RADINKA JOURNAL OF SCIENCE
AND SYSTEMATIC LITERATURE REVIEW

ISSN 2988-5825

https://www.rjupublisher.com/ojs/index.php/RJSLR

Bacteria Contaminants in Meatballs Sold in The Market Padang City Raya

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Abstract

Meatballs are a processed meat product that has high nutrition. The maximum shelf life for meatballs made from meat is 1 day (12 hours). Storing meat foods at inappropriate temperatures, such as room temperature, greatly facilitates the growth of microbes. The marketplace for meatballs in traditional markets in the city of Padang is still sold on the streets at open-air temperatures, and without using sufficient refrigeration temperatures. The possibility of contamination with microorganisms is very large. This research aims to determine the suitability and quality of meatballs sold at Pasar Raya Padang City by examining the Total Plate Count (TPC) and identifying the types of bacteria that contaminate meatballs sold at Pasar Raya Padang City. The research results showed that the amount of microbial contamination in meatballs varied, namely 1.9x10 6, 7.5x10 6, 5.6x10 6, 1.8x10 6, 8.6x10 6, and 9.2x10 6. The results of this study showed that the meatball samples had microbial contamination greater than 10 5 CFU/gr or did not meet SNI standards. Based on macroscopic and microscopic morphological identification as well as biochemical reactions, all isolates were classified as bacteria from the genus Bacillus.

Keywords: Meatballs, Bacillus bacteria, contaminant

Acknowledgments: Thank you to the head of the Biology education study program who has permitted this research.

For citation:
Sutra, B., Indriati, G., & Megahati S, R. R. P. (2024). Bacteria Contaminants in Meatballs Sold in The Market Padang City Raya. Radinka Journal of Science and Systematic Literature Review, *2*(1), 240-248.

Acknowledgments: Thanks to all parties who have supported the implementation of this research. I hope this research can be useful.

Introduction

Food and beverage products that will be consumed by the public must be based on food health standards or requirements. Food and drink are basic needs for humans, which require good management so that they can be beneficial for the body. Food is a good medium for the growth of various kinds of microorganisms. Microorganisms can decompose proteins, ferment carbohydrates, and make fats and oils smell rancid. Although many microorganisms are not dangerous to humans, some microorganisms can cause damage, cause disease, or produce toxins in food and can cause poisoning (Hernández-Cortez et al., 2017). Meat is a food ingredient that is very vulnerable to contamination by exposure to microbes. Meat has a high risk of bacterial contamination, good handling is very necessary to extend the shelf life of meat (Papadochristopoulos et al., 2021); (Nethra et al., 2023).

According to (Arief, 2017), microbial contamination that is often contained in meatballs includes *Staphylococcus aureus, Escherichia coli*, *Salmonella sp*, *Bacillus cereus,* and *Clostridium perfringens*. According to (Laksanawati et al., 2024), Meatballs are a processed meat food that has high nutrition, the maximum shelf life is 1 day (12 hours). The nutritional content of meatballs consists of a minimum protein content of 9%, a maximum fat content of 2%, a maximum water content of 70%, and a maximum ash content of 3%. The nutritional content of these meatballs is very good for microbial growth, which is why meatballs made from meat have a relatively short shelf life. Storing meat foods at inappropriate temperatures, such as room temperature, can facilitate the growth of microbes. To overcome this, storing food using refrigerated temperatures such as in a refrigerator is very important. The development of microorganisms and the formation of toxins are related to time, place, and temperature. Therefore, it is best to store food for a long time using freezing temperatures (Teuteberg et al., 2021).

According to (Kumar et al., 2023) sales locations in open spaces, such as on the side of the road, are susceptible to contamination by bacteria. Data from the Padang City Health Service there was an outbreak (extraordinary event) of food poisoning due to consuming meatballs with a total of 13 victims, of which 12 people were treated outpatient and 1 person was hospitalized. The Padang city market is the same as most traditional markets in Indonesia, namely that it tends to be damp and lacks cleanliness. Seeing the condition of the market location which is muddy, and damp, and the meatballs being sold in an open location, it is easy for contamination to occur. This research aims to determine the suitability and quality of meatballs sold at Pasar Raya Padang City by examining *the Total Plate Count* (TPC) and identifying the types of bacteria that contaminate meatballs sold at Pasar Raya Padang City.

Methodology

Study This was carried out in the Microbiology Laboratory of Feed Science Technology Unand, West Sumatra, and identification of contaminating bacteria was carried out at the Regional Veterinary Investigation and Testing Center II Bukit Tinggi. This research is a descriptive study, namely a study that aims to determine the total microbial contamination in meatballs and to see the types of bacteria that contaminate meatballs sold in the Padang City market. The sampling technique was carried out by purchasing samples randomly at the Padang City Grand Market, totaling 6 samples from each market trader. Each trader takes the same number of samples. Next, bacteriological testing was carried out using the TPC (*Total Plate Count*) method, macroscopic and microscopic morphological identification, and biochemical identification.

In study tools used namely: incubator, autoclave, spectrophotometer, filter lamp, Erlenmeyer flask, 100 ml measuring cup, test tube, petri dish, measuring pipette, mortar, test tube rack, aluminum foil, tube needle, drill glass, electric stove, microscope, object glass, bunsen, dropper pipette, oven, incubator, rubber suction cup, label paper, tissue and camera as documentation, mask, gloves Used as material namely: meatball samples, NA (*Nutrient Agar*), 70% alcohol, 0.9 % NaCl, 3% H_2O_2, Safranin and crystal violet.

Sampling Place

Samples of meatballs were taken at the Raya market in Padang City where the meatballs used as samples were raw meatballs sold by market traders. Research sampling was carried out randomly.

Sterilization of Tools and Materials

Before tools and materials are used, all tools and materials are sterilized first by wrapping the tools and materials in the newspaper then put into an autoclave with a temperature of 121 ^{o}C and a pressure of 15 psi (per square inch) for 15 minutes, as for tools that are not heat resistant then sterilized using 70% alcohol.

Sample Preparation

The meatball sample was weighed 5 grams and cut into small pieces, then ground using a mortar until smooth. In the first dilution, the mashed meatballs were put into an Erlenmeyer tube containing 225 ml of Aquades and then homogenized. 1 ml of the homogenized solution was pipetted and poured into a test tube containing Aquades. 9 ml was then homogenized again (dilution 10^{-1} to a dilution level of 10^{-5}). The homogenized dilution taken as much as 1 ml will be cultured in NA (Nutrient Agar) media.

Total Plate Count (TPC) Check

Samples of all dilutions were taken as much as 1 mL and then placed into a sterile petri dish. Nutrient Agar media which is still in a liquid state at a temperature of ± 45˚C is poured into a petri dish containing the sample solution. The petri dish is shaken clockwise so that the solution is mixed evenly and then allowed to solidify. All Petri dishes were incubated at 37˚C for 48 hours. then count the number of colonies that grow directly.

Bacterial Isolation

Samples of all dilutions were taken as much as 1 mL, then poured into a petri dish, then NA media was poured in and homogenized. All Petri dishes were incubated at 37˚C for 48 hours. If bacterial growth forms from the petri dish that has been incubated, the bacteria are taken using a loop needle for culture and then inoculated on new NA media to obtain a single colony. Single colonies were streaked into sterile NA medium and stored as stock cultures for further testing.

Characterization of Bacteria

A total of one cycle of bacteria was implanted into the NA medium of a petri dish using the streak quadrat method. Bacteria growing in Na medium in petri dishes were then observed for colony morphology using a monocular microscope including the shape of the bacterial colony, the edges of the bacterial colony, and the elevation of the bacterial colony as in Figure 5. Morphological characteristics of bacterial colonies (Hadioetomo, 1993).

Gram stain

If gram-positive bacteria can be seen in purple, it shows that the bacteria can bind crystal violet, and if gram-negative bacteria can be seen in pink, it shows that the bacteria are not able to bind crystal violet and are only colored by safranin.

Bacterial Biochemical Test

a. Prepare 15 types of biochemical test medium
b. Then inoculate the isolate that has been marked gram-positive and negative, then inoculate with the method of stabbing ose until the bottom tube (liquid medium) and etched zig Zag on the surface of the solid candy medium.
c. After that, incubate in an incubator at 37 ^{0}C for 18-24 hours
d. The next step is carried out reading biochemical tests through several test stages, namely TSAI Test (*Triple Sugar Iron Agar*), SIM Test (*Sulphid Indol Moltility*), Urea Test, Citrate Test, Sugar Test (lactose, glucose, sucrose, maltose), MR Test (*Methyl Red*), VP Test (*Voges-Proskauer*), O/F Test (Oxidation Fermentation), and Nitrate Test.

Data analysis

Data analysis was carried out by describing *the Total Plate Count* (TPC) results and based on the results of bacteriological analysis of meatballs sold at Pasar Raya Padang City. The number of colonies that grew on each plate was counted to obtain data in the form of the number of bacterial colonies (CFU/gram). The analyst will be presented with a Standards Plate Count (SPC) in table form to make reading easier.

Results and Discussion

1. Analysis of microbial contamination in meatballs is seen from the total number of colonies that grow

Based on the results of bacterial contamination tests on meatballs sold at Pasar Raya Padang City, it was found that the number of bacterial colonies that grew was different at each sample location where the meatballs were taken. The largest number of bacterial colonies growing there is on location trader meatball near rubbish namely 9 2 colony.

Results Analysis of bacterial contamination of meatballs can seen in Table 1.

Table 1. Analysis of Microbial Contamination in Meatballs.

No	Sample	Growing colonies	Total Bacterial Colonies (cfu/gram)
1	Near coconut milk traders (Sp 1)	19	1.9×10^{6}
2	Near the chili seller (Sp 2)	75	7.5×10^{6}
3	Near the vegetable seller (Sp 3)	56	5.6×10^{6}
4	Near the fruit seller (Sp 4)	18	1.8×10^{6}
5	Near fish traders (Sp 5)	86	8.6×10^{6}
6	Near trash (Sp 6)	92	9.2×10^{6}

2. Observation of the morphology of bacteria in meatballs sold at Pasar Raya Padang City, including the color of the bacterial colonies, the shape of the colonies bacteria, and the edges of bacterial colonies. The research results can be seen in Table 2.

Table 2. Morphology of bacterial colonies in meatballs

		Bacterial Colony Morphology			
No	Sample	Color	Form	Edge	Elevation
1	Sp_1 1	White	Round	Slippery	Arise
2	Sp_1 2	White	Irregular And spread	Notched	Arise
3	Sp_2 1	White	Round	Slippery	Arise
4	Sp_3 1	White	Round	Slippery	Arise
5	Sp_3 2	White	Irregular And spread	Notched	Arise
6	Sp_4 1	White	Irregular	Notched	Arise
7	Sp_4 2	White	Round	Slippery	Arise
8	Sp_4 3	White	Irregular And spread	Notched	Arise
9	Sp_5 1	White	Round	Slippery	Arise
10	Sp_5 2	White	Irregular	Notched	Arise
11	Sp_6 1	White	Irregular	Notched	Arise
12	Sp_6 2	White	Round	Slippery	Arise

3. Based on the results of macroscopic observations of contaminating bacteria in meatballs, all colonies in isolates generally have the morphological characteristics of a round, irregular shape and are spread out with smooth edges and some have grooves, while the elevation of all colonies is raised and the bacterial colonies are white. The research results can be seen in Table 2 Morphological microscopic identification of bacteria in meatballs sold at Pasar Raya Padang City, including Gram staining, Endospores, and the shape of bacterial cells. The research results can be seen in Table 3.

Table 3. Results of the Gram staining test and morphological microscopic identification of bacteria in meatballs

		Bacterial Colony Morphology		
No	Sample	Grams	Endospores	Cell shape
1	Sp_1 1	Positive	There is	Basil
2	Sp_1 5	Positive	There is	Basil
3	Sp_2 1	Positive	There is	Basil
4	Sp_3 1	Positive	There is	Basil
5	Sp_3 2	Positive	There is	Basil
6	Sp_4 1	Positive	There is	Basil
7	Sp_4 2	Positive	There is	Basil
8	Sp_4 3	Positive	There is	Basil
9	Sp_5 1	Positive	There is	Basil
19	Sp_5 2	Positive	There is	Basil
11	Sp_6 1	Positive	There is	Basil
12	Sp_6 2	Positive	There is	Basil

Results study show that all colonies of isolates obtained the same results, namely all isolates of Gram-positive bacteria, producing endospores and bacillus-shaped cells.

4. Biochemical identification of meatball bacteria sold at Pasar Raya Padang City, including the TSIA (*Trepton Soya Iron Agar)* test, SIM (Sulfide *Indole Motility*) test, and urea test. Citrate test, lactose test, glucose test, cycle test, maltose test, MR (*Methyl Red*) test, VP (*Voges-Proskauser*) test, oxidation test, fermentation test, nitrate test, arabinose test, xylose, and gelatin test. The research results can be seen in Table 4.

Table 4. Results Biochemical identification of meatball bacteria.

No	Sample	Bacterial Colony				
		Sp $_{1}$ 1	Sp $_{1}$ 5	Sp $_{2}$ 1	Sp $_{3}$ 1	Sp $_{4}$ 1
1	TSIA	m/k	k/k	m/k	k/k	m/k
2	driver's license	+	+	+	+	+
3	Urea	-	-	-	-	-
4	Citrate	-	-	-	-	-
5	Lactose	-	-	-	-	-
6	Glucose	+	+	+	+	+
7	Sucrose	-	-	+	-	-
8	Mannitol	-	-	+	+	+
9	MR	+	+	+	+	+
10	VP	+	+	-	+	-
11	Oxidation	+	+	+	+	+
12	Fermentation	-	-	-	-	-
13	Nitrate	-	-	+	-	+
14	Arabinose	-	-	-	-	-
15	Xylose	-	-	-	-	-

Based on on Table 4 above can be seen that after biochemical testing was carried out with several test stages, different results were obtained. The TSIA test on isolates Sp $_{1}$ 1, Sp $_{2}$ 1, and Sp $_{4}$ 1 showed red color in the Slant section and yellow color in the butt section of the TSIA media. This shows that the bacteria are alkaline and that there is lactose and sucrose fermentation. Meanwhile, isolates Sp $_{1}$ 5 and Sp $_{3}$ 1 showed a yellow color on the slant and butt of the TSIA media. This shows that the bacteria are acidic and that there is lactose and sucrose fermentation. The results of biochemical tests using TSIA media showed positive results that all of these bacteria could use this media. in the SIM test, MR test, and gelatin test all isolates showed positive reactions. In the citrate test, urea test, lactose test, oxidation test, fermentation test, arabinose test, and xylose test, all isolates showed negative reactions. In the glucose test, only isolates Sp $_{1}$ 1 and Sp $_{2}$ 1 reacted positively. In the sucrose test, isolate Sp $_{2}$ 1 reacted positively. In the mannitol test isolates Sp $_{2}$ 1, Sp $_{3}$ 1 and Sp $_{4}$ 1 reacted positively.

The possibility of increased microbial contamination comes from Microbial contamination during the meatball-making process (Amqam, Natsir, et al., 2021); (Amqam, Manyullei, et al., 2021); (Tóth et al., 2021). The source of contaminants is possibly from the tools or ingredients added (tapioca, shell flour mangosteen fruit, spices, or other ingredients) in the processing process which can also come from unhygienic processing methods (Granados-Chinchilla et al., 2018). Contamination may occur if the finished food being produced is in direct contact with a surface table or food processing equipment during the previous preparation process contaminated by pathogenic microbes (Valero et al., 2016).

Meatballs are very susceptible to bacterial growth (Mouton, 2018); (Rebezov et al., 2021). This is caused by using meat as a basic ingredient, providing raw materials, and manufacturing processes, with the presentation of meatballs still using traditional methods and not paying attention to hygienic practices in the process. Meatball snacks that are often sold around or around the side of the road will be more easily contaminated by bacteria. Recontamination by Microbes can be caused by several things such as traveling traders having a place that is more open, less consistent temperature control, and less washing of serving utensils clean (Carrasco et al., 2012). One of the microbes that is an indicator of sanitation is coliform bacteria (Wen et al., 2020).

Pathogenic bacteria *Escherichia coli* is used as an indicator of food contamination by dirt as well as other microorganisms in the digestive tract and their presence indication of problems in the food processing process (Mohamed & Habib, 2023). This bacteria is a foodborne disease or what is called a disease caused by consuming food and drinks contaminated with viruses, bacteria, and parasites and is dangerous for humans, and it can even cause death. This bacteria can be found in ruminants, pigs, dogs, cats, and poultry (Demirbilek, 2018),; (Hamame et al., 2022); (Almansour et al., 2023). E. coli is included in the agent pathogen of foodborne illness because some strains of E. coli are pathogenic in humans and animals (Pokharel et al., 2023); (Yang et al., 2017); (Indraswari et al., 2021). Foodborne illnesses associated with various food products such as meat, vegetables, fruit, and other foods (Amit et al., 2017), The way of contamination is through hands, the evisceration process, pollution indirectly through water pollution, and product packaging (Muhib et al., 2023). On poultry products, E. coli has been used to assess the microbiological safety, and sanitary conditions during processing, and maintain the quality of health products communities around the world

Conclusion

Based on the results of research that has been carried out, it shows that the amount of microbial contamination in meatballs varies, namely 1.9×10^{6}, 7.5×10^{6}, 5.6×10^{6}\, 1.8×10^{6}, 8.6×10^{6} and $9.2 \times 10^{6.}$ The results of this study show that the meatball sample has microbial contamination greater than 10^{5} CFU/gr or does not meet SNI standards. Based on macroscopic and microscopic morphological identification as well as biochemical reactions, all isolates were classified as bacteria from the genus *Bacillus.*

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