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Research Article

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Mathematical Formulation to Differentiate between Naturally Occurred and Artificially Added Formaldehyde in the Ice Stored of Lizardfish (*Saurida tumbil*)

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ABSTRACT

The practice of formaldehyde abuse as a food preservative, especially in fishery products, is commonly occurred. However, the differentiation of the formaldehyde origin in fish is difficult to be performed. Meanwhile, physical observation is not sufficient to determine the additional formaldehyde in fishery products. This study aimed to formulate a quantitative method to detect the origin of formaldehyde in fish. The formulation was developed based on the differences in some chemical properties (Formaldehyde (FA), Trimethylamine (TMA) and Trimethylamine oxide (TMAO) of fish treated with and without formaldehyde addition. The fish used as samples were beloso fish/lizardfish (*Saurida tumbil*). The fish were prepared with and without the addition of formaldehyde and then stored in ice for 21 days. The content of FA, TMA, and TMAO of gill and flesh during storage were analyzed every 3 days to determine the difference in the trend of distribution patterns of two fish treatments. The data were statistically processed to produce two mathematical formulas to identify the origin of formaldehyde. The results revealed that detection of the origin of formaldehyde in fish could be performed using two approaches: the diffusion rate approach (validation 75-100%) and the ratio of formaldehyde, TMA, and TMAO (validation 96.47%).

Keywords: *formaldehyde, fishery products, food preservative, mathematical formulation, Surida tumbil*

ABSTRAK

Praktik penyalahgunaan formaldehida sebagai bahan tambahan pangan illegal, khususnya pada produk perikanan masih sering terjadi. Oleh karena itu, metode untuk membedakan kandungan asal formaldehida pada produk perikanan baik yang terbentuk secara alami atau yang sengaja ditambahkan secara illegal masih sulit untuk dilakukan. Disamping itu, pengamatan secara secara fisik sulit untuk dilakukan untuk menentukan terdapatnya penambahan formaldehida pada produk perikanan. Penelitian ini bertujuan untuk membuat formulasi metode kuantitatif untuk mendeteksi asal formaldehida pada produk perikanan. Formulasi yang dikembangkan berdasarkan oleh perbedaan beberapa senyawa kimia pada produk perikanan seperti (Formaldehida (FA), Trimetilamin (TMA) dan Trimetilamin oksida (TMAO)). Ikan yang digunakan sebagai sampel adalah ikan beloso/lizardfish (*Saurida tumbil*). Ikan dipreparasi dengan penambahan formaldehida sintetis dan tanpa penambahan kemudian disimpan dalam es. Kandungan FA, TMA, dan TMAO organ insang dan daging ikan selama penyimpanan dianalisis untuk mengetahui perbedaan kecenderungan pola distribusinya pada kedua perlakuan. Data diolah secara statistik hingga dihasilkan dua rumusan untuk mengidentifikasi sumber formaldehida. Hasil penelitian menunjukkan bahwa deteksi asal formaldehida pada produk perikanan dapat dilakukan dengan menggunakan dua pendekatan yaitu pendekatan laju difusi (validasi 75 – 100%) dan rasio dari senyawa formaldehida, TMA dan TMAO (Validasi 96,47%).

Kata kunci: formaldehida, produk perikanan, bahan tambahan pangan, formulasi matematika, *Saurida tumbil*

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1. Introduction

Formaldehyde at certain concentration has been known to pose harmful effects on consumers health. Consumption more than 0.2 mg/kg body weight/day of food with formaldehyde content potentially harm the consumers health due to the carcinogenic effects (Wang et al., 2007). On the contrary, the abuse of formaldehyde addition as a preservative to fishery products in some countries is still happened. Bangladesh (Bhowmik et al., 2017; Jaman et al., 2015), Malaysia (Noordiana et al., 2011; Aminah et al., 2013), Nepal (Joshi et al., 2015) and Taiwan (Yeh et al., 2013) conducted formaldehyde analysis on fish samples in the market and found a number of samples had formaldehyde content. Indonesia through Perka BPOM. No. 7/2018 states that formaldehyde is a compound that should not be added to food for any reason. However, this legal basis has not been able to eliminate the practice of abuse of formaldehyde.

Identifying the origin of formaldehyde in fish is difficult due to the formation of natural formaldehyde as a by-product of metabolic process in several fish species (Wahed et al., 2018). The concentration of natural formaldehyde in fish varies depend on the species, characteristics of the fish, and the environment (Zhang et al., 2017). Several types of fresh fish had a high natural formaldehyde content, such as opah fish/*Lampris guttatus* (Barokah et al., 2020), nomei (*Harpodon nehereus*) (Jaman et al., 2015; Shen et al., 2015), beloso (*Saurida tumbil*) (Anissah et al., 2019; Nurhayati et al., 2019) and squid (*Loligo* sp.) (Yeh et al., 2013).

Research on quantitative identification of the formaldehyde origin is still limited. Hoque et al. (2018)) formulated an equation to detect the formaldehyde content in two fish species by knowing the concentration of additional formaldehyde. However, this formula could not predict the origin of formaldehyde in a fish sample. Anissah et al. (2021) stated that the ratio of TMA and TMAO in the flesh of *Saurida tumbil* fish is a marker for the addition of formaldehyde. Shen et al. (2015) described the difference in the formaldehyde content in the external and internal organs of *Harpodon nehereus* fish that were treated with and without formaldehyde. These indications were analyzed; therefore, a quantitative method could be formulated to detect the formaldehyde origin.

There is no difference in chemical structure between natural and synthetic formaldehyde, on the other hand there are several types of fish that contain natural formaldehyde in varying concentrations. It causes problems in monitoring and controlling the formaldehyde abuse. Those were the reasons that encourage a research to formulate the origin of formaldehyde detection method. The previous studies showed that the addition of formaldehyde would change the composition of abundance TMA, TMAO and formaldehyde in fish organs (Anissah et al., 2021). This proportion change can be formulated mathematically and used as an indication to determine the origin of formaldehyde. The purpose of this study is to develop a quantitative formulation to detect the formaldehyde origin in fish.

2. Material and Methods

2.1. Sample Preparation and storage trials

The formulation of the detection method for the formaldehyde origin was developed based on the data of beloso fish (*Saurida tumbil*) as the sample. *Saurida tumbil* was known as demersal species with relatively high concentration of natural formaldehyde. The fish sample was provided from Cituis fish landing, Tangerang Regency, Banten Province. The fish were treated with formaldehyde immersion in three replications. The samples were soaked in a 3% formaldehyde solution for 30 minutes with a ratio of fish weight and formalin solution of 1: 3 (w/v) (Ariyani et al., 2019). Then, the samples were stored in ice for 21 days. The ice temperature during storage were maintained in 4^{o}C. Fish without artificially added formaldehyde and stored with the same procedure were used as a comparison.

2.2. Chemical analysis

The concentration of formaldehyde, TVB, TMA, and TMAO of flesh and gills were determined every 3 days during storage. The extraction of fish organ samples was carried out by TCA 7.5%. Extracted samples of gills and flesh were analyzed for formaldehyde content (mg/kg) (Benjakul et al., 2004) . TVB (Conway method) was analyzed in flesh during the storage (Özoğul & Özoğul, 2000) . Finally, TMA and TMAO of gills and flesh were analysed with deuterium oxide (D2O) solvent and were analyzes using proton chemical shift analysis (1 H-NMR) following Shumilina et al. (2016) method. In order to determine the accuracy of

the formula, the proportion of applicable formulation to obtained data was calculated.

2.3. Mathematical formulation and data analysis

The data used for the mathematical formulation were the concentration of formaldehyde, TVB, TMA, and TMAO of internal organs (flesh) and external organs (gills) of fish on ice storage at 0 to 21 days. Formaldehyde concentrations in gills and flesh of fish in two treatments were compared and used to determine the proportion of formaldehyde distribution per organ during storage. The difference between the results of this comparison were generalized and used to create a formula for determining the source of formaldehyde based on differences in diffusion rate. Determination of the constant value (k) was done by simulating the data obtained with scenarios of 3 and 6 days test intervals. The value that differs between the two treatments of fish samples was taken as a constant value. The composition of TMA, TMAO and formaldehyde in fish changed with the artificially addition of formaldehyde (Anissah et al., 2021). The abundance of TMA and TMAO data of fish organs in the two treatments along storage period were combined with formaldehyde concentration data. These data were processed to determine the differences in the abundance patterns of these three parameters on fish with the artificially added formaldehyde and naturally occurred formaldehyde. The existence of these differences were formulated mathematically into a formula to identify the source of formaldehyde in fresh fish. The validation of formula was conducted with calculation the fish samples that have treated and untreated with formaldehyde solution. The validation level was calculated : Confidence level = (correct data/sum of all simulations)x100%. Where the correct data is the data included in the formula which shows the same conclusions as reality. For example, the calculation results show the conclusion that there is additional formaldehye for samples that have been treated (formalin added), then the simulation is considered correct.The statistical analysis of collected data was done using SPSS 22 software.

3. Results and Discussion

The approach used to formulate the formaldehyde detection method were based on the differences in physical and chemical properties that may occur in fish without and with the addition of formaldehyde. The addition of formaldehyde as a fish preservative has been done by spraying or immersing fish in a solution of formaldehyde at a certain concentration. Physically, fish with formaldehyde addition are thought to have a diffusion direction from external to internal organs during the storage period, whereas natural formaldehyde will diffuse in reverse. No data have been obtained regarding the adsorption rate of fish because of formaldehyde addition. The chemical approach is based on the process of breaking down the TMAO into DMA, TMA and formaldehyde because of the presence of enzymes and bacteria in the deterioration phase of fish quality. The addition of formaldehyde in fish will inhibit the growth of bacteria because of its antibacterial properties. The TMAO and TMA are the major factors (97,44%) to discriminate the origin of formaldehyde in fish sample (Anissah et al., 2021). Based on those approach, the formulation of formaldehyde origin detection method was based on two assumptions: the difference in the diffusion rate of the formaldehyde solution and the ratio of the formaldehyde, TMA and TMAO content.

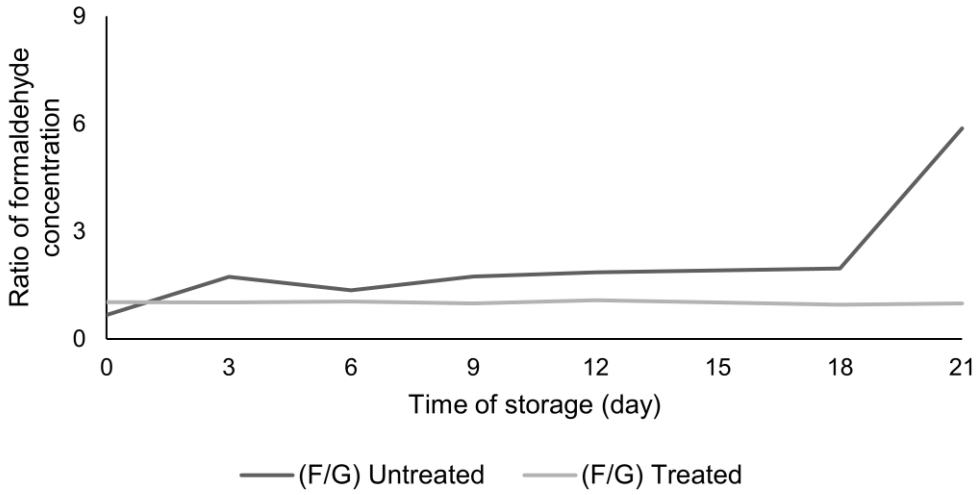
3.1. Method to distinguish formaldehyde origin based on the difference of diffusion rate

The results show that there were significant differences between time and among organs (inside and outside) of fish without and with the addition of formaldehyde. The results of formaldehyde concentration comparison in internal (flesh) and external (gill) organs in fish with and without the addition of formaldehyde showed significant differences ($p < 0.5$) (Figure 1). The fish samples (*Saurida tumbil*) with and without the addition of formaldehyde in the ice storage for six days showed a TVB value of less than 30 mg /100g and for 21 days storage was 86.67±10.05 mg/100g (Ariyani et al., 2019). The natural formaldehyde content of *Saurida tumbil* was found in the stomach contents at concentration of 15 mg/kg (Nurhayati et al., 2019).

The ratio of formaldehyde concentration in tissue and gill of the treated sample showed relatively stable during storage for 21 days. Meanwhile, the sample without the addition of formaldehyde showed the opposite results. It shows that in treated samples, formaldehyde was spread quickly after it was added. All of organs relatively had the same formaldehyde concentration. The other hand, formaldehyde concentration of untreated samples were

difference between inner and outer organs. It seems likely in all of the storage period. This trend of formaldehyde concentration during storage was then mathematically formulated (i) as followed:

$$k = 100\ x\ \left(\frac{d_b}{i_d} - \frac{d_a}{i_a}\right)$$



Note :
F: flesh
G: gill

Figure 1. The ratio of formaldehyde concentration in flesh and gill of fish during storage

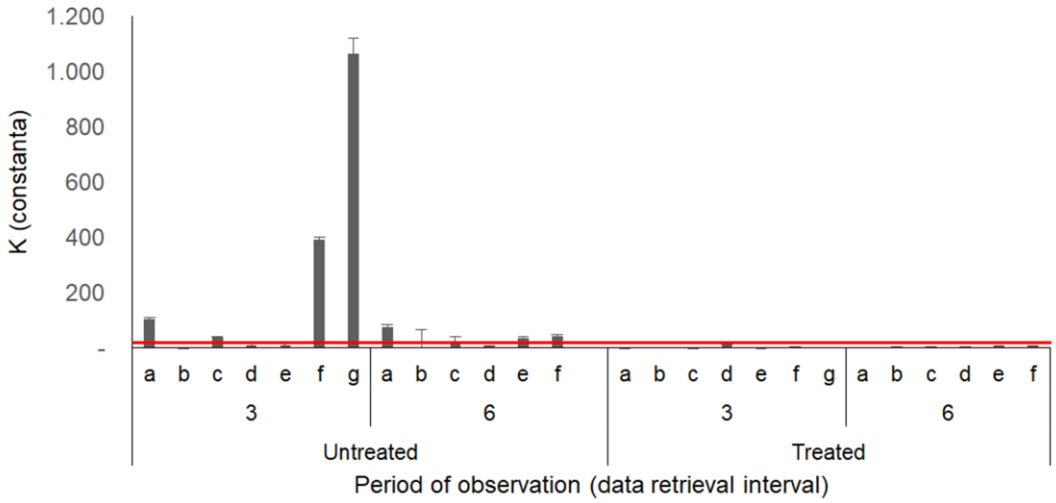
where :
k = constant value
da,b = concentration of formaldehyde in flesh at a and b time (mg/kg)
ia,b = concentration of formaldehyde in gills at a and b time (mg/kg)
a,b = time of first and second analysis (3 or 6 days interval)

The results of the formula application on the formaldehyde data of the treated and untreated samples are shown in Figure 2. K value in untreated samples were higher than treated samples. The k value is set at 20. The value of k> 20 indicates no addition of formaldehyde, while k< 20 suggests the addition of formaldehyde.

This formula (i) is expected to be easily applied by interested parties such as fishery product quality supervisors and law enforcement authorities. Therefore, the applications of the formula were simulated only at intervals 3 and 6 days of analysis. The results show that two timescales (storage) could be used as a time span for sampling analysis with different validation level (Table 1). The analysis with retrieval interval analysis in 6 days showed higher validation level than 3 days. The limitation of TVB was set on <30 mg/100 g because this value was the limit of TVB content in fresh fish that fit for consumption (Barokah et al., 2020). Furthermore, the formula could be applied by user with a measurement period of 3 and 6 days with TVB value < 30mg/100g.

Table 1. Validation level of formulation (i) application on different data retrieval interval

Data retrieval interval (day)	TVB (mg/100g)	Validation Level (%)
3	<30	75.00
	<87	78.57
6	<30	100.00
	<87	91.67



Note :
a-g : k simulation codes for formula (i)

Figure 2. Determination of k values for the formaldehyde sources differentiation based on changes in diffusion rates with a measurement range of 3 and 6 days

3.2. Method to differentiate formaldehyde origin based on comparing the TMA and TMAO content of fish flesh

During the deterioration process of fish quality, TMAO will decompose into TMA, DMA and formaldehyde in the presence of enzymes and bacteria. The amount of TMAO will be inversely proportional to TMA and formaldehyde while the amount of TMA is proportional to formaldehyde. The presence of artificially added formaldehyde will inhibit the ımposition of TMAO so that the :entration of formaldehyde and TMAO will be measured in high levels, while TMA will be detected in low amounts. One of the factors that could be used as a marker of the formaldehyde addition to fish samples was the difference in the concentration ratio of TMA and TMAO (Anissah et al., 2021). The formaldehyde, TMA and TMAO concentrations of treated and untreated *Saurida tumbil* fish tissue were plotted and expressed empirically (ii) as a formula for detecting the formaldehyde origin as followed:

$$k = \frac{Formaldehyde\ concentration\ (mg/kg)}{TMA/(TMAO + TMA)}$$

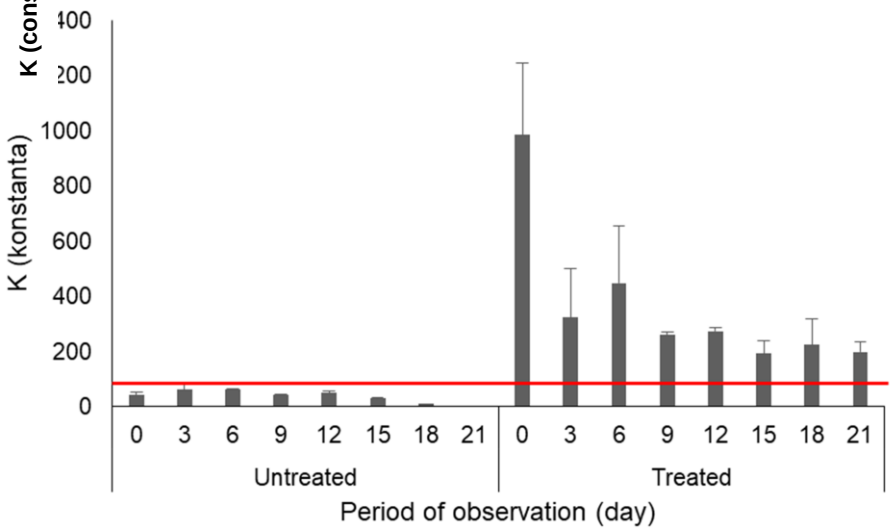


Figure 3. Determination of k values for differentiation of formaldehyde sources based on differences in the ratio of TMA and TMAO

Where :
k = constant value
TMA = TMA content of fish flesh
TMAO = TMAO content of fish flesh

The simulation results with treated and untreated samples show the different value of k (Figure 3). The k value was set in 100 to differentiate k treated and untreated samples. k value above 100 indicated the treated samples, while untreated samples show a k value below 100. Moreover, the k value obtained in the calculation of formula (ii) was higher than 100; therefore, it indicates an addition of formaldehyde. In contrast, the k value of less than 100 shows no addition of formaldehyde. Therefore, based on simulated data, the fish samples stored up to 21 days in ice storage (temperature 4°C, TVB <85 mg / 100g) could be analyzed for formaldehyde origin using the formula (ii) with validation level of 96,47%. Differences in species, size, method and time of storage will affect the validity of using this formula. Further research needs to be done to develop these two formulations so that they can be used for fish in general.

4. Conclusion

Detection of the origin of formaldehyde in fish could be done using two approaches: the diffusion rate (validated 75-100%) and the ratio of formaldehyde, TMA, and TMAO (validated 96,47%). Further research needs to be carried out with other types and categories of fish to validate the proposed formulas..

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