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The effect of cooking methods to the existence of *Bacillus* sp. spores in beef

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ABSTRACT

The aim of this research was to determine the existence of *Bacillus* sp. spores in beef after being given the cooking methods. This research used posttest-only control group design by using four treatments and five repetitions. This research used 50 grams of beef obtained from traditional market in Sedati, Sidoarjo and given 1 mL of *Bacillus* sp. spore solution according to 0.5 *McFarland* standards. The kind of treatments were steaming, grilling, frying, and using autoclave. The result showed that cooking method by steaming, grilling, and frying could not kill *Bacillus* sp. spores in beef. This was proven by the growth of *Bacillus* sp. colonies in Nutrient Agar (NA) taken aseptically from the beef sample after being treated. The characteristic were large size, flat, uneven edges, and give a distinctive smell of sour smell, also showed rod-shaped and spores form in the microscopic examination by spore staining test. Nonetheless, the cooking method by using autoclave could kill *Bacillus* sp. spores in beef. This was proven by there were no *Bacillus* sp. growth in Nutrient Agar (NA). Based on those results, it could be concluded that autoclave is effective to kill *Bacillus* sp. spores in beef. The results were analyzed by Chi Square test with SPSS version 20 for Windows.

Key words : *Cooking method, Bacillus sp. spores, Beef*

Introduction

Food is the most basic and essential need for human beings. Food security in Indonesia needs more serious attention because pathogenic microorganisms can grow well on food origins of livestock, therefore it often cause some problems such as anthrax, food borne disease, meat borne disease, waterborne disease (Erni, 2009). Beef is one of the animal's products that act as a source of protein that taking a responsibility as a building and regulating substance in our body system. Beef protein contains complete amino acid composition and many essential nutrients needed for health, thereby it potentially becomes a good medium for microbial growth, such as coliform bacteria (Erni, 2009). The damage of meat is mainly caused by the growth of the decomposing bacteria and pathogenic bacteria (Erni, 2009). Signs of the damage of meat are changes in odor (rot and rancid odor), color, taste, and the mucus. Meat damage contains pathogenic bacteria (bacteria that cause disease) and spore-forming bacteria (Erni, 2009). Bacterial spore is the form of bacteria that attempts to secure themselves from bad influences from the outside. Anthrax is a bacterial disease that attacks herbivores and other mammals, including humans. The bacteria that causes anthrax is *Bacillus anthracis* (Kementerian Pertanian Indonesia, 2016).

Heating is one of the best ways of beef processing, which is known as cooking process. Cooking

process is required before consuming the beef. The aims of the cooking process are intended to obtain a better flavor, aroma and texture, killing vegetative microbes and activating all enzymes. The several kinds of cooking methods are boiling and steaming at 100 °C, grilling, roasting, baking and frying at 150 – 300 °C (Sundari, 2015). In Indonesia, most of the community is still not aware of *Bacillus anthracis* and they don't understand it the most about the proper way of beef processing to kill bacterial spores. Those are the main reasons why there is still a lot of beef contaminated by bacteria such as *Bacillus anthracis*, even though the beef has passed the cooking phase.

Materials and Methods

This research was conducted on March – April 2019 in the Microbiology Laboratory BSL-2 Universitas Airlangga. The microscopic examination was done in Bacteriology and Microbiology Laboratory Faculty of Veterinary Medicine Universitas Airlangga. This research was using beef obtained from traditional market in Sedati, Sidoarjo. The criteria were among others, silver side part, 50 grams weight per each sample with a size of 12 x 9 cm and around 1 cm thick. The beef was given 1 mL of *Bacillus* sp. spore solution according to 0.5 *McFarland* standards obtained from Microbiology Laboratory BSL-2, Universitas Airlangga Surabaya.

Sample Preparation

The beef was obtained 200 grams and divided into 4 section, and then given 1 mL of *Bacillus* sp. spore solution according to 0.5 *McFarland* standards was inoculated into each beef sample by injection using disposable syringe. The samples classified into four treatment groups, the first group was inoculated beef cooked by steaming (I), inoculated beef cooked by grilling (II), inoculated beef cooked by frying (III), and inoculated beef cooked by autoclave (IV).

Cooking Treatments

Inoculated beef samples in the group I were treated by steaming method approximately at 100 °C temperature for 5 minutes. Inoculated beef samples in the group II were treated by grilling method approximately at 120 °C temperature for 4 minutes. Inoculated beef samples in the group III were treated by frying method approximately at 160 °C temperature for 3 minutes. Inoculated beef samples in the group IV were treated by using autoclave method approximately at 121 °C temperature with 1.5 atm for 15 minutes. After being treated, each sample was immediately identified the presence of *Bacillus* sp. spores by isolating in Nutrient Agar (NA) media by streak method.

Bacterial Isolation on Nutrient Agar (NA)

Bacterial isolation in Nutrient Agar (NA) is done by streak method. Ose that has been touched or affixed to the sample is scratched on the surface of the isolating media by making zig-zag lines or as long as possible or as many dotted lines as possible by not touching or tangent to the lines that have been formed before. Bacteria that has been isolated will be incubated at 37 °C for 24 - 48 hours.

Results

The result from 20 samples showed 15 samples were positive and 5 samples were negative. The research result was explained as follows: (1) five samples which treated by steaming were positive; (2) five samples which treated by grilling were positive; (3) five samples which treated by frying were positive; (4) five samples which treated by using autoclave were negative. Samples with positive results are seen with the presence of *Bacillus* sp. growth in Nutrient Agar (NA). The growth of bacteria in Nutrient Agar (NA) showed the characteristics as follows, yellowish white color, uneven edges and rough surface. Samples with negative results

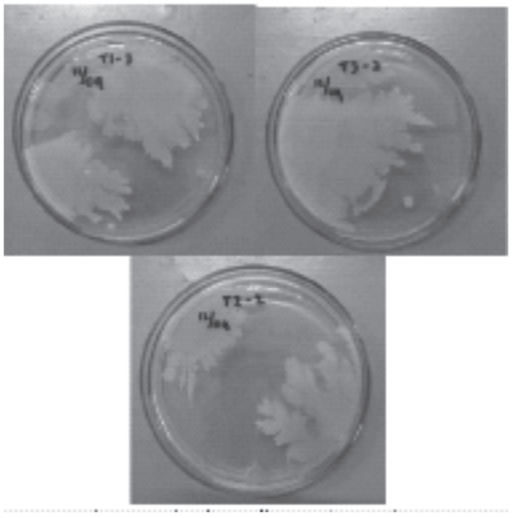

Fig. 1. The growth of *Bacillus* sp. in Nutrient Agar (NA) taken from beef sample after being treated. (1) By steaming (2) By grilling (3) By frying.

are seen with there were no *Bacillus* sp. growth in Nutrient Agar (NA). Bacterial inoculation on Nutrient Agar (NA) was aseptically taken from beef that had been given those several cooking treatments. Bacterial inoculation was carried out immediately after being given cooking treatment. After that, the bacteria that had been isolated were incubated approximately at 37 °C for 24 - 48 hours. The growth of bacteria in Nutrient Agar (NA) showed the characteristics as follows, yellowish white color, uneven edges and rough surface.

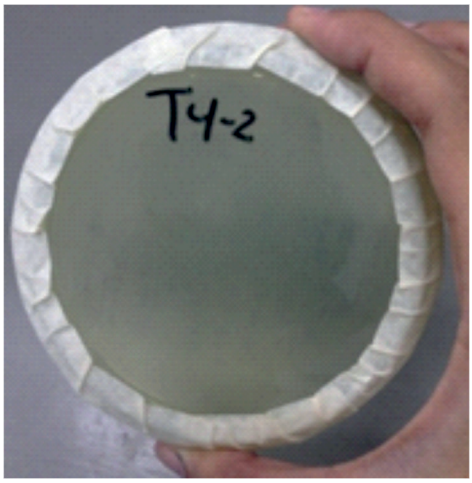

Fig. 2. There were no *Bacillus* sp. growth in Nutrient Agar (NA) taken from beef sample after being treated by using autoclave.

The result of the bacterial growth in beef (Table 1) showed that five repetitions of each treatment such as steaming, grilling and frying were positive, which means *Bacillus* sp. spores were still alive in the sample, but the five repetitions by using autoclave showed negative results which means there were no *Bacillus* sp. spores in the sample.

Disscusion

The processing of beef cooking by steaming method at 100 °C temperature in 5 minutes, grilling at 120 °C in 4 minutes and frying at 160 °C in 3 minutes could not kill *Bacillus* sp. spores. The processing of beef by steaming, grilling and frying methods were carried out by five repetitions for each method, which means there were 15 samples in total. The total 15 samples showed positive results after being treated by steaming, grilling and frying, hence there was *Bacillus* sp. spores in the samples.

The treatment of beef cooking by using autoclave at 121 °C temperature with 1.5 atm in 15 minutes could kill *Bacillus* sp. spores. Spores are the survival form of several bacteria such as *Bacillus* sp. in the unfavorable environmental conditions. Spores are more resistant to the heat than the vegetative cells. *Bacillus* sp. spore has a capability to survive in the heat that often used to processing raw food. After it is known that spores are resistant to the heat, the presence of spores must be essentially considered in the food processing. The endospores that produced by *Bacillus* sp. have a high resistance to the physical and chemical factors, as well as the extreme temperature (Hatmanti, 2000). If there are still any *Bacillus* sp. spores in the food after being cooked, the spores will become the vegetative cells, pathogen and toxigent that cause food poisoning. The disease due to *Bacillus* sp. is the food poisoning that included in the raw food which is mostly caused by *B. subtilis* and *B. cereus*. The spores will be gradually contaminated food, including the meat products and eggs. The toxin that produced by *B. cereus* will cause diarrhea or vomit (Tallent, 2012).

The processing of beef by using autoclave at 121 °C temperature with 1.5 atm for 15 minutes did not show *Bacillus* sp. spores in the samples after being treated. This was evidenced by there were not *Bacillus* sp. spores in Nutrient Agar (NA). Autoclave is considered good to kill bacterial spores because autoclave is using high temperature steam to sterilize the object (Hernando, 2015). The using of autoclave in industry life is to processing beef in the form of

Table 1. The Result of *Bacillus* sp. spores in Beef

Cooking Methods	Results		Total
	Positive	Negative	
Steaming approximately at 100 °C in 5 minutes	5	0	5
Grilling approximately at 120 °C in 4 minutes	5	0	5
Frying approximately at 160 °C in 3 minutes	5	0	5
Autoclave approximately at 121 °C with 1.5 atm in 15 minutes	0	5	5
Total	15	5	20

corned beef with the aim to kill all the contaminating bacteria include the spores. The vegetative cell of bacteria dies at 60 – 80 °C of temperature, while the bacterial spore dies at over 100 °C of temperature (Schlegel, 1994). The advantage of autoclave is capable to sterilize tools and materials until there are no living organisms at all. Autoclave is using high temperature and pressure to give the greater strength to kill the bacterial cells compared to the normal heat air (Permatasari, 2013). The temperature and length of the beef processing time affected the ability to kill *Bacillus* sp. spores in beef (Hernando, 2015).

Conclusion

The cooking methods by steaming, grilling, and frying could not kill Bacillus sp. spores in beef. Nonetheless, the cooking method by using autoclave could kill *Bacillus* sp. spores in beef. Autoclave is good to kill *Bacillus* sp. spores in beef, because autoclave is using high pressure and high temperature.

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