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RESEARCH ARTICLE

TRIMETHYLAMINE (TMA) PROFILE OF WHITE SNAPPER FILLET (*LATES CALCARIFER*) AFTER GIVING LIQUID SMOKE OF COCONUT SHELL

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ABSTRACT

The research on trimethylamine (TMA) profile of white snapper fillet (*L. calcarifer*) after giving liquid smoke of coconut shell has been conducted. The research objective was to determine liquid smoke concentration and soaking time, analyzing TMA concentration of *L calcarifer* fillet without and with giving liquid smoke of coconut shell during storage. White Snapper (*L. calcarifer*) is filleted, then wash ed with flowing fresh water. A part of fillet is soaked with liquid smoke and another part without liquid smoke. Both drained for 2 h at 40°C, afterward entered into sterofoam box and covered with sterile poly propylene plastic then stored in refrigerator at 4°C until analyzed every 2 days. Determination of liquid smoke concentration and soaking time through optimum curve, TMA concentration analysis follows colorimetric method (Dyer, 1945; Tozawa, 1971). The results indicated concentration of 5% optimum liquid smoke and optimum soaking time 10 minute. TMA concentration of fillet samples without or with soaking a liquid smoke at the beginning of analysis was not detected, but detected on day 2,4,6,8, 10 respectively 0.2465; 0.2567; 2.9674; 4.2435; 6.2696 mg TMAN/100 g and the fillet sample soaked with liquid smoke is 0.00009; 0.2492; 2.9612; 4.2391; 6.2606 mg TMAN/100 g. The results of analysis data either without fillet samples or with soaking a liquid smoke, TMA concentration has significant influenced to storage time.

INTRODUCTION

Fish is protein source for most people but it is easily damaged foodstuff than another animal meats. Indonesian which has tropical climate at environment temperature is very supportive to microorganisms growth so with its activities the fish release odors, which can cause a short shelf life and not economically profitable. The microbial activity, especially the development of specific spoilage organisms (SSO) that cause lipid oxidation and protein degradation resulting in breaking variety of chemical components formed a new compound responsible to the change in odors, taste and texture of fish meat (Hernandez, *et al*., 2009). The fish damage follow the rigor mortis stages, autolysis (loss of freshness) and decomposition by bacteria. These stages sooner or later is occurred depending on species, physiological condition of fish, microbial contamination. Besides, time and temperature is also the most important factor to control and maintain the fish freshness quality (Adams and Moss, 2008). Trimethylamineoxide was normal component contained in the sea fish. TMA fresh fish was found only in the lowest number or nothing. Lindsay *et al*. (1994) stated that TMA is not produced in significant quantitie in the beginning stages of storage but will appear 3 or 4 days and afterward the production rate of TMA parallel with bacteria pattern proliferation. According to (Davidek and Davidek., 1995) TMA includeed in biogenic amines group are formed in nonfermented food products during storage. If the growth of its bacteria inhibited, trimethylamine oxide will degraded by intrinsic enzyme activity of fish which is reduced into TMA. Fish with TMA concentrations less than1.5 mg TMAN/100 g is considered have good quality while between 10-15 mg TMAN/100 g is considered as an acceptable limits (Huss, 1988). Fish is considered rotten if they are above 30 mg TMAN /100 g (Bonnel, 1994). Palm, *et al*. (2011) stated that smoking process is process of the inclusion a volatile compounds from smoking source into the fish meat. With traditional smoking some hazardous components contained therein may sometimes not well controlled, but with liquid smoke this can be minimized as well as may be also acquired a desired taste, flavor and texture. According to Darmadji (1996), liquid smoke from coconut shell has advantages in inhibiting the growth of spoilage bacteria and pathogens. There are 40 compound components in liquid smoke with 7 dominant component that is 2-Methoxyphenol (guaiacol), 3.4-Dimethoxyphenol, Phenol, 2-methoxy-4 methylphenol, 4-Ethyl-2-methoxyphenol, 3-methylphenol and 5-Methyl-1.2.3-trimethoxybenzene (Budijanto *et al.,* 2008). Zuraida, (2009) stated that liquid smoke of coconut shell can inhibit the

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growth of *S. aureus* and *P. aeruginosa* pathogenic bacteria and phenols components (phenol, metalphenol and guaiacol) and acid components (derivative of benzoic acid)are components identified in liquid smoke of coconut shell that acts as anti bacterial. Inbasekar, (2011) reported the TMA concentration increased significantly in fresh fish stored with intestine than fresh fish stored without intestine. Likewise, Okoro (2010) concluded that TMA concentration of sea fish in *Liza falcipinnis* (Mullet) species are stored at room temperature for 12 hours was 4.8 mg TMAN/100 g, temperature 4°C for 6 days was 6.80 mg TMAN/100 g. Horsfall, M. Jnr. (2006) conducted a research on the formation of TMA against three sea fish species (*Tilapia spp, Mugil ceph alus* and *Carassius auratus*) were stored at freezing temperatures (-4°C) for 20 days and analyzed every 5days. In conclusion, TMA concentration was < 0.001–7.12 for *Tilapia spp.* < 0.001 –6.45 to *Mugil cephalus* and < 0.001–7.28 to *Corossious aura tus*. These data indicated that TMA concentration increased with the increase of storage time. According to Sotelo*, et al.*, (1995) TMA is more easily formed at temperatures above 0°C while FA and DMA at temperatures between -5°C and -10° C. This research was conducted to understand effect of liquid smoke of coconut shell to trimethylamine (TMA) concentration on the *L. calcarifer* fillet.

MATERIALS

Distilled water,no.1 Whatman filter paper,tissue paper, trichlo roacetic (TCA), trimethylamine-Hidrogen chloride (TMA-HCl), Toluen ($C_6H_5CH_3$), Picric Acid ($C_6H_3N_3O_7$), Hydroxide Potassium (KOH), formaldehyde (CHOH), buffer pH 4 and buffer pH 7, Sulphate Natrium (Na_2SO_4). All material used have p.a. quality are obtained from Merck. The liquid smoke of the result pyrolysis of coconut shell (grade-2) was obtained from PT Tropica Nucifera Industry Yogyakarta. Fresh fish was obtained from the fisherman after fishing at night with the best quality in the fish auction (TPI) Depok Yogyakarta.

METHODS

Twenty fresh *L. calcarifers* in average weight of 300-400 g was selected. During transport to the laboratory, the fish put into sterofoam box given ice grain. In laboratory, the fish is filleted then washed with flowing fresh water. Some of fillet were soaked with liquid smoke at optimum concentration and soaking time, the other part without soaked with liquid smoke. Both were drained for 2 h at 40°C, afterward it put into stero foam box and covered with sterile polypropylene plastic and stored in refrigerator set at 4°C until analyzed each 2 days. The liquid smoke concentration is determined by dilution with distilled water and made variations of concentrations from 0 % to 30 % the concentration range was 5%. Data every variation of concentration was made a curve to obtain optimum concentration. Soaking time was determined by data of optimum concentration and made variations of soaking time from 0 minutes to 30 minutes, with time range of 5 minutes. Data in every variation of soaking time was made a curve to obtain an optimum soaking time. Preparation of free protein tissue extracts is weighed 50 g of sample and added with 100 ml of 10% trichloro acetic acid. Furthermore, homogenized with blender at high speed for 1 minute. Homogenate was filtered with Whatman no.1 filter paper using Buchnerd's funnel. Four (4) hooded reaction tube is prepared, each tube is filled with sample of 1ml; 2ml; 3ml and 4 ml. Each tube was added with distilled water until total volume was 4 ml, added again 1 ml of 10% formaldehyde,10 ml Toluene and 3 ml KOH (25%). Pipette 8 ml for toluene base layer and move it into another test tube containing 0.4 g of sodium sulfate (anhydrous), pipette 5 ml of this solution and put into clean test tube and add 5 ml of picric acid solution. Samples were then put into glass cuvettes and measured its absorbance at 410 nm.

RESULTS AND DISCUSSION

The determination results of liquid smoke concentration.

Data each concentration variation were obtained through measurement of pH value on each concentration variation, the results are shown in Table 1.

Table 1. Data of concentration variation of liquid smoke

No	concentration	pH
1	0%	6.5173
2	5%	7.226
3	10%	7.0032
4	15%	6.8312
5	20%	5.9322
6	25%	5.8652
7	30%	5.4332

In the concentration range of 5% indicated that at liquid smoke concentration of 0 % to 5 %, increased from 6.5173 into 7.226 while at liquid smoke concentration of 10 % the pH values begins to reduce into 7.0032 and then more decreases with the increase liquid smoke concentration. These data were made optimum curve in order to obtain optimum concentration of 5 % liquid smoke.

The determination results of soaking time

Data of each soaking time variation are obtained by measuring of pH value from soaking time variation, are shown in Table 2.

Table 2. Data of soaking time variations

No	soaking time	pH
1	0 min	6.0789
2	5 min	5.546
3	10 min	5.844
4	15 min	5.8303
5	20 min	5.7445
6	25 min	5.7653
7	30 min	5.5812

In the time range of 5 minutes indicated that at 0 min to10 min the pH value were 6.0789; 5.546; 5.844. and with the increase of soaking time the pH value was more decreases. These data are then made optimum curve so that obtained data of optimum soaking time at the tenth minute.

Concentration analysis results (TMA) of *L. calcarifer* fillet during storage

The TMA concentration analysis results of sample *L. calcarifer* fillet is presented in Table 3.

Table 3. Data of *L. calcarifer* fillet TMA during storage period

Day	(mg TMAN /100 g)	
	L calcarifer fillet without liquid smoke	*L calcarifer* fillet soaked with liquid smoke
0	-	-
2	0.2465	0.00009
4	0.2567	0.2492
6	2.9674	2.9612
8	4.2435	4.2391
10	6.2696	6.2606

At the beginning of TMA concentration storage periode of *L calcarifer* fillet without liquid smoke or fillet soaked with liquid smoke cannot be detected and therefore cannot display TMA concentration data. After day 2, the TMA concentration was obtained on fillet without liquid smoke of 0.2465 mg TMAN/100 g and increases steadily until the end of storage in days 10 is 6.2696 mg TMAN/100 g. Similarly, in fillet samples soaked in liquid smoke, TMA concentration was detected on day 2 was 0.00009 mg TMAN/100 g and increased steadily until the end of storage in day10 was 6.2606 mg TMAN/100 g.

Storage effect toward TMA concentration of filet *L.calcarifer* without liquid smoke

TMA resulted by Oxide TMA reduction caused by spoilage bacterial (such as *Shewanella putrifaciens*) and enzymatic activity (TMAse demethylase). This TMAO is depended on anaerobic conditions associated with lactate catabolism (Ruiter, 1971; Ringo, *et al*., 1984). At the beginning of storage period the TMA concentration was not detected, it can be explained that at the time the formation of TMA has not occurred on the sample used. This supported by the statement (Lindsay, *et al.,* 1994) that TMA has not formed in significant quantities in the first stages of storage but will appear in 3 or 4 days and after that the formation rate of TMA parallel to the bacterial pattern proliferation. The increase of TMA generated by proteolytic bacterial activity will be accompanied by the increase of pH (Rodriguez Losada, Aubourg & Barros - Velazquez, 2004). Similarly reported in the results of fillet *L. calcarifer* pH measurement under the same conditions, indicated the increase in pH during storage period (Krisen *et al*. 2014). The TMA concentration in this research to the end of storage was still under the acceptance limit of 10-15 mg TMAN/100 g (Sikorski, *et al*. 1989).

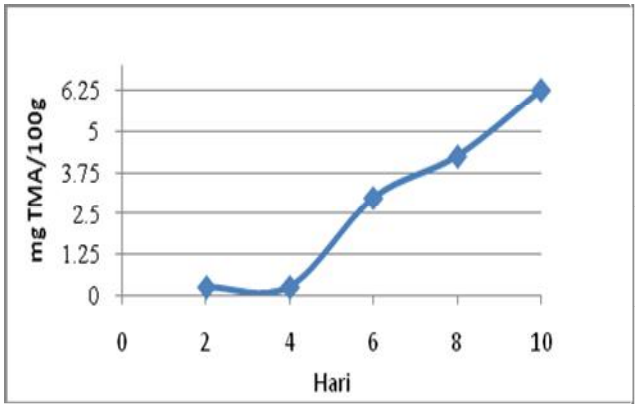

Figure 1. Fillet *L.calcarifer* TMA profile without Liquid Smoke during storage

Storage effect toward fillet *L. calcarifer* TMA concentration soaked in liquid smoke

As in the fillet without liquid smoke,TMA concentration of sample fillet are soaked in the liquid smoke at the same conditions in the beginning of storage was not detected. However on day 2 was found its concentrations is lower than TMA concentration on filet without liquid smoke that is 0.00009 mg TMAN/100 g. This is presumably due to the influence of active compounds components contained in the liquid smoke on fillet soaked with liquid smoke that have anti bacterial and antioxidant properties. According to (Girrard, 1992; Zuraida, 2009) antibacterial compounds supported antibacterial properties in the liquid smoke distillate is acidic and phenolic compounds.

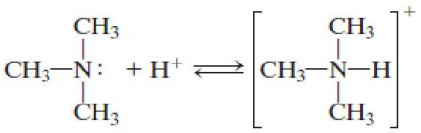

Figure 2. Trimethylamine reaction with Acid

Trimethylamine (TMA) on sample fillet is nitrogen bases easily protonated to form cations trimetilammonium, with acetic acid components in the liquid smoke. This trimethylamine then is also a weak base would bind proton (H) released by phenolic compounds so resulted in the formation of fenolic salt. On the day 4 the increase in concentration is continued lately, but after that there is increase in concentration doubled until the end of storage period in the day 10. From the result of data analysis either filet without liquid smoke or liquid smoke, TMA concentration has significant influence to the time.

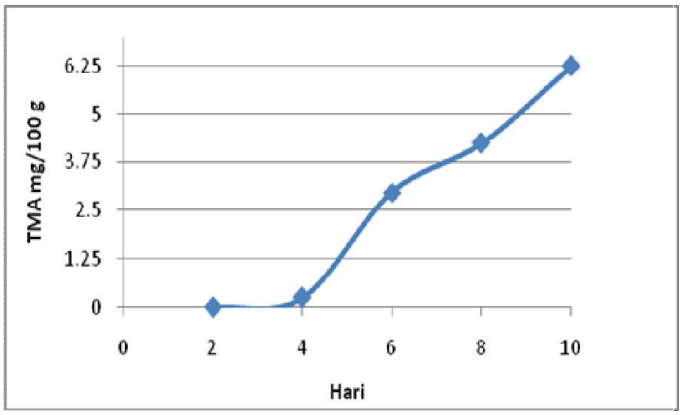

Figure 3. Fillet *L calcarifer* TMA Profile with liquid smoke during storage

Conclusion

- The optimum liquid smoke concentration was obtained at 5% and optimum soaking time at 10 minutes. In these conditions, liquid smoke giving a delay of 2 days to increase concentration of TMA filet *L calcarifer*.
- The results of data analysis of sample fillet TMA concentration without or with using the liquid smoke has significant influence to the storage time.

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