Occurrence of polycyclic aromatic hydrocarbons (PAHs) carcinogen in Indonesian commercial goat satay

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Abstract. This study aimed to investigate the carcinogen content of polycyclic aromatic hydrocarbons (PAHs) in commercial charcoal grilled goat satay. Twenty-one portions of commercial raw goat satay and charcoal grilled goat satay samples were obtained from 4 restaurants which are the largest, well-known, and 15-55 years old goat satay restaurant in Batu Municipality, East Java. Sampling of 2 goat satay types (goat satay without sliced fat and goat satay with sliced fat) was carried out 3 replications in 3 consecutive weekends where many tourists visited. The results showed that the temperature of wood charcoal for grilling commercial raw goat satay in various satay restaurants ranged 486.833-548.300 °C with a grilling duration of 2-5.5 minutes and grilling distance of 0.5-2 cm. The weight of raw goat satay for a serving (10 skewers) ranged 144.000-194.000 g and after grilling the weight ranged 97.333-148.000 g. Total natural antioxidant activity (Dipheny-picrylhydrazyl, DPPH), fat content and moisture content of raw goat satay with sliced fat and without sliced fat reached up to 36.661 and 34.124%; 13.440-26.100% and 1.5-6.305%; 57.940-66.385% and 71.530-76.810%, respectively. Benzo(a)pyrene (BaP), the only type of PAHs that is carcinogenic, for charcoal grilled goat satay with sliced fat and without sliced fat in various satay restaurants, ranged from not detected to 6.330 mg/kg and not detected to 0.773 mg/kg, respectively. The levels of BaP in charcoal grilled goat satay in several satay restaurants exceeded the safety limit by National Agency of Drug and Food Control (NA-DFC) of Republic Indonesia. Benzo(a)anthracene (BaA) and pyrene (Pyr) were detected in all grilled goat satay samples in various satay restaurants. Whereas, phenanthrene (Phe), acenaphthene (Acp) and naphthalene (Nap) were not detected in all grilled goat satay samples in various satay restaurants. The conclusion of our study was that the commercial charcoal grilled goat satay that are sold in Batu municipality contains PAH, especially the carcinogenic B(a)P. Therefore practical mitigation efforts are needed for producers to reduce and prevent the formation of carcinogenic B(a)P in commercial charcoal grilled goat satay.

Keywords: polycyclic aromatic hydrocarbons, benzo(a)pyrene, goat satay, satay restaurants, carcinogen
1. Introduction

The attention of consumers who are aware of food safety and health will eventually focus on natural, organic, safer and healthier foods [1]. Along with the increase in consumer welfare and education level, it is expected that consumers will prefer to buy healthier and safer food. Included in consumer decisions for buying goat satay. Exposure to PAHs (polycyclic aromatic hydrocarbons) in foods including processed meats causes the majority of cancers in humans based on a number of epidemiological studies in recent years [2]. In Indonesia, satay is a dish inherited from our ancestors and one of the delicious national dishes, where every state guest of the country who comes to the national palace will always be treated to satay. Sate has become a food treat for all people in society and can be easily made or purchased because besides being cheap, there are also many satay stalls and itinerant satay sellers in Indonesia. Sate is also still a sign of the luxury of a party dish by Indonesian people.

Behind the large number of consumers and producers or sellers of satay in Indonesia, it turns out that not much research has been reported on satay in Indonesia. Especially research on the safety and health of commercial satay consumed by consumers. The grilling process of satay turns out to be able to give rise to several carcinogenic compounds, including PAHs that are harmful to health if consumed in the long term. PAHs is formed in food, especially in meat and fish grilled over smoky and open charcoals. PAHs are deposited on the surface of grilled meat or fish due to pyrolysis or incomplete combustion of drippings from inside the meat or fish that come into direct contact with flame [3]. PAHs are also deposited on the surface of grilled meat even though they are undirect contact with the flame because the fat that drips and undergoes pyrolysis will produce PAHs that can contaminate grilled meat. The formation of PAHs when grilling using wood charcoal is proven to depend on the meat fat level, grilling time and grilling temperature [4]. Incomplete combustion of carbon-containing materials (oil, wood, garbage or charcoal) are the main cause of PAHs formation [5]. The properties of PAHs are nonpolar, lipophilic, formed from the pyrolysis (above 200 °C) of organic matter, especially fat [6, 7]. The reactions involved in the PAHs formation process are radical reactions of oxidation and decomposition [8]. The exact mechanism of PAH formation in grilled meat is not well understood. They argue that when meat is in direct contact with flame, there will be pyrolysis of fat from the meat and PAHs will form on the surface of the meat [9]. When grilling, the fat in the meat dripping onto the charcoal can produce heavy PAHs with an N$_3$ aromatic ring. Charcoal grilling is a method of intense heat cooking that produces PAHs in the meat [10]. Charcoal is carbon residue from wood and plant material which is burned or carbonated at a temperature of around 600-800°C for 4–5 days, and then the furnace is closed until the burning stops. Charcoal is flammable and is widely used as a heat source for grilled food and barbecues [9].

PAHs exposure can cause breast, lung and stomach cancers in humans [7]. The World Health Organization (WHO) and The International Agency for Research on Cancer (IARC) have categorized one type of PAHs, benzo(a)pyrene (BaP) as a carcinogenic [11,12]. Since 2018, the National Agency of Drug and Food Control (NA-DFC), Republic of Indonesia has set a maximum BaP limit of 5.0 µg/kg or ppb for heat treated meat and heat treated meat products [13]. Since 1984, the US Environmental Protection Agency (US-EPA) has determined 16 major PAHs that are frequently found in monitored environmental samples and have high mutagenicity and genotoxicity in humans. The sixteen PAHs are: naphthalene (Nap), acenaphthene (Acp), acenaphthylene (Acy), fluorine (Flr), anthracene (Ant), phenanthrene (Phe), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k] fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), benzo[g,h,i]perylene (BgP), and indeno[1,2,3-cd]pyrene (IcP) [14].

The carcinogenic process of PAHs begins when PAHs are ingested and subsequently undergoes enzymatic metabolism to produce reactive species capable of covalently bonding to DNA (eg. diol epoxides). Eventually the DNA will be damaged in its replication, mutation and tumorgenesis [6, 15, 16, 17]. Abrogation of protein synthesis in humans consuming PAHs occurs due to the epoxide metabolite (7,8-diol-9,10-epoxide benzo(a)pyrene) of BaP covalently bound to N-amino guanine or a phosphatase group from nucleic acid to change the structure of DNA and RNA. If the body is unable to provide feedback to normalize again, cell mutagenesis will occur and can cause cancer [18].
component could have genotoxic effects and increase the risk of colorectal cancer. Taking into account the risks associated with the consumption of PAHs, it is important to investigate mitigation methods that limit their formation and exposure [19].

The aim of this study was to investigate the content of 6 types of PAHs in commercial goat satay grilled with wood charcoal. Apart from that, it is also trying to investigate the precursors that cause the emergence of PAHs in commercial goat satay.

2. Methods

2.1. Study locations and respondents
This study used a survey method at 4 restaurants which are the largest, well-known, and 15-55 years old goat satay restaurants in Batu Municipality, East Java, based on the report of Rosyidi et al. [20]. Sampling was done 3 times in each peak season, namely during the weekend holidays where many tourists visit to Batu Municipality. This municipality is one of the most popular tourist cities in East Java. The Batu Municipality location, which is on the slopes of Panderman and Arjuna Mounts, makes this city the most popular tourist destination. Throughout 2019, the number of tourists visiting and vacationing in Batu Municipality reached 6,047,460 people [21].

2.2. Interview and Sampling
Samples were taken in the form of raw goat meat as the main raw material and ready-to-eat commercial goat satay. During sampling, observation and recording of the charcoal temperature, the grilling time, the meat weight for a portion of goat satay, the weight of post-grill goat satay, the size of each goat meat dice, the grilling equipment, the marination spices used before grilling, the turning process during grilling satay meat, the fan process used an electric fan or a hand fan, and a complementary ingredient for the satay dish were also carried out. In addition, interviews were also conducted with the owner of the satay restaurants regarding the history, the price/species/age of the goat, the amount of income on weekdays and weekends, the number of employees and the price of a serving of goat satay.

Raw goat satay and commercial charcoal grilled goat satay samples from each restaurant and each sampling replicate were tightly wrapped in polyethylene (PE) plastic and stored in tightly closed aluminum foil containers to minimize oxidation and dehydration. Furthermore, the samples were stored in an ice bag during the trip and when arrived in the laboratory, each sample was immediately homogenized by a blender (Philips) until it was smooth and even. The samples were repackaged and labeled in polyethylene (PE) plastic and contained in a tightly closed container lined with aluminum foil and stored in a freezer at -23°C until chemical analysis was carried out.

2.3. Chemical analysis of raw goat satay
The raw goat satay sample was prepared for chemical analysis as follows: crude fat content was determined by Soxhlet analysis, crude protein content was determined by Kjedahl analysis, total organic matter, moisture and ash content were determined by gravimetric method. The carbohydrate content of raw goat meat is determined by the formula 100% - (ash + moisture + fat + protein). Fat oxidation in raw meat was confirmed by the tio barbituric acid (TBA) method. The antioxidant activity was determined by the free radical scavenging method using diphenylpicril hydrazil (DPPH) radicals.

2.4. Analysis of PAHs in charcoal grilled goat satay
Commercial goat satay samples were prepared for analysis of 6 types of PAHs using gas chromatography (GC). Analysis of 6 types of PAHs were determined using the slightly modified Chen and Chen method [22]. A total of ± 5 g of sample was added with 0.5N NaOH as much as 10 ml. It was heated in a waterbath at a temperature of 80°C for ± 3 hours. It was cooled, added 10 ml of distilled water, then extracted with 20 ml of diethyl ether: petroleum ether (1: 1). The top layer is taken, then concentrated in a waterbath with a temperature of 50°C. One milliliter of dichloromethane was added and injected as much as 1 µL of the sample and the standard on GC-2010 Shimadzu. The PAHs standard
used was a mixture of 6 types of PAHs standards, namely naphthalene, acenaphthene, phenanthrene, pyrene, benzo(a)anthracene and benzo(a)pyrene with serial concentrations.

2.5. Data analysis
Statistical analysis was performed for all measurement data using the SPSS procedures (version 25.0) using descriptive statistics. This method presents data in charts, graphs, measures of central tendency of data and distribution data.

3. Results and discussion

3.1. Weight of raw goat satay, grilling temperature and time, and cooking loss of goat satay
As the data presented in Table 1, commercial goat satay producers or restaurants used fresh goat meat and fat cuts around 144-194 g and 146,333-165,667 g for a serving of goat satay (10 skewers) with sliced fat and without sliced fat, respectively. When the survey was conducted in early 2021, the price of goat carcass that was bought by producers from local butchers was around IDR 100,000-IDR 130,000 per kg or an average of IDR 111,250 per kg. So, the capital was only for a serving of goat satay for goat satay (10 skewers) with sliced fat and without sliced fat ranged IDR 16,020-IDR 21,582.5 and IDR 16,279.546-IDR 18,430.454, respectively. The selling price for a serving of goat satay with sliced fat and without sliced fat ranged from IDR 30,000-IDR 45,000 and IDR 40,000-IDR 55,000, respectively. The goat satay producers preferred goat to lamb and were under a year old or all of their teeth are milk teeth. The goat satay producers also preferred female goats if there was stock at regular local butchers. This was because based on their years of experience that female goats have less fat than male goats or sheep. Rosyidi et al. had also reported so [20]. Goat meat has a characteristic tend to be dark red in color, rough texture, flavor and aroma different from lamb [23]. People in Asian countries and developing countries consume a lot of goat meat [24].

A portion of post-grilled goat satay (10 skewers) weighs for goat satay with sliced fat and without sliced fat ranged 97.333-148 g and 104.333-137.667 g, respectively. Goat satay with sliced fat tended to experience a higher cooking loss than goat satay without sliced fat. This was because the sliced fat experience dripping when grilling. The cooking loss for a portion of goat satay with sliced fat and without sliced fat ranged 12.736-39.292% and 5.922-36.382%, respectively. Complete data is presented in Table 1.

The temperature of wood charcoal for grilling the raw meat satay tended to have no significant differences between non-fat and with fat satay in various satay restaurants. The temperature of wood charcoal ranged 486.833-548.300 °C and the distance between charcoal and meat ranged 0.5-2 cm. Satay restaurants that used electric fans and hand fans tended to have a wood charcoal temperature that was not significantly different from satay restaurants that only used hand fans. The grilling time both the raw goat satay without and with sliced fat in various satay restaurants ranged from 2.5-5.5 minutes. The grilling duration of the satay depended on the amount of wood charcoal used, the speed and frequency of the hand fan. The degree of doneness for the goat satay without and with sliced fat fat in various satay restaurants was very welldone, dry and slightly burnt. This was in accordance with the consumers preferences who prefer very welldone for the degree of doneness of satay. An indication of welldone satay according to satay traders was that there has been a darker color change or a dark blackish brown color on the surface of the meat. Medium for the degree of doneness satay was slightly brown and the inside was still red. Its texture was not too dry, the outside and the inside of the meat was brown. Very welldone satay was blackish brown with a dry texture [25]. The average thickness of the raw satay meat was 1.59 cm and the coal spacing was 4.23 cm. The mean internal temperature of medium and welldone satay was 61.89 °C and 73.31 °C, respectively with the mean times of 2 minutes 43 seconds and 5 minutes (300.4 seconds), respectively [26]. Medium, welldone and very welldone goat satay had an internal temperature of 62.07; 73.30; and 85.17 °C, respectively with grilling time of 3, 5 and 7 minutes, respectively.
| Variables of study                        | Unit | Raw and charcoal grilled goat satay with sliced fat | Raw and charcoal grilled goat satay without sliced fat |
|-----------------------------------------|------|--------------------------------------------------|------------------------------------------------------|
|                                        |      | RM. Mesir | Sate Hoplate | Sate Pak Djumari | RM. Cairo | RM. Mesir | Sate Hoplate | Sate Pak Djumari | RM. Cairo |
| **Raw goat satay**                      |      |           |             |                 |           |           |             |                 |           |
| Meat weight*                            | g    | 160.33 ± 0.577 | 194.000 ± 1.000 | 191.667 ± 1.528 | 144.000 ± 2.646 | 164.000 ± 1.000 | 165.667 ± 0.577 | 146.333 ± 0.577 |
| Antioxidant activity (DPPH)             | %    | 18.985 ± 0.164 | 36.661 ± 0.164 | 28.805 ± 0.164 | 22.750 ± 0.164 | 31.424 ± 0.164 | 34.124 ± 0.082 | 14.567 ± 4.418 |
| Levels of malondialdehyde                | mg/kg | 0.593 ± 0.016 | 2.525 ± 0.004 | 0.008 ± 0.000 | 0.737 ± 0.012 | 1.256 ± 0.016 | 0.402 ± 0.064 | 1.315 ± 0.042 |
| Total organic matter                     | %    | 46.770 ± 0.065 | 53.836 ± 0.536 | 52.250 ± 0.581 | 40.420 ± 0.246 | 33.320 ± 0.126 | 38.410 ± 0.572 | 52.680 ± 0.574 |
| Ash                                     | %    | 0.920 ± 0.000 | 1.205 ± 0.355 | 1.075 ± 0.125 | 0.510 ± 0.490 | 0.105 ± 0.045 | 0.915 ± 0.054 | 1.065 ± 0.025 |
| Moisture                                | %    | 64.165 ± 0.055 | 63.850 ± 0.530 | 57.940 ± 0.090 | 66.385 ± 0.205 | 76.810 ± 0.420 | 75.680 ± 0.100 | 71.530 ± 0.340 |
| Fat                                     | %    | 18.360 ± 0.550 | 14.990 ± 0.080 | 26.100 ± 0.060 | 11.440 ± 0.240 | 1.500 ± 0.240 | 2.260 ± 0.190 | 6.305 ± 0.275 |
| Protein                                 | %    | 15.907 ± 0.321 | 18.830 ± 0.140 | 14.240 ± 0.210 | 18.275 ± 0.635 | 18.995 ± 0.185 | 20.050 ± 0.190 | 20.145 ± 0.145 |
| Carbohydrate                            | %    | 0.648 ± 0.458 | 1.125 ± 0.625 | 0.645 ± 0.485 | 1.390 ± 1.120 | 1.620 ± 0.040 | 0.915 ± 0.285 | 0.955 ± 0.735 |
| Charcoal grilled goat satay             |      |           |             |                 |           |           |             |                 |           |
| Charcoal temperature                    | °C   | 532.700 ± 17.730 | 486.833 ± 17.500 | 533.667 ± 6.665 | 512.933 ± 4.524 | 548.300 ± 40.493 | 523.867 ± 6.576 | 512.067 ± 6.529 |
| Grilled satay weight*                   | g    | 97.333 ± 0.577 | 145.667 ± 0.577 | 148.000 ± 1.000 | 125.667 ± 2.887 | 104.333 ± 0.577 | 134.333 ± 0.577 | 137.667 ± 0.577 |
| Cooking loss                            | %    | 39.292 ± 0.506 | 24.912 ± 0.661 | 22.782 ± 0.143 | 12.736 ± 0.524 | 36.382 ± 0.195 | 18.913 ± 0.320 | 5.922 ± 0.284 |
| Coagulability                           | %    | 60.708 ± 0.506 | 75.088 ± 0.661 | 77.218 ± 0.143 | 87.264 ± 0.524 | 63.618 ± 0.195 | 81.087 ± 0.320 | 94.078 ± 0.284 |
| Levels of 6 types of PAHs of grilled goat satay |    |           |             |                 |           |           |             |                 |           |
| Benzo(a)pyrene (BaP)                    | mg/kg | 0.387 ± 0.008 | nd           | nd               | 6.330 ± 0.006 | nd           | 0.773 ± 0.009 | nd               |
| Benzo(a)anthracene (BaA)                | mg/kg | 2.238 ± 0.012 | 3.151 ± 0.008 | 14.204 ± 0.009 | 17.770 ± 0.007 | 7.196 ± 0.068 | 2.139 ± 0.011 | 5.014 ± 0.003 |
| Pyrene (Pyr)                            | mg/kg | 8.316 ± 0.009 | 356.930 ± 0.012 | 368.970 ± 0.008 | 438.670 ± 0.006 | 1.062 ± 0.026 | 490.240 ± 0.007 | nd               |
| Phenanthrene (Phe)                      | mg/kg | nd           | 1.297 ± 0.007 | nd               | nd           | nd           | nd           | nd               |
| Acenaphthene (Acp)                      | mg/kg | nd           | nd           | nd               | nd           | nd           | nd           | nd               |
| Naphthalene (Nap)                       | mg/kg | nd           | nd           | nd               | nd           | nd           | nd           | nd               |

*:* Means within a row with different superscript differ significantly at p<0.05.; nd: not detected; DPPH: diphenyl-picrylhydrazil; *: 10 skewers per serving
The color of the meat on brown and slightly black, respectively. Color is produced due to a chemical reaction between phenol and O$_2$ and between protein and carbonyl in grilled food. The higher the phenol and carbonyl levels in the flame, the meat will be more golden or brownish [25].

The temperature of the charcoal in various satay restaurants ranged 486.833-548.300 °C and the distance between the flame and meat ranged 0.5-2 cm were the main precursors for the formation of PAHs in goat satay. Direct contact between beef satay and open flame during gas grilling encourages the formation of PAHs [27]. The PAHs compounds are produced through the pyrolysis process (incomplete combustion) as long as the meat is grilled by charcoal and when the fat from the meat drips onto the flame, it will produce significant levels of PAHs during direct heating of the meat with charcoal. PAHs production is influenced by the concentration of fat in the meat and the proximity of the food to the heat source [28]. The PAHs and HCA (heterocyclic aromatic amines) concentrations were significantly lower in beef satay grilled with a gas grill at the lowest temperature (150 °C) than at higher temperatures (200, 250, 300 and 350 °C). The PAHs and HCA formation increases with increasing grilling temperature. BaP levels increase at grilling temperatures of 300 and 350 °C and levels exceed the maximum limits required by the Commission Regulation (EU) 2015/1125. Grilling meat at high temperatures significantly causes the formation of PAHs and HCAs [29]. The exact mechanism of PAHs formation is still not well understood. The current opinion is that PAHs are also formed through free radical reactions, cyclization and intramolecular addition of small molecules (pyrosynthesis). Pyrolysis that occurs when meat is grilled at high temperatures results in the condensation of smaller organic compounds containing nitrogen (amino acids and proteins) to form PAHs which generally involve a Diels Alder-type rearrangement reaction [30]. The mixture of carcinogenic compounds PAHs and HCA that is formed when grilling satay at high temperatures is very worrying and poses a risk to consumer health [31, 32].

### 3.2. Fat content, lipid oxidation value and antioxidant activity of raw goat satay

The fat content of goat meat, which is the main raw material for goat satay, was certainly higher in goat satay with sliced fat than without sliced fat. Table 1 presents data for the fat content of goat meat for goat satay with sliced fat and without sliced fat ranged 13.440-26.100% and 1.5-6.305%, respectively. As a component of muscle membranes or adipose tissue, fat in muscle is stored as triacylglycerol located between muscle fibers [33]. The fatty acid composition of lamb and goat meat is similar. Of the total fatty acids, 45% are MUFA, monounsaturated fatty acids and 10% are PUFA, polyunsaturated fatty acids [34]. The levels of saturated fatty acids (SFA), MUFA, PUFA and total omega in lamb and mutton per 100 g were 1.730 g and 1.464 g; 2.066 g and 1.413 g; 0.603 g and 0.673 g; 0.157 g and 0.224 g, respectively. Total levels of omega 3 in beef and lamb are more than in chicken or pork [35]. The meat contains pro-oxidants that are susceptible to oxidation, such as PUFA, cholesterol, protein and pigments [36, 37]. The higher amounts of unsaturated fatty acids lead to meat oxidative processes [33]. The fat melting point is determined by the presence or absence of double bonds and the chain length of the constituent fatty acids. The melting point of SFA or long chain fatty acids is higher than that of unsaturated fatty acids or short chain fatty acids [38].

Fat is more associated with the formation of PAHs because the lipophilic component is the main precursor for PAHs. Through lipid oxidation, the fat in beef satay reacts with protein and other amino compounds to form a brown substance. The color is similar to the melanoidin produced in the reaction of Maillard [27]. The PAHs levels are higher in grilled meat at high temperatures due to the pyrolysis of fat [39]. The smoke formed during grilling carries PAHs from the pyrolysis of fat and contaminates the surface of the satay [40]. During grilling, the PAHs deposition together with the smoke arose which enveloped the beef satay [41].

The transformation of oxidants and reducing agents in the oxidative process is caused by the transfer of one or more electrons from reductant, an electron donor to oxidant, an electron acceptor. Pathological diseases in humans and undesirable changes in food systems are significantly associated with oxidative reactions involving reactive oxygen species (ROS) and reactive nitrogen species (RNS) [42]. Synthetic
or natural antioxidants are added in the meat product formulations to prevent or delay the oxidation reaction so that their shelf life is longer [43].

The thiobarbituric acid reactive substances (TBARS) method was used to determine the presence of fat oxidation in raw goat satay and is expressed as the amount of malondialdehyde (MDA). The TBARS are a compound that can react colorimetrically by spectrophotometry with abundant MDA in the final stage of fat oxidation [44]. The MDA is widely used to estimate lipid peroxidation in membrane and biological systems. The loss of nutritional value and safety of meat and meat products is mostly due to lipid peroxidation [45]. The lipid oxidation value in the TBARS method tends to be too high because TBARS does not only react with MDA but with other oxidized molecules [44]. The other oxidized molecules are volatile compounds of secondary oxidation products such as propanal, hexanal and pentanal which are responsible for off-odor and off-flavor [43, 44, 46]. The aldehydes, ketones, alkanes and others are stable secondary oxidation products formed in the termination phase which are responsible for off-flavor and rancid odour [33]. Lipid peroxidation and chemical spoilage of muscle cause the MDA in cooked goat meat to be higher than raw [47].

As the data presented in Table 1, the TBA value was detected in all raw goat meat samples. It indicated lipid oxidation in all goat meat samples prior to grilling. The TBA values of raw goat satay with sliced fat and without sliced fat ranged 0.008-0.737 mg malondialdehyde/kg and 0.402-1.315 mg malondialdehyde/kg, respectively. Raw goat satay without sliced fat with a lower fat content tends to be higher for the TBA value than raw goat satay with sliced fat with a higher fat content. The TBA values were also not always low in raw goat meat with high endogenous antioxidants. The oxidative processes and biochemical components in the muscle tissue system depend on the nutritional composition of animal feed. Imbalance or instability of the function of the biological system of muscle occurs due to less than optimal oxidative potential in the muscle tissue system which results in the formation of free radicals and secondary oxidation product compounds in muscle and meat [42]. The levels of phospholipids, which are in cell membranes and contain a lot of unsaturated fatty acids in meat and meat products are higher so they are more susceptible to lipid peroxidation [48]. The prooxidant levels, enzyme activity, pH, temperature and composition of protein and lipid fractions in meat affect its oxidative stability [49]. The oxidative processes phenomenon in meat that causes secondary compounds formation that can endanger human health [50]. Deterioration of meat initially occurs due to oxidative processes during muscle conversion into meat (slaughter and post-slaughter stages) which disrupt the balance of pro-oxidant and antioxidant systems in vivo. This oxidative process continues during meat processing or during meat storage [33]. During postmortem conditions, meat oxidation occurs and causes changes in color pigments and lipids so that the color, aroma and flavor of the meat are no longer attractive. Feed nutrition, genetics, production management, and environmental conditions of livestock greatly affect oxidative processes in muscle tissue [42].

The endogenous antioxidant activity of raw goat satay with sliced fat and without sliced fat as measured by the diphenyl-picyrhydrazyl (DPPH) method ranged 18.985-36.661% and 14.567-34.124%, respectively. It presents in Table 1. The results of our study showed that the natural antioxidant activity in raw goat satay tended to be higher in raw goat satay with sliced fat than without sliced fat. The antioxidant activity of various biological systems is generally measured by the cheap, fast and simple DPPH method. The DPPH is a stable hydrophobic free radical compound that is less able to attack macromolecules such as proteins in solution [47]. The results of our study, the antioxidant activity in raw goat meat was higher than the study by Susanto et al. who reported that male and female “Kacang” goats had an average antioxidant activity (DPPH) of 12.085% and 12.651%, respectively [51]. Our study results were also lower than those of Mirzaei et al. who reported that goat meat has antioxidant activity (DPPH) of 42% [47].

Endogenous antioxidants in meat include the protein peptide histidine (carnosine and anserine) because they have strong antioxidant properties as potential free radical scavengers and metal chelating agents. The molecular structure of protein will change when the meat is heated so that its antioxidant activity is lower [47]. The levels of carnosine and anserine in the loin of goat meat were higher than the rump, namely 62.25 and 81.93 mg/100 g compared to 49.54 and 66.32 mg/100 g [24].
The composition of fat and protein fractions in meat affects the effectiveness of its natural antioxidants [52]. The oxidative stability in meat is influenced by fat content and fatty acid composition, where higher fat content and PUFA/SFA ratio will result in rapid and high lipid oxidation [33]. The protein fraction contains myoglobin and ferrous iron, whose concentration can affect the rate of meat oxidation [53]. The ubiquinone, glutathione, lipoic acid, spermine, carnosine and anserine are some of the meat endogenous antioxidants that have been studied [37]. The antioxidant peptides can bind metals and have the potential to act as hydrogen donors to stop free radical chain reactions in the body [54]. The most abundant natural antioxidants in meat were carnosine and anserine [55]. The antioxidant properties and antiglycation activity in muscle are found in the dipeptides carnosine and anserine [56]. Every 100 g of beef and lamb contains carnosine about 365 mg and 400 mg, respectively [55]. The most important natural antioxidant in meat is carnosine because it can be completely absorbed into the plasma [57]. The another natural antioxidant present in meat is coenzyme Q10 (ubiquinone) [58]. Every 100 g of beef and lamb contains about 2 mg of ubiquinone [55]. The another natural antioxidant in meat is glutathione which also plays a role in the immune response and contributes to the 'meat factor' in increasing iron absorption. Every 100 g of beef contains about 12–26 mg of glutathione [59]. The red meat contains 2 and 10 times more glutathione than poultry and fish, respectively. Another natural antioxidant in meat is vitamin E or alpha-tocopherol which also plays a role in boosting the immune system and helps prevent blood clots. Each 100 g of lamb and mutton contains about 0.44 mg and 0.20 mg, respectively [60].

There are three types of specific action of antioxidant compounds, namely (1) scavenging free radicals and reactive species in the initiation, propagation and termination phases (when hydroperoxide breakdown); (2) chelating transition metals and (3) donating electrons in the propagation and termination phases to stabilize fat molecules [61].

3.3. Protein, carbohydrate, moisture and ash content of raw goat satay

The protein content tended to be higher in raw goat satay without sliced fat than with sliced fat. The protein content of raw goat satay with sliced fat than without sliced fat ranged 14.240-18.830% and 18.995-20.145%, respectively. Our study results were not much different from those of Susanto et al. who reported male and female 'Kacang' goat meat had an average protein content of 19.98% and 18.60%, respectively [51]. The essential amino acids of 'Kacang' goat meat with the highest levels were leucine, lysine, arginine, threonine, valine, isoleucine, histidine, phenylalanine, cystine, tryptophan, and methionine of 75.8; 66.2; 62.1; 56.2; 47.2; 36.5; 29.6; 29.6; 22.3; 21.4; and 20.1 mg/kg, respectively [51].

According to Faustman et al. [53], the rate of oxidation in meat is influenced by the protein fraction, namely myoglobin which catalyzes lipid oxidation. Myoglobin is more abundant in red meat and in darker, more heme pigments and reactive iron in myoglobin. Free radicals and oxidative deterioration of fatty acids are formed as a result of oxygen-dependent lipid oxidation [50]. The functional groups on the side chains of amino acids are the main targets of protein oxidation processes caused by reactive oxygen species (ROS) or secondary products of fat oxidation catalyzed by myoglobin or metals [62]. Lipid oxidation and protein oxidation have a similar mechanism through a free radical or ROS chain reaction which is initiated by the separation of a hydrogen atom from the protein and a carbon-centered protein radical is formed [49]. Lipid oxidation and protein oxidation are very interacting, where aldehydes from fat oxidation are pro-oxidant agents and react with proteins to cause physical changes. Alkoxyl radicals and hydroxyl derivatives are formed when hydrogen atoms from proteins absorb peroxyl radicals, which are the product of fat oxidation to form alkylperoxides. The iron released during protein oxidation due to the conversion of myoglobin to metmyoglobin can catalyze the lipid oxidation in meat. Lipid oxidation will be faster if there is more iron and myoglobin in meat [46]. Besides that, the rate of oxidation of unsaturated fatty acids increases in the presence of superoxide and hydrogen peroxide anions, which are intermediate products of metmyoglobin formation [53]. The positive correlation between lipid and protein oxidation is reflected in the reciprocal transfer of ROS and non-reactive species (such as hydrogen peroxide and hydroperoxides) between fats and proteins [63]. The
protein oxidation in meat causes changes in meat color and texture, essential amino acids, digestibility and functionality of meat proteins disappear due to the formation of protein carbonyl and protein cross-linking and loss of sulfhydryl groups [64].

The moisture of raw goat satay tended to be higher in goat satay without sliced fat than with sliced fat. The moisture of raw goat satay with sliced fat than without sliced fat ranged 57.940-66.385% and 71.530-76.810%, respectively. The presence of moisture and other materials can affect the PAHs formation. Moisture provides a source of oxygen when heating thus preventing incomplete combustion and causing an inhibitory effect [65].

There was no significant differences between the ash and carbohydrate contents of the raw goat satay with sliced fat and without sliced fat in various satay restaurants. The ash and carbohydrate contents of goat meat ranged from 0.920-1.095% and 0.645-1.620%, respectively. The information is still very limited and scarce for the formation of PAHs from amino acid and sugar precursors compared to fat precursors [30]. The formation of polycyclic aromatic compounds (PAC) due to the influence of amino acids (asparagine, proline, and tryptophan) with thermal reactions at temperatures much higher than the usual cooking temperature [66]. The formation of PAHs due to the pyrolysis of amino acids (proline) and carbohydrates (glucose) after the Maillard reaction [67].

3.4. PAHs content of charcoal grilled goat satay
As shown in the data in Table 1, the PAHs levels for benzo(a)pyrene (BaP) in charcoal grilled goat satay with sliced fat in various satay restaurants tended to be higher than grilled goat satay without sliced fat. The BaP levels in charcoal grilled goat satay with sliced fat and without sliced fat in various satay restaurants ranged from not detected to 6.330 mg/kg and not detected to 0.773 mg/kg or ppm (parts per million). Our study results were higher (mg/kg or ppm) than the report of Adiyastiti and Suryanto [25] and Darmadji [68] who reported BaP only in µg/kg or ppb (parts per billion). This was due to the limitations of the gas chromatography capacity in the laboratory where we analyzed PAHs levels, LPPT University of Gajah Mada, Yogyakarta. The BaP content of charcoal grilled goat satay was higher than gas grilled goat satay. The BaP content of charcoal and gas grilled goat satay with a grilling time of 3, 5, and 7 minutes were 2.55; 4.16; 5.02 ppb and 0.68; 2.71; 1.65 ppb, respectively. This was because charcoal, the result of wood pyrolysis in which there is a PAHs content so that it will contribute to increasing the BaP content [25]. The BaP content of charcoal and gas grilled goat satay were 1.76 ppb and 0.78 ppb, respectively [68]. The commercial charcoal grilled chicken satay in Yogyakarta contained a BaP of 1.76 ppb and 0.78 ppb, respectively [68]. The BaP was identified as a marker of PAHs carcinogens in grilled beef and chicken [71]. The BaP levels were higher because of the horizontal heat source which cannot prevent the fat dripping from the meat on the flame [72]. On the surface of the meat is more likely to form PAH than on the inside. PAHs compounds were produced through the pyrolysis process (incomplete combustion) as long as the meat were charcoal grilled and when the fat from the meat drips onto the flame, it will produce significant levels of PAHs during direct heating of the meat with charcoal. Fat content and grilling distance greatly affect the level of PAHs formed [28].

Our study data presented in Table 1 shows that BaP appeared in charcoal grilled goat satay with sliced fat when the natural antioxidant activity was lower (18.985 and 22.750%), lower fat content (13.444%), higher water content (66.385%) and lower total organic matter (40.420%) in raw goat meat. Conversely, in grilled goat satay without sliced fat, BaP appeared when the natural antioxidant activity in raw goat meat was higher (34.124%). This was thought to be the result of the wood charcoal used and the addition of an excessive dose of antioxidants from the marinated spices just before grilling which contain spices, including garlic. All of the respondent's satay restaurants, used goat curry soup (‘gule’) and ground garlic as a marinade just before grilling the raw goat satay. Ahmad Kamal et al. found the same thing with us, marinated beef satay contained significantly higher PAHs than unmarinated
controls. Sugar, salt, coriander, lemon grass, turmeric, galangal, fennel, cumin, and cooking oil were the ingredients for the marinade they use [29]. After marinating (24 hours) there was an increase in PAHs in meat because PAHs were fat-soluble [73]. The higher PAHs levels were detected in grilled meat that were marinated before grilling [74]. The bioactive compounds at low concentrations can act protectively, whereas at high concentrations they act as pro-oxidants and can be degraded at high temperatures [75, 76]. The fat content, temperature, cooking time, cooking process, type of fuel used, and distance to the heat source greatly affect variations in PAH levels in meat [71].

The wood charcoal used as a heat source for grilling satay in various satay restaurants of respondents turned out to contain PAHs which can contaminate goat satay. A large number of volatile compounds and PAHs were released from charcoal [77]. As the combustion time increased after the first ignition of charcoal, PAHs emissions were reduced in the smoke generated from charcoal burning [78]. The release of PAHs (naphthalene and phenanthrene) from the wood charcoal occurs when it is re-burned because the charcoal still contains tar when carbonated at low temperatures [79]. Re-combustion of charcoal also produces benzene, a volatile organic compound in the smoke of about 8.01% [80]. Benzene is the main precursor for the formation of PAHs [81] and can cause eye and respiratory system irritations with headache [82].

Garlic which is used as a marination seasoning before grilling the satay has antioxidant capacity due to the sulfhydryl and phenolic compounds it contains. The sulfhydryl compounds in garlic and onions act as free radical scavengers and hydrogen peroxide, which can capture electrophilic compounds that inhibit the formation of PAHs [27]. The main antioxidant bioactive compounds of phytochemicals are carotenoids, phenolic, and essential oils compounds. Vegetables contain lots of phenolic compounds [83]. The main phenolic compounds are flavonoids whose structures vary widely among vegetables [84]. The mixing meat with natural antioxidants in small sizes or ground was more effective in preventing the oxidation of lipid and protein. Grinding and homogenizing meat with natural antioxidants and food ingredients enrich the contact between sensitive compounds and antioxidants [49].

The results of our study indicated that the excessive concentration of antioxidants due to the already high endogenous antioxidants plus the natural antioxidants from marinated seasonings before grilling the satay can actually trigger the formation of PAHs. The dose or concentration of antioxidants greatly affects its capacity to inhibit the formation of PAHs, where a moderate dose can inhibit it and conversely at higher doses it supports the formation of PAHs in grilled meat [75, 85, 86, 87, 88, 89]. The antioxidant bioactive compounds in spices and heating temperature greatly affect their capacity to inhibit the formation of PAHs [75, 88, 90]; the absence of certain PAHs precursors in the seasoning itself and the type of raw food material [90, 91].

The BaP levels in both the grilled goat satay with sliced fat or without sliced fat in several satay restaurants exceeded the safe limit set by BPOM RI, the National Agency for Drug and Food Control (NADFC), Republic of Indonesia [13]. BPOM RI has set a safe limit for BaP in heated processed meat of 0.005 mg/kg (BPOM, 2018). WHO and IARC have categorized the only type of PAHs, namely BaP, as carcinogenic or class 1 [11].

PAHs type benzo(a)anthracene (BaA) was detected in all grilled goat satay samples in various satay restaurants as shown in the data presented in Table 1. BaA levels tended to be higher in grilled goat satay with sliced fat than without sliced fat. BaA levels in grilled goat satay with sliced fat than without sliced fat in various satay restaurants ranged from 2.238–15.770 mg/kg and 2.139–7.196 mg/kg, respectively. When grilling meat, BaA and BaP can survive on the surface of the meat because they are both heavy PAHs which are more stable [5]. IARC has classified BaA as possibly carcinogenic or class 2B [11]. Possibly carcinogenic, meaning it can occur or not depending on existing precursors. In the European Union, BaA is a high molecular weight PAHs monitored for food safety. In order to monitor the presence of PAHs that contaminate food, there are 15 types of PAHs that are prioritized by the US-EPA (United States Environmental Protection Agency) while the European Union (EU) in 2005 only prioritizes 8 types of PAHs or called PAH8 which have high molecular weights, namely: benzo(a)pyrene (BaP), benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), chrysene (Chr), indeno[1,2,3-cd]pyrene (IcP), benzo(g,h,i)pyrene (BgP), and dibenzo(a,h)anthracene (DhA).
Aaslyng et al. [69] noted that in the European Union (EU) since 2002, BaP has been used as a marker for carcinogenic PAHs in food. Currently, the European Union (EU) uses PAH4 as a marker, because in foods containing PAHs, BaP is not always detected. The PAH4 are BaA, Chr, BaP and BbF, the amount of which is limited to 30 µg/kg [5].

PAHs type of pyrene (Pyr) was also detected in almost samples of grilled goat satay in various satay restaurants. The levels of Pyr in grilled goat satay with sliced fat than without sliced fat in several satay restaurants ranged from 8.316-438.670 mg/kg and not detected up to 490.340 mg/kg, respectively. IARC has classified Pyr as non-carcinogenic or class 3 [11]. So it was still safe for consumption even though Pyr was present in commercial grilled goat satay.

PAHs type of phenanthrene (Phe) was not detected in almost samples of grilled goat satay in various satay restaurants. Phe were detected only in samples of grilled goat satay with sliced fat from one satay restaurant and in samples of grilled goat satay without sliced fat from one satay restaurant. Although Phe was present in these commercial grilled goat satay, it was still safe for consumption. This is because the IARC has classified Phe as non-carcinogenic or class 3.

PAHs type of acenaphthene (Acp) and naphthalene (Nap) were not detected in all grilled goat satay samples in various satay restaurants. IARC has classified Acp as non-carcinogenic or class 3 and Nap as possibly carcinogenic or class 2B [11]. The Acp and Nap are mild PAHs which are more volatile and evaporate quickly [5]. Our study results were not the same as those of Ahmad Kamal et al. and Perello et al. who found Phe, Acp and Nap to be PAHs that were mostly formed in grilled meat [29, 92]. During the heating process, cyclohexane which is a degradation product of lipid oxidation is oxidized to a benzene ring structure and reacts with C₄ carbon compounds to form Nap [73].

4. Conclusions
The goat satay producers prefered a female goats than a male goats and/or a sheep and was under a year old. The weight of fresh got meat for a serving of grilled goat satay with sliced fat and without sliced fat (10 skewers) ranged 144-194 g and 146.333-165.667 g, respectively. In various satay restaurants, after grilling goat satay with wood charcoal at a temperature between 486.833-548.300 °C for 2-5.5 minutes and a distance of 0.5-2 cm, a serving of grilled goat satay with sliced fat and without sliced fat experienced cooking losses ranged 12.736-39.292% and 5.922-36.382%, respectively. In various satay restaurants, the levels of raw goat satay fat, lipid oxidation, antioxidant activity, protein, carbohydrates, moisture and ashes for raw goat satay in sliced fat and without sliced fat ranged 13.440- 26.100% and 1.5-6.305 %; up to 2.523 and 1.315 mg melanaldehyde/kg; up to 36.661 and 34.124%; 14.240-18.830% and 18.995-20.145%; 0.645-1.620%; 57.940-66.385% and 71.530-76.810%; 0.920-1.095% and 0.645-1.620% and 0.920-1.095%, respectively. In various satay restaurants, the levels of BaP, BaA and Pyr for grilled goat satay with sliced fat and without sliced fat ranged from not detected to 6.330 mg/kg and not detected to 0.773 mg/kg; 2.238-15.770 mg/kg and 2.139-7.196 mg/kg and 8.316-438.670 mg/kg and not detected up to 490.340 mg/kg. The BaP levels in grilled goat satay in several satay restaurants exceeded the safe limit set by BPOM RI. BaA and Pyr were detected in almost samples of grilled goat satay in various satay restaurants. Phe, Acp and Nap were not detected in almost grilled goat satay samples in various satay restaurants.

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