highest constituent (41%) was acetic acid, followed by phenol (26%). Other constituents with similarity more than 95% belong to phenolics, i.e. 3-methylphenol, 2-methylphenol, 2-methoxyphenol and 3, 5-dimethylphenol.

Toxicity of the redistilled liquid smoke: Data of the toxicity test toward brine shrimp larvae and the corresponding LC_{50} are exhibited in Fig. 2. There are increasing numbers of dead larvae with the increasing concentrations of the liquid smoke. Afterwards, the values are derived in the form of relation between-log concentration of the liquid smoke and percent of dead larvae. The LC_{50} value of 0.2147% is obtained from a line equation $y = -1.6662x + 4.9456$ with $R^2 = 0.8527$.

TVB-N values: TVB-N values of pomfret fish that have been preserved for 30 min using the redistilled liquid smoke are shown in Fig. 3. The concentrations of the liquid smoke were 0, 8, 9 and 10% and the TVB-N values were determined every 8 hours at room-temperature storage. Increasing TVB-N values are caused by the action of degrading bacteria on the fish muscular tissues that produces mainly ammonia, trimethylamine and dimethylamine. With prolonging the storage time, the TVB-N values also increasing. Analysis on hour-8 shows that immersion in 10% concentration of liquid smoke produces the lowest TVB-N value (4 mg/100 g) among the three other treatments, as well as those observed at hour-16. Analysis on hour-24 shows that TVB-N value for the control (38 mg/100 g) has exceeded the maximum permissible value, whereas those treatments with the 8-10% concentration of liquid smoke are still below the threshold. All treatments have exceeded permissible TVB-N values at hour-32. Until 24 h, pomfret fish that have been immersed in the liquid smoke showed TVB-N

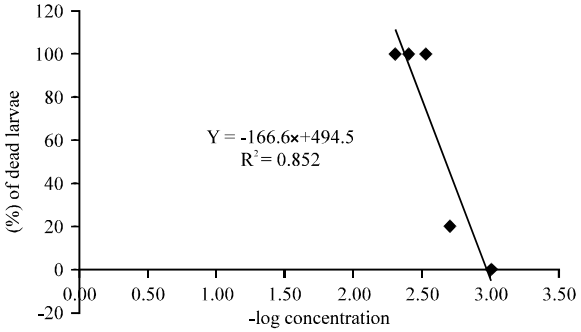

Fig. 2: Relation between -log concentration of liquid smoke and percent of dead larvae

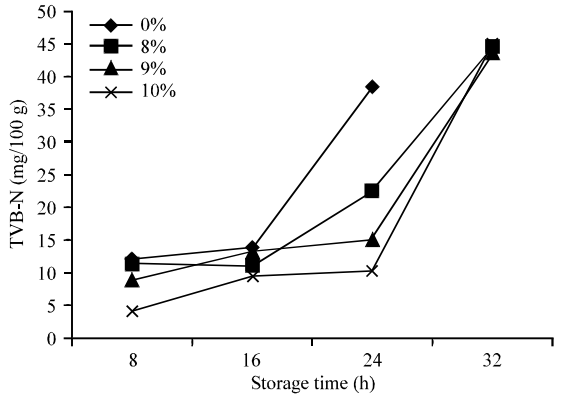

Fig. 3: Total volatile base nitrogen (TVB-N) values of pomfret fish preserved with various concentrations of liquid smoke 8%, 9%, 10% and control

values that are lower than that without liquid smoke treatment, indicating that the fish are well preserved by the redistilled liquid smoke.

Antibacterial properties: Two species for assay on antibacterial activity were used, namely *S. aureus* (representing Gram positive) and *E. coli* (representing Gram negative). Antibacterial activity of the liquid smoke can be seen from the clear zone formed surrounding the corresponding well (Fig. 4). The clear zone formed on *S. aureus* is more distinct as compared with that on *E. coli*. This is related to the effects of the liquid smoke on the bacterial growth. Based on the diameter of formed clear zone, the liquid of oil-palm shells has antibacterial activity in strong category. The strength of antibacterial activity is designated as follow: inhibition area >20 mm is very strong, inhibition area of 10-20 mm is strong, inhibition area of 5-10 mm is moderate and inhibition area <5 mm is in weak category (Davis and Stout, 1971).

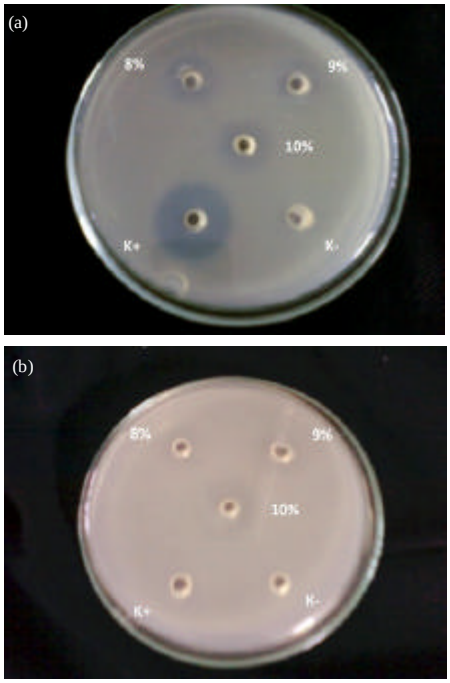

Fig. 4(a-b): Antibacterial activity of redistilled liquid smoke derived from oil-palm shells toward (a) *S. aureus* and (b) *E. coli*

Table 2: Inhibition indices of liquid smoke of oil-palm shells as compared with commercial antibiotics

	Average of inhibition index	
Treatment	*Staphylococcus aureus*	*Escherichia coli*
Blank	0.0[a]	0.0[a]
8% liquid smoke	1.2[b]	0.8[b]
9% liquid smoke	1.2[b]	1.1[c]
10% liquid smoke	1.4[b]	1.2[c]
Chloramphenicol 100 ppm	1.8[c]	0.0[a]

Value with different letter in the same column is significantly different at $p<0.05$

Data of the formed clear zone diameter were further converted into inhibition index (Table 2). The values of inhibition index exhibited by the liquid smoke of concentration 0, 8, 9 and 10% are also compared with that of 100 ppm chloramphenicol. The average of inhibition index by the liquid smoke toward *S. aureus* shows higher value than that toward *E. coli* for the same concentration. The value is also compared with inhibition by common antibiotics chloramphenicol 100 ppm. Test on Duncan's multiple range gives three categories of results, designated as a, b and c. The same letter means that the treatments do not give significantly different results. On *S. aureus*, the use of 8, 9 and 10% liquid smoke give the same inhibition effect (letter b); however, it is significantly

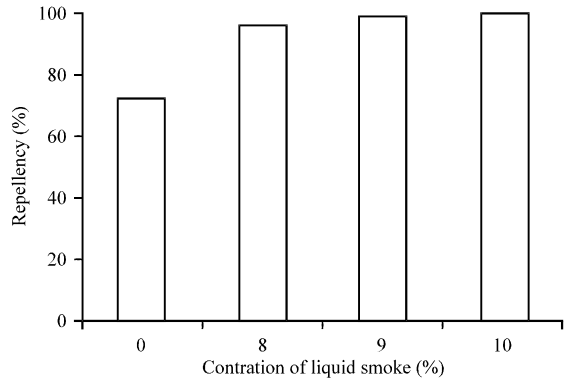

Fig. 5: Fly repellency on the dried-fermented fish with different application of redistilled liquid smoke

Table 3: Significance of fly repellency based on Duncan's multiple range

Level of liquid smoke (%)	Average of repellency
0	71.75[a]
8	95.02[b]
9	98.27[c]
10	99.69[d]

Value with different letter in the same column is significantly different at $p<0.05$

different with that of chloramphenicol 100 ppm (letter c) and the blank (letter a). The 8% concentration of liquid smoke (letter b) gives inhibition differently with that of 9 and 10% concentration (letter c) toward *E coli*. The use of chloramphenicol 100 ppm. does not inhibit the growth of this particular bacteria, just similar to the blank (letter a).

Repellency to flies: Observation on repellency of the liquid smoke toward flies is shown in Fig. 5. Increasing repellency is linearly correlated with the increasing concentration of the applied liquid smoke. The best repellency of the liquid smoke applied on the fermented salted fish was analyzed using Duncan's multiple range (Table 3). The first step was to determine the highest value of all treatments. The highest value was 99.69, obtained from concentration of 10% liquid smoke. The next step considers the letters corresponding to the treatment. All of the average of repellency is followed by different letter. This means that every treatment has significant effect, differently from one to another. Therefore, the average of the highest repellent (10% concentration of liquid smoke) is the best treatment.

DISCUSSION

Physical and chemical properties and toxicity: From the appearance aspect, color of the redistilled liquid smoke is no longer dark. The redistillation turns the previous dark color liquid smoke to bright yellowish-brown. This physical property shall give good effect for its utilization in foodstuff.

The redistilled liquid smoke of oil-palm shells mainly contains organic acids and phenolics. Lower organic acids are weak acids with pK_a of approximately 5. However, the organic acids are more acidic as compared with the phenolics, particularly due to resonance stabilization of the carboxylic anions (RCO_2^-). These acidic compounds play important role in creating the low pH of the liquid smoke (pH 3.2). In addition, the low pH value represents a high quality of liquid smoke particularly in its utilization as food preservatives (Wijaya *et al*., 2008; Theron and Lues, 2011). Furthermore, compounds of PAHs family are not identified, indicating that the redistillation of the liquid smoke at 80°C is effective in eliminating the carcinogenic PAHs content. GC-MS analysis on liquid smoke is also performed in a previous study. Budijanto *et al*. (2008) on his investigation on the safety of liquid smoke derived from coconut shells for food products shows that there are 40 constituents identified by GC-MS analysis. There are phenolic components, such as phenol, 2-methoxy phenol, 3,4-dimethoxy phenol, 2-methoxy-4-methyl phenol. Dihydroxy benzoic acid, methoxybenzoic acid and hydroxyl benzoic acid are present as minor components.

Level of organic acids, total phenolics and pH of our study are different with the study by Darmadji and Triyudiana (2006) which also used liquid smoke redistilled below 100°C, producing 12.34% organic acids and 1.1% total phenolics. Therefore, the redistilled liquid smoke from oil-palm shells gives lower organic acids (9.14%) but higher total phenolics (2.6%). The difference is due to cellulose-hemicellulose and lignin contents in the raw materials, from which the pyrolysis process would determine the liquid smoke quality. There seems to be a clear correlation between lignin and phenolics contents. The liquid smoke from oil-palm shells is also less acidic as compared with the liquid smoke from bamboo (3.08), *Leucaena* wood (2.9) and corn cobs (3.0) (Swastawati *et al*., 2007). Acids level is also affected by distillation temperature (Darmadji, 2002).

Three main components of the liquid smoke from our study are carboxylic acids, phenolics and carbonyls. Hemicelluloses are wood component that firstly undergo pyrolysis to produce furfural, furan, acetic acid and its homologues. Hemicelluloses consist of pentosans ($C_5H_8O_4$) and hexosans ($C_6H_{10}O_5$), with the average proportion depend on the raw materials. Pyrolysis of the pentosans produces furfural, furan and their derivatives, as well as carboxylic acids. Together with cellulose, pyrolysis of hexosans also produces acetic acid and its homologs. Decomposition of hemicelluloses happens at 250-300°C. Lignin in pyrolysis process will produce phenolics that will contribute to smoke aroma in smoked products. Phenolics are produced from lignin

decomposition at 300-450°C (Girard, 1992). Further process in the pyrolysis of cellulose will produce acetic acid and carbonyl compounds, such as acetaldehyde, glioxal and acreolin. Pyrolysis of lignin will produce phenol, guaiacol and syringol, together with their homologs and derivatives (Darmadji, 2002).

Phenolics contribute to the quality for modifying color, flavor, aroma and odor (Sengul *et al*., 2009). Level of total phenolics in the liquid smoke of oil-palm shells 0.26% (b/b) is more than 20 times higher than that of liquid smoke of teakwood, pine wood and bamboo (Wijaya *et al*., 2008). Phenolics with low boiling points are good as bacteriostatics. Organic acids are stronger in inhibiting bacterial growth as compared with phenolics; however, combination of these two categories of organic acids will give rise to higher inhibition. Phenolics, as healthy phytochemicals, are commonly used in food industries as antioxidant and antibacterial (Esekhiagbe *et al*., 2009).

Toxicity of the liquid smoke using BSLT method revealed that no larvae survived on the concentrations of 0.3, 0.4 and 0.5%. LC_{50} in this study is 0.2147%. A compound is designated to have acute toxicity if the LC_{50} is below 0.1% (Carballo *et al*., 2002). Therefore, based on this assay, the redistilled liquid smoke of oil-palm shells is not considered as toxic material.

Potency as antibacterial in fresh fish preservation: Redistilled liquid smoke in this study act as bacteriostatics (inhibit the growth without lethal effect) on *E. coli* and bacteriocidal/bacteriolitic (lethal effect) on *S. aureus*. Inhibition on bacterial growth by an antibacterial agent can be due to the inhibition of synthesis of cell wall, proteins, nucleic acids and disruption of the functions of cell membranes (Jawetz *et al*., 2010). Resistance of *E. coli* which is higher than that of *S. aureus* toward antibacterial agents may be related to the structure of cell membranes. *E. coli* is a member of Gram negative bacteria with layered membrane structure, among which are lipoproteins, lipopolysaccharides and peptidoglicans (Madigan *et al*., 2010).

Organic acids are food preservatives that function as acid-taste elicitor and reduce bacterial growth by reducing pH within the food to pH level that can inhibit bacterial growth. Principle of the inhibition by organic acids is that the undissociated part of the acids (anion moieties) can penetrate into the cell wall of the bacteria and disrupt the normal physiological function of the cells (Theron and Lues, 2011).

Phenolics in the liquid smoke of *pelawan* (*Tristania obovata*) wood actively attack on bacterial vegetative cells, can penetrate and disrupt cell walls and precipitate proteins in the microbe cells (Panagan and Syarif, 2009). Inhibition by phenolics may be due to interaction through hydrogen bonding with some important proteins as enzyme constituent (Saravanakumar *et al*., 2009).

Fish putrefaction is commonly indicated by high TVB-N value. The increase in TVB-N values is due to bacterial action as proved by inclination of bacterial population. Immersion treatments in liquid smoke solutions are able to reduce the rate of volatile base formation as compared with the control. The higher the concentration of the liquid smoke, the better the ability to inhibit the formation of the volatile bases. The maximum TVB-N level for fresh fish in Japan and Australia is 30 mg N% (Siagian, 2002). Dwiyitno and Riyanto (2006) in the experiment using liquid smoke of coconut shells for preserving fresh mackerel is able to maintain TVB-N value below the maximum level until 12-hour storage with treatments using 7.5 and 10% concentrations.

Based on BSLT, the LC_{50} for liquid smoke of oil-palm shells is 0.22%, meaning that it is safe to be incorporated in foodstuff. Safe limit for phenol in food products is 0.0006-0.5% or 0.06-5000 mg kg^{-1} (Girard, 1992). Therefore, immersion in the redistilled liquid smoke (0.18-0.26%) will not exceed the determined safe limit. Phenol content will also decrease in further fish processing such as washing and heating for products to be consumed.

Potency as fly repellent: Fly infestation on dried-fermented fish during sundrying may be due to some volatile bases such as ammonia and hydrogen sulfide. These two compounds are originated from protein degradation caused by proteolytic enzymes during autolysis and fermentation processes (Ariyani *et al*., 2007). However, immersion in liquid smoke solution has elicited odorous phenolics dislikable to flies. In addition, the presence of 30% salt is indirectly functioning as insecticide (Ariyani *et al*., 2007). Some phenolics detected in the redistilled liquid smoke of oil-palm shells, namely cresol, creosol, guaiacol, syringol, eugenol and xylenol, may gave specific aroma. Furfural as the only member of aldehydes that is also detected in this particular liquid smoke has been reported as a disinfectant (Madigan *et al*., 2010). Furfural gives pleasant aroma that reduces aroma of the phenolics that have rather sharp odors. This compound gives specific aroma of the liquid smoke that function as repellent. Action of the liquid smoke is not lethal as compared to insecticides which are

working as contact insecticide. Hence, insects that do not like poison with repellent action can quickly avoid the baits (Yuliani *et al*., 2005).

CONCLUSION

Liquid smoke of oil-palm shells redistilled at 80°C gives clear yellowish-brown in color with specific aroma of volatile constituents. With chemical specifications of organic acids 9.1%, total phenolics 2.6% and pH 3.2, the redistilled liquid smoke is potential as a safe preservative for fresh fish. By 30 min immersion in the liquid smoke solution, the fish freshness can be longer than that without liquid smoke treatments, i.e. up to 24 h at room temperature storage. The redistilled liquid smoke of oil-palm shells is bacteriostatics in nature and shows strong inhibition toward bacterial growth. The best treatment as antibacterial is toward *S. aureus* which represents Gram positive. Immersion in the solution of redistilled liquid smoke functions as antibacterial and fly repellent for dried-fermented fish. The fly-repellency of the treated dried-fermented fish is excellent, with the repellency above 95%. Thus, the redistilled liquid smoke of oil-palm shells can be applied as safe fish preservative and can be combined with other preservation methods such as cooling and salting.

REFERENCES

AOAC, 2005. Official Methods of Analysis. 18th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Ariyani, F., S. Haryati, M. Wahyuni and S.H. Wisudo, 2007. Penggunaan ekstrak bahan alami untuk menghambat infestasi lalat selama penjemuran ikan jambal asin. J. Pascapan. Bioteknol. Kelautan dan Perikanan, 2: 117-127.

Budijanto, S., R. Hasbullah, S. Prabawati, Setyadjit, Sukarno and I. Zuraida, 2008. Identification and safety test on liquid smoke made from coconut shell for food product. Indones. J. Agric. Postharv. Res., 5: 32-40.

Carballo, J.L., Z.L. Hernandez-Inda, P. Perez and M.D. Garcia-Gravalos, 2002. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnol., Vol. 2.

Darmadji, P. and H. Triyudiana, 2006. Proses pemurnian asap cair dan simulasi akumulasi kadar benzopyrene pada proses perendaman ikan. Agritech, 26: 94-103.

Darmadji, P., 2002. Optimasi proses pembuatan tepung asap. Agritech, 22: 172-177.

Davis, W.W. and T.R. Stout, 1971. Disc plate methods of microbiological antibiotic assay. Applied Microbiol., 22: 659-665.

Dwiyitno and R. Riyanto, 2006. Studi penggunaan asap cair untuk pengawetan ikan kembung (*Rastrelliger neglectus*) segar. [Study on the use of liquid smoke for preservation of fresh mackerel (*Rastrelliger neglectus*)]. J. Pasca Panen Bioteknol. Kelaut. Perik., 1: 143-148.

Esekhiagbe, M., M.M.U. Agatemor and C. Agatemor, 2009. Phenolic content and antimicrobial potentials of *Xylopia aethiopica* and *Myristica argentea*. Macedonian J. Chem. Chem. Eng., 28: 159-162.

Fitrial, Y., M. Astawan, S.S. Soekarto, K.G. Wiryawan, T. Wresdiyati and R. Khairina, 2008. Antibacterial activity of water lily extract toward diarrhea-causing pathogenic bacteria. J. Teknol. Indust. Pangan, 19: 158-164.

Girard, J.P., 1992. Smoking. In: Technology of Meat and Meat Products. Girard. J.P. (Eds.). Ellias Harwood, New York..

Halim, M., P. Darmadji and R. Indrati, 2006. Aktivitas biopreservatif asap cair cangkang sawit dalam menghambat bakteri patogen dan pembusuk [Activities biopreservatif shell liquid smoke oil in inhibiting pathogenic bacteria and spoilage]. Agrosains, 19: 67-79.

Jawetz, E., G.E. Melnick and C.A. Adelberg, 2010. Medical Microbiology. 25th Edn., McGraw-Hill, New York, ISBN-13: 978-0071624961.

Madigan, M.T., J.M. Martinko, D.A. Stahl and D.P. Clark, 2010. Brock Biology of Microorganism. 13th Edn., Benjamin Cummings, San Fransisco, California, ISBN-13: 978-0321649638.

Manilal, A., S. Sujith, G.S. Kiran, J. Selvin and C. Shakir, 2009. Cytotoxic potentials of red alga, *Laurencia brandenii* collected from the Indian coast. Global J. Pharmacol., 3: 90-94.

Nurhayati, T.D. and K. Sofyan, 2005. Tempurung kelapa sawit (TKS) sebagai bahan baku alternatif untuk produksi arang terpadu dengan pyroligneous/asap cair. J. Il. Teknol., 3: 39-44.

Panagan, A.T. and N. Syarif, 2009. Uji daya hambat asap cair hasil pirolisis kayu pelawan (*Tristania abavata*) terhadap bakteri *Escherichia coli*. J. Penel. Sains, 6: 30-32.

Saravanakumar, A., K. Venkateshwaran, J. Vanitha, M. Ganesh, M. Vasudevan and T. Sivakumar, 2009. Evaluation of antibacterial activity, phenol and flavnoid contents of *Thespesia populnae* flower extracts. Pak. J. Pharm. Sci., 22: 282-286.

Sengul, M., H. Yildiz, N. Gungor, B. Cetin, Z. Eser and S. Ercisli, 2009. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci., 22: 102-106.

Siagian, A., 2002. Mikroba patogen pada makanan dan sumber cemarannya Il. Kelaut, 4: 214-222.

Swastawati, F., T.W. Agustini, Y.S. Darmanto and E.N. Dewi, 2007. Liquid smoke performance of lamtoro wood and corn cob. J. Coastal Dev., 10: 189-196.

Tamaela, P., 2003. The antioxidant effect of coconut shell liquid smoke to inhibit lipid oxidation on smoked skipjack (*Katsuwonus pelamis*) steak during storage. Ichthyos, 2: 59-62.

Theron, M.M. and J.F. Lues, 2011. Organic Acids and Food Preservation. CRC Press, Boca Raton, ISBN: 1420078429.

Waterhouse, A.L., 2002. Determination of Total Phenolics. In: Current Protocols in Food Analytical Chemistry, Wrolstad, R.E., (Ed.). John Wiley and Sons, New York, ISBN: 0555003302. pp: 1- 4.

Wijaya, M., E. Noor, T.T. Irawadi and G. Pari, 2008. Karakterisasi komponen kimia asap cairdan pemanfaatannya sebagai biopestisida. Bionature, 9: 34-40.

Yanti, A.R. and E. Rochima, 2009. The effect of drying temperature on chemical characteristics of liquid smoked *Clarias gariepinus* fillet at room temperature. J. Bionatura, 11: 21-36.

Yuliani, S., S. Usmiati and N. Nurdjannah, 2005. Efektivitas lilin penolak lalat (repelen) dengan bahan aktif limbah penyulingan minyak nilam. J. Pascapan, 2: 1-10.

Zuraida, I., Sukarno and S. Budijanto, 2011. Antibacterial activity of coconut shell liquid smoke (CS-LS) and its application on fish ball preservation. Int. Food. Res. J., 18: 405-410.