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Research Article

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Investigation of Microbial Contamination of Rare, Medium, and Well-Done Beef Steak Samples

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Abstract

Background: Population growth is accompanied by the need for more protein sources. Correspondingly, providing adequate food alongside compliance with hygiene issues has always been of concern. On the other hand, since *Escherichia coli* and *Salmonella* spp. are among the bacteria causing foodborne diseases, hundreds of millions of people worldwide experience foodborne and waterborne diseases, preparing food free from contamination with such bacteria has been a constant concern in the food industry.

Methods: The present study examined 12 samples of raw meat, Rare steak, Medium steak, and Well-Done steak in terms of microbial contamination using total count, *E. coli* and *Salmonella* tests, respectively.

Results: Based on the results of the microbial tests, all the raw and cooked meat samples were less than the national standard in terms of bacterial contamination. The highest number of bacterial colonies were counted in raw meat (3.67×10^5 cfu/g) and the lowest number in Well-Done steaks (1.17×10^4 cfu/g). Further, no significant difference was observed between the three types of steak ($P > 0.05$).

Conclusion: By examining the effects of two parameters, temperature and time, on the level of bacterial contamination of the tested meat steaks, the results of microbial tests showed that Well-Done steak samples had a lower level of bacterial contamination than the other two types.

Keywords

Total count, *Escherichia coli*, *Salmonella*, Steak

Introduction

Meat and its products are among the most important food sources in the daily diet of people in developed and developing countries, whose consumption is affected by many factors. Since the pattern of meat production and consumption increases proportionally with the availability of income, the demand for meat in developing countries has continuously increased [1]. Meat is a nutrient-rich food containing proteins, essential fatty acids, vitamins, and minerals, and contains more than 15% energy, 40% protein, and 20% fat, and is therefore easily perishable because it provides a suitable environment for the growth of different microorganisms [2]. Contamination of raw meat occurs easily from external sources during slaughter, handling, and processing through knives, tools, clothes, hands, and air [3]. Improper storage and distribution during meat processing may lead to contamination with spoilage microorganisms and foodborne pathogens, which constitute the highest meat safety risks [4]. Contaminated meat and meat products seriously cause various biological, chemical, physical, and especially microbial risks [5].

To prevent rapid meat spoilage, physical parameters such as temperature, pH, and water activity should be regulated to minimize microbial growth on beef during production and distribution [6]. If the storage temperature is incorrect, the shelf life of fresh meat can be significantly reduced by microbial growth [7]. The main groups of bacteria responsible for meat spoilage are Enterobacteriaceae, lactic acid bacteria, and *Pseudomonas* spp. [8]. Meat is a dynamic product with biochemical activity, the degree of its pleasantness for the consumer is affected by various factors such as animal health, nutrition, animal rearing environment, as well as pre-and post-slaughter measures [9]. There is insufficient information on microbiological survival rates in meat products after heating. Thorough cooking can generally destroy most bacteria in raw meat, including pathogenic bacteria. Nevertheless, food poisoning can still occur if there are subsequent lapses in food safety practices. For example, raw meat may be contaminated with spores of certain pathogenic meat bacteria (such as *Clostridium perfringens*), and the spores are not easily destroyed at normal cooking temperatures. The heat of cooking can further activate the spores to germinate and become vegetative cells that can multiply rapidly in foods that have been left at room temperature for a long period. Consuming foods containing high levels of vegetative cells of *C. perfringens* may result in foodborne illnesses [10]. The degree of thermal inactivation of bacteria has been evaluated through experiments at an isothermal temperature that has been partially performed with bacteria in meat, but mostly in other food matrices or liquid media [11]. The process of cooking meat leads to an increase in temperature, and applying this temperature to the meat creates a desirable flavor or aroma. As a result, roasted, smoked, or grilled meat products are prepared, which are consumed on a large scale globally and have different flavor and aroma characteristics [12]. Another purpose of cooking meat is to reduce microbiological risks (increasing shelf life and inactivating anti-nutrient enzymes) and improve the digestibility and bioavailability of nutrients. Cooking time and temperature are crucial for forming quality characteristics of cooked meat [13]. Boiling, fire-roasting, and oven-roasting are equally effective in ensuring safety from these pathogens, however, some pathogenic microbes are resistant to heat even after pasteurization. Therefore, preventing microbial spoilage in meat products requires more effective methods such as sterilization, ultra-high temperature heating, ohmic heating, microwave heating technology, and dielectric heating [14]. Cooking technologies can be used to achieve physical changes in the structure of muscles, increase softness, denature muscles, dissolve proteins and create textural changes and make rare meat [15]. Well-done meat will appear slightly dry and grayish-brown, while medium-low cooking will make rare and reddish-pink meat. Increasing the cooking temperature or time usually reduces the redness and increases the browning of the meat [16]. Ready-to-Eat meat and poultry products that are intended to be consumed again without additional preparation include a wide range of products and processes from well-done, non-preserved, or dried meat to various meat combinations. The consumption of such products has increased significantly due to food variety, along with the ease of consumption, which provides a significant part of the food demand of society, especially for young people and teenagers [17]. On the other hand, in recent years, to a large extent, people have become interested in consuming functional (healthy) foods, especially those that contain meat, including meat products such as steaks. Since the composition of meat along with its cooking method is one of the factors affecting the taste quality of meat products [18], the experiences and satisfaction of eating meat can be changed by the skills of meat preparation and cooking [19]. The temperature of cooking is critical to ensure safety and achieve a favorable dining experience [13]. The fate of a pathogen in food and, in turn, the level of human exposure is related to consumer behavior in the kitchen [20]. Steak is cooked in different ways and is divided into three types of Rare, Medium, and Well-Done according to the cooking time and temperature. Fatty acids, usually, short-chain fatty acids, are associated with the flavor of cooked meat. Rare meat is affected by the amount of water and fat remaining after cooking the product, while the degrees of doneness also affect the meat's juiciness [21]. The present study evaluated the microbial load of steak in three different cooking modes, including Rare, Medium, and Well-Done, to investigate the effect of cooking method and heating time on the microbial load of the prepared steaks.

Materials and Methods

The steaks were prepared from a beef meat fillet. After thorough washing, the meat samples were cut into smaller pieces of 180 g, 2 cm thick, and 4 cm in diameter. The full composition of the ingredients for flavoring the meat pieces is given in Table 1.

After mixing the ingredients (Table 1) and finishing the meat resting process under sterile conditions for 12 h, an APW Grill Machine was used to cook the steak pieces prepared according to three recipes (Rare, Medium, and Well-done). The samples were transferred to the laboratory in sterile aluminum foils for testing. The temperature and time needed to cook Rare, Medium, and Well-Done steaks are given in Table 2.

Preparation of test samples, initial suspension, and decimal dilutions

To prepare the initial suspension from the samples sent

Table 1: Ingredients for flavoring and preparing steak.

Ingredients	Weight (g)
Meat	1000
Oil	50
Mustard sauce	50
Soy sauce	10
Salt	3
Spices (including garlic powder and turmeric)	3
Steak seasoning (paprika, onion powder, red pepper, and sea salt)	35

Table 2: Temperature and time required to cook steak types.

Types of steak	Temperature (°C)	Time (min)[a]
Rare	120	2
Medium	150	4
Well-Done	170	6

a: The specified times are considered for cooking both sides of the steaks.

to the laboratory, the sterile knife, spatula, and forceps were used to collect 10 - 25 g and transfer them to pre-sterilized glass containers with lids. To dilute the samples, 0.1% peptone water was used in the first stage and Ringer's solution in the subsequent stages of dilution.

It should be noted that the volume of diluents used should be nine times the weight of the sample. The solution prepared in the above order has a dilution of 10^{-1}. To prepare the next decimal dilutions, 9 ml of diluent was poured into the test tubes, followed by autoclaving (121°C, 15 min, 15 psi). To prepare the second dilution (i.e., 10^{-2}), after homogenizing the initial suspension with a pipette, 1 ml of it was added to the first test tube containing 9 ml of diluent. Subsequent dilutions were prepared in the same way [22].

Microbial tests

The total plate count (Total Aerobic Bacterial Count)

Inoculation and incubation: Using a sterile pipette, 1 ml of the initial suspension was added to a sterile plate containing Plate Count Agar (PCA) culture medium. The PCA is a non-selective culture medium used for cultivation with the pour-plate method according to the Iranian National Standard No. 5272-1 [23]. After the specified incubation period, plates with less than 300 colonies were selected and *E. coli* was searched and counted using the Most Probable Number (MPN) method.

E. coli is a bacterium that generates gas by fermenting lactose at 44 °C and produces indole from tryptophan. The purpose of counting *E. coli* colonies was to determine the most probable number of this bacterium per milliliter or gram of the sample. The present study used the selective enrichment medium of Lauryl sulfate broth.

An aliquot of the sample or the initial suspension was inoculated in the selective enrichment broth medium (peptone water). The inoculated medium was incubated at 37 °C for 48 h and gas production was checked after 24 and 48 h. An aliquot of the medium in test tubes containing gas or turbidity was inoculated into the tube containing the selective medium of EC (*E. coli*) broth. Then, tubes containing inoculated EC broth were incubated at 44 °C for 48 h and checked for gas production after 24 to 48 h. The tubes containing gas were re-inoculated into the tube containing peptone water without indole. Then, the inoculated tubes were incubated at 44 °C for 48 h and checked for indole formation. The test tubes that produce turbidity or gas in the selective medium, gas broth in EC broth and indole in peptone water at 44 °C are considered positive for the presence of *E. coli* in a certain amount of weight or volume of the sample.

The 9-tube MPN test

Three test tubes containing selective enrichment medium (peptone water) with a double concentration were inoculated by a certain amount of initial suspension. Three test tubes containing selective enrichment medium (peptone water) with normal concentration were inoculated by a certain volume of initial suspension and then under the same conditions, three other test tubes containing selective enrichment medium with normal concentration were inoculated with a certain volume of decimal dilutions. The addition of other decimal dilutions to the selective medium at normal concentration was continued until ensuring that the test tubes of the last dilution gave negative results. The test tubes were incubated at 37 °C for 48 h and checked for gas formation after 24 and 48 h. EC broth medium was inoculated by any double-concentration medium that produced turbidity or gas, as well as by any normal-concentration medium that produced gas [23].

Salmonella testing

Salmonella is a bacterium that forms specific or indeterminate colonies onto selected solid media; if the test is performed by [23], *Salmonella* shows biochemical and serological reactions. It is necessary to search and identify *salmonella* in four consecutive steps as follows:

1- Pre-enrichment in non-selective broth medium

2- Enrichment in selective broth medium

3- Cultivation in solid medium and identification

4- Confirmation of the test

An aliquot of the sample was inoculated into Buffered Peptone Water (BPW) and incubated at 37 °C for 18 h. In the second stage, an aliquot of the medium from the first step was inoculated into two media of Rappaport Vassiliadis soya broth (RVS broth) and Muller-Kaufmann Tetrathionate/Novobiocin broth (MKTn broth). The inoculated RVS broth medium was incubated at 41 °C and the inoculated MKTn broth medium at 37 °C for 24 h.

The two cultures obtained were inoculated on selective solid medium Xylose lysine deoxycholate agar (XLD agar), followed by incubation at 37 °C for 24 h. To confirm the test, the suspected *salmonella* colonies were sub-cultured and the confirmatory tests were performed using corresponding biochemical and serological tests. To prepare the initial suspension, 25 g of the sample was added to 225 ml of BPW enrichment medium. Therefore, the ratio of the sample to the pre-enrichment medium was one-tenth by weight/volume. If the amount of the sample is more than 25 g, the diluent is added to the extent that the one-tenth weight/volume ratio is established at the end. The initial suspension was incubated at 37 °C for 18 h. Using a sterile pipette, 0.1 ml of the obtained culture was then transferred to a test tube containing 10 ml of RVS medium and 1 mL of the obtained culture was transferred to a test tube containing 10 ml of MKTn broth medi-

Table 3: The mean number of bacteria under the influence of various steak cooking methods.

Bacteria	Unit	Raw meat	Rare steak	Medium steak	Well-Done steak
Total	CFU/g	3.67×10^5	1.27×10^4	1.23×10^4	1.17×10^4
E. coli	MPN/g	3.83	0.299	0.290	0.200
Salmonella	Per 25 g of sample	-	-	-	-

um. After 24 h of incubation, a loop of the culture obtained from the RVS broth was inoculated onto the surface of a large plate containing the first selected solid medium, the surface of which had been dried so that discrete colonies formed on the surface of the agar. The second selected solid medium was cultured in the same way as the above method using a new sterile culture colony. After incubation for 24 h, a loop of MKTn broth medium was linearly cultured on the surface of two selected solid media, followed by incubation at 37 °C for 24 h. After 24 h of incubation, the plates were checked for the presence of specific and indeterminate *Salmonella* colonies. After growing on XLD agar medium, the specific *Salmonella* colonies have a black center and a slightly red-colored translucent zone, which changes color by an indicator [23]. All tests were performed in triplicate.

Results and Discussion

The total plate counts

In the investigation of the total bacterial count under the influence of different steak preparation methods (Figure 1), it was observed that the preparation methods had a significant effect on the total bacterial count ($P < 0.05$). Among these, the highest number of bacterial colonies was counted in raw meat (3.67×10^5 cfu/g) and the lowest number in Well-Done steaks (1.17×10^4 cfu/g). However, the results showed no statistically significant difference in the number of total bacterial colonies among the heat-treated steaks (Rare, Medium, and Well-Done). The results also indicated that the number of aerobic bacteria in the prepared steak samples was acceptable within the standard range [23].

(Figure 1^{a-b}) Columns with different letters represent statistically significant differences ($n = 3$, $P<0.05$)

Testing the level of contamination with *E. coli*

In examining the effect of using different methods of steak preparation (Rare, Medium, and Well-Done) on the average number of *E. coli* bacteria (Figure 2), the results showed that the steak preparation methods significantly affected the number of *E. coli* bacteria compared to the raw meat sample and caused a decrease in the number of colonies ($P < 0.05$). Although no statistically significant changes were seen in the counting of the mentioned bacteria in the proposed methods, in general, the use of the thermal process with different temperatures and times had a significantly decreasing effect on the mean number of *E. coli* bacteria compared to raw meat.

(Figure 2^{a-b}) Columns with different letters represent statistically significant differences ($n = 3$, $P < 0.05$)

The results showed that while the number of *E. coli* bacteria in raw meat was 3.83 MPN/g, the application of different thermal processes reduced this number to less than 0.3 MPN/g, so this reduction was statistically significant ($P < 0.05$), within the safe range compared to the existing standard. In examining the number of these bacteria, like the total bacterial count, increasing the temperature and heating reduced the number of bacteria, but not statistically significant. On the other hand, the lowest number of *E. coli* bacteria was seen in Well-Done steaks (0.2 MPN/g) [23].

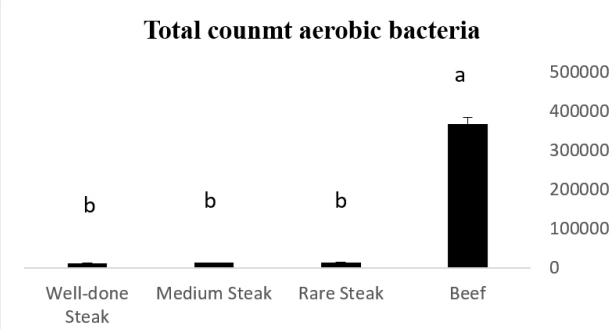

Figure 1: The mean count of aerobic bacteria in raw meat and prepared steaks (cfu/g).

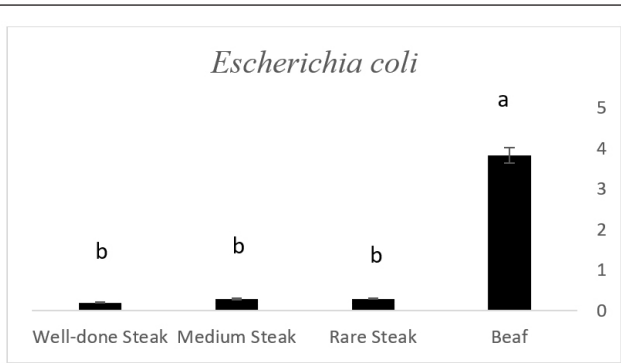

Figure 2: The mean count of *E. coli* bacteria in raw meat and prepared steaks (MPN/g).

Salmonella count

According to the existing standard [23], the maximum acceptable number of *Salmonella* bacteria per 25 g of a sample or square centimeter must be negative. The results of determining the effect of the cooking method on the mean number of *salmonella* bacteria showed that since the used raw meat was negative for this bacterial contamination, the application of thermal processes with different temperatures and times in the preparation of steaks had no effect on this amount and number, and *Salmonella* has been reported as negative in all prepared steak samples. According to the results of investigating the effect of different methods of preparing and cooking steak on the mean number of *E. coli* and *Salmonella* bacteria (Table 1), it can be said that the application of more intense heat methods (higher temperature and more time), which was gradually seen in the preparation of steak from Rare to Well-Done method, reduced the total number of bacteria and *E. coli* bacteria. The number of these bacteria was significantly lower in all prepared products compared to raw meat. Among the suggested methods of preparing steak, there was no statistically significant difference in terms of counting total bacteria, *E. coli*, and *Salmonella*. As it was said, the application of extreme thermal methods numerically caused a non-significant decrease in the (average) number of bacteria.

Bacteria are at their best when the internal and external characteristics of the culture medium are optimal for their growth. The intrinsic properties of food, such as pH, and water activity, and external properties, such as the characteristics of the environment in which the food is stored, both affect the growth and activity of bacteria. Water activity is not the

same as the moisture content of the food, but is a measure that determines between 0 and 1 the availability of water in the food, on which the growth and survival of bacteria depend. Controlling these factors (for example, controlling food storage temperature) can prevent bacterial overgrowth [10]. According to the results and considering that the average number of bacterial colonies counted and the products produced are within the standard range, it is obvious that the type of steak consumed depends on other factors such as sensory properties. In a study by [24], participants acknowledged that they prioritized food availability and taste over food safety. Among the regular occurrence of cross-contamination events during slaughter and meat storage, it should be noted that another case of inadvertent contamination by consumers during food preparation is insufficient and undercooked food [25]. Bergsema et al. [26] and De jang et al. [27], demonstrated the persistence of the invisibility of pathogens during consumer-style cooking methods. The fate of a pathogen in food and, in turn, the level of human exposure is related to consumer behavior in the kitchen [20]. Kang et al. [28] investigated the role of the steak cooking method on consumer satisfaction and dissatisfaction and reported that cooking is a process of adaptation to personal tastes and is influenced by cooking self-efficacy. Poor quality of cooked beef is generally attributed to deficiencies in cooking skills [22]. Eating experiences and satisfaction can be modified by meat preparation and cooking skills [19]. Since foods such as kebabs and steaks are prepared manually from red meat, the cooking heat may be insufficient to eliminate possible primary or secondary contamination (during meat preparation). The results of the present study suggest that the steaks prepared mechanically and automatically have less microbial load and fewer contamination cases. Cooking had an instantaneous impact at the discount of microbial infection in steak samples so that everyone cooked samples had no infection ensuing of their inedibility. In this regard, none of the nine cooked steak samples studied in this research was declared as inedible. The contamination level of raw steak samples was higher than the heated samples. This result seems reasonable due to the non-heating of the raw samples tested. Aycicek et al. [29] showed that the leading cause of raw steak contamination was *Salmonella* strains. This bacterium is sensitive to heat, and accordingly, after cooking, all contaminated raw samples have lost *salmonella* and other pathogenic bacteria and can be consumed. The findings show that even in the case of primary or secondary contamination during meat processing, the heat during cooking is sufficient to sterilize the steaks, and foods such as kebabs, hamburgers, and steaks can be easily consumed [30] in line with the present research. It should be noted that the research results do not include toxigenic bacteria, such as *Staphylococcus aureus* and *E. coli*, that cause food poisoning through heat-stable toxins [30, 31]. Another result obtained from the present research was the cooking temperature and time of steaks. The higher the cooking temperature and time of the steaks, the lower the contamination load. Microbial load decreased in Rare, Medium, and Well-Done steak samples, respectively, so a significant difference was observed between them compared to raw meat.

Conclusion

The results of the microbial tests of all the tested samples revealed that the level of contamination for all the samples was lower than the standard limit and therefore their consumption was unimpeded. Based on the findings of the present study, considering the frequency of observed bacterial contamination, the extent of foodborne diseases, the problems in the field of food hygiene and its importance in the health and economy of society, and since the lack of knowledge and lack of compliance with basic health principles can be the most important reason for these contaminations and endanger public health and safety, it seems necessary to train working people in the field of correct control of health issues and supervision in the preparation, transportation, storage, and supply stages to prevent the transmission of microbial contamination.

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None.

Conflict of Interest

None.

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