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The effect of burning time on the amount of bacteria *Staphylococcus aureus contamination of grilled meatballs sold in Darusssalam, Banda Aceh*

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Abstract

This study aims to determine the effect of heating time in the combustion process on *Staphylococcus aureus* contamination in grilled meatballs. A total of 9 samples of meatballs obtained from 3 sellers of grilled meatballs were divided into 3 treatments, namely burning at temperatures ranging from 56˚C-59˚C for 1 minute, 3 minutes, and 5 minutes, with 3 repetitions. Determination of the number of microbes was carried out using the Total Plate Count (TPC) method. The research data were analyzed using a two-way ANOVA analysis of variance followed by Duncan's test. The results showed that the duration of burning affected the amount of *Staphylococcus aureus* contamination in grilled meatballs. The best combustion was shown at 5 minutes, with a microbial count of 3.6 × 101 CUF/g. The results of the analysis of variance (ANOVA) showed that there was a significant difference (P<0.05) in the average value of *Staphylococcus aureus* microbial contamination in grilled meatballs given treatment. Based on the calculation results, it can be concluded that 5 minutes of burning time is the most effective for decontaminating the amount of *Staphylococcus aureus* microbial contamination in grilled meatballs.

Keywords: Burning time, grilled meatballs, Total Plate Count, Staphylococcus aureus

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Introduction

Food is a necessity the most basic human and must fulfilled to survive. Preservation of the food to be consumed can be presented in various types and ways of different processing (Permatasari et al., 2021). Fast foods are increasingly popular because they have good taste and cheap prices. One of the most delicious snacks that the public is interested in is grilled meatballs (Pertiwi, 2019). This food is made using flour and minced and shaped chicken become round, boiled and smeared with special seasoning, then grilled on top of charcoal (Mayaserli and Anggraini, 2019). The process of making grilled meatballs is considered very good because it goes through the boiling process and burning. However, contamination of pathogenic bacteria in this food can also occur when actions in presentation are not done perfectly (Prananda et al., 2019).

Food contaminated by pathogenic bacteria such as Salmonella, Clostridium perfringens, and *Staphylococcus aureus* can come from equipment, air, animals, waste, water, fruit, humans, and additives such as flour and seeds grains (Lestari et al., 2018). One of the common pathogenic bacteria as an indicator of food processing the unhygienic is *Staphylococcus aureus* (Rahayu et al., 2014). Based on SNI (Indonesian National Standard) (2009), *Staphylococcus aureus* contamination limits are allowed on processed meat products of 102 CFU/g. *Staphylococcus*

bacteria aureus can grow at optimum temperatures of 30˚C–37˚C (Irawan et al., 2015). Contamination by *Staphylococcus aureus* bacteria is very often reported in food derived from meat and eggs (Amyati and Wijayanti, 2020). This is due to the high levels of protein contained in the product, so that be a good medium for the growth of pathogenic bacteria (Pertiwi, 2019).

Staphylococcus aureus are bacteria that cannot tolerate warming up. *Staphylococcus aureus* will be off by heating at a temperature of 66°C for 10 minutes (Rihastuti and Soeparno, 2014). Processed grilled meatballs using temperature and heat not perfect can be the cause of the high bacterial contamination of the snacks (Oktaviani et al., 2016). Besides the ability to infect, *Staphylococcus aureus* too capable of producing enterotoxins can cause poisoning to people who consume them (Martanda, 2019).

Materials and Method

Place and Time of Research

The sampling location is Darussalam, Banda Aceh, from January 2022 to February 2022. Inspection samples were carried out in the Microbiology Laboratory Faculty of Veterinary Medicine Unsyiah.

Research Samples.

As many as 9 samples were taken from three meatball traders. Three samples were taken from each trader. Sampling was carried out only once.

Research Tools and Materials

The tools used are plastic sterile, test tube, Erlenmeyer tube, Petri dish, beaker, Bunsen lamp, micropipette, microtypes, gloves, label paper, matches, 1 ml sterile pipette, incubator, and stomachers. The materials used are grilled meatball sample specimens, Buffered Peptone Water (BPW) 0.1%, and Mannitol Salt Agar (MSA).

Research methods

This research method is carried out based on field experimental method with ANOVA approach and for testing carried out based on SNI 2897:2008 standard. Sample treatment

Sample treatment

The meatball sample was burned at a temperature ranging from 56˚C-59˚C with burning times which are different, namely 1 minute, 3 minutes, and 5 by using charcoal that comes from coconut shell.

Sample Inspection Methods Total Plate Count (TPC) Methods The research was carried out using the *Staphylococcus aureus* bacteria analysis through the Total Plate Count (TPC) method with Buffer Peptone Water (BPW) solvent and using selective media Mannitol Salt Agar (MSA). According to (SNI 7338:2009), the principle of TPC is showing the number of microbes on a product such as milk, meat, and eggs and its processing by counting the number of bacterial colonies grown in the Nutrient agar.

Calculation of the Number of Microorganisms

Colony counts were carried out on agar nutrien showing growth between 25-250 colonies. If the growing colony is less than 25 or more than 250, then calculations are carried out at retail higher. Number of colonies less than 25, recorded according to the actual number of lowest dilution level.

Microorganisms quantity calculation formula

Number of microorganisms (CFU/ml) = Number colony x Dilution factor.

$$\text{Dilution factor} = \frac{1}{Dilution\ rate}.$$

Sample Inspection Procedure

Sterilization of tools and materials done to avoid contamination bacteria from the tools and materials used so that it can interfere with research results. Sterilization is done by Autoclave with a temperature of 121˚C and a pressure of 1 atm for 15 minutes.

Sample dilution was carried out with how to mix 25 grams of grilled meatballs with 225 ml of 0.1% BPW solution. Comparison of sample and 0.1%

BPW solution is 1:9. Baked meatballs and 0.1% BPW solution put in sterile plastic then homogenized with a stomacher. Next poured into a test tube so that to a 10^{-1} dilution. Then move as much as 1 ml of suspension from a dilution of 10^{-1} with a sterile pipette into 9 ml 0.1% BPW solution to get 10^{-2} dilution. The same way is done up to a 10^{-3} dilution. The results of each the dilution are taken as much as 1 ml and put in a Petri dish in duplicate. After that added 15-20 ml MSA in each Petri dish already contains suspension and is shifted horizontally by forming a figure eight so that the suspension is evenly mixed. Media so that it is allowed to solidify and so on incubated at 34-36˚C for 24 hours.

Data analysis

Analysis of the data used analysis variance of two-way pattern ANOVA to find out the resulting average difference sample group and proceed with the test Duncan.

Results and Discussion

Staphyloccus aureus contamination on Grilled Meatballs. Calculation of the amount of contamination of *Staphylococcus aureus* was performed with using Mannitol Salt Agar media (MSA), which is a selective medium differential for the isolation and identification of bacteria *Staphylococcus* sp (Hayati et al., 2019). Media This can inhibit the growth of bacteria Gram negative due to the salt content of NaCl with a high concentration (7.5%-10%), thereby inhibiting the growth of other bacteria (Boerlin, 2003).

Growth of *Staphylococcus aureus* on MSA media showed colonies that were yellow, round, shiny, elevation convex, and smooth colony surface can be seen in Figure 1. This is appropriate with research conducted by Dewi (2013), regarding the isolation and identification of bacteria *Staphylococcus aureus*. The ability of *Staphylococcus aureus* ferments mannitol causes an increase in acid levels in media, so as to be able to change indicators pH that changes the MSA medium from colorless red to yellow (Rahmi et al., 2018).

Isolates suspected to be *Staphylococcus aureus*, then Gram-stained catalase test as a confirmatory test. Results Gram stain shows bacteria that purple (Gram positive) and shaped cocci. (Figure 2). Purple color in bacterial cells due to the structure of the Gram cell wall positive consists of thick peptidoglycan, so that it can retain the dye primer (crystal violet) (Karimela et al., 2017). This is in accordance with the opinion of Jawetz et al. (2005), that *Staphylococcus aureus* It is a -shaped Gram-positive bacterium cocci, arranged in groups or pair like grapes, facultative anaerobic and non-motile.

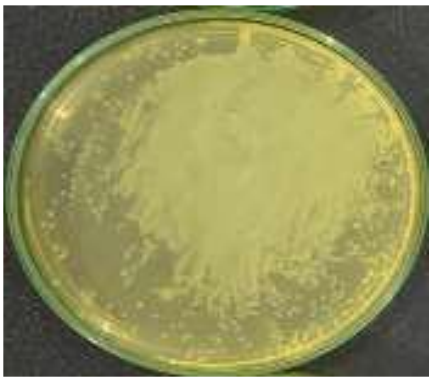

Figure 1. Microbial colonies on media Mannitol Salt Agar (MSA) grilled meatballs.

In the catalase test, these bacteria were able to hydrolyzes H2O2 to H2O and O2 indicates the characteristics of *Staphylococcus* (Figure 3). It fits with the opinion of Malelak (2015), which stated that the catalase test on bacteria cocci-shaped can distinguish between Streptococcus and *Staphylococcus* bacteria, where *Staphylococcus* bacteria will give positive results in the catalase test capable of break hydrogen peroxide (H2O2) bonds into water and gas bubbles.

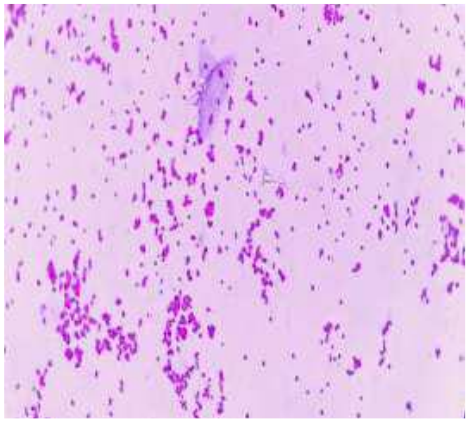

Figure 2. Image of the results of Gram staining of Staphylococcus aureus bacteria using a microscope with 1000x magnification. Description: a) bacteria in the form of cocci.

Figure 3. Description of the catalase test results for Staphylococcus aureus bacteria. Information: a) Positive result of the catalase test, b) Negative control of the catalase test.

Staphylococcal Qualitative Analysis aureus on grilled meatballs

Bacterial contamination research results *Staphylococcus aureus* on grilled meatballs indicates the presence of a significant amount of contamination quite high in these foodstuffs. It can be observed from the amount of contamination microbes in the control group on each traders are 2.5 x 10^4 , 3.1 x 10^4, and 2,6 x 10^4. Treatment with burning for 1 minute, 3 minutes and 5 minutes at temperatures ranging from 56˚C-59˚C known can reduce the amount of contamination of *Staphylococcus aureus* on grilled meatballs (Table 1).

Table 1. The results of calculating the average contamination microbes in the given grilled meatballs combustion treatment for 1 minute, 3 minutes, and 5 minutes.

Burning Duration	Microbial Contamination Rate (CFU/g)		
	Merchant 1	Merchant 2	Merchant 3
Control	$2,5 \times 10^4$	$3,1 \times 10^4$	$2,6 \times 10^4$
1 minute	$1,3 \times 10^4$	$1,9 \times 10^4$	$1,5 \times 10^4$
3 minute	$3,5 \times 10^3$	$4,4 \times 10^3$	$3,9 \times 10^3$
5 minute	$2,9 \times 10^1$	$3,6 \times 10^1$	$3,1 \times 10^1$

Data shown in Table 1. indicates that the amount of contamination *Staphylococcus aureus* bacteria on baking for 1-3 minutes at temperature ranging from 56˚C-59˚C cannot yet eliminates most of the bacteria. Use at this temperature is based on material and temperature used by traders to prepare grilled meatballs. Generally, traders use charcoal from coconut shell with less fire hot. In the treatment group 1-3 minutes has shown a decrease in numbers significant microbe, but still showed more colony growth higher than the requirements of SNI 08-1-1-7388: 2009 ie 1 x 102 CFU/g.

Based on this can It is known that the internal combustion process 1 and 3 minutes with temperatures ranging from 56˚C-59˚C, not yet efficient in minimizing the amount *Staphylococcus aureus* contamination in meatballs fuel, and the product is not yet feasible for consumed. This is because *Staphylococcus aureus* is included group of thermophilic microorganisms resistant to heating (Saimah et al., 2016). At a longer burning time (5 minutes), the amount of *Staphylococcus aureus* contamination decreasing until it reaches $3,6 \times 10^1$ so this snack can be declared already fit for consumption.

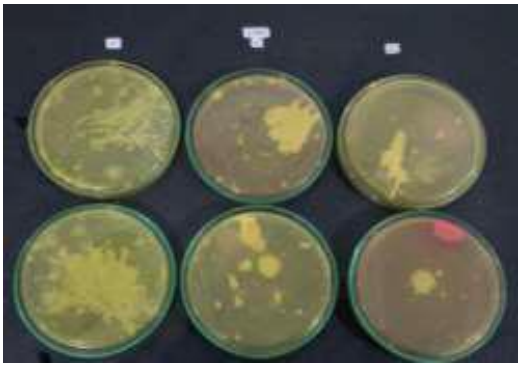

Figure 4. Microbial colonies on burning 5 minutes on Mannitol medium Salt Agar (MSA) grilled meatballs

Effect of burning time on the amount of contamination *Staphylococcus aureus* on meatballs burn

The results of the analysis of pattern two Anova test direction of the amount of contamination *Staphylococcus aureus* shows value the significance is 0.000 (p-value < 0.05). Based on the results of the analysis, it can be concluded that respectively treatment (time of burning) have a significant effect to the average amount of bacterial contamination *Staphylococcus aureus* on grilled meatballs. While in the follow-up test using Duncan's test showed that result significantly different between each treatment (burning time) that is for 1 minutes, 3 minutes and 5 minutes.

Based on Table 2 average The highest microbial contamination was observed in Baked meatballs are in treatment 1 namely burning process for 1 minute 5.49 log CFU/g and the average pollutant value The lowest microbial is found in the process burning for 5 minutes of 1.64 log CFU/g. While the Anova test analysis two-way pattern on groups (traders) shows its significance value 0.083 (pvalue > 0.05) gives effect the same as the average number *Staphylococcus aureus* contamination. In the k group (traders) there is no need Duncan's further test was carried out because of group (trader) does not exist difference.

Table 2. Average ANOVA calculation results microbial contamination in grilled meatballs which was given combustion treatment for 1 minutes, 3 minutes and 5 minutes.

Burning Duration	Microbial Contamination Rate (CFU/g)		
	Merchant 1	Merchant 2	Merchant 3
Control	$2,5 \times 10^4$	$3,1 \times 10^4$	$2,6 \times 10^4$
1 minute	$1,3 \times 10^4$	$1,9 \times 10^4$	$1,5 \times 10^4$
3 minute	$3,5 \times 10^3$	$4,4 \times 10^3$	$3,9 \times 10^3$
5 minute	$2,9 \times 10^1$	$3,6 \times 10^1$	$3,1 \times 10^1$

Description: a,b,c,d Different superscripts in the same column shows significant difference (P<0.05)

Burning is one procedure that are often performed for decontaminate the microbial count of the product food and equipment. General combustion can kill pathogenic bacteria, though have not been able to kill spores (Mailia et al., 2015). Some bacteria are heat tolerant too requires temperature and burning time which takes longer to be eliminated optimum pathogen. the longer the time in the combustion process it gets smaller (5 minutes) the growth of viable bacteria increases, and vice versa with temperature combustion, the higher the temperature used, the smaller the growth bacteria (Yudhistira et al., 2017).

Burning temperature and time have a major effect on the amount of contamination *Staphylococcus aureus* bacteria in meatballs burn. In the control group that was not given combustion treatment can be observed the amount of contamination the number of growing microbes very high, namely 5.49 log CFU/g. According to Ray and Bhunia's statement (2008), that In general, several types of bacteria, yeast, mold, and the virus will experience death when heated at 66oC for 10 minutes, one of them is bacteria *Staphylococcus aureus*. Heating process caused some damage important structural and functional components from cells (Wu, 2008). Microbes treated with combustion at time and temperature certain heating will experience a heatsock phase, sublethally injured or death.

The heating temperature will cause open bacterial cell walls resulting in Plasmids enter cells rapidly called the heat-shock phase. In the sublethal phase or death, the cytoplasmic membrane remains intact on injured bacterial cells but lose permeability function. These cells will loss and change conformation of the cellular material in injured cells which causes bacteria to produce Autolytic enzymes cause cell lysis bacteria (Ray and Bhunia, 2008). Happening Bacterial death due to the combustion process characterized by loss of

cell permeability bacteria. In sublethal bacteria Usually there is damage to the cell wall cell membrane, ribonucleidacid (RNA), deoxyribonucleid acid (DNA), ribosomes and enzymes present in the bacteria (Saimah et al., 2006).

In addition to the effect of burning time, the amount of microbial contamination is also affected by several factors such as processing environment, processing, presentation, and environment presentation (Malelak et al., 2015). In this study, the presentation process and conditions presentation environment of the three merchants Grilled meatballs are still considered bad. Sale Grilled meatballs are done at the same location adjacent to public roads, and canals disposal of water containing a lot of garbage. Bacterial contamination of food products can occur from intermediary animals, traders, equipment and environment (Khoiriyah, 2011). Number of bacterial contaminations in traders 2 observed to be higher than traders 1 and 3, this happens because Merchant 2 does not store meatballs inside sealed container for easier exposure by reservoir animals, dust and vehicle fumes (Mayaserli and Anggraini, 2019)

This is in accordance with research Prananda et al. (2019), total bacterial contamination *Staphylococcus aureus* on grilled meatballs exceeds the SNI limit because there is the number of traders is still lacking attention to cleanliness of equipment and serving of grilled meatballs. Usual equipment used by grilled meatball traders to be source of bacterial contamination if not cleaned to the maximum that will be increase the number of pathogenic bacteria, especially the part that is in direct contact with the meatball fuel, like a knife used to cut raw materials, reused for cutting cooked food without cleaned first and left in an open place (Mayaserli and Anggraini, 2019). Plus, handling food that does not wash hands first first and directly use hands that contain microbes can also triggers the growth of pathogenic bacteria in meatballs the fuel causing the displacement microbes from hands to food, and will multiply in food (Hariyati et al., 2018).

In addition to the risk of infection, the presence of bacteria such as Staphylococcus aureus in food products also poses the potential for intoxication (Nurmila and Kusdiyantini, 2018). Detectable in food products when bacterial contamination reaches a minimum level of 1 x 105 CFU/g (Salasia et al., 2009), enterotoxins produced by Staphylococcus aureus exhibit resistance to the heat used in cooking. Consequently, although heating can eliminate the bacteria, the enterotoxins can still induce food poisoning (Rahayu et al., 2014). Symptoms observed in individuals consuming food products containing Staphylococcus aureus enterotoxins include abdominal pain, vomiting, and diarrhea (Lestari et al., 2018).

Conclusion

The efficacy of reducing Staphylococcus aureus bacterial contamination in grilled meatballs was observed with a burning time of 5 minutes and a temperature range of 56˚C-59˚C. The contamination of Staphylococcus aureus in grilled meatballs is influenced by factors related to hygiene and sanitation.

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