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Determination of Endogenous Formaldehyde in Moonfish (*Lampris guttatus*) During Frozen Storage

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ABSTRACT

Formaldehyde is a carcinogenic compound that harmful to health and often misused as a food preservative in Indonesia. However, in most of the foods including fish, formaldehyde can be formed endogenously or naturally during storage. Recently, Moonfish (*Lampris guttatus*) from Indonesia has been exported. Meanwhile, during delayed transportation to importing countries, moonfish need to be kept frozen up to several months This condition leads to the formation of formaldehyde. The aim of this research is to study the pattern of endogenous formaldehyde formation in moonfish during the frozen storage period. The study was conducted by observing the formation of endogenous formaldehyde as well as the formation of DMA (dimethylamine), TVB (total volatile base), TMA (trimethylamine), and TMAO as parameters related to the formation of endogenous formaldehyde in moonfish. The parameters were observed periodically shortly after the fish died until six months in frozen storage. The results showed that the content of formaldehyde, TVB, TMA, and DMA in moonfish during six months of frozen storage increased every month. The formation of natural formaldehyde in the moonfish was inline with quality degradation. Moonfish is still suitable for consumption based on its freshness level up to one month in frozen storage, but based on the formation of formaldehyde, the level of consumption should consider the calculation of maximum daily intake based on specified reference dose.

INTRODUCTION

Formaldehyde is classified as a carcinogenic compound by International Agency for Research Cancer with oral reference dose (rfd) of 0,2 mg/kg of body weight **(IARC 2004)**. In Indonesia, formaldehyde is unfortunately often misused as a preservative agent in some products of food, such as meatball, noodle, fruits and fish. National Adrenal Diseases Foundation of United Stated data showed that the incident caused by food poisoning ranked in the first place which is higher (66.7%) than other poisoning cases such as drugs, cosmetics, etc. and One of those food poisonings is caused by formaldehyde intake **(Paratmanitya and Aprillia, 2016)**. However, formaldehyde can be formed naturally due to deterioration process through the reduction reaction with endogenous enzyme as a catalyst **(Riyanto *et al*,. 2006; Rachmawati *et al.*, 2007; Murtini *et al.*, 2014)**. Based on World Health Organization data **(WHO, 1989)**, formaldehyde originally found in fruit and vegetable between 3,3 mg/kg to 60 mg/kg, in meat ranged from 8 to 20 mg/kg and in dairy products ranged from 1 to 3,3 ppm. Meanwhile, in fish and crustaceans, formaldehyde could be found in between 1 to 98 mg/kg.

Natural formaldehyde in fish could be formed through reduction of trimethylamine oxide (TMAO) into equimolar formaldehyde (FA) and dimethylamine (DMA) with endogenous enzyme catalyst of TMAOase once the deterioration of fish begin **(Huss, 1995; Sotelo and Rahbein, 2000; Liu *et al.*, 2010)**. TMAO content on fresh marine fish ranged from 1 and 100 mg/kg, on the other hand, TMAO content on fresh aquaculture fish is between 5 to 20 mg/ 100 g **(Hebard *et al.*, 1982; Nielsen *et al.*, 2001)**. TMAO is a compound that acts as osmoregulator in fish, and its content is affected by fish species, age, body size and environmental factor. In addition to TMAO content, formaldehyde content on fish is also affected by fish quality, temperature, time of storage and oxygen existence **(Sotelo *et al.*, 1995; Riyanto *et al.*, 2006; Rachmawati *et al.*, 2007)**. Furthermore, **Leelapongwattana *et al.*, (2008)** reported that frozen storage will increase the natural formaldehyde formation in fish due to oxygen lack which leads to increasing of TMAOase activity to degrade TMAO into formaldehyde and DMA. Therefore, the research on natural formaldehyde formation pattern on fish which are kept in frozen condition is needed to be conducted.

Moonfish or opah fish (*Lampris guttatus*) in Indonesia are known as pelagic non targeted fish or bycatch product of tuna fishing. The research on biological and ecological aspect of the fish is rarely done **(Hawn *et al.*, 2002; Seki *et al.*, 2004)**. **Wegner *et al.* (2015)** found that moonfish lives solitary and spatially distributed in almost of all sub tropic and several cold waters. This fish is classified as predator epi-mesopelagic fish that lives in the open ocean up to 300 m in depth and also known as “first hot blood fish” which has the ability to increase its metabolism in the deep sea with a body temperature between 7^{O} – 9^{O}C above its environment and In Indonesia, moonfish can be found in Indian Ocean of south of Indonesia **(Novianto *et al.*, 2015)**.

Though is not the main targeted fish in tuna fishing, demanding of moonfish as a frozen product in the worldwide market such as Mauritius, Taiwan, Japan, America, and Malaysia, has become increased every year **(Indonesia Fisheries Quarantine , Quality Control and Safety Agency, 2017)**. However, in 2016 Indonesian Government found 4,7 ton of frozen Moonfish landed at Muara Baru fishing port Indonesia contained formaldehyde up to 250 ppm **(Tribunnews, 2016)** . There is no explanation whether the existence of formaldehyde in moonfish is naturally formed or by illegally added by fishermen. Consequently, information related to natural formaldehyde formation in moonfish became a necessity. The research aims to obtain information of natural formaldehyde formation in moonfish during deterioration process and also to know the feasibility level and safety level of moonfish consumption. The result of the research hopefully could be used as a scientific basis for controlling, supervising, and policy-making concerning the content of formaldehyde in the moonfish.

MATERIALS AND METHODS

Sample Preparation

The study was conducted using moonfish (*Lampris guttatus*) samples collected from Indonesian tuna fishing vessel. Four samples of moonfish with average weight of 18.57 ± 3.14 Kg were captured from Indian Ocean by Indonesian tuna fishing vessels and were eviscerated and kept in frozen condition in the vessel for one week until landed at Benoa fishing port in Bali. The location of the moonfish capture was at coordinates of 17°32'0"S and 111^{o}20'0"E (Figure 1). The samples were kept frozen in the vessels at -18^{o}C to maintain the frozen condition and were brought to the Laboratory in Jakarta by airplane. In the Laboratory, after eviscerating the meat samples were cut and devided into 4 groups as replication and then packed in the vacuum plastics before stored in a freezer with temperature of -18^{o}C for 6 months same as the moonfish usually was stored before has been exported. The analyses were done at point 0 just right after the fish landed and continued to be analyzed periodically on the same date every month for six month storage.

Analysis Methods

Parameters analyzed in this study were proximates (protein, fat, ash and moisture), total volatile base Nitrogen (TVB-N), (trimethylamine (TMA), trimethylamine oxide (TMAO), formaldehyde and dimethylamine (DMA). Proximates (protein, fat, ash, and moisture) were analyzed only at point 0 after one week the sample stored on the ship and before the fish were stored at frozen condition at the laboratory **(AOAC 2005)**. The TVB, TMA, and TMAO analysis were performed by using Conway plate **method (Ozogul and Ozogul, 2000; Hebard *et al.*, 1982)**. Formaldehyde and DMA content were analyzed using UV-Vis Spectrophotometer (Pekin Elmer Lambda 25 UV/Vis Spectrophotometer) at 412 nm **(Benjakul *et al.*, 2004)**. The pattern of TVB, TMA, TMAO, DMA, and formaldehyde formation in the moonfish were observed monthly to 6 months of frozen storage.

Statistical Analysis

The data were tabulated and presented as mean value with standard deviation. The data were then analyzed using statistical software of Statistical Package for Social Science (SPSS Windows version 17.0) at one-way ANOVA test and mean comparison of formaldehyde, TMA, TVB, TMAO and DMA content based on storage time at frozen condition **(Aminah *et al.*, 2013)**. Prior to one-way ANOVA statistical analyisis, data normality ware tested using Kolmogorov - Smirnov test. Correlation amongst parameters (TVB, TMA, TMAO, Formaldehyde, and DMA) was analyzed by Pearson correlation analysis **(Barokah *et al.*, 2018).**

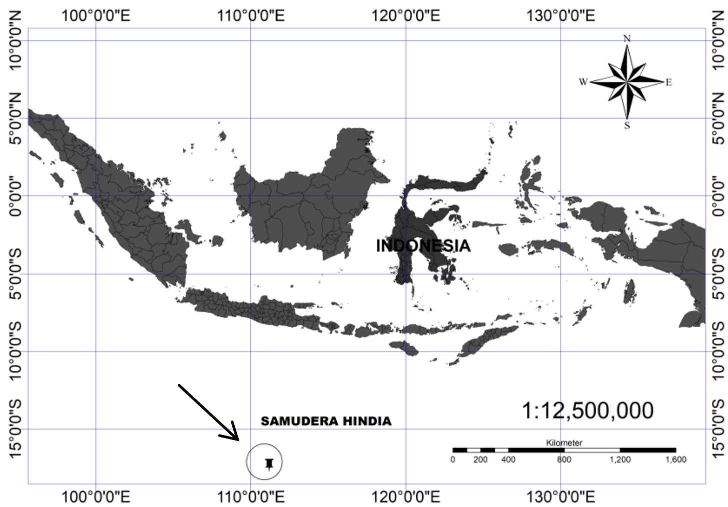

Figure 1. Fishing ground of Moonfish (*Lampris guttatus*) by Indonesian fishing vessel

RESULTS AND DISCUSSION

Chemical Composition of Moonfish (*Lampris guttatus*)

The proximates showed that protein, fat and ash content of moonfish in this study were higher than the contents reported by **Nurnadia *et al*. (2011)**. The difference of proximate content of the moonfish flesh might be caused of the differences of fishing ground. The moonfish used in this study were caught in Indian Ocean, while the moonfish used in **Nurnadia *et al.* (2011)** were captured around Malaysian waters. According to **Aziz *et al*. (2013)** fish habitat affects the chemical content in the meat such as proximate, amino acids and fatty acids.

Table 1. Mean value of proximates of moonfish (*Lampris guttatus*)

Samples	Moisture (%)	Protein (%)	Ash (%)	Fat (%)
Moonfish from Indian Ocean (this study)	75.22 ± 0.39	24.71 ± 0.25	1.28 ± 0.34	0.96 ± 0.22
Moonfish from Malaysian Waters (Nurnadia *et. al*, 2011)	74.61 ± 0.94	19.61 ± 1.39	1.16 ± 0.22	6.89 ± 2.76

The chemicals of fish meat that plays an important role in the formation of formaldehyde in fish after death are fat and protein **(Liu *et al,*.2010)**. Based on the protein content, moonfish used in this study could be classified as high protein fish as classified by Standsby (1967) that fish with protein content more than 20% can be classified as high protein fish. Furthermore, **Seibel and Walsh (2002)** stated that in fish protein complexs there are TMAO degradation from choline compounds which then decompose into formaldehyde and DMA during deterioration process. Later on, the high protein content of the moonfish samples used in this study seems to be a precursor in the formation of natural formaldehyde. Meanwhile, fat content of fish meat is also correlated indirectly to formaldehyde formation of fish during the deterioration process **(Jacobsen *et al*., 2010).** This is happen because free fatty acid can decompose into choline and continued to TMAO. However, the process can not contribute significantly in the formation of natural formaldehyde **(Sotelo *et al.* 1995)**. According fat content of 0.96% (Table 1), the moonfish is actually classified as low fat content or lean fish which might contribute insignificantly to the formation of natural formaldehyde in moonfish.

Trimethylamine Oxide (TMAO) and Trimethylamine (TMA) Content of Moonfish (*Lampris guttatus*) during frozen storage

Trimethylamine oxide (TMAO) which is commonly present in marine fish and mussels has also play an important role in the physiological function of the fish osmoregulation system during their life. The TMAO content in fish varies depending on species, habitat, and response to the seasonal changes **(Sotelo and Rehbein, 2000; Seibel and Walsh, 2002)**. After death, TMAO on fish will break down into TMA due to bacterial activity and equimolar DMA with formaldehyde catalyzed by TMAOase as enzyme activator (Summers *et al*, 2016).

The results showed that TMAO content of moonfish increased in the first month of frozen storage, then tended to slightly decrease after second month (Figure 2.). However, TMAO content changed insignificantly ($p > 0.05$). Variation of change the TMAO concentration among the sample could be considered as one of the cause. The increase of TMAO content in the first month of storage is expected by the presence of lipoprotein compounds in moonfish meat matrix which can be described through enzymatic activity during deterioration process **(Nurhayati *et al.*, 2019)**. According of **Yasuhara and Shibamoto (1995)**, in the phase of deterioration of fish and during storage of low temperature, enzymatic degradation of lipoprotein in fish compounds can still take place. Therefore it is still possible that lipoprotein compound decomposes into choline and then changes into TMAO by enzyme dehydrogenase. This leads to the increasing TMAO content. The rate of TMAO degradation in fish depends on several factors including fish species, storage conditions, storage temperature, and fish meat integrity (Parkin and Hultin, 1982).

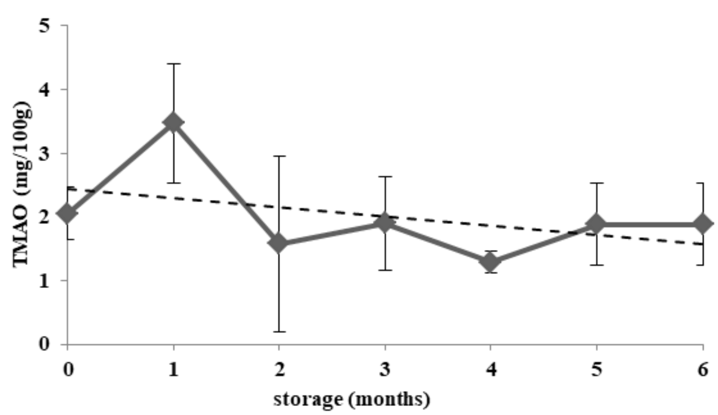

Figure 2. TMAO content of moonfish (*Lampris guttatus*) during 6 months of frozen storage

The TMA content of moonfish tended to increase steadily, especially in the first 4 month of frozen storage, and showed a significant different ($p < 0.05$) after six months of frozen storage (Figure 3). These results indicated that at frozen temperature storage the process of deterioration by bacterial activity can still possibly continue. This is in line with the study of **Gram and Dalgaard (2002)** which states that the rate of TMA formation in fish meat was strongly influenced by storage temperature, fish meat integrity and microbial activity. Furthermore,TMA can be formed in the storage of freezing temperature through the non-enzymatic degradation of TMAO by the activity of several microorganisms such as *Aeromonas* spp., *Photobacterium phosphoreum*, *Shewanella putrefaciens* and *Vibrio* spp which are anaerobic bacteria. The anaerobic microorganism could be still active in freezing storage or in the condition of absence of oxygen and is still be able to decompose TMAO to TMA. As shown in Figure 3, the formation of TMA was slightly slower after 4 months of storage at frozen condition. This might happen due to less optimum of microbial activity to decompose TMAO compounds in frozen conditions. In addition, the stability of storage temperature can also lead to fluctuations of TMA content in the moonfish.

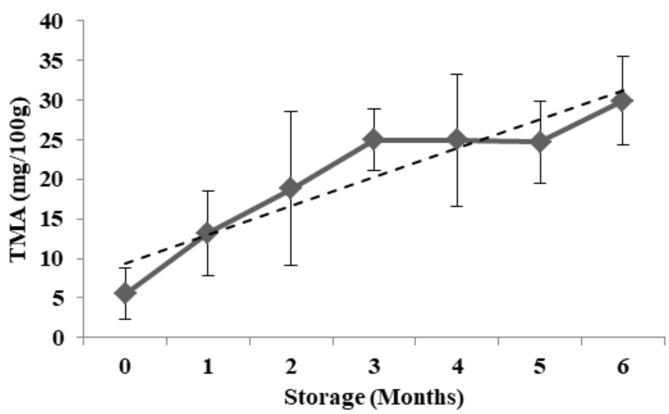

Figure 3. TMA content of moonfish (*Lampris guttatus*) 6 months of frozen storage

Formaldehyde Formation on Moonfish and its Correlation with TVB and DMA

Formaldehyde, TVB and DMA content in moonfish were increased significantly ($p<0.05$) during frozen storage for 6 months and concentration of formaldehyde was higher than TVB and DMA (Figure 4). Formaldehyde, TVB, and DMA concentration in moonfish in this study are positively correlated with the storage time and described by forming a linear regression model. This happened because the absence of oxygen under freezing conditions can increase the activity of TMAOase and degrade TMAO into formaldehyde and DMA **(Sotelo *et al.*, 1995; Leelapongwattana *et al*., 2008)**.

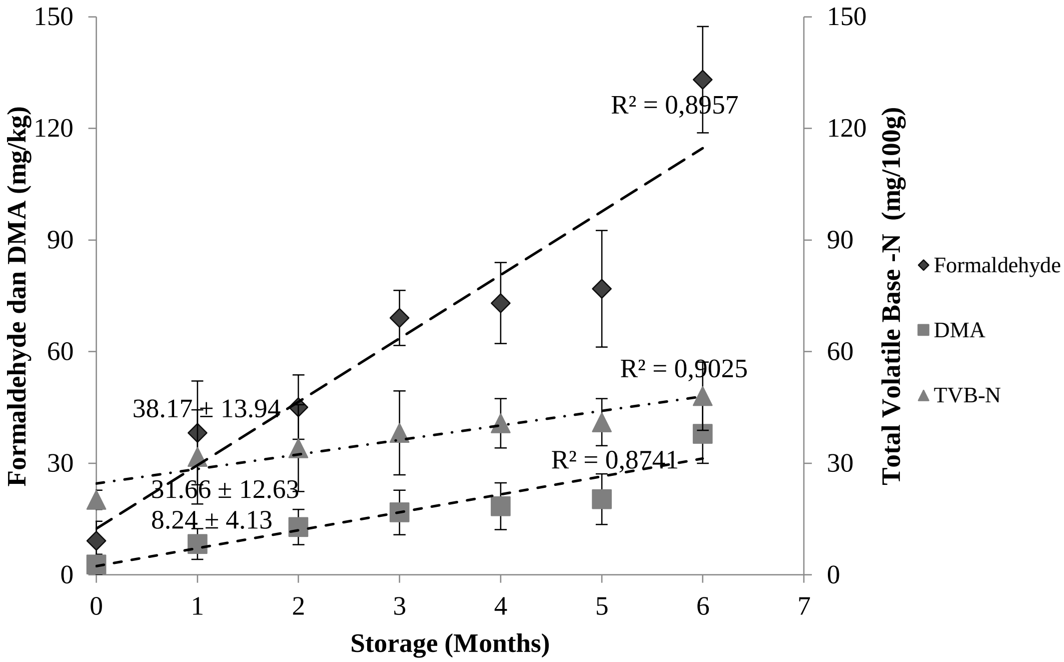

Figure 4. Formaldehyde, DMA and TVB-N formation patterns in moonfish (*Lampris guttatus*) during 6 months of frozen storage

The initial formaldehyde content in moonfish was 9.15 mg/kg at the first point before the fish were stored in frozen condition in the laboratory (Figure 4). Assuming that formaldehyde content is absence in life fish, the formaldehyde found in moonfish which was landed one week after captured before stored in the laboratory might be related to on board lack of handling. This could happen considering that moonfish is a non-targeted fish in tuna fishing activity. Lack of handling or improper handling of fish can accelerate fish deterioration and affects the formation of natural formaldehidye in fish **(Rachmawati *et al.*, 2007)**.

The formaldehyde content continues to rise to 133 mg/kg after sixth months in frozen storage. The increase of formaldehyde in moonfish was also reported in 2016 which was found in moonfish capture from Indian Ocean and distributed in Jakarta. The formaldehyde content of Jakarta moonfish stored at freezing temperature (-18^{o}C) reached to 250 mg/kg **(Tribunnews, 2016)**. The content has greatly exceeded the safety limits of formaldehyde exposure to food products which was stipulated by the IARC and Indonesian government regulations .

Dimethylamine (DMA) which is naturally formed as a by-product of TMAO reduction by endogenous enzymes also produces formaldehyde **(Sotelo and Rehbein, 2000; Murtini *et al.*, 2014)**. The content of DMA varies within the species of fish depending on the content and activity of TMAO or TMAO demethylase enzyme which converts TMAO to DMA and formaldehyde equimolar quantitatively **(Huss, 1995)**. The results showed that DMA of moonfish continues to increase in frozen storage conditions. The increase in DMA content is in line with the increase of formaldehyde levels in the moonfish. The increase in DMA content is caused by autolysis reaction of DMA during frozen storage and it is associated with membrane fish muscle, therefore its production will be higher in fish in which handling and temperature were abused **(Khidhir, 2011)**. However, the content of DMA formed in moonfish is lower than its formaldehyde content. This is probably due to other pathways forming natural formaldehyde in moonfish in addition to TMAO use degradation derived from the decomposition of fatty fish meat. According to **Leelapongwattana *et al.* (2008)**, the fat is directly correlated with the total microbial growth in fish flesh. This happens because, at certain stages of the microbial decay process, the lipase enzyme is produced in large quantities which further enzymes will break down the fat into free fatty acids, glycerol, and other compounds. Further decomposition of free fatty acids into choline is then described to be TMAO by dehydrogenase enzyme and continues to formaldehyde formation.

The results showed that the content of TVB moonfish in this study continues to increase along with the length of storage time. This is in line with the statement of **Liu *et al.* (2010)** that TVB is a protein degradation compound that produces a number of volatile bases such as ammonia, histamine, trimethylamine hydrogen sulfide and dimethylamine. Therefore the formation of TVB in moonfish during frozen storage has increased in accordance with TMA, formaldehyde and DMA formation. In addition, an increase in TVB levels may be due to frozen storage conditions, especially the decreased or even the absent of oxygen levels which is in turn will increase the activity of proteolytic enzymes to decompose the volatile basic found in the moonfish meat. Correlation analysis of formaldehyde formation with DMA and TVB degradation parameters showed significant positive correlation. This suggests that the rate of quality deterioration is closely bound to the risk of exposure to formaldehyde in the moonfish. Formation of natural formaldehyde in fish can take place during the decomposition process, with the lower the quality, the natural formaldehyde levels can also be high **(Murtini *et al*., 2014)**. The moonfish in this study can still be said in fresh condition when kept frozen up to one month. This is because the content of TVB in moonfish is still less than the limit of TVB content required **(Gulsun *et al*., 2009)** and Indonesian National Standard (SNI) 4110: 2014 which is less than 30 mgN%. These mean that based on the freshness level, moonfish which is stored until one month at frozen temperature is still feasible to be consumed. However, from safety point of view, precaution should be taken for consuming moonfish which has been stored for one month in frozen condition due to formaldehyde level that might impact to consumer's health. More accurate review might be necessary to ensure consumers' health since formaldehyde level has already exceeded the tolerance limit for consumption

CONCLUSION

Formaldehyde in moonfish can form naturally under frozen storage conditions for six months of storage reaching concentrations up to 133 mg / kg with a TVB-N content of more than 30 mg/100g. Thus, frozen moonfish that have been stored for six months are no longer suitable for consumption because they have a risk level of danger that can have an impact on consumers' health and their quality has declined. Moonfish is still suitable for consumption based on its freshness level up to one month in frozen storage, but based on formation of formaldehyde, the level of consumption should consider the calculation of maximum daily intake based on specified reference dose.

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