ASSESSMENT OF NUTRITIVE VALUE AND HYGIENIC STATE OF LIVER (KIBDA) AND SLICED MEAT SANDWICHES IN NEW VALLEY GOVERNORATE

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ABSTRACT

In the present work, 50 samples of each ready-to-eat (RTE) sandwiches of the liver (kibda) and sliced meat were collected at random from the points of sale in El-Kharga city, New Valley Governorate, Egypt. The hygienic (coliforms, fecal coliforms, *E. coli*. yeast, and mould counts) and nutritional (moisture, protein, fat, ash, gross energy, and cholesterol content) quality were assessed. All samples were sensory accepted. The coliforms were detected in 52 and 50%; fecal coliforms in 10 and 2%; and *E. coli* in 4 and 2% of the examined RTE sandwiches of kibda and sliced meat, respectively. Pathogenic *E. coli* strains were identified from the liver (3 strains) and sliced meat (1 strain) samples. The average yeast count was 4.20±0.0.25, and 3.46±0.17; while that of mould was 3.18±0.13 and 2.90±0.07 $\log_{10}$ cfu/g, respectively. The average moisture contents (%) were 55.62±0.43 and 43.50±0.68; protein (%) were 24.29±0.47 and 24.45±0.60; fat (%) were 10.41±0.25 and 16.13±0.43; and ash (%) were 2.75±0.08 and 1.41±0.06, respectively. The average gross energy contents (Kcal/100g) were 190.90±3.30 and 243.0±4.6, respectively. The average total cholesterol contents (mg/100g) were 60.12±6.93 and 50.45±6.02, respectively. In conclusion, although nutritious, RTE sandwiches under investigation may pose public health concerns (pathogenic bacteria and cholesterol), especially those of liver (kibda).

***Key Words*:** Quality, Microbial, Nutritional, Sandwiches, Sliced meat, Liver (Kibda), Ready-to-Eat.

INTRODUCTION

Several modern trends and changes in food consumption and socio-economical patterns lead to an increasing demand consumption of ready-to-eat (RTE) foods, including sandwiches (Ritson and Hutchins, 1995; Hyebin *et al.,* 2014).

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Egyptian fried liver sandwiches known as "Kibda" and to a less extent sliced meat sandwiches are among the popular takeout/takeaway foods in Egypt. Egyptian liver sandwiches are prepared mainly from imported frozen liver (Abd-El-Malek, 2014). The demand for such sandwiches increased in Egyptian society and received real consumers' preferability; owe their appeal to their fresh taste and appearance, low cost, and nutrient value (provide consumers with protein, fat, carbohydrates and energy). They have become a diet staple to solve the problem of a shortage of fresh meat of high

price which is not available for many families with limited income (Shaltout *et al.,* 2019).

In Egypt, RTE meat sandwiches may, however, represent a public health hazard because of using raw materials of poor quality, inadequate personnel hygiene and post-cooking long holding that encourages heavy bacterial loads; rendering the food to be of inferior quality or unfit for human consumption (El-Ziqaty *et al.,* 2016). Subsequent to the heat treatment, RTE sandwiches can be contaminated with mesophilic gram-negative rods (e.g., *Enterobacteriaceae* and *E. coli*), and yeasts and molds. Many factors such as bad handling, storage and display may increase the microbiological contamination of final RTE meat sandwiches at the point of sale (Angelidis *et al.,* 2006). Flies, insects and rodents are commonly attracted to such sites. The majority of food vendors are uninformed of good hygiene practices (GHP) (Mensah *et al.,* 2002).

The presence of coliforms, fecal coliforms or *E. coli* in meat meals indicates inadequate processing and/or post-processing contamination (soiled hands, utensils and contaminated water). A large number of coliforms suggests poor product quality and the likelihood of enteric pathogens, posing threats to public health (Trout and Osburn, 1997).

Fecal coliforms and *E. coli* had been used as an indicator for fecal contamination (Clarence *et al.,* 2009). *E. coli* is commonly non-virulent, but some strains have adopted pathogenic or toxigenic virulence factors (Gi *et al.,* 2009 and Datta *et al.,* 2012). At present, *E. coli* was recognized as a serious food-borne pathogen associated with numerous outbreaks of disease (Scotter *et al.,* 2000).

Beside palatability and wholesomeness, today's consumer expectations are directed toward the nutritional values of the food. The chemical assessment of gross composition (moisture, protein, lipid, ash percentage) has

therefore become a necessity (Andree *et al.,* 2010).

Cholesterol is an active metabolite within the cells of organ meats constituting a major component of cell membranes and nerves, its high levels, however, is a leading risk factor for human cardiovascular diseases (Nollet and Toldra, 2011; Tabas, 2002).

Owing to the increasing demand for RTE meat sandwiches and the large population consuming them, it is necessary to assess their hygienic condition and nutritional quality. This work had been planned to secure the hygienic (Coliforms, Fecal coliforms, *E. coli*, and Yeast & mold), and nutritive quality (protein, fat, ash, caloric value and total cholesterol content) of RTE liver "Kibda" and sliced meat sandwiches obtained from the point of sale at EL-Kharga city, New Valley Governorate, Egypt.

MATERIALS AND METHODS

1. Collection of samples
Samples of ready-to-eat sandwiches of liver and sliced meat (50 of each of them) were randomly collected in sterile polyethylene bags separately from the point of sale at EL-Kharga city, New Valley Governorate, Egypt, and directly transferred to the laboratory of Meat Hygiene, Faculty of Veterinary Medicine, Assiut University under a chilled condition in an insulated ice-box, where samples were subjected to sensory evaluation followed by preparation for bacteriological and chemical analysis.

2. Sensory evaluation of the samples
The evaluation was focused on detection of any faults in appearance, odor or texture with the general acceptability.

3. Preparation of samples (BAM, 1998)
The meat content of the sandwich was collected under aseptic conditions in sterile mortar where they were thoroughly mixed.

4. Microbiological examination of samples

4.1. Preparation of the dilutions

Ten grams of the mixed sample was weighed under aseptic conditions in a sterile poly-ethylene bag then 90 ml of sterile 0.1% peptone water was added, and the contents were homogenized by Stomacher (Seward 400) for 2 minutes; then ten-fold serial dilutions were prepared in tubes with 9ml sterile peptone water.

4.2. Coliforms, fecal coliforms, and *E. coli* count (MPN/g) (AOAC, 1980).

Coliforms were counted in Lauryl Sulphate Tryptose (LST) broth (35±0.5ºC, 48h) followed by Brilliant Green Lactose 2%Bile (BGLB) broth (35±0.5ºC, 48h); fecal coliforms in E.C. broth (45±0.5ºC, 48h in water bath); and *E. coli* on Eosine Methylene blue (EMB) agar (35±0.5ºC, 24h; nucleated colonies with or without metallic sheen). The number /g was calculated from MPN tables for the 3 tubes dilutions.

4.3. Identification of *E. Coli* isolates:

Suspected isolates of *E. coli* were purified and delivered to the Lab of Microbiology, Benha University, Egypt for both biochemical and serological identification. Biochemically identification was according to MacFaddin (2000). IMVC, urease, TSI, and sugars fermentation were among the tests performed. Serological identification was performed by slid agglutination according to Kok *et al.* (1996) using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan).

4.4. Total yeast and mould count (FAO, 1992)

Sterile melted and tempered (45ºC) Malt Extract Agar was used for the count. The plates were incubated at 25°C for up to 5 days; then the colonies were counted. The mould and the yeast count/g were calculated and recorded.

5. Proximate Analysis

Moisture, crude protein, fat, and ash percentages were determined; and gross energy value were mathematically calculated.

5.1: Determination of moisture percentage (AOAC, 2012)

Twenty grams of the well homogenized wet sample was dried at 65°C in Drying Oven (Blue Pard Scientific Instrument Co LTD, Taiwan) for 24 hr then at 105°C for 6 hr.

$$\text{Moisture \%} = \frac{W1 - W2}{W1} \times 100$$

W1= Weight of the sample before drying
W2= Weight of the sample after drying

5.2. Determination of protein percentage "Macro Kjeldahl Method" (AOAC, 2000)

For analysis ½ gram of dried sample was used. The obtained nitrogen percentage was multiplied by the factor of 6.25 to obtain the protein percentage.

$$\text{Nitrogen \%} = \frac{(50 - R) \, X \, 0.0014 \, X \, 100}{Weight \, of \, sample}$$

$$\text{Protein \%} = (N \, \% \, X \, 6.25)$$

5.3. Determination of fat percentage "Ether Extract Method" (AOAC, 2012):

Soxhlet method was used with slight modification; briefly, 1 gram of the dried sample was weighed onto dry filter paper of known weight then wrapped. The sample was then extracted with petroleum ether (60/80) for about 16 hrs.

$$\text{Fat\%} = \frac{W1 - W2}{a} \times 100$$

W1= weight of the filter paper with the sample before extraction.
W2 = weight of the filter paper with the sample after extraction.
a = weight of the sample.

5.4. Determination of ash percentage (AOAC, 2012):

One gram of the dried sample, in a dry clean porcelain crucible of known weight, was ignited in a muffle furnace at 550-600ºC for 6 hours until grayish-white ash was obtained.

$$\text{Ash\%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

N.B. The obtained results on dry weight basis were converted to wet weight basis using the equation of Jurgens and Bregendahl (2007) as following:

$$\text{Nutrient wet basis\%} = \frac{\text{Nutrient dry basis\% } \times \text{Dry matter\%}}{100}$$

5.5. Calculation of gross energy value:

The gross energy value was calculated according to the equation of Merrill and Watt (1973):

Gross energy value (kcal/100g) = (Protein% x 4) + (Fat% x 9) + (Carbohydrate% x 4) with slight modification excluding the carbohydrates percentage.

6. Determination of total cholesterol content:

For total cholesterol determination, 1.25 grams of wet sample was used in three steps including the extraction of fat (Bligh and Dyer, 1959); preparation of the extracted lipid for cholesterol determination (Naeemi *et al.,* 1995); and Enzymatic determination of cholesterol (Pasin *et al.,* 1998) using diagnostic cholesterol reagent (CHOD-PAP, Ref: 230001, Spectrum, S.A.E.). Absorbance was measured using the spectrophotometer (Unico 2100UV, USA) at wavelength 546nm.

$$\text{Cholesterol "mg/100 g"} = \frac{A\ sample}{A\ standard} \times 200$$

A sample= absorbance of sample.

A standard= absorbance of standard.

7. Statistical analysis

Statistical analysis was performed using Graph Pad-Prism. The results were expressed as mean ± standard error. One-way ANOVA followed by Duncan's Multiple Range Test was used to compare the obtained data. The mean difference was considered significant at $p < 0.05$.

RESULTS

Table 1: Statistical results of microbial count (MPN/g) of examined RTE sandwiches samples (n=50 each).

Item	Coliforms		Fecal coliforms		*E. coli*		Yeast		Mould	
	+ve[1] (%)	Count[2]	+ve[1] (%)	Count[2]	+ve[1] (%)	Count[2]	+ve[1] (%)	Count[3]	+ve[1] (%)	Count[3]
Liver (kibda)	26 (52%)	>1100 (3.6->1100)	5 (10%)	21 (3-43)	2 (4%)	5.5 (3.6-7.3)	35 (70%)	4.20± 0.25[a]	42 (84%)	3.18± 0.13[a]
Sliced meat	25 (50%)	150 (3-1100)	1 (2%)	3.6	1 (2%)	3.6	33 (66%)	3.64± 0.17[a]	46 (92%)	2.90± 0.07[a]

[1] Positive samples; [2]Median value (MPN/g); [3]Mean value ($\log_{10}$ cfu/g)

In the same column means with different superscripts are significantly different (P<0.05).

Table 2: Prevalence of *E. coli* isolates from the examined RTE sandwiches samples.

E. coli strain	Liver (kibda)		Sliced meat		Strain characterization
	No.	%	No.	%	
O91: H21	-	-	1	2	EHEC
O111: H2	1	2	-	-	EHEC
O26: H11	2	4	-	-	EHEC

Table 3: Mean values of proximate composition (%) of examined RTE sandwiches (n= 50 each).

Item	Moisture	Protein	Fat	Ash
Liver (kibda)	55.62±0.43[a]	24.29±0.47[a]	10.41±0.25[b]	2.75±0.08[a]
Sliced meat	43.50±0.68[b]	24.45±0.60[a]	16.13±0.43[a]	1.41±0.06[b]

In the same column means with different superscripts are significantly different (P<0.05)

Table 4: Statistical results of the energy content of the examined RTE sandwich samples (n= 50 each).

Item	Gross energy (Kcal/100g)	EP (%)[1]	EF (%)[2]
Liver (kibda)	190.90±3.3[b]	51.05±0.77[a]	39.53±1.39[b]
Sliced meat	243.0±4.6[a]	40.58±1.02[b]	59.42±1.02[a]

[1]Calories percentage derived from protein; [2] Calories percentage derived from fat
In the same column means with different superscripts are significantly different (P<0.05)

Table 5: Mean values of total cholesterol content (mg/100g) of examined RTE sandwich samples (n= 50 each).

	Liver (kibda)	Sliced meat
Total cholesterol	60.12±6.93[a] (8.98-264.2)	50.45±6.02[a] (13.77-175.9)

In the same row means with different superscripts are significantly different (P<0.05)

DISCUSSION

With the tremendous increase in consumption of RTE meat sandwiches, it is important to know about the hygienic and nutritional quality. Consumers are looking for RTE foods that are fresh, healthy, safe, and nutritious (Fang, 2005). In the current study, the light was spot on the quality of RTE kibda and sliced meat sandwiches collected from the points of sale at El-Kharga city, New Valley Governorate, Egypt.

Hygienic quality:
Cooked meat is excellent media for the growth of bacteria, molds and yeasts. Meat meals can be exposed to several ways of contamination through improper preparation and handling which constitute the most direct and harmful source of microbiological contamination (Ehirl *et al.,* 2001).

Organoleptic assessment of samples revealed all were accepted with no obvious faults detected. The results showed that coliforms were detected in 52 and 50% of the examined RTE kibda and sliced meat sandwiches with a median count of >1100 and 150 MPN/g; Fecal coliforms in 10 and 2% of the samples with a median value of 21 and 3.6 MPN/g; and *E. coli* in 4 and 2% of the samples with a median count of 5.5 and 3.6 MPN/g, respectively. Liver (kibda) sandwiches revealed a higher incidence and count of coliforms, fecal coliforms and *E. coli* (Table1).

No standards for microbiological criteria of RTE sandwiches were released by the Egyptian authorities according to our

knowledge. So, the criteria presented by the Centre for Food Safety (2014) in Hong Kong, showed the permitted level of hygiene indicator organisms in ready-to-eat food (*Escherichia coli* (cfu/g): "<20 satisfactory", "20 - $\leq 10^2$ borderline", ">10^2 unsatisfactory", was in use. With regard to that, *E. coli* count recorded in sandwiches under study was satisfactory (<20 cfu/g) for all examined samples with the high count recorded for liver sandwiches.

At New Valley Governorate, Sotohy *et al.* (2019) found a lower coliform count (2.25±0.13 $\log_{10}$ cfu/g) and a lower incidence of *E. coli* (3.3%). However, El-Ziqaty *et al.* (2016) at Alexandria and Gaafar *et al.* (2019) in Qalubiya governorate found a lower count of coliforms (6.8x10^2 and 1.9x10^2 cfu/g, respectively), but a higher incidence of *E. coli* (32 and 5.8%, respectively) in sandwiches of kibda collected from different vending shops and restaurants. A lower total coliform count was also declared by Abdu-Elaziz *et al.* (2018) "23.2 MPN/g from liver sandwiches at Ismailia Governorate. A lower incidence of coliforms (26.5%), but a higher of *E. coli* (49%) was recorded by Ibrahim *et al.* (2019) in kibda sandwiches obtained from different small restaurants and street vendors at Alexandria Governorate.

El-Fakhrany *et al.* (2019) recorded similar *E. coli* incidence (4%) in RTE liver sandwiches obtained from different shops and markets in El-Fayoum, Egypt. Meanwhile, El-Shenawy *et al.* (2016), Shaltout *et al.* (2016), and Shaltout *et al.* (2017) declared higher incidence of *E. coli* (40, 33.3, and 40%, respectively) in liver sandwiches from different fast-food services at Qalubiya Governorate.

The serological identified strains of *E. coli* were O111:H2 (1 strain "2 %") and O26:H11 (2 strains "4 %") from kibda sandwiches; and O91:H21 (1 strain "2%") from sliced meat sandwiches (Table 2). El-Ziqaty *et al.* (2016) and Ibrahim *et al.* (2019) at Alexandria Governorate identified different *E. coli*

serotypes with varied incidences from RTE liver sandwiches.

The presence of *E. coli* could be referred to post-cooking fecal contamination. Entero-pathogenic *E. coli* constitute public health hazards as it may give rise to food poisoning and gastroenteritis among adult consumers (Lues *et al.*, 2006).

Contamination of food with fungi is common in the in-contact environment under unsatisfactory hygienic conditions (ICMSF, 2005). Mould contamination was recorded in 84 and 92% of the examined liver and sliced meat sandwich samples, with an average count of 3.18±0.13 and 2.90±0.07 $\log_{10}$ cfu/g, respectively. On the other hand, Yeast was found in 70 and 66% of the examined sandwich samples with an average count of 4.2±0.25 and 3.64±0.17 $\log_{10}$ cfu/g, respectively (Table 1). A higher incidence but lower count of mould was assumed in sandwiches of sliced meat, while a higher incidence and count of yeast was in kibda sandwich samples.

The obtained results of mould and yeast counts were consistent with Abdu-Elaziz *et al.* (2018) "3.13 and 3.7 $\log_{10}$ cfu/g, respectively" at Ismailia Governorate; but varied from that of El-Ziqaty *et al.* (2016), found lower count (7.2 x 10^2 and 1.6 x 10^3 cfu/g) but the higher incidence (100%) of mould and yeast, respectively from street vended liver sandwiches at Alexandria Governorate. Elgazzar *et al.* (2019) recorded a higher incidence of mould (96.2%) and lower of yeast (61.5%) from RTE fried liver sandwiches collected from different supermarkets and restaurants with various sanitation levels in Mansoura city, Egypt.

Morshdy *et al.* (2018) at Zagazig city and Sotohy *et al.* (2019) at New Valley Governorate estimated total yeast and mould count of 3.57 and 3.6 $\log_{10}$ cfu/g respectively, from RTE liver sandwiches collected from different localities with various sanitation levels.

Nutritional quality:

The nutritive value of ready-to-eat meat sandwiches is generally derived from their high protein content which contains all essential amino acids. As well, fat is an essential component for sensory perception and supplies fatty acids that cannot be synthesized by humans. They also supply consumers with vitamins, minerals, carbo-hydrates and calories (Vasut and Robeci, 2009).

The results in Table (3) declared that the average moisture contents (%) of liver and sliced meat sandwiches were 55.62±0.43 and 43.50±0.68, respectively. Sandwiches of kibda represented higher moisture content (P<0.05). The protein average value (%) was 24.29±0.47 and 24.45±0.60, while the fat average (%) was 10.41±0.25 and 16.13±0.43, respectively (Table 3). Protein content was parallel in both types of samples, however higher fat content was recorded in samples of sliced meat sandwiches (P<0.05). The average value of ash (%) was 2.75±0.08 and 1.41±0.06, respectively (Table 3). Samples of livers showed the higher ash content (P<0.05), which may correlate to additives during preparation.

The average gross energy value (Kcal/100g), based only on protein and fat content, of the kibda sandwich samples was 190.9±3.3; with the highest percentage of energy (51.05±0.77 %) provided from protein. for sliced meat sandwiches the average gross energy value was 243.0±4.6; with the highest percentage of energy provided from fat (59.42±1) (Table 4). Sliced meat samples showed higher gross energy content (P<0.05).

The results of chemical analysis were close to that of El-Dashlouty *et al.* (2015) for RTE liver sandwiches obtained from street vendors at Behiera Governorate (moisture 57.34%, protein 23.28%, fat 7.8%, ash 2.61%, and calories 199Kcal/100g).

Average total cholesterol contents (mg/100g) were 60.12±6.93 and 50.45±6.02 in examined kibda and sliced meat sandwich samples,

respectively (Table 5). Sliced meat sandwiches are declared lower total cholesterol content. WHO (1990) recommended cholesterol intake should be limited to should not exceed 300 mg/day from various food sources including meat and meat products constitute a major part. These limitations referring to not only to the amount of fat, but also to the fatty acid composition and the cholesterol level in food (Chizzolini *et al.,* 1999).

In conclusion, the obtained results revealed a higher incidence of coliforms, fecal coliforms, *E. coli* and yeasts in kibda sandwiches. As well, the total cholesterol level was higher in sandwiches of kibda. Sliced meat sandwiches recorded a fairly lower incidence of fecal coliforms and yeasts, as well as a lower count of mould. Protein content was fairly parallel in both types of samples. Ready-to-eat sandwiches under investigation may represent public health issues especially those of kibda. Sandwiches of sliced meat showed somewhat better quality (lower incidence of fecal coliforms and lower total cholesterol content). Food vendors should be informed about good hygienic practices during food preparation and handling at the point of sale. As well, post-cooking holding for a long time should be avoided and plenty of uncontaminated green salad should be supplied with the sandwiches. Consumers should be informed about the hazards and benefits of such meals and Egyptian standards for ready to eat sandwiches need to be set.

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تقدير القيمة الغذائية والحالة الصحية لساندويتشات الكبدة وساندويتشات شرائح اللحم في محافظة الوادي الجديد

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تم في هذا البحث جمع عدد ٥٠ عينة من كل من سندويتشات الكبد (كبدة) وشرائح اللحوم، بشكل عشوائي من أماكن البيع بمدينة الخارجة ـ محافظة الوادي الجديد ـ مصر. تم تقييم الجودة الصحية (عدد القولونيات، القولونيات البرازية، الإشريكية القولونية، الخميرة، والفطريات) والغذائية (الرطوبة، البروتين، الدهون، الرماد، الطاقة الإجمالية، ومحتوى الكوليسترول). كانت جميع العينات مقبولة من الناحية الحسية، وأظهرت النتائج تواجد القولونيات في ٥٢ و٥٠٪؛ القولونيات البرازية في ١٠ و٢٪؛ والإشريكية القولونية في ٤ و٢٪ من سندوتشات الكبدة وشرائح اللحم، على التوالي. تم التعرف على سلالات متعددة من الإشريكية القولونية الممرضة من عينات الكبدة (٣ سلالات) وشرائح اللحوم (سلالة واحدة). كان متوسط عدد الخميرة ٤,٢٠ ± ٠,٢٥، و٣,٤٦ ± ٠,١٧؛ بينما كان متوسط عدد الفطريات ٣,١٨ ± ٠,١٣ و٢,٩٠ ± ٠,٠٧لو١٠خلية/جم، على التوالي. كان متوسط محتوى الرطوبة ٥٥,٦٢ ± ٠,٤٣ و٤٣,٥٠ ± ٠,٦٨؛ البروتين ٢٤,٢٩ ± ٠,٤٧ و٢٤,٤٥ ± ٠,٦٠؛ الدهون ١٠,٤١ ± ٠,٢٥ و١٦,١٣ ± ٠,٤٣؛ والرماد٢,٧٥ ± ٠,٠٨ و١,٤١ ± ٠,٠٦ ٪، على التوالي. كان متوسط محتوى الطاقة الإجمالية (كيلو كالوري/ ١٠٠جم) هو١٩٠,٩٠ ± ٣,٣٠ و٢٤٣,٠ ± ٤,٦، على التوالي وكانت اعلى نسبة طاقة مصدرها البروتين. كان متوسط محتوى الكوليسترول الكلي (ملجم / ١٠٠ جم) هو ٦٠,١٢ ± ٦,٩٣ و٥٠,٤٥ ± ٦,٠٢، على التوالي. خلصت الدراسة إلى أن الساندويتشات الجاهزة للأكل محل الدراسة ذات قيمة غذائية عالية الا انها قد تشكل قلقًا على الصحة العامة (بكتيريا التسمم الغذائي ونسبة الكوليسترول)، خاصةً تلك المتعلقة بساندويتشات الكبدة.

الكلمات الكاشفة: الجودة، ميكروبية، غذائية، ساندويتشات، شرائح لحم، كبدة، جاهزة للأكل.