Optimization of stingray (*Trygon sephen*) smoking to extend shelf life and consumer preferences using response surface methodology

H Suprapto¹²*, S Kumalaningsih¹, W Wignyanto¹, I Santoso¹, and S Sucipto¹³

¹Doctoral Program in Agroindustrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang
²Department of Agricultural Product Technology, Faculty of Agriculture, Mulawarman University, Samarinda
³Halal and Qualified Industry Development (Hal-Q-ID), Faculty of Agricultural Technology, Brawijaya University, Malang

E-mail: supraptohadi74@gmail.com

Abstract. Smoking is one of the preservation processes that are widely used in fishery products. To date, the fish smoking process has not been standardized so that the quality and shelf life of products are varied. Therefore, the optimization of fish smoking is essential. This study aims to determine the optimization of the smoking process of stingrays to obtain smoked stingrays which have a long shelf life and high sensory value that consumers like. This study used the Response Surface Method (RSM) using three independent variables, including the soaking time of stingrays in liquid smoke (2-4 hours), smoking temperature (60-80 °C), and smoking time (3-5 hours). The storage time (days) and consumer preferences (value) were measured in this study. The results showed that stingrays soaked in liquid smoke for 3 hours, heated to 86.8 °C for 3 hours, showed the best results with a shelf life of up to 9 days and most preferred by consumers. This result can be made as a recommendation for the standardization of stingray smoking.

1. Introduction

Fisheries play an important role in supporting the supply of protein for the Indonesian people. However, protein derived from fish for the Indonesian community has been unevenly distributed. Fish preservation is a suitable method to be applied in fisheries products distribution from the production center to other regions. Some ways of preserving fish include salting, drying, freezing, and smoking. This preservation process prevents fish damage and increases the economic value—the fishermen's income and the surrounding community increase. Stingray resources are quite abundant in Indonesia. The catch of stingrays increased every year. Based on statistical data on Indonesian fisheries, in 2014,
the total volume of stingrays captured and landed by fishermen throughout Indonesia reached 35,784 tons [1].

Like other types of fish, stingrays have complete nutritional contents, such as protein, carbohydrates, fats, vitamins, and minerals. After being caught, stingrays easily damaged either chemically or microbiologically if it does not get the appropriate treatment. To extend the shelf life of these fish, modern and traditional methods can be used. Modern preservation methods such as freezing and canning require expensive technology and costs so that they are not affordable for most traditional fishers. Therefore, one of the famous and inexpensive methods of preserving fish is smoking. This method is intended not only for prevention, but that role has now shifted towards the formation of flavour and colour of smoked fish [2].

So far, the fish smoking method can produce attractive colour and flavour characteristics [3,4]. The acceptance of smoked fish is mainly based on sensory attributes than others [5,6]. Several analyses on chemical compositions contained in the smoke have been conducted in several studies [7,8,9 10,11]. Important factors affecting the quality of smoked fish is the temperature and time of smoking. Smoking makes colour changes and appear more attractive to meat but causes a significant decrease in protein [12].

The smoking process can be done directly by burning or heating the smoke source material, which is in conjunction with the heat in direct contact with the fish or by using liquid smoke. The use of liquid smoke has several advantages; namely, its content is clear, easy to apply and control, more uniform, the density can be regulated, and safer [10,13,14]. This condition relates to the quality and shelf life of smoked fish. Therefore, it is essential to have smoking efforts which are simultaneously carried out using appropriate technique.

Because the relationship between smoking conditions that consist of smoke concentration, smoke density, soaking time in the smoke solution, heating rate, and time with the changes that occur are quite complicated, it is necessary to correlate and predict the effective method needed [15,16]. Response Surface Methodology (RSM) is a method that is useful for modeling and analyzing problems in a response that is influenced by the optimizing of several variables to the response. It is necessary to optimize the smoking process simultaneously to extend the shelf life and consumer preferences of the smoked fish product. Thus, this study aims to determine the optimization of the smoking process of stingrays to obtain smoked stingrays, which have a long shelf life and high sensory value and are liked by consumers.

2. Materials and Methods

2.1. Materials
Fresh stingray fish (Trygon shepen) were obtained from Central Fish Auction Center in Brondong Region, Lamongan, East Java province, Indonesia. The fish with a weight of 250 grams, was stored in a cool box with a temperature of 20 °C. Food grade 2 liquid smoke from coconut shell was purchased at Madania market in Bantul, Yogyakarta, Indonesia. All chemicals and microbiological were of analytical grade and obtained from Merck (Germany), Sigma-Aldrich (UK).

2.2. Smoking process
The stingray fish was cleaned, removed of the gut and gill, washed, soaked in 25% of the liquid smoke (2 - 4 hours), drained, then was placed in the smoked chamber. A chamber smoking with dimension 80 cm x 60 cm x 100 cm, adjust smoking temperature (60 – 80 °C) and smoking time (3 – 5 hour) to be considered after the condition in the smoking chamber suitable with temperature treatment, equipped with a thermocouple and automatic timer. The final product was then cooled and placed desiccator for further study.
2.3. **Shelf life assessment**
Shelf life assessment is done by observing the appearance of smoked stingrays every day. Smoked stingrays were placed in an opened container at the laboratory and observed daily to change odour. Smoked stingrays were considered damage if their odour is acid and rancid. In addition, the bacterial content was also tested (the total plate count method). Smoked stingrays were not considered for consumption if off odour and bacterial content more than 5.0 x 10^5 CFU/g, it is referred to [17].

2.4. **Consumer preferences assessment**
As many as 30 consumers were tested for their preferences to smoked stingrays. The assessment method is by determining the quality level based on a scale number 1 as the lowest value and number 9 as the highest value with the use of the scoring sheet. This assessment refers to [18].

2.5. **Experimental design**
A three-level, three variables central composite rotatable design was employed for optimization for three important independent variables the soaking time on the liquid smoke (X1: 2 hours, 3 hours, and 4 hours), smoking temperature (X2: 60 °C, 70 °C, and 80 °C), and smoking time (X3: 3 hours, 4 hours, and 5 hours). Regression analysis was performed on the data of response variables such as the shelf life (Y1) and consumer preferences (Y2).

2.6. **Statistical analysis**
The least-square multiple regression methodology was used to enquire the relationship between the independent and dependent variables.

3. **Results and Discussion**

3.1. **Fitting the response surface models**
The central composite design was used to build a second-order response surface model. According to a design created using Design Expert 7.1.6, as many as 20 experiments were performed in duplicate and the obtained results are shown in Table 1.

3.2. **Shelf life**
Shelf life is a stable condition of food products that remains safe and can maintain specific sensory, chemical, physical, and microbiological properties. Information about storage stability can be used to determine the product shelf life after the process. In general, there are three critical components related to storage stability, namely microbiological changes (especially for products with a short shelf life), chemical and sensory changes [5,19,20]. The effect of several treatments on shelf life is shown in Figure 1.

Figure 1 describes the interactive effects of the independent variables (soaking time, temperature, and heating time) on the shelf life of smoked stingray storage. The longer the soaking time in liquid smoke, the more phenolic compounds are absorbed into the stingray meat, so that the storage stability is longer. The slow oxidation rate during storage may be due to the effectiveness of the antioxidant smoke. The smoking process can inhibit the growth of microbes as a function of phenolic compounds [21].

The longer the smoking time in liquid smoke, the more chemical compounds in liquid smoke stick to the stingray's meat, extending the shelf life of the smoked fish. Coconut shell smoke distillate can preserve food ingredients due to acidic, phenolic, and carbonyl compounds. In general, the chemical composition of coconut shell liquid smoke is phenol, carbonyl, and acid [22]. These compounds are bioactive compounds that can as antioxidative and antimicrobial, so that it can extend shelf life of smoked fish.
Table 1. The central composite design matrix and the experimental data for the responses.

| Treatment No. | Code level X1 | Code level X2 | Code level X3 | X1 (hour) | X2 (°C) | X3 (hour) | Y1 (day) | Y2 |
|---------------|--------------|---------------|--------------|----------|--------|----------|---------|----|
| 1             | -1           | -1            | -1           | 2        | 60     | 3        | 3       | 4.14 |
| 2             | 1            | -1            | -1           | 4        | 60     | 3        | 5       | 4    |
| 3             | -1           | 1             | -1           | 2        | 80     | 3        | 7       | 6    |
| 4             | 1            | 1             | -1           | 4        | 80     | 3        | 6       | 6    |
| 5             | -1           | -1            | 1            | 2        | 60     | 5        | 5       | 5    |
| 6             | 1            | -1            | 1            | 4        | 60     | 5        | 6       | 6    |
| 7             | -1           | 1             | 1            | 2        | 80     | 5        | 8       | 4    |
| 8             | 1            | 1             | 1            | 4        | 80     | 5        | 8       | 3    |
| 9             | -1.68        | 0             | 0            | 1.3      | 70     | 4        | 4       | 5    |
| 10            | 1.68         | 0             | 0            | 4.7      | 70     | 4        | 5       | 5    |
| 11            | 0            | -1.68         | 0            | 3        | 53.2   | 4        | 3       | 2.43 |
| 12            | 0            | 1.68          | 0            | 3        | 86.8   | 4        | 9       | 8.29 |
| 13            | 0            | 0             | -1.68        | 3        | 70     | 2.3      | 6       | 3.86 |
| 14            | 0            | 0             | 1.68         | 3        | 70     | 5.7      | 8       | 5    |
| 15            | 0            | 0             | 0            | 3        | 70     | 4        | 8       | 7.57 |
| 16            | 0            | 0             | 0            | 3        | 70     | 4        | 8       | 8    |
| 17            | 0            | 0             | 0            | 3        | 70     | 4        | 8       | 8.29 |
| 18            | 0            | 0             | 0            | 3        | 70     | 4        | 8       | 7.29 |
| 19            | 0            | 0             | 0            | 3        | 70     | 4        | 9       | 8    |
| 20            | 0            | 0             | 0            | 3        | 70     | 4        | 8       | 8.29 |

The temperature treatment and heating time of stingrays that have been soaked in liquid smoke also significantly affect the shelf life of smoked stingrays that produced. The higher the temperature and the longer the heating time will further extend the shelf life, although several nutritional compounds are lost due to heating. Samples were processed at 10 °C had more microbiological content than those processed at 5 °C, and have acceptability limit until 40 days [23]. Optimization of the combination of liquid smoke, temperature, and time is expected to obtain durable smoked fish products, but not many nutrients are damaged.

3.3. Consumer preferences

The sensory properties consist of scores of colour, aroma, taste, texture, and acceptability of smoked stingrays. The effect of several treatments on consumer preferences of smoked was presented in Figure 2. The results of the overall consumer preferences assessment (the colour, aroma, taste, and texture) of smoked stingrays show that the scores obtained were 2.43 - 8.29. Figure 2 shows that each treatment interaction has a significant effect. The soaking time stingrays in liquid smoke before the smoking process greatly affects consumer preference. Colour can be of great interest to producers or retailers because of the effect on consumer purchases [24].
The interaction of the smoking process that is temperature and time also influences consumer preferences. Short smoking time and low temperature will make the fish texture too soft so that consumers do not like it. Likewise, long heating times and temperatures that are too high will make the fish texture hard. According to Soeparno, that cooking temperature affects the colour of the cooking meat. For example, the interior colour of beef cooked at 60 °C is a bright red colour, at a temperature of 70-80 °C or higher in gray-brown colour [25]. On the other hand, that tenderness was affected by the heating temperature [26,27].

Figure 1. (a) Effect of soaking time on liquid smoke and temperature of smoking to the shelf life; (b) effect of soaking time on liquid smoke and smoking time to the shelf life and (c) effect of temperature of smoking and smoking time to the shelf life.
Figure 2. (a) Effect of soaking time on liquid smoke and temperature of smoking to the consumer preferences; (b) effect of soaking time on liquid smoke and smoking time to consumer preferences and (c) effect of temperature of smoking and smoking time to consumer preferences.

4. Conclusions
There is an effect of several variables of the smoking process (i.e., soaking time in liquid smoke, temperature, and time) on the shelf life and consumer preferences. The treatment with soaking time, heating temperature, and heating time, respectively at 3 hours soaked in liquid smoke, smoked at 86.8 °C for 4 hours is an optimal process. The advantage of this method is the use of a faster time of heating, extend the shelf life, and most preferred by consumers.

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