THE MICROBIOLOGICAL ASSESSMENT OF READY-TO-EAT-FOOD (LIVER AND KOFTA SANDWICHES) IN TANTA CITY, EGYPT

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A B S T R A C T

Contamination of ready-to-eat foods sold by street vendors and restaurant premises rendering them unacceptable for human consumption has become a global health problem. This study aimed to examine the quality and safety of liver and kofta sandwiches in Tanta city. 140 samples were analyzed including liver and kofta sandwiches with their ingredients (fried liver, grilled kofta, bread and salad). The mean total aerobic plate count, coliform count, staphylococci count, fungal count, proteolytic and lipolytic count in all liver sandwich samples ranged from 3.73 to 5.99, 1.84 to 3, 2.36 to 2.76, 2.26 to 3.31, 2.61 to 4.60 and 2.56 to 3.70 log. cfu/g. respectively; and in kofta sandwich samples, they ranged from 4.26 to 6.26, 2.51 to 4.59, 2.42 to 4.19, 2.46 to 3.35, 3.24 to 5.61 and 2.53 to 4.98 log. cfu/g. respectively. Based on the microbiological Guidelines for Ready-to-eat Food by Centre for Food Safety, the level of contaminations was within acceptable microbiological limits for 80% of liver sandwiches; while approximately half of kofta sandwiches were of unsatisfactory microbiological quality due to high APC. The presence of *E. coli* in some RTE samples concluded that street foods are highly unsafe and unfit for human consumption. It was recommended that the generally acceptable microbial guideline value for APC of RTE foods set at $<10^6$ cfu/g be adapted locally until more precise microbial criteria for this food type could be developed through an appropriate scientific process.

Keywords: Kofta, Liver, *Staphylococcus, E. coli*

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1. INTRODUCTION

Ready to eat (RTE) food can be described as the status of food being ready for immediate consumption at the point of scale, it could be raw or cooked, and can be consumed without further treatment [1]. There is an increase in the consumption of ready-to-eat fast food because of a change in social patterns, which characterized by increased mobility, large numbers of itinerary workers and less family centered activities. Thus, good manufacturing practices of foods taken outside the home such as good sanitation or sanitary measure and proper food handling have been transferred from individuals/families to the food vendor who rarely enforces such practice [2]. Unfortunately, increasing numbers of local foodborne diseases continue to be implicated with food service institutions that prepare and sell Egyptian RTE foods [3-6]. Steps to improve local surveillance of quality and safety of RTE foods are needed to help ensure public health. The benefits in cost and convenience derived from RTE foods should always be coupled with safety assurance. Street vended and restaurant food can contribute to food security of those involved in its production, particularly, suppliers of raw produce, food processors, and consumers [7]. The cross-contamination of such foods with pathogenic microorganisms could occur during the processing of ready fillings as

well as during the preparation of fillings and sandwiches ([8-10]. Although the microbial quality of many RTE foods has been the subject of numerous investigations in more developed countries [11-14], in Egypt there is a paucity of information on the microbial quality and safety of this type of food products. In developing countries like Egypt, where effective food safety controls by concerned regulatory agencies are yet to be realized, evaluation of food microbial hazards and their indicators would help provide criteria for setting functional microbial guideline values. The aims of this study are to assess the microbial quality of liver and kofta sandwiches in Tanta city and to highlight the public health implication of consuming heavily contaminated sandwiches.

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 140 samples of ready to eat sandwiches with their ingredients, comprising of ten each of liver sandwiches, fried liver, bread of liver sandwiches, combined kofta sandwiches, grilled kofta, bread of kofta sandwiches and salad for kofta sandwiches, were obtained from street vended and restaurant premises in Tanta city, Egypt. The samples were collected over two months period in July and August. Samples were purchased into sterile specimen containers, and were taken in cold packs under aseptic condition to the laboratory for microbiological analysis within one hour of collection.

2.2. Sample preparation

The samples were prepared and examined according to the technique recommended by [15] as follows: 25 gm liver and kofta were removed aseptically from each sample and transferred to a sterile polyethylene bag. Then, 225 ml of 1% sterile peptone water were aseptically added to the content of the bag and homogenized at 200 rpm for 1-2 minutes to prepare the initial dilution 1/10 which was used for the preparation of other serial dilutions.

2.3. Microbiological analysis

The spread plate technique was used to prepare duplicate plates for determination of aerobic plate counts (APC), fungal counts and coliform counts [16], Proteolytic and Lipolytic counts [17]. After incubation, duplicate agar plates between 30 and 300 colonies were counted, and then mean counts were calculated.

2.4. Isolation and identification of pathogenic bacteria

Salmonella spp. were isolated and identified according to [18]. *E. coli* were isolated and identified according to [19].

3. RESULTS

The mean microbial population of the liver sandwich samples analyzed is presented in Table 1. The total aerobic plate count in all liver sandwich samples were in the range of 3.73 to 5.99 log CFU/g. Generally, the fried liver recorded the highest (5.99) number of bacterial growth especially in restaurant samples as shown in Table 1. Also, when all elements where combined restaurant samples still had the highest total aerobic plate count (5.42) as shown in Table 1. The total coliform count ranged from 1.84 to 3.69, with restaurant samples having the highest count (Table 1). The range of staphylococci count was 2.36 to 2.76 with restaurant sandwich samples recording the highest; however, no significant differences were found between the street vended and restaurant samples (Table 1). The fried liver samples had the highest total proteolytic and lipolytic counts, while bread samples had significantly smaller counts as shown in Table 1. Furthermore, the total fungal count ranged from 2.26 to 3.31, with restaurant fried liver samples having the highest count, this is shown in Table 1. Table 2 shows the mean of different bacterial and fungi counts in Kofta Sandwich samples obtained from

Table 1. Mean of different bacterial and fungi Counts (log CFU/g) in Liver Sandwich samples obtained from street vended and restaurant premises

Samples	Source	Aerobic Plate Counts	Coliform Counts	Staphylococci Counts	Proteolytic Counts	Lipolytic Counts	Fungal Counts
Combined Sandwich	Street Vended	5.06bc	2.72^{ab}	2.73^{a}	4.01^{a}	3.00^{ab}	2.66^{b}
Combined Sandwich	Restaurant	5.42^{ab}	3.69^{a}	2.76^{a}	3.65^{ab}	3.20^{ab}	2.67^{b}
Fried Liver	Street Vended	5.21^{bc}	2.39^{bc}	2.63^{a}	4.60^{a}	3.70^{a}	2.50^{b}
Fried Liver	Restaurant	5.99^{a}	2.95^{ab}	2.45^{a}	3.93^{a}	3.42^{ab}	3.31^{a}
Bread	Street Vended	3.73^{d}	1.84^{c}	2.36^{a}	2.61^{c}	2.56^{c}	2.35^{b}
Bread	Restaurant	4.67^{c}	2.26^{bc}	2.38^{a}	3.41^{ab}	2.59^{c}	2.26^{b}

abcd= Values in the same column bearing different letters are significantly different ($P<0.05$).

Table 2. Mean of different bacterial and fungi Counts (log CFU/g) in Kofta Sandwich samples obtained from street vended and restaurant premises

Samples	Source	Aerobic Plate Counts	Coliform Counts	Staphylococci Counts	Proteolytic Counts	Lipolytic Counts	Fungal Counts
Combined Sandwich	Street Vended	5.78^{a}	4.58^{a}	2.78^{cd}	5.15^{a}	3.03^{bc}	2.46^{b}
Combined Sandwich	Restaurant	6.01^{a}	3.71^{b}	3.62^{ab}	5.33^{a}	3.73^{b}	3.25^{ab}
Grilled Kofta	Street Vended	5.83^{a}	2.92^{bc}	ND	5.61^{a}	4.46^{ab}	2.77^{ab}
Grilled Kofta	Restaurant	6.26^{a}	2.89^{bc}	4.19^{a}	5.37^{a}	4.98^{a}	2.85^{ab}
Bread	Street Vended	4.90^{b}	2.51^{c}	2.96^{bc}	3.24^{b}	4.13^{ab}	2.51^{b}
Bread	Restaurant	4.62^{b}	2.96^{bc}	2.42^{d}	3.84^{b}	3.68^{ab}	2.74^{ab}
Salad	Street Vended	4.92^{b}	3.60^{b}	$3.16b^{c}$	3.36^{b}	2.53^{c}	3.35^{a}
Salad	Restaurant	5.70^{a}	4.58^{a}	2.88^{cd}	3.41^{b}	3.74^{ab}	3.07^{ab}

abcd= Values in the same column bearing different letters are significantly different ($P<0.05$).
ND denotes “Not detected”

Table 3. Microbiological Quality (CFU/g) of samples vended in streets and restaurant of Tanta city on the basis of Aerobic Plate Count (APC).

Samples	Source	Satisfactory $< 10^5$	Acceptable $10^5 - < 10^6$	Unsatisfactory $\geq 10^6$	Unacceptable/ (NA) potentially hazardous
Combined Liver Sandwich (10)	Street Vended	5 (50%)	3 (30%)	2 (20%)	E. Coli (4) *Salmonella* spp. (ND)
Combined Liver Sandwich (10)	Restaurant	2 (20%)	6 (60%)	2 (20%)	E. Coli (ND) *Salmonella* spp. (ND)
Combined Kofta Sandwich (10)	Street Vended	1 (10%)	5 (50%)	4 (40%)	E. Coli (3) *Salmonella* spp. (ND)
Combined Kofta Sandwich (10)	Restaurant	2 (20%)	2 (20%)	6 (60%)	E. Coli (ND) *Salmonella* spp. (ND)

NA denotes APC "Not applicable"
ND denotes "Not detected"

street vended and restaurant premises. Generally, the total aerobic plate count ranged from 4.62 to 6.26 log CFU/g., with restaurant grilled kofta samples having the highest count (Table 2). In addition, combined restaurant samples still had higher total aerobic plate count (6.01) as shown in Table 2. The total coliform count in all kofta sandwich samples were in the range of 2.5 to 4.58 log CFU/g. with street vended sandwich samples and restaurant salad having the highest count (4.58) as shown in Table 2. The range of staphylococci count was 2.42 to 4.19 with restaurant grilled kofta samples recording the highest. The bread and salad samples had significantly smaller total proteolytic and lipolytic counts, while the grilled kofta samples recording the highest counts (Table 2). The total fungal count ranged from 2.46 to 3.35, with restaurant sandwich samples having the highest count as shown in Table 2. Based on the microbiological guidelines for some ready-to-eat foods by Centre for Food Safety, the microbiological quality of ready to eat sandwiches has been placed into category three, where the aerobic colony count at $<10^5$ is rated as satisfactory, 10^5 to $<10^6$ as acceptable, $\geq 10^6$ as unsatisfactory (Table 3). Overall 50% and 20% of street vended and restaurant liver sandwiches were satisfactory, 30% and 60% were acceptable, and 20% and 20% were of unsatisfactory microbiological quality. Meanwhile, 10% and 20% of street vended and restaurant kofta sandwiches were satisfactory, 50% and 20% were acceptable, and 40% and 60% were of unsatisfactory microbiological quality (Table 3). Unsatisfactory results were due to high total aerobic plate count. The overall contamination rate of *E. Coli* in liver and kofta sandwiches from street vendors was 40% and 30%. Nevertheless, no samples were found to be contaminated by *Salmonella* spp.

4. DISCUSSION

The food we eat carries some form of microbial association [20]. Microorganisms affecting food comes from natural micro flora or are introduced by manufacturing steps ranging from harvesting, processing storage and distribution. In some cases these micro flora have no effect on the food and can be consumed without consequence, but those that are introduced during course of processing depending on type and level of contamination can spoil the food and cause food borne illnesses.

This study revealed that sandwich samples collected from street vended and restaurant premises in Tanta city had significant growth of microorganisms, but the microbial load of sandwich sample gotten from some locations were higher than others to the extent that it may pose a threat to the health of regular consumers. Overall, the total viable count of bacterial population in all liver and kofta sandwich samples ranged from 3.73 to 5.99 log. cfu/g. (Table 1), and from 4.26 to 6.26 log. cfu/g. (Table 2), respectively. Generally, the liver and kofta samples recorded the highest (5.99 and 6.26 log. cfu/g. respectively) number of bacterial growth especially in restaurant premises as shown in (Table 1 & 2). Although no significant differences in aerobic plate counts were found between the street vended and restaurant combined samples ($P < 0.05$), the combined restaurant samples of both liver and kofta sandwiches still had higher total viable bacterial count (5.42 and 6.01 log. cfu/g. respectively) than street vended combined samples as shown in Table 1 & 2. However, significant differences in aerobic plate counts between street vended and restaurant foods were only observed in liver, bread (Table 1) and salad samples (Table 2). The high bacterial count in cooked foods would indicate that they were contaminated during after-cooking handling procedures, demonstrating an overall lack of hygiene. Other studies have shown that during the preparation of raw meat and vegetables in kitchens, numerous surfaces can become contaminated, the contaminating microorganisms can survive for considerable periods of time [21, 22]. In these instances, the cross contamination may occur. The total coliform count in all liver and kofta sandwich samples ranged from 1.84 to 3.69 log. cfu/g. (Table 1), and from 2.51 to 4.59 log. cfu/g. (Table 2), respectively; with combined street vended kofta samples having the highest count (Table 2). Coliforms are mainly found in water, soil and fecal matter as they are widely distributed in water, soil and vegetation [23]. They belong to the family Enterobacteriaceae; that do not form spores; they also ferment lactose to produce acid and gas [24]. They are among the most common bacteria that cause disease. The presence of these organisms in ready-to-eat food (sandwiches) depicts a deplorable state of poor hygiene and sanitary practices employed in the processing and packaging of this food product [25]. The range of staphylococci count was 2.36 to 2.76 log. cfu/g. (Table 1), and 2.42 to 4.19 log. cfu/g. (Table 2), in all liver and kofta sandwich samples respectively; with restaurant kofta samples recording the highest (Table 2). *Staphylococcus* is a Gram-positive bacterium; they are facultative anaerobe growing better in the presence of air [26]. Their presence in food indicates human contact such as poor personal hygiene and poor manufacturing practices of the food vendor [2]. They produce enterotoxins that can withstand high temperature, which on ingestion can cause vomiting, and diarrhea [27]. They also can withstand high sodium chloride concentration [24]. Although death from *Staphylococci* food poisoning is rare [28] it can cause death in small children and the immunocompromised. The presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in these products might have contaminated the stored products from source because of handling by retailers. Improper handling and improper hygiene might lead to the contamination of food and this might eventually affects the health of the consumers [29-33]. The total fungal

count in all liver and kofta sandwich samples ranged from 2.26 to 3.31 log. cfu/g. (Table 1), and from 2.46 to 3.35 log. cfu/g. (Table 2), respectively; with street vended salad having the highest count, this is shown in Table 2. Previous study [34] has shown that salads and sandwiches had the highest yeast and molds counts (median 3.91 and 3.93 log cfu g-', respectively. The fungi have been reportedly isolated from ready-to-eat-food in other studies [35-38]. The presence of fungi in vegetable and sandwich samples may be because of improper storage causing this foods stuff especially the salad to become humid therefore supporting the growth of these fungi. The vegetables have high water content or water activity this may encourage spoilage if not well preserved. These fungi produce important metabolite called aflatoxin, which has been shown to be highly toxic to man and all domestic and laboratory animals [39]. The total proteolytic count in all liver and kofta sandwich samples ranged from 2.61 to 4.60 log. cfu/g. (Table 1), and from 3.24 to 5.61 log. cfu/g. (Table 2), respectively; with street vended kofta samples having the highest count (Table 2). Meanwhile, lower lipolytic count ranged from 2.56 to 3.70 log. cfu/g. (Table 1), and from 2.53 to 4.98 log. cfu/g. (Table 2), was recorded in same sandwich samples, respectively. Proteolytic and lipolytic microorganisms can be responsible for a variety of odor and flavor problems in foods. Some of the common bacteria are strongly proteolytic and/or lipolytic [40-43] and cause serious defects in dairy, meat, poultry, and seafood products when high counts (10^6 per g or ml or above) are reached during refrigerated storage [44,45]. In case of unpackaged cooked products putrefaction results from the growth and protein degradation by the proteolytic Gram-positive bacteria [46]. The proteolytic count and lipolytic count of food can be helpful in drawing attention to unsatisfactory manufacturing practices.

Using the microbiological Guidelines for Ready-to-eat Food [47], this study has shown that the majority of ready-to-eat liver sandwiches (80%) collected from street vended and restaurant premises in Tanta city were of satisfactory or acceptable microbiological quality (Table 3). However, 60% and 40% samples of kofta sandwiches collected from restaurant and street vended premises respectively, were of unsatisfactory microbiological quality according to published microbiological guidelines [47]. Unsatisfactory results were due mainly to high APC. High APC, may indicate that the cooking process was inadequate, that post cooking contamination had occurred, that the length of time and temperature control in storage or display facilities was inadequate to prevent bacterial growth, or that a combination of these factors was involved. Some of the APCs of the grilled and fried sandwich samples analyzed exceeded the typical guideline APC value set at $<10^6$ cfu/g for RTE food products. Fried liver appeared to be the samples that recorded relatively lower APC values than the grilled kofta samples tested (Table 3). Traditionally, this meat product is cooked instantaneously by panfrying in very hot oil for the shortest time possible to avoid toughening of meat and excessive caramelization of the sugar ingredient of the product that may lead to unavoidable surface charring. [48] Cited that the combination of drying and frying should have a synergistic effect on the lowering of the total microbial counts in these products. Burnt surfaces of grilled kofta have been reported to significantly hamper heat transfer in the food and eventually prevent further cooking of the inner portions of meat pieces being heated [49]. Having grilled meat products that are overcooked on the surface yet undercooked inside may be a result of the immediate oxidation of proteins and fats on the surface of the food that are exposed to high temperature at the start of the grilling procedure [50].

On the other hand, all the examined samples from restaurant were acceptable; however, four liver sandwich samples and three kofta

sandwich samples collected from street vendors were of unacceptable quality due to the presence of *E. coli*. *E. coli* were the most predominant bacteria isolated from cooked meals [51]. The presence of *E. coli* in RTE foods is undesirable because it indicates that the food has been prepared under poor hygienic conditions.

Nevertheless, despite the high rates of contamination of street vended foods with *E. coli*, No samples tested in this study were found to contain *Salmonella* spp. This result is consistent with the low prevalence observed in other surveys conducted in Australia [52] and South Africa [53]

Conclusion

These findings demonstrate that ready-to-eat food sold in Tanta city constitutes a likely potential hazard to human health. The presence of *E. coli* in RTE samples indicated fecal contamination, thereby suggesting possible risk of infection involved in the consumption of such food. Effective training of all food handlers and managers, may lead to an improvement in hygienic practices and implementing a hazard analysis system in street vended and restaurant premises. While there is still no microbial guideline value for APCs of Egyptian RTE foods, the adoption of the generally accepted APC guideline value of $<10^6$ cfu/g of food sample may be appropriately used until more comprehensive APC guideline values for Egyptian RTE foods are be established.

5. REFERENCES

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التقييم الميكروبيولوجي للأطعمة الجاهزة "ساندوتشات الكبدة والكفتة" بمدينة طنطا – مصر

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الملخص العربي

ان تلوث الاطعمة الجاهزة التي تباع من قبل الباعة الجائلين و المطاعم والتي يجعلها غير قابلة للاستهلاك الأدمي أصبح مشكلة صحية عالمية ولذلك تهدف هذه الدراسة إلى فحص جودة وسلامة ساندوتشات الكبدة والكفتة والتي تباع بمدينة طنطا حيث تم فحص عدد 140 عينة تشمل ساندوتشات الكبدة والكفتة الجاهزة للأكل بالإضافة الى مكوناتها ، الكبدة والكفتة المطهية والخبز والسلطة وقد أظهرت النتائج أن متوسط العد الكلي للبكتيريا الهوائية والكوليفورم و البكتريا العنقودية والفطريات والبكتريا المحللة للبروتين وكذلك البكتريا المحللة للدهون في جميع عينات ساندوتشات الكبدة قد تراوحت من 3,73-5,99، 1,84-3، 2,36-2,76، 2,26-3,31، 2,61-4,60، 2,56-3,70 لوج وحدة ميكروبية / جرام على الترتيب. وتراوحت في ساندوتشات الكفتة من 4,26-6,26، 2,51-4,59، 2,42-4,19، 2,46-3,35، 3,24-5,61، 2,53-4,98 لوج وحدة ميكروبية / جرام على التوالي. واستنادا إلى المبادئ المكروبيولوجية للأطعمة الجاهزة من قبل مركز وسلامة الأغذية كان مستوى التلوث في ثمانون بالمائة من ساندوتشات الكبدة ضمن الحدود المكروبيولوجية المقبولة بينما كان ما يقرب من نصف ساندوتشات الكفتة ذو جودة مكروبيولوجية غير مرضية وذلك بسبب ارتفاع مستوى العد الكلى للميكروبات الهوائية كما أفادت النتائج عزل ميكروب إشرشيا كولاي من بعض العينات المجمعة من الباعة الجائلين مما جعلها غير آمنة وغير صالحة للاستهلاك الآدمي وقد أوصت الدراسة أنه يمكن استخدام العد الكلى بالميكروبات الهوائية أقل من 10^6 وحدة ميكروبية / جرام كقيمة استرشاديه للأطعمة الجاهزة المحلية حتى يمكن وضع معايير ميكروبية أكثر دقة لهذه الأنواع من الأطعمة من خلال دراسات علمية مناسبة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):187–197, ديسمبر 2013)