

## Optimisation of liquid smoke from water hyacinth (*Eichhornia crassipes* (Mart.) Solms) to preserve eels (*Sybranchus bengalensis* McClell)

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### Abstract

Antibacterials and antioxidants are important properties of liquid smoke due to the acid, phenol, and carbonyl contents, especially for fish preservation, where these additives help in quality maintenance and shelf life extension of fishery products. Therefore, this study aimed to observe the optimisation of steaming and storage time in the liquid smoke preservation on eels. These animals were soaked with water hyacinth liquid smoke, where 35 mL of the solution was used to drench 50 g of eels at 30% concentrations. The liquid smoke used in this study was re-distilled to separate the polycyclic aromatic hydrocarbons (PAH) from the liquid smoke. The data obtained from these tests were analysed using the Response Surface Methodology (RSM), while the total plate count (TPC) and total volatile bases (TVB) were used as response variables. The results were in line with the Indonesian National Standard (SNI) No. 2725.1:2009 regarding the maximum limit for smoked eels' TPC, where the values ranged between  $2 \times 10^4$  -  $2 \times 10^5$  CFU/g. The model parameters were also obtained by observing the surface regression of the TPC response with an  $R^2$  value of 87.0%. According to SNI No. 2354.8.2009 (BSN, 2009b), the TVB analysis indicated the range of values between 2.530-3.998 mg N/100 g, at approximately 140 hrs (6 days). This showed that temperature and time affected the acquisition of the TVB test by 61.6%. Therefore, the water hyacinth liquid smoke was recommended as an eels preservative based on the TPC and TVB values obtained.

## 1. Introduction

Eels are freshwater fish commonly found in Indonesia, which have a fairly high nutritional content including protein (15%), fat (26%), moisture (61%), and minerals (1%) (Belitz *et al.*, 2009). Most of these fishes contain water content (62.81%), protein (16.20%-17.50%), carbohydrates (0.13%-1.39%), fat (17.00-17.92%), ash (1.34%), as well as vitamins A (2068.55-3316.38mg.100g-1) and E (0.21-0.224%). The fish also has mineral contents such as magnesium (121-145.35 ppm), zinc (20.9-24.44 ppm), iron (30.99-48.08 ppm), and calcium (0.48-0.52%) (Wijayanti and Setiyorini, 2018). The traditional fish-smoking process uses coconut shells or burnt wood, which negatively affects the released polycyclic aromatic hydrocarbons (PAH) content. This subsequently indicates the importance of determining alternative smoking agents for food preservatives (Sokamte *et al.*, 2020). One of these alternatives involves the use of liquid smoke derived

from biomass pyrolysis, which mostly produces acid, phenol, and carbonyl compounds due to the thermal decomposition of lignin, cellulose, and hemicellulose. These compounds are found to contribute to the provision of aroma, colour, and flavour, as well as antioxidant and antibacterial contents, which allow liquid smoke to act as a food preservative agent. Furthermore, the presence of phenols and etherphenols in the liquid smoke obtained from lignin decomposition (at 100-900° C) plays a role in providing food aroma, antioxidant activity, and preservatives. This aroma is produced from syringol (1.6-dimethoxy phenol) and guaicol (2-methoxy phenol) compounds, as well as their derivatives (Yao *et al.*, 2020). The degradation of lignin also generates aromatic hydrocarbons, which contain benzene ring and bonded hydroxyl groups. In addition, these compounds often bond to other groups, including acids, ketones, aldehydes, and esters (Zhang *et al.*, 2018).

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Water hyacinth is a typical wild plant in Indonesia, which mostly grow in lakes or rivers. According to Yao *et al.* (2020), the water hyacinth contained hemicellulose (20-40%), cellulose (20-50%), and lignin (20-40%), which made the plant a very good source of liquid smoke through the pyrolysis process (Bote *et al.*, 2020). Furthermore, several studies showed that the water hyacinth liquid smoke did not contain benzo(a)pyrene PAH, making it a safe food additive (Ratnani *et al.*, 2021). The response surface methodology is one of the attractive methods to optimise the process variable due to directly searching for the best values in specific factor ranges. It also determines the correlation between responses and input variables (Hadiyanto and Sutrisnorhadi, 2016; Singh and Tirkey, 2021). However, the study on optimising eels preservation through water hyacinth liquid smoke is still limited. Although the preservation is affected by steaming temperature and storage time, the optimum value of smoked eels conversion based on TPC and TVB is still hampered. Therefore, this study aimed to optimise the liquid smoke treatments on eels fish preservation, especially the steaming and storage time, to the response of TPC and TVB analyses.

## 2. Materials and methods

### 2.1 Materials

The water hyacinth pyrolysis conducted at 400°C for 4 hrs yielded the liquid smoke employed in this study. Due to PAHs being carcinogenic and hazardous to food, the liquid smoke produced was re-distilled to remove these compounds. Figure 1 shows the liquid smoke products used for food applications. The swamp eels and agar media, water hyacinth liquid smoke, aquadest, as well as ethanol were used as the TPC test materials, while the perchlorate smoke, phenolphthalein indicator, NaOH, H<sub>3</sub>BO<sub>4</sub>, HCl, and K<sub>2</sub>CO<sub>3</sub> were utilised in the TVB analysis.



Figure 1. Liquid smoke from water hyacinth

### 2.2 Liquid smoke production

The production of liquid smoke was conducted by the preparation of water hyacinth raw materials at 2-3 cm length, subsequently placed in a pyrolysis reactor. This process was conducted by introducing 550 g dry water hyacinth with 10% water as a raw material into a type 316 stainless steel pyrolysis reactor, which had a length

and internal diameter of 360 and 240 mm under an atmospheric vacuum. After passing through the condenser, the liquid smoke was distilled to obtain a pure result, as shown in Figure 1. (Maulina and Silia, 2018).

### 2.3 Application to eels fish

The liquid smoke from the distilled water hyacinth was diluted to 30% and used to soak the eels, which were observed for a storage period between 24-120 hrs. Subsequently, these smoked eels were observed for their TPC and TVB contents (Desvita *et al.*, 2020). The following are the phases of smoked eels processing: (1) The eels were weeded to clean the internal and external contents using running water and extraction processes, (2) Soaking in a 10% (w/v) saline solution for 1 hr, then washing with running water and draining for 10 mins, (3) After 30 mins of steaming, the food was cooled to room temperature, (4) The eels were immersed in a 30% liquid smoke solution with a water solvent and subsequently drained, and (5) The final stage of the eels preservation procedure was drying, carried out in an oven at 80°C for 8 hrs using liquid smoke (Khamidah *et al.* 2019).

### 2.4 Total plate count analysis

The equipment used for this microbiological test were autoclave, petri dish, incubator, analytical balance, test tube, drying oven, dropper pipette, spirit lamp, and Erlenmeyer flask (Swastawati *et al.*, 2019). Approximately 1 mL of the crushed sample was mixed with 9 mL of buffered peptone water. Subsequent ten-fold dilutions (up to 10<sup>-4</sup> dilution) were prepared and placed in a petri dish, mixed with a plate count agar (PCA) media, and tightly closed. The plates were incubated. The colonies were counted and reported.

### 2.5 Total volatile bases analysis

The TVB analysis was carried out using the Indonesian National Standard (SNI) No. 2354.8.2009 (BSN, 2009), where the test was conducted by mixing 5 g of sample with 7% TCA solution, at a ratio of 1:3. Stirring for 1 min, the sample was made homogeneous and separated through a filter paper, leading to the production of a clear solution. Moreover, the channels were prepared by draining 1 mL of boric acid, where the outer sources on the right and left sides were each added with 1 mL of K<sub>2</sub>CO<sub>3</sub> solution and an unmixed clear sample, respectively. These were incubated for 2 hrs at 37°C and internally titrated using the HCl with a concentration of 0.02 N till a slightly pink colour was achieved. The next step was also calculated using the following formula (Ariestya *et al.*, 2016).

$$\text{TVB (mg N \%)} = (\text{Sample titration (mL)} - \text{blank (mL)}) \times \text{N HCl} \times 14.007 / \text{Sample weight (g)} \times 100$$

## 2.6 Determination of water content

The eel sample was one of the other aquatic biota processed towards a final product, such as fresh and frozen fish, as well as other processed fishes used for human consumption. The principle of testing the WC (water content) was carried out by using water hyacinth liquid smoke as a sample and smoothening the smoked eels with a blender until the particles were eligible to pass through a 20 mesh sieve. Subsequently, 5 g of the obtained samples were placed into a cup before being transferred to a non-vacuum oven at 105°C for 16-24 h. The determination of the weight of water was also gravimetrically calculated based on the differences in the sample weights (before and after being dried) until a fixed result similar to the National Standard Indonesia (SNI) no-01-2354.2-2006 is achieved (BSN, 2006a).

## 2.7 Sensory evaluation

At the smoked eels preservation stage, a sensory and preference test was carried out using a 1-9 value with 20 panellists that are expected to obtain the final results of likes and dislikes. This was based on inserting the treated smoked eels into a coded plastic seal before descriptively assessing the panel, likes, and tests (National Standardization Agency of Indonesia (BSN), 2009; Arestya et al., 2016).

## 2.8 Optimisation of the preservation process has used liquid smoke made from water hyacinth using the response surface methodology

The response surface methodology was used with Minitab statistics to optimise the eels preservation process. This aimed to search and obtain variable combinations with the potential to produce the best reaction. In this study, the steaming temperature and storage period were the independent variables, designed at 40-70°C and 24-120 hours to achieve an optimal procedure in the eels preservation process, respectively (Khamidah et al., 2019; Rahmawati et al., 2020).

## 2.9 Statistical analysis

The data obtained from each analysis were used as the results of the TPC and TVB tests, where the parameter assessment was carried out at the Chemical Engineering Process Laboratory of Unwas, through the Response Surface Methodology (RSM) technique (Rahmawati et al., 2020). The independent variables

were the temperature and storage time, with their range and levels shown in Table 1. This experimental design was prepared by using Minitab 14 program, subsequently producing 13 one-time trials without repetition, as shown in Table 2.

## 3. Results and discussion

### 3.1 Total plate count analysis on smoked eel

The quality of the preserved eels was observed using the water hyacinth liquid smoke from the TPC and TVB values. The Total Plate Count is one of the parameters used to detect the number of bacteria in food products. This was carried out by counting the developed bacterial colonies through agar media. To determine the quality and shelf life of a food product, fresh fish is also found to be an important criterion. Therefore, the TPC analysis was conducted by counting the number of developed bacteria on an agar medium at 37°C for 24 hrs, to determine the freshness of the fish (Valø et al., 2020). Liquid smoke is often used to maintain the freshness of a fish through the process of soaking. This technique contained phenolic compounds, organic acids, and carbonyl groups. These acids were found to be bactericidal/bacteriostatic due to having the capability to control the growth of several bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Escherichia coli*. Meanwhile, the phenols functioned as antioxidants and preservatives, each observed with fungicidal, herbicidal, and insecticidal properties. These compounds were observed as great antibacterial agents at a high boiling point in a smoking technique. The phenol, carbonyl, and organic acids within a liquid smoke were found to also play an important role in fish preservation, making the process safer than the traditional technique (Keryanti et al., 2020).

The carbonyl compounds within this smoking technique are also known to contribute to lower microbiological growth. This is because the alginate coating did not exhibit antibacterial properties. However, this coating was reportedly described to decrease bacterial growth when applied to aerobic-based fishes. This was due to acting as a barrier to oxygen transfer. Based on the dominant role of bacteria, the quality of a fish is found to decline at death through changes in smell, appearance, and texture. To avoid these undesirable changes, fish were often preserved using

Table 1. Range and level of eel preservation variables

Independent variable	Range and levels				
	Star point ( $-\alpha/1.682$ )	Level low (-1)	Level medium (0)	Level high (+1)	Star point ( $+\alpha/1.682$ )
Steaming temperature (°C)	35	40	55	70	76
Storage time (hours)	4	24	72	120	140

Table 2. Response TPC and TVB on eel preservation with liquid smoke

Code variable		Independent variable		Dependent variable			
X <sub>1</sub>	X <sub>2</sub>	Steaming temperature (°C)	Storage time (hours)	Response (Y) TPC CFU/g	Prediction TPC CFU/g	Response (Y) TVB (mg N/100 g)	Prediction TVB (mg N/100 g)
-1	-1	40	24	50.000	18.949	3.33	3.07
1	-1	70	24	200.000	164.911	3.99	3.54
-1	1	40	120	150.000	157.589	3.80	3.97
1	1	70	120	200.000	203.551	2.97	2.94
-1.414	0	34	72	70.000	80.895	3.46	3.46
1.414	0	76	72	200.000	216.605	2.78	3.06
0	-1.414	55	4	20.000	61.072	2.95	3.32
0	1.414	55	140	200.000	186.428	3.76	3.60
0	0	55	72	100.000	90.000	3.17	3.15
0	0	55	72	100.000	90.000	3.13	3.15
0	0	55	72	100.000	90.000	3.13	3.15
0	0	55	72	100.000	90.000	3.23	3.15
0	0	55	72	50.000	90.000	3.18	3.15

liquid smoke (Wijayanti *et al.*, 2020). In this study, the TPC response is presented in Table 2. This was in line with the requirements of SNI No. 2725.1-2009 (BSN, 2009c) concerning the microbiological quality standard of smoke fish. These results indicated that the maximum TPC and bacterial contamination limit were  $1 \times 10^5$  CFU/g, respectively (Setiawati 2014; Widawati and Budiyananto 2014). Based on this study, the results obtained between 0-6 days or 4-140 hours were also in line with the SNI, where the TPC values ranged from  $2 \times 10^4$  -  $2 \times 10^5$  CFU/g (Indiarto *et al.*, 2020).

According to Faisal *et al.* (2018), the meatballs preserved with liquid smoke had a longer shelf life than those without the technique. This indicated the importance and versatility of liquid smoke, which also could maintain the quality of snakehead fish on microbiological testing, especially on TPC and coliform values at 4°C for 10 days. As a preservative for tilapia, this technique was reportedly effective at a concentration of 12.5%. This was due to the phenolic compounds acting as antibacterial agents. In addition, bacteria were observed to have decreased, indicating that the highest value of microorganisms at the liquid smoke level was  $8 \times 10^6$  CFU/g. To preserve catfish, a previous study showed that the liquid smoke from durian skin could control bacteria at a TPC value of  $4.243 \pm 0.012$  CFU/g. However, these values were still relatively low in all treatments, as the smoked catfish products were not adequately stored. This indicated that longer immersion led to a lower TPC value. The Kesambi leaf liquid smoke was also observed as an ingredient for smoking Se'i Ikan, where the TPC values on the 4th and 5th days were  $4.5 \times 10^3$  and  $5.8 \times 10^4$ , respectively (Mardyaningsih *et al.*, 2016).

### 3.2 Model development for TPC value prediction

Based on the Analysis of Variance (ANOVA) test regarding the TPC assessment, the observations of temperature and time were carried out. The results showed a p-value of  $0.157 > \alpha$  (0.05), indicating the acceptance of the  $H_0$ , which stated that the regression model was suitable. For the simultaneous regression parameter test, the p-value of  $0.554 > 0.05$  was obtained, indicating the acceptance of the  $H_0$ , which stated that the independent variables (xi) did not have a significant effect on the response factor (y). Therefore, the ANOVA of the steaming temperature and storage time did not have a positive effect on TPC. This was not in line with several previous studies, such as Galam (Salim and Rahmi, 2018), which stated that temperature had significant effects on the TPC value ( $p < 0.05$ ). The results were also different from Ratnawati and Hartanto (2010) and Zuraida *et al.* (2011), which showed that 20-day storage of liquid-smoked fish balls significantly reduced ( $p < 0.05$ ) the water content of the samples. The TPC value in smoked roa fish was subsequently observed at  $3 \times 10^2$  CFU/g, with statistical analysis showing a significant difference between the test and the sample at seawater washing, compared to freshwater ( $p < 0.05$ ) (Damongilala, 2009). From storage at room temperature for 0, 7, 14, and 21 days, the TPC values for smoked roa fish were  $2.24 \times 10^3$ ,  $1.42 \times 10^4$ ,  $6.75 \times 10^3$ , and  $7.76 \times 10^4$  CFU/g, respectively. Furthermore, the total number of smoked trout bacterial colonies was recommended by the SNI (2725.1:2009), where the maximum limit for the TPC value was  $1.0 \times 10^5$  CFU/g (Dotulong *et al.*, 2018). Similar results were also obtained in smoking filet fish, with significance ( $p$ -value  $< 0.05$ ) observed in the statistical TPC value (Ceylan *et al.*, 2018).

The model parameters were statistically determined using the Response Surface Methodology (RSM), based on the designed experimental dataset. This indicated that the predicted model should be validated with the dataset to ensure estimation suitability. Therefore, the model and experimental points should have a high coefficient of determination ( $R^2$ ) (Hadiyanto and Sutrisnorhadi, 2016; Hadiyanto *et al.*, 2017). The model parameters were obtained by observing the TPC surface regression with an  $R^2$  value of 87.0%. This indicated that temperature and time affected TPC by 87.0%, with the remaining 13.0% being caused by other factors. Therefore, the process of prediction was found to be less suitable. This lack of determination estimated that the distribution of the TPC data was not a suitable test in this study. The simultaneous regression parameters carried out on liquid-smoked eels are described in Equation 1 and Table 3.

$$y = 90000 + 47981X_1 + 44320X_2 + 29375X_1^2 + 16875X_2^2 - 25000X_1X_2 \quad (1)$$

### 3.3 Optimisation TPC on smoked eel

Using water hyacinth liquid smoke, the optimum conditions for obtaining information on the number of colonies (TPC) in the eels are shown in Figure 2, where the minimum and maximum (dark green) situations were estimated. This was due to the distribution of the TPC values for smoked eels. In this study, several factors causing bad data distribution were less effective through the utilised tools, indicating the necessity to repeat the experiments to obtain more accurate data. However, the TPC value generally met the SNI standards set for smoked, pindang, and fresh fish, respectively. This was to ensure that the liquid-smoked eels were fit for consumption. The most optimal temperature was also obtained at 34°C and 54 hrs, with a TPC value of 18.428 CFU/g

### 3.4 Total volatile base analysis on smoked eel

The TVB value is an index of food quality deterioration, which indicates that enzyme activity encourages an increased process of autolysis, subsequently leading to the smoked eels rot. Besides bacterial activity, enzymatic events are also found to be the cause of quality deterioration, leading to the index

known as the TVB content. Based on this study, the eels were preserved in liquid smoke and tested for their TVB value. As an indicator to determine damages to the smoked fish, TVB was observed as the breakdown of protein at the final stage. Moreover, the bacterial colonies obtained an energy source from the degradation of protein into amino acids, peptone peptides, polypeptides, and dipeptides, with the nitrogen elements subsequently forming TVB. During decomposition, several compounds generating a foul odour were obtained, such as hydrogen sulfide, mercaptans, indole, skatole, putrescine, and cadaverine. This indicated that the maximum TVB in salted and smoked fish should not be more than 100 mg N/100 g. Drying temperature also increased the activity of enzymes in protein hydrolysis, indicating that this nutritional content was not denatured at 70°C, leading to the maximum TVB not being formed through the nitrogen element (Hardianto and Yunianta 2015).

Liquid smoke is useful as a preservative due to containing several beneficial compounds, namely organic acids and phenols, which are bactericidal/bacteriostatic based on controlling the growth of bacteria, such as *Bacillus subtilis*, *Pseudomonas fluorescensi*, *Staphylococcus aureus*, and *Escherichia coli*. The phenol compounds obtained from the pyrolysis of lignin also had preservative potentials, as well as being an antioxidant with fungicidal, herbicidal, and insecticidal properties. According to (Valø *et al.*, 2020), the phenol compounds produced by liquid smoke purification were a great antibacterial agent at a high boiling point. Based on the Indonesian National Standard (SNI) no. 2354.8.2009 (BSN, 2009), the maximum TVB value was 30 mg N/100 g. According to Table 2, these values ranged between 2.530-3.998 mg N/100 g at approximately 140 hrs (6 days). This showed that dry water hyacinth liquid smoke was used as a preservative for eels based on the SNI. This was not in line with the study of liquid-smoked tuna from corn cobs and coconut shells, whose TVB value ranged between 24.63-28.38 mg N/100 g. Based on the addition of liquid smoke from corn cobs, the TVB value was found to be higher than that of coconut shells (Hardianto and Yunianta 2015).

Table 3. Estimated regression coefficient on smoked eel TPC and TVB

Term	TPC			TVB		
	Coef	T	P	CoefS	T	P
Constant	90000	6.588	0.000	3.14660	3.775	0.000
Temperature	47981	4.443	0.003	0.14088	-1.346	0.220
Time	44320	4.104	0.005	0.07376	0.705	0.504
Temperature * Temperature	29375	2.536	0.039	0.05845	0.521	0.618
Time * Time	16875	1.457	0.188	0.17495	1.559	0.163
Temperature * Time	-25000	-1.637	0.188	-0.37450	-2.531	0.039
	R-Sq = 87.0%	R-Sq(adj) = 77.8%		R-Sq = 61.6%	R-Sq(adj) = 34.2%	



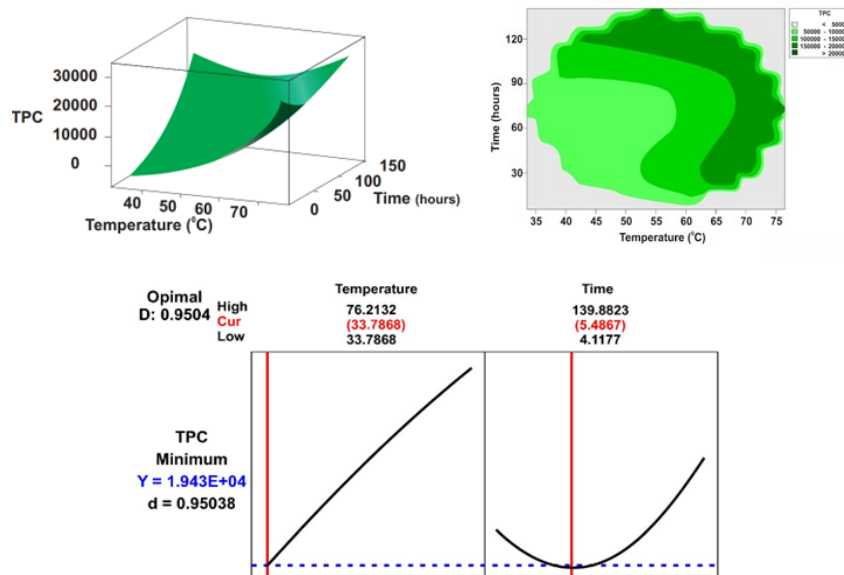


Figure 2. Response surface and contour of smoked eel TPC

At room temperature for 0-21 days, the TVB value for roa fish was reportedly greater between 41.58-59.64 mg N/100 g, which increased with more bacterial activities. This was because one of the decomposition products was classified as a volatile base. According to the SNI, the standard TVB value for processed fish ranged between 100-120 mg N/100 g. In this study, the TVB result was in line with the pH data of smoked roa fish, which were related to the activities of the naturally present bacteria and enzymes (Ceylan *et al.*, 2018; Dotulong *et al.*, 2018). The damage inhibition activity by the liquid smoke compounds (especially phenol and organic acids) was shown in this process, indicating that the acid, phenol, and carbonyl values were 41.45, 2.44, and 56.10%, respectively. Therefore, the damage resistance of this process was maximised. This was found to be smaller than the liquid smoke of corn cobs and coconut shells, subsequently corroborated by the result that pH affected TPC and TVB. Using liquid smoke, a study was conducted on the fumigation of masmin, which was a traditional fish product often smoked and dried in India (Nithin *et al.*, 2020). This indicated that liquid smoke was diluted with water at a liquid smoke and water ratio of 1:3, as salt was added to derive the right taste at 45°C for 90 mins. The results showed that the TVB provided significant effects during the 12th day of storage, with no sample values subsequently exceeded.

### 3.5 Model development for total volatile base value prediction

Based on this study, the regression model (Lack of Fit) obtained a p-value of  $0.000 < \alpha (0.05)$ , showing the acceptance and rejection of  $H_0$  and  $H_1$ , respectively. This indicated the unsuitability of the model. Meanwhile, the p-value of  $0.554 > (0.05)$  was obtained for the simultaneous regression parameter test, indicating the

acceptance of  $H_0$ , which showed that the independent variables ( $x_i$ ) did not have a significant effect on the response factor ( $y$ ). This was in line with the study on masmin fumigation. The model parameters were also obtained by observing the surface regression TVB response, with an R-value of 61.6%. This indicated that temperature and time affected the acquisition of the TVB test by 61.6%, with the remaining 38.4% being caused by other factors. Although the determination value was quite large, it was still not maximised. This was because the estimated data distribution from the TVB test did not have good analytical results. Based on Table 3, the simultaneous regression parameters obtained are the following.

$$y = 3.14660 - 0.14088X_1 + 0.07376X_2 + 0.05845X_1^2 + 0.07376X_2^2 - 0.3745X_1X_2 \quad (2)$$

### 3.6 TVB optimisation on smoked eel

The response surface of the TVB test is shown in Figure 3, which indicates that the result was not optimal and minimal. Similar to the TPC analysis, the TVB value also met the SNI, which stated that the model produced was not optimal. Therefore, increased accuracy and the use of more precise test equipment were still needed. The most optimal temperature was subsequently obtained at 76°C and 134 hrs, with a TVB value of 2.76 CFU/g.

The smoking study between 70-150°C for 1-4 h was reportedly carried out on several aquatic animals, such as peeled scales, eels, mackerel, tuna, as well as red and skinned fishes. This indicated that a minimum smoking temperature of 85°C was set to kill the microorganisms. Also, the shelf life of the fish was between 3-10 days, which was subsequently extended by refrigeration (Belitz *et al.*, 2009). The use of liquid smoke was also used to replace the traditional processing of Cakalang (*Katsuwonus pelamis L.*; *Cakalang fufu*), one of the Tuna products in North Sulawesi. Moreover, the

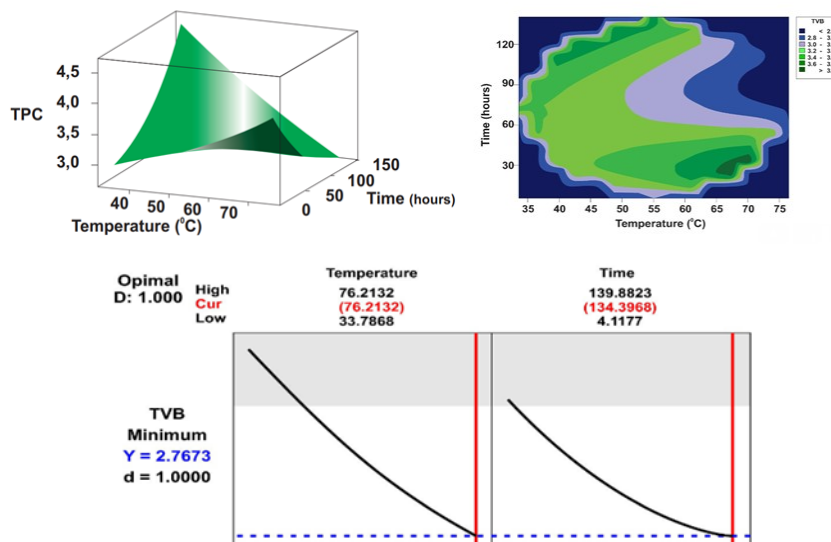


Figure 3. Response surface and contour of smoked eel TVB

traditional processing of smoked skipjack tuna contained quite high PAH, where the product was soaked for 20 mins using liquid smoke concentrations of 0.4, 0.6, and 0.8%, respectively. This was subsequently heated at 70-80°C for 4 hrs. In the study, the best results obtained were fresh fillets heated at 70-80°C for 1 hr, which were dipped in 0.8% liquid smoke and reheated at 70-80°C for 4 hrs. This produced a moisture and phenol content of 47.63 and 12.62%, respectively. It also produced pH, appearance, taste, aroma, and texture values of 4.8, 7.2, 8.3, 7.7, and 6.46, respectively, as well as a PAH <math>< 0.25</math> ppb (Berhimpon *et al.*, 2018). This indicated that the liquid smoke concentration and the interaction between the two treatments had no significant effect ( $P \geq 0.05$ ) on water content, pH, PAH, aroma, appearance, and texture. However, the method of liquid smoke application had a very significant effect ( $P \leq 0.01$ ) on water content, taste, and texture. These conditions were almost similar to this present study, which focused on the smoking of eels through the water hyacinth liquid smoke.

The chemical components of water hyacinth liquid smoke, such as acid (41.45%), phenol (2.44%), and carbonyl (56.10%), were determined using GCMS, which indicated that the material did not contain benzo (a)pyrene, leading to its bold utilisation as a food additive. The pyrolysis of water hyacinth also produced similar results to those of coconut shells, as well as palm oil starch and durian wastes (Ratnani *et al.*, 2021). The amount of phenol in liquid smoke was affected by the lignin and pyrolytic temperature in the material. According to several previous studies, the liquid smoke produced by hemicellulose pyrolysis created furfural (a furan derivative) at 200-250°C, as well as a long succession of carboxylic/acetic acids and homologues. At 280-350°C, cellulose pyrolysis also produced acetic acid and its homologues, as well as a tiny quantity of furan and phenol. In addition, the pyrolysis of lignin

created phenols and ethers at 400°C.

### 3.7 Determination of water content

The water content analyses are shown in Table 4, where WC was achieved at a steaming temperature of 76°C and a storage time of 72 hrs. The best water content value was also observed at 10.4%. Based on the study of coconut shells on fish balls, the treatment of liquid smoke obtained poor data, with 74.56 and 74.27% observed before and after the analysis (Zuraida *et al.* 2011). This indicated reduced water content levels in food products treated with liquid smoke. The addition of liquid smoke also caused moisture loss and insolubility in connective tissues, leading to the texture becoming rubbery and dry. Based on BSN (2009), the maximum water content in smoked fish was 60%. However, the smoked eels obtained in this study met the requirements of the SNI, which indicated that the water content of the fresh fish was more than 60%. The WC level of wild eels was also found to be higher than the cultivated types due to the protein content contained in them. This indicated that higher water content led to greater protein composition.

### 3.8 Sensory evaluation

The sensory evaluations of the smoked eels are shown in Table 5, where the panellists highly preferred the appearance, texture, and aroma at a steaming temperature and storage time of 55°C and 72 hrs, respectively. This was because the smoked eels had a whole, clean, and shiny appearance under all the treatment conditions. The aroma was also fragrant and delicious with no foul odours, while the texture was chewy, dry and dense. Moreover, the value of the specifications for sample no. 11 (with 55°C and 72 hrs) became the panellists' choice, which was in line with the sensory test of tilapia meat based on the appearance,

smell, and texture (Ariestya et al., 2016). According to the skipjack tuna, the coconut shell liquid smoke produced a higher score in appearance and aroma compared to that of rice husks. Meanwhile, the taste, texture, and presence of mould did not show any difference. The aggregated score for all treatments on skipjack tuna was observed between 7-9. This was due to the assumption that the formation of the colour originated from the absorption of smoke dyes, oxidation and polymerisation of compounds, as well as the reaction with proteins. Therefore, a condensation reaction occurred between the carbonyl and the amine, causing the appearance of a characteristic smoke colour (Swastawati et al. 2014).

Table 4. The water content of the water hyacinth liquid-smoked eel

No Sample	Temperature, °C	Time, hours	Moisture content, %
1	40	24	11.5
2	70	24	11.4
3	40	120	16.0
4	70	120	13.0
5	34	72	12.5
6	76	72	10.4
7	55	4	16.3
8	55	140	11.3
9	55	72	13.6
10	55	72	14.1
11	55	72	14.4
12	55	72	13.8
13	55	72	14.3

#### 4. Conclusion

The results showed that the analysis carried out was quite encouraging due to the TPC ranging between  $2 \times 10^4$  to  $2 \times 10^5$  CFU/g. A p-value of  $0.157 > (0.05)$  was also

obtained, indicating the acceptance of  $H_0$ , which stated that the regression model with an  $R^2$  value was 87.0%. This showed that temperature and time affected the TPC by 87.0%. Furthermore, the TVB analysis ranged between 2,530-3,998 mg N/100 g at a storage mass of approximately 140 hrs (6 days), indicating that dry water hyacinth liquid smoke was used as a preservative for eels. The regression model (Lack of Fit) also obtained a p-value  $0.000 < (0.05)$ , indicating that temperature and time affected the TVB test gain by 61.6%. The best moisture content and sensory test values were subsequently obtained at 76/55°C and 72 hrs each, respectively. This indicated that the steaming temperature and storage period of water content at 76°C and 72 h still met the standards for smoked fish.

#### Conflict of interest

The authors declare no conflict of interest.

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Table 5. The sensory evaluation of water hyacinth liquid-smoked eel.

No Sample	Steaming temperature, °C	Time, Hours	Specification		
			Appearance	Texture	Odour
1	40	24	7	3	5
2	70	24	7	3	7
3	40	120	9	5	9
4	70	120	7	5	7
5	34	72	9	7	7
6	76	72	5	5	7
7	55	4	7	7	9
8	55	140	9	5	7
9	55	72	9	9	7
10	55	72	9	7	9
11	55	72	9	9	9
12	55	72	9	9	7
13	55	72	9	9	9



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