



## **ANALYSIS OF BENZO( $\alpha$ )PYRENE ON CHICKEN SATE WITH METHODS High Performance Liquid Chromatography (HPLC)**

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### **Abstract**

Satay is a processed food product from the combustion process. The combustion process can produce carcinogenic compounds. The carcinogenic compound is Polycyclic Aromatic Hydrocarbons (PAH). PAH is a group of compounds that can be formed due to incomplete combustion of the reaction between meat fat dripping over organic and inorganic substances such as charcoal, oil and gas. Benzo( $\alpha$ )pyrene is a member of the five-ringed PAH which is a group of organic compounds that are mutagenic and carcinogenic. the combustion process will produce smoke with high benzo( $\alpha$ )pyrene contaminants. HPLC was chosen to carry out an analysis of benzo( $\alpha$ )pyrene because it has many advantages including the simple and sensitive way it works.

This study aims to analyze the levels of benzo( $\alpha$ )pyrene in satay. The type of research used is experimental laboratory, many samples used were 8 samples, namely 1 sample in the form of raw meat as a control material and 7 samples were burned for 5 minutes.

Keywords: Chicken satay, PAH, Benzo( $\alpha$ )pyrene, HPLC.

### **1. INTRODUCTION**

Chicken is the most favorite food in the world, chicken is liked by the people because it is an easy way to process it, besides chicken can also be made as an appetizer, main dish and closing. Chicken can be added various kinds of ingredients such as vegetables, fruits, and seeds. How to cook it can be fried, baked, steamed, boiled <sup>(7)</sup>. One of the results of processed chicken, namely chicken satay is chicken produced through the combustion process, wherein the combustion process produces cancerous carcinogenic compounds. These carcinogenic compounds are Polycyclic Aromatic Hydrocarbons (PAH) <sup>(2)</sup>. PAH is a group of compounds that can be formed due to incomplete combustion of the reaction between meat fat dripping over organic and inorganic substances such as charcoal, oil and gas, then the results of this reaction are smoke that will stick to the meat <sup>(15)</sup>.

The Environmental Protection Agency (EPA) determined 15 types of dangerous PAHs from 100 known types of PAH, one of which was benzo( $\alpha$ )pyrene <sup>(2)</sup>. Lukitaningsih and Sudarmanto <sup>(9)</sup> say that, PAH compounds have the potential to spread widely through food and these compounds are carcinogenic. According to Darmadji (2009), the combustion process will produce smoke with high benzo( $\alpha$ )pyrene contamination. Benzo( $\alpha$ )pyrene is a member of the five ringed PAH which is a group of organic compounds that are mutagenic and carcinogenic, and are products of incomplete combustion at temperatures of 300 ° C and 600 ° C <sup>(11)</sup>.

According to the research of Marlina, et al. <sup>(10)</sup>, the level of samples of grilled fish that were burned directly using charcoal without wrapping beforehand was a sample which had the highest benzo ( $\alpha$ ) pyrene content of 10.30 mg / kg.

From the problems, it is expected that this program can examine the analysis of benzo ( $\alpha$ ) levels in chicken satay using the High Performance Liquid Chromatography (HPLC) method, to monitor or prevent cancer due to exposure to benzo( $\alpha$ )pyrene.

## 2. RESEARCH METHODOLOGY

### a. Research methods

The type of research conducted is laboratory experimental research supported by literature. The design of this study is the variation in the time of burning chicken satay (3 minutes, 5 minutes, 8 minutes) by examining an object by collecting data, analyzing data, and interpreting the results to obtain information to draw conclusions and decision making.

### b. Time and Place

The location of the study was conducted at the SIG Laboratory or PT. Saraswanti Indo Genetech. Research time in March until April 2019.

### c. Tools and Materials

The instruments used in this study were a set of HPLC instruments, glassware, analytical scales, evaporators, microfilterers. The materials used in this study were broiler chicken, chicken satay, standard benzo ( $\alpha$ ) pyrene, 60% acetonitrile, hexane, 0.4 M NaOH.

### d. Procedure

Skinless chicken part of the chest is cut 100 grams per sample. Then put it on a skewer for 20 grams / stick after that all samples are dipped in spices (sweet soy sauce and oil) for 5 minutes. And burned on wood charcoal with a burning time of 3 minutes, 5 minutes and 8 minutes. For control samples of raw chicken meat the chest is weighed 100 grams.

A total of 100  $\mu$ L standard benzo ( $\alpha$ ) piren 100  $\mu$ g / mL were pipetted, then put into an amber vial and then added 900  $\mu$ L of acetitrile 60% (benzo ( $\alpha$ ) concentration of 10  $\mu$ g / mL). Then take 10  $\mu$ L of the standard benzo ( $\alpha$ ) piren 10  $\mu$ g / mL and then insert it into the amber vial add 990  $\mu$ L of 60% acetonitrile and homogenize (benzo ( $\alpha$ ) concentration of 10  $\mu$ g / L). From the solution that has been made, a standard series of benzo ( $\alpha$ ) piren is made with a concentration of 1-30  $\mu$ g / L as many as five points and inject it into the flouresance HPLC set.

As much as 5 grams of the sample were weighed and put into a 50 ml tube adding 10 mL of 0.4 M NaOH and ethanol: H<sub>2</sub>O (9: 1). Homogenize the solution and warm for 30 minutes at 600C. Then the solution is extracted with a 10 mL hexane solution twice. Steam hexane using the evaporator. Then add 1 mL acetonitrile and strain with a 0.45  $\mu$ m membrane filter.

Put the sample into the amber vial and then inject it into the fluorescent detector HPLC. The columns used were inertsil ODS-3 C18 (150mm x 4.5 mm) mobile phase and the flow rate was acetonitrile 80% and 1.2 mL / minute with excitation wavelengths and emissions were 280 nm and 410 nm.

## 3. RESULT AND DISCUSSION

Tabel 3.1 Standart of Benzo( $\alpha$ )pyrene

No	Concentration ( $\mu$ g/L)	Retention time (minute)	Wide Area (mV)
1.	0,1	5,041	159566
2.	1	5,020	980691
3.	5	5,009	3932041
4.	15	4,998	11998681
5.	30	4,989	24468180
<i>Slope (a)</i>			848200,57
<i>Intercept (b)</i>			999,99
R			0,9999

Linearity is used to obtain test results that are proportional to the concentration of analytes. This equation must meet the requirements, namely the correlation coefficient (r) can be accepted if  $r \geq 0.9999$  <sup>(4)</sup>. For intercept (a) is the intersection point between a line with the Y axis in the diagram whereas, slope is the slope size on a line whose value shows how the contribution given variable X to variable Y <sup>(4)</sup>. The line equation formed on the standard benzo(α)pyrene curve is  $y = 848200,57x + 999.99$ , with  $r = 0,9999$  because  $r \geq 0,9999$  then the result of the equation is accepted. This equation will then be used to determine the level of benzo(α)pyrene in the sample.

Tabel 3.2 Level Benzo(α)pyrene On the Sample

Sample	RT (minute)	Wide Area	Level
Kontrol	4,981	-	-
Sampel 1	5,129	213.050,1325	0,05
Sampel 2	5,129	255.460,161	0,06
Sampel 3	4,455	509.920,332	0,12
Sampel 4	4,981	552.330,1325	0,13
Sampel 5	5,129	804.790,5515	0,19
Sampel 6	5,129	764.380,503	0,18
Sampel 7	4,455	213.050,1325	0,05

In this study the results of combustion of control chicken satay did not detect benzo(α)pyrene levels, this is because the control used is raw chicken meat, so benzo(α)pyrene will not be formed because there is no high temperature combustion process. This is in line with the research conducted by Ahmed <sup>(6)</sup> which states that benzo(α)pyrene can be in meat when cooked at high temperatures so that it will accumulate in the lipid component due to its lipophilic properties. 7 samples were burned 5 minutes after analysis using HPLC, the results were obtained that each sample contained benzo(α)pyrene but, the results obtained from all existing samples, did not exceed the standard levels of benzo(α)pyrene set by BPOM which is 10 ppb. This shows that the sample is safe for consumption.

The results of benzo(α)pyrene levels in the sample can be affected by cooking time. This is because when the time is longer, the temperature will increase and the fat pyrolysis process will occur longer. Fat pyrolysis itself is a reaction between the fat of chicken meat that drips on the charcoal which will then evaporate and stick to the meat so that it will affect the increase in levels of benzo(α)pyrene <sup>(15)</sup>.

Samples are burned using wood charcoal. Charcoal is the result of incomplete combustion so that it contains PAH compounds. The use of wood charcoal can increase the level of benzo(α)pyrene this because charcoal is the result of wood pyrolysis. Wood itself contains lignin with the composition of propane guaiakol and syringyl propane which in the event of pyrolysis will produce a mixture of complex phenol compounds, PAHs and carbonyl compounds <sup>(14)</sup>. In a study conducted by Adiyastiti <sup>(15)</sup>, states the production of PAH in cooked meat using charcoal is affected by the concentration of fat and the proximity of the meat to the heat source

From the levels of benzo(α)pyrene, it can be analyzed that in the sample there are levels of benzo(α)pyrene. This is consistent with the research of Adiyastiti <sup>(15)</sup> which states the formation of benzo(α)pyrene can be influenced by the type of fuel used, fat pyrolysis and combustion temperature and also influenced by the method used to cook meat and the extent to which meat is cooked.

Another factor that affects the increase in levels of benzo( $\alpha$ )pyrene is the type of fuel used. Research conducted by Muyela<sup>(3)</sup> which states the concentration of benzo( $\alpha$ )pyrene in cooked meat using charcoal is much higher than that cooked with gas, namely the results of benzo( $\alpha$ )pyrene 24  $\mu\text{g} / \text{kg}$  cooked meat using charcoal and 5 , 7  $\mu\text{g} / \text{kg}$  of cooked meat using gas fuel. The use of gas fuel is safer because fire from gas will produce carbon dioxide and carbon less than charcoal fuel<sup>(15)</sup>.

If the processed food is consumed continuously it will cause DNA replication errors, so that DNA will experience mutations that can cause cancer<sup>(6)</sup>.

#### **4. CONCLUSION**

There are levels of benzo ( $\alpha$ ) pyrene in 7 samples, but the levels are still within safe limits for consumption.

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