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Microbiological Quality of Ready-to-Eat Liver Sandwiches (Kibda)

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Abstract: This study was undertaken to evaluate the microbiological quality of ready-to-eat (RTE) liver sandwiches known as "Kibda" from vending shops and different restaurants in Assiut city, Egypt. Microbiological analysis of 100 samples of examined RTE liver sandwiches resulted in total bacterial counts from 1×10^2 to 2×10^7 cfu/g with average 1×10^6 cfu/g and Enterobacteriaceae counts ranged from 1×10^2 to 2×10^5 cfu/g with average 1×10^4 cfu/g, while, total fungal counts from 1×10^2 to 5×10^6 cfu/g with an average 4×10^5 cfu/g. Coagulase positive *Staphylococcus aureus*, *Bacillus cereus*, Shigella and *Salmonella Typhimurium* were detected in 40, 20, 23 and 7% of examined samples, respectively. *S. aureus* was the most common pathogen detected in examined samples (mean counts 6×10^2 cfu/g), while, mean values of *B. cereus* were 8×10^2 cfu/g. Three isolates of *S. aureus* were positive for enterotoxin production. Also, 39 isolates related to family Enterobacteriacae could be isolated. The obtained results indicate that consumption of RTE liver sandwiches may cause a public health hazard to the consumer. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving should be taken.

Key words: Ready-To-Eat Liver Sandwiches · Microbiological Quality · *Staphylococcus aureus* · *Salmonella typhimurium*

INTRODUCTION

Egyptian fried liver sandwiches known as "Kibda" are one of the most popular fast food in Egypt. As much people are eating out, the commercially available sandwiches may be of their choice. The low price makes these sandwiches popular among poor people [1].

Egyptian liver sandwiches are prepared mainly from imported frozen liver (sometimes locally fresh liver) which is minced, very small pieces of green pepper, salt and a variety of spices are added. Then the mixture is arranged inside a loaf of traditional Egyptian bread and cooked.

Ready-To-Eat (RTE) liver sandwiches provide a source of readily available and nutritious meals for the consumer. These foods are well appreciated by consumers because of their taste, low cost, nutrient value and ready availability for immediate consumption [2].

However, questions have been raised about the safety and microbiological quality of these sandwiches [3]. Such prepared foods are considered to be susceptible to post-preparation contamination by pathogenic bacteria [4]. Moreover, various RTE meats are becoming increasingly popular in the world and could be easily contaminated with various pathogens [5].

Furthermore, RTE liver sandwiches do not undergo any further treatments to assure their safety before consumption, therefore risk of contamination with many pathogens as *S. aureus* and *B. cereus* must be considered [6].

Therefore, this study aimed to evaluate the microbiological quality of the ready-to-eat liver sandwiches known as Kibda from vending shops and different restaurants in Assiut city, Egypt.

MATERIALS AND METHODS

Collection of Samples: One hundred samples of RTE liver sandwiches were randomly collected from local retail establishments in Assiut city as restaurants and street vendors. The samples were collected under aseptic conditions, wrapped in sterile plastic bags, sealed, labeled and kept in ice boxes [7].

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Preparation of Samples: Ten grams of sample were weighed under aseptic condition, homogenized with 90 ml of sterile distilled water by using mortar and pistol. Serial dilutions were prepared and spread plate technique [7] was used on appropriate selective media.

Microbial Analysis: Liver samples were analyzed for total bacterial count (TBC) on standard plate count agar (OXOID, CM0463) [8], Enterobacteriaceae on MacConkey agar (Biolife, CB 5502), *B. cereus* on *B. cereus* selective agar (MYPA), *S. aureus* on Mannitol salt agar (Biolife, CB 6204) and Yeast and Moulds on Malt extract agar Base (HIMEDIA, M137). The standard procedure [9] was followed for microbial analysis with above respective media. All plates were incubated under aerobic conditions at 36±1°C for 24- 72 hrs. The mean number of colonies counted was expressed as colony forming units (CFU)/ per gram.

For detection of *Salmonella spp.*, the homogenized mixture was incubated for 24 hours at 37°C for pre-enrichment of the organism. Following enrichment in selenite cystine (SC) and Tetrathionate (TT) broths, presumptive *Salmonella spp.* was detected by streaking on xylose lysine deoxycholate agar (XLD agar) (Biolife, CB 5502) [10].

Identification of *S. aureus*: *S. aureus* was identified by morphological examination, biochemical identification, catalase activity test, detection of haemolysis, mannitol test, coagulase test [11], thermostable nuclease test "D-Nase activity" and demonstration of *S.aureus* enterotoxins by ELISA [12]. By using ELISA, the absorbance was measured at 450 nm in an ELISA plate reader (ELX800, BioTek Instruments, Bad Friedrichshall, Germany).

RESULTS AND DISCUSSION

Microbiological quality problems of RTE liver sandwiches (Kibda) depend greatly on low initial quality of raw liver and other ingredients, inefficient cooking process and improper sanitary practices for personnel and for cooking/processing utensils [13]. Even though some ingredients reach a temperature that is ideal to ensure that the food is cooked thoroughly, cross-contamination during preparation has been traced back to the use of uncooked vegetables and unhygienic handling [14].

Total Bacterial Counts (TBC): The obtained results presented in Table (1) revealed that total bacterial count (TBC) ranged from 1×10^2 to 2×10^7 cfu/g with an average 1×10^6 cfu/g. Similar findings were recorded in another study in Alexandria city where microbial investigation of 30 samples of roasted liver revealed that the average count/gm of total bacterial counts was 8×10^6 cfu/g [15]. On contrary, high incidence of TBC (4×10^7 cfu/g) was recorded in a related study in Assiut city [16]. On the other hand, El- mossalami *et al*. [17] found lower incidence (3.6×10^3 cfu/g) of TBC of liver sandwiches in another research in Alexandria city.

As recorded in Table (2), out of 100 RTE liver sandwiches samples assessed in this study, 4 (4%) gave unsatisfactory level for TBC according to microbiological guidelines [18].

Enterobacteriaceae: Members of the family *Enterobacteriaceae* have been considered a potent cause of foodborne outbreaks [19]. In our study, *Enterobacteriaceae* counts ranged from 1×10^2 to 2×10^5 cfu/g with average 1×10^4 cfu/g (Table 1).

Table 1: Mean microbial profile of RTE liver sandwiches sold in Assiut City, Egypt (CFU/g)

Item	Total Bacterial Count	Enterobacteriaceae Count	*B. Cereus*	*S. aureus*	*Salmonella Typhimurium*	*Shigella*	Total Fungal Count
Liver Sandwiches (n = 100)	1×10^6	1×10^4	8×10^2	6×10^2	Detected (7%)	Detected (23%)	4×10^5

Table 2: Comply the examined RTE liver sandwiches to the microbiological guidelines, (2014) for TBC and Enterobacteriaceae counts

Item	Range cfu/g	Category	No. of Samples	%
TBC	($<10^5$)	Satisfactory	40	40
	10^5 -< 10^7	Borderline	56	56
	$\geq 10^7$	Unsatisfactory	4	4
Enterobacteriaceae	$<10^2$	Satisfactory	79	79
	10^2-<10^4	Borderline	15	15
	$\geq 10^4$	Unsatisfactory	6	6

Table 3: Coagulase reaction and enterotoxin production of *S. aureus* in examined RTE liver sandwiches

No. of isolates	Identified bacterium	Further Identification	Enterotoxin production
3	*S. aureus*	Coagulase +ve / DNase -ve	--------
1	*S. aureus*	Coagulase +ve / DNase +ve	C
2	*S. aureus*	Coagulase +ve / DNase +ve	--------
2	*S. aureus*	Coagulase -ve / DNase -ve	--------
1	*S. aureus*	Coagulase +ve / DNase +ve	B
1	*S. aureus*	Coagulase +ve / DNase +ve	A

Table 4: Mean of positive *S. aureus* and *B. cereus* from examined RTE liver samples

Test Organism	% positive	Range cfu/g	Mean of the positive samples cfu/g
Coagulase-positive *Staphylococcus*	8% (8 samples)	1 X10 -1 $X10^4$	6 $X10^2$
B. cereus	20% (20 samples)	1 -4 $\times10^3$	8 $\times10^2$

According to microbiological guidelines for foods [18], out of 100 examined RTE liver sandwiches samples, 6 samples (6%) were considered unsatisfactory (unfit for human consumption) (total enterobacteriaceae count < 10^4). Also, 15 samples (15%) were classified as borderline (count 10^2 – <10^4). Besides, 79 samples (79%) fell in the category satisfactory (count < 10^2) as shown in Table (2).

Similar findings found by Mohamed [16] who recorded that mean value of *B. cereus* was 1 $\times10^3$ cfu/g. While, lower incidence of Enterobacteriaceae group (5 $x10^3$ cfu/g) was detected in 60% of 10 examined liver samples [20].

The presence of Enterobacteriaceae in heat treated food as RTE liver sandwiches indicates inadequate cooking or post-processing contamination [18]. The presence of these microbes in liver sandwiches can be linked to improper handling and processing, use of contaminated raw materials or the use of dirty processing utensils like knife and trays [21].

Cooked sliced meats are regarded as high-risk foods [22]. Large outbreaks of infectious intestinal diseases have occurred as a result of consumption of cooked meats, but were mainly due to post cooking contamination [23].

Total Fungal Count (TFC): As observed in Table (1), minimum count of total fungal counts was 1 $\times10^2$ cfu/g while, maximum was 5 $\times10^6$ cfu/g with a mean 4 $\times10^5$ cfu/g. In another study, the presence of TFC in the selected street foods was in the range of 3.93 -8.0 $\times10$ cfu/g. It might be due to the use of unhygienic dusty surroundings [24].

The presence of yeast/ mould in the food sample is due to its disperse in the form of spores which are abundant in the environment and can be introduced through dust and soil [25]. Their presence in these food samples is a serious public health concern as these fungi may be associated with the production of mycotoxin [26].

Higher-than desirable numbers of mould and yeast in tested samples may have arisen from non fresh bread and/or ingredients with low microbiological quality [27].

Coagulase-Positive Staphylococci: Coagulase-positive *S. aureus* was detected in 8 (8%) samples with the range and mean of positive samples tabulated in Table 4.

S. aureus was the most common pathogen detected in examined samples with mean count 6 $\times10^2$ cfu/g (Table 1).

Coagulase positive Staphylococcus is considered as an indicator of poor hygiene/handling procedures. The results indicated that 60% of samples fell into the guideline categories of "Satisfactory" and 8% classified as "Unsatisfactory" for Coagulase positive Staphylococcus (Table 5).

Concerning *S. aureus*, 40% were positive for Coagulase Positive Staphylococcus (CPS) strains. *S. epidermidis* predominated among the isolates (25%). Further isolates included *S. anginosus* (5%) and *S. mitis* (5%).

The obtained results of our study disagree with a related study conducted in Alexandria city where *S. aureus* was isolated with a higher percentage (80%) with mean value of (4.8 $\times10^3$ cfu/g) [17].

In a related study, Staphylococcal counts ranged from 3 $\times10^2$ to 1 $\times10^6$ cfu/g of food examined. *S. epidermidis* predominated among the isolates (40%). Further isolates included *S. xylosus* (20%), *S. warneri* (20%), *S. saccharolyticus* (15%) and *S. hominis* (5%) [28].

El-Sherbeeny *et al*. [29] examined 114 street-vended ready-to-eat Egyptian food samples, their study revealed that 41, 37, 26 and 3% of the samples were contaminated with *S. aureus, B. cereus, Cl. perfringens* and Shigella, respectively. On other hands, *Vibrio parahaemolyticus* and *Salmonella* were not detected.

Table 5: Comply the examined RTE liver sandwiches to the microbiological guidelines, (2014) for *S. aureus* and *B. cereus*

Bacteria	Satisfactory	Borderline	Unsatisfactory
S. aureus	60 Samples (60%)	32 Samples (32%)	8
B. cereus	80 Samples (80%)	20 Samples (20%)	Nil

S. aureus entered into the street foods during handling, processing or vending. It also due to the fact that it forms the normal microflora present on the skin and in the nose and throat of most healthy people. So contamination of ready-to-eat foods with coagulase-positive staphylococci is largely as a result of human contact [30].

Our data shown in Table (3) revealed that three isolates of *S. aureus* were positive for enterotoxin production, namely staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin C (SEC).

S. aureus enterotoxins are considered one of the common causes of food poisoning worldwide, with outbreaks caused by mishandling of foods after heat treatment [31, 32]. Although, cooking destroys the bacteria, the toxin produced by *S. aureus* is heat stable and may not be destroyed even by heating [33].

One of the most important toxin threats in warfare or bioterrorism is Staphylococcal enterotoxin B (SEB). Staphylococcal enterotoxin B is a toxin associated with incidences of massive food poisoning [34].

Bacillus Cereus: A total of 100 RTE liver sandwiches samples tested for *B. cereus*, only 20 samples (20%) were positive with counts ranged from 1×10 to 4×10^3 cfu/g & mean value was 8×10^2 cfu/g. According to Microbiological Guidelines for foods [18], 80 samples (80%) were satisfactory (i.e. test results indicating good microbiological quality for *B. cereus*) while 20 samples (20%) categorized as borderline (Table 5).

Similar results (22%) of *B. cereus* were detected in a related study in South Africa [35]. Also, the results of this study concerning *B. cereus* were in harmony with those obtained in a similar study in Assiut city, (1×10^3 cfu/g) [16]. On the contrary, higher incidence (72%) of *B. cereus* was recorded by El- mossalami *et al.* (17) with mean value of 3.7×10^3 cfu/g.

In general, the presence of *B. cereus* in food is of great significance since this organism produces heat-sensitive (diarrheal) and heat- stable (emetic) toxins associated with food poisoning [36].

Salmonella: Our results illustrated in Table (1) showed that *Salmonella typhimurium* were detected in 7% of examined RTE liver samples. On the contrary, salmonella failed to be isolated in other studies [15, 27].

Ready-to-eat foods should be free of Salmonella as consumption of food containing this pathogen may result in food borne illness. The presence of this organism indicates poor food preparation and handling practices such as inadequate cooking or cross contamination. Consideration may also be given to investigating the health status of food handlers who may have been suffering from salmonellosis or asymptomatic carriers of the organism [27].

Shigella: Our data illustrated in Table (1) showed that Shigella could be detected in 23 of examined RTE liver samples (23%).

Shigella is responsible for less than 10% of reported foodborne illnesses per year, infecting approximately 300,000–450,000 people annually [37, 38].

However, in practice, Shigella is rarely isolated from processed products. Most outbreaks result from contamination of raw or previously cooked foods during preparation at home or in foodservice establishments. Generally, the source of contamination can be traced to a carrier whose personal hygiene is poor [39].

Micrococcus spp: *Micrococcus spp*. could be detected in 2 RTE liver sandwiches (2%). This result disagreed with other records obtained in a related study conducted by Odu and Akano [40] who could isolate *Micrococcus spp*. with higher incidence (9.1%).

CONCLUSION

Out of 100 examined RTE liver sandwiches in Assiut City, Egypt, 52% were classified as satisfactory and 48% were categorized as unsatisfactory. Therefore, the results of our study indicate that hygienic conditions of some processed RTE liver sandwiches were very poor and may constitute a considerable hazard to human health. So using of high quality raw materials, efficient heat treatment, adequate cleaning and sanitization of utensils should be applied.

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