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Effect of Lemon Marination on *Polycyclic Aromatic Hydrocarbons* (PAH) and Quality of Chicken Satay

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Abstract: This study aims to determine the effect of different lemon marinates on Polycyclic Aromatic Hydrocarbons (PAHs) and quality of chicken satay. The percentages of lemon used 0%, 5 %, 10 % and 15 %. Chicken meat was marinated for 10 minutes in a homogeneous. The study used a Nested Completely Randomized Design. Parameters used were physical quality (pH, cooking loss and WHC), chemical quality (moisture, fat and protein content) and *Polycyclic Aromatic Hydrocarbons* (PAHs). Data were analyzed using Analysis of Variance (ANOVA), if the results of statistical analysis significant or very significant differences were obtained Duncan's test (UJBD) and descriptive PAH. The results of statistical analysis showed that the use of lemons marinates with different proportions had a very significant effect (P<0,01) on pH, cooking loss, WHC and moisture, fat content had a significant effect (P<0,05) but had no significant effect (P>0,05) on protein content. Chicken satay with 15% lemon marinates showed non-detectable results PAH. The study concluded that lemon marinates can reduce the content of PAH carcinogens and affect the quality of chicken satay. The lowest levels of PAHs were found in the chicken satay that was marinated with a 15% lemon marinates.

Keywords: Chemical quality; Chicken satay; Physical quality; *Polycyclic Aromatic Hydrocarbobs* (PAH)

Introduction

The Republic of Indonesia is the world's largest archipelagic country, with more than 1,700 islands and the world's fourth most populous country. Indonesia's population will be 270.20 million people, with an annual population growth rate of 1,25%. The significant population growth has led to an increase in the consumption of animal protein in Indonesia, one of which is chicken products. The consumption of chicken meat in 2017 reached around 3,93 million tons or around 15,07 kilograms per capita per year (Khalil et., 2019). Chicken meat is a source of animal protein that is consumed by many Indonesians. The factors that cause chicken meat to be in demand by the public are its delicious taste, affordable price, and good nutritional content. The nutritional content of 100 grams of chicken meat is 23,06% protein, 7% fat, 0,98% minerals and 135 kcal calories (Horbańczuk and Wierzbicka, 2016).

One of the processed chicken products is satay. Satay is made from pieces of meat cut into small pieces and skewered with bamboo and then grilled over charcoal, coconut shells, or a gas-fired grill (Saputro et al., 2020). Satay is a delicious food to eat, but behind the delicious taste, this type of preparation can be bad for the health of the body. The processing of chicken satay done at high temperatures, which causes the formation of carcinogenic. Polycyclic Aromatic Hydrocarbons (PAHs) are carcinogenic compounds, that can be formed due to incomplete combustion can cause cancer in human. Benzo(α)pyrene (BaP) is a type of PAHs that is highly carcinogenic and is most often produced as a result of combustion (Rozentale, 2015).

The main source for the formation of PAHs is the high temperatures during the combustion process. PAHs formed by incomplete combustion of inorganic substances (charcoal, oil, gas) and organic matter. Meat burned with wood charcoal, if the burnt food comes

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direct contact with hot coals, the drippings from the meat will produce PAHs which will be deposited on the surface. The fat dripping of charcoal generates PAHs that may combine with smoke and contaminate the food. Cooking with charcoal using a long heating time causes the formation of PAH carcinogens (Nowakowska, 2014). The external temperature of the coals wood charcoal when roasting satay ranges from 270-300^{o}C (for medium maturity) and 300-350^{o}C (for very ripe maturity) (Dutta et al., 2022).

The formation of PAHs can be influenced by several factors, including the length of time the meat is cooked, the temperature at which it is cooked, the type of meat and the processing treatment. Marination is the processing of meat by immersing it in marinade ingredients before further processing. This treatment used improve quality of processed products. Marination can affects the resulting product, if the marinating time is too long, the texture of meat will become soggy and crumbly. PAHs found in food are mainly caused by their type of the cooking time, temperature and treatment (Siddique et al., 2021). The ingredients used marinades are varied and include sugar, salt (NaCl), coriander, lemongrass, onion and different types of acid. One of the acidic marinades that can be applied to meat is lemon (Sampaio, 2021). The addition of acidic ingredients in the marinade can reduce the formation of PAHs. Marination meat with lemon (*Citrus limon* L) and tamarind can prevent the formation of carcinogenic Polycyclic Aromatic Hydrocarbon (PAH) compounds (Nor et al., 2022).

The compounds can be attenuated or decrease if done by pre-treatment using different techniques. Marination chicken meat with mixing 394 g of lemon with 3,940 ml of distilled water was able to lower the meat's pH and improve the meat's tender texture (Jinap et al., 2018). Lemon marinade decrease carcinogenic compounds can cause cancer in humans up to 40%. The activity of cooked meat can be overcome by pre-treating meat to be processed with a marinade. The application lemon juice marinade prior to the meat cooking process has been shown reduce the content of carcinogenic PAH in the fried meat patty. The presence of lemon (*Citrus limon* L) has the potential inhibit cancer cell proliferation, delay tumourigenesis, and comprehensive agent of carcinogenesis (Cordeiro et al., 2020).

Based on the above description, this research was conducted to determine the effect of lime marination on carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) and the quality (physical and chemical) of satay. This research was conducted to determine the best treatment to reduce carcinogenic PAHs by lemons margination to produce quality and safe chicken satay for consumption.

Method

The object of the research was chicken satay. The design of the research that was used can be seen in Figure 1.

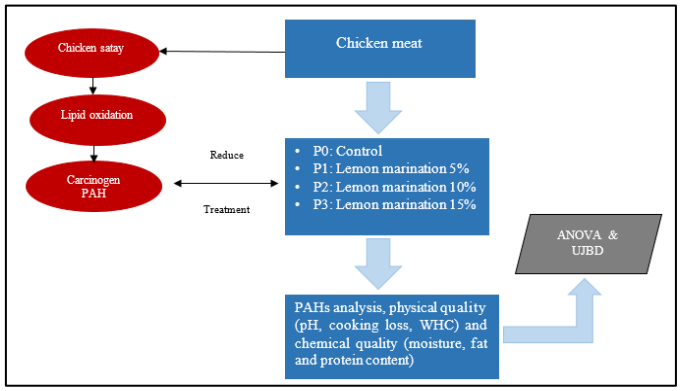

Figure 1. Design the research

Data were analysed using analysis of variance (ANOVA) when statistical results showed significant or very significant differences with Duncan's test (UJBD) and PAH analysis descriptive. The research sample consisted of 4 treatments, each treatment consisted of 5 replications, so there were 20 experimental units. Sample P0 control 0%, P1 lemon marination 5%, P2 lemon marination 10% and P3 lemon marination 15%.

The materials used in this study were chicken breast, charcoal and coconut shells, marinade and peanut sauce. The tools used were bamboo skewers, analytical balances, pH meters, gas chromatography FID, digital scales, blander, freezers, spoons, aluminium foil, plastic, styrofoam and blue ice. The process of chicken satay uses lean chicken breast meat cut lengthwise into thin slices of 5 x 2 x 1cm. Marinade the meat in lemon juice for 15 minutes. The meat is then skewered, there are four pieces of meat on a skewer. Chicken satay is grilled for 7 minutes over charcoal and coconut shells. Collecting data in this study using laboratory analysis. Testing the quality of chicken satay was observed included physical test (pH, cooking loss and Water Holding Capacity (WHC)), chemical quality (moisture, fat and protein content) and PAH analysis.

Result and Discussion

Physical Quality

The research data on physical quality of chicken satay include pH, cooking loss and WHC with different percentages of lemon marinade, can be seen in Table 1.

Table 1. The average physical quality of chicken satay

Sample	pH	Cooking loss	WHC
P0	6,55 ± 0,06^{c}	18,36 ± 0,59^{c}	29,71 ± 0,31^{a}
P1	6,47 ± 0,08^{bc}	17,08 ± 0,69^{bc}	30,92 ± 0,47^{b}
P2	6,43 ± 0,06^{b}	16,08 ± 0,70^{b}	31,93 ± 0,54^{c}
P3	6,27 ± 0,07^{a}	13,35 ± 0,88^{a}	33,09 ± 0,46^{d}

Note: Different superscripts (a-d) in the same line shows a very significant different (P<0,01).

The results of the analysis showed that effect lemon marination at different percentages had a very significant effect (P < 0,01) on the pH, cooking loss and WHC of chicken satay. Data from Table 1. showed that average pH of chicken satay decreased with increasing lemon. The decrease pH of the chicken satay is due to an acid-base reaction between the citric acid in lemon and the protein meat. Lemon is one of the ingredients in the marinade that contains citric acid. Citric acid is an organic acid compound that is acidic and can release hydrogen ions (H+) when dissolved in water. The greater concentration of hydrogen ions (H+) released, the greater decrease in pH of meat (Sari and Patriani, 2021).

The process of chicken satay with marinated acid can lower the pH and increase the acidity of the product, which results in a fresher flavor, a softer texture and a longer shelf life for the product. The citric acid in lemons not only has the ability to lower pH of the fiber, but also aids in breakdown of meat, increasing meat's ability to absorb spices (Gunanda et al., 2021). The use of organic acids in the marinade causes a decrease pH of grilled chicken. Grilled chicken marinated in lemon marinade significantly decreased the pH compared to the control treatment without marination or soaking. Lemon marinated grilled chicken had a pH of 5,24 lower than the control sample of 5,48 (Jinap et al., 2017). The process of chicken tikka (spicy grilled chicken pieces) is done by lemon marination. Lemon juice (*Citrus limon* L) is often used flavor and marinate foods. Marinating chicken meat using 20% concentration of lemon juice at 4^{o}C before burning resulted in lower pH compared to the control treatment (Kumar et al., 2015).

Marinating lemons can reduce cooking losses in satay meat due to the direct effect of acid in the marinade solution on meat. Lemons (*Citrus limon* L) can help open the pores on surface of the meat, increasing the ability of the meat to absorb liquid (Biyatmoko et al., 2018). The acid in marinade solution can help the meat absorb more liquid. This increases the meat's ability to hold water. The process heating meat prevents liquid inside from coming out easily, reducing cooking losses. The cooking loss values for the acidic marinades were lower than control. The cooking loss for the chicken meat marinated with 5% apple cider vinegar was 20,24%, the marinade with 10% apple cider vinegar was 15,19%, while the cooking loss of tmeat in the control treatment was 37,81%. The cooking loss of chicken meat decreased with the increase of acid concentration (Unal et al., 2020). Application of marinating meat with a solution of organic fruit citric acid (apples, grapes, oranges, persimmons, lemon) can improve the quality of meat. Marinated with 0.5%, 1% and 1.5% citric acid solution, the lowest cooking loss value was obtained for meat marinated with highest acid concentration (Sengun et al., 2021).

The Water Holding Capacity (WHC) is the ability of the meat to hold water when it is heated. Citric acid can help the protein in meat retain water increasing the WHC in meat. The process of meat marination with citric acid can lower the pH value of the meat. The content of citric acid can help change the protein structure in meat, allowing it to hold more water (Kalahrodi et al., 2021). The WHC value increases due to changes in the relationship between protein and water and changes in myofibril bonds. Protein in meat can interact with water through hydrogen bonds. The process of burning satay causes the protein to experience denaturation or changes in protein structure which causes a loss of protein and water. Changes in myofibril bonds or protein structure in animal muscles during the combustion process cause actin and myosin bonds to weaken so that WHC increases due to the weakening of myofibril bonds which allows air to bind more easily (Kristiawan et al., 2019).

This study shows that chicken satay with 15% lemon marinade has the highest average 33,09%. The effect of marinating chicken breast pieces with lemon juice showed a higher WHC value compared to the control treatment without marination. The WHC value of chicken breast pieces soaked in 10% lemon juice solution at 4^{o}C was 25,67%, while the control treatment without marination obtained a lower WHC value of 22,59% (Augustyńska-Prejsnar et al., 2019). Chicken breast meat with the addition of acid marinade can increase WHC. Hard processing tends to damage the protein structure in meat, thereby reducing the meat's ability to hold water (Zhang et al., 2020).

Chemical Quality

The research data on chemical quality of chicken satay include moisture, fat and protein content with different percentages of lemon marinade, can be seen in Table 2. The results of the analysis showed that effect lemon marination at different percentages a significant effect (P < 0,05) on the moisture and fat content. The protein content no effect (P > 0,05). Moisture content is one of the parameters for monitoring meat quality which has an impact on the texture, taste, and stability of the product. Moisture content in meat is the percentage of water contained in the meat. Food products with a high

moisture content will deteriorate at a faster rate due to the increased activity of microorganisms (Anwar et al., 2021). Lemon contains a citric acid has hygroscopic properties or can absorb moisture and increase the water content in meat (Islam et al., 2016). Lemons marinade solution or adding lemon slices to meat causes moisture to be absorbed and the water content in the meat to increase. The citric acid found in lemons helps open the pores on the surface of the meat, increases the ability of the meat to absorb water, and retains moisture in the meat (Gomez-Salazar et al., 2018).

Table 2. The average chemical quality of chicken satay

Sample	Moisture	Fat	Protein
P0	61,19 ± 0,77^{a}	4,50 ± 0,34^{c}	32,50 ± 1,12
P1	62,14 ± 0,12^{ab}	3,93 ± 0,61^{bc}	31,94 ± 1,07
P2	62,23 ± 1,25^{b}	3,81 ± 0,47^{ab}	31,39 ± 0,33
P3	63,46 ± 0,59^{c}	3,44 ± 0,48^{b}	31,23 ± 0,35

Note: Different superscripts (a-c) in the same line shows a significant different (P<0,05), while those with no notation show no effect.

The results of this study moisture content of chicken satay with different percentages of marinating lemons 61,19% - 63,46%. The addition of natural antioxidants such as tea, cherries, and lemon to the chicken before the roasting process showed an average value of moisture content of 59.36% - 78.68%. The control treatment had a lower moisture content than the green tea margination. The control treatment for meat burned using wood charcoal without marination obtained a moisture content of 64.94%, while meat burned using wood charcoal with marination was 67,41% (Yao et al., 2020). The moisture content is affected by differences in the concentration of solvent and dissolved substances known as osmosis. Osmosis is a process of diffusion of water through a permeable membrane differentially from an area of high concentration to an area of low concentration (Rumondor et al., 2023). Lemon as a marinade can interact with meat protein. The citric acid in lemons can destroy protein bonds and produce a soft texture, so it can increase the water content in the meat.

The moisture content of chicken satay is influenced by several factors, namely the type and quality of chicken, cooking method, temperature, and storage as well as the effect of adding spices or other ingredients (Sobral et al., 2020). The decrease in water content in the meat satay is influenced by the cooking factor which causes the liquid in the meat to drip out during the roasting process (Dutta et al., 2022). The process of roasting satay at high temperatures causes more water evaporation from the meat, thus increasing the possibility of a loss of water content (Khan et al., 2022).

Marinating lemons can reduce the fat content in chicken satay. Chicken meat marination with the acid contained in the lemon will react with the protein and fat content. The reaction between the acids and protein in meat can help break down the protein structure and soften the meat fibers, thus making the meat more tender, besides that the reaction between the acids can also help reduce the fat content (Ribeiro et al., 2019). The addition of natural antioxidants such as tea, cherries, lemon onions to chicken meat before the roasting process shows an average value of fat content of 2,38% - 8,10%. The high citric acid concentration resulted in a lower fat content in meat compared to the non-marinated control (Yao et al., 2020). Fat content can be affected by the cooking process, temperature and cooking time. High temperatures will melt the fat and tend to damage the texture of the satay, besides that cooking methods with high temperatures and long enough times can increase the risk of forming carcinogenic compounds which have a bad impact on the body (Domingo et al., 2017).

The protein content decreased as the marination percentage of the given lemon increased. Protein is a macronutrient that forms important building blocks in the formation of biomolecules. The addition of natural antioxidants such as tea, cherries, and lemon to the chicken before the roasting process showed an average value of protein content 14.94% - 25.81%. The control treatment for meat burned using wood charcoal without marination obtained a protein content of 24.94%, while meat burned using wood charcoal with marination was 23,34% (Yao et al., 2020). Protein levels can be affected by the type of meat, cooking, and the addition of other ingredients. The heating process is closely related to temperature and heating time, temperatures and a long heating process will cause denaturation of the nutritional content of meat such as protein and other nutrients (Nor et al., 2022). Chicken meat soaked in a solution of lemon juice for a long time, the acid in the lemon juice can help break down the protein structure in the meat into fragments or small molecules such as peptides or amino acids, this can make the measurement of protein levels in meat decrease slightly (Kumar et al., 2015).

PAH Analysis

The research data on PAH analysis of chicken satay with different percentages of lemon marinade, can be seen in Table 3. Polycyclic Aromatic Hydrocarbons (PAHs) are the largest class of chemical carcinogens. PAHs in food are caused by the pyrolysis process of organic matter such as fat at temperatures > 200 C (Duan et al., 2016). The dripping lipid content and direct contact on the surface of the grilled satay can form PAHs which are volatile and then deposited on the surface of the meat when the smoke rises (Kim et al., 2016). The main PAHs that are often found in the environment can be classified into 16 types, but the PAHs that are often

used as an indicator of the level of food safety are PAH4 consist of Benzo(a)pyrene, Chrysene, Benzo(b)fluoranthene, and Benz(a)anthracene (BaA) (Racovita et al., 2020).

Table 3. PAH analysis

Type PAH	Sample (mcg/kg)			
	PO	P1	P2	P3
Acenapthylene	nd	nd	nd	nd
Indeno (1.2.3-cd)pyrene	nd	nd	nd	nd
Benzo(g.h.l)perylene	nd	nd	nd	nd
Dibenzo(a.h)anthrace	nd	nd	nd	nd
Benzo(a)pyrene	5.610	5.520	4.642	3.721
Chrysene	3.763	3.214	2.954	2.032
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(b) fluoranthene	2.369	2.212	1.832	1.093
Benzo(a)anthracene	3.216	2.763	2.325	1.942
Pyrene	1.093	nd	nd	nd
Fluorenthene	nd	nd	nd	nd
Anthracene	nd	nd	nd	nd
Phenanthrene	nd	nd	nd	nd
Fluorene	0.282	nd	nd	nd
Acenapthene	nd	nd	nd	nd
Naphthalene	nd	nd	nd	nd

Note: PAH4 in the 15% lemon marinade (P3) resulted in lower levels of carcinogens compared to other treatments.

The short-term impact of exposure to PAHs on the human body will cause eye irritation, nausea, vomiting, and diarrhea. Anthracene and Naphthalene cause direct skin irritation such as allergies (Wang et al., 2013). PAHs of the Benzo(a)pyrene and pyrene types are indicated to cause cancer and DNA damage (Zhang et al., 2020). Exposure activity associated with human cancer was observed in the bladder, esophagus, respiratory system, kidney, intestine, and lung. The following is a picture of the impact of PAH exposure on the human body which can be seen in Figure 2.

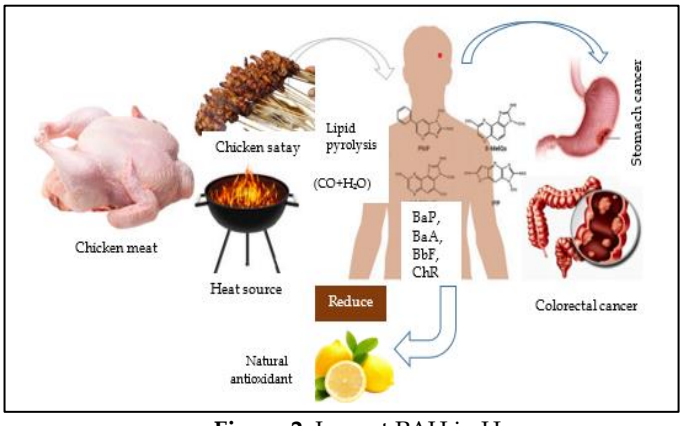

Figure 2. Impact PAH in Human

The results showed that PAH4 in the 15% lemon marinade treatment resulted in lower levels of carcinogens compared to other treatments. The LOD of PAH testing in this study was >0,004 mcg/kg. The marination process with natural antioxidants such as lemons can reduce carcinogen levels. Lemon contains citric acid, vitamin C, vitamin E, essential oils, polyphenols, bioflavonoids, and flavonoids (Paw et al., 2020). The addition of acidic marinade to charcoal grilled chicken can inhibit PAHs. Vitamin C and vitamin E in lemons function to prevent body cells from damage caused by free radicals. Free radicals are one of the causes of cell damage that triggers the formation of PAH compounds. Vitamins can capture free radicals and prevent chain reactions that can damage body cells (Lee et al., 2020). Phenol contained in lemons can reduce the radical (1,1-diphenyl-2-picrylhydrazyl) DPPH. Phenol in lemons can attribute electrons to the DPPH radical, then convert into a stable. Phenol can also reduce the occurrence of fat oxidation by neutralizing reactive oxygen which can trigger the formation of PAHs (Wang et al., 2019). PAH reduction including sugar, synthetic antioxidants, natural antioxidants, and microwave pre-treatment. (Saputro et al., 2022). The use of some fruit juices, spices and tea can limit the formation of PAHs in smoked or grilled meats before the cooking process (Lu et al., 2018). Lemon (Citrus limon L) as a natural antioxidant is able to prevent the formation of PAH carcinogens in grilled meat (Nor et al., 2022).

Conclusion

Lemon-marinated chicken satay has good quality in terms of physical quality (pH, cooking loss, WHC) and chemical quality (moisture, fat and protein content). Marinating lemons can reduce PAH levels in chicken satay. The lowest levels of PAHs were found in the chicken satay that was marinated with a 15% lemon marinates.

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Author Contributions
Conceptualization, Djalal Rosyidi and Lilik Eka Radiati; conducting research, Citra Nurma Yunita; writing—original draft preparation, Citra Nurma Yunita; writing—review and editing, Citra Nurma Yunita; supervision, Djalal Rosyidi and Lilik Eka Radiati;

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Conflicts of Interest
The authors declare no conflict of interest

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