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Antimicrobial Activity of Corncob Liquid Smoke and its Aplication to Smoked Milkfish (*Chanos chanos* Forsk) Using Electric and Mechanical Oven

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Abstract. The application of corncob liquid smoke to milkfish had been conducted using electric and mechanical oven in this study. The purpose of this research was to evaluate the ability of antibacterial corncob liquid smoke in various concentrations (5; 7.5; and 10%) to inhibit the growth of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Vibrio harveyi,* and *Vibrio parahaemolyticus*. The carbonyl compounds of corncob liquid smoke was analysed by Gas Chromatography/Mass Spectrometry. Major component of carbonyl found in corncob liquid smoke were phenol; 2 methoxy phenol; anhydride formic acid and 2,6, dimethoxy phenol. The antimicrobial activity of corncob liquid smoke was effective to inhibit the growth of four microbial pathogen *Escherichia coli*, *Staphylococcus aureus*, *Vibrio harveyi,* and *Vibrio parahaemolyticus*. Examination of *t-test* independent sample to the proximate composition of smoked milkfish by corncob liquid smoke using mechanical and electric oven in terms of moisture; protein; and lipid content showed significantly different (P<0,05) result.

Keywords: Corncob liquid smoke; Carbonyl compound; Antimicrobial activity; Proximate; and Milkfish.

1. Introduction

Fish smoking was traditionally used as a means of preservation of fish by wood combustion. Nowadays, liquid smoke are used as preservation of smoked fish to increase the quality of smoked fish and food safety point of view. Liquid smoke have several advantage over traditional method in terms of environmental pollution, cost, product uniformity, and safety. It is affects the organoleptic properties of smoked fish in many ways, by improving the shelf life, by its antimicrobial and antioxidant activities [1], [2], [3]. Moreover, antimicrobial activity may be better evaluated and handled. The most suitable liquid smoke for elaboration of particular foods may be chosen not only from the sensory but also the safety point of view. A potential application for liquid smoke could be contribute to product safety by controlling the growth microbial pathogens [4]. It contains phenol, acetic acid, syringol, guaiacol, catechol, eugenol, and organic acid that could destroy the walls of bacteria cells [5]. The objective of this study was to determine the inhibitory effect of corncob liquid smoke against four microbial pathogens such as *Escherichia coli*; *Staphylococcus aureus*; *Vibrio harveyi;* and *Vibrio parahaemolyticus* and chemical characteristics of smoked milkfish using commercial liquid smoke i.e. proximate value (moisture, protein, and lipid content).

2. Material and Method

2.1. Carbonyl Compound in Corncob Liquid Smoke

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Samples (5% liquid smoke in hexane) was homogenized into 12 mL of methanolic KOH solutions, then centrifuged at 3000 rpm for 5 minutes. Smoke components were identified and quantified by Gas Chromathography/Mass Spectrometry. The peaks were identified by comparison of retention times.

2.2. Antimicrobial Activity in Corncob Liquid Smoke

Paper discs were dipped into corncob liquid smoke extract with different concentration 5%; 7.5%; and 10%. The samples were placed into petri dish that contained microbial pathogen (*Escherichia coli*, *Staphylococcus aureus*, *Vibrio harveyi,* and *Vibrio parahaemolyticus*). Then incubated with upside down position during 24 hours at 30^0C. The diameter of inhibition zone that formed on the petri dish was measured by measuring the diameter of the barriers that formed around the paper disc. The inhibition zone was detected between diameter of the barriers that formed around the paper disc and the diameter of paper disc.

$$\frac{\text{Potential antimicrobial in liquid smoke}}{\text{Diameter control that similar concentration}} \text{ x } 100\%$$

2.3. Moisture Content

Fish samples were dried by using an oven for ± 24 hours at 100^0C ± 5 until the weight of samples remained constant [6]. The percentage of moisture content was calculated based on :

$$\frac{\text{dry weight}}{\text{wet weight}} x100\%$$

2.4. Crude Protein

Protein content were determined by using Kjeldahl Method [6]. Protein content formula are as follows :

$$\%\ \text{N} = \frac{\text{ml HCl x N HCl x 14,008}}{\text{mg of sample}} x100\%$$

$$\%\ \text{protein} = \%\ \text{N x } 6,25$$

Note:

N HCl: Normality of standart HCl (0,02N)

ml HCl: Standart volume HCl for titration

14,008: Weight from nitrogen atom

6,25: Conversion factor of nitrogen in fish protein

2.5. Lipid Content

Determination of lipid content used was Soxhlet method, where the principle lipid separation from material was using organic solvent chloroform [8]. Lipid which have extraction in flask flowing N for the purpose to evaporate organic solvent in flask. The percentage of lipid content were calculated using the following formula :

$$\frac{\text{gr sample - gr of sample before extraction}}{\text{gr of sample with flask after extraction}} x100\%$$

2.6. Statistical Analysis

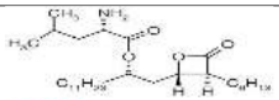
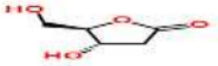
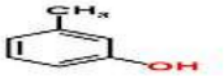
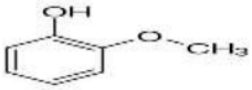
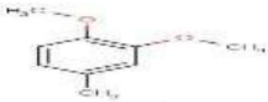
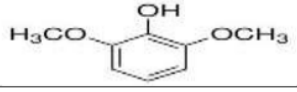
The collected data from two replications was subjected to a *t* –test to determine the differences between traditional and liquid smoke treatment (independent variable) for each parameter using the SPSS statistical package 16. Significantly different treatment means were further separated using the LSD (Least Significant method and significance was reported at P <0.05 with means ± deviation standard (SPSS 16 Program).

3. Result and Discussion

3.1. Carbonyl Compound in Corncob Liquid Smoke

From the pyrolisis process, corncob liquid smoke produces some carbonyl compound that can be seen in Table 1.

Table 1. Carbonyl Compound in Corncob Liquid Smoke (%)

No.	Carbonyl	Retention Time	Peak Area (%)	SI	Molecule Structure
1.	Anhydride formic acid	3,246	12,52	96	
2.	Dihydro2,3H furanone	10,158	4,51	91	
3.	Phenol	12,581	18,93	94	
4.	2 Methyl phenol	15,215	3,67	93	
5.	3 Methyl phenol	15,958	9,26	93	
6.	2 Methoxy phenol	16,450	15,74	94	
7.	2 Ethyl phenol	19,022	9,10	94	
8.	4 Methyl benzene, 2 Methoxy, 1 Hidroxy	19,923	9,43	92	
9.	2 Methyl benzene, 1,4 dimethoxy	22,621	6,35	88	
10.	2,6, Dimethoxy phenol	24,718	10,49	96	

Source: Research report (2013)

As can be seen in Table 1 that major of carbonyl compound in corncob liquid smoke were dominated by phenol derivatives such as: phenol; 2 methoxy phenol; anhydride formic acid and 2,6, dimethoxy phenol. [7], reported that corncob liquid smoke were dominated by three compound namely propanoic acid, 2 furan methanol and phenol. These compound are important for their attributed as an antimicrobial and antioxidant [8]. High content of phenol is possible to give preservative effect to the product because if its capability to kill bacteria, fungi, and antioxidative properties to prevent rancidity. Phenolic compound give an impart to shelf life of smoked milkfish [9]. Result of [10] showed analysis of carbonyl and phenol derivatives on smoking process were dominated by furancarboxaldehyde; furanmethanol; 2-metyhl-2-cyclopenten-1-one; phenol; 2 methyl phenol; 3 methyl phenol; guaiacol; 4 methyl-guaiacol; and syringol.

3.2. Antimicrobial Activity

The antimicrobial agent of corncob liquid smoke was found effective to inhibit the growth *Escherichia coli, Staphylococcus aureus, Vibrio harveyi* and *Vibrio parahaemolyticus* (Table 2).

Table 2 showed that 5% of corncob liquid smoke was less effective as it only found to inhibit the growth of bacteria in the range between 17.78%-46.12%. But 7.5 to 10% of corn cob liquid smoke was found

effective to inhibit the microbial activity especially against *Vibrio haveryi* and *Vibrio parahaemolyticus*. Based on [4], the antibacterial activity of four commercial smoke condensates was able to inhibit the growth of the investigated pathogens *Aeromonas hydrophila*, *Yersinia enterocolitica*, and *Listeria monocytogenes* at low temperature.

Table 2. Antimicrobial Activity in Corncob Liquid Smoke

No	Liquid smoke concentrate (%)	Replication	Potential Antimicrobial (%)			
			Escherichia coli	*Staphylococcus aureus*	*Vibrio harveyi*	*Vibrio parahaemolyticus*
1.						
	5	1	30.55	17,78	46,12	35,5
	7.5		32.22	25.55	60.55	41.67
	10		58.33	46.67	61.67	47.22
2.						
	5	2	21.67	22.78	27.22	41.67
	7.5		30.00	37.78	38.33	50.55
	10		63.88	41.67	55.55	75.55
3.						
	5	3	20.55	27.22	20.55	46.12
	7.5		23.88	35.00	36.12	49.45
	10		51.67	46.12	80.55	52.78

Source: Research report (2013).

3.3. Proximate Analysis

The results of proximate analysis on raw materials and smoked fish indicated variation on chemical composition (Fig. 1). Proximate composition of smoked milkfish using corncob liquid smoke and dried by electric and mechanical oven showed that moisture content, protein and lipid were significantly different (P<0,05). It was indicated that the changes on the proximate composition of smoked fish was affected by the chemical compound of liquid smoke and the different method of drying by using electric and mechanical oven.

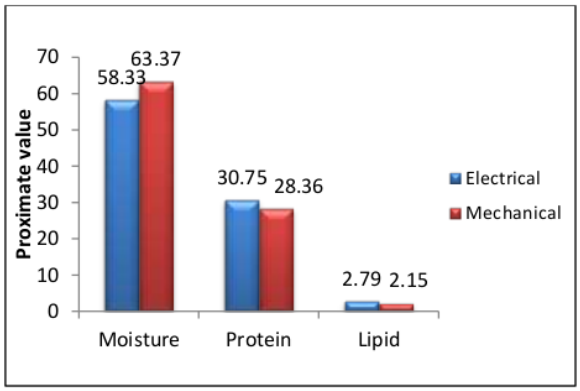


Fig. 1: Proximate value of smoked milkfish using corncob liquid smoke

Based on [11], the two different commercial smoke flavourings led to changes in the physicochemical characteristics such as protein, lipid and moisture content, on smoked beef tongue by liquid smoke give moisture content of 59.58%; 59.37%; 58.92%; 56.38%; and 52.93% respectively. Lipid content on smoked skipjack by coconut shell liquid smoke from 0 to 6 day were 1.11% to 0.92%; 6.97 to 6.87%. Protein content of smoked little tuna by coconut shell liquid smoke were 31.09 up to 36.95% [12], [13].

4. Conclusion

Corncob liquid smoke was found able to inhibit the growth of pathogenic bacteria: *Escherichia coli, Staphylococcus aureus*, *Vibrio harveyi* and *Vibrio parahaemolyticus* at room temperature. Electric oven was found more effective in maintaining the quality of smoked milkfish comparing to the mechanical oven as it completed by temperature control and air circulation system.

5. Acknowledgement

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