International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.15, pp 317-331, 2017

Effect of Combination Treatment of Concentration Liquid Smoke, Immersion Duration, Packaging and Long Storage different Levels of Antibacterials Nila Fish Fillet (*Oreochromis niloticus*)

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Abstract : This study aims to determine of antibacterials inhibititory power diameter (DDH) of fillet of tilapia (*Oreochromis niloticus*) given preservation with liquid smoke derived from a combination of liquid smoke treatment concentration, soaking time, types of packaging and long storage are different. This study was conducted experimentally using factorial experiment with a completely randomized design patterns (RAL) 5 x 3 x 3 x 5 with 3 replicates in order to obtain 675 experimental units. A factor consists of the concentration of liquid smoke consisting of control (smokeless liquid / 0%), 5% and 10%, 15% and 20%; factor B consists of soaking time with liquid smoke is composed of three (3) levels ie soaking time 5 minutes, 10 minutes and 15 minutes; factor C consists of the type of packaging consists of three (3) levels ie without packaging (control), packaging polyethylene (PE) and packaging of polypropylene (PP) and factor D consists of the storage time (days) consists of 5 (five) levels ie 0 , 3,6,9 and 12 days. The parameters measured were the levels ofantibacterials diameter inhibition (DDH) . Results of research on the analysis of variance showed (1) .there was an interaction of treatment with different concentrations of soaking time, soaking time with the storage time, the concentration difference with storage time while the combination of two other treatments showed no significant difference (no interaction). In the treatment of three treatment difference immersion, the concentration of liquid smoke with storage time showed real interaction to the diameter of the inhibition (DDH) Escherichia coli, while the combination of the other three treatments were not significantly different (no interaction). Four combined treatment showed no inhibition of the interaction of the diameter (DDH) *Escherichia coli* which is antibacterial liquid smoke on fillet of tilapia. (2). diameter inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia tertinngi contained in four combination treatment that liquid smoke concentration of 20 percent, a 15-minute soaking time, type of packaging polyethylene (PE) and storage for 3 days amounted to 16.697 mm / ppb.(3). the diameter of the power bland (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in three treatment combinations contained in the treatment liquid smoke concentration of 20 percent, a 15-minute soaking time and storage time of 12 days amounted to 16.693 mm / ppb.(4). diameter inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in the treatment of liquid smoke concentration of 20% at 10 minutes soaking time of 12 626 mm / ppb.(5). the diameter of inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in the treatment of liquid smoke concentration of 20% in the duration of storage for 12 days amounted to 14 693 mm / ppb.(6). the diameter of inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highs on a combination of old submerged for 15 minutes and for 12 days storage time high of 8.6 mm / ppb.

Key words : fish fillet, immersion, concentration, packaging, storage, antibacterial

I. Introduction

Among the species of freshwater fish are now being developed and grown in the provinces of West Sumatra are Tilapia (*Oreochromis niloticus*). The potential of aquaculture land estimated area of 12,300 hectares [1]. This is because these fish easy life, fast breeding, the meat is white and it was quite tasty. Processing methods can be developed against the fish is a fish processing fish. Result fillet processing such as fillets of fish including food very quickly decompose (high perishable food). As perishable foodstuffs, then the quality of the fish must be maintained as much as possible to get into the hands of consumers. For that we need good handling and preservation and processing into products ready to be eaten but durable power longer. One way of processing that has long been known to the public is the curing of fish.

Fumigation is a technique of embedding and incorporating various chemical compounds of smoke into foodstuffs [2]. Fogging was intended to extend the shelf life of a material, but in line with the increase in public acceptance of the product smoke then that goal began to turn to the flavor, which gives aroma and distinctive taste and prevents rancidity of the meat due to the oxidation of fat. Fumigation can be done traditionally or in modern [3]. Traditional fumigation can be done in the cold and heat by burning wood or sawdust, where the smoked fish direct contact with the smoke. While modern fumigation using liquid smoke (steam dispersion in the fluid as a result of condensation of smoke from wood pyrolysis) as media smoking. Generally wider community, especially the coastal communities do fumigation with traditional fumigation techniques. Though the technique of curing it has a lot of shortcomings, among other things take a long time, is not efficient in the use of firewood, the uniformity of the product to produce color and flavor desired difficult to control, environmental pollution, and the most dangerous is the residual tar and hydrocarbon compounds polycyclic aromatic (*benzo(a)pyren*) deposited in food that can be harmful to health. In areas producing smoked fish, in order to meet the source of the smoke (wood) many people who cut down trees, even be protective coastal mangroves were not spared from logging target. These circumstances make alternative use of firewood has to be considered as well as fogging technique was time to be replaced with modern fumigation.

The use of liquid smoke broader application to replace the traditional way of curing. With the provision of liquid smoke aroma smoke on fish would be more practical because only by spraying or dipping the fish in a solution of liquid smoke, followed by heating. The development of liquid smoke more rapidly in the preservation of foodstuffs, due to the costs required for timber and equipment manufacture more efficient smoke, harmful components can be separated or reduced before being used in food as well as the composition of the liquid smoke is more consistent for repeated use [4].

Modern fogging is fumigation with the gas phase (gas phase smoke) or fumigation with liquid smoke (liquid smoke). Fumigation with the liquid smoke made by soaking the product in liquid smoke that has been disbursed through the process of pyrolysis and distillation [4]. Fumigation this way can improve the quality of products in terms of health because of carcinogenic compounds such as benzo (a) pyren contained in the liquid smoke can be absorbed and reduced in number, while the tar can be separated by using sedimentation and filtration [5].

Some research on the production and use of liquid smoke has been carried out include the determination of the temperature and time of pyrolysis of rubber wood to produce liquid smoke quality [6], the study of raw materials cinnamon at a temperature pyrolysis 400^{o}C produce quality liquid smoke [7] and the dominant containing compound acetic acid and phenol [8], a materials research cinnamon with temperature pyrolysis of 400^{o}C at concentrations of 1500 ppm showed antioxidant supreme amounted to 35.091% [9], the determination of antibacterial properties of liquid smoke produced from several kinds of soft wood [10], the preservation of the tongue smoke with liquid smoke produced from teak [11]. Budaraga research results *et al*, [12] to get the dominant content of liquid smoke coconut husks, coconut shell and cinnamon contains acetic acid and phenol. Further research Budaragaet.al.,[13] to get the cytotoxic properties (the ability to kill Artemiasalina) liquid smoke cinnamon at 400^{o}C temperature pyrolysis of 19.048%. These studies all utilize hardwood and softwood separately. Whereas softwood with low lignin content will be very effective to extend the lasting power of fish and produce flavor which is not typical [14]when combined with other wood (hardwood).

Based on the above research, the cinnamon is ideal to use as a preservative. The results of further research Budaraga *et al*, [15] to get the purification of liquid smoke cinnamon on the distillation temperature of 140^{o}C have undetectable levels of benzo (a) pyrene. Further research Budaraga *et al*, [16] to get the liquid smoke

toxicity cinnamon purified by precipitation during the 3-day 83.75%. Results antioxidant liquid smoke cinnamon in a manner different purification produces antioxidants that are strong enough (<50 ppm) Budaraga *et al*, [17]. Furthermore, the results of research Budaragaet.al.,[18] to get the measurement results on the antibacterial properties of *Escherichia coli* liquid smoke cinnamon purified by precipitation for 3 days resulted in inhibition diameter 34.129 mm/ppb. Their immersion in liquid smoke concentration cinnamon right would affect the levels of antioxidants and so far there is no information about it.

The next process followed by drying the fillets of tilapia resulting in decreased water levels expected product microbial activity is inhibited, resulting in a longer lasting power products. During this time the nature of the community is still traditional fish processing, fish fillet products in the form of beef jerky is usually not packaged properly so easily contaminated by microorganisms which will result in reduced power durable besides that do not pay attention shelf. Besides the water content of the product is still relatively high. To obtain a lower water content, then fillet products were not made in the form of a thick but in the form of thin slices. It is intended that the liquid smoke cinnamon can more rapidly penetrate into slices of fillet of tilapia, as well as the drying process faster. With the form of the product in the form of thin slices of fillet, hoped no bones were shipped, all the edible parts and form a thin more attractive for consumers. Contamination with microbes and other damage can be prevented by packing with a plastic bag. It remains no information about the type of packaging and storage right on levels of phenol and carbonyl tilapia fillet stuffed with liquid smoke. The results of the study [19] showed no packaging was good at cooking spices during storage will cause a loss of quality. The purpose of this study to determine the levels of antibacterials diameter inhibition smoked fillet of tilapia given combined treatment of liquid smoke concentration, soaking time, types of packaging and storage time are different.

II. Raw and Methods

The materials used for the manufacture of fish fillets ie tilapia black bought at the market bottom of the crocodile with an average weight of 250 grams / fish, alcohol 70%, salt, water and liquid smoke cinnamon purified by distillation temperature of 140^{o}C and chemicals for analysis of phenol and carbonyl. The tools used in this study are: a. Equipment for the manufacture of preservative solutions flask, glass beaker, beakers, pipettes, and mixer.b. Equipment for the manufacture of fish filet was basins, pans, mixers, stainless steel knives, water heating, cutting boards, work desks, spray equipment, pan drainer, freezer, and analytical scales. c. Equipment for drying of tilapia fillets: briquette stove heat resistance [20], a drying oven tool length 240 x width 100 x height 80 cm measurement device 200^{o}C [21]. d. Equipment for packaging and storage: storage shelves, polyethylene, polypropylene plastic, paper labels, paper plates for a fillet. Another tool used in this study such as, refrigerator coolers, freezers, flask, cup petridist, electric stove, filter paper, oven, burette, incubators, ovens, porcelain dish, desiccator, filter, thermometer, erlenmeyer125 ml and 500 ml, beaker, filter paper, soxhlet, vortex, test tubes, micro burette, pipette, pipette volumetric flask of 250 ml.

2.1.Metode Research

The experimental design used in this study using factorial pattern in a completely randomized design (CRD) is a combination of liquid smoke concentration with soaking time, types of packaging and storage in order to obtain 5 x 3 x 3 x 5 x 3 trial replications = 675 experimental units. The first factor consisted of 5 (five) level is the concentration of liquid smoke control, 5% and 10%, 15% and 20%; The second factor of soaking with liquid smoke is composed of three (3) levels ie soaking time 5 minutes, 10 minutes and 15 minutes; The third factor type of packaging consists of three (3) levels ie without packaging, packaging polyethylene (PE) and polypropylene packaging (PP) and the factor of the place of storage time (days) consists of 5 (five) levels ie 0,3,6,9 and 12 days. The observed data in the form of phenol and carbonyl analyzed by analysis of variance on the real level of 5%, when dilanjutnya significantly different by Tukey's test at 5 percent significance level [22].

2.2. Implementation Research.

2.2.1.Preparation liquid smoke.

Before the pickling process fillet of tilapia with liquid smoke cinnamon purified by distillation temperature of 140^{o}C first prepare liquid smoke subsequent dilution with distilled water. The concentration of preservative liquid smoke used is smokeless liquid (control), 5%, 10%, 15% and 20%.

2.2.2.Make fillet of tilapia and preservation with liquid smoke

The process of making fillets of tilapia and preservation with liquid smoke cinnamon well as packaging and storage done in this study are as follows: In the conduct of research activities begins with the preparation of materials and tools such as a desk, knives, cutting boards that have been sterilized with alcohol 70% and cinnamon liquid smoke that has been purified. Prepared aqudest (control), liquid smoke concentration of liquid smoke 5%, 10%, 15% and 20%, Tilapia been in fresh condition refers to the SNI [23] on the specifications of fresh fish and SNI [24] on the requirements of the raw material with the characteristics raw materials are clean, free of any odor indicating decay, is free of signs of decomposition and forgery, free from other natural properties that can reduce the quality and not harmful to health. Organoleptic characteristics of the raw material has a freshness: a) appearance: intact, convex eyes, bright white cutlet; b) The smell: specific fresh fish; c) texture: Solid, compact and elastic, with a weight of 250 ± 10 grams. As for how to manufacture fillets of tilapia as follows: Cultivated using fresh fish that has passed through the phase freezing (rigor mortis) and cleanliness is always maintained by weeding the scales of a fish, discarding the entrails, feces, and lining the wall of the stomach is black, then do the washing up clean to remove any remaining dirt, blood, loose scales and slime. Already clean then performed an incision behind the gill fins to the back of the head; front heads toward keekor incision along the dorsal fin using a stainless steel knife and a knife made parallel so separated from the ribs when taking fillet.

Turn the fish, cut off the back fin gills until the head backward; The cut of the tail toward the head. Open the fillet by cutting towards the head with a knife close to the ribs, cutting through the bone of thorns. Furthermore fillet obtained immediately put into the freezer -20°C as soon as possible. To prevent a decline in quality, cleanliness fillet is always maintained and in working to make fillets have to really pay attention to sanitary aspects such as using gloves, head, working table knife would have been made sterile by sprayed and rinsed with alcohol before starting the job.

In this study using fish fillets in the form of block ie boneless fillets. Avoid contamination which can easily infiltrate into the meat tissue and muscle meat that has been open to the whole fish. In the process of handling for each stage of work to keep the fish stay fresh is to protect from the sun, wind, other heat source to increase the temperature of the fish and once made fillet put in the freezer. To reduce drip (water from the muscle tissue is lost in the frozen product melted) fillet do immersion in pure saline solution 15% for 20 seconds.

This fillet construction work done quickly but carefully to avoid spoilage, contamination and defects due to carelessness which may adversely affect the product and to anticipate these things put in feezer. Waste obtained from pemfilettanbe removed from the processing to avoid contamination of the product. In blocks, fillets transported easily stored and handled SNI [25].Furthermore, fish blocks are cut in the form of stick (size of ± 5 x 10 cm with a thickness of ± 2 cm) and are given treatment liquid smoke is a concentration of 5%, 10%, 15%, 20% and control (without liquid smoke) and combined with the long immersion different ie 5 minutes, 10 minutes and 15 minutes. After completion of the immersion, the fillet is removed and drained and winds up dry fillet surface. Fillet of tilapia further arranged on the shelves of the oven so evenly, and dried at 70 ° C for 6 (six) hours.

After the fillets of tilapia smoked dry due to heating, fillet cooled at room temperature for ± 20 minutes to cool placed in a clean container styre form and hygienic [26], and then inserted into the packaging polyethylene (PE), polypropylene (PP) and without packaging shall be retained and held at room temperature observations began days 0, 3 days, 6 days, 9 days and 12 days to antibacterials diameter inhibition (Gariga.et.al, [27]danHarmita, *et .al*.,[28]

III. Result and Discussion

Antibacterial Test Inhibitory Power Diameter (DDH) *Escherichia coli*

In the analysis of variance DDH *Escherichia coli* fillet of tilapia showed that the combination of two treatments showed an interaction such as long soaking with different concentrations, dipping time with the storage time, the concentration difference with storage time while the combination of two other treatments showed no significant difference (no interaction). In the treatment of three treatment difference immersion, the

concentration of liquid smoke with storage time showed real interaction to the diameter of the inhibition (DDH) *Escherichia coli* (P <0.05), while in the other triple combination treatment was not significantly different (no interaction). Four combined treatment showed no interaction (P> 0.05). The average value of the diameter of the inhibition (DDH) fillet of tilapia in the treatment of different concentrations of liquid smoke, duration of storage, types of packaging and different storage time is presented in Table 1 and Figure 1 below.

Table 1. Values DDH *Escherichia coli* (mm/ ppb) fillet of tilapia based on differences in the concentration of liquid smoke, prolonged submersion, types of packaging and storage

Type	**Time (K)**	**Concentration (L)**	**Timestorage (S) (day)**					**Mean (L)/(K)**
Packaging	soaking (minute)	liquid smoke (%)	0 (S_0)	3 (S_1)	6(S_2)	9(S_3)	12(S_4)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	5 (K1)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	2,327	8,327	5,147	11,147	7,623	6,914
		15 (L_3)	5,333	11,333	8,260	14,260	10,710	9,979
		20 (L_4)	8,333	14,333	10,117	16,117	12,707	12,321
	Mean 5 minute		3,199	6,799	4,705	8,305	6,208	5,843
Control		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
(non	10 (K2)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
packaging)		10 (L_2)	4,327	10,327	7,147	3,623	9,623	7,009
(KK)		15 (L_3)	7,333	13,333	10,260	6,710	12,710	10,069
		20 (L_4)	10,333	16,333	12,117	9,707	14,707	12,639
	Mean 10 minute		4,399	7,999	5,905	4,008	7,408	5,944
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	15 (K3)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	6,327	3,147	9,147	5,623	11,623	7,173
		15 (L_3)	9,333	6,260	12,260	8,710	14,710	10,255
		20 (L_4)	12,333	9,117	14,117	10,707	16,707	12,596
	Mean 15 minute		5,599	3,705	7,105	5,008	8,608	6,005
	Mean	0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	concentration	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
	liquid smoke	10 (L_2)	4,327	7,267	7,147	6,798	9,623	7,032
		15 (L_3)	7,333	10,309	10,260	9,893	12,710	10,101
		20 (L_4)	10,333	13,261	12,117	12,177	14,707	12,519
	Mean	5 (K_1)	5,843					
	time	10(K_2)	5,944					
	soaking (minute)	15(K_3)	6,005					
	Mean time storage		4,399	6,167	5,905	5,774	7,408	5,930
	Mean packaging control (KK)		5,931					
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	5 (K1)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	2,317	8,317	5,137	11,137	7,613	6,904
		15 (L_3)	5,323	11,323	8,250	14,250	10,700	9,969

		20 (L_4)	8,323	14,323	10,107	16,107	12,697	12,311
	Mean 5 minute		3,193	6,793	4,699	8,299	6,202	5,837
Packaging		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
PP	10 (K2)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	4,317	10,317	7,137	3,613	9,613	6,999
		15 (L_3)	7,323	13,323	10,250	6,700	12,700	10,059
		20 (L_4)	10,323	16,323	12,107	9,697	14,697	12,629
	Mean 10 minute		4,393	7,993	5,899	4,002	7,402	5,938
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	15 (K3)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	6,317	3,137	9,137	5,613	11,613	7,163
		15 (L_3)	9,323	6,250	12,250	8,700	14,700	10,245
		20 (L_4)	10,323	16,323	12,107	16,588	14,697	12,629
	Mean 15 minute		3,128	1,877	4,277	2,863	5,263	3,482
	Mean	0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	concentration	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
	liquid smoke	10 (L_2)	4,317	7,257	7,137	6,788	9,613	7,022
		15 (L_3)	7,323	10,299	10,250	9,883	12,700	10,091
		20 (L_4)	6,215	10,215	7,405	16,601	9,131	8,314
	Mean	5 (K_1)	5,837					
	time	10(K_2)	5,938					
	soaking (minute)	15(K_3)	3,482					
	Mean time storage		3,571	5,554	4,958	5,054	6,289	5,085
	Mean packaging control (PP)		5,086					
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	5 (K1)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	2,297	8,297	5,117	11,117	7,593	6,884
		15 (L_3)	5,303	11,303	8,230	14,230	10,680	9,949
		20 (L_4)	8,303	14,303	10,087	16,087	12,677	12,291
	Mean 5 minute		3,181	6,781	4,687	8,287	6,190	5,825
Packaging		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
PE	10 (K2)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	4,297	10,297	7,117	3,593	9,593	6,979
		15 (L_3)	7,303	13,303	10,230	6,680	12,680	10,039
		20 (L_4)	10,303	16,303	12,087	9,677	14,677	12,609
	Mean 10 minute		4,381	7,981	5,887	3,990	7,390	5,926
		0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000
	15 (K3)	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
		10 (L_2)	6,297	3,117	9,117	5,593	11,593	7,143
		15 (L_3)	9,303	6,230	12,230	8,680	14,680	10,225
		20 (L_4)	6,202	10,202	7,391	8,588	9,118	8,300
	Mean 15 minute		3,120	1,869	4,269	2,855	5,255	3,474
	Mean	0 (L_0)	0.000	0.000	0.000	0.000	0.000	0.000

	concentration	5 (L_1)	0.000	0.000	0.000	0.000	0.000	0.000
	liquid smoke	10 (L_2)	4,297	7,237	7,117	6,768	9,593	7,002
		15 (L_3)	7,303	10,279	10,230	9,863	12,680	10,071
		20 (L_4)	6,202	10,202	7,391	16,697	9,118	8,300
	Mean	5 (K_1)	5,825					
	time	10(K_2)	5,926					
	soaking (minute)	15(K_3)	3,474					
	Mean time storage		3,560	3,283	3,385	2,282	4,215	3,345
	Mean packaging control (PE)		5,075					
	CV = 1,96							

Description: Figures followed by different letters in the same row or column showed significant differences (P <0.05).

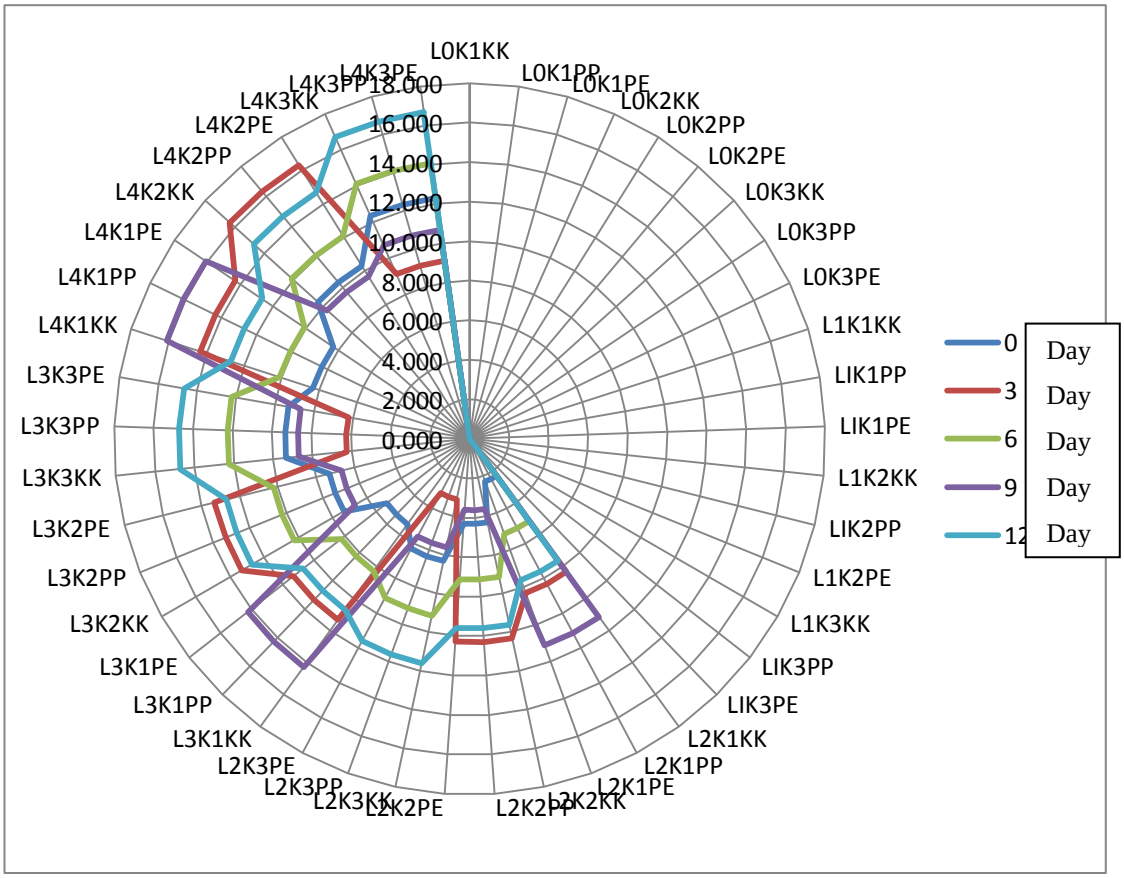

Figure 1. Values DDH *Escherichia coli* (mm/ppb) fillet of tilapia based on differences in the concentration of liquid smoke, prolonged submersion, types of packaging and storage time.

Based on Table 1 and Figure 1 shows that the Diameter Power Inhibition (DDH) *Escherichia coli* fillet of tilapia given combined treatment of different concentrations of liquid smoke (L), prolonged submersion (K), the type of packaging (KK, PE and PP) and storage time (S) ranged from 0.00 to 16.697 mm / ppb. The diameter of inhibition contained in the combination treatment of liquid smoke to 20 percent, a 15-minute soaking time, type of packaging polyethylene (PE) and storage for 3 days amounted to 16.697 mm / ppb. The amount of inhibition is suspected due to the use of liquid smoke concentration is high enough so that the ability to inhibit the growth of microbes, especially *Escherichia coli* becomes higher. According to ItaZuraidaet.al.,[29] stating liquid smoke can hinder the development of P. aeruginosa and S. aureus. Liquid smoke MIC of 0.40 °/o on S. aureus and 0:22 °/o for P. aeruginosa.

Furthermore, the average value of the interaction of DDH *Escherichia coli* (mm /ppb) fillet of tilapia based treatment differences soaking time, the concentration of liquid smoke to the storage time is presented in Table 2 and Figure 2 below.

Table 2.Average value interaction DDH *Escherichia coli* (mm / ppb) fillet of tilapia based on differences in the concentration of liquid smoke, long soaking and storage time.

Time (K)	**Concentration (L)**	**Time storage (S)**					**Mean**	**Interaction**
soaking (minute)	**liquid smoke (%)**	**0 (S^0)**	**3 (S^1)**	**6(S^2)**	**9(S^3)**	**12(S^4)**	**L*S**	**L*S**
	0 (L^0)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
5 (K^1)	5 (L^1)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
	10 (L^2)	2.3233 ^{y}	8.3133 ^{o}	5.1333 ^{u}	11.133 ^{i}	7.6100 ^{p}	6.903	-3.383
	15 (L^3)	5.3200 ^{u}	11.320 ^{i}	8.2467 ^{0}	14.247 ^{d}	10.697 ^{j}	9.966	-3.446
	20 (L^4)	8.3200 ^{o}	14.320 ^{d}	10.103 ^{k}	16.103 ^{b}	12.693 ^{f}	12.308	-2.788
Mean 5 minute		3.193	6.791	4.697	8.297	6.200	5.835	-2.198
Interaction (K^1*S)		4.392	7.992	5.691	9.291	7.217	6.916	
	0 (L^0)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
10 (K^2)	5 (L^1)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
	10 (L^2)	4.3133 ^{v}	10.313 ^{k}	7.1333 ^{q}	3.6100 ^{w}	9.6100 ^{l}	6.996	0.422
	15 (L^3)	7.3200 ^{q}	13.320 ^{e}	10.247 ^{k}	6.6967 ^{r}	12.697 ^{f}	10.056	0.374
	20 (L^4)	10.320 ^{k}	16.320 ^{b}	12.103 ^{g}	9.6933 ^{l}	14.693 ^{c}	12.626	0.576
Mean 10 minute		4.391	7.991	5.897	4.000	7.400	5.936	-1.179
Interaction (K^2*S)		5.592	9.192	6.891	5.217	8.417	7.062	
	0 (L^0)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
15 (K^3)	5 (L^1)	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.0000 ^{z}	0.000	0.000
	10 (L^2)	6.3133 ^{s}	3.1333 ^{x}	9.1333 ^{m}	5.6100 ^{t}	11.610 ^{h}	7.160	-1.414
	15 (L^3)	9.3200 ^{m}	6.2467 ^{s}	12.247 ^{g}	8.6967 ^{n}	14.697 ^{c}	10.241	-1.441
	20 (L^4)	12.320 ^{g}	9.1033 ^{m}	14.103 ^{d}	10.693 ^{j}	16.693 ^{a}	12.582	-0.867
Mean 15 minute		5.591	3.697	7.097	5.000	8.600	5.997	1.019
Interaction (K^3*S)		6.792	4.891	8.091	6.017	9.617	7.081	
Mean	0 (L^0)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
consentration	5 (L^1)	0.000	0.000	0.000	0.000	0.000	0.000	0.000
liquid smoke	10 (L^2)	4.317	7.253	7.133	6.784	9.610	7.019	-1.458
	15 (L^3)	7.320	10.296	10.247	9.880	12.697	10.088	-1.504
	20 (L^4)	10.320	13.248	12.103	12.163	14.693	12.505	-1.026
Interaction (L)		5.592	7.358	6.891	6.841	8.417		
Mean								
time		4.392	6.160	5.897	5.766	7.400	5.923	
soaking (minute)								
Interaction (K*L*S)		-1.200	1.547	-1.200	1.649	-1.200		

Description: Figures followed by different letters in the same row or column showed significant differences (P <0.05).

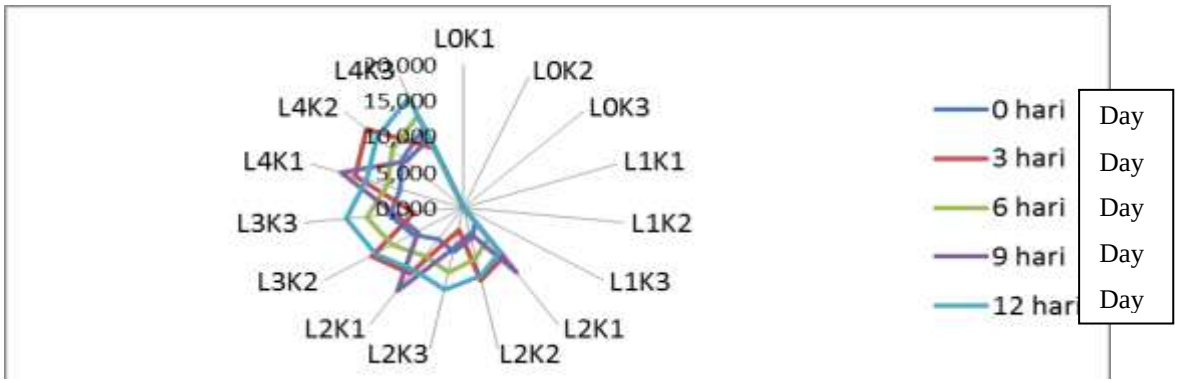

Figure 2.Average value interaction DDH *Escherichia coli* (mm / ppb) fillet of tilapia based on differences in the concentration of liquid smoke, long soaking and storage time.

In Table 2 and Figure 2 shows the DDH *Escherichia coli* (mm / ppb) fillet of tilapia on a combination of three (3) treatment of different concentrations (L), prolonged submersion (K) and storage (S) ranged from 0.00 to 16.693 mm / ppb. The bland power capability found in giving treatment liquid smoke concentration of 20 percent, a 15-minute soaking time and storage time of 12 days amounted to 16.693 mm/ppb. The amount of liquid smoke inhibition is suspected due to the high concentration of liquid smoke is used, then the immersion liquid so that the smoke will be absorbed perfectly on fillet of tilapia giving figures inhibitory effect on the bacteria *Escherichia coli* higher. According Pszczola[5]bahw liquid smoke can act as bacteriostatic caused due to formaldehyde, but the activity of this compound alone is not enough as the cause of all the observed effects. The combination of functional components phenol and organic acid content is high enough to work synergistically to prevent and control the growth of microbes. The content of the high levels of acid can inhibit microbial growth because microbes can only grow in a low acid levels. Furthermore Prananta[30] suggested a phenol with a high boiling point in the smoke is also a high antibacterial substance.

Furthermore, the average value of the interaction of DDH *Escherichia coli* (mm / ppb) fillet of tilapia by soaking time difference treatment with liquid smoke concentrations presented in Table 3 and Figure 3, below.

Table 3. The value of the average interaction DDH E Coli (mm / ppb) fillet of tilapia by different concentrations of liquid smoke with a soaking time

Time Soaking (K)	**Concentration (L)**					**Mean**	**Interaction**
(minute)	**0 (L0)**	**5 (L1**	**10(L2)**	**15(L3)**	**20(L4)**	**L**	**L*K**
5 (K^1)	0.0000 ^{i}	0.0000 ^{i}	6.9007 ^{h}	9.9660 ^{e}	12.308 b	5.835	-6.151
10(K^2)	0.0000 ^{i}	0.0000 ^{i}	6.9960 ^{g}	10.056 ^{d}	12.626 a	5.936	-6.292
15(K^3)	0.0000 ^{i}	0.0000 ^{i}	10.056 ^{d}	10.241 ^{c}	12.583 a	6.576	-5.634
Mean (K)	0.000	0.000	7.984	10.088	12.506	6.116	
Interaction (K*L)	0.000	0.000	0.191	0.017	0.017	0.048	

Description: Figures followed by different letters in the same row or column showed significant differences (P <0.05).

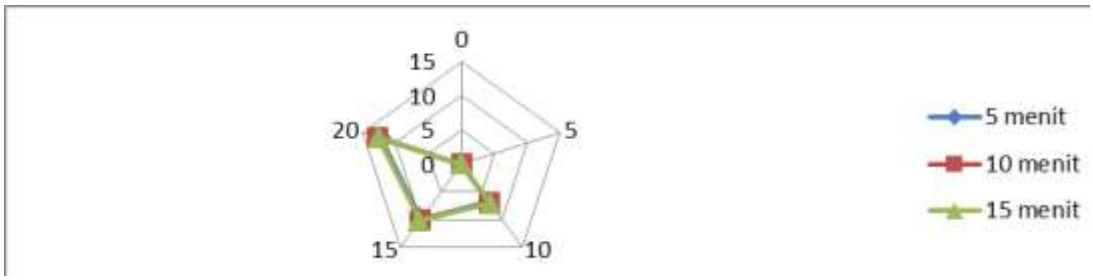

Figure 3. The value of the average interaction DDH E Coli (mm / ppb) fillet of tilapia by different concentrations of liquid smoke with a soaking time

In Table 3 and Figure 3 shows the value of a negative interaction on the line of soaking treatment with a concentration of 0%, 5%, 10%, 15% and 20% against *Escherichia coli* DDH tilapia fillets. Means that both factors are not the same response. While the column shows the value of positive interaction between the concentration of liquid smoke 0%, 5%, 10%, 15% and 20% with prolonged submersion. Values of positive interaction means both treatment factors together provide a response to the DDH Escherichia coli. In Table 3 and Figure 3 shows the greatest inhibition of liquid smoke to the development of *Escherichia coli* in fillets of tilapia are treated liquid smoke concentration of 20% at 10 minutes soaking time of 12 626 mm / ppb. The amount of liquid smoke inhibition against the bacteria *Escherichia coli* is suspected because of the concentration of liquid smoke used is quite high, offset by soaking time long enough so that the absorption of liquid smoke on fillet of tilapia increasingly effective, so the inhibition becomes high. According to Rodiah.et.al.,[31] that the main component in liquid smoke consists of acid, phenol derivatives, and carbonyl. The chemical elements can take the role of flavor (aroma), color forming, antibacterial and antioxidant. Liquid smoke can be used as a preservative for antibacterial and antioxidant properties .. Phenol compounds and acetic acid in liquid smoke can inhibit the growth of bacteria Pseudomonas fluorescence, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus. Substances present in liquid smoke is a material that is bacteriostatic and bacteriocidal. Compounds that very act as antimicrobials are phenolic compounds and acetic acid. Liquid smoke lowers the pH so that it can slow the growth of microorganisms [10]. On dilution of 10 times, liquid smoke can inhibit the growth of bacteria Pseudomonas fluorescence, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus[10].

Furthermore, the average value of the interaction of DDH *Escherichia coli* (mm / ppb) fillet of tilapia by treatment with different concentrations of liquid smoke storage time is presented in Table 4 and Figure 4 below.

Concentration (L)	**Time storage (S) (day)**					**Mean**	**Interaction**
(%)	0 (S^0)	3 (S^1)	6(S^2)	9(S^3)	12(S^4)	S	S*L
0 (L^0)	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.000	0.000
5 (L^1)	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.0000 ^{l}	0.000	0.000
10(L^2)	4.3133 ^{k}	7.2533 ^{h}	7.1333 ^{i}	6.7844 ^{j}	9.6100 ^{g}	7.019	-1.297
15(L^3)	7.3200 ^{h}	10.296 ^{e}	10.247 ^{e}	9.8800 ^{f}	12.697 ^{c}	10.088	-1.316
20(L^4)	10.320 ^{e}	13.248 ^{b}	12.103 ^{d}	12.163 ^{d}	14.693 ^{a}	12.505	-0.981
Mean (L)	4.391	6.159	5.897	5.765	7.400	5.922	
Interaction (L*S)	-4.992	-6.768	-6.519	-6.385	-8.017	-6.536	

Description: Figures followed by different letters in the same row or column showed significant differences (P <0.05).

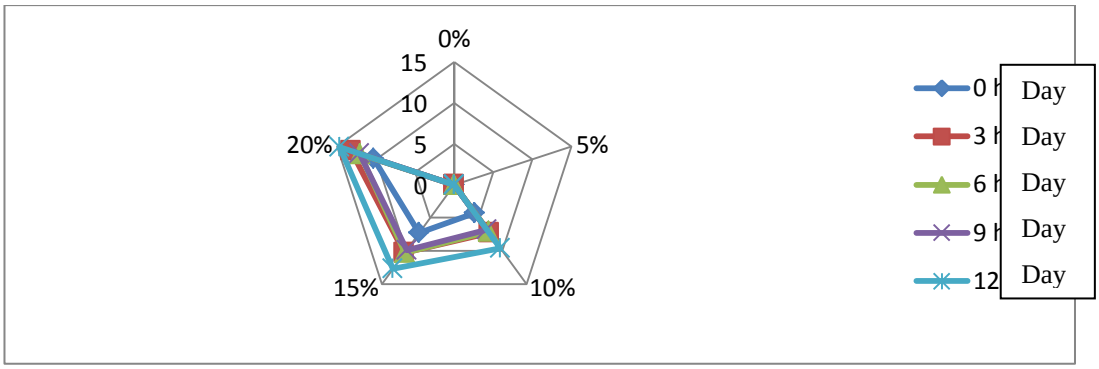

Figure 4. The average interaction DDH E Coli (mm / ppb) fillet of tilapia based treatment of different concentrations of liquid smoke with storage time.

In Table 4 and Figure 4 shows the value zero interaction on line treatment of liquid smoke concentration 0% and 5% with storage time against *Escherichia coli* DDH fillet of tilapia while the value of negative interaction at a concentration of 10%, 15% and 20% with storage time. In the column shows the value of a negative interaction on the storage time 0 days, 3 days, 6 days, 9 days and 12 days. Values of zero interaction means both treatment factors together provide a response to the DDH Escherichia coli. While the value of a negative interaction means that both factors are not the same response. In Table 4 and Figure 4 show the greatest inhibition of liquid smoke to the development of *Escherichia coli* in fillets of tilapia are treated liquid smoke concentration of 20% in the duration of storage for 12 days amounted to 14 693 mm / ppb. The amount of liquid smoke inhibition against the bacteria *Escherichia coli* is suspected because of the concentration of liquid smoke used is quite high, balanced with storage time long enough so that the absorption of liquid smoke on fillet of tilapia increasingly effective, so the inhibition becomes high. Result research Marasabessy[32] that the fish is smoked until the fourth day of storage in LDPE and PP plastic packaging is still acceptable, while the smoked fish are packaged in HDPE plastic has been rejected because it was overgrown with mold exceeding the tolerance limit. Furthermore Marasabessy[32] that during storage decreased levels of water, Aw and phenols, while total fungi and bacteria, TVB, and TBA increased in smoked fish in all packaging.

Furthermore, the average value of the interaction of DDH *Escherichia coli* (mm /ppb) fillet of tilapia by treatment with different concentrations of liquid smoke storage time is presented in Table 5 and Figure 5 below.

Table 5. The average value of interaction DDH *Escherichia coli* (mm / ppb) fillet of tilapia by soaking time difference treatment with liquid smoke with storage time

Concentration (L)	**Time storage (S) (day)**					**Mean**	**Interaction**
(%)	0 (S^0)	3 (S^1)	6(S^2)	9(S^3)	12(S^4)	(S)	S*L
5 (L^1)	3.1907 ^{o}	6.7907 ^{f}	4.6967 ^{k}	8.2967 ^{b}	6.2000 ^{g}	5.835	-0.937
10(L^2)	4.3907 ^{l}	7.9907 ^{c}	5.8967 ^{h}	4.0000 ^{m}	7.4000 ^{d}	5.936	0.284
15(L^3)	5.5907 ^{i}	3.6967 ^{n}	7.0967 ^{e}	5.0000 ^{j}	8.6000 ^{a}	5.997	-1.503
Mean (L)	6.159	5.897	5.766	7.400	7.400	5.922	
Interaction (L*S)	-0.073	0.260	-0.073	-0.061	-0.073	-0.004	

Description: Figures followed by different letters in the same row or column showed significant differences (P <0.05).

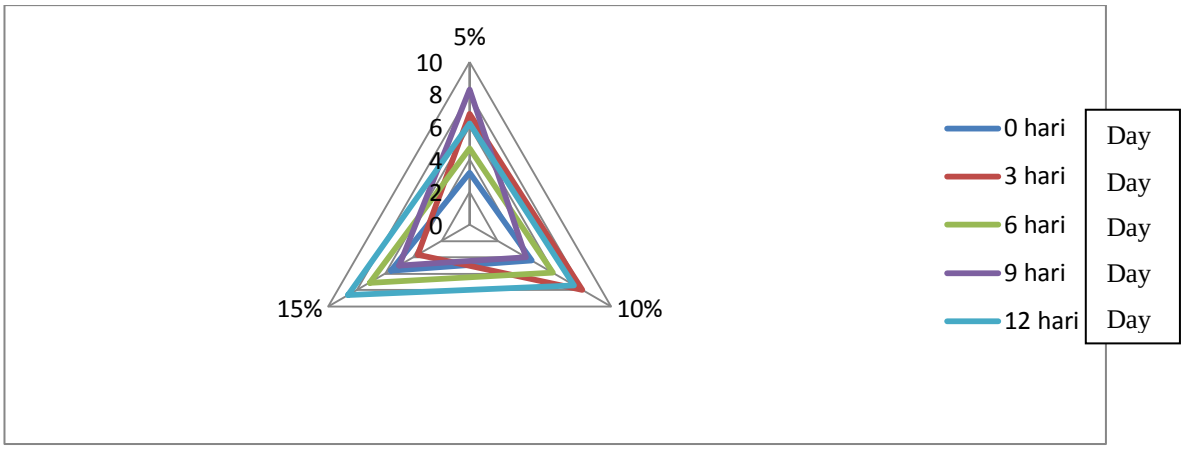

Figure 5.Average value interaction DDH *Escherichia coli* (mm/ppb) fillet of tilapia by soaking time difference treatment with liquid smoke with storage time

The average value of the diameter of the inhibition (DDH) *Escherichia coli* fillet of tilapia in Table 5 and Figure 5 are given two treatments, namely a combination of soaking time 15 minutes and duration of storage for 12 days give DDH *Escherichia coli* in fillets of tilapia high of 8,6 mm/ppb and a statistically significant interaction. Results DDH value *Escherichia coli* tilapia fillet smallest in the treatment of prolonged submersion for 5 minutes at storage time 0 days amounted to 3.19 mm / ppb. The size of the diameter of the inhibition (DDH) *Escherichia coli* in fillets of tilapia on a long submerged for 15 minutes on a fillet of tilapia on the concentration of liquid smoke 20% estimated by the longer immersion in liquid smoke 20% and the longer the storage will be more effective at inhibiting the growth of bacteria Escherichia DDH *Escherichia coli* so high coli. Inhibitory power for allegedly formed in the fillet of tilapia contained liquid smoke containing acid components, carbonyl and phenol.

Diameter higher inhibition often with an increased concentration of liquid smoke that is given to the tilapia fillets. *Escherichia coli* bacteria, including gram-negative bacteria which have properties more resistant to antimicrobials than the gram-positive bacteria. This is due to *Escherichia coli* (gram-negative) had a lipopolysaccdayde layer attached to the outer membrane with a hydrophobic bond. According to Brooks *et al*., [33] that the outer membrane has the ability to get rid of hydrophobic molecules and protective cells.

The test results showed inhibition of antimicrobial activity against Escherichia coli. The higher the concentration of liquid smoke used causes the greater the inhibition zone. Immersion fillet of tilapia with liquid smoke allegedly contains acidic compounds, carbonyl and phenol. Usually, phenolic groups have antimicrobial properties [34]. Acid group also has antimicrobial effects because the compound can diffuse into the cell. After the nucleic acid chelate, consequently the transcription process will be interrupted and inactivate genetic material that inhibits bacterial growth. The mechanism of inhibition may react by: (a) to react with the cell membrane, (b) inactivation of enzymes essential and (c) the destruction or inactivation of the function of the genetic material [35].

The resilience of the bacterium *Escherichia coli* (gram-negative) on fillet of tilapia given immersion liquid smoke with different dipping time seem varied, but showed higher inhibition pattern of liquid smoke is used, the pattern of inhibition higher. According to Madigan [36], the cell wall of gram-positive are highly reactive -COOH group that is bonded antimicrobial compound and diffuses into the cell. This leads to antimicrobial compounds both hydrophilic and hydrophobic to diffuse into the cell. In the cell walls of gram-negative bacteria are lipopolysaccdayde layer that selectively causing antimicrobial compound is harder to diffuse into the cell. Liquid smoke on fillet of tilapia can inhibit the growth of gram-negative bacterial lipopolysaccdayde layer because there is the side that is hydrophilic carboxyl, amino, phosphate, and hydroxyl [37]. Gram-negative cell walls have a structure dindingsel layered consisting of lipopolysaccdayde, peptidoglycan and lipoproteins. According to Madigan[36], lipopolysaccdayde layer is a barrier to hydrophobic antimicrobial compounds.

IV. Conclusion

1. There was an interaction of treatment with different concentrations of soaking time, soaking time with the storage time, the concentration difference with storage time while the combination of two other treatments showed no significant difference (no interaction). In the treatment of three treatment difference immersion, the concentration of liquid smoke with storage time showed real interaction to the diameter of the inhibition (DDH) Escherichia coli, while the combination of the other three treatments were not significantly different (no interaction). Four combined treatment showed no inhibition of the interaction of the diameter (DDH) *Escherichia coli* which is antibacterial liquid smoke on fillet of tilapia
2. Diameter inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia tertinngi contained in four combination treatment that liquid smoke concentration of 20 percent, a 15-minute soaking time, type of packaging polyethylene (PE) and storage for 3 days amounted to 16.697 mm / ppb.
3. The diameter of the power bland (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in three treatment combinations contained in the treatment liquid smoke concentration of 20 percent, a 15-minute soaking time and storage time of 12 days amounted to 16.693 mm / ppb.
4. Diameter inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in the treatment of liquid smoke concentration of 20% at 10 minutes soaking time of 12 626 mm / ppb.
5. The diameter of inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highest in the treatment of liquid smoke concentration of 20% in the duration of storage for 12 days amounted to 14 693 mm / ppb.
6. The diameter of inhibition (DDH) of liquid smoke against *Escherichia coli* in fillets of tilapia highs on a combination of old submerged for 15 minutes and for 12 days storage time high of 8.6 mm / ppb

Acknowledgment

We would like to thank Directorate General of High Education, Ministry of Education, in correspond to Implementation Agreement of Competitive Research Grant, National Priority, Rector of Ekasakti University, The Head of LPPM Ekasakti University, The Dean of Agriculture Faculty Ekasakti University, Laboratory team and staff that have provided help in this activity.

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