

Effect of Smoking Materials on Histamine and 5-Hydroxymethyl-2-Furfural in Mackerel (*Scomber scombrus*) Product

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ABSTRACT: Hot-smoking is one of the smoking methods often used to preserve foods by exposing food to smoke from burning or smoldering material such a wood. During the storage of smoked products, measurement of 5-hydroxymethyl-2-Furfural (HMF), an important intermediate, is widely used as an indicator of Maillard reaction. The presence of HMF is responsible for characteristic of color due to greatly influence essential food quality and health effect. Effect of smoking materials and times on histamine formation and HMF content of smoked mackerel was determined. Fillets, which were soaked in 20% (w/w) brine solution for 30 min, were smoked with corn cob and bagasse for 1.5, 2, 2.5 and 3 h. All samples were stored in a sealed polyethylene bag at room temperature for 14 days. Chemical composition of fresh product was primarily analyzed. Moisture, protein and salt contents were 69, 11 and 29% (w/w), respectively. TVB-N and histamine contents were 0.74 and 0.40 mg/100 g, respectively. The pH value was 4.29. Fillets smoked by corn cob for 1.5 and 2 h showed high scores of overall acceptability, which was 7.60 and 7.47, respectively. Histamine and HMF formation was lower in fish smoked with corn cob than in fish smoked with bagasse for 2 h.

Keyword: mackerel; histamine; smoked; corn cob; bagasse

INTRODUCTION

Atlantic mackerel (*Scomber scombrus*) including sea fish (Scombridae) found in the cold sea water of the North Atlantic Ocean are called “SABA” fish in Thailand. Atlantic mackerel are iridescent blue green above with a silvery white underbelly [1]. Histamine, a non-volatile substance of amines, is found in fish with dark meat, especially scombroid. The toxic poisoning from scombroid is called “scombrotxin”. Histamine is present in fresh, canned and cooked products. Fresh products typically have barely detectable levels of histamine. However, the toxin can resist at high temperatures of food processing [2]. The mechanism of histamine formation is caused by bacteria producing decarboxylase enzyme. The decarboxylase pulls carbon dioxide out of

the amino acid Steiner's head by using the decarboxylation [3]. The total volatile basic nitrogen fraction (TVB-N) is often used as a quality parameter in the fish industry to assess spoilage and fishy smell. Marine fish contain small amount of trimethylamine oxide, which can be measured from TVB-N. Pretreatment of headed and gutted fish including salt treatment can keep the quality of fish prior to processing because salt help reduce water activity (a_w) of food resulting in inhibition of microbial growth. This can reduce histamine produced by microorganism [4] and prolong the shelf life of products.

Smoking methods consist of cold- and hot-smoking. Temperature of the smoke is in the range of 12-45 °C during cold-smoking and 40-100 °C during hot smoking. In hot-



smoking, the process may be carried out in different stages. The temperature of products smoked with hot-smoking method reach up to 85 °C. Various pre-treatments prior to smoking, such as salting and drying and/or after treatments, are applied in industries [2,18]. Smoking is often used to preserve foods by exposing it to smoke from smoldering material such a wood. Heating processes can affect the quality of product which leads to consumer dissatisfaction. Maillard reaction or non-enzymatic browning reaction can be a main cause of color change and quality degradation of food products during processing and storage [9,18]. Maillard reaction is strongly dependent on the food matrix composition as well as the technological conditions of the reaction [9]. During the processing and storage of smoked products, measurement of 5-hydroxymethyl-2-Furfural (HMF) or furosine, an important intermediate, is widely used as an indicator of Maillard reaction. The presence of HMF is responsible for characteristic of color due to greatly influence essential food quality and health effect. The objective of the study was to determine the effects of smoking materials and time on the chemical compositions, histamine formation and 5-hydroxymethyl-2-furfural (HMF) content of smoked mackerel.

MATERIALS AND METHODS

Raw materials

Fresh Atlantic mackerels (*Scomber scombrus*) were obtained from local market in Thailand and placed in an ice box with a fish to ice ratio of 1:2 (w/w) (approximate at 0 °C) prior to transporting to the laboratory. Average weight and length of fish obtained was around 0.46-0.61 kg and 28-30 cm, respectively. Sodium chloride (NaCl) and smoking materials (corn cob and bagasse), was supplied from a local market.

Sample preparation

Fish used in this experiment must not be in ice over 4 h of harvesting. Fish were immediately

headed, gutted and filleted without skin removal. After that, the fillets were washed and soaked in 20% (w/v) brine solution for 30 and 60 min. All of samples were smoked using smoking hood (SO10A-OFM, Thailand) with different types of materials (corn cob and bagasse), the temperature of smoke at 80 °C at 0.5, 1, 1.5, 2, 2.5 and 3 h.

Chemical analysis

Chemical composition (moisture, protein, salt, pH, histamine and total volatile base-nitrogen) were analyzed for the initial freshness of samples. Protein and moisture contents were determined according to AOAC, (2000) [5] and pH value was investigated using pH meter (pH-510, Eutech Instruments, Singapore). Salt (NaCl) content in fish muscle was determined using the volumetric method and the salt content was then calculated as percentage of the sample [6].

Histamine determinations

Histamine contents in fish muscle were investigated by the colorimetric method [7] with some modifications. Around 10 g fish muscle was added with 2.5% trichloroacetic acid and transferred into an ion exchange column with weakly acidic cation exchange resin (Amberlite-GC 50). Derivatives of samples were purified with diazo reagent and followed by the measurement of absorbance at 495 nm. The absorbance of sample and standards was measured using UV-visible spectrophotometer (UV-1610 Shimadzu, Japan) with glass cuvettes. Histamine was estimated from the standard curve of absorbance versus concentration of histamine in the range 0-80 µg/ml.

Total volatile base nitrogen

TVB-N assay of fish muscle was analyzed by TCA-extract steam distillation method. [8] The results of TVB-N were then calculated using the formula as follows:

$$\text{TVB-N (mg/100g)} = \frac{(V_s - V_a) \times 14 \times N_{\text{HCl}}}{W_s}$$

which TVB-N of the samples (mg/100 g), V_s is the consumption amount of hydrochloric acid by the titrated boric acid absorbing liquid (mL), V_a is the consumption amount of hydrochloric acid by the titrated blank absorbing liquid (mL), N_{HCl} is the concentration of the hydrochloric acid (mol/L), and $A = 14$ is the mass of the nitrogen amount with 1 mL hydrochloric acid standard titration solution (1 mol/L).

5-Hydroxymethyl-2-Furfural (HMF)

The following assays were performed using the methods as mentioned in Cohen et al. [9], and then 5 ml of 95 % ethyl alcohol was added to 5 g of sample. The mixture was centrifuged at 1000 g for 15 min. 2 ml supernatant of the centrifuged sample was introduced into 16 ml screw cap tube. Two ml of 12% (w/w) trichloroacetic acid (TCA; Sigma, Germany) and 2 ml of 0.025 M thiobabitoric acid (TBA; Carlo Erba, Italy) were subsequently added and mixed thoroughly. The tubes with sample were then placed in the water bath (Memmert Model W 600, Denmark) at 40 °C (± 0.5 °C). After incubating for 50 min, the tubes were cooled immediately using tap water and the absorbency was measured at 443 nm. A calibration curve of HMF (Aldrich, Germany) was utilized to quantify the HMF concentration.

Determination of physical property

Hardness value of samples was measured by a Texture Analyzer (TA.XT2, England) using a cylinder probe with diameter 2 mm (P/2). The condition used for the present study was pretest speed at 3.0 mm/s; speed in the sample at 2.0 mm/s; past test speed at 10.00 mm/s; and distance of 7.0 mm.

Sensory evaluation

Sample was soaked in 20% (w/v) brine solution for 30 min and smoked with smoking materials (corn cob and bagasse), were grilled at 80 °C for 30 min prior to sensory evaluation using 30 semi-trained panelists with a 9-point

hedonic scale (1 = not likely to 9 = very much likely).

Statistical Analyses

Data were analyzed using Analysis of Variance (ANOVA) with three replications following the Complete Randomized Design. The Duncan's multiple range test was further used to determine the difference of means, the relationship and degree of influence of smoking material types and time on other parameters, such as histamine content, TVB-N and HMF content, texture analysis colorimetry and sensory evaluation in Atlantic mackerel (*Scomber scombrus*).

RESULTS AND DISCUSSION

Chemical composition of fresh fillets

Chemical composition of sample fillets (moisture and protein content) including pH value, salt, histamine and TVB-N) is given in Table 1. Histamine content of control referring to initial freshness of fish was 0.40 ± 0.01 mg/100 g. This was consistent with the previous study indicating 0.07-1.24 mg/100g of histamine content in fresh Jack mackerel stored at 0°C [10] However, FDA reported a level of histamine content (less than 8-40 mg/100 g), which was classified in mild poisoning [3]. This study confirmed that the fillet samples obtained were fresh due to low content of the histamine.

Histamine formation at fillets soaked in 20 % brine concentration (w/v) for 30 min was investigated. At 20% (w/v) of brine solution, the activity of bacteria creating the decarboxylase enzyme could be stopped. This was consistent with FAO [3], indicating that high concentration of salt (17.5 - 25% w/v) retarded decarboxylase activity leading to histamine formation. Salt concentration at 17% (w/v) or higher could be used for food application and minimize the histamine formation in seafood products [4]. However, levels of histamine in seafood products were limited at 50 ppm [2].



Total volatile base-nitrogen (TVB-N) is a measure of the total amount of a variety of nitrogen containing substances, which were fishy smell produced by bacteria [10]. The result showed that TVB-N was 3.07 mg/100g in 20% (w/w) salt solution for 30 min resulting in fishy smell. Several authors found that fresh Jack Mackerel had TVB-N value of 19-21 mg/100 g [11].

Physical property of fillets soaked in 20% (w/v) brine solution for 30 min

Physical property of texture analysis indicated that hardness of fillets soaked in 20 % brine concentration (w/v) for 30 min was 0.26 N, which was higher than that of fresh fillets (0.22 N).

Table 1 Chemical composition of fillets Atlantic mackerel (*Scomber scombrus*).

Chemical Component	Quantity
Moisture (%)	68.63±2.70
Protein (%)	10.85±0.57
Salt (%)	28.80±0.10
pH	4.29±0.05
Histamine (mg/sample 100 g)	0.40±0.01
TVB-N (mg/100g)	0.74±0.04

Sodium chloride plays an important role influencing on extract protein gel [12]. Our preliminary study found that the suitable smoking time was 1.5 and 2.0 h as determined from sensory evaluation

(Table 2). The result showed that the fillets smoked for 1.5 and 2.0 h had high scores of overall acceptability, which were 7.60 and 7.47, respectively.

Table 2 Sensory evaluation of fillets soaked in 20% brine solution (w/v) for 30 min and smoked at various times.

Duration time (h)	Color ^{ns}	Flavor ^{ns}	Taste ^{ns}	Texture ^{ns}	Overall liking
0.5	n/a	n/a	n/a	n/a	n/a
1	5.93±1.94	6.07±2.19	6.27±1.79	5.47±2.26	5.60±2.26 ^b
1.5	6.80±2.04	6.53±1.96	6.87±1.81	6.53±1.88	7.60±2.35 ^a
2	6.80±1.42	6.60±1.30	6.27±1.49	6.73±1.62	7.47±1.41 ^a
2.5	5.67±2.13	6.00±1.85	6.00±2.00	5.80±2.27	5.80±1.97 ^b
3	5.87±1.92	6.47±1.96	6.13±1.81	6.00±2.03	5.73±1.44 ^b

Mean ± SD. Values in the same column with different letters are statistically different ($p \leq 0.05$). Sensory evaluation used is a 9 point hedonic scale (1 = not likely to 9 = very much likely).

Effect of smoking materials and time on histamine content during storage

The fillets were smoked with corn cob and bagasse at 80 °C for 1.5 and 2.0 h and then stored in a sealed polyethylene bag for 14 days at room temperature. As compared between fillets smoked with corn cob and bagasse, it indicated that smoking materials and times affected histamine content for 14-day storage (Figure 1). Histamine content of fresh fillets (control) increased dramatically during storage as observed from the deep slope of straight line (slope = 0.5491; $R^2 = 0.9869$), while histamine content of fillets smoked with corn cob for 2.0 h showed the highest efficiency of phenolic antioxidant substances to inhibit histamine formation as observed from a slight increase in straight line during storage (slope = 0.0426; $R^2 = 0.7707$). Higher amounts of histamine content could be reduced in fillets smoked by corn cob than in those smoked by bagasse due to their chemical compositions of smoke consisting of phenol and formaldehyde [13].

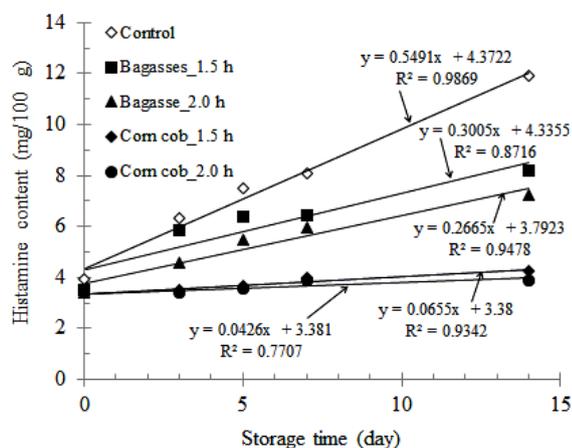


Figure 1 Effect of smoking materials and times on histamine formation during 14-day storage.

These components have high antioxidant capacity, which could inhibit microbial growth, enzyme activity and chemical change in smoked products [14]. Several studies indicated that phenolic compounds obtained from smoke of corn cob were 335 ppm, while

phenolic compounds of smoke obtained from bagasse were only 0.012 ppm [15].

Effect of corn cob and bagasse used for smoking on the HMF content during storage

As non-enzymatic browning is one of the major causes of color change in food products during processing and storage, the effect of smoking types and times on the accumulation of HMF was investigated in the present study. Effect of corn cob and bagasse used for smoking fillets on the HMF content is shown in Figure 2. After smoking with different types of materials (corn cob and bagasse), HMF of fillets smoked with corn cob increased slightly and remained constant as measured from the slope (0.2861; $R^2=0.9524$), while that of fillets smoked with bagasse increased significantly during storage (slope = 0.6003; $R^2 = 0.9883$). This was probably due to the sugar components in bagasse. Sugar cane bagasse typically contains sucrose, glucose and fructose, which are the substrates of the Maillard reaction [15].

However, it should be noted that smoking time could affect HMF content in smoked fillets. After smoking, the HMF content of fillets smoked with corn cob was lower than that of fillets smoked with bagasse. This was probably due to smoking temperature. In hot-smoking process, the temperature of product reached up to 80 °C, where HMF could be formed as a result of non-enzymatic browning such a Maillard reaction in smoked products. As discussed previously, rate of HMF increased with increasing temperature and subsequently increased brown pigment formation [16]. Although the fillets smoked with corn cob and bagasse were stored in a sealed bag at the room temperature, the HMF content increased throughout the storage. This was because the HMF was an intermediate substance of Maillard reaction leading to melanoidins and brown color development [17].



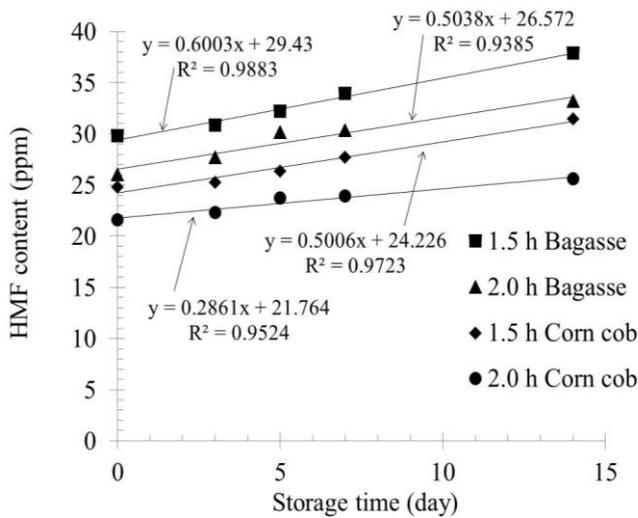


Figure 2 Effect of corn cob and bagasse on HMF content of smoked fillets.

CONCLUSION

The optimal brine concentration at 20% (w/v) for 30 min could stop the histamine outbreak in seafood fillets resulting in consumer safety. The formation of histamine was also significantly affected by the smoking materials and times. The results indicated that smoking time at 1.5 and 2 h showed high scores of overall acceptability for smoked products. After the storage, the fillets smoked with corn cob for 2.0 h showed the highest efficiency to inhibit histamine formation and the HMF content as observed from the changes in contents of histamine and HMF during storage.

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