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Microbiological and nutritional profils of Egyptian beef luncheon

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ABSTRACT

This work was done to evaluate the nutritional and microbiological profile of beef luncheon produced in Egypt. Seventy beef luncheon samples were collected from ten different sources (LU01 to LU10) in the local market in Cairo, Egypt. The nutritional profile included (moisture, protein, fat, pH, and preservatives contents). The microbiological profile included (total bacteria, lactic acid bacteria, coliform group, yeast & mold, Staphylococci, in addition to some pathogens i.e., Salmonella/Shigella, Listeria sp., Campylobacter sp., and Bacillus cereus). The moisture, protein, and fat percentages ranged from 56.8 to 63.6%, 11.8 to 14.4%, and 18 to 25.1 %, respectively. LU03 showed the highest protein and lowest fat content (14.4 and 18%, respectively). Potassium sorbate, potassium benzoate, and nitrite concentrations ranged from 650 to 1180 ppm, 430 to 630 ppm, and 9 to 80 ppm, respectively. Natamycin and Nisin were detected in 40 and 20% of tested samples. Regarding the microbiological evaluation, lactic acid bacteria were absent in 30% of tested samples. The total bacterial count in the tested sample ranged from 2.49 to 4.74 Log10 cfu /g. Salmonella/Shigella, Listeria sp., and Campylobacter sp. were not detected in all tested samples, while Bacillus cereus was found in only three sources, while with percentages of 85.7 % in LU01 and LU02 while100% in LU03. The chemical composition of the studied beef luncheons was around the acceptable value according to Egyptian specifications. All tested preservatives were in the allowed range of Codex Alimentarius, except potassium sorbate and natamycin in 40% of the studied samples. The microbial content in most of the studied samples was higher than the permissible limits, while all samples were free of pathogenic bacteria tested. The study recommends emphasizing the importance of following sanitation and hygienic practices in the production of beef luncheons.

Keywords: beef luncheon; safety; quality; preservatives

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INTRODUCTION

One of the most popular and widely used as fast-food items is the beef luncheon. It is seen as a quick and simple way to cook meat dishes and as a solution to the problem of shortage of fresh meat. As many Egyptian families with limited incomes believe that fresh meat is very expensive, they cannot buy expensive fresh meat **(Johler and Guldimann, 2021).** Meat is classified as a highly perishable food due to its high content of moisture, protein, vitamins, and minerals, as well as a wide range of endogenous bioactive substances including carnitine, taurine, carnosine, ubiquinone, and creatine **(EOSQ, 2005; Abd-Allah, et al., 2012).**

There is great interest in studying the safety of meat and meat products, not only at the international level but also at the national level, as the Food Safety Authority was established in 2017. Microbiological quality is an indicator of the potential occurrence of food infections and food poisoning as well as new microbial risks such as antimicrobial resistance. Microbial contamination depends on the microbial load of the meat and the various ingredients as well as the manufacturing steps and handling processes (**Johler and Guldimann, 2021**). *Campylobacter*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, and *Shigella* were frequently implicated in food-borne illnesses and should be considered in assessing health risks to humans from the consumption of meat products (**WHO, 2007; Xavier 2017**). Total bacterial count, lactic acid bacteria, total coliform, and yeast & molds were also used to assess the microbiological quality of processed meat, including beef luncheon (**Zafar, et al., 2016).** The presence of aerobic bacteria like most isolates *Staphylococcus* sp., *E. coli*, *Bacillus* sp., may be due to poor hygienic measures during processing and handling of meat products. So, consumption of these products could be associated with possible risk of infection, suggesting the need for the institution of strict hygienic measures during handling of meat products. Therefore, measures to assure the quality of the raw ingredients, besides controlling the environmental and the hygienic conditions throughout the processing ought to be applied for the offering of safe products. Contamination by pathogenic microorganisms is one of the most important challenges faced by producers of processed meat products. A variety of human health issues, as well as financial losses for producers owing to product recalls from stores, can be brought on by the presence of food borne viruses in beef products **(Alirezalu, et al., 2020).** Since they may be ingested without additional processing and are known to be effective growth substrates for pathogenic bacteria, ready-to-eat (RTE) meats are particularly concerning **(Maricica, et al., 2014)**. *E. coli*, *Salmonella*, and coagulase-positive *Staphylococcus aureus* are the three most significant bacterial pathogens in beef products that cause foodborne illness **(Maricica, et al., 2014)**.

The objective of the current study is to assess the microbiological and chemical quality of different beef luncheon products distributed in Cairo city, Egypt.

MATERIALS AND METHODS

1. Samples collection

Seventy beef luncheon samples were collected from ten different sources (LU01 to LU10; seven samples from each one) in the local market in Cairo, Egypt. Samples from LU05, LU06 and LU08 were from unknown brands, whereas the others were from commercial known brands. Samples were kept at 4°C in an ice tank during collection and transportation to the laboratory. Samples were analyzed within 24 h of reception.

2. Chemical analysis

The chemical composition (Moisture, Protein, Fat and pH) of beef luncheon was performed according to (**AOAC, 2012)** Natamycin content in collected beef luncheon was assayed using UV-spectrophotometer method **(Chen, et al., 2008).** Determination of both potassium benzoate and potassium sorbate was achieved according to the method described by (**AOAC, 2000**). While nitrite concentration was done using sulfanilic acid method (**Ugalde-Benítez, 2012**).

The nisin producing by bacteria Lactococcus lactis subsp. lactis ATCC 11454 were evaluated against different microbial culture. **(Park, et al., 2019).**

3. Microbiological examination

Collected samples were prepared for all microbiological examinations by weighing 10 g (25 g for *Salmonella* and *Shigella*) in a sterile Stomacher bag and then homogenized in a Stomacher with 90 ml of sterile 0.1% (w/v) peptone water for two minutes. Further decimal dilutions were prepared in volumes of 9 ml of 0.1% (w/v) peptone water. The microbiological analyzes were performed using the pour plate method **(APHA, 2001).**

1.3. Total bacterial count

Total bacterial count was determined using pour plate technique. After hardening of medium, the plates were incubated at 30°C for 24-48 h. After incubation period, colonies were counted (**ICMSF, 2002**).

2.3. Lactic acid bacteria

Lactic acid bacteria were counted on de Man, Rogosa, Sharpe (MRS) agar, incubated at 30°C for 72 h under anaerobic conditions (**ICMSF, 2002**).

3.3. Coliform group

Detection of coliform group was done in two stages, the first step was to detect the presence of acid and gas "presumptive test" using MacConkey broth medium at 37°C for 24-48 h. where the second step is to ascertain the presence of coliform bacteria by "confirmed test" which done on Eosin Methylene Blue (EMB) agar plates at 37°C for 24-48 hours according to **APHA (1998).**

4.3. *Staphylococcus* spp.

Staphylococci were counted in Baird-Parker agar medium and cultured for 72 hours at 37°C. (**APHA,1998; Merck, 2010**).

5.3. Yeasts & Molds

Total yeasts and molds were determined on potato dextrose agar (PDA). Molds colonies were counted after 3-5 days of incubation at 25°C. (**APHA, 1998; Atlas, 2010**).

6.3. *Salmonella*/*Shigella* spp.

Detection of *Salmonella*/*Shigella* sp was done by adding 25 g of tested sample to 225 mL peptone water and mixing well, one mL of diluted sample was added to 20 mL tetrathionate broth, and then 0.1 mL of prepared solution were cultured on S.S agar medium and incubated at 37 ° C for 24-48 h (**Atlas, 2010**).

7.3. *Bacillus cereus*

Detection of *Bacillus cereus* was done by using PEMPA agar and incubated at 37ºC for 24 h. Peacock blue colored colonies (3-5 mm) surrounded by blue zone of egg yolk hydrolysis against green/greenish yellow background were presumed to be *B. cereus* (**Rasool, et al., 2017**).

8.3 *Campylobacter* spp.

Two grams of each sample were added to 10 mL of thioglycolate medium with Skirrow supplement (SR 69, Oxoid) and incubated at 37°C for 24 h under microaerophilic conditions (6%O2+10%CO2). Both *Campylobacter* blood agar base media (10% sheep blood) and *Campylobacter* blood free selective agar base were streaked with a loopful of each enriched

broth. Using gas production kits made specifically for *Campylobacter* sp., the plates were incubated inverted for 48 h at 43°C under microaerophilic conditions (**Maher, et al., 2003**).

9.3. *Listeria* spp.

Twenty-five grams from each sample were upped to 225 mL of listeria enrichment broth medium. and incubated at 30°C for 7 d. After that, 0.1 mL of the inoculated enrichment broth culture was streaked on Palcam agar media incubated at 35°C for 24-48 h (**Hitchins, 2003**).

4. Statistical analysis

SPSS for statistical software (version 13; SPSS Institute Inc., Chicago, IL) was used for all statistical analysis in this investigation. All data are shown as mean ± standard deviation (SD). (**SPSS, 2001**). The data obtained from seven samples were analyzed by a one-way ANOVA using 'Proc Mixed' In all cases, the level of statistical significance was of $P < 0.05$.

RESULTS AND DISCUSSION

1. Chemical composition of beef luncheon

Beef luncheon meat is used in Egypt as a ready-to-eat processed meat product and as a source of protein. The protein percentage in the beef luncheon samples collected from 10 sources in Cairo ranged from 11.8 ±0.05 to 14.4 ±0.06% with a significant difference ($p < 0.05$) as seen in **(Table 1).** According to the Egyptian standard specification **(EOSQ, 2005)**, the protein content of the Egyptian luncheon should be around 16%. In a previous study achieved by **(Sabry ,2016)**, the protein content of the Egyptian beef luncheon ranged from 12.2 ±0.04 to 15.74 ±0.3%. The results in this study confirm what was mentioned in previous studies that it must be ensured that the manufacture of luncheon meat in Egypt is due to the importance of protein as a source of essential amino acids that the body cannot synthesize **(Sabry, 2016)**.

Increasing the moisture content of beef luncheon is one of the most important factors that encourage microbial growth and lead to spoilage. According to Egyptian standared specfication for beef luncheon the moisture content should be around 60%. From the obtained moisture percentage results in **(Table 1).**, it was found that the moisture content of sixty percent of the samples ranged between 58.8 ±0.30 and 52.7 ±0.12 % (did not exceed 60%). On the other hand, samples collected from sources LU01, LU03, LU05, and LU06 contained moisture content of more than 60%. In harmony with the obtained results, previous studies indicated that the moisture content of beef luncheon samples collected from different supermarkets in Egypt ranged from 57% to 66% **(Sabry, 2016)**. Also, **Smith, et al., 2004)** mentioned that the average moisture percentage has ranged from 56.97 ±0.69 to 64.52 ±0.31 %. It can be demonstrated that the moisture percentage in all tested samples was within acceptable limits according to the Egyptian standard specifications. Concerning the Fat% and PH value in the beef luncheon collected in this study, it was found to be within the permissible limits of the ESS, as the fat percentage ranged from 18 ±0.01 to 25.1±0.16% with a significant difference ($p < 0.05$) as shown in **(Table 1).**,. In general, the beef luncheon samples from LU03 source have a high protein value (14.4 %) with a low-fat value (18%).

2. Preservatives in beef luncheon

Potassium sorbate (E202), potassium benzoate (E212), natamycin (E235), nisin (E234), and nitrite (E250) were estimated I n this study **(Table 2)** as common five preservatives added to beef luncheon. Potassium sorbate, potassium benzoate, and nitrite were found in all luncheon samples collected from the ten sources. The concentrations of potassium sorbate in beef samples ranged from 650 ±24 ppm in LU01 to 1180 ±30 ppm in LU05. Moreover, 40% of the beef

luncheon samples contained potassium sorbate at concentrations higher than those allowed in Codex LU03, LU05, LU06 and LU08 **(Table 2).** The contents of potassium benzoate, nisin, and nitrite were lower than the allowed concentration stated by Codex standard 192-1995 **(Table 2).** Potassium benzoate ranged from 430 ±11 to 630 ±70 ppm. Nisin was detected at concentrations less than the permissible limits in 20% of the studied samples (LU04 and LU10). Remarkably, natamycin was detected in 40% of tested samples at concentrations exceeding the permissible Codex (20 ppm). In general, it can be noticed that the percentages of the investigated preservatives in the collected beef luncheon samples were within the acceptable limits according to Codex specifications, except 40% of the content of potassium sorbate and natamycin.

Table 1. Proximate composition[*] of beef luncheon collected from different sources.

Source	Moisture %	Protein %	Fat %	pH
LU01	$61.2^{b} \pm 0.20$	$13.5^{b} \pm 0.31$	$20.1^{d} \pm 0.10$	$5.0^{c} \pm 0.13$
LU02	$56.8^{d} \pm 0.11$	$11.8^{c} \pm 0.05$	$22.5^{b} \pm 0.13$	$6.1^{a} \pm 0.04$
LU03	$62.8^{a} \pm 0.09$	$14.4^{a} \pm 0.06$	$18.0^{e} \pm 0.01$	$5.5^{b} \pm 0.80$
LU04	$57.6^{d} \pm 0.08$	$13.2^{b} \pm 0.10$	$25.0^{a} \pm 0.13$	$5.2^{c} \pm 0.11$
LU05	$63.6^{a} \pm 0.10$	$13.5^{b} \pm 0.14$	$19.8^{d} \pm 0.06$	$6.0^{a} \pm 0.12$
LU06	$60.8^{b} \pm 0.05$	$14.0^{a} \pm 0.40$	$21.2^{c} \pm 0.23$	$5.8^{a} \pm 0.32$
LU07	$58.8^{c} \pm 0.30$	$12.7^{bc} \pm 0.54$	$23.0^{b} \pm 0.05$	$4.8^{c} \pm 0.20$
LU08	$52.7^{e} \pm 0.12$	$12.3^{bc} \pm 0.21$	$25.1^{a} \pm 0.16$	$6.2^{a} \pm 0.08$
LU09	$58.4^{c} \pm 0.17$	$14.0^{a} \pm 0.89$	$20.7^{c} \pm 0.12$	$4.2^{d} \pm 0.03$
LU10	$58.01^{c} \pm 0.12$	$13.1^{b} \pm 0.12$	$23.8^{b} \pm 0.05$	$5.9^{a} \pm 0.04$
Standard Levels[#]	**About 60**	**About 16**	**Not more than 30**	**----**

[*]Values are presented as mean ± standard deviation (SD), *n= 7*. Values with the same letter within the same column are insignificantly differed ($p > 0.05$).

[#] According to **EOSQ, (2005)**

Table 2. Preservatives[*] of beef luncheon collected from different sources.

Source	Preservatives agents (ppm)				
	Potassium sorbate	**Potassium benzoate**	**Natamycin**	**Nisin**	**Nitrite**
LU01	$650^{f} \pm 24$	$460^{c} \pm 22$	$53^{a} \pm 6$	Nd	$36^{d} \pm 1$
LU02	$980^{d} \pm 19$	$550^{b} \pm 17$	$62^{a} \pm 11$	Nd	$20^{e} \pm 6$
LU03	$1120^{c} \pm 10$	$540^{b} \pm 15$	$40^{b} \pm 7$	Nd	$42^{c} \pm 1$
LU04	$950^{d} \pm 12$	$470^{c} \pm 8$	Nd	$11.0^{a} \pm 0.3$	$9^{f} \pm 3$
LU05	$1180^{c} \pm 30$	$500^{b} \pm 19$	Nd	Nd	$38^{d} \pm 2$
LU06	$1090^{b} \pm 32$	$480^{c} \pm 5$	Nd	Nd	$62^{b} \pm 2$
LU07	$990^{d} \pm 22$	$600^{a} \pm 92$	Nd	Nd	$43^{c} \pm 8$
LU08	$1050^{a} \pm 12$	$630^{a} \pm 70$	Nd	Nd	$52^{c} \pm 3$
LU09	$1000^{a} \pm 10$	$430^{c} \pm 11$	$45^{b} \pm 4$	Nd	$80^{a} \pm 3$
LU10	$890^{e} \pm 11$	$610^{a} \pm 34$	Nd	$14.0^{a} \pm 0.2$	$15^{e} \pm 1$
Codex standard[#] (ppm)	**1000**	**1000**	**20**	**25**	**80**

[*]Results are presented as means ± standard deviation (SD), *n=7*, Nd= Not detected at the studied conditions
Values with the same letter within the same column are insignificantly differed ($p > 0.05$).

[#]General standard for food additives (**Codex Alimentarius, 2019**).

3. Microbiological quality of the beef luncheon product.

Contamination of meat products with microorganisms occurs due to several reasons, including manual processing and non-application of sanitary requirements in manufacturing, handling, and packaging **(Zafar et al., 2016).** The microbiological criteria applied in the present study were total bacterial count, lactic acid bacteria (LAB), total coliform, yeast & molds, and *Staphylococcus* sp., *Bacillus cereus*, *Salmonella* and *Shigella*, *Campylobacter* sp., and *Listeria* sp. All tested the luncheon samples were positive for the total aerobic bacterial count, total coliform, yeast & molds, and *Staphylococcus* sp. **(Fig.1, 3, 4, and 5).** The total bacterial count in the tested sample ranged from 2.49± 0.1 to 4.74± 0.2 Log_{10} cfu /g in LU08 and LU04, respectively **(Fig. 1).** The obtained results were similar to those recorded by **Shaltout et al., (2016)** who stated that the total aerobic bacterial count (log cfu/g) in beef luncheon samples, collected from Giza city in Egypt, were 4.2 ± 0.1 Log_{10} cfu /g,and also **Mousa et al., (2014)** obtained higher results as they stated that the total aerobic bacterial counts were 5.8 Log_{10} cfu /g in the beef luncheon in Egypt.

There is no significant difference (*p>0.05*) between the total bacterial count of samples collected from LU05, LU06, and LU08, which had the highest total bacterial counts compared to the other sources **(Fig. 1).** These samples (LU05, LU06, and LU08) exceeded the permissible limit recommended by the Egyptian Organization for Standardization and Quality (**EOSQ, 2005**) which stated that the permissible limit of total plate count was 10^4 cfu/g.

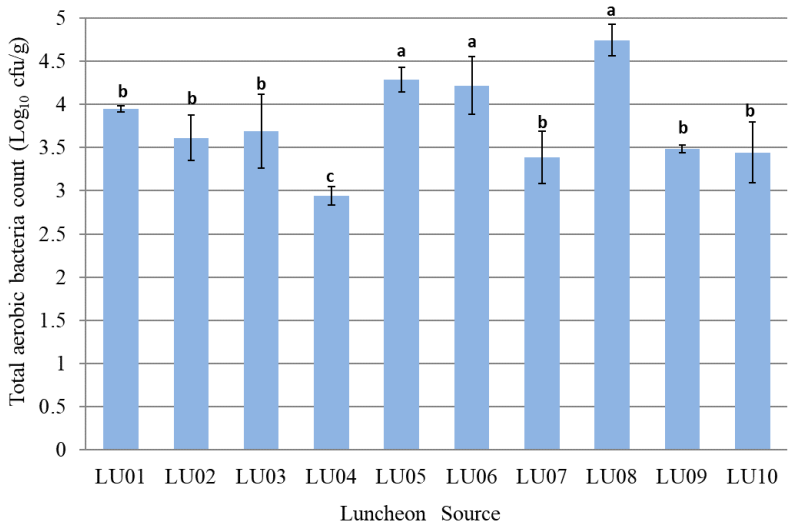

Figure 1: Total aerobic bacterial count (*n=7*, ±SD) in luncheon samples from different sources. Columns with the same litter are insignificantly differed (*p > 0.05*).

LAB were absent in 30% of tested samples (LU05, LU08 and LU10) **(Fig. 2).** The dominant bacteria in the samples source LU04 were considered as lactic acid bacteria, where the means LAB in these samples were 3.51 ±0.2 Log_{10} cfu /g with significant difference (*p<0.05*) compared to the other sources. LAB counts were ranged from 1.3 ±0.2 to 2.0 ±0.5 Log_{10} cfu /g without significant differences (*p>0.05*).

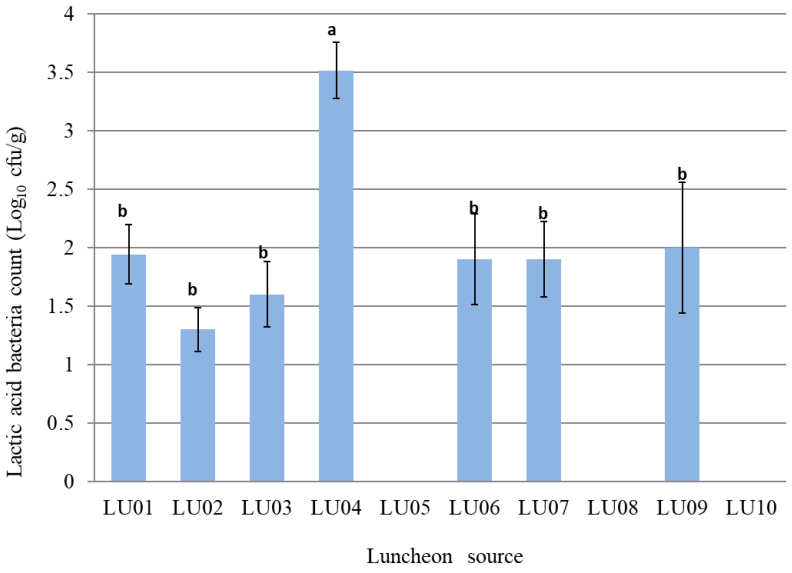

Figure 2: Lactic acid bacteria count (*n=7*, ±SD) in luncheon samples from different sources. Columns with the same litter are insignificantly differed ($p > 0.05$)

The maximum and minimum level of coliform counts were 3.87 ±0.7 and 1.49 ±0.6 with an average of 2.61 ±0.5 Log_{10} cfu /g. Samples from LU02 and LU08 sources only are the samples in which the total number of coliforms was less than the limits allowed in the Egyptian standard specifications (**EOSQ, 2005**), which stated that the permissible limit of total coliform count was 10^2 cfu/g. Generally, the presence of coliforms in meat products indicates that the hygiene requirements necessary to ensure the safety of these products are not applied. In this regard, **(Synge et al., 2013)** stated that 80% of the beef luncheon samples collected from Giza city contained coliform. Also, (**Abd-Allah and Ismail ,2012)** indicated that 100% of semi-dry Egyptian salted meat basterma samples collected from the local markets in Assiut city contained coliform.

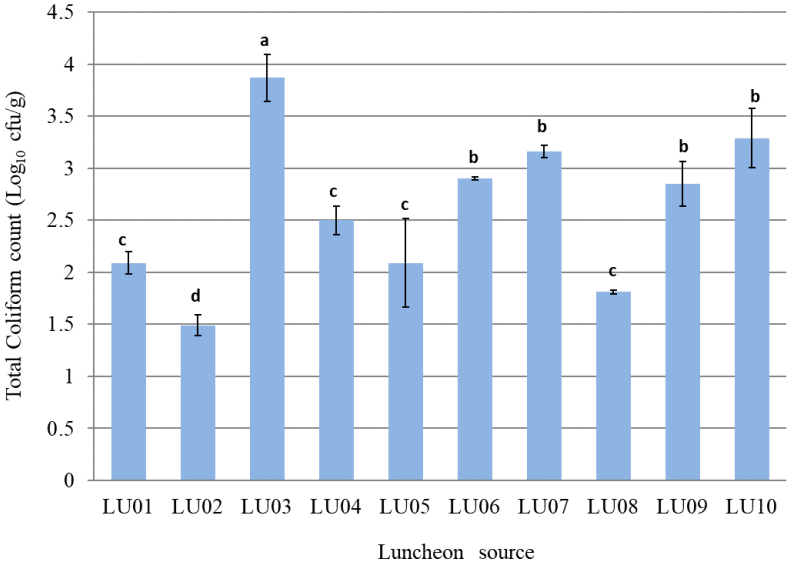

Figure 3: Total coliform count (*n=7*, ±SD) in luncheon samples from different sources. Columns with the same litter are insignificantly differed ($p > 0.05$)

Staphylococci are found naturally on the skin of humans and animals, as well as in festering wounds, and are considered part of the normal flora of the human nose. Therefore, its presence in meat products indicates its arrival through the environment, especially humans, represented by manufacturers and dealers of these products. **(Shawish, et al., 2016)** Figure 4 showed that the *Staphylococcus sp.* count in the collected beef luncheon samples ranged between 2.0 ±0.22 and 3.74 ±0.23 with a mean value of 2.58 ±0.40 Log_{10} cfu /g. These values were in harmony with those mentioned by **Acco, et al., (2003).** According to the **EOSQ (2005)**, beef luncheon should be free from *Staphylococcus*.

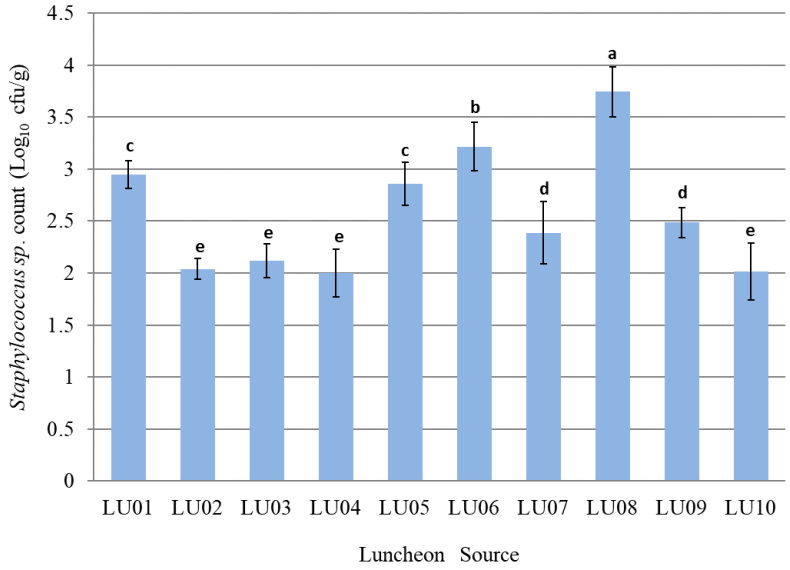

Figure 4: *Staphylococcus* sp. count (*n=7*, ±SD) in luncheon samples from different sources. Columns with the same litter are insignificantly differed ($p > 0.05$)

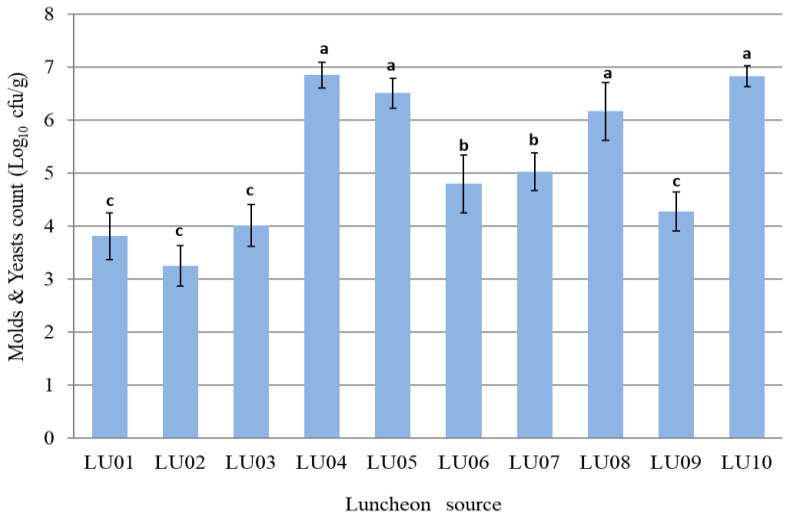

Figure 5: Yeast and Mold count (*n=7*, ±SD) in luncheon samples from different sources. Columns with the same litter are insignificantly differed ($p > 0.05$).

The yeasts and molds count of the collected beef luncheon samples ranged between 3.52 ±0.23 and 6.85 ±0.23 with an average of 5.15 Log_{10} cfu /g. The samples containing the lowest yeast and mold counts are LU01, LU02, LU03 and LU09. Referring to Table2, it can be noticed that these samples contain natamycin as preservative act as antifungal agent. Generally, all tested samples exceeded the permissible limit recommended by **EOSQ (2005)** which stated that the permissible limit of mold and yeast count was 10^2 cfu/g.

Salmonella /*Shigella* sp., *Campylobacter* sp., and *Listeria* sp. Did not detected in all tested samples. *Bacillus cereus* was detected in LU01, LU02, and LU03 by ratio of 85.7, 85.7, and 100%, respectively,

Table 3. Detection ratio of pathogenic bacteria in all the beef luncheon sources.

Pathogenic bacteria	% detection rate									
	LU01	**LU02**	**LU03**	**LU04**	**LU05**	**LU06**	**LU07**	**LU08**	**LU09**	**LU10**
***Salmonella /Shigella* spp.**	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	85.7	85.7	100	-	-	-	-	-	-	-
***Campylobacter* spp.**	-	-	-	-	-	-	-	-	-	-
***Listeria* spp.**	-	-	-	-	-	-	-	-	-	-

(-) not detected under studied conditions.

CONCLUSION

From the results obtained in this study, the beef luncheon meat that is traded in the study areas in Cairo is acceptable from the perspective of nutritional value, where the percentages of moisture, protein, and fat were within the permissible limits of the Egyptian standard specifications. The preservative content of the studied beef luncheon was within the limits allowed by the Codex organization, except for 40% of the samples in terms of potassium sorbate and natamycin content. Concerning the microbiological quality, the studied luncheon meat was proven to be free of *Salmonella* /*Shigella* sp., *Bacillus cereus*, *Campylobacter* sp., *Listeria* sp. However, the content of the studied samples of total aerobic bacteria, coliforms, *Staphylococcus*, yeasts, and molds was not satisfactory, as the results recorded higher values than the permissible values in most of the samples.

Therefore, the present study recommends the importance of applying the rules of sanitation and personal hygiene and maintaining good manufacturing practices during all production periods. The study also confirmed shedding light on the extreme danger of street food, as the current study demonstrated that beef luncheon samples from unknown brands had an unacceptable and very low microbiological quality compared to commercial brands' luncheons. Therefore, the National Food Safety Authority must consider the establishment of mandatory requirements for producers and handlers of meat and meat products, especially luncheon meat.

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