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QUANTIFICATION OF THE CONTAMINATION OF SOME MEAT AND POULTRY PRODUCTS SOLD IN SHARKIA GOVERNORATE

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ABSTRACT

During the period , June to November 2008 the microbiological and chemical quality and the hygiene of some meat and poultry products, ready-to-eat (RTE) sold and consumed in Zagazig city were evaluated. A total of 108 local meat and poultry samples produced by 3 national Companies (36 from fresh sausage, 18 from chicken luncheon, 36 minced meat, and 18 from chickens) were tested. The microbiological quality was determined by total aerobic viable counts, total mesophilic and psychrophilic bacteria, total coliform, and faecal coliform, molds, yeasts, and pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*).

The numbers of total fungi, aerobic bacteria and *Staphylococcus aureus* in Halwany chicken luncheon gave 2×10^2 fp/g , 3×10^6 and 5.2×10^2 cfu/g, respectively, which were higher than the limit set by the Egyptian Standards (ES), and free from faecal coliform.

Halwany and Americana sausage showed higher counts for *Staphylococcus aureus*, total coliform, and aerobic bacteria exceeding the ES limits. Halwany sausage was better than Americana concerning faecal coliform and *Staphylococcus* spp. while the later showed higher values for fungi, yeasts psychrophilic, and mesophilic bacteria. Minced meat products found to harbour considerable densities of microorganisms, Halwany and Americana gave almost the same values which exceed the ES. Chilled chicken from Sharkia Chicken Company gave high total coliform reached 19 times of the acceptable level and the aerobic bacteria reach 3.5×10^7 cfu/g .

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All meat products were free from *Listeria monocytogenes* as well as faecal coliform except Americana sausage (50% positive for faecal coliform). *Salmonella* spp. were detected in five meat products and only Halwany minced meat was free. Higher protein content was recorded in chicken samples, while the lower was found in sausage, with a pH values ranged between 5.2-6.1.

The fat content in all meat samples was lower than ES, with less moisture content, 50.28% for Halwany luncheon while it reached 70.2% in chicken. Most products tested are not acceptable according to the presence of *Salmonella* spp., *Staphylococcus aureus*, and heavy load of coliform and faecal coliform counts. So, it must be rejected before distributed to the consumers. The results obtained indicate the need to improve the processing and handling of these products, to meet the ES requirements.

Keywords: Meat, poultry, food poisoning, pathogenic microorganisms, *Salmonella*, *Listeria*, *Staphylococcus*.

INTRODUCTION

The study of the microbial quality of some meat and poultry ready-to-eat products is important from the public health point of view. Since, meat and chicken products are subjected to contaminate with saprophytic as well as food-poisoning and/or pathogenic microorganisms. Contamination may occur during slaughter, handling, processing, packaging, storing,.....etc. This indicates that such products are highly perishable.

Foodborne pathogens have been estimated to cause 76 million illness and approximately 9000 deaths each year (Mead *et al.*, 1999). Food safety is one of the most important issues marketing any kind of food, meat and poultry products are no exceptions. Processed raw beef and poultry meat naturally harbor bacteria, most of these bacteria are responsible for their spoilage, but are not pathogenic to humans. However, these products can harbor bacteria capable of causing human disease and/or food poisoning. A number of foodborne pathogens have been isolated from these, *Salmonella* serotypes, *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter jejuni* which are of a major concern.

The aerobic bacterial load has been studied in chickens (Kotula and Pandya, 1995) was 8 log10/cfu/g, while the number in meat food samples (Stagnitta *et al.*, 2006) ranged between 10^3 to 10^6 cfu/g, and in ready- to- eat foods (Mosupye and Holy, 1999) was 3.4 (±0.4) log_{10} cfu/g). Regarding the food-poisoning or the pathogenic microorganisms the acceptable level of *Salmonella* spp., *Listeria monocytogenes* was reported by Legnani *et al.* (2004) to be zero % of the samples.

Of the 102 samples of foods of animal origin **Pacini *et al.* (1996)** found that meat and meat products had the highest incidence of contamination with different pathogens, *L. monocytogenes* was the main pathogen occurring in meat and meat products.

The objective of the present studies was to determine the occurrence and levels of pathogenic and non-pathogenic microorganisms present in meat products sold and consumed in Sharkia governorate.

MATERIALS AND METHODS

Sampling

In this study, different meat and poultry products were obtained from Zagazig city supermarkets, Sharkia governorate, Egypt. One hundred and eight samples of meat and poultry products i.e, ready-to-eat (RTE) locally produced by 3 national Companies i.e, Halwany minced meat; Americana minced meat; Halwany sausage; Americana sausage; Halwany chicken luncheon and Sharkia Company Chickens were randomly collected between 1st June 2008 and 31st November 2008. These samples were aseptically kept in sterile polythene pouches, sealed and transported in ice packs then chemically and microbiologically analysed.

Experimental Approaches

Twenty five grams of each homogenized sample(or other wise stated) were separately transferred to a 500 ml Erlynmayer flask containing 225 ml of (1%) sterilized peptone water (Difco, 1989), thoroughly mixed and serial dilutions were prepared except for *Listeria monocytogenes* detection where only 1 g sample size was deemed to be representative of the sample. Appropriate dilutions from each of the prepared samples were inoculated into the appropriate nutrient and selective media. Enumeration was carried out using three replicates from each dilution. The samples were tested for

hazardous microorganisms (*Salmonella* spp., *Listeria monocytogenes and Staphylococcus* spp.), spoilage microorganisms (proteolytic bacteria, lipolytic bacteria, fungi and yeasts), and for total aerobic viable counts, total mesophilic bacteria, psychrophilic bacteria, total coliforms and faecal coliforms in addition to the chemical analyses

Microbiological Analyses

Appropriate dilutions prepared from each sample were used for inoculating various nutrient and selective media. The microbial determinations applied were: total aerobic viable counts, total mesophilic bacterial counts, and psychrophilic bacterial counts were estimated using glucose yeast extract nutrient-agar medium (Difco, 1989). Plates were counted after incubation at 37° C for 48 h, at 28° C for 48 h and at 5° C for 5-7 days for counting total aerobic viable counts, total mesophilic bacterial counts, and psychrophilic bacterial counts, respectively.

Total coliform and faecal coliform counts were estimated on MacConkey-Agar (Difco, 1989) using pouring plate technique. Suitable plates were counted after 24 h at 37°C and 44.5°C for total coliform and faecal coliform counts, respectively.

The numbers of *Staph. aureus* were determined on Baird Parker-Agar Medium (Baird Parker and Devenport, 1965) and the plates were incubated at 37°C for 48 h. representative colonies on a suitable plates were transferred to Nutrient- Agar slants and the cultures were subjected to the following tests: Gram staining, catalase production (Baily and Scott, 1962), sugar fermentation and coagulase production (Morrison *et al.*, 1962).

Searching for *Salmonella* spp. 25 g of each sample were inoculated into two pre-enrichment media namely peptone water and tetrathionate broth (Difco, 1989) after incubation for 24 h at 37 °C each. Then the resulting colonies were streaked on Difco Brilliant Green - Agar plates and examined after 18-25 h (Georgela & Boothroyd, 1965 and Khan & McCaskey, 1973) where presumptive *Salmonella* spp. appear as pink colonies surrounded by bright red medium. For purification, colonies were examined and streaked on MacConkey-Agar plates and Difco Triple Sugar Iron-Agar slants and

incubated at 37°C for 18-24 h. After being isolated and purified, suspected *Salmonella* spp. isolates were subjected to the following biochemical confirmation: Gram staining, catalase production, lactose fermentation, H_2S production

For the enrichment of *Listeria monocytogenes, Listeria* Enrichment Broth (UVM1; Oxoid, 1998) as a primary enrichment followed by transferring to *Listeria* Enrichment Broth (UVM2; Biolife, 1991). Isolation was performed using Palcam-Agar supplemented by *Listeria* Palcam supplement (Biolife, 1991) by a loopful from UVM2 streaked onto Palcam-Agar and incubated at 30°C for 48 h (Vannetten *et al.*, 1989). Five typical colonies (dark brown or black with brown halo) were picked up, streaked onto Trypticase Soy Agar supplemented with 0.6% yeast extract and incubated at 37°C for 24 h. Pure separate colonies were inoculated into tubes of Trypticase Soy Broth supplemented with 0.6% yeast extract and incubated at 37 °C for 24 h. The presumptive *Listeria* colonies brown green coloured colonies with a black halo were subjected to the following biochemical confirmation tests: Gram staining, catalase production, urease and suger fermentation.

Proteolytic and lipolytic bacteria were estimated on milk agar and butter-fat agar, respectively (James and Natalie, 1987).

Yeasts and Fungi were counted according to (YGCB-Agar, Perkoppovà, 1984) and (Baruah and Barthakur, 1997), respectively.

All foodstuff samples under investigation were subjected to chemical determinations including the following parameters, pH-values , moisture content , protein content according to A.O.A.C. (2000) while, fat content was determined according to A.O.A.C. (2002).

RESULTS AND DISCUSSION

Microbial Counts

Fig. 1 shows that ten different microbial counts of six meat and chicken products sold and consumed in Sharkia governorate. These meat products were three Halwany products namely Halwany chicken luncheon (Fig.1.a), Halwany sausage (Fig.1.c) and Halwany minced meat (Fig.1.e). In addition to the previous company there are two

different products from Americana company (Americana sausage {Fig.1.d} and Americana minced meat {Fig.1.f}) and one more chicken product from Sharkia Chicken Company (Fig.1.b) were used.

Nowadays, food and any additives are more strictly regulated than any before. In the United States, the Food and Drug Administration (FDA) is in charge of the approval of any additive used in food, whilst the European counterpart is the Scientific Committee for Food (SCF). Here, the Egyptian Standars (ES) issued by the Egyptian Organization for Standard and Quality (EOSQ, 2005) is the authority responsible for food safety. The EOSQ also takes into account in its legislation the recommendations made by other international regulatory bodies, such as the Codex Alimentaries Commission (CAO) and the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (La Vecchia, 1998). Our results will be compared with the EOSQ 2005, regulations.

Concerning chicken luncheon obtained from Halwany Company (Fig.1.a), three different microbial counts fungi, *Staph. aureus* and aerobic bacteria were higher than those recommended by Egyptian Standards (ES), since the counts were 2×10^2 fp/g , 5.2×10^2 cfu/g and 3.0×10^6 cfu/g , respectively. These counts should be zero in both fungi and *Staph. aureus* while, only 10^4 cfu/g in case of aerobic bacteria according to the ES . These results were nearly in agreement with those obtained by Zahran *et al.* (2008) who found that the total aerobic counts for chicken luncheon were 1.33×10^7 cfu/g. On the other hand, Halwany chicken luncheon was free from faecal coliform and recorded only 1.7×10^2 cfu/g for total coliform. The recommended ES for total coliform in chicken luncheon was 10^2 cfu/g which was lower than those obtained in this study.

The results presented in Fig. 1-b show the microbial counts in chilled chicken obtained from Sharkia Chicken Company. It was conspicuous that the numbers of *Staph. aureus* , faecal coliform , total coliform and acrobic bacteria were 2.1×10^2 , 0 , 1.9×10^3 and 3.5×10^7 cfu/g , respectively. Faecal coliform was absent while total coliform show higher numbers (19 times) the acceptable level (1.0×10^2 cfu/g). These results were comparable with those

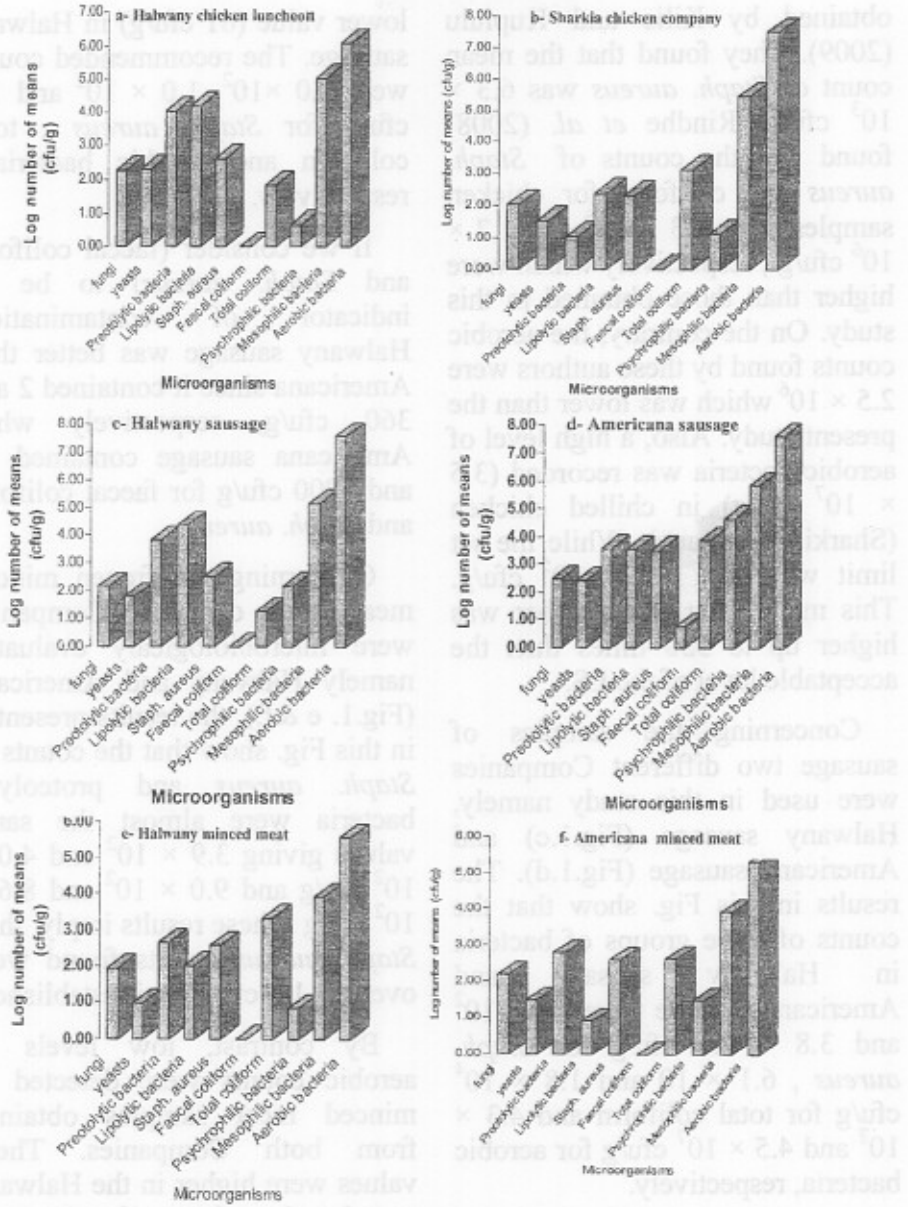

Fig. 1. Microbial counts in different meat products common in Sharkia governorate collected between June and November 2008

obtained by Kilic and Kuplulu (2009). They found that the mean count of *Staph. aureus* was 6.3×10^3 cfu/g. Rindhe *et al.* (2008) found that the counts of *Staph. aureus* and coliform for chicken samples were 1.3×10^6 and 1.7×10^6 cfu/g , respectively which were higher than those obtained in this study. On the contrary, the aerobic counts found by these authors were 2.5×10^6 which was lower than the present study. Also, a high level of aerobic bacteria was recorded (3.5×10^7 cfu/g) in chilled chicken (Sharkia Company). While the set limit was only 1.0×10^5 cfu/g. This means that this number was higher up to 350 times than the acceptable level of the ES.

Concerning the samples of sausage two different Companies were used in this study namely, Halwany sausage (Fig.1.c) and Americana sausage (Fig.1.d). The results in this Fig. show that the counts of three groups of bacteria in Halwany sausage and Americana sausage were 3.6×10^2 and 3.8×10^3 cfu/g for *Staph. aureus* , 6.1×10 and 1.8×10^4 cfu/g for total coliform and 4.3×10^7 and 4.5×10^7 cfu/g for aerobic bacteria, respectively.

It seems that all of these values were almost higher than those recommended by ES except in the case of total coliform which gave a lower value (61 cfu/g) in Halwany sausage. The recommended counts were 1.0×10^2, 1.0×10^2 and 10^6 cfu/g for *Staph. aureus* , total coliform and aerobic bacteria , respectively.

If we consider (faecal coliform and *Staph. aureus*) to be an indicator of contamination, Halwany sausage was better than Americana since it contained 2 and 360 cfu/g, respectively while Americana sausage contained 16 and 3800 cfu/g for faecal coliform and *Staph. aureus*.

Concerning the frozen minced meat, two different Companies were microbiologicaly evaluated namely Halwany and Americana (Fig.1. e &f.), the results presented in this Fig. show that the counts of *Staph. aureus* and proteolytic bacteria were almost the same values giving 3.9×10^2 and 4.0×10^2 cfu/g and 9.0×10^2 and 8.6×10^2 cfu/g .These results imply that, *Staph. aureus* counts found were over the 10^2 cfu/g limit established.

By contrast, low levels of aerobic counts were detected in minced meat samples obtained from both Companies. These values were higher in the Halwany samples than those of Americana samples giving 5.6×10^5 cfu/g and 2.1×10^5 cfu/g, respectively. Total aerobic counts found in this study

were below 10^6 cfu/g limit. Counts of mesophilic aerobic counts were lower than those obtained by Salihu *et al.* (2010) they found that the total mesophilic aerobic bacteria counts from 216 samples of ground beef were 4.5×10^9 cfu/g .

In case of lipolytic bacteria it was also conspicuous from Fig. (1.e and f) that the counts of this bacteria were higher in Halwany minced meat giving 6.4 times than that of Americana minced meat. On the other hand, Americana minced meat samples showed higher counts in psychrophilic bacteria counts giving 12.7 times over than those of Halwany minced meat.

The results presented in Table 1 show the prevalence of different microbial groups in six meat products. It is obvious that all meat products under this investigation were free from *L. monocytogens* as well as faecal coliform expect Americana sausage samples which gave 50% positive results with faecal coliform. This result was in agreement with those obtained by Mangia *et al.* (2006) they found that no count of *Listeria* in sausage samples. On contrary the present results were disagree with those obtained by Ryu *et al.* (1992) they reported that *L. monocytogenes* occurred in 34% of beef sausage sample in Japan.

Similar results were obtained by Soriano *et al.* (2001) who reported that *L. monocytogenes* was not detected in any of beef product samples under investigation. Also Zahran *et al.* (2008) found that *L. monocytogenes* counts were undetectable (<100) in minced meat, these results support our findings.

On the contrary, *Staph. aureus* was detected in all meat products used in this study with a percentage of 100%. Abd El- Aziz (1987) found that 80 % of examined beef sausage samples were positive for *Staph. aureus* and these results were lower than those found in this study. Moreover, Ranucci *et al.* (2004) found that *Staph. aureus* was isolated in 47.1% of the sausage samples.

A variable percentages of total coliform as well as *Salmonella* spp. were observed in the forementioned meat products. It was also conspicuous from Table 1 that the presence of total coliform was in the range between 66.7% for Halwany sausage to 100% for either chilled chicken, Halwany minced meat or Americana minced meat.

Table 1. Percentage of samples contaminated with food poisoning and / or pathogenic bacteria

Test / Samples	Halwany chicken luncheon	Sharkia Company chicken	Halwany sausage	Americana sausage	Halwany minced meat	Americana minced meat
Total coliform	83.3	100	66.7	83.3	100	100
Faecal coliform	0	0	0	50	0	0
Staphylococcus aureus	100	100	100	100	100	100
L. monocytogenes	0	0	0	0	0	0
***Salmonella* spp**	16.7	16.7	33.3	33.3	0	16.7

Table 2. Chemical analysis of some meat products collected between June and November 2008

Test / Samples	Protein %	Moisture %	MPR	Fat %	pH value
Halwany luncheon	19.38	50.28	2.59	8.73	5.9
Sharkia Company Chicken	23.36	72.20	3.10	0.93	6.1
Halwany sausage	15.47	61.47	3.98	21.39	5.3
Americana sausage	14.40	63.60	4.45	20.48	5.2
Halwany minced meat	16.78	67.30	4.03	14.39	5.3
Americana minced meat	17.19	66.17	3.86	13.68	5.5

MPR[1] : Moisture protein ratio.

The percentage of samples contaminated with *Salmonella* spp. were ranged between 0% for Halwany minced meat to 33.3% for either Halwany sausage or Americana sausage. Therefore five products from 6 would be consider to be contaminated with *Salmonella* spp. with a variable proportions and only one product (Halwany minced meat) was free from *Salmonella* spp.

Our results were higher than those obtained by Matticke *et al.* (2002) who found that 7.5% of 53 frozen beef sausage samples were contaminated with *Salmonella* spp. While Simmons *et al.* (2003) reported that all 20 frozen chicken samples examined were negative for *Salmonella* spp. Also, Cohen *et al.* (2007) found that *Salmonella* spp. was detected in 2.1% of chicken meat.

Concerning chemical analysis of some meat products, the data in Table 2 revealed that the higher protein content was found in chicken samples obtained from Sharkia Campany Chickens, while the lower content of protein was found in sausage samples obtained from Americana Company. On the other hand protein content of Halwany sausage was higher (15.47%) than that of Americana sausage (14.40%) as well as than that it was established by ES (15%).

The moisture content (%) of the six meat products under investigation it was clear that the lowest content of moisture (50.28%) was detected in Halwany luncheon while the highest moisture (72.2%) was found in chilled chicken (Sharkia Company). Moreover, the moisture content in Halwany luncheon was also lower than that given by the standard specifications (Table, 2).

In addition, Halwany sausage and Americana sausage recorded moisture content of 61.47% and 63.60%, respectively. Similar results were obtained by Reyes-Cano *et al.* (1994) they reported that the content of moisture of Guerrero, Distrito Federal, Morelos and Oaxaca beef Cecina productions were 61.5%, 62.9%, 64.5 % and 64.7%, respectively.

In the case of minced meat, no difference between Americana and Halwany companie was detected. Both products have almost the same content of moisture giving 67.30% and 66.17% in Halwany and Americana products, respectively. These levels of moisture contents were lower than that of ES (70%).

The data in Table 2 reveal also that the values of pH were ranged between 5.2 to 6.1 in the six different meat products under investigation. The highest pH values was obtained with chilled chicken, while the lowest value was found with Americana sausage. Mangia *et al.* (2006) found that pH value ranged from 5.59 to 5.76, these values were nearly comparable with the present results.

Concerning the fat content, it was obvious that fat content was as lower as 8.73% in Halwany luncheon. This value was lower than that recorded by ES which it should be 30% when the product was free from any extenders while it should be 35% when it combined with extenders. Also, fat content in sausages were 21.39% and 20.48% for Halwany and Americana Companies, respectively. These values were below the established limit which was 30%, while these results nearly agree with those found by Aly (2006) who reported that moisture, protein and fat content were 70.1%, 15.7% and 21.1%, respectively.

Fat content in minced meat were 14.39% and 13.68% in Halwany and Americana Companies. These results were not stuffed with several fats to increase the weight of the expected since most of minced meat product. These results were less than those mentioned by the standard limit i.e., 20%.

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التقدير الكمي لتلوث بعض منتجات اللحوم والدواجن المباعة بمحافظة الشرقية

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قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق

تم تقدير الجودة الميكروبية والكيميائية والصحية لبعض المنتجات (اللحوم والدواجن) الجاهزة للاكل والتي تباع في مدينة الزقازيق وذلك خلال الفترة من يونيو الي أغسطس لسنة ٢٠٠٨ . وتم اختبار ١٠٨ منتج محلي للحوم والدواجن والتي انتجت بواسطة ٣ شركات (٣٦ عينة سجق ، ١٨ عينة لانشون فراخ ، ٣٦ عينة لحم مفروم ، ١٨ عينة فراخ).

لقد تم تحديد الجودة الميكروبية من خلال تقدير العدد الكلي للبكتيريا الهوائية الحية، والبكتيريا المحبة لدرجات الحرارة المتوسطة والمحبة للبرودة وبكتيريا مجموعة القولون وبكتيريا القولون البرازية وكذلك الفطريات والخمائر الي جانب تقدير البكتيريا المرضية المتمثلة في *Staphylococcus* spp. ، *Salmonella* spp. ، *Listeria monocytogenes*.

ولقد وجد أن العدد الكلي للفطريات والعدد الكلي للبكتيريا والعدد الكلي لبكتيريا *Staphylococcus* في لانشون الفراخ الخاص بشركة حلواني كانت ٢ × ١٠^{٢} ، ٣ × ١٠^{٦} ، ٥,٢ × ١٠^{٢} خلية /جرام علي التوالي وهذه الأعداد كانت أعلي من تلك الموجودة في المواصفات المصرية القياسية للانشون . اما بالنسبة للسجق امريكانا وحلواني فكان العدد الكلي لبكتيريا *Staphylococcus* وبكتيريا مجموعة القولون والعدد الكلي للبكتيريا الحية أعلي من المسموح به في المواصفات القياسية المصرية .

أيضا كان العدد الكلي لبكتيريا *Staphylococcus* والقولون البرازية لسجق حلواني أقل من سجق امريكانا ، وكان العدد الكلي للفطريات والخمائر وأيضا البكتيريا المحبة لدرجة الحرارة المتوسطة والبكتيريا المحبة للبرودة ذات أعداد عالية بالنسبة لسجق امريكانا. ولقد تجاوزت اعداد الميكروبات لمنتج اللحم المفروم حلواني وأمريكانا عن الموجودة في

المواصفات القياسية المصرية وكانت اعداد بكتيريا القولون في الفراخ المنتجة من الشركة الشرقية للدواجن تعادل ١٩ مرة أعلي من المستويات المقبولة وكان العدد الكلي للبكتيريا هو ٣,٥ × ١٠^{٧} خلية /جرام .

كما وجد ان كل منتجات اللحوم خالية من بكتيريا *Listeria* وبكتيريا القولون البرازية فيما عدا سجق امريكانا حيث كان ٥٠% من المنتج يحتوي علي بكتيريا القولون البرازية . ولقد تم تحديد بكتيريا *Salmonella* في ٥ منتجات بينما لحم مفروم حلواني كان خاليا من بكتيريا *Salmonella* وكانت أعلي نسبة من البروتين في الفراخ واقلها في السجق وتتراوح قيمة pH من ٥,٢- ٦,١ وكان محتوي الدهون في جميع العينات اقل من المواصفات القياسية المصرية وانخفضت الرطوبة الي ٥٠,٢٨% في اللانشون بينما وصلت الي ٧٠,٢% في الفراخ.

كان اختبار معظم المنتجات غير مقبول نظرا لوجود *Salmonella* spp ، *Staphylococcus* ،ومحتواها العالي من بكتيريا مجموعة القولون ومجموعة القولون البرازية ولذلك يجب رفضها قبل توزيعها للمستهلكين وبالتالي تشير النتائج المتحصل عليها إلى ضرورة تحسين كل من عمليات الإنتاج والتداول لهذه المنتجات نظراً لعدم مطابقتها للمواصفات القياسية المصرية .