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Bacterial and chemical quality of raw meat and ready-to-eat cooked meat

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ABSTRACT

A grand total of ninety random samples of raw meat, grilled kofta, and kabab (30 of each) were collected from different meat restaurants in Benha city, Kaliobia governorate, Egypt. The study aimed to determine the effect of grilling on bacteriological quality of the examined samples through the detection of APC, Total Enterobacteriaceae count, Total Coliform count, isolation and identification of Enteropathogenic E.coli and S. aureus. The mean values of APC, Total Enterobacteriaceae counts, and Total Coliform counts of the examined samples were 3.25×104± 0.47×104, 2.45×104± 0.31×104, and 1.37×104± 0.18×104 for raw meat, 1.09×104± 0.22×104, 7.96×103± 1.58×103, and 4.14×103± 0.79×103 for grilled kofta, and 7.42×103± 1.05×103, 4.64×103± 0.70×103, and 2.29×103± 0.43×103 for kabab, respectively. The incidence of EPEC was 30%, 10%, and 3.3% in the examined samples of raw meat, grilled kofta, and kabab, respectively. S.aureus was detected in 26.7%, 6.7%, and 3.3% of the examined samples of raw meat, grilled kofta, and kabab, respectively. Also, the study aimed to determine their chemical quality through detection of pH, TVN, and TBA values. In conclusion, the examined raw meat samples were more contaminated with the highest level of microorganisms compared to those of grilled meat products and the bacteriological and chemical examination of those samples is a good indicator to detect the quality and hygienic condition of them

1. INTRODUCTION

Meat is an essential source of nutrients for people need healthy diet as it is rich in proteins with good amino acid balance and high in some minerals and vitamins than from others (Fidel Toldra, 2017). Ready-to-eat (RTE) meat products are highly desired owing to their high biological value, fair price, good flavor, and easy to serve (Mosupye et al., 1998).On the other hand, there is major evidence that meat products can easily be contaminated with various types of microorganisms (such as Enterobacteriaceae, E.coli, and S.aureus) and facilitate the growth of spoilage and pathogenic bacteria if not properly handled and stored, leading to loss of quality and possible public health issues (Vernozy-Rozand et al., 2002). The contamination of food relates to factors such as improperly washed utensils and equipment, poor hygiene, a dirty environment, and the presence of animals in the cooking area. The key hazards were the insufficient exposure of foods to time/temperature, extensive handling of foods by cooking after preparation, and left cooked foods open until served (Oranusi et al., 2007).

Enterobacteriaceae have an epidemiological importance as some of their members are pathogenic and may cause serious infections and food poisoning outbreaks to human being. Furthermore, the total Enterobacteriaceae count can be taken as indicator of possible enteric contamination in the absence of coliform organisms (Mosupye and Van Holy, 2000). Member of Gram-negative bacteria, e.g. E.coli is widely used as a surrogate indicator; its presence in food usually suggests direct and indirect fecal contamination (Clarence et al., 2009). In general, Enteropathogenic strains of E.coli are implicated in many cases of infantile diarrhea. In such cases, the severity of symptoms is severe to mild, ranging from undetectable to serious and potentially life-threatening (Varnam and Evans, 1991).

Staphylococcus aureus is Gram-positive cocci can resist drying and radiation. They create certain enzymes that are associated with the invasiveness of Staphylococcus and many extracellular substances, some of which are heat-stable enterotoxins, rendering the food dangerous even though it has a normal look (Pre Scott et al., 2005). Enterotoxins from Staphylococcus aureus is a common cause of food poisoning that occurs after various foods contaminated with Staphylococcus aureus are ingested. Symptoms of rapid onset include nausea and violent vomiting with or without diarrhea (Argudin et al., 2010).

pH value of meat has been linked to the chemical characteristics of meat, so by direct measurement of their pH, the early finding of meat spoilage is obtained. The pH measurement of spoiled meat may be considered an indirect measurement of the accumulation of ammonia, which suggests muscle deterioration (Gill, 1983).

Total volatile nitrogen (TVN) may be commonly used during storage as an indicator of protein decomposition by microorganisms and tissue enzymes (Greer and Murray, 1991).

Thiobarbituric Acid (TBA) values could be a useful quality index for the assessment of rancidity during the storage of lipid-rich food (Hassan and Omama, 2011).

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The effect of heat plays an important role in influencing the bacterial and chemical quality of meat and meat products and preventing the incidence of foodborne diseases by decreasing and preventing the presence of pathogenic microorganisms such as Enterobacteriaceae, E.coli, and S.aureus. So, for meat to be healthy for human consumption, any cooking method must achieve an internal temperature of at least 71.1°C (Kilsby et al., 2000).

The study aimed to determine the effect of grilling on bacteriological quality of the examined samples through the detection of APC, Total Enterobacteriaceae count, Total Coliform count, isolation and identification of Enteropathogenic E.coli and S. aureus.

2. MATERIAL AND METHODS

2.1. Collection of Samples

Ninety random samples of raw meat, grilled kofta, and kabab were purchased from different meat restaurants (30 of each) in Benha city, Kaliobia governorate, Egypt. Each sample was kept in a separated sterile plastic bag and preserved in an icebox then labeled and transferred to the laboratory under complete aseptic conditions without undue delay and the collected samples were examined as quickly as possible to determine the effect of grilling on their bacteriological and chemical profiles and *their* fitness for human consumption.

2.2.Bacteriological examination:

2.2.1. Preparation of samples (ISO 4833-1, 2013)

Accurately 225 ml of 0.1 % sterile peptone water were applied to 25 gm. of the sample and thoroughly mixed for 1.5 minutes using a sterile blender, from which ten-fold serial dilutions were prepared.

2.2.2. Determination of Aerobic Plate Count (ISO 4833-1, 2013)

Using sterile plate count agar at 37°C for 24 hours. On plates containing 30-300 colonies, APC / g was calculated.

2.2.3. Determination of Total Enterobacteriaceae Count (ISO 4833-1, 2013)

Using Violet Red Bile Glucose agar at 37°C for 24 hours. Every purple colony was then recorded, and the count of Enterobacteriaceae / g was determined.

2.2.4. Determination of Total coliform count (ISO 4832, 2006)

Using Violet Red Bile agar medium at 37°C for 24 hours. All dark red colonies measuring 0.5 mm in diameter were counted and the average number of colonies was estimated.

2.2.5. Detection of Enteropathogenic E.coli

2.2.5.1. Enrichment

MacConkey broth tubes with inverted Durham's tubes were inoculated with the original homogenate and incubated at 37°C for 24 hours. Positive MacConkey broth tubes with inverted Durham's tubes (acid and gas production) were incubated at 44±0.5°C for 24 hrs (Eijkmann test).

2.2.5.2. Selective plating

Using positive MacConkey broth tubes onto Eosin Methylene Blue agar medium (EMB) at 37°C for 24 hours. The suspected colonies were apparently metallic green in color.

2.2.5.3. Morphological examination (ISO, 1995) and Motility test (Mac Faddin, 2000)

2.2.5.4. Biochemical identification (Kreig and Holt, 1984)

E.coli shown positive results with indol, methyl red, nitrate reduction and sugar fermentation test (lactose, sucrose, and arbinose), while negative results shown with Voges Proskaur, citrate utilization, H2S, gelatin liquefaction and sugar fermentation test (inositol).

2.2.5.5. Serodiagnosis of E.coli

According to Kok et al. (1996) by using rapid diagnostic E.coli antisera sets (DENKA SEIKEN Co., Japan) for *diagnosis of the Enteropathogenic types.*

2.2.6. Determination of Staphylococcus aureus (FDA, 2001)

Using Baired Parker agar plate at 37°C for 48 hours. Suspected colonies appear as black shining convex colonies, 1-1.5 mm in diameter with narrow white margin and surrounded by a clear zone extending into opaque medium.

2.2.6.1. Morphological examination (ISO, 1995)

2.2.6.2. Biochemical identification (Mac Faddin, 2000)

S. aureus showed positive results with catalase test, coagulase test , ADH, D-Nase activity and growth at 10% NaCl while negative results with indol test, oxidase and bile esculin test.

2.3. Chemical examination

2.3.1. Determination of pH (Pearson, 2006)

2.3.2. Determination of Total Volatile Nitrogen "TVN" (ES: 63-9/ 2006)

2.3.3. Determination of Thiobarbituric Acid Number "TBA" (ES: 63-10/2006)

2.4. Statistical analysis

Data were analyzed using the descriptive statistic SPSS (Version 20). Differences in mean of analyzed data were considered significant at $P < 0.05$.

3. RESULTS

3.1. Aerobic plate count

The results in a table (1) recorded that APC (cfu/g) of the examined samples of raw meat, grilled kofta, and kabab were 3.25×104± 0.47×104, 1.09×104± 0.22×104, and 7.42×103± 1.05×103, respectively.

Table 1 Statistical analytical results of Aerobic plate count (APC) (cfu/g) in the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

Meat status	Min.	Max.	Mean ± S.E*
Raw meat	9.4×10^3	1.7×10^5	$3.25\times10^4\pm 0.47\times10^4$ a
Grilled Kofta	7.1×10^3	6.5×10^4	$1.09\times10^4\pm 0.22\times10^4$ b
Kabab	4.6×10^3	1.8×10^4	$7.42\times10^3\pm 1.05\times10^3$ c

S.E* = Standard error of mean. Mean values with different superscripts in the same column were significantly differed ($p < 0.05$)

3.2. Total Enterobacteriaceae count

The results achieved in table (2) declared that the mean values of total Enterobacteriaceae counts (cfu/g) in the examined samples of raw meat, grilled kofta, and kabab were 2.45×104± 0.31×104, 7.96×103± 1.58×103, and 4.64×103± 0.70×103, respectively.

Table 2 Statistical analytical results of *Enterobacteriaceae* counts (cfu/g) in the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

Meat status	Min.	Max.	Mean ± S.E*
Raw meat	8.0×10^3	1.2×10^5	$2.45\times10^4\pm 0.31\times10^4$ a
Grilled Kofta	5.3×10^3	4.4×10^4	$7.96\times10^3\pm 1.58\times10^3$ b
Kabab	3.9×10^3	9.7×10^3	$4.64\times10^3\pm 0.70\times10^3$ c

S.E* = Standard error of mean. Mean values with different superscripts in the same column were significantly differed ($p < 0.05$)

3.3. Total coliform count

The results are given in table (3) revealed that the mean values of total Coliform count (cfu/g) in the examined samples of raw meat, grilled kofta, and kabab were 1.37×104± 0.18×104, 4.14×103± 0.79×103, and 2.29×103± 0.43×103, respectively.

Table 3 Statistical analytical results of Total Coliform counts (cfu/g) in the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

Meat status	Min.	Max.	Mean ± S.E*
Raw meat	4.9×10^3	6.5×10^4	$1.37\times10^4\pm 0.18\times10^4$ a
Grilled Kofta	3.0×10^3	2.3×10^4	$4.14\times10^3\pm 0.79\times10^3$ b
Kabab	1.7×10^3	5.2×10^3	$2.29\times10^3\pm 0.43\times10^3$ c

S.E* = Standard error of mean. Mean values with different superscripts in the same column were significantly differed ($p < 0.05$)

3.4. Enteropathogenic E.coli

The results in a table (4) declared that 13 (43.3%) isolates of E. coli were isolated from examined samples represented as 9 (30%) from raw meat with serotypes O26:H11 and O111:H2 EHEC (10%, 6.7%, respectively), O84:H21 and O113:H4 EPEC (3.3%, 3.3%, respectively), O127:H6 ETEC (6.7%) and O159 EIEC (3.3%), 3 (10%) from grilled kofta with serotypes O26:H11 and O111:H2 EHEC (3.3% for each one) and O127:H6 ETEC (3.3%) and 1 (3.3%) from kabab with serotype O26:H11 EHEC.

Table 4 Prevalence of Enteropathogenic *E. coli* isolated from the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

E.coli strains	Raw Meat		Grilled kofta		Kabab		Strain Characteristics
	No.	%	No.	%	No.	%	
O26 : H11	3	10	1	3.3	1	3.3	EHEC
O111 : H2	1	3.3	1	3.3	-	-	EHEC
O84 : H21	1	3.3	-	-	-	-	EPEC
O113 : H4	1	3.3	-	-	-	-	EPEC
O127 : H6	2	6.7	1	3.3	-	-	ETEC
O159	1	3.3	-	-	-	-	EIEC
Total	9	30	3	10	1	3.3	

% was calculated according to the total number of samples. EPEC = Enteropathogenic *E.coli* EIEC = Enteroinvasive *E.coli* EHEC = Enterohaemorrhagic *E.coli* ETEC = Enterotoxigenic *E.coli*

3.5. Staphylococcus aureus

The results in a table (5) declared that the mean values of S. aureus counts (cfu/g) in the examined samples of raw meat, grilled kofta, and kabab were 3.91×103± 0.54×103, 8.07×102± 1.13×102, and 5.14×102± 0.69×102, respectively. There are 11 isolates of S. aureus that were isolated from the examined samples represented as 8 (26.7%) from raw meat, 2 (6.7%) from grilled kofta, and 1 (3.3%) from kabab.

Table 5 Statistical analytical results of *Staphylococcus aureus* counts/g in the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

Meat status	+ve samples		Min.	Max.	Mean ± S.E*
	No	%			
Raw meat	8	26.7	> 10	9×10^3	$3.91\times10^3\pm 0.54\times10^3$ a
Grilled Kofta	2	6.7	> 10	2×10^3	$8.07\times10^2\pm 1.13\times10^2$ b
Kabab	1	3.3	> 10	7×10^2	$5.14\times10^2\pm 0.69\times10^2$ c

S.E* = Standard error of mean. Mean values with different superscripts in the same column were significantly differed ($p < 0.05$)

3.6. Chemical profile

The results in table (6) revealed that mean values of pH in the examined samples of raw meat, grilled kofta and kabab were 5.77 ± 0.01, 5.68 ± 0.01, and 5.62 ± 0.01, respectively, the mean values of TVN (mg%) in the examined samples of raw meat, grilled kofta, and kabab were 6.79 ± 0.48, 5.23 ± 0.41, and 3.87 ± 0.35, respectively and the mean values of TBA (mg/kg) in the examined samples of raw meat, grilled kofta and kabab were 0.32 ± 0.03, 0.18 ± 0.01, and 0.14 ± 0.01, respectively

Table 6 Statistical analytical results of pH, TVN & TBA values in the examined samples of raw meat and grilled meat products in different meat restaurants (n=30).

Meat status	pH	TVN	TBA
Raw meat	5.77 ± 0.01[a]	6.79 ± 0.48[a]	0.32 ± 0.03[a]
Grilled Kofta	5.68 ± 0.01[a]	5.23 ± 0.41[b]	0.18 ± 0.01[b]
Kabab	5.62 ± 0.01[a]	3.87 ± 0.35[b]	0.14 ± 0.01[b]

S.E* = Standard error of mean. Mean values with different superscripts in the same column were significantly differed ($p < 0.05$)

4. DISUCSSION

Meat and meat products are known to be a good source of high biologically important protein, vitamins, and some minerals. On the other hand, due to the presence of spoilage bacteria and/or food-borne pathogens, they pose public health risks (Gracey et al., 1999).

APC in the examined samples shown in table (1) were 3.25×104± 0.47×104 for raw meat, 1.09×104± 0.22×104 for grilled kofta and 7.42×103± 1.05×103 for kabab. The obtained results were almost similar to those recorded by Saad et al. (2011), who found that APC was 3.92×104 ± 0.57×104 in the examined samples of grilled beef kofta. Higher results were, however, obtained by Shaltout et al. (2013), who found that the APC was 1.5× 107 ± 0.43×107 in the examined samples of cooked kofta.

The key sources from which microorganisms reach food are the place of preparation, cooking and serving utensils, raw materials, time and temperature abuse of cooked foods, and personal hygiene of food handlers (Rane-Sharmila, 2011).

The mean values of total Enterobacteriaceae count (cfu/g) in the examined samples shown in table (2) were 2.45×104± 0.31×104 for raw meat, 7.96×103± 1.58×103 for grilled kofta and 4.64×103± 0.70×103 for kabab. The obtained results were nearly similar to those recorded by Elwi (1994) who found that the mean values of enterococci count were 15×103/g and 45×102/g in the examined samples of cooked meat and cooked kofta, respectively. However, higher findings were recorded by Shaltout et al. (2013) who found that the mean Enterobacteriaceae counts of examined samples of kofta were 1.5×107 ± 0.48×107 cfu/g. Both pathogenic and spoilage bacteria are found in the Enterobacteriaceae family that can be isolated from refrigerated food. Incidence of Enterobacteriaceae was 100% in samples of minced meat (Lindberg et al., 1998).

The mean values of total Coliform count (cfu/g) in the examined samples shown in table (3) were 1.37×104± 0.18×104 for raw meat, 4.14×103± 0.79×103 for grilled kofta, and 2.29×103± 0.43×103 for kabab. The current results were agreed with those reported by Abd-El-Fatah-Rabab (2015) who found that the mean values of the Coliform count were 5.54×103± 0.96× 103 in the examined beef kofta samples. Higher findings were obtained by Al-Tawwab (2004) who recorded that the mean values of the Coliform count were 3.5×107 ± 6.4×106 in the examined RTE kofta samples.

The presence of Coliform bacteria in a large number can be responsible for the poorer quality of meat products contributing to economic losses and the likelihood of enteric pathogens posing threats to public health (Trout and Osburn, 1997).

The prevalence of isolated E.coli from the examined samples of raw meat and grilled meat products were shown in table (4), the results revealed that 13(43.3%) isolates of E.coli were isolated from examined samples represented as 9

(30%) from raw meat with serotypes O26:H11 and O111:H2 EHEC (10%, 6.7%, respectively), O84:H21 and O113:H4 EPEC (3.3%, 3.3%, respectively), O127:H6 ETEC (6.7%) and O159 EIEC (3.3%), 3 (10%) from grilled kofta with serotypes O26:H11 and O111:H2 EHEC (3.3% for each one) and O127:H6 ETEC (3.3%) and 1 (3.3%) from kabab with serotype O26:H11 EHEC. Higher findings were obtained by Al-Mutairi (2011) who isolated several strains of virulent E.coli from 80% of examined samples of beef kofta such as (O166, O78, O126, O55, O26, O20, O25:K11, O119, O125:K70, O146 & O126) while 20% were un-typable.

Pathogenic strains of E.coli are believed to be the main causes of traveler's diarrhea and gastrointestinal disease. EPEC is also a food-borne disease that in developing countries produces potentially fatal infant diarrhea (Alonso et al., 2011).

The mean values of S. aureus counts in the examined samples shown in table (5) were 3.91×103± 0.54×103 for raw meat, 8.07×102± 1.13×102 for grilled kofta, and 5.14×102± 0.69×102 for kabab. There are 11 isolates of S.aureus that were isolated from the examined samples represented as 8 (26.7%) from raw meat, 2 (6.7%) from grilled kofta, and 1 (3.3%) from kabab. Nearly similar results were obtained by Shafik-Nagwa (2013) who found that the mean values of S. aureus counts (cfu/g) was 5.4×102 ± 1.2×102 for the examined samples of kofta. However, higher findings were recorded by Al-Kour (2001), who found the mean values of S. aureus counts (cfu/g) were 4.13×103 ± 1.25×103/g in the examined ready to eat meat samples.

The presence of S. aureus in food indicates its contamination from food handlers and inadequately cleaned equipment (ICMSF, 1996). Enterotoxins from Staphylococcus aureus are common cause of food poisoning that occurs after various foods contaminated with S. aureus are ingested. Symptoms of rapid onset include nausea and violent vomiting with or without diarrhea (Argudin et al., 2010).

The results given in table (6) revealed the mean values of pH, TVN, and TBA in the examined samples. The mean values of pH in the examined samples of raw meat, grilled kofta, and kabab were 5.77 ± 0.01, 5.68 ± 0.01, and 5.62 ± 0.01, respectively.

pH limits of beef meat were 6.4 is considered unaccepted, 6.2 considered slightly accepted and less than 6.2 considered accepted while pH 6.5 might be considered an indicator for the beginning of meat spoilage (Pearson and Duston, 1994).

The mean values of TVN (mg %) in the examined samples of raw meat, grilled kofta, and kabab were 6.79 ± 0.48, 5.23 ± 0.41, and 3.87 ± 0.35, respectively.

The increase in TVN values during storage in meat and meat products can be correlated with the breakdown of protein by the action of microbial strains and proteolytic enzymes (Yassien-Nessrien, 2003).

The mean values of TBA (mg/kg) in the examined samples of raw meat, grilled kofta, and kabab were 0.32 ± 0.03, 0.18 ± 0.01, and 0.14 ± 0.01, respectively. The rancidity of food items offers a distinctive sensory value that makes TBA values a good indicator of food rancidity (Salem-Amany, 1992).

5. CONCULOSIONS

The current study comes to the fact that raw meat and grilled meat products have been contaminated by various types of microorganisms due to several reasons, primarily poor hygiene, and contamination after cooking. In addition, the samples of raw meat examined were more contaminated with the highest level of microorganisms compared to those of grilled meat products which were exposed to heat treatment by grilling. Therefore, to improve the hygienic quality of raw meat and grilled meat products, proper cooking of meat, avoiding post-cooking contamination, and high-quality raw materials must be taken into consideration. Also, separation of raw unprocessed meat from RTE meat products, good hygienic practices, and application & implementation of HACCP system especially during preparation and serving should be applied.

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